SPATIAL AND TEMPORAL INFLUENCES ON THE PHYSIOLOGICAL CONDITION OF INVASIVE SILVER CARP

BY

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THESIS

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ABSTRACT

Bighead (*Hypophthalmichthys nobilis)* and silver carp (*H. molitrix*) (hereafter, Asian carp) are invasive species that have the potential to negatively influence non-native freshwater systems. Following their introduction to the United States in the early 1970s, Asian carp have become established in the Mississippi River Basin, which includes the Illinois, Ohio, and Wabash rivers, and their range is expanding. The establishment of Asian carp has the potential to negatively influence the community structure of native species. For example, Asian carp are efficient, filter-feeding planktivores that may negatively affect native obligate or facultative planktivorous fishes. Currently, factors motivating or controlling the spread of Asian carp have not been well defined, and little is known about the potential consequences of recently discovered hybrids between bighead and silver carp. Improving our understanding of how Asian carp interact with their environment can help provide drivers determining their movement and spread, and what may happen should Asian carp spread into new habitats.

The overall goal of my thesis was to test for abiotic and biotic factors that influence stress and nutrition in wild-caught silver and bighead carp across spatial and temporal scales. To accomplish this goal, I performed three distinct field studies involving wild silver carp, bighead carp, and their hybrids. For each of my studies, I sampled blood from wild-caught silver and bighead carp from large rivers in Illinois (Illinois, Mississippi, Ohio, and Wabash). Blood samples were analyzed for metrics of stress and nutrition that provide insights into the health and condition of free-swimming fish sampled across different habitats. My results suggested that silver carp nutrition varied across rivers and time periods, stress varied across time periods, and there were few effects of abiotic and biotic factors on silver carp nutrition. My results also

showed that hybrid Asian carp nutrition was intermediate between parental silver and bighead carp. In concert, my research suggests that silver carp spread may be limited by factors at broad spatial and temporal scales, as opposed to local abiotic and biotic interactions, and decreased hybrid nutritional status may further limit Asian carp spread.

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To my family and friends

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CHAPTER 1

GENERAL INTRODUCTION

When examined at a broad spatial scale, there are a number of elements that contribute to our understanding of community structure and dynamics at a particular location (Chase & Leibold, 2003; Hubbell, 2001; Tilman, 2004; Tonn, 1990). Community structure can be shaped by a series of filters as local assemblages are affected by processes such as space and time, which may act at larger scales (Tonn, 1990). At smaller scales, limiting resources (i.e., food and habitat) can be a driver of competition and predation (Tonn & Magnuson, 1982). Another factor of community structure includes differences among species and among compositional groups within niches. These factors are important for determining an individual's specific role in a system, the biodiversity within a given habitat, and the outcome of species interactions, as shown by species' abundances and/or distributions (Chase & Leibold, 2003; Tilman et al., 1997). Collectively, a number of different components have been encompassed to describe community structure, dynamics, and the complex interactions and interrelationships among species (Tonn, 1990).

Complex interactions determining community structure may be also be influenced by diet and habitat (Chase & Leibold, 2003; Tilman, 2004; Werner & Gilliam, 1984). Food is a critical limiting resource that provides nutrients and energy (Werner & Gilliam, 1984; Wootton, 1998). Time is another limiting resource that is required for reproduction, shelter from unfavorable physical conditions, and evasion from predators (Wootton, 1998). Together, these can affect an individual's preferred niche and habitat choice. Niche preference can differ throughout an individual's life as habitat and desired food change, body size increases (Werner & Gilliam,

1984), and species interactions may become complex in a community where niche preferences are plastic. The structure of a community can also change due to intra- or inter-specific competition. Diet and habitat shifts are not the only processes that affect community dynamics; species distributions are also affected by processes that occur at greater spatial and temporal scales (Tonn, 1990).

Processes that dictate community structure at a particular location are often in a state of non-equilibrium. Community interactions are driven by several processes at local levels (i.e., limiting resources) and at greater spatial and temporal scales (i.e., climate change, physiological and life history adaptations, richness and composition of existing assemblages) (Tonn, 1990). There is an important distinction between regional and local spatial scales. For example, climate change can occur at a regional spatial scale, which may cause a shift in limiting resources by reducing food availability and/or altering habitat, forcing organisms to find another niche (Tonn, 1990). Species abundance and distribution may also vary at regional and local scales, depending upon existing species and niche availability, with some communities shaped by the organisms present, and others being controlled by the environment (Tonn, 1990). While species richness is linked to local and regional spatial scales, it is unknown from which spatial scale it originates (Cornell & Lawton, 1992). A particular location can give a snapshot of the interactions of the community at a specific point in time, but to understand all of the processes that affect species assemblages and structure, several observations need to be made. Community interactions are complex and fluid, therefore it is critical to examine the processes affecting assemblages at several local locations across a broad spatial and temporal extent, as species interactions may change at different scales or across seasons.

Temporal variation across large rivers

Large river ecosystems are intriguing and understudied when addressing questions about community structure, as they experience substantial seasonal variation that may play a role in influencing community dynamics. For example, flooding events, seasonal water temperature variation, and photoperiod can all impart changes on riverine ecosystems within a given year (Lowe, Likens, & Power, 2006). Annual floods affecting flow regimes can alter habitat suitability, food availability, nutrient cycling, and production processes. Seasonal temperature changes are also typically concomitant with flooding events, and act as a trigger for many fish species to spawn (Bayley, 1995). Temperature may vary considerably along environmental gradients based on channel morphology and longitudinal position along the reach within the river or stream (Schlosser, 1990). Together, the natural, seasonal variation that rivers exhibit can act as important drivers of community structure, and are particularly important when considered in conjunction with changes to river habitats initiated by anthropogenic processes.

Humans have long been modifying and disturbing large river-floodplains for their benefit, so much so that it is often difficult to recognize the original processes that regulated systems (Bain, Finn, & Booke, 1988; Bayley, 1995; Koel & Sparks, 2002). Through practices such as levying or impounding for commercial navigation, power generation, flood control, or agriculture purposes, modifications have been made to channel morphology, flow regimes, and physical-chemical attributes, with many parameters showing high inter-annual variation (Schlosser, 1990). Because of this, it is important to use multiple time points when studying large rivers due to their unpredictability. It is also interesting to note that species exhibiting r-selected life-history characteristics (i.e., high annual growth and mortality rates) have evolved to quickly colonize large areas, such as river-floodplains. The variability of the flood regime may

make r-selected species successful, as those species can adapt to human alterations and inter-annual changes (Bayley, 1995). As humans continue to modify natural river ecosystems, this may alter niches to allow for an increase in the establishment potential of invasive species.

Invasive species

The introduction of nonnative species can create serious ecological effects, which may have negative consequences for communities within river ecosystems (Kolar et al., 2007; Pimentel, Zuniga, & Morrison, 2005; Ricciardi & MacIsaac, 2011). Invaders previously shown to cause the strongest ecological influences include those with the following characteristics: generalist predators or omnivores; those with the capability to reach high abundances and are highly fecund; those introduced to systems where functionally similar species do not exist; and those with a history of strong influences on other invaded regions (Ricciardi & MacIsaac, 2011). Invasive species can impart change to native animals by disrupting community structure through cascading impacts on food webs, either bottom up or top down. For example, threadfin shad (*Dorosoma petenense*) were introduced to a California lake and caused a diet shift for three species of native planktivores, from zooplankton to nearshore benthos (Ricciardi & MacIsaac, 2011). Invasive grass (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) have negatively affected large rivers from the bottom up by reducing natural aquatic vegetation and reducing water quality, respectively (Pimentel et al., 2005). Ecosystem changes caused by invasive species have been implicated in the risk status of about 400 of the 958 species listed as endangered or threatened under the Endangered Species Act, with 44 native fish species included on that list (Pimentel et al., 2005). The declines in native species due to the community alterations caused by exotic organisms may lead to their extirpation or extinction (Gurevitch &

Padilla, 2004; Pimentel et al., 2005). Other influences of non-native species include ecological effects, predation, disease transfer, competition, habitat modification, and hybridization (Ricciardi & MacIsaac, 2011).

Invasive species also have the potential to spread well beyond their point of introduction, possibly influencing communities at broad spatial scales. According to Neubert and Parker (2004), the faster an exotic species disperses across the landscape, the more dangerous the invasion is to host communities. As such, determining factors that influence or drive the spread of invasive species can have implications for control, spread, impact, and resource allocation for managers working to contain or control invasive species (Arim, Abades, Neill, Lima, & Marquet, 2005; Crowl, Crist, Parmenter, Belovsky, & Lugo, 2008; Neubert & Parker, 2004). Currently, the process of invasion has been demonstrated to follow a similar sequence of events, regardless of the taxa of the invader (Arim et al., 2005). First there is an initial establishment (low spread), followed by an expansion phase (increasing spread rates), and finally a saturation phase where the rate of spread plateaus (Arim et al., 2005), with propagule pressure acting as an important predictor of invasion success (Leung & Mandrak, 2007). However, the underlying processes controlling the rate of dispersal of individual invasive species is often not well defined. Factors accelerating the rate of invasive species expansion include increases in anthropogenic control over transport capacity and economic globalization (García-Berthou, 2007; Hulme, 2009). This allows for exotic species to establish beyond their native range, with the potential to become numerically and ecologically dominant compared to native populations (Carroll, 2007). Invasive species may not have a natural control (i.e., no natural predators), resulting in negative effects on native species (Shea & Chesson, 2002). Without a natural predator, the availability of food may be altered or depleted, at which point an organism (either native or invasive) may be forced to

disperse (Hansson, 1991). In addition to factors that drive the rate of spread, there are some abiotic factors that potentially interact simultaneously, creating confounding results. One example is physical habitat, such as temperature, which may inhibit or promote the growth of an invader as it varies seasonally and spatially (Shea & Chesson, 2002). Another factor that may prevent the spread or negative effects of invasive species is biodiversity: the greater the species diversity in a community, the less susceptible it may be to invaders (Stachowicz, Whitlatch, & Osman, 1999). Greater species richness has positive effects on biomass accumulation and overall resource use across trophic levels, resulting in little change in those responses over time and improved ecosystem resiliency (Duffy, 2009). While several potential drivers or limiters of invasive species spread exist, there are still unknowns about the ecological and evolutionary processes that allow for invasion (Hastings et al., 2005). One way to better understand the process of dispersal is to quantify physiological parameters (S. J. Cooke & Suski, 2008; Ricklefs & Wikelski, 2002). An organism's capability to disperse and persist in a new habitat is related to its health and fitness. Dispersal rates are often correlated with population growth rates, which are affected by propagule pressure and local conditions in a habitat (Hansson, 1991; Leung & Mandrak, 2007). An organism's health and condition may also vary temporally with seasonal changes in environmental conditions. Quantifying nutritional and stress physiological parameters of an exotic species may help to identify abiotic and biotic factors influencing their spread and allow for predictions of suitable habitat conditions.

Nutrition

Two common metrics used to quantify physiological properties of individual organisms are nutrition and stress. These metrics have previously been shown to vary with biotic and

abiotic components of the environment, and can provide insights into population and community structure and organismal abundance. Assessing fish nutritional status using blood chemistry is valuable because nutritional condition represents a relationship between fitness and energy stores (Congleton & Wagner, 2006). Nutritionally deficient organisms may experience reduced foraging ability, coupled with poor growth and low survival (Wagner et al., 2010). Several studies have quantified the nutritional health of individual fish using blood chemistry indices, and a number of key parameters have been identified to convey nutritional status (Congleton & Wagner, 2006; Gingerich, Philipp, & Suski, 2010; Wagner & Congleton, 2004). For example, alkaline phosphatase (ALP) is a cell-membrane-associated glycoprotein found in all tissues, and elevated ALP activities in blood are likely related to an increased processing of energy substrates by the liver (Congleton & Wagner, 2006). Similarly, protein can respond to changes in body condition, with nutritionally-deprived individuals showing depressed protein reserves (Farbridge & Leatherland, 1992). Lipase has been shown to increase in fasted fish, with activities decreasing in response to food intake. Triglyceride and calcium concentrations may become elevated after feeding, and cholesterol responds to changes in the body energy reserves. Together, these metrics provide stable indications of the overall energetic status of an individual, as it may take several days before changes in blood-chemistry responses to food deprivation occur (Congleton & Wagner, 2006). These nutritional metrics may also change seasonally, as temperature directly influences the metabolic scope of fishes (Tonn, 1990). More importantly, reductions in energy due to limited food intake may result in a primary or secondary stress response of an individual.

Stress

When an animal perceives a threat or stressor in the environment, it typically exhibits a stress response (Barton, 2002). There are many factors that may impact the stress response of a fish, such as environmental factors (temperature or water quality), ontogenetic stage, or genetics (Schreck, 2007). Quantification of the stress response in wild, free-swimming fishes can be used as a tool for testing the internal condition of an organism. Stress is metabolically expensive as it causes cellular and molecular damage and it can be energetically expensive for an individual to return to homeostasis. This may cause a loss of performance at an organismal level, which may subsequently compromise population growth (Kassahn, Crozier, Pörtner, & Caley, 2009). For the purpose of this thesis, stress is defined as "displacement from homeostasis". Stress can divert energy away from reproduction, development, or growth in organisms (Kassahn et al., 2009). Plasma corticosteroid (cortisol) is a primary stress response to various physical, chemical, and psychological stressors for vertebrate animals, with increased plasma glucose levels acting as a secondary stress response by increasing metabolic rate (Kassahn et al., 2009; Wagner & Congleton, 2004). Plasma cortisol concentrations elevate quickly, but have not been shown to increase within the first three minutes of a stressful event (Romero & Reed, 2005). Investigating stress physiology across multiple spatial and temporal scales can be useful to define interactions of individual organisms and populations with their habitats, and aid in the management of communities and ecosystems.

Macrophysiology

Physiology is a discipline that aims to link an organism and its population to the environment (S. J. Cooke & Suski, 2008; Ricklefs & Wikelski, 2002). Quantifying the stress and

nutritional physiology of individuals represents a key component in testing for large-scale ecological patterns and processes, and is essential in developing a broad-scale perspective on the interaction between variation in biodiversity and ecosystem functioning (Chown, Gaston, & Robinson, 2004). This focus on large-scale physiological variation and its implications is also known as a macrophysiological approach (Chown et al., 2004). Macrophysiology is defined as the "investigation of variation in physiological traits over large geographical and temporal scales and the implications of this variation" (Chown, Gaston, Kleunen, & Clusella-Trullas, 2009; Chown & Gaston, 2008). Wide-ranging patterns and processes are valuable in providing a new awareness into the cause of physiological variation because it is typically not as obvious at a small scale (Gaston et al., 2009). Encompassing regional and landscape perspectives to investigate the consequences of population variation across space and within assemblages is an advantageous tool to promote further learning and understanding of physiology (Chown et al., 2009). Quantification of animal health, stress, and condition through blood chemistry can be valuable in promoting a supplementary comprehension of what underlies the link between physiology and invasive species, community structure, competition, spread, and distribution.

Asian carp

Bighead (*Hypophthalmichthys nobilis)* and silver carp (*H. molitrix*) (hereafter, Asian carp) are invasive species that have exhibited negative impacts on non-native freshwater systems (Irons, Sass, McClelland, & Stafford, 2007; Pegg & McClelland, 2004; Sampson, Chick, & Pegg, 2009). Asian carp are successful invaders due to their opportunistic feeding habits and life history traits. They grow rapidly, can tolerate a broad spectrum of environmental variables, have short generation times with high reproductive success, and can easily disperse (DeGrandchamp

et al., 2008). Asian carp have been recorded in several natural waters in the United States, and their range is currently expanding. Both species are reproductively successful and considered established in the Mississippi River Basin, which includes the Illinois, Ohio, and Wabash rivers. They are also successful in ponds, lakes, smaller rivers, and backwaters (Kolar et al., 2007). As a generalist species, Asian carp are planktivorous filter-feeders, with their diets primarily consisting of phyto- and zooplankton. Because of their feeding habits, a trophic alteration can arise from inter-specific competition between Asian carp and other native fishes that are planktivorous as adults or as juveniles (Herborg et al. 2007). Previous studies suggest that there is a nutritional similarity between bigmouth buffalo (*Ictiobus cyprinellus*) and Asian carp, as well as a diet overlap with gizzard shad (*Dorosoma cepedianum*), as these fish are also pump-filter feeders of phyto- and zooplankton (Irons et al., 2007; Sampson et al., 2009). Kolar et al. (2007) speculates that competition for planktonic resources could lead to the decline of native fish species. This is in accordance with Irons et al. (2007), who suggested that the relative masses of native gizzard shad and bigmouth buffalo have been negatively influenced by the introduction and establishment of Asian carp. The presence of Asian carp may also result in interference competition with native fishes for limited habitat. More specifically, native fishes may be forced into finding different, suboptimal habitats, which can have population-level effects (Kolar et al., 2007). Decreases in growth would affect maturation and reproductive rates, creating negative consequences for the overall fitness of native fish species (Kolar et al., 2007). The establishment of Asian carp has the potential to negatively influence species community structure, with the possibility of further adverse repercussions in new areas of establishment. Factors impacting their spread include: natural spread, the use of these fish as live bait in new waters, release of ballast water, spread by commercial fishers, release or escapement from

livehaulers, or escapement from ponds after flooding events (Kolar et al., 2007). Nutrition and stress may provide insights about the health and fitness of individuals, which can be used to understand potential dispersal and continued survival of these invaders in their current habitats. It is critical to learn more about potential drivers of bighead and silver carp population dynamics through the study of their physiology at an individual level across several areas where they have already spread and established.

Hybridization

Hybridization is a naturally occurring process for several varieties of wild freshwater taxa (Epifanio & Nielsen, 2001; Lamer et al. 2010) and can affect the community structure of an ecosystem. There are a number of performance traits that may be affected by hybridization including growth, size at maturity, metabolic activities, and survival rates (Davies et al., 2012; Hubbs, 1955; Hutchings & Fraser, 2008; Rosas, Barton, Copsey, Barbier de Reuille, & Coen, 2010). Hybrid crosses may also exhibit inferior or superior performance compared to their parents, which can affect the overall fitness of the population as hybridization continues (Rosas et al., 2010). Although it is uncommon for bighead and silver carp to hybridize in their native ranges (Kolar et al., 2007; Lamer et al., 2010), a growing proportion of hybrid Asian carp have been documented in backwaters of the Illinois and Mississippi rivers (Lamer et al., 2010). Several studies have documented first generation (F_1) hybrids, as well as advance-generation interbreeding (second or subsequent introgression and backcrossing) of hybrids (post-F_1 hybrids) (Epifanio & Nielsen, 2001; Lamer et al., 2010). There is documentation that post-F_1 hybrids demonstrate reduced fitness and growth, reduced disease resistance, reduced jumping ability, and a lower food conversion efficiency compared to their counterparts (Lamer et al., 2010). It is

critical to incorporate physiology through the examination of nutritional parameters in Asian carp and their hybrids for a greater understanding of the underlying processes of the genetic variation within these fish (S. J. Cooke & Suski, 2008). A reduction in fitness and growth may result in decreased competition for food with native fishes, as hybrid Asian carp may not forage as successfully. This can benefit native species showing high dietary overlap with Asian carp, including gizzard shad and bigmouth buffalo (Irons et al., 2007; Sampson et al., 2009). There is also potential for this introgression to serve as a natural control of Asian carp in U.S. waters (Lamer et al., 2010), which may reduce the spread and further distribution of these fish.

CHAPTER 2

SPATIAL AND TEMPORAL INFLUENCES ON THE PHYSIOLOGICAL CONDITION OF INVASIVE SILVER CARP[1]

Abstract

We quantified nutritional and stress parameters (alkaline phosphatase (ALP), cholesterol, protein, triglycerides, cortisol, and glucose) in invasive silver carp (*Hypophthalmichthys molitrix*) inhabiting four large rivers over three distinct time periods in the Midwestern United States. Examining the basic biology and ecology of an invasive species is crucial to gain an understanding of the interaction between an organism and its environment. Analyzing the physiological condition of wild-caught silver carp across broad spatial and temporal scales is essential because stress and nutritional parameters can link individuals to their habitats and vary among populations across environments. During each time period, we collected blood samples from individual silver carp in the Illinois River and portions of the Mississippi, Ohio, and Wabash rivers in Illinois. We tested for relationships between silver carp nutrition and stress across rivers, reaches within rivers, and time periods. Principal component analyses separated physiological parameters into a stress component (cortisol, glucose) and two nutritional components representative of short-term feeding (ALP, protein, triglycerides) and body energy reserves (cholesterol, protein). Akaike's Information Criterion (AICc) suggested that time period had the greatest influence on stress. Stress levels were consistent in all four rivers, and declined across time periods. Akaike's Information Criterion also suggested that interactions of time period and river had the greatest influence on short-term feeding and body energy reserves.

[1]This chapter will appear in its entirety in Conservation Physiology and is referred to later in this thesis as "Liss, Sass, & Suski, In Press". Liss, S.A., Sass, G.G., & Suski, C.D. (In Press). Spatial and temporal influences on the physiological condition of invasive silver carp. *Conservation Physiology*.

There was no specific pattern across time periods within each river, nor was there a pattern across rivers. Our results provide a better understanding of nutritional and stress condition in invasive silver carp across a broad landscape and temporal scale, with implications for managing and predicting the spread of this species.

Introduction

Riverine ecosystems are spatially and temporally dynamic, and this variability may influence habitat quality for fishes. For example, hydrologic regimes, nutrient loading, seasonal variation, and human activity can interact, or act independently, to generate lotic habitat characteristics that vary across an extreme range of conditions (Karr, Toth, & Dudley, 1985; McClelland et al., 2012; Pyron & Neumann, 2008). Water temperature may vary spatially or seasonally along environmental gradients caused by channel morphology and location within the river, which may influence the density, diversity, and/or richness of aquatic communities (Schlosser, 1990). Warmer water temperatures may induce growth or spawning (Bayley, 1995; DeGrandchamp, Garvey, & Csoboth, 2007) and increase feeding rates (Kitchell, Stewart, & Weininger, 1977), while cooler temperatures may lead to decreased feeding and lower metabolic rates (Bohl, 1980). Seasonal variation in flow rates can cause lotic environments to vary from slow to fast-moving, which may also provide suitable environmental conditions for larval production in fish (Lohmeyer & Garvey, 2008). In addition to the influence of broad-scale factors, habitat parameters that operate at smaller scales can also influence resident fish and aquatic communities. Competition for resources (e.g., food availability, thermal habitat, refuge) may occur within high quality lotic habitats, with a competitive advantage offered to individuals that obtain access to these preferred habitats (Huey, 1991).

Access to habitat of varying quality may influence decisions by individual fish that relate to reproduction and/or movement, which may ultimately influence abundance, distribution, and mortality. Resource allocation is often shaped by habitat quality, with high quality habitat providing sufficient food accessibility (maximizing fitness) and low quality habitat potentially forcing an animal to disperse and find sufficient resources (Homyack, 2010; Huey, 1991). For example, the energy required for optimal growth and recruitment can be influenced by available resources, including access to food, nutritional status, and the energy expenditure of parents (Encina & Granado-Lorencio, 1997; Homyack, 2010). Similarly, variation in thermal regimes may reduce foraging activity (Burel *et al.*, 1996) or prey availability (Burdis & Hoxmeier, 2011; Wahl, Goodrich, Nannini, Dettmers, & Soluk, 2008), which may restrict food access. Related to habitat quality is the notion of allostasis, and the influence that habitat quality can have on allostatic load, which, in turn, can influence abundance, distribution, and mortality. Essentially, individuals strive to maintain a balance between daily and seasonal environmental variation, and unpredictable events that can affect energy requirements. Higher quality habitats may provide environmental conditions that minimize an organism's allostatic load, and, in turn, can reduce energetic expenditures (McEwen & Wingfield, 2003). Thus, understanding how fish populations interact with habitat of varying quality at broad temporal and spatial scales can provide insights into population-level trends, including energy use, movement, dispersal, reproduction, and mortality.

Physiological tools are an integral way to quantify organism-environment interactions, particularly when considered over broad spatial scales (Chown et al., 2004; Chown & Gaston, 2008; Homyack, 2010), and can link individuals to the habitats in which they reside (S. J. Cooke & Suski, 2008; Ellis, McWhorter, & Maron, 2012; Ricklefs & Wikelski, 2002). In particular,

aspects of physiology that quantify the stress and nutritional status of individuals are particularly insightful. Stress and nutritional metrics represent how behavior and physiology interact within dynamic environments to influence individual homeostasis and balance energy requirements that are not reflected in simply quantifying habitat occupancy (Ellis et al., 2012; Raubenheimer, Simpson, & Tait, 2012). More importantly, changes at the population level (e.g., abundance, distribution) occur in response to changes in individuals (Ellis et al., 2012; Ricklefs & Wikelski, 2002), making the quantification of animal health, stress, and condition through blood chemistry valuable for understanding links between physiology, community structure, competition, and movement.

Silver carp (*Hypophthalmichthys molitrix*) are relatively recent invaders to North America (early 1970s) (Chick & Pegg, 2001; Kolar et al., 2007), and represent an excellent model organism for asking questions regarding the impacts of broad- and small-scale habitat variation on the stress, condition, and distribution of wild organisms. Silver carp have exhibited rapid population growth and spread since their introduction, making them the dominant species in several riverine ecosystems in the Midwestern United States (Kolar *et al.*, 2007; Sass *et al.*, 2010). More importantly, silver carp can have multiple spawning bouts per year, have few natural predators as adults, and feed low on the food chain (primarily prioritizing planktonic prey), all of which may be responsible for their success as invaders (Chick & Pegg, 2001; Kolar et al., 2007). Currently, we do not know how various broad- and small-scale parameters influence the health and condition of silver carp, or how these different factors can influence the movement, activity, and spread of this invasive species. Information on stress and condition at broad scales can have important implications for understanding not only how organisms interact with their environments, but also on factors influencing their spread and distribution across the

landscape. Therefore, our objective was to quantify variation in blood-based nutritional and stress parameters of silver carp across broad spatial and temporal scales.

Materials and Methods

Field Analysis

We collected fish in association with two long-term fish monitoring programs coordinated by the Illinois River Biological Station: the Long-Term Resource Monitoring Program (LTRMP) and the Long-Term Illinois, Mississippi, Ohio, and Wabash River Fish Population Monitoring Program (LTEF) (Gutreuter *et al.*, 1995; Tyszko *et al.*, 2012). Ancillary water quality data, including Secchi disk transparency, water temperature, dissolved oxygen (DO), surface velocity, and river stage, were also collected during fish sampling. Water temperature and DO were collected with a handheld YSI (Model 85), and velocity was measured with an Acoustic Doppler Velocimeter (ADV), as per LTEF sampling protocols (Tyszko *et al.*, 2012). River stage information was obtained from the U.S. Army Corps of Engineers river gage readings (http://rivergages.mvr.usace.army.mil/WaterControl/new/layout.cfm). We selected four reaches with established populations of silver carp in each of the Illinois, Mississippi, and Ohio rivers, and five reaches within the Wabash River for fish collection. These four rivers are all part of the Mississippi River Basin and have extensive agricultural land use, which has increased nutrient levels basin-wide (Donner, 2003). Reaches sampled in the Illinois River included Alton, Chillicothe, La Grange, and Meredosia (Fig. 1.1). In the Mississippi River, reaches consisted of Chain of Rocks, Kaskaskia, Pool 20, and Pool 25 (Fig. 1.1). The Confluence, Pool 52, Pool 53, and Smithland were sampled in the Ohio River, and Mount Caramel, New Harmony, Palestine, Terre Haute, and Vincennes reaches were our sites in the Wabash River (Fig. 1.1). Sampling

was divided into three time periods (Mid-summer = June 15 – July 31; Late-summer = August 1 – September 15; Early fall = September 16 – October 31) (Table 1.1). We sampled blood from up to 14 silver carp in each selected reach from the four rivers during each time period (Table 1.1).

We collected silver carp primarily using pulsed-direct current (DC) electrofishing according to methods outlined in Gutreuter *et al.* (1995) and Tyszko *et al.* (2012). Some of the silver carp analyzed leapt on board the boat prior to being stunned by electricity and were sampled for blood in a manner identical to those that were stunned by electricity and labeled accordingly. Immediately after capturing a fish, we collected blood from the caudal vessel using either a 2.5 or 3.8 cm needle (BD PrecisionGlide needles, gauge 22) with a 1 mL syringe (BD Slip Tip Sterile Syringes, volume 1 mL) pre-rinsed in heparin saline (Houston, 1990). We drew blood from all individuals, regardless of collection method, in less than three minutes to obtain a baseline value of stress not influenced by sampling procedures (Ellis et al., 2012; Romero & Reed, 2005). This also qualifies as a baseline nutritional status as nutritional values have not been shown to change in under three minutes (Congleton & Wagner, 2006; Gingerich et al., 2010). One mL of whole blood was collected and placed into a microcentrifuge tube and spun at 6600 RPM for at least three minutes to separate plasma from red cells. Plasma was removed using a transfer pipette and placed into two additional 1.5 mL microcentrifuge tubes. Plasma and red cells were then flash frozen in a dry shipper charged with liquid nitrogen (Suski, Killen, Kieffer, & Tufts, 2006). Samples were transported to the University of Illinois at Urbana-Champaign and stored in a < -75° C freezer until processing. We also recorded total length (mm) and weight (g) for each fish.

Laboratory Analysis

We analyzed plasma triglycerides (mg dL^{-1}), cholesterol (mg dL^{-1}), alkaline phosphatase (ALP) (U L^{-1}), and cortisol (ng mL^{-1}) using commercially available kits; EnzyChrom Triglyceride Assay Kit (ETGA-200), EnzyChrom Cholesterol Assay Kit (ECCH-100), and QuantiChrom Alkaline Phosphatase Assay Kit (DALP-250), respectively (BioAssay Systems, California, USA), and Cortisol EIA Kit (ADI-900-071) (Enzo Life Sciences, Pennsylvania, USA). Plasma glucose (mg dL^{-1}) was determined enzymatically with a microplate spectrophotometer (Spectra Max Plus 384 Model #05362, Molecular Devices, California, USA) following the procedure of Lowry and Passonneau (1972). We measured protein (g dL^{-1}) with a hand-held protein refractometer (AST model 1250, Thomas Scientific, New Jersey, USA) (Wells & Pankhurst, 1999), certified for use in the range of 0-12 g dL^{-1}. The cortisol EIA kit we used has been identified as accurate and precise when used for fishes (Sink, Lochmann, & Fecteau, 2008), and has a lower detection limit of 0.0567 ng mL^{-1}. Individuals below sensitivity limits were treated as being equal to the lowest detection limit value for the kit (0.0567 ng mL^{-1}) (Haddy & Pankhurst, 1999; Ramsay et al., 2006). Cholesterol, ALP, protein, and triglycerides have been shown to represent either a short-term, recent feeding nutritional component or a long-term body energy reserve nutritional component (Congleton & Wagner, 2006; Guerreiro, Peres, Castro-Cunha, & Oliva-Teles, 2012; Wagner & Congleton, 2004). Cortisol and glucose are associated with the stress response of teleost fishes (Barton, 2002; Congleton & Wagner, 2006; Wagner & Congleton, 2004).

Statistical Analyses

We used a multivariate principal component analysis (PCA) to reduce the dimensionality of the data and to quantify relationships between nutritional and stress parameters of silver carp among rivers, reaches within river, and over time (Smith, 2002; Vega *et al.*, 1998); our sample sizes met PCA minimums recommended by Green (1991). Principal components (PCs) with eigenvalues > 1 were used for analysis. Selected PCs underwent varimax factor rotation to maximize the amount of variation explained by each factor (Gingerich & Suski, 2011; Kaiser, 1960). Components with eigenvectors > 0.4 or < -0.4 contributed maximally to each principal component (Gingerich & Suski, 2011; Kaiser, 1960). Positive factor loadings (> 0.0) indicated a positive correlation with the raw data and negative factor loadings (< 0.0) indicated a negative correlation with the raw data. Rotated PCs were then treated as independent response variables in subsequent analyses. The fit of predictor variables (river, reach, time period, total length, and their interactions) were compared against each PC and assessed using biologically relevant models chosen *a priori*. Reaches were nested within rivers (as denoted by "[]" in Table 1.2) because the effects of reach can only occur within a single level of another variable (i.e., the river where the reach is located). Variables were crossed (as denoted by "*" in Table 1.2) to determine interactions between variables.

Competing models were compared using Akaike's Information Criterion corrected for small sample size (AICc) to quantify and rank the best approximating model (Grueber, Nakagawa, Laws, & Jamieson, 2011; Hegyi & Garamszegi, 2011; Symonds & Moussalli, 2011). Based on the ΔAICc, a value $0 \leq \Delta\text{AICc} \leq 2$ shows substantial support that a model is the best fit to the data, while an $\Delta\text{AICc} > 10$ shows little support (Burnham & Anderson, 1998; Suski & Ridgway, 2007; Symonds & Moussalli, 2011). Following best fit model determination for each

rotated PC, a mixed-model analysis of variance (ANOVA) for the best-fitting model was compared using a Tukey's multiple comparison *post-hoc* analysis to aid with visualization of trends in the data (Demsar, 2006). We performed all statistical analyses using JMP version 10.0 (SAS Institute, North Carolina, and USA). Rejection of the null hypothesis (α) for all tests was 0.05. All values are reported as means ± S.E.M. where appropriate. We used the null hypothesis of no variation in stress or nutrition (i.e., physiological parameters) for silver carp across rivers, across reaches within rivers, or across time periods.

Results

Silver carp total length, weight, and the six nutritional and stress parameters were highly variable among rivers and time periods (Table 1.3). Total length and weight were strongly correlated ($p < 0.05$, $r^2 = 0.90$), therefore only total length was included as a variable in analyses. Out of the 504 cortisol readings for silver carp, 297 fish had cortisol values below the detection limit of the kit (Table 1.3). Out of the 513 fish sampled, 110 fish leapt onto the boat. Environmental parameters were also variable among rivers and time periods (Table 1.4).

Principal components analysis produced three factors with eigenvalues > 1, which described 71 % of the total variation in the physiological parameters tested (Table 1.5). The first principal component (PC1) explained 28 % of the variation and was characterized by positive factor loadings for triglycerides, ALP, and protein (Table 1.5), characteristic of short-term feeding. The second principal component (PC2) explained 25 % of the variation and was characterized by positive factor loadings for cholesterol and protein (Table 1.5), describing long-term body energy reserves. The third principal component (PC3) explained 18 % of the variation

and was characterized by positive factor loadings for cortisol and glucose (Table 1.5), representative of stress.

Variation in PC1 was best explained by the model comprised of time period, nested within river (Table 1.2). The ANOVA identified significant differences in PC1 scores across each of the time periods per river [$F_{(3, 487)} = 8.33$, $p < 0.0001$]. *Post-hoc* analysis showed that, in the late-summer, silver carp PC1 scores peaked in the Ohio River, with negative short-term feeding scores in the other two time periods (Fig. 1.2a). Similarly, PC1 scores in the Wabash River peaked in the late-summer with lower scores in the other two sampling periods (Fig. 1.2a). In the Illinois River, PC1 scores in the early fall were lowest relative to the mid- and late-summer time periods. PC1 scores across time periods in the Mississippi River did not differ (Fig. 1.2a). Variation in PC2 was also best explained by the model comprised of time period, nested within river (Table 1.2). The ANOVA for the best fit model of PC2 confirmed significant variation across each of the time periods per river [$F_{(3, 487)} = 7.40$, $p < 0.0001$]. *Post-hoc* analysis showed silver carp sampled from the Mississippi River had PC2 scores that peaked in the early fall relative to sampling periods earlier in the year (Fig. 1.2b). Wabash River PC2 scores were also significantly greater in the early fall compared to the mid-summer time period. There were no significant differences in PC2 scores across time periods in the Illinois or Ohio rivers (Fig. 1.2b). Variation in PC3 was best explained by the model that consisted of time period only (Table 1.2). There was significant variation across time periods for PC3 [ANOVA, $F_{(2, 495)} = 14.50$, $p < 0.0001$]. *Post-hoc* analysis determined that across all rivers, silver carp had the lowest stress scores during the early fall time period relative to samples collected earlier in the year (Fig. 1.2c).

Discussion

Understanding the physiology of invasive species is important to address problems facing conservation and management, and how to control these species (S. J. Cooke et al., 2013). Knowledge of nutrition and stress levels can assist in developing strategies to control the spread of silver carp, and help define predictive relationships between habitat characteristics and individual performance in river basins not yet invaded. For wild-caught silver carp sampled at broad spatial scales, the majority of the variability in the data showed that indices of short-term feeding (PC1; triglycerides, protein, and ALP in plasma), were most strongly influenced by time period, considered independently for each river. Previous research has shown that triglycerides in plasma rise following feeding, protein responds to changes in nutritional status (e.g., food consumption, growth, body condition), and elevated concentrations of ALP are related to the processing of energy substrates by the liver (Congleton & Wagner, 2006; Guerreiro et al., 2012; Wagner & Congleton, 2004). More importantly, all three of these metrics vary according to fasting and re-feeding patterns in fishes (Congleton & Wagner, 2006; Hanson, Ostrand, & Glenn, 2012). For example, Wagner and Congleton (2004) demonstrated that plasma ALP and protein concentrations declined in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) during fasting, but recovered after feeding was resumed. Hanson and Cooke (2009) also showed that triglyceride values in smallmouth bass (*Micropterus dolomieu*) declined because of fasting during the parental care period of spawning. Mechanisms explaining our observed feeding variability across time periods may include changes in food availability, energetic constraints, or both (Wootton, 1998).

Annually, biotic and abiotic characteristics of rivers can vary significantly. According to Wahl *et al.* (2008), zooplankton densities in the Illinois River peak in late spring, while Burdis

and Hoxmeier (2011) observed peak zooplankton densities in May and June in the Upper Mississippi River. This variation in zooplankton data may explain trends in the PC1 scores for silver carp from the Illinois River, as scores were greatest in the mid- and late-summer, potentially during periods of peak zooplankton concentrations. According to Tyszko *et al.* (2012), silver carp catch per unit effort (CPUE, number hr^{-1}) also peaked in the late-summer, which may indicate that feeding is optimal for silver carp in the Illinois River during this time period, regardless of density. Silver carp in the Mississippi River showed consistently low PC1 scores across all three time periods sampled, indicating low feeding rates across all seasons. Despite this uniformity, CPUE of silver carp in the Mississippi River varied across time periods, by as much as three-fold (Tyszko *et al.*, 2012). This potentially indicates that silver carp feeding levels are not influenced by density in this river. The complex morphology of rivers has also been shown to vary seasonally in terms of nutrient loading, species composition, and productivity, all of which may influence food availability in the late-summer, and, in turn, drive PC1 scores (Amoros & Bornette, 2002; Houser & Richardson, 2010). For silver carp in the Midwestern US, variation in prey abundance may explain observed patters in short-term feeding indices.

Energetic constraints, related to water temperature and/or reproduction, may be another mechanism explaining patterns in short-term feeding (PC1) across time periods. Optimal metabolic conditions, food intake, growth, and nutrient use for fishes are affected by water temperature; fish generally consume more food during periods of elevated temperatures to balance metabolic costs and optimize growth (Burel et al., 1996; Guerreiro et al., 2012; Kaushik & Médale, 1994). Water temperature also affects metabolic rates and maintenance energy requirements for carp, similar to other teleost fishes (Kaushik, 1995; Spataru & Gophen, 1985).

For silver carp in the Illinois River, short-term feeding indices were lowest during the early fall when water temperatures were coolest. In contrast, silver carp in the Ohio and Wabash rivers showed the greatest values for short-term feeding indices during late-summer sampling period, which was the period of highest water temperature for these two locations. Silver carp CPUE was also greatest in the late-summer for the Ohio and Wabash rivers (Tyszko *et al.*, 2012), which could suggest that feeding is optimal for silver carp in these rivers during this time period, regardless of density. Reproduction may decrease feeding rates, is metabolically expensive, depletes energy stores (Encina & Granado-Lorencio, 1997), and may result in reductions in short-term feeding indices (Encina & Granado-Lorencio, 1997; Sumpter, Le Bail, Pickering, Pottinger, & Carragher, 1991). Asian carp have been shown to utilize environmental cues such as elevated flow, temperature, or river stage to initiate spawning (DeGrandchamp *et al.*, 2007), with peak larval production typically occurring in the spring (Lohmeyer & Garvey, 2008). Silver carp in the Missouri River, for example, have been observed to reproduce as early as March (Papoulias, Chapman, & Tillitt, 2006). Surface velocity was highest across all four rivers during the mid-summer, suggesting that conditions would be suitable for silver carp spawning during this sampling period. This coincides with decreased short-term feeding scores in the Ohio and Wabash rivers during the mid-summer time period. Regardless of the mechanism driving short-term feeding, it was the principal component showing the majority of the variability in silver carp feeding and changed temporally and spatially.

Similar to what was observed for indices of short-term feeding, time period considered independently for each river was the strongest predictor of variation in silver carp body energy reserves (cholesterol and protein in plasma; PC2). Previous studies have shown that plasma protein concentrations respond to changes in body condition, with nutritionally-deprived

individuals showing reduced protein reserves relative to feeding individuals (Farbridge & Leatherland, 1992). Cholesterol is a lipid present in animal tissue that is positively correlated with body energy reserves (Congleton & Wagner, 2006; Hasler et al., 2011). More importantly, both of these metrics have been shown to vary in response to the feeding history of individual fish. For example, Lambert and Dutil (1997b) showed that protein is an energy reserve that can be mobilized during periods of low energy in Atlantic cod (*Gadus morhua*), while Hanson *et al.* (2012) demonstrated that plasma protein concentrations declined in juvenile Chinook salmon from depletion of energy reserves during smoltification. Congleton and Wagner (2006) also suggested that protein and cholesterol responded to body energy reserves rather than directly to food intake as these metrics declined slowly in fasted fishes and did not recover after re-feeding in juvenile Chinook salmon.

Seasonal differences in food availability and cyclical changes in energy allocation may explain variation in energy stores in silver carp. River temperature can fluctuate greatly, potentially influencing feeding conditions, food availability and quality, and subsequently fish body composition through the allocation of energy resources (Burel et al., 1996; Lambert & Dutil, 1997a, 1997b; Mogensen & Post, 2012). Fishes have the ability to apportion energy to lipid stores to prepare for periods of scarce resources, such as in winter (Mogensen & Post, 2012). Cooler water temperatures in the fall and winter can reduce activity and feeding rates of fishes (Burel et al., 1996; Johansen, Erkinaro, & Amundsen, 2011), which can minimize energy expenditures and allow for increased energy storage (Morales, Perez-Jimenez, Furne, & Guderley, 2012). Silver carp have also been observed to situate themselves in positions of low water velocity to conserve energy (Calkins, Tripp, & Garvey, 2012). Body energy reserves for silver carp in the Mississippi and Wabash rivers were greatest during early fall sampling, which

was the period of coolest water temperatures recorded for these two rivers. Interestingly, silver carp CPUE rates were also lowest in the early fall in the Mississippi River (Tyszko *et al.*, 2012). This may contribute to why body condition was greatest, as fewer organisms were competing for resources, allowing silver carp to store energy during this time period in this river. Similarly, Spataru and Gophen (1985) observed that silver carp body condition was greatest in autumn and winter relative to other seasons of the year. Analogous to our patterns observed in short-term feeding, high body energy reserves in early fall may also be related to bioenergetics. The cool water temperatures observed during this time period for all four rivers may have resulted in lower metabolic demands and the absence of energy-consuming spawning events (Burel *et al.*, 1996; Papoulias *et al.*, 2006). Interestingly, body energy reserves of silver carp in the Illinois and Ohio rivers did not differ across time periods despite changes in water temperature of almost 40 % and 30 % (respectively) during this study. Irrespective of the mechanism(s) changing body energy reserves, our study provides evidence that body energy reserves in silver carp may peak in the early fall, as observed by the greatest scores in the Mississippi and Wabash rivers during this time period.

Values of PC3 for silver carp (cortisol and glucose in plasma) were highly influenced by time period, independent of river or reach. Because PC3 was comprised exclusively of stress-related variables, our results indicate that stress levels change seasonally, and in synchrony, across broad spatial scales. Plasma corticosteroid (cortisol) is produced during the primary stress response to various physical and psychological stressors for vertebrate animals. Increased plasma glucose levels provide energy as part of the secondary stress response (Barton, 2002; Schreck, 2007; Wagner & Congleton, 2004). Several types of disturbances may trigger the stress response in fishes including chemical, physical, or perceived stressors (i.e., temperature,

nutrition, water quality) (Barton, 2002). For example, season and temperature can exert a strong influence on the hormonal rates of reactions to stress, with greater temperatures causing biological reaction rates to increase (McLeese, Johnsson, Huntley, Clarke, & Weisbart, 1994; Pottinger & Carrick, 2000; Schreck, 2007), and potentially elevating cortisol concentrations (Schreck, Contreras-Sanchez, & Fitzpatrick, 2001). Stream-dwelling fishes have been observed to undergo shifts in distribution from summer habitats to overwintering areas to avoid metabolic stress (Schlosser, 1991). Stress levels have also been shown to correlate positively with reproductive activity (Akar, 2011) and negatively with nutritional status (Mommsen, Vijayan, & Moon, 1999). Our results showed that the lowest silver carp stress levels occurred during the early fall, regardless of river, which was the period of lowest water temperature for all rivers sampled. Similar results were reported by Jessop *et al.* (2013) who showed that both species-specific and environmental variables were important in reptile and bird responses to stressors across broad scales. Reduced stress levels in silver carp may also be caused by favorable water quality and temperatures (Pottinger & Carrick, 2000), natural, seasonal fluctuations in concentrations of cortisol that are consistent across broad spatial scales, or both (Koldkjaer, Pottinger, Perry, & Cossin, 2004; Pickering & Christie, 1981). Elevated indices of stress in the mid- and late-summer may also be correlated with the general timing of silver carp spawning in these rivers (Kolar *et al.*, 2007; Papoulias *et al.*, 2006). Additionally, according to Tyszko *et al.* (2012), CPUE for silver carp was greatest in the late-summer in all four rivers sampled, which may have contributed to the higher stress scores. Our results clearly demonstrate that stress in silver carp is driven by time period, and is consistent among populations separated across broad spatial scales.

Interestingly, variables previously shown to influence fish nutrition and stress were not significant drivers of variation for silver carp in our study. Principal components describing short-term feeding (PC1), body energy reserves (PC2), and stress (PC3), were not affected by total length or by reaches within rivers. Previous studies have shown that total length can influence short-term feeding and body energy reserves in fish (Hurst & Conover, 2003; Johansen et al., 2011; Pangle, Sutton, Kinnunen, & Hoff, 2004). The capacity for energy storage is often related to body size, where lipid and protein content comprise a greater portion of total body mass in larger individuals (Pangle *et al.*, 2004). In juvenile striped bass (*Morone saxatilis*), lipid reserves were greater in larger individuals attributable to an increase in lipid mass throughout the growing season (Hurst & Conover, 2003). Direct competition between different age and size classes because of diet overlap may also exist, with larger fish having a greater advantage in accessing food, as observed in Atlantic salmon (*Salmo salar*) (Johansen *et al.*, 2011). In response to stressors, previous research has also shown that total length and the ontogenetic stage of silver carp can affect the stress response (Laiz-Carrión *et al.*, 2012; Schreck, 2007). Total length may not have contributed to a best fit model because of the number of individuals caught (513), and, even though size varied from 252 mm to 900 mm, the majority of fish sampled were around the same size (mean 577 mm ± 6 mm). Our findings are in accordance with Encina and Granado-Lorencia (1997), who suggested that seasonal differences in condition, nutrition, and somatic energy content of juvenile and mature *Leuciscus pyrenaicus* were better explained by variation in the environment (e.g., food quantity and quality) than size.

Similarly, our results also demonstrated that variation in PC scores did not change across reaches within the same river. Previous research has shown that species richness may vary from upstream to downstream locations in rivers, with species richness generally greater downstream

(Karr *et al.*, 1985). Earlier studies on the Illinois River showed significant differences in fish communities across reaches (McClelland *et al.*, 2012), suggesting that reach-scale factors have the potential to impact species assemblages and community structure. However, we did not observe any influence of reach-scale effects on silver carp nutrition and stress. One potential reason may be due to our sampling protocol. Our study was conducted in only one year, which was an epic flood year (Olson & Morton, 2012a), and this single sampling year might not account for potential inter-annual variability influencing silver carp nutrition and stress. For example, Wagner *et al.* (2010) observed that health indicators of Lake Whitefish (*Coregonus clupeaformis*) sampled across several locations in Lake Michigan varied across years. Additionally, the rivers we sampled have been greatly altered by anthropogenic influences (i.e., agriculture use, levees, and dams), potentially homogenizing the effects of reach-scale factors at the spatial scales examined.

Despite the findings of our study, there are a number of avenues for future studies to explore related to this topic. For example, the influence of fine-scale habitat features, along with community structure and species diversity and richness, on the health and condition of fishes should be investigated. Examining specific, quantifiable habitat and community characteristics and relating them to nutritional and stress parameters may provide a better comprehension of the link between physiology and invasive species, community structure, and competition, and how interacting factors may influence movement. Other prospective research could address inter-annual variability by continuing similar work over several years for a more comprehensive understanding of silver carp nutritional and stress relationships at a broad spatial and temporal scale, similar to McClelland *et al.* (2012) and Wagner *et al.* (2010). When combined, results from these future studies can help predict the response of Asian carp to habitats not yet invaded,

and help predict how Asian carp will perform in different regions should their distributions spread.

CHAPTER 3

INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON STRESS AND NUTRITION IN INVASIVE SILVER CARP

Abstract

Community structure and dynamics in aquatic ecosystems are influenced by a variety of abiotic and biotic factors. These factors also affect local community resilience to nonnative species invasions. I compiled habitat characteristics, zooplankton concentrations, fish abundances, and species composition and richness data from a fish population monitoring program in the La Grange Reach, Illinois River, Illinois, USA (LGR), to test for their effects on several physiological parameters in invasive silver carp (*Hypophthalmichthys molitrix*). I collected blood samples and quantified nutritional and stress metrics (alkaline phosphatase (ALP), cholesterol, protein, triglycerides, cortisol, glucose) from individual silver carp inhabiting the LGR across three distinct time periods. Simple linear regression analyses identified significant relationships between abiotic and biotic characteristics and silver carp nutritional and stress parameters. Competing regression models were compared with Akaike's Information Criterion (AICc) and then *post-hoc* analyses were performed to visualize trends in the data. Akaike's Information Criterion described best fit models for two of the six physiological metrics tested. Silver carp ALP activities and glucose concentrations in plasma were positively influenced by gizzard shad (*Dorosoma cepedianum*) relative abundances, water temperature, cladoceran concentration, and suspended solids. For the other indices (cholesterol, protein, triglycerides, cortisol), AICc modeling described every abiotic and biotic variable as a best fit model. Thus, there were no distinct explanatory variables, suggesting the predictor variables I tested failed to identify patterns in these physiological parameters. My results provide a better

understanding of the local-scale interactions of abiotic and biotic factors on seasonal silver carp stress and nutrition, with implications for managing and predicting the spread of this species.

Introduction

Several theories contribute to our understanding of community structure and dynamics, with several hypotheses describing the range of complex interactions and interrelationships among species and their habitats (Chase & Leibold, 2003; Hubbell, 2001; Tilman, 2004; Tonn, 1990). Community interactions may be driven by local scale factors including biotic processes (i.e., competition for limiting resources, species richness/composition) or abiotic processes (i.e., habitat complexity) (Tonn & Magnuson, 1982; Tonn, 1990). Greater species richness can improve ecosystem resiliency (Duffy, 2009), but is inadequate for explaining what maintains ecosystem processes on its own (Hooper & Vitousek, 1997; Tonn & Magnuson, 1982). Species composition (i.e., functional groups, classified by physiological and morphological differences) is also imperative because compositional differences have the potential to exert strong influences on ecosystem processes (Tilman et al., 1997; Tonn & Magnuson, 1982). Importantly, the ability of assemblages to resist non-native invasions may be influenced by species richness and composition, competition, predation, and environmental variability (i.e., abiotic factors) (Gido & Brown, 1999; Ricciardi & MacIsaac, 2011; Stachowicz et al., 1999). A local scale study can give a snapshot of community and species interactions at a specific point in time, but those interactions may be complex and fluid. As such, it is crucial to examine abiotic and biotic variation across multiple temporal scales.

Large river ecosystems are intriguing and understudied when addressing questions about community structure. Large rivers experience substantial seasonal and habitat variation that may

play a role in influencing community dynamics at a local scale. Habitat alterations may also occur through processes such as levying or impounding for commercial navigation, flood control, or agriculture purposes, which can affect channel morphology, flow regimes, and physical-chemical attributes (Schlosser, 1990). These changes can lead to large inter-annual variation (i.e., more frequent water-level fluctuations, flooding, and disturbances to the ecosystem) (Koel & Sparks, 2002). Together, these alterations may impact local species diversity by allowing for colonization opportunities and influencing the establishment potential for invasive species (Gido & Brown, 1999). Physical habitat, such as water temperature, may inhibit or promote the individual growth of an invader as it varies across time and space (Shea & Chesson, 2002). As a result, it is important to use multiple time points annually when studying large rivers due to their unpredictability. An organism's health and condition may also vary seasonally with changes in habitat conditions, and fish may exhibit behavioral and physiological responses to temperature (Lowe et al., 2006). Quantifying the nutritional and stress physiology of an invasive species may help to identify abiotic and biotic factors influencing their success and to allow for predictions of suitable habitat conditions.

Physiology is a discipline that aims to link an organism and its population to the environment (S. J. Cooke & Suski, 2008; Ricklefs & Wikelski, 2002). Individual nutrition and stress have previously been shown to vary with biotic and abiotic conditions of the environment, and can provide insights into population and community structure and dynamics. Assessing fish health using blood chemistry is valuable, as nutritional condition represents a relationship between fitness and energy stores (Congleton & Wagner, 2006). Nutritionally deficient organisms may experience reduced foraging ability, poor growth, reduced swimming performance or decreased survival, relative to individuals that are better fed (Gingerich et al.,

2010; Wagner et al., 2010). The metabolic scope of an individual is directly influenced by environmental factors (temperature, oxygen, salinity) (Tonn, 1990; Wootton, 1998), which can alter the energy requirements for fish at different times of the year. During periods of low nutritional inputs, fish may not have sufficient energy to allocate to reproduction, potentially reducing fitness (Wootton, 1998). Baseline cortisol levels have the potential to predict relative fitness, but a direct relationship between baseline cortisol and fitness is not always reliable or consistent, making assumptions between these variables tenuous (Bonier, Martin, Moore, & Wingfield, 2009). Reductions in energy due to limited food intake may also result in the induction of a stress response in an individual, making the quantification of stress a tool that can help define the health of an organism. More importantly, stress is metabolically expensive, can potentially cause a loss of performance at an organismal level, and can negatively impact fitness through reducing the energy available for activity and inhibiting reproduction, which may subsequently compromise population growth (Kassahn et al., 2009; Schreck, 2010). Quantifying the interactions of abiotic and biotic factors and their relationship with organismal stress and nutrition is imperative in understanding how those various factors are perceived and dealt with by individuals (Wingfield, 2013). Further, such studies can promote a supplementary comprehension of what underlies the link between physiology, invasive species, and community structure and dynamics.

Silver carp (*Hypophthalmichthys molitrix*) (SVCP) are planktivorous filter-feeders that have successfully established in the Mississippi River Basin (MRB), and they have the potential to disperse and invade neighboring watersheds depending upon the suitability of habitats (S. L. Cooke & Hill, 2010; Herborg et al., 2007; Kolar et al., 2007). More importantly, silver carp have the potential to negatively affect freshwater ecosystems (Calkins et al., 2012; Irons et al., 2007;

Kolar et al., 2007; Sass et al., 2010). For example, due to diet overlap, the presence of silver carp has caused a negative impact on the body condition of native gizzard shad (*Dorosoma cepedianum*) (GZSD) and bigmouth buffalo (*Ictiobus cyprinellus)* (BMBF) (Irons et al., 2007; Sampson et al., 2009). Currently, however, we do not know how these abiotic and biotic factors impact nutritional condition and stress in invasive silver carp at a local scale. Therefore, the objective of my study was to quantify the impact of local-scale abiotic, biotic, and community-related parameters on the stress and nutritional condition of wild-caught, invasive silver carp. I hypothesized that silver carp nutritional condition would decrease, and stress levels would increase, with increased biotic and community-related parameters associated with greater competition. I also hypothesized that silver carp nutritional condition would increase, and stress levels would decrease, with the increased quality of abiotic habitat features, due to greater food availability.

Materials and Methods

Field Analysis

I collected silver carp in association with two long-term fish monitoring programs coordinated by the Illinois River Biological Station (IRBS): the Long-Term Resource Monitoring Program (LTRMP) and the Long-Term Illinois, Mississippi, Ohio, and Wabash River Fish Population Monitoring Program (LTEF) (Gutreuter et al., 1995; Tyszko et al., 2012). The LTRMP and LTEF programs are designed to sample and enumerate the entire fish community of large rivers using a standardized series of protocols that date back to 1989 and 1957, respectively (Irons et al., 2007; McClelland et al., 2012). As part of these sampling protocols, I collected silver carp from the La Grange Reach, Illinois River (LGR), located near

Havana, Illinois (LTRMP Field Station 6, latitude: 40^{o} 18 minutes N, longitude: -90^{o} 3 minutes W) from June through October, 2011. This reach comprises a 129 km stretch of the Illinois River extending from the La Grange Lock & Dam at river km 126 upstream to the Peoria Lock & Dam at river km 255 (Fig. 2.1). The LGR is a highly variable habitat with extensive anthropogenic modifications (e.g., impoundments, agriculture, altered flood regimes) and a high abundance and biomass of established, invasive silver carp (Koel & Sparks, 2002; Sass et al., 2010). Sampling was divided into three time periods: mid-summer = June 15 – July 31; late-summer = August 1 – September 15; early fall = September 16 – October 31. I sampled blood from up to 12 (range 10 – 12) silver carp in the LGR during each of the three time periods in 2011. While there are limitations to performing this study at one location (i.e., spatial and temporal correlation, replication), an ecosystem response is of theoretical and practical interest because it allows for the inclusion of abiotic and biotic processes that are typically difficult to incorporate in an artificial system (Carpenter, Chisholm, Krebs, Schindler, & Wright, 1995; Carpenter, 1989; Schindler, 1998). The La Grange Reach expands for 129 km and experiences variability in climate, fish community structure, and primary production (Koel & Sparks, 2002; McClelland et al., 2012), and we were able to sample fish experiencing a range of abiotic and biotic characteristics over the three distinct time periods.

Similar to Liss et al. (In Press), I collected silver carp primarily using pulsed-direct current (DC) electrofishing according to methods outlined in Gutreuter et al. (1995) and Tyszko et al. (2012). About half of the silver carp analyzed in the current study leapt on board the boat prior to being immobilized by electricity. Silver carp exhibit a jumping behavior when startled (Green & Smitherman, 1984), which can make their capture by electroshocking difficult. Fish that voluntarily leapt on board were sampled for blood in a manner identical to those that were

stunned by electricity. Immediately after capturing a fish, I collected blood from the caudal vessel using either a 2.5 or 3.8 cm needle (BD PrecisionGlide needles, gauge 22) with a 1 mL syringe (BD Slip Tip Sterile Syringes, volume 1 mL) pre-rinsed in heparin saline (Houston, 1990). I drew blood from all individuals, regardless of collection method, in less than three minutes to obtain a baseline value of stress and nutrition that would not be influenced by sampling procedures (Congleton & Wagner, 2006; Ellis et al., 2012; Gingerich et al., 2010; Romero & Reed, 2005). One mL of whole blood was collected and placed into a microcentrifuge tube and spun at 6600 RPM for at least three minutes to separate plasma from erythrocytes. Plasma was removed using a transfer pipette and placed into two additional 1.5 mL microcentrifuge tubes. Erythrocytes and plasma were then flash frozen in a liquid nitrogen charged dry shipper (Suski et al., 2006). Samples were transported to the University of Illinois at Urbana-Champaign and stored in a < -75° C freezer until processing. I also recorded total length (mm) and weight (g) for each fish.

Water quality parameters within the LGR were collected from fixed sites every two weeks during fish sampling periods in 2011, and from a set of randomly selected locations at quarterly intervals, according to established protocols (APHA, 1992; Soballe & Fischer, 2004). The collected parameters included temperature, Secchi disc transparency, dissolved oxygen (DO), pH, turbidity, conductivity, velocity, total phosphorus (TP), total nitrogen (TN), suspended solids, and chlorophyll *a*, which are used as a representation of habitat quality. In 2011, zooplankton collections were conducted monthly during May – November in the LGR. For each sample, 30 L of water were pumped through a 55 µm filter and this procedure was replicated three times. We examined the integrated zooplankton community and concentration throughout the entire water column by attaching a weight to a hose and lifting that hose from the bottom to

the surface of the water while pumping. Zooplankton samples were preserved in a sugar-buffered formalin solution and transported to the IRBS for analysis. We enumerated and identified the macrozooplankton samples (55 μm filter) with a zooplankton counting wheel. Zooplankters were identified as rotifers to Genus, cladocerans to Genus, and copepods to Family. An estimate of the number of zooplankton in each sample was determined by dividing the sample concentrate volume, by the volume of subsamples required to reach 100 zooplankton, and multiplied by the number of zooplankton counted in the subsample(s). This number was then divided by the volume filtered to get an estimate of the number of zooplankton in 1 L of river water for each sample. Each predictor variable was averaged across all sampling episodes within a time period, resulting in a single mean parameter value per time period.

Fish species richness, community composition richness, and catch per unit effort (CPUE, number hr^{-1}) for all fish captured by electrofishing during each time period were quantified in accordance with LTRMP protocols (http://www.umesc.usgs.gov/ltrmp.html) (Ickes & Burkhardt, 2002). Community composition, quantified as the number of functional groups present, was defined according to functional groups identified in Poff and Allen (1995). To develop these functional groups, diet information was taken from Pflieger (1975), Becker (1983), and the Ohio Department of Natural Resources (http://www.dnr.state.oh.us/Home/species_a_to_z/AZFish/tabid/17913/Default.aspx) and used to assign fish to the following functional feeding groups; benthic invertivores, general invertivores, herbivore-detritivores, omnivores, piscivores, planktivores, and surface/water column invertivores (Poff & Allan, 1995). A list of all fish species collected during this time period is available at http://www.umesc.usgs.gov/data_library/fisheries/fish_page.html. Again, each

predictor variable was averaged across all sampling episodes within a time period, resulting in a single mean parameter value per time period.

Laboratory Analysis

I analyzed plasma alkaline phosphatase (ALP) (U L^{-1}), cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}), and cortisol (ng mL^{-1}) using commercially available kits; QuantiChrom Alkaline Phosphatase Assay Kit (DALP-250), EnzyChrom Cholesterol Assay Kit (ECCH-100), and EnzyChrom Triglyceride Assay Kit (ETGA-200), respectively (BioAssay Systems, California, USA), and Cortisol EIA Kit (ADI-900-071) (Enzo Life Sciences, Pennsylvania, USA). Plasma glucose (mg dL^{-1}) was determined enzymatically with a microplate spectrophotometer (Molecular Devices, Spectra Max Plus 384, Model #05362, California, USA) following the procedure of Lowry and Passonneau (1972). Protein (g dL^{-1}) was measured by a hand-held protein refractometer (AST model 1250, Thomas Scientific, New Jersey, USA) (Wells and Pankhurst, 1999), which is certified for the use in the range of 0-12 g dL^{-1}. The cortisol EIA kit used has been identified as accurate and precise when used for fishes (Sink et al., 2008), and has a lower detection limit of 0.0567 ng mL^{-1}. Individual cortisol values that were below the sensitivity limit of the kit were treated as being equal to the lowest detection limit value for the kit (0.0567 ng mL^{-1}) (Haddy & Pankhurst, 1999; Ramsay et al., 2006). Cholesterol, ALP, protein, and triglycerides have been shown to represent either a short-term, recent feeding nutritional component or a long-term body energy reserve nutritional component (Congleton & Wagner, 2006; Guerreiro et al., 2012; Wagner & Congleton, 2004). Cortisol and glucose are associated with the stress response of teleost fishes (Barton, 2002; Congleton & Wagner, 2006; Wagner & Congleton, 2004).

Statistical Analyses

I used simple linear regression to quantify relationships between silver carp nutritional and stress parameters (response variables) and total length, water quality parameters, zooplankton data, species abundance (CPUE), species richness, and species composition (predictor variables) over the three time periods sampled. To quantify variation in environmental parameters across time periods, I used an analysis of variance (ANOVA). Prior to regression analyses, I developed a correlation matrix of the different water quality predictor variables to identify correlated terms and avoid colinearity within the data. Following colinearity analyses, I identified three abiotic (habitat) variables that were representative of the different water quality parameters measured: water temperature, TP, and suspended solids. More specifically, water temperature was inversely correlated with conductivity, DO, and Secchi disc transparency ($p < 0.05$) and positively correlated with turbidity, chlorophyll *a*, and suspended solids ($p < 0.05$). Total phosphorus was negatively correlated with Secchi disc transparency, chlorophyll *a*, DO, and pH, ($p < 0.05$) and positively correlated with TN, velocity, conductivity, and suspended solids ($p < 0.05$). Suspended solids correlated negatively with pH, DO, and Secchi disc transparency ($p < 0.05$) and correlated positively with TP, chlorophyll *a*, turbidity, and temperature ($p < 0.05$) (Table 2.1). Together, water temperature, TP, and suspended solids represented the 11 water quality parameters analyzed. Each predictor variable was treated as a nested parameter within time period because measurements from all sampling episodes were averaged in each of the three time periods. Competing regression models were then compared using Akaike's Information Criterion, corrected for small sample size (AICc), to quantify and rank the best approximating model for each blood variable (Grueber et al., 2011; Hegyi &

Garamszegi, 2011; Symonds & Moussalli, 2011). Based on the ∆AICc, a value $0 \leq \Delta AICc \leq 2$ shows substantial support that a model is the best fit to the data, while an $\Delta AICc > 10$ shows little support (Burnham & Anderson, 1998; Suski & Ridgway, 2007; Symonds & Moussalli, 2011). Following best fit model determination for each nutritional and stress parameter (defined as $\Delta AICc < 4$), *post-hoc* analyses were performed to visualize trends in the data. A mixed-model ANOVA for the best-fitting categorical predictor models was compared using a Tukey's multiple comparison *post-hoc* analysis to aid with visualization of trends in the data (Demsar, 2006). For continuous predictor variables, I used a regression analysis with a line of best fit for determination of trends. I performed all statistical analyses using JMP version 10.0 (SAS Institute, North Carolina, and USA). Rejection of the null hypothesis (α) for all tests was 0.05. All values were reported as means ± S.E.M. where appropriate. I used the null hypothesis of no variation in stress or nutrition (i.e., physiological parameters) for silver carp across abiotic or biotic habitat characteristics nested within time periods.

Results

Silver carp total length, weight, and the six measured nutritional and stress parameters were highly variable across the three time periods in the LGR (Table 2.2). Total length and weight were strongly correlated ($p < 0.0001$, $r = 0.94$), therefore only total length was included as a variable in the analyses. Out of 32 cortisol readings for silver carp, nine fish had cortisol concentrations below the detection limit of the kit and were treated as being equal to the lowest detection limit value for the kit (Haddy & Pankhurst, 1999; Ramsay et al., 2006). Abiotic and biotic environmental parameters also varied across the three time periods (ANOVAs, $p < 0.05$; Table 2.3). Mean water temperatures were twice as warm in the mid- and late- summer relative

to early fall sampling period (ANOVA, $p < 0.05$; Table 2.3). Mean cladoceran concentrations were almost 10-fold greater in the mid- and late-summer relative to the early fall (ANOVA, $p < 0.05$; Table 2.3). Average relative abundances of GZSD were lowest in the early fall compared to the other sampling periods.

Variability in ALP activities in the plasma of wild-caught silver carp was best explained by the CPUE of GZSD, and models that included water temperature and cladoceran concentration also provided substantial support (ΔAICc values 2.61 and 3.29, respectively) (Table 2.4). The CPUE of GZSD, water temperature, and cladoceran concentration explained 71, 69, and 68 % of the variability in ALP, respectively. Alkaline phosphatase activities were positively correlated with the significant predictor variables from the regression analyses ($p < 0.05$), and ANOVA indicated that ALP values were significantly greater in the mid- and late-summer months, compared to the early fall [$N = 32$, $F_{(2, 31)} = 35.57$, $p < 0.0001$].

Variability in the plasma glucose concentration of silver carp was best explained by suspended solids, with cladoceran concentration, water temperature, and the CPUE of GZSD also showing substantial support (ΔAICc values of 0.92, 1.14, and 3.67, respectively) (Table 2.5). The r^2 value for glucose and suspended solids was 0.38, for glucose and cladoceran concentration was 0.36, for glucose and water temperature was 0.36, and between glucose and the CPUE of GZSD was 0.31. Plasma glucose concentrations were positively correlated with each of the predictor variables from the regression analyses ($p < 0.05$), and ANOVA indicated that plasma glucose concentrations were significantly greater in the mid- and late-summer periods, compared to the early fall [$N = 32$, $F_{(2, 31)} = 9.06$, $p < 0.001$].

All competing AICc models had ΔAICc < 4.0 for cortisol, cholesterol, and triglycerides (Tables 2.6, 2.7, 2.8, respectively). The r^2 value for the best fit model (planktivore richness) and

cortisol was 0.11. Cholesterol had an r^2 value of 0.02 with the best fit model of total length, and triglycerides and CPUE of GZSD had an r^2 of 0.04. This indicated that no parameter provided strong support for trends in the data, and therefore did not provide a substantial explanation. Similarly, 10 of the 14 competing AICc models had ΔAICc < 4.0 for the plasma protein concentration in silver carp, again indicating that support for most parameters in explaining variation in protein was similar and weak (Table 2.9). The best fit model (total length) and protein had an r^2 value of 0.13. As a result, these models do not provide a meaningful interpretation of these physiological variables.

Discussion

For wild-caught, invasive silver carp collected in the La Grange Reach of the Illinois River, ALP activities in plasma were positively influenced by water temperature, cladoceran concentration, and the CPUE of GSZD, particularly during the mid- and late-summer sampling periods. Alkaline phosphatase is a nutritional parameter, and previous research has shown that elevated quantities of ALP are related to the processing of energy substrates by the liver. Decreased ALP activities have been observed in starving fish (Congleton & Wagner, 2006; Sandnes, Lie, & Waagbo, 1988). Previous research with Atlantic salmon (*Salmo salar*) also documented a positive correlation between ALP activities in plasma and water temperature (Sandnes et al., 1988). There is substantial diet overlap between GZSD and SVCP, as both are filter-feeding fishes that consume zooplankton, including cladocerans (Sampson et al., 2009; Shuang-lin & De-shang, 1994). Previous literature has documented an increase in cladoceran concentrations during warmer water temperatures throughout the summer in the Illinois River (i.e., mid- and late-summer), thereby elevating potential food resources for filter feeding fishes (Wahl et al., 2008). This is analogous to my results, which show that cladoceran concentrations

were more than 10-fold greater in the mid- and late-summer relative to the early fall. Thus, quantities of ALP in the plasma of silver carp likely correlate positively with GZSD CPUE because feeding conditions were optimal for both species during those time periods. Both species were potentially consuming cladocerans at high rates relative to other times of the year, and GZSD numbers may have increased to take advantage of the ample food supply. In addition, water temperature plays a major role in the feeding rates of fish. Metabolism increases with warmer water temperature, elevating the rate at which fishes must acquire food to balance metabolic costs and optimize growth (Burel et al., 1996; Guerreiro et al., 2012; Kaushik & Médale, 1994; Kitchell et al., 1977). Specifically for silver carp, feeding is reduced in temperatures below 15^{o} C (Kolar et al., 2007). In my study, the mean temperature was about 15^{o} C in the early fall, which may be why silver carp ALP activities were significantly lower during this time period. Together, my results demonstrate that variation in silver carp feeding rates, quantified by ALP activities in plasma, were likely driven by cladoceran concentrations, as well as elevated water temperature, in the mid- and late-summer.

Plasma glucose for silver carp correlated positively with suspended solids, cladoceran concentrations, water temperature, and the CPUE of GZSD. Plasma glucose is a reliable indicator of stress and correlates with a wide range of environmental stressors, including water temperature, sediments, and salinity (Menge & Sutherland, 1987; Silbergeld, 1974; Wells & Pankhurst, 1999). Season and water temperature can exert a strong influence on the hormonal rates of reaction to stress, with greater temperatures causing biological reaction rates to increase (McLeese et al., 1994; Pottinger & Carrick, 2000; Schreck, 2007). In my study, water temperatures recorded during the mid- and late-summer sampling periods averaged over 31° C; a temperature shown to induce physiological disturbances in silver carp (Kolar et al., 2007).

Warmer water temperatures can also increase the maintenance energy cost of an individual (Von Oertzen, 1985), which can elicit a stress response (Akar, 2011). Elevated volumes of suspended solids in the water may reduce visibility or cause abrasions, potentially resulting in an increase in stress for fish (Redding, Schreck, & Everest, 1987). Silver carp also have specialized, sponge-like gill rakers that allow them to consume very small suspended particles (3 – 4 μm) (Calkins et al., 2012; Irons et al., 2007; Kolar et al., 2007). Elevated concentrations of suspended solids may therefore increase the potential for silver carp gill rakers to become clogged, which may trigger a stress response. My results indicate that, while silver carp feeding likely was not impaired during the mid- or late-summer (i.e., our proxy of silver carp feeding, ALP, did not decrease with increased GZSD CPUE), they may still be eliciting a stress response due to the density of GZSD as potential competitors. This may explain the positive relationship between CUPE of GZSD and plasma glucose in my data. Finally, concentrations of plasma glucose have been shown to decline in fasted fishes (Congleton & Wagner, 2006). My study used baseline physiological metrics (i.e., blood was drawn in under three minutes), indicating that it should be possible to use glucose as a reliable indicator of nutrition (Gingerich et al., 2010; Romero & Reed, 2005). As such, this may indicate increased silver carp feeding (by proxy of increased glucose concentrations in my data) in the mid- and late-summer compared to the early fall. This is also in accordance with my results of silver carp ALP values (similarly acting as a representation for feeding), which indicated increased feeding in the mid- and late-summer relative to the early fall. Regardless of the mechanism, concentrations of glucose in the plasma of silver carp were positively correlated with suspended solids, water temperature, the CPUE of GZSD, and cladoceran concentrations during sampling.

A surprising number of blood-based physiological metrics did not have a single, clear best-fit model to explain trends in the data, indicating that variation in the response variables was not explained by the predictors we tested. For example, none of the models had ΔAICc > 4 for cortisol, triglycerides, or cholesterol and protein had all models with ΔAICc < 5. There are a number of possible explanations for my observations. One potential explanation is due to a lack of scope or variability in the observed physiological parameters because of limited sample size. For example, plasma cortisol concentrations had a mean of 19.3 and a S.E.M. of 5.2, with a range of 0.06 to 102.5, showing little population-level variation. Similarly, plasma protein concentrations ranged from 3.0 to 4.8, with a S.E.M. of 0.08 and a range from 3.0 to 4.8. Cortisol was collected in less than three minutes from all fish, and is therefore reflective of background stress levels in the population that are considered resting or stress-free (Barton, Morgan, & Vijayan, 2002; Romero & Reed, 2005). Similarly, protein is a nutritional parameter and can respond to changes in body condition (Farbridge & Leatherland, 1992), which clearly was not fluctuating in silver carp in my system based on the lack of variability in the data. Alternatively, the timing of my sampling may not have been ideal to observe variation in some of the parameters measured. Triglyceride concentrations, for example, increase after feeding (Congleton & Wagner, 2006). Silver carp are constantly filter-feeding at a very low trophic position (Dong & Li, 1994; Sampson et al., 2009; Shuang-lin & De-shang, 1994), suggesting that there may not be a time when their triglyceride concentrations would decrease. Similarly, cholesterol concentrations are representative of body energy reserves and decrease in fasted fish (Congleton & Wagner, 2006; Hasler et al., 2011). At a local scale, the duration of my study may not have been long enough to document the shift of energy from feeding to body reserves. Future studies should corroborate this hypothesis by sampling across a greater duration of time,

sampling throughout the year (from January through December) as opposed to June through October. Results from my study clearly show that, for a large number of nutritional and stress metrics quantified, there were no distinct best fit models to describe variation in the nutrition or stress of silver carp.

Several abiotic and biotic environmental factors quantified during sampling also failed to predict patterns for stress and nutrition in silver carp. These predictor variables have previously been shown to influence the structure and dynamics of a community through resource availability and competition, community-level performance, and/or habitat complexity. In my study, TP, overall species abundance, species composition, and species richness were not drivers for any of the stress or nutrition metrics of wild silver carp in the LGR. Environmental characteristics have been shown to alter life history characteristics in fishes (Schlosser, 1990). Previous research has shown that concentrations of TP drive productivity in freshwater systems, and can be used as a predictor of planktonic community structure and abundances (Beisner et al. 2006; Jeppesen et al. 2000; Mainstone & Parr, 2002; Schindler, 1974). Higher concentrations of TP should result in greater planktonic concentrations, (which are a major food source for silver carp (Buck, Baur, & Rose, 1978; S. L. Cooke, Hill, & Meyer, 2009; Kolar et al., 2007)); thus, elevated concentrations of TP should also translate to more food for silver carp. In my study, the concentration of TP was comparable with other rivers in the MRB (Donner, 2003), and would classify the La Grange Reach as eutrophic (S. L. Cooke et al., 2009). Therefore, increased volumes of TP in the water should have resulted in fish being in a better nutritional condition, but it was not a significant driver of cholesterol, protein, or triglycerides (proxies of nutritional performance) for silver carp in my study. Total phosphorus in aquatic systems has also led to an increase in species richness and diversity (Jeppesen et al., 2000; Pegg & McClelland, 2004).

Community composition is another important driver of ecosystem processes in aquatic and grassland plant communities (Tilman et al., 1997; Tonn & Magnuson, 1982) that can influence productivity, biomass accumulation, and resource availability. More specifically, species-rich environments have greater resource use across trophic levels, making them less prone to invasion (Duffy, 2009; Gido & Brown, 1999). Based on the community richness, composition, and overall abundance data generated in my study, the LGR can be considered species-rich. The lower (downstream) reaches in the Illinois River (including the La Grange Reach) have a greater species richness and abundance relative to the upstream reaches in the Illinois River (north of the Peoria Lock & Dam) (McClelland et al., 2012; Pegg & McClelland, 2004). This is common as large river floodplains exhibit a more diverse environmental landscape downstream, allowing for greater productivity (Schlosser, 1991). In spite of this distinction, silver carp have been able to invade, establish, and their populations have grown exponentially (Sass et al. 2010). This is in accordance with my study for silver carp in the LGR, as concentrations of cholesterol, cortisol, protein, and triglycerides in silver carp were not driven by species richness, composition, or overall abundance. My study provides evidence, that for several stress and nutritional metrics quantified, silver carp are not limited by the abiotic or biotic habitat characteristics I tested.

Studies have documented that silver carp are successful invaders (Calkins et al., 2012; Chick & Pegg, 2001; McClelland et al., 2012; Sass et al., 2010). Interestingly, other research has demonstrated the impact of local scale on the abundance of silver carp in the Illinois River (McClelland et al., 2012). In my study, four of the six physiological parameters quantified in silver carp were not distinct explanatory drivers of variation based on a suite of abiotic and biotic habitat characteristics tested. This may suggest that silver carp stress and nutrition were not generally correlated with abiotic and biotic factors of the La Grange Reach, Illinois River, or the

suite of environmental variables we examined were not associated with silver carp stress or nutrition. During my study, the MRB faced extreme flooding, which may have influenced my results (Olson & Morton, 2012b). Additionally, in other invaded habitats with lower productivity or different species diversity, the abiotic and biotic environmental parameters may be more influential. Despite these caveats, and while my results may suggest no definitive driver of variation for important physiological metrics, results from this study further emphasize the successfulness of invasive silver carp in the wild.

CHAPTER 4

PHYSIOLOGICAL CONSEQUENCES OF HYBRIDIZATION: BACKCROSSING DECREASES NUTRITIONAL PERFORMANCE IN INVASIVE ASIAN CARP

Abstract

Hybridization can be a naturally occurring process that has the potential to influence several aspects of individual fish performance (fitness, growth, size at maturity, metabolic activities, and survival rates). Hybrid offspring may exert evolutionary pressures on parental species by exhibiting hybrid vigor (increased fitness) or by demonstrating inferior performance (outbreeding depression) compared to their parents. I examined the nutritional performance of invasive Asian carps in the Illinois River, Illinois, USA, using parental bighead carp (*Hypophthalmichthys nobilis*), parental silver carp (*H. molitrix*), and their reciprocal hybrids by quantifying a suite of nutritional physiological parameters (alkaline phosphatase (ALP), calcium, cholesterol, lipase, protein, triglycerides). Individuals were separated into four distinct genetically-identified groups based on single nucleotide polymorphisms (SNPs) (parental silver carp, parental bighead carp, advanced group hybrids, first generation group hybrids). A mixed-model analysis of variance (ANOVA), followed by *post-hoc* analyses, were performed to visualize trends in the data. Significant relationships existed between genetic identification groups and three of the six physiological metrics. Parental silver carp were in better nutritional condition than parental bighead carp, as they had the greatest concentrations of triglycerides, protein, and lipase activities in plasma. Parental bighead carp exhibited the lowest concentrations overall. The nutritional status of advanced group hybrids was more similar to parental silver carp, as advanced group individuals showed identical concentrations for two of the three parameters tested (protein, lipase). First generation group individuals were more

nutritionally analogous to parental bighead carp; these individuals maintained identical concentrations relative to parental bighead carp for all three metrics tested (triglycerides, lipase, protein). My results provide a needed understanding of the impacts of Asian carp hybridization on nutritional performance in the wild, and may have management implications for predicting the future spread and potential fitness of these hybrid invaders.

Introduction

A species refers to any distinct population division of any type of vertebrate wildlife or fish, that can reproduce when mature (Waples, 1991). Hybridization can be defined as the genetic crossing of two or more divergent and classifiable species, and is a naturally occurring process prevalent in taxa that coexist in the same niche space or occupy similar habitats (Davies et al., 2012; Epifanio & Nielsen, 2001; Hubbs, 1955). There are a number of performance traits that may be affected by hybridization including growth, size at maturity, metabolic activities, and survival rates (Davies et al., 2012; Hubbs, 1955; Hutchings & Fraser, 2008; Rosas et al., 2010). Hybrid offspring may also affect speciation processes by exhibiting inferior performance (reduced vigor, fitness, condition) or, in some cases, superior performance (improved vigor (heterosis), fecundity, accumulation of adaptive traits) relative to parental species (Rosas et al., 2010; Soltis, 2013). For example, there have been documented declines in the performance and fitness of wild populations of fishes due to interbreeding with conspecifics that have escaped confinement, or escaped heterospecific organisms (Hutchings & Fraser, 2008; McGinnity et al., 2003; Muhlfeld et al., 2009). Similarly, there have been documented decreases in the metabolic rates of hybrids (Davies et al., 2012) and decreases in their growth relative to pure parental species (Hutchings & Fraser, 2008). Other studies have documented the anthropogenic

promotion of desired traits (growth, survival, disease resistance) from crossbreeding heterospecific parents (Bryden, Heath, & Heath, 2004; Green & Smitherman, 1984; Lamer et al., 2010). Should hybridization continue, interbreeding may lead to a "hybrid swarm" (complete merging of two taxa) or to the extinction of one taxon (Scribner, Page, & Bartron, 2001). Nevertheless, relatively little is known about the influences of hybridization on nutritional condition in fishes.

Individual nutritional condition can provide excellent insights into the performance of hybrid organisms, as nutritional metrics describe a balance between fitness and energy stores (Congleton & Wagner, 2006). Nutritionally deficient individuals may experience negative effects on performance traits such as growth, feeding, fitness, and/or survival relative to their counterparts that are well fed (Gingerich et al., 2010; Wagner et al., 2010), and individual nutrition can vary with the conditions of the current habitat (i.e., temperature, habitat quality) or due to the interaction between an organism and its environment (i.e., ability to obtain prey, ability to avoid predation). Additionally, physiology is a valuable tool to link an organism and its population to the environment (S. J. Cooke & Suski, 2008; Ricklefs & Wikelski, 2002). Thus, quantifying the nutritional status of hybrids can provide insights into the performance of hybrid populations relative to their parents and may provide critical information for the management of invasive species that tend to hybridize, such as Asian carp.

Bighead (*Hypophthalmichthys nobilis)* and silver carp (*H. molitrix*) (hereafter, Asian carp) coexist in the wild and are a U.S. federally injurious group of invasive species that have become the dominant fishes in the Upper Mississippi River Basin (Kolar et al., 2007; McClelland et al., 2012; Sass et al., 2010). More importantly, previous research suggests that Asian carp may negatively affect invaded freshwater habitats (Calkins et al., 2012; Irons et al.,

2007; Kolar et al., 2007; Sass et al., 2010). In sympatry, Asian carp partition food resources based on size preferences, with silver carp more proficient at consuming smaller prey items (DeGrandchamp et al., 2008; Dong & Li, 1994; Kolar et al., 2007; Sampson et al., 2009). Recent research has proven that Asian carp hybridize in native and non-native habitats (Lamer et al., 2010; In Review), with a conservative estimate of about 23 % of individuals in the Mississippi River Basin (MRB) existing as hybrids between parental silver carp and parental bighead carp. The physiological performance of hybrids relative to their parents has not been quantified, and testing for differences in performance (i.e., hybrid vigor, outbreeding depression) could have significant implications for the spread, control, and future of these invaders. Therefore, Asian carp serve as an ideal model organism to test and better understand the performance characteristics of hybridization in wild organisms, as well as, to make predictions about how hybridization can affect the future trajectory of these invasive fishes. My objective was to define the influence of hybridization on the nutritional performance of wild-caught, invasive Asian carp in the Upper Mississippi River Basin.

Materials and Methods

Field Analysis

Fish collection methods were based on those described in Lamer et al. (2010). Seventy-seven *Hypophthalmichthys* individuals were collected near Morris, Illinois (river km 423), on the Illinois River. Asian carp hybridization has been documented in the Illinois River (Lamer et al., 2010), making this an ideal site to collect *Hypophthalmichthys*. All Asian carp were captured by commercial harvesters contracted by the Illinois Department of Natural Resources using monofilament trammel and gill nets (7.62 - 10.16 cm inner bar mesh, 2.4 - 5.3 m deep). The

duration of each net set varied, and all fish captured were used for nutritional and genetics analysis regardless of putative identification (Lamer et al., 2010).

Asian carp were initially identified in the field based on gill raker appearance (comb-like-bighead carp, sponge-like-silver carp, twisted or club-like-hybrid carp) (Kolar et al., 2007; Lamer et al., 2010). Ventral keel length and an overlap of pectoral fin over the pelvic fin (or lack of overlap) were not used as identifying characteristics in the field as they are not considered reliable (Lamer et al., 2010). Following removal from gill nets, I collected blood from the caudal vessel using either a 2.5 or 3.8 cm needle (BD PrecisionGlide needles, gauge 22) with a 1 mL syringe (BD Slip Tip Sterile Syringes, volume 1 mL) pre-rinsed in heparin saline (Houston, 1990). One mL of whole blood was collected and placed into a microcentrifuge tube and spun at 6600 RPM for at least three minutes to separate plasma from erythrocytes. Plasma was extracted using a transfer pipette and placed into two additional 1.5 mL microcentrifuge tubes. Plasma and erythrocytes were then flash frozen in a dry shipper charged with liquid nitrogen (Suski et al., 2006). Samples were transported to the University of Illinois at Urbana-Champaign in liquid nitrogen where they were stored in a < -75° C freezer until processing.

Following blood sampling, 1 cm × 1 cm fin clips were biopsied from the distal end of the caudal fin from all fish and placed into a labeled microcentrifuge tube containing 95 % EtOH. Microcentrifuge tubes were individually wrapped in aluminum foil, placed on wet ice, and transported to the laboratory for storage at -60° C pending electrophoretic analysis (Lamer et al., 2010). Total length (mm) and weight (g) were also recorded for each fish. Strong positive correlations between total length and weight for fishes are very common; therefore, only total length was included as a variable in the analyses.

Laboratory Analysis

I analyzed plasma alkaline phosphatase (ALP) (U L^{-1}), calcium (mg dL^{-1}), lipase (U L^{-1-}), cholesterol (mg dL^{-1}), and triglycerides (mg dL^{-1}) using commercially available kits; QuantiChrom Alkaline Phosphatase Assay Kit (DALP-250), QuantiChrom Calcium Assay Kit (DICA-500), QuantiChrom Lipase Assay Kit (DLPS-100), EnzyChrom Cholesterol Assay Kit (ECCH-100), and EnzyChrom Triglyceride Assay Kit (ETGA-200), respectively, (BioAssay Systems, California, USA). We measured protein (g dL^{-1}) with a hand-held protein refractometer (AST model 1250, Thomas Scientific, New Jersey, USA) (Wells & Pankhurst, 1999), which is certified for use in the range of 0-12 g dL^{-1}. Alkaline phosphatase is a cell-membrane-associated glycoprotein found in all tissues, and elevated ALP activities in blood are likely related to an increased processing of energy substrates by the liver (Congleton & Wagner, 2006). Similarly, protein can respond to changes in body condition, with nutritionally-deprived individuals showing depressed protein reserves (Farbridge & Leatherland, 1992). Lipase has been shown to increase when acting as a transporter of nutrients during triglyceride hydrolysis. Lipase may also increase in fasted fish, with activities decreasing in response to food intake. Triglyceride and calcium concentrations may become elevated after feeding, and cholesterol responds to changes in the body energy reserves. Together, ALP, calcium, cholesterol, lipase, protein and triglycerides in plasma have been shown to represent fish nutritional status (Congleton & Wagner, 2006; Guerreiro et al., 2012; Wagner & Congleton, 2004).

Deoxyribonucleic acid (DNA) was extracted from fish tissues using the Agencourt DNAdvance genomic DNA extraction kit (Beckman Coulter, Massachusetts, USA), according to the manufacturer's instructions. Genomic DNA was eluted from the magnetic particles in 150 µL of elution buffer. Reactions were performed in 96-well plates. Genomic DNA was tested for

quantity and quality using the Qubit 2.0 Fluorometer (Life Technologies, New York, USA) and agarose gel electrophoresis, respectively. DNA aliquots were genotyped at 57 species-diagnostic single nucleotide polymorphisms (SNPs) (Lamer et al., In Review) using the MassARRAY 4 analyzer system (Sequenom Inc., California, USA) to call SNP genotypes. The posterior distribution of individual assignment was delineated into hybrid categories implementing the algorithm as computed by NewHybrids version 1.1 beta (Anderson & Thompson, 2002). Initially, the following classes were set using "Jefrey's-like priors" (Anderson & Thompson, 2002) in NewHybrids: parental species (bighead carp (BH) or silver carp (SV)), F_1 hybrids (F_1), first-generation backcrosses (BxBH or BxSV), second generation backcrosses (Bx2BH or Bx2SV), F_2 hybrids (F_2), third generation backcrosses (Bx3BH or Bx3SV), fourth generation backcrosses (Bx4BH+ or Bx4SV+), and individuals with an advanced cross that is difficult to assign (F_x). Initially, 100,000 replicates were used with a burning time of 50,000 steps.

Statistical Analyses

I used a one-way analysis of variance (ANOVA) to quantify the influence of genetic grouping on total length and all physiological indices of nutritional condition. Prior to analysis, I separated individuals into four distinct groups based on genetic identification. The groups were as follows: an advanced group with 46 individuals (Bx2BH, Bx2SV, Bx3SV, Bx4BH+, Bx4SV+, F_x), a first generation group with nine individuals (BxBH, BxSV, F_1), a parental silver carp group with 16 individuals (SV), and a parental bighead carp group with six individuals (BH) (Table 3.1). Statistical analyses were also performed without the F_1 hybrids from the first generation group and without the F_x hybrids from the advanced group, as these hybrids may exhibit different characteristics than the other hybrids in their respective groups (Lamer et al.,

2010; Muhlfeld et al., 2009), but there were minimal effects on the results; therefore, I chose not to remove these F_1 individuals from analyses. I used a Tukey's multiple comparison *post-hoc* analysis where appropriate to test for differences in mean values among the groups for each nutritional parameter (Demsar, 2006). I performed all statistical analyses using JMP version 10.0 (SAS Institute, North Carolina, and USA). Rejection of the null hypothesis (α) for all tests was 0.05. All values are reported as means ± S.E.M. where appropriate. We used the null hypothesis of no variation in nutrition (i.e., physiological parameters) among parental silver carp, parental bighead carp, or their hybrid crosses.

Results

Total length, weight, and the six measured nutritional parameters were highly variable among *Hypophthalmichthys* genetic identification groups (Table 3.2). Of the seven conditional indices examined, three showed significant variation across the different genetic groups used in this study ($p < 0.05$). Plasma protein was over 35 % greater in the parental silver carp group than in the fist-generation and parental bighead carp groups, but did not differ from the advanced group ([ANOVA ($n = 77$, $f_{(3, 76)} = 13.05$, $p < 0.0001$)]; Fig. 3.1a). Concentrations of plasma triglycerides did not differ between the parental silver carp and advanced groups, but triglyceride concentrations in the parental silver carp group were more than two and a half times greater than the parental bighead carp and first generation groups ([ANOVA ($n = 77$, $f_{(3, 76)} = 14.17$, $p < 0.0001$]; Fig. 3.1b). The lowest quantities of plasma lipase were observed in parental bighead carp, with no significant differences observed among the other three groups. Lipase activities in parental silver carp and the advanced group were over four-fold greater than in parental bighead carp ([ANOVA ($n = 77$, $f_{(3, 76)} = 3.60$, $p < 0.0175$]; Fig. 3.1c). Total length, plasma ALP,

calcium, and cholesterol did not differ significantly among genetic identification groups (all $p > 0.05$).

Discussion

For wild-caught, invasive Asian carp existing in sympatry in the Illinois River, parental silver carp exhibited significantly greater concentrations of triglycerides, protein, and quantities of lipase in plasma relative to parental bighead carp. Triglycerides are the primary energy stores in fishes, and they have been shown to decline during starvation and subsequently increase after feeding (Congleton & Wagner, 2006; Morales et al., 2012). Conversely, lipase is an enzyme that increases in fasted fish, as animals break down lipids for use during periods of starvation, and decreases to very low levels in fed fish. More specifically, lipase is involved in the early steps of triglyceride hydrolysis and it functions to break down triglycerides for mobilization (Babin & Vernier, 1989; Congleton & Wagner, 2006; Mohammadizadeh, Afkhami, Bastami, Ehsanpour, & Soltani, 2012; Wagner & Congleton, 2004). Triglyceride hydrolysis has the potential to increase the amount of lipase in plasma because it acts as a transporter of nutrients (Babin & Vernier, 1989; Mommsen et al., 1999). Plasma protein is an important nutritional requirement for fish maintenance and growth and has been shown to decrease in response to food deprivation in fishes (Congleton & Wagner, 2006; Kaushik, 1995; Svobodová et al., 2009; Wagner & Congleton, 2004). Parental silver carp feeding indices (e.g., triglycerides and protein concentrations) were greater than the feeding indices in parental bighead carp, indicating that parental silver carp were in significantly better nutritional condition relative to parental bighead carp. The amount of lipase in plasma of parental silver carp was also greater than in parental bighead carp. I chose to interpret lipase as an indicator of good nutritional condition because of

its function as a transporter of nutrients (Babin & Vernier, 1989; Mommsen et al., 1999) rather than as an indicator of starvation. These nutritional differences are likely related to gill raker morphology and feeding preferences. Silver and bighead carp are pump, filter-feeding fishes that use their gill rakers to retain food particles from the inhaled water (Bitterlich, 1985; Calkins et al., 2012; Kolar et al., 2007; Sampson et al., 2009; Sass et al., 2010). Bighead carp gill rakers are long, straight, and comb-like allowing them to consume suspended food particles down to 17 µm (Kolar et al., 2007; Lamer et al., 2010; Sampson et al., 2009), while silver carp gill rakers are hard and sponge-like in appearance, allowing them to consume food particles as small as 3 – 4 µm (De-Shang & Shuang-Lin, 1996; Omarov, 1970). The Illinois River is a highly disturbed ecosystem that receives intense agricultural nutrient inputs (categorizing it as eutrophic) and also has dense concentrations of phytoplankton, zooplankton, and bacteria (Donner, 2003; Houser & Richardson, 2010; Koel & Sparks, 2002; Wahl et al., 2008). It is likely that the elevated plasma triglyceride concentrations and lipase activities in parental silver carp relative to parental bighead carp may be occurring because silver carp are able to ingest more miniscule food particles from the river (down to 3 – 4 µm), while bighead carp are confined to eating larger food particles no less than 17 µm (De-Shang & Shuang-Lin, 1996; Omarov, 1970; Opuszynski & Shireman, 1991). Parental silver carp have access to a wider range of prey and are able to consume food on a near-continuous basis as they swim, which may explain why parental silver carp have elevated concentrations of plasma triglycerides and lipase activities. In contrast, bighead carp consume larger food particles that may be more energy-rich, but are likely less abundant (Mittelbach, 2002; Wahl et al., 2008). Additionally, previous literature has shown that bighead carp are the initial invaders into new habitats. After bighead population increases begin to stabilize, silver carp invade and their population densities increase exponentially. Silver carp become the more

successful species in the habitat, and exploit more resources compared to bighead carp, because of their increased feeding breadth (Irons et al., 2007; Sass et al., 2010). My results indicate that, for the Illinois River, when in sympatry, parental silver carp are in better nutritional condition than parental bighead carp, with plausible differences likely driven by gill raker morphology and feeding habits.

Interestingly, first generation group individuals (BxBH, BxSV, F_1) and advanced group backcrosses (Bx2BH, Bx2SV, Bx3SV, Bx4BH+, Bx4SV+, F_x) had nutritional conditions that were similar to either parental bighead or silver carp. Individuals from the first generation group exhibited concentrations of triglycerides and protein in plasma analogous to parental bighead carp, while offspring from the advanced group had plasma concentrations of protein comparable to parental silver carp; plasma lipase activities in hybrids were intermediate between parental groups. Previous research has documented that hybrid individuals can be inferior or superior to their parents (Rosas et al., 2010). For example, hybrid vigor (i.e., heterosis) occurs when hybrid offspring have increased growth, size, and/or yield: offspring, compared to the parents (Birchler, Yao, Chudalayandi, Vaiman, & Veitia, 2010; Chen, 2010; Rosas et al., 2010). Hybrid vigor may also result in a "hybrid swarm" if hybrids can survive and interbreed with other hybrid individuals, or backcross with parental species, leading to the complete merging of two taxa (Scribner et al., 2001). Hybrid offspring between parental silver and bighead carp typically possess gill rakers that are twisted, wavy, fused, or deformed, which is intermediate to the phenotypic gill raker varieties that exist for the parental groups described above (Kolar et al., 2007; Lamer et al., 2010). This intermediate gill raker phenotype in hybrid Asian carp may be because the further backcrossed a species becomes, the gene conversion can lead to an unequal expression of maternal and paternal alleles in the offspring (Chakraborty, 1989; Chen, 2010),

with the fraction contributed from each parent rarely 50 % (Mallet, 2007). This could explain why individuals in the first generation group are nutritionally identical to parental bighead carp based on the triglycerides, lipase, and protein concentrations documented in my study. It may also indicate that individuals in the advanced group are becoming more genetically similar to the parental silver carp group based on their nutritional plasma lipase activities and concentrations of protein. The twisted, club-like gill rakers of first generation group hybrids likely function more like parental bighead carp gill rakers and prevent first generation group hybrids from accessing the smaller-sized food particles that parental silver carp can consume. In contrast, the intermediate gill raker phenotype of the advanced group likely begins to function more like parental silver carp, which may be why two of the three nutritional metrics were identical for those groups. The majority of the individuals randomly sampled in my study were hybrids (with the highest number of hybrids separated into the advanced group), suggesting a high occurrence of hybridization in the wild. This is similar to the findings of Lamer et al. (2010) who found a conservative estimate of about 23 % of individuals existing as hybrids between parental silver carp and parental bighead carp in the MRB. My results suggest that it is unlikely for a hybrid swarm to occur. First generation group hybrids are more related to parental bighead carp and displayed decreased feeding indices of triglycerides and protein compared to parental silver carp. Advanced group individuals (although potentially becoming more nutritionally similar to parental silver carp) still exhibited inferior recent feeding indices relative to parental silver carp, demonstrated by decreased triglyceride concentrations. Previous findings have shown that silver carp comprise a greater density of biomass than bighead carp in the Illinois River (Garvey et al., 2011). This may further indicate improved feeding capabilities of silver carp and their more closely-related hybrids, similar to my findings. Irrespective of the differential causes in hybrid

nutrition, my study provides clear evidence that individuals in the advanced group are more comparable to parental silver carp (and are therefore in better nutritional condition) compared to first generation group hybrids that are more analogous to parental bighead carp in this reach of the Illinois River.

Previous research with hybrid individuals has specifically examined the condition of F_1 hybrids (first hybrid progeny of two taxa) because of their potential to show the greatest vigor or inferiority (Muhlfeld et al., 2009; Rosas et al., 2010). Because my study was performed in a natural setting, and morphological identification of parental bighead, parental silver, and hybrid carp is difficult in the field (Lamer et al., 2010), there were only two F_1 hybrid individuals and uneven numbers of hybrids across each genetic identification group, which may have impacted my results. Additionally, I only sampled one known location of *Hypophthalmichthys* hybrid individuals at one single point in time in the Illinois River. Future studies should incorporate greater temporal and spatial scale sampling because Asian carp are hybridizing in several rivers throughout the MRB (Lamer et al., 2010) and rivers can experience substantial seasonal variation (potentially affecting the nutritional condition in fishes) (Bohl, 1980; Gorman & Karr, 1978; Liss, Sass, & Suski, In Press; Lowe et al., 2006). Despite these caveats, my study illustrates that nutritional performance can decline in backcrossed Asian carp hybrid individuals in the wild.

Diverse environments may offer performance advantages to different genetic groups. Based on my data, for *Hypophthalmichthys* living in sympatry in oligotrophic habitats (those showing reduced phytoplankton concentrations), I would expect parental silver carp and advanced group individuals to outperform parental bighead carp and first generation group individuals. The breadth of the feeding capability for silver carp in the wild is greater than that of bighead carp, as silver carp are able to consume smaller food particles (De-Shang & Shuang-

Lin, 1996; Shuang-lin & De-shang, 1994). For Asian carp living in sympatry in a eutrophic environment (replete with phytoplankton), I would also expect parental silver carp and advanced group hybrids to outperform parental bighead carp and first generation group hybrids, as demonstrated by the results from my study. Silver carp numbers and biomass are increasing exponentially and they are the dominant fish species in their invaded habitats (Garvey et al., 2011; Sass et al., 2010). This may indicate that silver are outperforming bighead carp, as indicated by their population growth. Bighead carp living in sympatry with silver carp may prioritize zooplanktonic prey of a higher nutritional value, representing an optimal foraging strategy, compared to the broad feeding preferences of silver carp (Mittelbach, 2002; Wahl et al., 2008). However, if silver carp densities reduce the amount of prey available to bighead carp, then silver carp may outperform bighead carp based on their feeding abilities. Regardless, my results clearly illustrate a decrease in individual nutritional condition in Asian carp hybrids, suggesting that if hybridization continues, it may reduce the future spread of these invaders.

CHAPTER 5

GENERAL CONCLUSION

There are several theories that contribute to our understanding of community structure and dynamics, which may be driven by a number of abiotic and biotic factors. The interactions of these factors can occur at broad landscape scales, local scales, or across temporal scales. Together, these community characteristics may affect the establishment potential of an invasive species. Once an invasive species becomes established, hybridization among closely related taxa may occur, further affecting the community structure of an ecosystem. A disconnect exists, however, between the establishment of an invasive species and the influence that landscapes, local-scale parameters, seasonality, and hybridization may have on the physiological performance of those invaders. Although correlations may exist among landscapes, local scales, seasons, and hybrids, few studies have attempted a holistic approach to test for the effects of these parameters on the stress and nutritional status of invasive species in the wild. My thesis research offers a multi-faceted approach to test for drivers of stress and nutrition in an invasive species, and testing for the effects of hybridization on nutritional performance using Asian carp (*Hypophthalmichthys* spp.) as model organisms.

The first two chapters of my thesis demonstrated that silver carp nutrition and stress levels varied across landscape, local, and temporal scales. Specifically at the landscape scale, two nutritional metrics (short-term feeding and body energy reserves) were driven by time period, considered independently for each river. Interestingly, silver carp stress was driven by time period alone and decreased throughout the sampling periods. My findings suggest that nutrition and stress are potentially influenced by food availability, reproductive events, and/or water temperatures. My results have important management implications, as I would predict that

silver carp are moving more during the summer months when their feeding and stress levels were elevated, and their body energy stores were depleted. Silver carp may be more mobile in the summer months in order to find adequate food resources and/or to find suitable habitats for reproduction. Thus, Asian carp could be targeted to reduce abundances just prior to or during reproductive periods for more effective control. It may also be possible to target these fishes during periods of lower water temperatures (i.e., early fall, winter) when they are not likely to be moving to new locations, as they exhibited the lowest stress levels and the greatest body energy reserve scores during this time period.

At a river reach (local) scale, a suite of abiotic and biotic environmental factors were not correlated with blood-based indices of stress and nutrition in silver carp. Hypothesized environmental factors that I expected would have a high likelihood of influencing the physiology of these fishes (total phosphorus, fish species richness, composition, or overall species abundance) were unrelated to silver carp stress or nutrition. My findings further emphasize the successfulness of invasive silver carp in the wild, which may have important implications for their control. When attempting to manage invasive silver carp, I would suggest taking the quantity of food available, water temperature and the concentration of suspended solids in the area of concern into consideration when predicting the potential spread of this species. A single abiotic (i.e., TP) or biotic (i.e., fish species richness/composition/abundance) environmental parameter should not be the only indicator used when attempting to identify the driver(s) of silver carp spread, nor should it be the only targeted parameter when trying to prevent its spread. I would also suggest incorporating a landscape scale (entire river) approach, as local scale habitat changes did not appear to affect these fish. Based on my findings, silver carp seem to be limited by factors at a broad-scale, as small-scale patterns in stress and nutrition were not evident.

The third chapter of my thesis demonstrated that hybridization between bighead and silver carp influenced the nutritional performance of these fishes. Parental silver carp were in better nutritional condition than parental bighead carp, as exhibited by greater lipase activities and concentrations of triglycerides and protein. This variation in nutritional condition is likely due to differences in gill raker morphology and feeding habits. I would expect silver carp to outperform bighead carp in oligotrophic waters (such as the Great Lakes) because of the breadth of their diet, and their ability to feed at a very low trophic position. I would also expect silver carp to outperform bighead carp in eutrophic waters, as suggested by my results. Silver carp population growth and densities are increasing exponentially, and they are the dominant species in most invaded habitats (Garvey et al., 2011; Sass et al., 2010), thus reinforcing their ability to outperform bighead carp. Hybrid carp groups displayed differences in nutritional condition as well. The advanced group hybrids were more nutritionally similar to parental silver carp, and the first generation group hybrids were more nutritionally comparable to parental bighead carp. Therefore, advanced generation hybrids were in better nutritional condition than first generation group individuals. I would expect hybridization to continue occurring between Asian carp as the majority of the individuals I sampled were hybrids. However, it is unlikely that a hybrid swarm will occur. My results suggested that both hybrid groups were nutritionally inferior to parental silver carp. Relative to parental silver carp, advanced group hybrids displayed decreased concentrations of triglycerides and first generation group hybrids showed lower triglyceride and protein concentrations. Collectively, my thesis research provides the framework for a holistic approach to quantify potential factors that may influence stress and nutritional performance, and I recommend that this approach be used across taxa to better understand the influences of these factors on the potential spread and distribution of a species.

FIGURES AND TABLES

Fig. 1.1. Map of the Illinois River and portions of the Mississippi, Ohio, and Wabash rivers in Illinois illustrating reaches sampled in 2011. The Illinois River consists of the Alton reach (river mile (RM) 0-70), Meredosia (RM 70-80), La Grange (RM 80-158), and Chillicothe (RM 158-231). The Mississippi River consists of Pool 20 (RM 343-364.5), Pool 25 (RM 242-273.5), Chain of Rocks (RM 165.5-200.5) and Kaskaskia (RM 117-165.5). The Ohio River consists of Smithland Pool (RM 848-918.5), Pool 52 (918.5-939), Pool 53 (RM 939-962.5), and the Confluence (RM 962.5-981). The Wabash River consists of Terre Haute (RM 315.5-351), Palestine (RM 351-385.5), Vincennes (RM 385.5-412), Mt. Carmel (RM 412-444.5), and New Harmony (RM 444.5-487).

Fig. 1.2

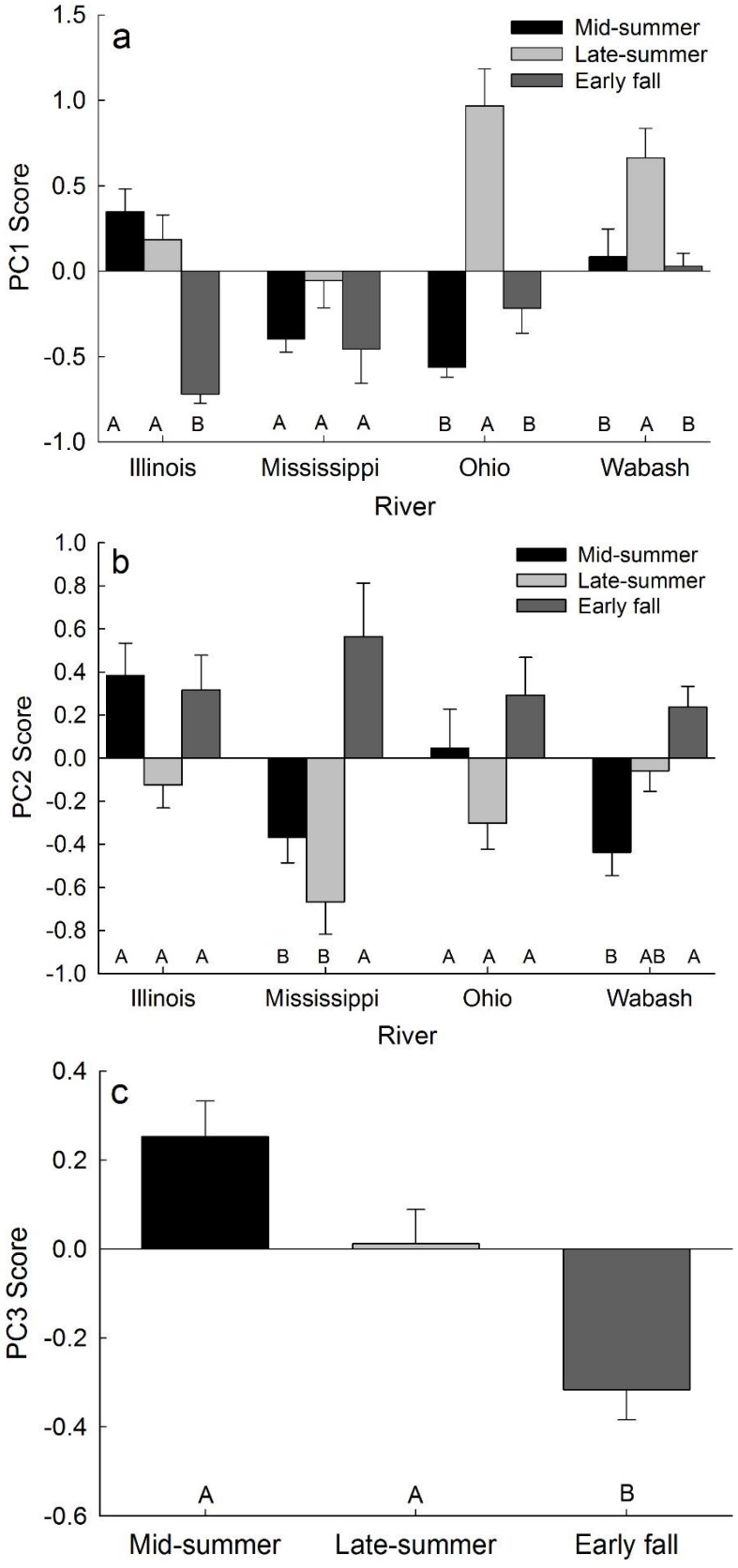
a
Mid-summer
Late-summer
Early fall
PC1 Score
1.5
1.0
0.5
0.0
-0.5
-1.0
A A B A A A B A B B A B
Illinois
Mississippi
Ohio
Wabash
River
b
Mid-summer
Late-summer
Early fall
PC2 Score
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
A A A B B A A A A B AB A
Illinois
Mississippi
Ohio
Wabash
River
c
PC3 Score
0.4
0.2
0.0
-0.2
-0.4
-0.6
A A B
Mid-summer
Late-summer
Early fall

Fig. 1.2 (continued)

Fig. 1.2a. Relationship for the best fit AICc ranked model linking PC1 (short-term feeding) scores for silver carp (*Hypophthalmichthys molitrix*) by time period, independent of river. Results of statistical analysis are reported on the figure with dissimilar letters indicating significant differences across time periods, with each river considered independently.

Fig. 1.2b. Relationship for the best fit AICc ranked model relating PC2 (body energy reserves) scores for silver carp (*Hypophthalmichthys molitrix*) by time period, independent of river. Results of statistical analysis are reported on the figure with dissimilar letters indicating significant differences across time periods, with each river considered independently.

Fig. 1.2c. Relationship for the best fit AICc ranked model explaining PC3 (stress) for silver carp (*Hypophthalmichthys molitrix*) by time period. Results of statistical analysis are reported on the figure with with dissimilar letters indicating significant differences across time periods.

Time Period	Sampling Dates	River	Number of Reaches	Total # Fish Sampled Per River Per Time Period
Mid-summer	June 15 – July 31, 2011	Illinois	4	53
		Mississippi	4	47
		Ohio	4	51
		Wabash	5	32
Late-summer	August 1 – Sept. 14, 2011	Illinois	3	30
		Mississippi	4	43
		Ohio	4	43
		Wabash	5	50
Early fall	Sept. 15 – October 31, 2011	Illinois	4	40
		Mississippi	4	34
		Ohio	4	40
		Wabash	5	50

Table 1.1. Silver carp (*Hypophthalmichthys molitrix*) sampling at broad temporal and spatial scales across mid-summer, late-summer and early fall time periods in the Illinois, Mississippi, Ohio, and Wabash rivers during 2011.

Principal Component	Model	AICc	Δ AICc	AICc Weight	Model Likelihood
PC1	Time Period[River]	1327.90	0.00	1.00	1.00
	Reach*Time Period[River]	1344.38	16.48	0.00	0.00
	Time Period	1366.63	38.73	0.00	0.00
	Reach[River]	1380.43	52.53	0.00	0.00
	River	1405.80	77.90	0.00	0.00
	River*Total Length	1414.76	86.86	0.00	0.00
	Total Length	1420.61	92.71	0.00	0.00
	Time Period*Total Length	1422.73	94.83	0.00	0.00
PC2	Time Period[River]	1377.57	0.00	1.00	1.00
	Time Period	1389.23	11.66	0.00	0.00
	River*Total Length	1402.61	25.04	0.00	0.00
	River	1412.81	35.24	0.00	0.00
	Total Length	1414.78	37.21	0.00	0.00
	Time Period*Total Length	1418.32	40.75	0.00	0.00
	Reach*Time Period[River]	1428.21	50.64	0.00	0.00
	Reach[River]	1431.08	53.51	0.00	0.00
PC3	Time Period	1383.35	0.00	1.00	0.90
	River*Total Length	1390.13	6.78	0.03	0.03
	River	1390.34	6.99	0.03	0.03
	Total Length	1390.76	7.41	0.02	0.02
	Time Period[River]	1391.08	7.73	0.02	0.02
	Time Period*Total Length	1405.30	21.95	0.00	0.00
	Reach*Time Period[River]	1418.16	34.81	0.00	0.00
	Reach[River]	1421.07	37.72	0.00	0.00

Table 1.2. Model selection results relating predictor variables to variation in PC scores for wild-caught silver carp (*Hypophthalmichthys molitrix*). PC1 corresponds to a short-term feeding score; PC2 represents a body energy reserve score, while PC3 represents a stress score. Fish were collected from four rivers across three time periods and sampled for blood immediately following collection. Models are ranked by differences in AICc values (ΔAICc), and the model with the lowest ΔAICc value is the best fit to the data, with AICc weight determining the best approximating model.

Table 1.3

River	Parameter	N	Min	Max	Mean	Median	Standard Error Mean
Illinois	Triglycerides (mg dL^{-1})	118	10.1	298.1	96.8	84.5	5.0
	Cortisol (ng mL^{-1})	122	0.06	102.5	10.2	2.3	1.7
	Cholesterol (mg dL^{-1})	123	44.3	592.0	218.0	211.4	5.8
	Glucose (mg dL^{-1})	123	18.8	73.2	39.6	38.2	1.0
	Protein (g dL^{-1})	123	2	5.6	3.7	3.7	0.0
	ALP (U L^{-1})	123	3	105	35	33	2.0
	Weight (g)	123	160	3890	1213	1120	49
	Total Length (mm)	123	311	709	495	487	5
Mississippi	Triglycerides (mg dL^{-1})	121	6.6	357.8	97	79.6	6.0
	Cortisol (ng mL^{-1})	120	0.06	288	12.6	0.1	3.3
	Cholesterol (mg dL^{-1})	124	57.4	557.3	190.5	182.7	6.6
	Glucose (mg dL^{-1})	124	11	78.6	40.0	38.1	1.3
	Protein (g dL^{-1})	124	2.0	5.3	3.5	3.5	0.1
	ALP (U L^{-1})	124	5.6	142.2	25.7	21.4	1.7
	Weight (g)	124	540	6450	1905	1465	107
	Total Length (mm)	124	387	872	599	525	10
Ohio	Triglycerides (mg dL^{-1})	134	4.7	527.2	109.0	77.4	7.7
	Cortisol (ng mL^{-1})	131	0.06	292.7	13.0	0.1	3.9
	Cholesterol (mg dL^{-1})	134	71.1	526.0	207.2	198.3	6.5
	Glucose (mg dL^{-1})	134	1.2	168.7	51.4	46.9	2.0
	Protein (g dL^{-1})	134	2	5.6	3.7	3.7	0.1
	ALP (U L^{-1})	134	6.8	146.8	26.8	22.5	1.5
	Weight (g)	134	140	7930	2931	2760	146
	Total Length (mm)	134	252	900	631	660	13
Wabash	Triglycerides (mg dL^{-1})	132	42.0	589.7	140.8	116.2	7.2
	Cortisol (ng mL^{-1})	131	0.06	97.1	9.1	0.1	1.7
	Cholesterol (mg dL^{-1})	132	79.1	369.7	168.4	164.4	3.6
	Glucose (mg dL^{-1})	132	16.5	98.7	40.7	38.3	1.2
	Protein (g dL^{-1})	132	2.9	5.6	3.9	4.0	0.0
	ALP (U L^{-1})	132	7.1	111.0	25.3	20.5	1.4
	Weight (g)	132	140	7000	2952	2335	149
	Total Length (mm)	132	262	866	615	595	12
Total	Triglycerides (mg dL^{-1})	505	4.7	589.7	111.6	90.5	3.4
	Cortisol (ng mL^{-1})	504	0.06	292.7	11.2	0.1	1.4
	Cholesterol (mg dL^{-1})	513	44.3	592	195.7	186.2	3.0

Table 1.3 (continued)

River	Parameter	N	Min	Max	Mean	Median	Standard Error Mean
	Glucose (mg dL^{-1})	513	1.2	168.7	43.1	40.3	0.8
	Protein (g dL^{-1})	513	2.0	5.6	3.7	3.7	0.0
Total	ALP (U L^{-1})	513	3.0	146.8	28.1	22.5	0.8
	Weight (g)	513	140	7930	2277	1810	69
	Total Length (mm)	513	252	900	577	563	6

Table 1.3. Sample size, minimum, maximum, mean, median, and standard error mean values for several nutritional and stress parameters of silver carp *Hypophthalmichthys molitrix*, sampled in the Illinois, Mississippi, Ohio, and Wabash rivers during 2011.

Time Period	Environmental Variable	Illinois River		Mississippi River		Ohio River		Wabash River	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Mid-summer	Secchi (cm)	29.09	0.43	23.68	0.93	24.49	0.61	12.00	1.03
	Surface Velocity (m s^{-1})	0.54	0.03	0.49	0.04	0.37	0.04	0.75	0.09
	Water Temperature (C)	28.45	0.18	29.34	0.14	25.35	0.08	23.33	0.09
	DO (mg L^{-1})	4.88	0.20	5.79	0.12	6.18	0.06	6.23	0.06
	River Stage (m)	3.88	0.25	6.57	0.32	10.44	0.21	5.07	0.02
Late-summer	Secchi (cm)	28.77	1.20	29.67	1.35	44.77	1.65	31.40	0.75
	Surface Velocity (m s^{-1})	0.27	0.04	0.46	0.04	0.18	0.02	0.21	0.02
	Water Temperature (C)	28.36	0.41	28.16	0.11	32.31	0.09	25.42	0.38
	DO (mg L^{-1})	6.06	0.33	6.66	0.15	6.47	0.25	10.00	0.28
	River Stage (m)	4.71	0.42	4.69	0.16	7.22	0.30	0.98	0.03
Early fall	Secchi (cm)	30.85	0.78	24.24	1.23	51.68	2.16	34.68	1.44
	Surface Velocity (m s^{-1})	0.24	0.01	0.33	0.05	0.22	0.02	0.39	0.03
	Water Temperature (C)	17.47	0.52	16.89	0.33	23.54	0.07	20.42	0.21
	DO (mg L^{-1})	8.04	0.10	8.51	0.06	8.57	0.11	10.07	0.17
	River Stage (m)	2.79	0.24	3.73	0.31	6.55	0.28	1.26	0.04

Table 1.4. Ancillary environmental data collected across rivers and time periods sampled in 2011. Values shown were averaged across time periods for all reaches. All parameters were measured in real time concurrently with fish sampling, except for river stage. River stage was acquired by the Army Corp of Engineers River Gauges website (http://rivergages.mvr.usace.army.mil/WaterControl/new/layout.cfm).

	PC 1	PC 2	PC 3
Triglycerides (mg dL^{-1})	**0.87**	0.027	-0.050
Cortisol (ng mL^{-1})	-0.12	0.10	**0.87**
Cholesterol (mg dL^{-1})	-0.16	**0.89**	0.066
Glucose (mg dL^{-1})	0.39	-0.28	**0.64**
Protein (g dL^{-1})	**0.43**	**0.76**	-0.16
ALP (U L^{-1})	**0.70**	0.032	0.10
% Variance Explained	28	25	18
Eigenvalue	1.7	1.5	1.1

Table 1.5. Principal components summarizing stress and nutritional characteristics for silver carp *Hypophthalmichthys molitrix* sampled from the Illinois, Mississippi, Ohio, and Wabash rivers during 2011.

Variables were loaded into three principle components (PC1, PC2, and PC3). Characteristics contributing maximally to each principle component are in bold and are greater than 0.4. Positive values for each principal component correlate positively with the stress and nutritional characteristics.

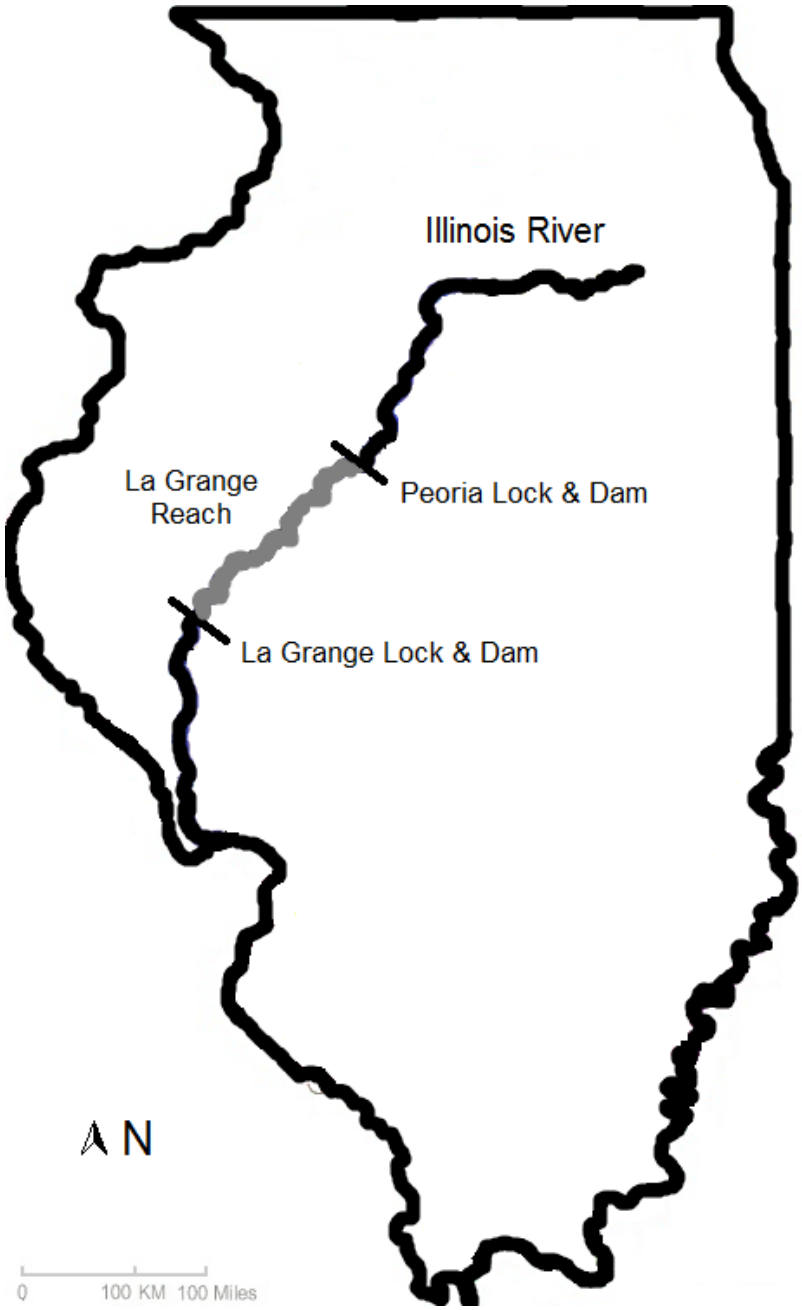

Fig. 2.1. Map of the Illinois River, Illinois, USA, showing the locations of sample collection for this study. The La Grange Reach (129 km) is located between La Grange Lock and Dam (L & D) and Peoria L & D.

Water Quality Parameter	Negative Correlation	Positive Correlation	No Correlation
Temperature	Conductivity DO Secchi Disc	Chlorophyll *a* Suspended Solids Turbidity	Total Nitrogen Velocity pH Total Phosphorus
Total Phosphorus	Chlorophyll *a* DO pH Secchi Disc	Conductivity Suspended solids Total Nitrogen Velocity	Temperature Turbidity
Suspended Solids	DO pH Secchi Disc	Chlorophyll *a* Temperature Total Phosphorus Turbidity	Conductivity Total Nitrogen Velocity

Table 2.1. Correlation matrices showing relationships between water quality predictor variables used in regression analyses. All correlations are significant at $\alpha = 0.05$.

Parameter	N	Min	Max	Mean	Median	Standard Error Mean
Triglycerides (mg dL^{-1})	30	14.8	233.4	93.4	86.6	7.9
Cortisol (ng mL^{-1})	32	0.06	102.5	19.3	7.8	5.2
Cholesterol (mg dL^{-1})	32	159.0	592.0	228.5	213.4	13.2
Glucose (mg dL^{-1})	32	21.5	63.3	39.6	38.6	1.9
Protein (g dL^{-1})	32	3.0	4.8	3.8	3.7	0.1
ALP (U L^{-1})	32	6.7	71.3	35.0	35.7	3.7
Weight (g)	32	800	2540	1196	1130	61
Total Length (mm)	32	450	617	494	487	6

Table 2.2. Sample size, minimum, maximum, mean, median, and standard error mean values for nutritional and stress characteristics of silver carp *Hypophthalmichthys molitrix* sampled in the La Grange Reach, Illinois River during 2011.

Parameter	Mid-summer	Late-summer	Early Fall
Secchi Disc Transparency (cm)	20.7 ± 0.6^{a}	20.9 ± 0.7^{a}	21.9 ± 0.6^{a}
Temperature (C)	30.9 ± 0.1^{a}	31.6 ± 0.2^{a}	15.1 ± 0.4^{b}
DO (mg L^{-1})	5.0 ± 0.2^{c}	7.97 ± 0.4^{b}	9.3 ± 0.1^{a}
pH	7.9 ± 0.02^{c}	8.2 ± 0.04^{a}	8.1 ± 0.02^{b}
Turbidity (NTU)	63.7 ± 2.6^{a}	66.4 ± 3.2^{a}	58.5 ± 2.9^{a}
Conductivity (µS cm^{-1})	774.8 ± 7.1^{a}	626.5 ± 8.9^{b}	753.6 ± 12.6^{a}
Velocity (m s^{-1})	0.2 ± 0.03^{a}	0.04 ± 0.01^{b}	0.12 ± 0.02^{a}
Total Phosphorus (mg L^{-1})	0.4 ± 0.02^{a}	0.4 ± 0.02^{a}	0.4 ± 0.03^{a}
Total Nitrogen (mg L^{-1})	2.9 ± 0.1^{a}	1.9 ± 0.08^{b}	2.3 ± 0.1^{b}
Suspended Solids (mg L^{-1})	65.0 ± 3.5^{a}	63.8 ± 3.5^{a}	57.2 ± 3.4^{a}
Chlorophyll *a* (µg L^{-1})	44.3 ± 2.6^{b}	85.9 ± 4.2^{a}	34.6 ± 1.8^{b}
Rotifers (rotifers L^{-1})	118.5 ± 10.1^{a}	90.8 ± 7.9^{a}	105.7 ± 17.0^{a}
Copepods (copepods L^{-1})	3.1 ± 0.3^{a}	0.9 ± 0.1^{c}	1.9 ± 0.3^{b}
Cladocerans (cladocerans L^{-1})	2.7 ± 0.3^{a}	2.8 ± 0.3^{a}	0.3 ± 0.1^{b}
Total Zooplankton (total zoop. L^{-1})	124.4 ± 10.1^{a}	94.5 ± 7.8^{a}	107.9 ± 17.1^{a}
Species Richness	38	49	45
Planktivore & Omnivore Richness	13	16	16
Plantivore Richness	3	2	3
CPUE_excluding SVCP (fish hr^{-1})	179.2	319.7	328.5
CPUE_GZSD (fish hr^{-1})	99.2	120.3	34.5
CPUE_SVCP (fish hr^{-1})	8.3	46.8	26.0

Table 2.3. Mean values for water quality parameters, zooplankton concentrations, and fish species richness and abundance for the La Grange Reach, Illinois River during 2011. CPUE refers to fish caught per electroshocking hour, GZSD refers to gizzard shad (*Dorosoma cepedianum*), SVCP refers to silver carp (*Hypophthalmichthys molitrix*), and excluding SVCP refers to the abundance of all other fishes caught during sampling. All values are reported as the means ± S.E.M. where appropriate. Results of statistical analyses are reported as superscript letters, with with dissimilar letters indicating significant differences of an individual parameter across time periods (ANOVA, $p < 0.05$).

Model	AICc	ΔAICc	Model Likelihood	AICc Weight
CPUE_GZSD (fish hr^{-1})	251.44	0.00	1.00	0.68
Temperature (C)	254.06	2.61	0.27	0.18
Cladocerans (cladocerans L^{-1})	254.73	3.29	0.19	0.13
Suspended Solids (mg L^{-1})	260.34	8.89	0.01	0.01
Planktivore Richness	279.92	28.48	0.00	0.00
CPUE_excluding SVCP (fish hr^{-1})	287.91	36.47	0.00	0.00
Planktivore & Omnivore Richness	288.70	37.25	0.00	0.00
CPUE_SVCP (fish hr^{-1})	289.95	38.51	0.00	0.00
Rotifers (rotifers L^{-1})	289.99	38.54	0.00	0.00
Copepods (copepods L^{-1})	290.64	39.19	0.00	0.00
Total Zooplankton (total zoop. L^{-1})	290.74	39.29	0.00	0.00
Total Length (mm)	291.01	39.57	0.00	0.00
Species Richness	291.05	39.61	0.00	0.00
Total Phosphorus (mg L^{-1})	291.06	39.62	0.00	0.00

Table 2.4. Model selection results relating predictor variables to variation in alkaline phosphatase (ALP) activities for wild-caught silver carp (*Hypophthalmichthys molitrix*). Fish were collected from the La Grange Reach, Illinois River across three time periods and sampled for blood immediately following collection. Models are ranked by differences in AIC values (ΔAICc), and the model with the lowest ΔAICc value is the best fit to the data, with AICc weight determining the best approximating model. CPUE refers to fish caught per electroshocking hour, GZSD refers to gizzard shad (*Dorosoma cepedianum*), SVCP refers to silver carp, and excluding SVCP refers to the abundance of all other fishes caught during sampling.

Model	AICc	ΔAICc	Model Likelihood	AICc Weight
Suspended Solids (mg L^{-1})	233.28	0.00	1.00	0.42
Cladocerans (cladocerans L^{-1})	234.21	0.92	0.63	0.26
Temperature (C)	234.42	1.14	0.57	0.24
CPUE_GZSD (fish hr^{-1})	236.95	3.67	0.16	0.07
CPUE_excluding SVCP (fish hr^{-1})	241.42	8.13	0.02	0.01
Planktivore & Omnivore Richness	242.17	8.89	0.01	0.00
Total Phosphorus (mg L^{-1})	246.19	12.91	0.00	0.00
Species Richness	246.62	13.33	0.00	0.00
Total Zooplankton (total zoop. L^{-1})	247.44	14.16	0.00	0.00
Copepods (copepods L^{-1})	247.57	14.28	0.00	0.00
Planktivore Richness	247.91	14.63	0.00	0.00
Rotifers (rotifers L^{-1})	248.09	14.80	0.00	0.00
CPUE_SVCP (fish hr^{-1})	248.10	14.82	0.00	0.00
Total Length (mm)	248.65	15.37	0.00	0.00

Table 2.5. Model selection results relating predictor variables to variation in plasma glucose concentrations of wild-caught silver carp (*Hypophthalmichthys molitrix*). Fish were collected from the La Grange Reach, Illinois River, across three time periods and sampled for blood immediately following collection. Models are ranked by differences in AIC values (ΔAICc), and the model with the lowest ΔAICc value is the best fit to the data, with AICc weight determining the best approximating model. CPUE refers to fish caught per electroshocking hour, GZSD refers to gizzard shad (*Dorosoma cepedianum*), SVCP refers to silver carp, and excluding SVCP refers to the abundance of all other fishes caught.

Model	AICc	ΔAICc	Model Likelihood	AICc Weight
Planktivore Richness	309.71	0.00	1.00	0.17
CPUE_GZSD (fish hr^{-1})	310.64	0.93	0.63	0.11
CPUE_SVCP (fish hr^{-1})	311.27	1.55	0.46	0.08
Rotifers (rotifers L^{-1})	311.28	1.56	0.46	0.08
Temperature (C)	311.44	1.72	0.42	0.07
Cladocerans (cladocerans L^{-1})	311.53	1.82	0.40	0.07
Copepods (copepods L^{-1})	311.59	1.88	0.39	0.07
Total Zooplankton (total zoop. L^{-1})	311.65	1.94	0.38	0.06
Species Richness	312.00	2.29	0.32	0.05
Suspended Solids (mg L^{-1})	312.12	2.40	0.30	0.05
Total Phosphorus (mg L^{-1})	312.15	2.44	0.30	0.05
Planktivore & Omnivore Richness	313.01	3.29	0.19	0.03
CPUE_excluding SVCP (fish hr^{-1})	313.10	3.38	0.18	0.03
Total Length (mm)	313.21	3.50	0.17	0.03

Table 2.6. Model selection results relating predictor variables to variation in cortisol concentrations for wild-caught silver carp (*Hypophthalmichthys molitrix*). Fish were collected from the La Grange Reach, Illinois River across three time periods and sampled for blood immediately following collection. Models are ranked by differences in AIC values (ΔAICc), and the model with the lowest ΔAICc value is the best fit to the data, with AICc weight determining the best approximating model. CPUE refers to fish caught per electroshocking hour, GZSD refers to gizzard shad (*Dorosoma cepedianum*), SVCP refers to silver carp, and excluding SVCP refers to the abundance of all other fishes caught.

Model	AICc	ΔAICc	Model Likelihood	AICc Weight
Total Length (mm)	371.91	0.00	1.00	0.08
CPUE_SVCP (fish hr^{-1})	372.28	0.38	0.83	0.07
Rotifers (rotifers L^{-1})	372.28	0.38	0.83	0.07
Copepods (copepods L^{-1})	372.29	0.38	0.83	0.07
Total Zooplankton (total zoop. L^{-1})	372.29	0.38	0.83	0.07
Species Richness	372.30	0.39	0.82	0.07
Total Phosphorus (mg L^{-1})	372.30	0.39	0.82	0.07
Planktivore Richness	372.31	0.41	0.82	0.07
Planktivore & Omnivore Richness	372.34	0.44	0.80	0.07
CPUE_excluding SVCP (fish hr^{-1})	372.35	0.45	0.80	0.07
CPUE_GZSD (fish hr^{-1})	372.45	0.54	0.76	0.06
Temperature (C)	372.46	0.56	0.76	0.06
Suspended Solids (mg L^{-1})	372.47	0.56	0.76	0.06
Cladocerans (cladocerans L^{-1})	372.47	0.56	0.76	0.06

Table 2.7. Model selection results relating predictor variables to variation in cholesterol concentrations for wild-caught silver carp (*Hypophthalmichthys molitrix*). Fish were collected from the La Grange Reach, Illinois River across three time periods and sampled for blood immediately following collection. Models are ranked by differences in AIC values (ΔAICc), and the model with the lowest ΔAICc value is the best fit to the data, with AICc weight determining the best approximating model. CPUE refers to fish caught per electroshocking hour, GZSD refers to gizzard shad (*Dorosoma cepedianum*), SVCP refers to silver carp, and excluding SVCP refers to the abundance of all other fishes caught.

Model	AICc	ΔAICc	Model Likelihood	AICc Weight
CPUE_GZSD (fish hr^{-1})	314.43	0.00	1.00	0.17
Temperature (C)	315.86	1.43	0.49	0.08
Cladocerans (cladocerans L^{-1})	315.88	1.45	0.48	0.08
Suspended Solids (mg L^{-1})	316.03	1.59	0.45	0.08
Planktivore Richness	316.18	1.75	0.42	0.07
Total Length (mm)	316.35	1.92	0.38	0.06
CPUE_SVCP (fish hr^{-1})	316.71	2.28	0.32	0.05
Rotifers (rotifers L^{-1})	316.71	2.28	0.32	0.05
Copepods (copepods L^{-1})	316.78	2.35	0.31	0.05
Total Zooplankton (total zoop. L^{-1})	316.79	2.36	0.31	0.05
Species Richness	316.85	2.42	0.30	0.05
CPUE_excluding SVCP (fish hr^{-1})	316.87	2.44	0.30	0.05
Total Phosphorus (mg L^{-1})	316.87	2.44	0.30	0.05
Planktivore & Omnivore Richness	316.89	2.46	0.29	0.05

Table 2.8. Model selection results relating predictor variables to variation in triglyceride concentrations for wild-caught silver carp (*Hypophthalmichthys molitrix*). Fish were collected from the La Grange Reach, Illinois River across three time periods and sampled for blood immediately following collection. Models are ranked by differences in AIC values (ΔAICc), and the model with the lowest ΔAICc value is the best fit to the data, with AICc weight determining the best approximating model. CPUE refers to fish caught per electroshocking hour, GZSD refers to gizzard shad (*Dorosoma cepedianum*), SVCP refers to silver carp, and excluding SVCP refers to the abundance of all other fishes caught.

Model	AICc	ΔAICc	Model Likelihood	AICc Weight
Total Length (mm)	37.58	0.00	1.00	0.11
Total Zooplankton (total zoop. L^{-1})	37.78	0.20	0.91	0.10
Copepods (copepods L^{-1})	37.78	0.20	0.91	0.10
Species Richness	37.81	0.23	0.89	0.10
Rotifers (rotifers L^{-1})	37.83	0.25	0.88	0.10
CPUE_SVCP (fish hr^{-1})	37.83	0.25	0.88	0.10
Total Phosphorus (mg L^{-1})	37.86	0.27	0.87	0.09
Planktivore & Omnivore Richness	38.60	1.02	0.60	0.07
CPUE_excluding SVCP (fish hr^{-1})	38.78	1.20	0.55	0.06
Planktivore Richness	39.19	1.61	0.45	0.05
Suspended Solids (mg L^{-1})	41.89	4.31	0.12	0.01
CPUE_GZSD (fish hr^{-1})	42.09	4.51	0.10	0.01
Cladocerans (cladocerans L^{-1})	42.12	4.54	0.10	0.01
Temperature (C)	42.14	4.56	0.10	0.01

Table 2.9. Model selection results relating predictor variables to variation in protein concentrations for wild-caught silver carp (*Hypophthalmichthys molitrix*). Fish were collected from the La Grange Reach, Illinois River across three time periods and sampled for blood immediately following collection. Models are ranked by differences in AIC values (ΔAICc), and the model with the lowest ΔAICc value is the best fit to the data, with AICc weight determining the best approximating model. CPUE refers to fish caught per electroshocking hour, GZSD refers to gizzard shad (*Dorosoma cepedianum*), SVCP refers to silver carp, and excluding SVCP refers to the abundance of all other fishes caught.

Fig. 3.1

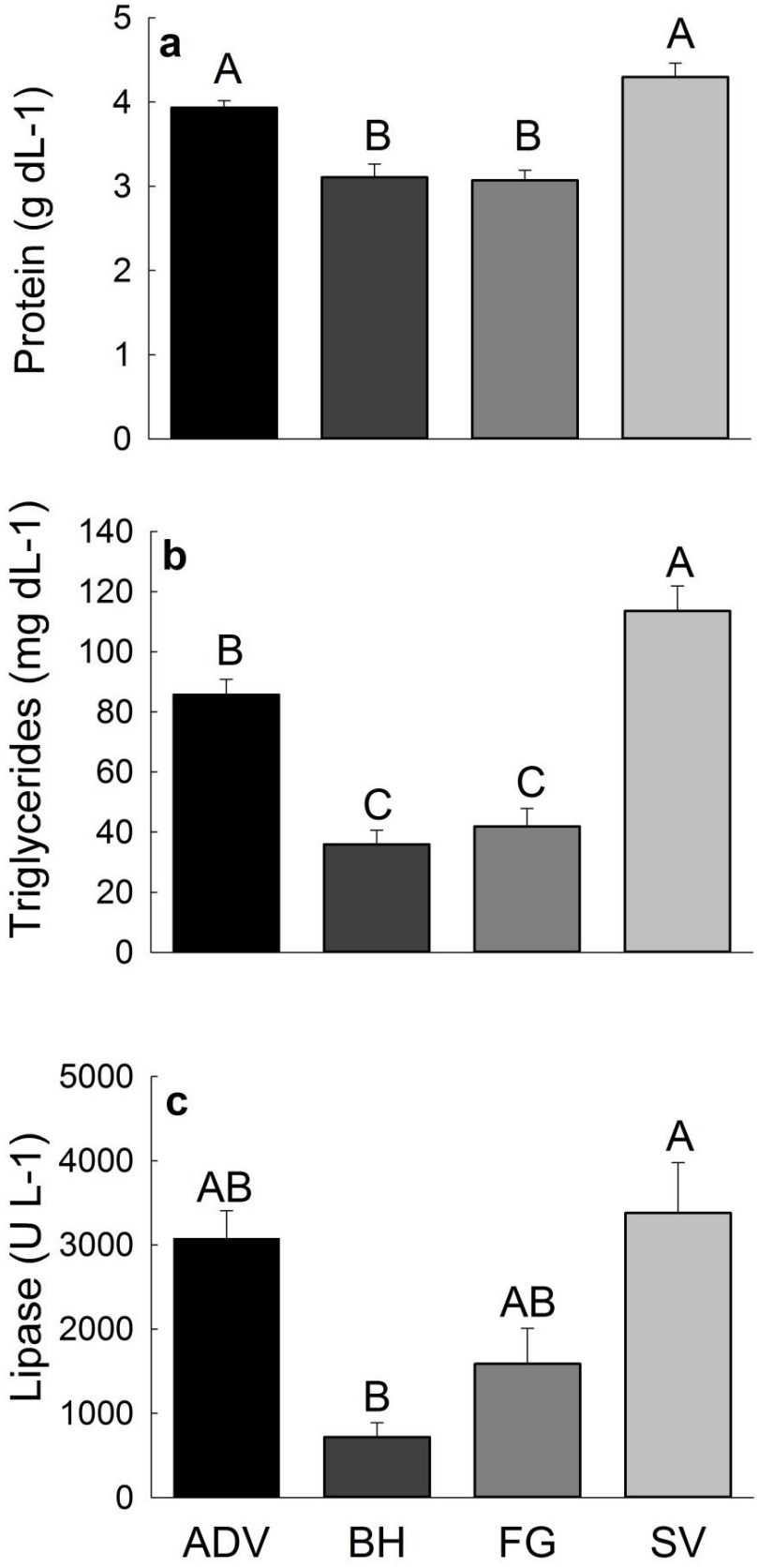
a
Protein (g dL-1)
5
4
3
2
1
0
A
B
B
A
b
Triglycerides (mg dL-1)
140
120
100
80
60
40
20
0
B
C
C
A
c
Lipase (U L-1)
5000
4000
3000
2000
1000
0
AB
B
AB
A
ADV
BH
FG
SV

Fig. 3.1 (continued)

Fig. 3.1a. Relationship between plasma protein (g dL^{-1}) and genetic identification grouping for Asian carp individuals (advanced generation group = ADV, parental bighead carp *Hypophthalmichthys nobilis* = BH, first generation group = FG, parental silver carp *H. molitrix* = SV). Results of statistical analysis are reported on the figure with dissimilar letters indicating significant differences across groupings ($\alpha = 0.05$). Error bars denote one standard error about the mean.

Fig. 3.1b. Relationship between plasma triglycerides (mg dL^{-1}) and genetic identification grouping for Asian carp individuals (advanced generation group = ADV, parental bighead carp *Hypophthalmichthys nobilis* = BH, first generation group = FG, parental silver carp *H. molitrix* = SV). Results of statistical analysis are reported on the figure with dissimilar letters indicating significant differences across groupings ($\alpha = 0.05$). Error bars denote one standard error about the mean.

Fig. 3.1c. Relationship between plasma lipase (U L^{-1}) and genetic identification grouping for Asian carp individuals (advanced generation group = ADV, parental bighead carp *Hypophthalmichthys nobilis* = BH, first generation group = FG, parental silver carp *H. molitrix* = SV). Results of statistical analysis are reported on the figure with dissimilar letters indicating significant differences across groupings ($\alpha = 0.05$). Errors bars denote one standard error about the mean.

Genetic Grouping	Genetic Classification	Number of Individuals	Total
Parental Bighead Carp	BH	6	6
Parental Silver Carp	SV	16	16
First Generation Group	F_1	2	9
	BxBH	4	
	BxSV	3	
Advanced Generation Group	Bx2BH	2	46
	Bx2SV	2	
	Bx3SV	16	
	Bx4BH+	11	
	Bx4SV+	12	
	F_x	3	

Table 3.1. Genetic identification group and the number of individuals from each genetic classification separated into the genetic identification groups. Parental species are bighead carp *Hypophthalmichthys nobilis* = BH, silver carp *H. molitrix* = SV, and their hybrids: F_1 hybrids = F_1, first-generation backcrosses = BxBH or BxSV, second generation backcrosses = Bx2BH or Bx2SV, F_2 hybrids = F_2, third generation backcrosses = Bx3BH or Bx3SV, fourth generation backcrosses = Bx4BH+ or Bx4SV+, and individuals with an advanced cross that is difficult to assign = F_x, sampled near Morris, Illinois, Illinois River, river km 423.

Table 3.2

Grouping	Parameter	N	Min	Max	Mean	Median	Standard Error Mean
Parental Bighead Carp	ALP (U L^{-1})	6	3.0	13.7	9.2	10.0	1.6
	Calcium (mg dL^{-1})	6	9.7	12.8	11.4	11.4	0.5
	Cholesterol (mg dL^{-1})	6	122.3	285.3	240.6	261.3	24.8
	Protein (g dL^{-1})	6	2.5	3.7	3.1	3.2	0.2
	Triglycerides (mg dL^{-1})	6	19.4	54.2	35.9	35.4	4.7
	Lipase (U L^{-1})	6	371.6	1498.3	718.3	560.7	170.0
	Weight (g)	6	4150	11480	6675	6175	1051
	Total Length (mm)	6	722	1029	840	828	44
Parental Silver Carp	ALP (U L^{-1})	16	1.7	39.0	13.3	9.8	2.6
	Calcium (mg dL^{-1})	16	9.7	14.0	11.7	11.3	0.4
	Cholesterol (mg dL^{-1})	16	198.9	622.7	311.0	239.0	32.5
	Protein (g dL^{-1})	16	3.5	5.8	4.3	4.2	0.2
	Triglycerides (mg dL^{-1})	16	44.6	163.9	113.6	107.1	8.3
	Lipase (U L^{-1})	16	637.5	8257.0	3379.7	3090.3	595.4
	Weight (g)	16	3140	9860	6089	5825	528
	Total Length (mm)	16	681	911	802	795	18
First Generation Group	ALP (U L^{-1})	9	4.4	14.4	9.2	9.0	0.9
	Calcium (mg dL^{-1})	9	10.4	11.8	11.1	11.2	0.2
	Cholesterol (mg dL^{-1})	9	201.9	293.2	248.2	248.0	11.5
	Protein (g dL^{-1})	9	2.6	3.6	3.1	3.1	0.1
	Triglycerides (mg dL^{-1})	9	18.0	67.3	41.9	48.4	6.0
	Lipase (U L^{-1})	9	584.3	4774.9	1588.3	1308.1	421.8
	Weight (g)	9	3020	9640	4767	3970	720
	Total Length (mm)	9	682	960	766	745	766
Advanced Generation Group	ALP (U L^{-1})	46	1.0	50.3	12.0	9.7	1.5
	Calcium (mg dL^{-1})	46	10.0	15.9	12.0	11.8	0.2
	Cholesterol (mg dL^{-1})	46	172.0	428.7	280.0	252.7	8.6
	Protein (g dL^{-1})	46	2.8	5.1	3.9	3.9	0.1
	Triglycerides (mg dL^{-1})	46	24.8	155.5	85.7	88.2	5.2
	Lipase (U L^{-1})	46	385.0	6951.1	3076.0	2911.1	330.8
	Weight (g)	46	3110	10620	5709	5510	222
	Total Length (mm)	46	653	951	787	785	9
Total	ALP (U L^{-1})	77	1.0	50.3	11.7	9.2	1.1
	Calcium (mg dL^{-1})	77	9.7	15.9	11.8	11.6	0.1
	Cholesterol (mg dL^{-1})	77	122.3	622.7	279.7	264.0	8.9

Table 3.2 (continued)

Grouping	Parameter	N	Min	Max	Mean	Median	Standard Error Mean
Total	Protein (g dL^{-1})	77	2.5	5.8	3.8	3.9	0.1
	Triglycerides (mg dL^{-1})	77	18.0	163.9	82.5	82.8	4.5
	Lipase (U L^{-1})	77	371.6	8257.0	2781.5	1568.9	252.6
	Weight (g)	77	3020	11480	5761	5460	209
	Total Length (mm)	77	653	1029	792	783	8

Table 3.2. Genetic identification group sample size, minimum, maximum, mean, median, and standard error mean values of several nutritional characteristics of parental bighead carp (*Hypophthalmichthys nobilis*), parental silver carp (*H. molitrix*), and their hybrid backcrosses sampled near Morris, Illinois, Illinois River, river km 423.

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