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VARIATION IN GILL RAKERS OF ASIAN CARP AND NATIVE FILTER-FEEDING
FISHES FROM THE ILLINOIS, JAMES AND WABASH RIVERS, USA.

A Manuscript Style Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of Master of Science

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College of Science and Health
Biology

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VARIATION IN GILL RAKERS OF ASIAN CARP AND NATIVE FILTER-FEEDING
FISHES FROM THE ILLINOIS, JAMES AND WABASH RIVERS, USA.

By Liza Walleser

We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Master of Science in Biology.

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ABSTRACT

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Populations of silver (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*) may be controlled by targeting the structure of gill rakers – how they filter food particles from the water column. Because species-specific differences in gill raker structure were not well understood, I investigated the morphology and spacing of these structures in Asian carp and compared them to those in gizzard shad (*Dorosoma cepedianum*) and bigmouth buffalo (*Ictiobus cyprinellus*) -- two species of filter-feeding fish competing with Asian carp for food in the upper Mississippi River basin. Stereomicroscopy and a novel approach of confocal microscopy were used to examine the morphologies of gill rakers from each species. Qualitative analyses indicated unique morphologies of rakers among all four species. Quantitative analyses of silver carp and gizzard shad indicated spacing of gill rakers in silver carp was correlated with fish length and did not generally differ among sampling sites or months. Spacing of gill rakers in gizzard shad was not correlated with fish length, but differed among sites and months. Thus, silver carp may be controlled with microparticles which target the length-dependent spacing of their gill rakers. Simultaneously, consumption of microparticles by gizzard shad may be minimized based on site-specific spacing of gill rakers.

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INTRODUCTION

A myriad of wildlife species have been transferred from their native ranges to new locations. Several species of Asian carp have been introduced into North America including silver carp *Hypophthalmichthys molitrix*, bighead carp *H. nobilis*, grass carp *Ctenopharyngodon idella*, and black carp *Mylopharyngodon piceus*. Asian carp were introduced to the United States primarily from regions of China (Jennings 1988, Fuller 1999). The native range of silver carp and bighead carp is within the borders of China, Russia, and North Korea (Fan 1990), where they are used in aquaculture for controlling algal growth and to increase fish production (Cremer and Smitherman 1980, Opuszynski *et al.* 1991). A current global problem is the escape or release of these carp from their holding facilities into natural rivers and lakes, where they pose a threat to native fish populations (Xie and Chen 2001).

In the United States, silver and bighead carp were introduced in the 1970s for wastewater treatment and improving quality of water used in aquaculture facilities (Chick and Pegg 2001, Kolar *et al.* 2007). By the 1980s they had escaped into the Mississippi River, chiefly through flooding of aquaculture facilities (Freeze and Henderson 1982), but also via emptied ballast water, release of bait fish, escape during live transfer, and intentional release of pets or live market fish (Kolar and Lodge 2002, Herborg *et al.* 2007, Irons *et al.* 2007, Keller and Lodge 2007, Cooke and Hill 2010, Hansen 2010). They subsequently invaded the Missouri, Ohio, and Illinois Rivers (Tucker *et al.* 1995, Chick and Pegg 2001, Irons *et al.* 2007, Delong 2010) and quickly established

reproducing populations (Xie and Chen 2001, Mandrak and Cudmore 2004, Kolar *et al.* 2007).

Because of their unique life history characteristics, silver and bighead carp have the potential to out-compete native species and alter the trophic structure of aquatic systems. In contrast to black, common, and grass carp, silver and bighead carp are efficient filter-feeders of plankton (Xie and Chen 2001, Kolar *et al.* 2007, Cooke and Hill 2010) and can decrease food availability for other native filter-feeding fish, including paddlefish *Polyodon spathula*, gizzard shad *Dorosoma cepedianum*, and bigmouth buffalo *Ictiobus cyprinellus* (Dong and Li 1994, Chick and Pegg 2001, Lu *et al.* 2002, Radke and Kahl 2002, Schrank *et al.* 2003, Delong and Thorp 2006, Irons *et al.* 2007, Sampson and Pegg 2009). In addition, because most fish are plankton-feeders at some stage of their life cycle, silver and bighead carp can decrease resource availability for many organisms other than those that are filter-feeders as adults (Chick and Pegg 2001, Delong 2010). This includes game fish such as walleye *Sander vitreus*, lake trout *Salvelinus namaycush*, bluegill *Lepomis macrochirus*, and black bass *Micropterus* spp. (*e.g.*, Hansen 2010). Moreover, their wide-spectrum diet, which includes detritus, bacteria, phytoplankton, and zooplankton (Xie and Chen 2001, Kolar *et al.* 2007, Cooke and Hill 2010), fuels very fast growth and reproduction and allows them to thrive in a diverse range of aquatic habitats (Xie and Chen 2001, Kolar *et al.* 2007, Cooke and Hill 2010). Moreover, rapid and prolonged growth makes them vulnerable to predators for a relatively short period (Kolar *et al.* 2007). Asian carp (hereafter, referring only to silver and bighead) have been difficult to manage because they are long-lived and mobile (DeGrandchamp *et al.* 2008). They are rapid colonizers (DeGrandchamp *et al.* 2008) and

tolerant of variable water temperature, flow, and low concentrations of dissolved oxygen (Conover *et al.* 2007). Consequently, one of the most critical concerns regarding Asian carp is the extent to which they will spread their range across North America, including the Laurentian Great Lakes (Chick and Pegg 2001, Mandrak and Cudmore 2004, Cooke and Hill 2010).

The Great Lakes are a valuable natural resource for the United States and Canada, providing potable water and producing over seven billion dollars annually in revenue from fisheries (ASA 2008 as cited in Hansen 2010). While supporting populations of numerous native vertebrate and invertebrate species, the Great Lakes have also been colonized by over 180 exotic species (Holeck *et al.* 2004), including the sea lamprey *Petromyzon marinus*, round goby *Neogobius melanostomus*, and zebra mussel *Dreissena polymorpha* (Conover *et al.* 2007). As an already stressed system, the effect of silver and bighead carp invasion into these lakes is uncertain, though considerable.

Introduction of additional filter-feeders into the Great Lakes, especially extremely efficient filter-feeders with a wide-spectrum diet, could have profound, negative effects on primary producers and consumers (Lazarro *et al.* 2003, Ke *et al.* 2009). For example, Irons *et al.* (2007) noted decreased condition of gizzard shad (–7%) and bigmouth buffalo (–5%) following the introduction of silver and bighead carp into La Grange Reach of the Illinois River. They suggested that the decrease in condition was most likely due to competition with Asian carp, which can lead to decreased body mass, fecundity, and overall health, and increased prevalence of disease among native fish. Negative effects on food webs and native fish due to Asian carp have also occurred in other locations

(Spataru and Gophen 1985, Burke *et al.* 1986, Xie and Yang 2000, Xie and Chen 2001, Lu *et al.* 2002, Xie 2003).

Various methods have been considered to control populations of Asian carp, including harvest, introduction of genetically modified fishes to wild populations (Nowak 2002, Conover *et al.*, 2007), and physical barriers between water bodies (USEPA 2004, DeGrandchamp *et al.* 2008, GLC 2010). National laws have also been implemented (*e.g.*, Lacey Act) to prohibit illegal trafficking of plants and animals, including Asian carp (Favre 2003, GLFC 2010). However, it is uncertain if any of these methods, whether mandatory or voluntary, can fully prevent the spread of Asian carp.

One additional method of control has included the distribution of piscicides to kill exotic fish. This approach has been used in the Chicago area waterways to prevent the Asian carp from gaining access to Lake Michigan (Kolar *et al.* 2007, USFWS 2009). Although this method is efficient in temporary extirpation of the carp in a given area, current piscicides used to control nuisance fishes do not have adequate specificity to only control Asian carp (ACC 2010). However, if a piscicide contained within a particle was primarily retained in the gill rakers of Asian carp, then it may provide selective control of these species while limiting exposure to non-target organisms.

Accordingly, the focus of this study was to better understand the filter-feeding abilities of Asian carp through examination of gill raker structures along the inner margin of gill arches that fish use to sieve food particles from water passing through their buccal cavity. The structure and function of gill rakers is highly variable among different species and little is known about the gill rakers of Asian carp of the upper Mississippi River basin. The objective was to describe the morphology and spacing of gill rakers in

silver carp and bighead carp relative to those of gizzard shad and bigmouth buffalo, two native filter-feeding fishes.

Qualitative analysis of morphology of gill rakers was conducted and compared among species with stereomicroscopy and a novel use of confocal microscopy (Chapter I). Gill rakers of silver carp and gizzard shad, two species that may have high levels of dietary overlap, were quantitatively compared. Size and spacing of gill rakers were examined relative to biological and environmental factors, such as fish size and site and time of collection (Chapter II).

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CHAPTER I

Running title: Confocal microscopy to describe gill rakers

Confocal microscopy as a useful approach to describe gill rakers of Asian carp and native filter-feeding fishes

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Abstract. To better understand potential diet overlap among exotic Asian carp native species of filter-feeding fish, confocal microscopy was used to document morphological differences in the gill rakers of silver carp *Hypophthalmichthys molitrix*, bighead carp *H. nobilis*, gizzard shad *Dorosoma cepedianum*, and bigmouth buffalo *Ictiobus cyprinellus* collected from the Illinois, Wabash, and James Rivers (USA) in 2011. The three-dimensional structure of gill rakers in each species was described in greater detail than that described by previous microscopic techniques. Compared to traditional methods of tissue analysis, confocal microscopy also preserved the natural state and structure of the gill arch.

Keywords: silver carp, bighead carp, gizzard shad, bigmouth buffalo, suspension feeding

INTRODUCTION

The ability of fish to acquire food is greatly influenced by the structure of gill rakers, whose species-specific lengths, shapes, and spacing along the concave margin of the gill arch optimize particle retention. Gill rakers of filter-feeding fish are typically long, thin projections that capture phytoplankton and zooplankton from the water column. Atypical morphologies of gill rakers have been noted in filter-feeding species of Asian carp, silver *Hypophthalmichthys molitrix* (Valenciennes) and bighead carp *H. nobilis* (Richardson). Both species possess an epibranchial organ (also known as the suprabranchial organ; Wilamowski 1972; Kolar *et al.*, 2007), which consolidates food particles retained by the gill rakers with mucus. In comparison to bighead carp, gill rakers of silver carp are fused together with a profuse mucus network to form a sponge-like filter (Jirasek *et al.*, 1981). Such modifications can greatly increase the efficiency of Asian carp filtering small food particles from the water.

Details of morphological differences in gill rakers among species can provide crucial information about corresponding feeding abilities of individuals and populations. In the Mississippi River basin, the diet of Asian carp overlaps with that of native filter-feeding species, such as gizzard shad *Dorosoma cepedianum* (LeSueur) and bigmouth buffalo *Ictiobus cyprinellus* (Valenciennes) (Sampson *et al.*, 2009). Similar morphologies in gill raker structure in these native fish and Asian carp may be related to competition for similar-sized food.

Structures of gill rakers are often described using microscopy. Traditional microscopy methodologies (*i.e.*, light microscopy) used to describe the morphology of

gill rakers require them to be dehydrated, rehydrated, embedded, sectioned, and mounted on slides. More advanced microscopic techniques (*i.e.*, scanning electron microscopy) require critical point drying. These desiccating processes could greatly distort the size and morphology of gill rakers. Some studies dissolve excess mucus to enhance analysis of gill rakers (*e.g.*, Hampl *et al.* 1983). For silver carp, where mucus may affect filtering abilities, it seems counterintuitive to remove or alter this characteristic. Moreover, light microscopy requires construction of serial sections (Hoogenboezem *et al.* 1981), which is labor intensive and can introduce errors. Many of the deficiencies of light microscopy may be overcome by using confocal microscopy. Not only does confocal microscopy require little preparatory work and supplies, it also lessens handling and possible degradation of the sample. Both light and confocal microscopy require storing samples in a formalin fixative, but only confocal microscopy takes advantage of the autofluorescent properties of formalin to highlight the three-dimensional characteristics of the object without reconstructing serial sections. To our knowledge, confocal microscopy has not been previously used to describe structures as large as gill rakers.

The goal of this study was to determine if confocal microscopy can be used to describe the morphology of gill rakers in filter-feeding fishes. Therefore, the gill rakers from silver and bighead carps, gizzard shad, and bigmouth buffalo were examined and described. A description of the gill rakers of bigmouth buffalo has not been previously published, nor have the gill rakers of these four species been assessed using the microscopic techniques applied in this study.

MATERIALS AND METHODS

In October 2010, silver and bighead carp (<100 mm fork length) were harvested from laboratory stocks maintained at the Upper Midwest Environmental Sciences Center (UMESC, U.S. Geological Survey, La Crosse, WI). In May, June, August, and November 2011, silver carp, bighead carp, gizzard shad, and bigmouth buffalo (>100 mm fork length) were collected by electrofishing in the Illinois River near Havana, Illinois, the Wabash River near Lafayette, Indiana, and the James River near Mitchell, South Dakota. Fish were euthanized by cranial concussion. All fish were handled and treated according to guidelines approved by the UMESC Animal Care and Use Committee. Gill rakers of each fish were excised on site and stored in Modified Davidson's Fixative (MDF) (Rowley Biochemical Institute: Danvers, Massachusetts). Following at least 24-hour storage in MDF, each gill arch was initially examined and imaged under stereomicroscopy (Nikon SMZ800 dissecting microscope) with a Leica EC3 camera (1-2X objective). Samples were then stored in MDF until subsequent examination with confocal microscopy.

For analysis under confocal microscopy, samples were removed from MDF and rinsed in tap water for 30 seconds before being placed in a chambered coverglass (2.5–4.5 mL 19.4 cm², Fisher Scientific, Pittsburg, PA, USA). Gill arches and rakers were entirely intact, though arches were trimmed as needed with a razor blade along gill filaments and outer edges of arch to fit the sample in the chamber cell. Deionized water was added to the chamber to completely submerge the sample. If necessary, lead weights

(round split shot) were placed on top of the sample to prevent floating. Gill rakers were imaged under a Nikon C1 Confocal microscope (Eclipse TE2000-U) with a fluorescein isothiocyanate (FITC) filter and EZ-C1 software Ver.3.40.

Z-series (stack of optical sections collected along the z-axis) of gill rakers from silver carp and bighead carp were collected with 12- μ m steps between images (2X objective lens). Z-series of gill rakers from gizzard shad were collected with 6- μ m steps between images (10X objective lens). Z-series of gill rakers from bigmouth buffalo were collected with 12- μ m steps between images (10X objective lens). Images of gizzard shad and bigmouth buffalo were displayed with maximum projections of the entire z-series; those of silver and bighead carp were displayed as individual frames from the z-stack. Images of gill rakers from all species were obtained with a medium pinhole, 5.80 gain, and increased saturation. Images were processed with ImageJ software 1.43u (National Institute of Mental Health, Bethesda, MD, USA). For a comprehensive review of confocal microscopy see Concello & Lichtman (2005).

RESULTS

Images of gill rakers from bighead carp from the dissecting microscope demonstrated they had long comb-like gill rakers with clear separation between adjacent rakers (Fig 1a, 1b). In contrast, the gill rakers of silver carp had an amalgamated appearance, making individual rakers difficult to distinguish (Fig. 1c, 1d). Using confocal microscopy, the images of bighead carp confirmed that gill rakers were closely set, but distinctly separate (Fig. 2a), as observed under the dissecting microscope. However, thick bases were observed connecting rakers to the gill arch, which then thinned to a point as they progressed away from the gill arch. Confocal imagery of silver carp demonstrated that the row of gill rakers along the outer margin of each gill arch had exit pores of various shapes and sizes (Fig. 2b). Pore shape was generally elliptical; however it ranged from approximate circles and elongated ellipsoids, to irregular polygons (Fig. 3). Average pore diameter varied from 40.4 μm to 275.0 μm . These pores were not evident from images obtained with stereomicroscopy, but were consistently revealed by confocal microscopy.

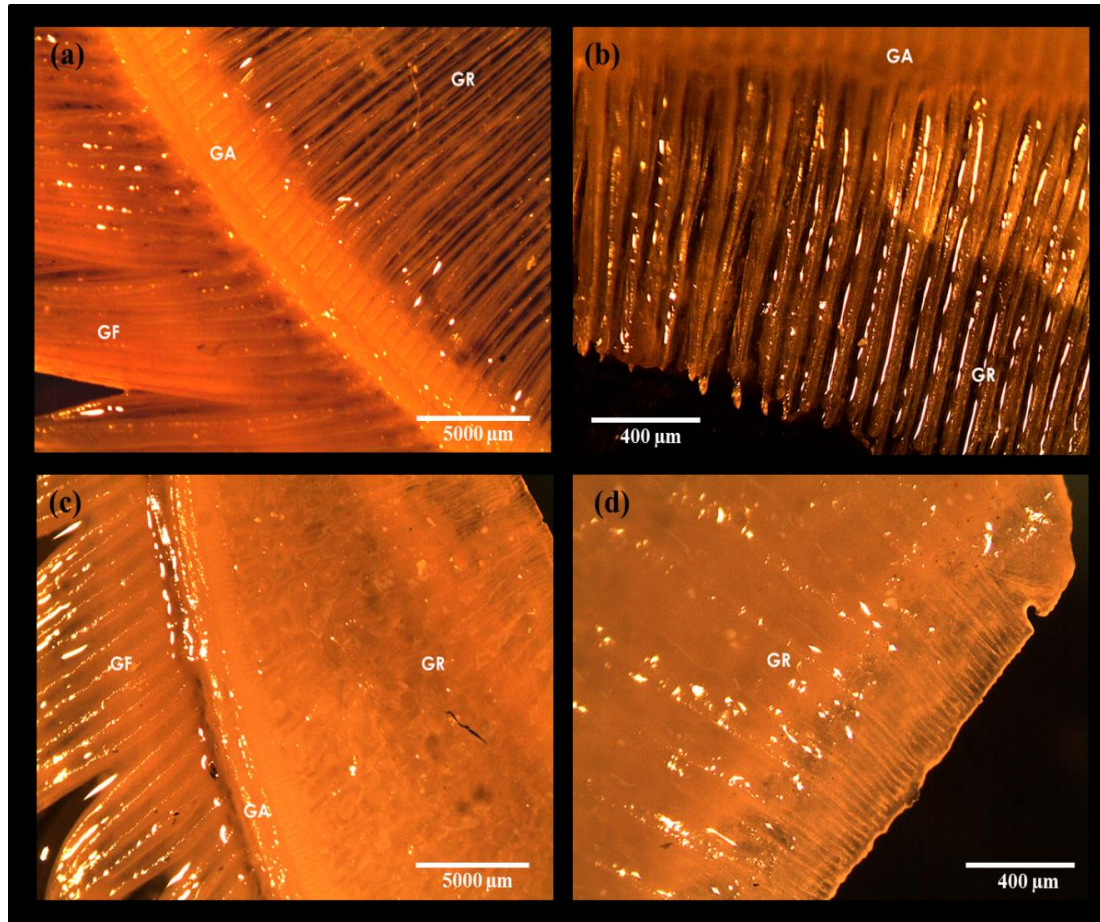


FIG. 1. Gill rakers (unstained) of laboratory harvested Asian carp imaged with dissecting microscope. (a) gill arch in bighead carp (1X), (b) gill rakers in bighead carp (2X), (c) gill arch in silver carp (1X), and (d) gill rakers in silver carp (2X). GA, gill arch; GR, gill rakers; GF, gill filaments. Image processing: (a) applied unsharp mask, (b) applied unsharp mask, increased gamma 0.25, (c) applied unsharp mask and increased contrast, and (d) applied unsharp mask, increased gamma by 0.2.

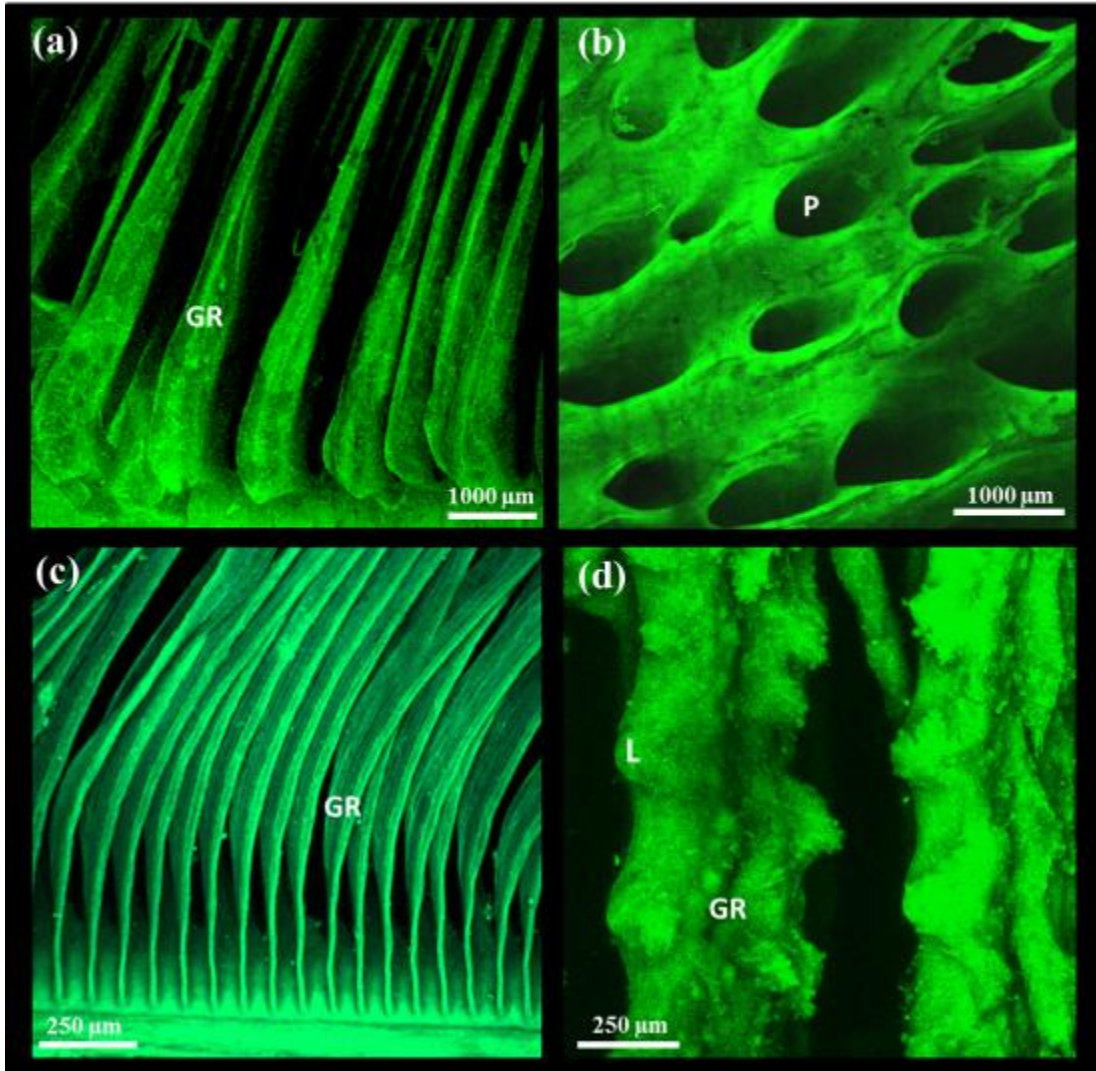


FIG. 2. Gill rakers imaged with confocal microscopy. Gill rakers of (a) bighead carp (2X), (b) silver carp (2X), (c) gizzard shad (10X), and (d) bigmouth buffalo (10X) (382mm). GR, gill raker; P, pore; L, lobe. Image processing: (a) applied unsharp mask radius 1.0 pixels and mask weight 0.50, decreased gamma 0.2, applied smooth mask, and increased contrast, (b) decreased gamma 0.20, increased contrast, (c-d) applied maximum projection and increased contrast.

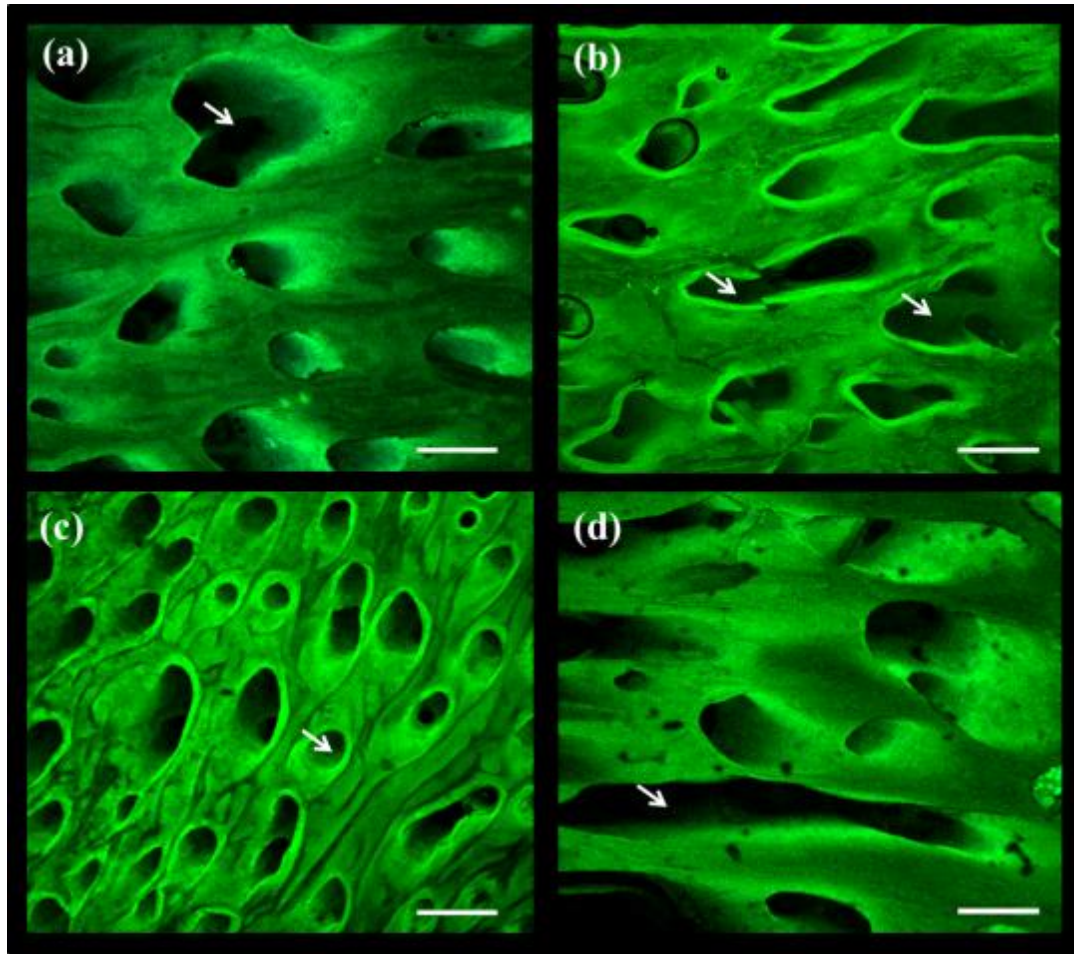


FIG. 3. Gill rakers imaged with confocal microscopy of silver carp from the (a) Illinois River in August (445 mm), (b) Illinois River in May (396 mm), (c) Wabash River in June (565 mm), and (d) Illinois River in August (405 mm). All scale bars equal 1000 μm . Arrows indicate examples of various pore shapes. Image processing: (a) decreased gamma 0.10, Gaussian blur 0.5, and increased contrast, (b) decreased gamma 0.15 and increased contrast, (c) decreased gamma 0.20, and (d) decreased gamma 0.30, Gaussian blur 0.5, and increased contrast.

Confocal microscopy demonstrated that gill rakers of gizzard shad stemmed off the gill arch at a perpendicular angle and had a filamentous morphology (Fig. 2c). Often the gill raker appeared to connect to the gill arch with a thickened stem on the lateral margin (facing viewer in image). In these instances, the width of the raker abruptly increased, and gradually decreased as filaments flowed back toward each other. However, there was significant variation among the images produced from gill rakers in gizzard shad (Fig. 4), likely a result of the angle in which they were positioned in the chamber cell. In some gizzard shad, gill rakers appeared as linear filaments with uniform thickness and containing nodule-like structures (Fig. 4b, 4c).

Gill rakers of bigmouth buffalo stemmed perpendicularly off the gill arch and were most similar in gross morphology to gill rakers of bighead carp and gizzard shad. However, using confocal microscopy, each raker was observed to have lobular projections along the surface (Fig. 2d). The primary vertical bar of each gill raker appeared to maintain a uniform diameter as it extended off the gill arch; rounded mounds projected off this surface. Mound projections had a diameter approximately one-third to one-half the diameter of the primary vertical bar and a height less than one-quarter the diameter of the primary vertical bar. The mounds appeared to have irregular surfaces and the overall structure projected out and slightly downward off the main raker bar. Mound projections were spaced at a consistent distance relative to others, and predominantly projected off the medial and lateral edges along the circumference of each raker. The lobated nature of gill rakers in this species appeared shifted vertically in one raker compared to the adjacent raker. This resulted in interdigitation of projections among two adjacent gill rakers.

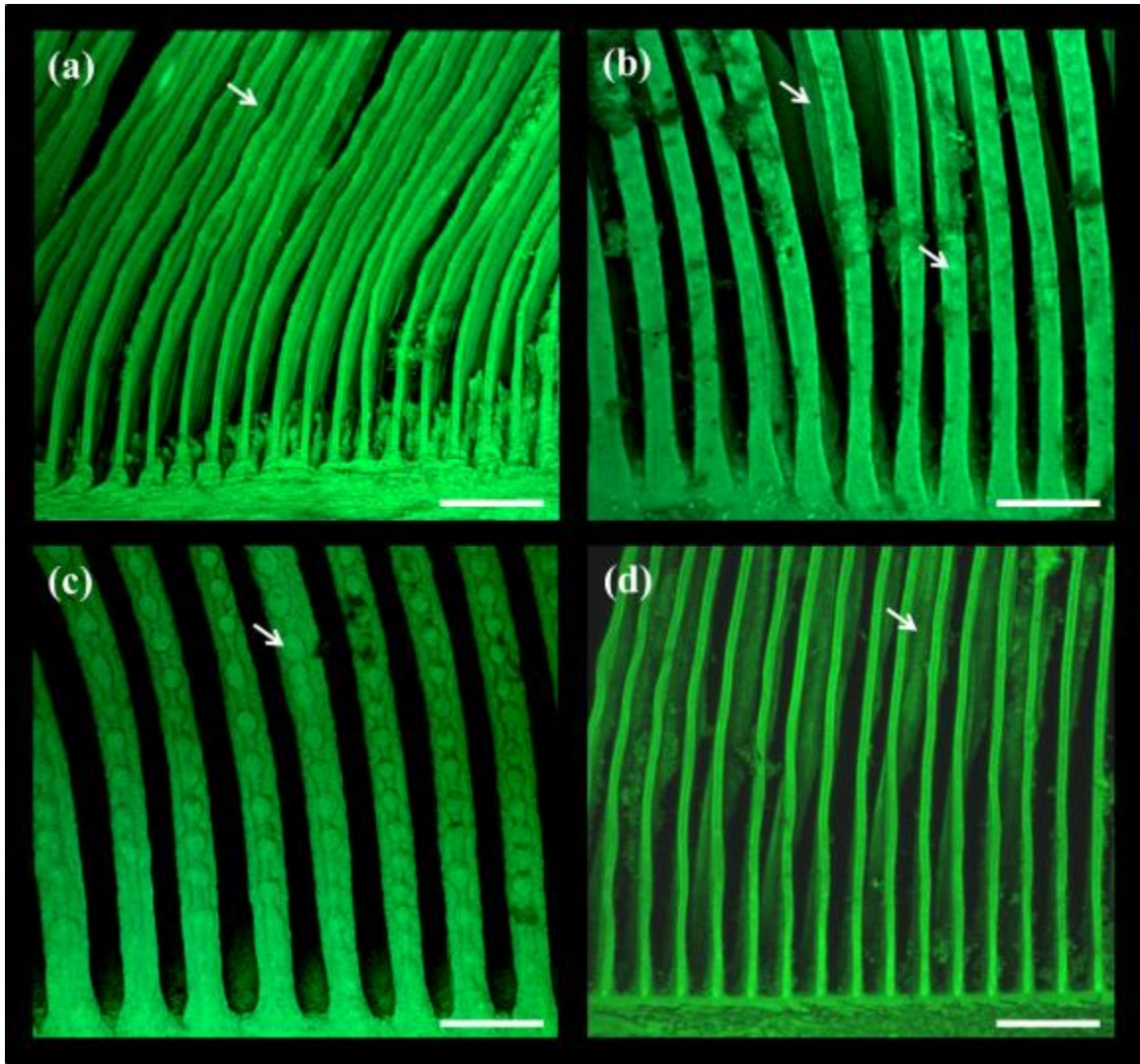


FIG. 4. Gill rakers imaged with confocal microscopy of gizzard shad from the (a) Illinois River in June (129 mm) (b) Wabash River in June (222 mm), (c) James River in November (282 mm), and (d) Illinois River in August (174 mm). Size is indicated by fork length. All scale bars equal 250 μm . Arrows indicate examples of filaments and nodules. Image processing: (a) decreased gamma 0.30 and increased contrast, (b) decreased gamma 0.30 and increased contrast, (c) decreased gamma 0.35, and (d) decreased gamma 0.30 and increased contrast.

DISCUSSION

The gill rakers of each species differed in thickness, shape, and spacing. Descriptions from previous literature generally supported the visual depiction of these structures obtained using confocal microscopy. However, by imaging these rakers completely intact and in three-dimensions with confocal microscopy, higher quality and more-detailed images were produced, compared to those previously available.

Jirasek *et al.* (1981) indicated that the gill rakers of silver carp were fused by mucus with “transverse and longitudinal primary trabeculae.” This resulted in “rounded, oval, and polygonal outlets of different diameters.” This description from stereomicroscopy supported our results from confocal microscopy. In some cases, it appeared this variation among pores could be attributed to the fusion of two or more individual pores. In other cases, a particular pore was distinctly separate from other pores but was also unique in regards to shape or size relative to that of other pores within the same fish. To our knowledge, the pore-laden gill rakers of silver carp are unique among the gill rakers of filter-feeding fishes present North America.

The depictions of gill rakers in bighead carp with confocal microscopy were also supported by previous literature, in that they have rakers long and separated as they stem off the gill arch (Cremer & Smitherman 1980; Jennings 1988). As described in Henderson (1976; as cited in Jennings 1988), gill rakers widened above the connection to the gill arch. However, direct overlap of this widened portion could not be determined

from the results of our study. Nonetheless, it is clear the morphology of these rakers is highly divergent of those observed in silver carp.

Observations of numerous, filamentous gill rakers in gizzard shad with confocal microscopy was supported by authors using alternative microscopic methods (Schmitz & Baker, 1969; Drenner *et al.*, 1984; Mummert & Drenner, 1986). The interraker spacing in gizzard shad appeared significantly smaller than the interraker spacing in bighead carp and bigmouth buffalo, as well as the pore diameters in gill rakers of silver carp – which is consistent with the literature (Kolar *et al.*, 2007). Images provided in this study detailed a filamentous morphology of gill rakers in gizzard shad, as opposed to simplified diagrams depicting rakers with filaments of uniform thickness in previous studies (*e.g.*, Mummert & Drenner, 1986). The gill rakers of some gizzard shad in this study appeared as filaments of uniform thickness, though it remains unclear if that is a true depiction of the rakers in these fish or if the lack of more-detailed structure was an artifact of the angle in which the sample was imaged. Regardless, varying morphologies observed for gill rakers of gizzard shad warrants further study to investigate (1) the existence and prevalence of thin, filamentous morphologies versus thick, linear morphologies, (2) how distinct morphologies may affect the abilities of gizzard shad to filter food, (3) the prevalence and function of nodule structures observed in gizzard shad with thick, linear gill rakers, (4) if such nodule structures could function in taste reception, and (5) if gill raker thickness varies among gizzard shad populations.

In contrast to the other species, there was little information in the literature on the morphology of gill rakers in bigmouth buffalo. Because bigmouth buffalo retain a wide range of prey types (Goodchild 1990), we hypothesized that the lobes on the surface of

each gill raker may be beneficial for capturing variable sizes of prey. Bigmouth buffalo consume cladocerans, copepods, and large-bodied rotifers, distinct from the small-bodied rotifers, plankton, and detritus typical of the diets of gizzard shad, silver carp, and bighead carp (Sampson *et al.*, 2009).

The interdigitation of projections on adjacent gill rakers in bigmouth buffalo may indicate a channel model of filter-feeding, or even a reducible channel model (Hoogenboezem *et al.* 1991). In the channel model of filtration, food particles are retained in channels between lobular protrusions off a single gill raker. In the reducible channel model, food particles can also be retained between gill rakers on adjacent gill arches due to attached striated muscle that allows gill rakers of one gill arch to insert between the spaces of gill rakers on an adjacent arch (Hoogenboezem *et al.* 1993). If bigmouth buffalo possess these abilities, they may adjust the spacing of the branchial sieve to improve retention of variously sized food particles. Results from confocal microscopy in this study prompt further research regarding bigmouth buffalo to better understand (1) the function of the lobated rakers (2) if the rakers are used in a channel model of sieving and, if so, (3) to determine whether or not they are reducible in size.

In this study, confocal microscopy provided a new perspective on the filtering structure of suspension-feeding fishes that was not possible with traditional light microscopy that only document the specimen in x- and y-dimensions. Confocal microscopy filters light through a small pinhole to individually scan all parts of a sample. These small parts are compiled to create a new image that incorporates the z-dimension. Fortunately, the semi-translucent nature of gill rakers allowed for enough light to pass through them to allow the use of this methodology, a feature that may not be applicable

to other biological structures. Compared to previously established methodologies to examine gill rakers, confocal microscopy may preserve the sample in the most natural conditions possible, while providing a superior level of detail and faster processing time. Not only does this new methodology require little preparatory work and supplies, it also lessens degradation of the sample. The gill arch can be left entirely intact, except for occasional trimming along features that are not the focus of the study (*i.e.*, gill filaments and edges of gill arch).

The methods developed in this study examined gill rakers maintained in an aqueous environment to preserve natural coatings, especially mucus, and preserve the delicate morphology of intricate structures. With these methods the gill arches of any fish could be removed and rapidly analyzed after the sample was appropriately fixed in a solution, such as MDF, to induce autofluorescence. The images from confocal microscopy provide high-resolution, three-dimensional depictions of gill raker structure and spacing that is not possible to see with stereomicroscopy or other forms of light microscopy. Moreover, this technology provides the user options to display the z-series collected from their sample to best depict the structure of the sample, including maximum projections, single images, or 360 degree rotating movies.

In conclusion, lack of an established methodology to assess size, spacing, and shape of gill rakers can be problematic in studying the feeding ecology of filter-feeding fishes. This ecological information is critical in estimating diet overlap and potential competition among filter-feeding species. Such information is especially important in the management of invasive species, like Asian carp, and the conservation of native species with which they share food resources. The methodologies developed in this study may

suggest a more efficient way to examine and document the morphologies of these important filtering organs via confocal microscopy.

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FIGURE CAPTIONS

Figure 1. Gill rakers (unstained) of laboratory harvested Asian carp imaged with dissecting microscope. (a) gill arch in bighead carp (1X), (b) gill rakers in bighead carp (2X), (c) gill arch in silver carp (1X), and (d) gill rakers in silver carp (2X). GA, gill arch; GR, gill rakers; GF, gill filaments. Image processing: (a) applied unsharp mask, (b) applied unsharp mask, increased gamma 0.25, (c) applied unsharp mask and increased contrast, and (d) applied unsharp mask, increased gamma by 0.2.

Figure 2. Gill rakers imaged with confocal microscopy. Gill rakers of (a) bighead carp (2X), (b) silver carp (2X), (c) gizzard shad (10X), and (d) bigmouth buffalo (10X) (382mm). GR, gill raker; P, pore; L, lobe. Image processing: (a) applied unsharp mask radius 1.0 pixels and mask weight 0.50, decreased gamma 0.2, applied smooth mask, and increased contrast, (b) decreased gamma 0.20, increased contrast, (c-d) applied maximum projection and increased contrast.

Figure 3. Gill rakers imaged with confocal microscopy of silver carp from the (a) Illinois River in August (445 mm), (b) Illinois River in May (396 mm), (c) Wabash River in June (565 mm), and (d) Illinois River in August (405 mm). All scale bars equal 1000 μm . Arrows indicate examples of various pore shapes. Image processing: (a) decreased gamma 0.10, Gaussian blur 0.5, and increased contrast, (b) decreased gamma 0.15 and

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Figure 4. Gill rakers imaged with confocal microscopy of gizzard shad from the (a) Illinois River in June (129 mm) (b) Wabash River in June (222 mm), (c) James River in November (282 mm), and (d) Illinois River in August (174 mm). Size is indicated by fork length. All scale bars equal 250 μm . Arrows indicate examples of filaments and nodules. Image processing: (a) decreased gamma 0.30 and increased contrast, (b) decreased gamma 0.30 and increased contrast, (c) decreased gamma 0.35, and (d) decreased gamma 0.30 and increased contrast.

CHAPTER II

Intra- and interspecific variation of spacing in gill rakers of silver carp

Hypophthalmichthys molitrix* and gizzard shad *Dorosoma cepedianum

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Abstract. Controlling populations of filter-feeding silver carp (*Hypophthalmichthys molitrix*) may be possible with better understanding of their feeding ability. Because feeding is dependent upon structure of gill rakers in filter-feeding fishes, gill rakers of silver carp were examined and compared to those of native filter-feeding gizzard shad (*Dorosoma cepedianum*). Intra- and inter-species variation of gill rakers was examined in fish collected from three locations over four months. Spacing of gill rakers in silver carp was correlated with fish length and did not generally differ among collections. This suggested that size of particles removed by silver carp is dependent on fish size, rather than spatial or temporal factors. Measurements of gill rakers in gizzard shad were not correlated with fish length, but differed among sites and months. This suggested that size of particles filtered by gizzard shad may be population-dependent and vary with location and time. These findings may suggest how to manage silver carp populations based on length of fish per site, corresponding spacing of gill rakers, and hypothesized retention of food based on particle size.

INTRODUCTION

Several species of Asian carp have been introduced to North America. Of these species, silver carp *Hypophthalmichthys molitrix* may potentially compete with native fish species and alter the trophic structure of aquatic systems. Silver carp are extremely efficient filter-feeders (Cremer and Smitherman 1980) and able to retain particles as small as 3.2 μm in diameter (Dong and Li 1996). Their success at consuming large quantities of plankton may decrease food availability for native filter-feeding organisms, including gizzard shad *Dorosoma cepedianum*, bigmouth buffalo *Ictiobus cyprinellus*, and paddlefish *Polyodon spathula* (Dong and Li 1994; Schrank et al. 2003; Irons et al. 2007; Sampson et al. 2009).

Gill rakers are the structure within the buccal cavity of suspension-feeding fish which function to collect small food particles from ingested water (Hoogemboezem 1991; Van den berg 1994; Sanderson et al. 2001). Because variable functions and morphologies exist among gill rakers of different species, it is important to consider these characteristics to assess species-specific feeding abilities. Particularly for suspension-feeders, who passively filter food items from water as it passes over the gills, it is important to understand the size of particles which may be retained or passed through the rakers. Although the spacing of rakers has been examined in numerous species, less is known about those in silver carp, particularly silver carp in the upper Mississippi River basin. The ontogenic change of raker spacing, and the possibility of spatial and temporal phenotypic plasticity, is also unknown. Knowledge about the feeding ecology of these

fishes ultimately improves assessment of interspecies interactions and implementation of management for invasive species.

The objective of this study was to compare gill raker morphology of silver carp to that of native filter-feeding fish, and to relate raker morphology to the size of particle most optimally consumed by each species. Gizzard shad were chosen as the native filter-feeding species for comparison because they have the greatest overlap with silver carp in the size of particle retained by filtration (Fig. 1). Although the size of particles retained by filter-feeding invertebrates (zooplankton, insects, and mussels) also overlaps with that of silver carp, based on biochemical differences in the mucus associated with feeding (Asakawa 1970; Hunt 1970) it was hypothesized that their feeding mechanisms are distinct. We examined characteristics of gill rakers we considered to be most likely to influence particle capture in silver carp and gizzard shad, and determined if these characteristics vary among locations and months.

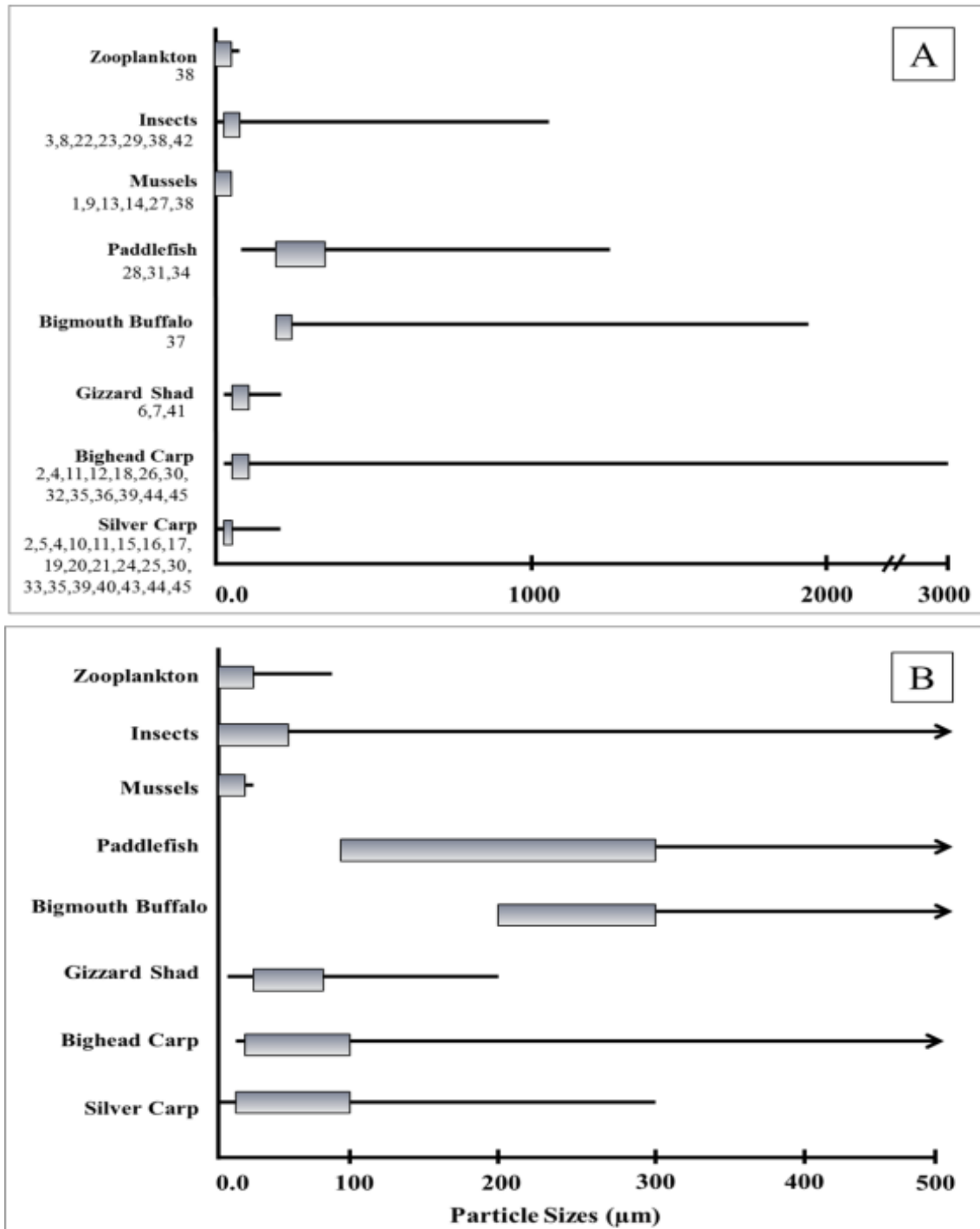


Fig. 1. Approximate size of particles (μm) retained in gill rakers of silver carp, bighead carp, gizzard shad, bigmouth buffalo, paddlefish, mussels, filter-feeding insects, and zooplankton: (A) complete scale of retention 0-3000 μm, (B) same as (A) but with focus

on particles sizes less than 500 μm . Line indicates range of literature values and box indicates estimated mean of most cited values. Literature cites silver carp with retention of 3.2 μm - 300 μm , bighead carp 5 μm -3000 μm , gizzard shad 10 μm - 200 μm , bigmouth buffalo 200 μm - 2000 μm , paddlefish 100 μm - 1300 μm , freshwater mussels 1 μm - 25 μm , filter-feeding insects 0.01 μm - 1000 μm , and zooplankton 0.5 μm - 100 μm . Number below organism name indicates the sources of data: 1. Baker and Levinton (2003), 2. Cremer and Smitherman (1980), 3. Cummins (1974), 4. Dabrowsky and Bartega (1984), 5. Dong and Li (1996), 6. Drenner et al. (1984), 7. Drenner et al. (1986), 8. Edler and Georgian (2004), 9. Helfrich et al. (1995), 10. Herodek et al. (1989), 11. Irons et al. (2007), 12. Jennings (1988), 13. Jorgensen et al. (1984), 14. Jorgensen (1996), 15. Kajak et al. (1977), 16. Kaul and Rishi (1993) (as cited in Kolar et al. 2007), 17. Kolar et al. (2005), 18. Kolar et al. (2007), 19. Kucklantz (1985), 20. Leventer and Teltsch (1990), 21. Ma et al. (2009), 22. Merritt and Cummins (1996), 23. Merritt and Wallace (1981), 24. Omarov (1970), 25. Opuszyński (1981), 26. Opuszyński et al. (1991), 27. Paterson (1986) (as cited in Mueller et al. 2004), 28. Rosen and Hales (1981), 29. Ross and Craig (1980) (as cited in Edler and Georgian 2004), 30. Sampson et al. (2009), 31. Sanderson et al. (1994), 32. Sieburth et al. (1978), 33. Smith (1989), 34. Smith (2004) 35. Spataru and Gophen (1983), 36. Spittler (1978) (as cited in Kolar et al. 2007), 37. Starostka and Applegate (1970), 38. Thorp and Covich (2010), 39. Voropaev (1968), 40. Voros et al. (1997), 41. Watson et al. (2003), 42. Wodsedalek (1912), 43. Xie (1999), 44. Xie and Chen (2001), 45. Xie and Liu (1994).

MATERIALS AND METHODS

Field

Silver carp and gizzard shad were collected by electrofishing from the Illinois River near Havana, Illinois, the Wabash River near Lafayette, Indiana, and the James River near Mitchell, South Dakota. The Illinois River was sampled during May, June and August 2011. The Wabash River was sampled in June 2011 and the James River was sampled in November 2011. A total of 136 fish were collected in five sampling events. Forty-eight silver carp were collected in the Illinois River (May: $n = 8$, June: $n = 20$, August: $n = 20$), five were collected from the Wabash River and seven from the James River. Forty-eight gizzard shad were collected from the Illinois River (May: $n = 8$, June: $n = 20$, August: $n = 20$), twenty were collected from the Wabash River and eight from the James River. Variations in sample numbers were due to high water levels and boat availability. Fish were euthanized after capture by cranial concussion. Fish fork length (mm) and weights (g) were measured. Right and left gill arches were removed on site and stored in Modified Davidson's Fixative (MDF). For each fish, length of the gill arch was measured, as well as the length of rakers at the longest point off the gill arch (Fig. 2).

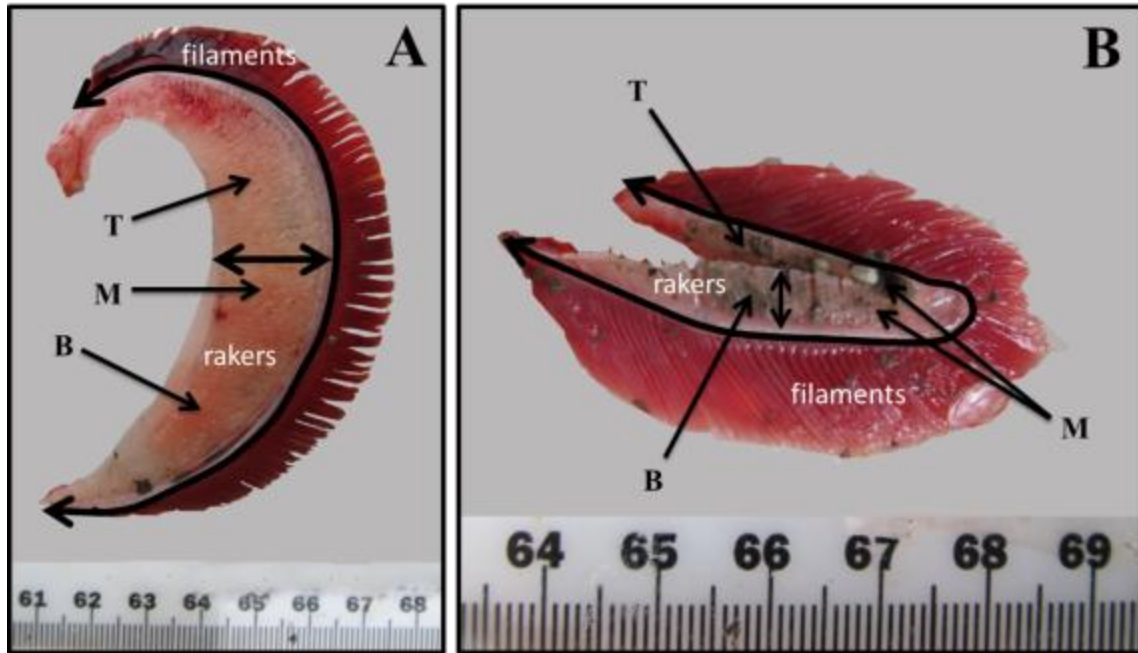


Fig. 2. Gill arch one in (A) silver carp and (B) gizzard shad. Gill rakers are on the concave margin of the gill arch and gill filaments are on the convex margin. Single-headed arrows point to the top (T), middle (M), and bottom (B) portions of gill raker sampled to determine intra- and interraker variation in gill raker morphology. Double-headed arrows designate measurements for gill arch length and raker length.

Laboratory

Following at least 24 hour storage in MDF, gill rakers were removed to measure microscopic morphology. Rakers were imaged with a Nikon C1 confocal microscope and EZ-C1 software Ver.3.40 following procedures in Chapter I. To test if gill raker spacing varied along the length of a gill arch, spacing was quantified at the top, middle, and bottom portions of all four gill arches from fish obtained from the Illinois River in May 2011 (Fig. 2). As no significant differences in gill raker spacing were detected within individual arches or among arches (see results section), during subsequent

sampling events only the first gill arches from each fish were harvested. For consistency, microscopic measurements were taken at approximately one-third the length of the gill arch, above the ventral connection to naturally occurring gill arches. All visible pores or interraker spaces of the gill rakers completely contained within one image (906 x 909 pixels for silver carp; 811 x 805 pixels for gizzard shad) from the confocal microscope were measured with ImageJ software 1.43u.

Because gill rakers of silver carp consisted of circular holes which created a net-like filtration system (Fig. 3a), filtration ability was quantified based on pore area, pore diameter, and passage ratio for each fish. These measurements were chosen because individual gill rakers were not visible; the mucus network completely enveloped rakers. Pore diameter was calculated based on the shortest distance across the each pore surface. Passage ratio was calculated based on the sum of all pore areas within the total area of one image. Passage ratio was taken to assess whether or not the percentage of pore area along the gill rakers of a gill arch could be better related to fish size, more so than pore area or diameter. Chemical dissolution of the mucus network could have been completed to reveal interraker spacing (e.g., Jirasek et al. 1983), but this was considered to be inappropriate given our desire to measure the intact filtration mechanisms of the buccal cavity in silver carp. Means of all measurements visible on one image, for each category, were calculated for each fish.

Because the gill rakers were clearly visible in gizzard shad, filtration ability was quantified based on interraker spacing. Measurements were taken between the outer edges of adjacent rakers, above the point where the raker stemmed off the gill arch (Fig. 3b). Means of all interraker spacings visible on one image were calculated for each fish.

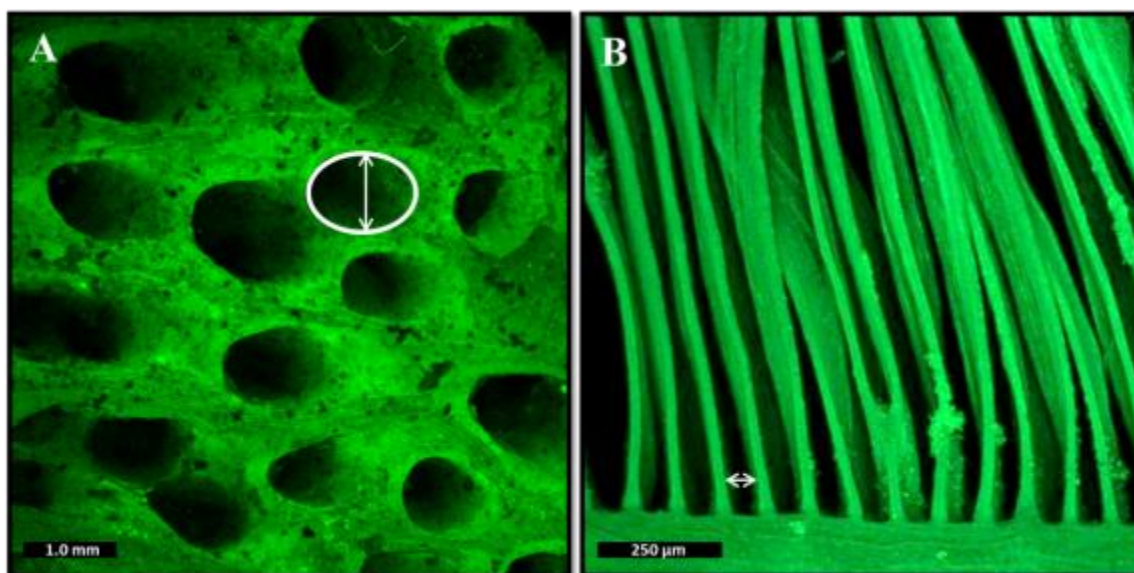


Fig. 3. Method of measuring gill rakers for silver carp and gizzard shad. (A) For silver carp the area (white circle) and the maximum width (white arrow) of pores were measured. (B) For gizzard shad the interraker distances (white arrow) were measured.

Statistical Analysis

Linear regression was used to test the relation between fork length and fish weight, pore area and pore diameter, as well as fork length and pore area, pore diameter, and passage ratio. Analysis of variance (ANOVA) was used to test for differences in fish size among sites and months, to test for differences in characteristics among and rakers and different locations within a raker, and to test for differences in raker characteristics between species. Fish length may be a factor influencing raker characteristics within a species, hence, pore measurements among sites and months were compared with analysis of covariance (ANCOVA) using fork length as the covariate. Because assumptions for ANCOVA could not be met with a two-way analysis, incorporating both species together, a one-way ANOVA were performed for each species separately.

A Kruskal-Wallis rank sum test was used to assess variation when data were not normally distributed, instead of ANOVA. Wilcoxon rank sum tests were used for pairwise comparisons when data were analyzed using the Kruskal-Wallis rank sum tests with α reduced to ≤ 0.01 . Welch two sample t-tests were used for interspecies comparisons of fish size and spacing of gill rakers. Only data from the Illinois River was included in any analyses among months, as that was the only study site which had repeated collections. All statistical analyses were performed with R version 2.9.2.

RESULTS

Silver Carp Body Size

The mean $\pm$ SE fork length and weight of all silver carp collected was 452.00 ± 9.88 mm and 1418.41 ± 95.90 g. With one exception, silver carp fork length and weight did not differ among sampling months or sites (Kruskal-Wallis test: all $p > 0.05$). Silver carp collected from the James River had smaller length and weight than those from the Illinois River (Wilcoxon test: $W = 308.5$, $p < 0.01$ and Wilcoxon test: $W = 296$, $p < 0.01$).

The mean $\pm$ SE length of gill arches in silver carp was 117.13 ± 2.5 mm; mean $\pm$ SE length of gill rakers was 17.23 ± 0.5 mm. Weight of silver carp was positively related to fork length ($R^2 = 0.95$, $F_{[1,58]} = 1101$, $p < 0.01$). Mean pore area was positively related to mean pore diameter ($R^2 = 0.83$, $F_{[1,58]} = 284.5$, $p < 0.01$). Fork length of silver carp was also positively related to mean pore area ($R^2 = 0.39$, $F_{[1,58]} = 36.81$, $p < 0.01$), mean pore diameter ($R^2 = 0.54$, $F_{[1,58]} = 69.4$, $p < 0.01$, Fig. 4), and the passage ratio ($R^2 = 0.1704$, $F_{[1,58]} = 11.91$, $p = 0.01$). Because pore diameter, rather than pore area or passage ratio, was best related to fork length only diameter measurements were included in subsequent analysis of pore sizes for silver carp.

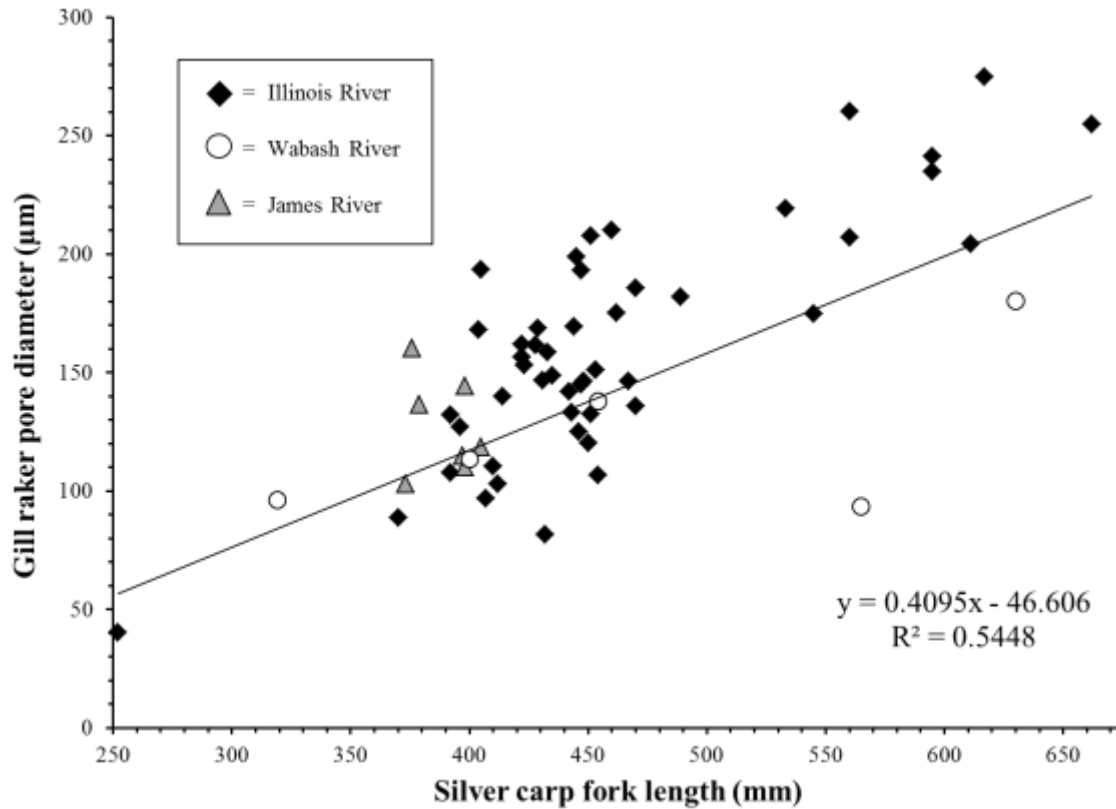


Fig. 4. Relation between fork length (mm) and pore diameter (μm) of gill rakers in silver carp.

Gizzard Shad Body Size

The mean $\pm$ SE fork length and weight of gizzard shad was 214.50 ± 6.83 mm and 177.09 ± 16.21 g. Weight of gizzard shad was positively related to fork length ($R^2 = 0.95$, $F_{[1,74]} = 1489.09$, $p < 0.01$). The mean $\pm$ SE gill arch length of gizzard shad was 47.55 ± 1.5 mm; mean $\pm$ SE raker length was 2.91 ± 0.13 mm. Size of spaces between gill rakers was not related to fork length ($R^2 = 0.004$, $F_{[1,74]} = 0.32$, $p = 0.57$) or weight ($R^2 = 0.01$, $F_{[1,74]} = 1.08$, $p = 0.30$) of the fish.

Fork length (Kruskal-Wallis test: $\chi^2_{[2]} = 17.48$, $p < 0.01$) and weight (Kruskal-Wallis test: $\chi^2_{[2]} = 16.79$, $p < 0.01$) of gizzard shad differed among sampling sites.

Gizzard shad from the Illinois River had fork lengths (Wilcoxon: $W = 302.5$, $p = 0.02$) and weights (Wilcoxon: $W = 302.5$, $p = 0.02$) similar to gizzard shad collected from the Wabash River. However, fork length and weight of gizzard shad from the James River was greater than those from the Illinois River and the Wabash River (Fork length, Wilcoxon test: $W = 41$, $p < 0.01$ and $W = 19.5$, $p < 0.01$; Weight, Wilcoxon: $W = 302.5$, $p = 0.02$). Gizzard shad fork length (Kruskal-Wallis test: $\chi^2_{[2]} = 9.30$, $p = 0.01$) and weight (Kruskal-Wallis test: $\chi^2_{[2]} = 9.30$, $p = 0.01$) also varied among sampling months. Fork length (Wilcoxon test: $W = 122$, $p = 0.03$ and $W = 112$, $p = 0.11$) and weight (Wilcoxon test: $W = 120$, $p = 0.04$ and $W = 112$, $p = 0.11$, respectively) were similar between May and June and May and August, however fork length (Wilcoxon test: $W = 105$, $p = 0.01$) and weight (Wilcoxon test: $W = 106$, $p = 0.01$) of fish collected in August was greater than in June.

Variation in First - Fourth Gill Arches

There were no significant differences in gill raker spacing among the four gill arches in silver carp ($F_{[3,48]} = 0.02$, $p > 0.99$) or gizzard shad ($F_{[3,48]} = 1.45$, $p = 0.24$). There also were no significant differences in spacing of gill rakers among the top, middle, and bottom locations along a single arch for silver carp ($F_{[3,48]} = 0.40$, $p = 0.67$) and gizzard shad ($F_{[2,48]} = 1.74$, $p = 0.19$) nor any interaction in gill raker spacing between gill arch number and location along a single gill arch for silver carp ($F_{[6,48]} = 0.15$, $p = 0.20$) or gizzard shad ($F_{[6,48]} = 1.50$, $p = 0.99$).

Interspecies Variation of Gill Rakers in Silver Carp and Gizzard Shad

Silver carp had consistently larger passages through rakers than gizzard shad (Fig. 5). Pore diameter in gill rakers of silver carp was greater than the distance between

gill rakers of gizzard shad at all sites and months sampled (Illinois River: $t_{[50.21]} = 17.16$, $p < 0.01$; Wabash River: $t_{[4.124]} = 6.08$, $p < 0.01$; James River: $t_{[7.89]} = 11.79$, $p < 0.01$; May: $t_{[7.20]} = 7.43$, $p < 0.01$; June: $t_{[19.63]} = 11.62$, $p < 0.01$; August: $t_{[20.42]} = 11.38$; $p < 0.01$). For example, mean pore diameter of silver carp collected in May was 171.21 μm and the average interraker spacing of gizzard shad collected in May was 28.18 μm ; mean pore diameter of silver carp collected from the Illinois River was 160.91 μm and interraker spacing of gizzard shad collected from the Illinois River was 33.76 μm .

Intraspecific Variation in Gill Rakers of Silver Carp

Mean pore diameter of gill rakers in silver carp differed among sites (ANCOVA: $F_{[2,56]} = 4.67$, $p = 0.01$), but did not differ among months in the Illinois River (ANCOVA: $F_{[2,44]} = 0.01$, $p = 0.99$). Fork length was a significant factor affecting the interraker spacing of silver carp collected among sites (ANOVA: $F_{[1,56]} = 74.59$, $p < 0.01$) and months (ANOVA: $F_{[1,44]} = 73.41$, $p < 0.01$). The 95% confidence interval for the mean pore diameter in silver carp ranged 80.69 to 185.75 μm (Table 1).

Intraspecific Variation in Gill Rakers of Gizzard Shad

Interraker spacing in gizzard shad from the Illinois River differed among sites (ANCOVA: $F_{[2,72]} = 16.60$, $p < 0.01$) and among months in the Illinois River (ANCOVA: $F_{[2,44]} = 21.16$, $p < 0.01$). Fork length significantly affected the interraker spacing of gizzard shad among months in the Illinois River (ANOVA: $F_{[1,44]} = 8.48$, $p < 0.01$), but not among sites (ANOVA: $F_{[1,72]} = 2.82$, $p = 0.10$). The 95% confidence interval for the mean space between gill rakers ranged 16.72 to 47.36 μm (Table 1).

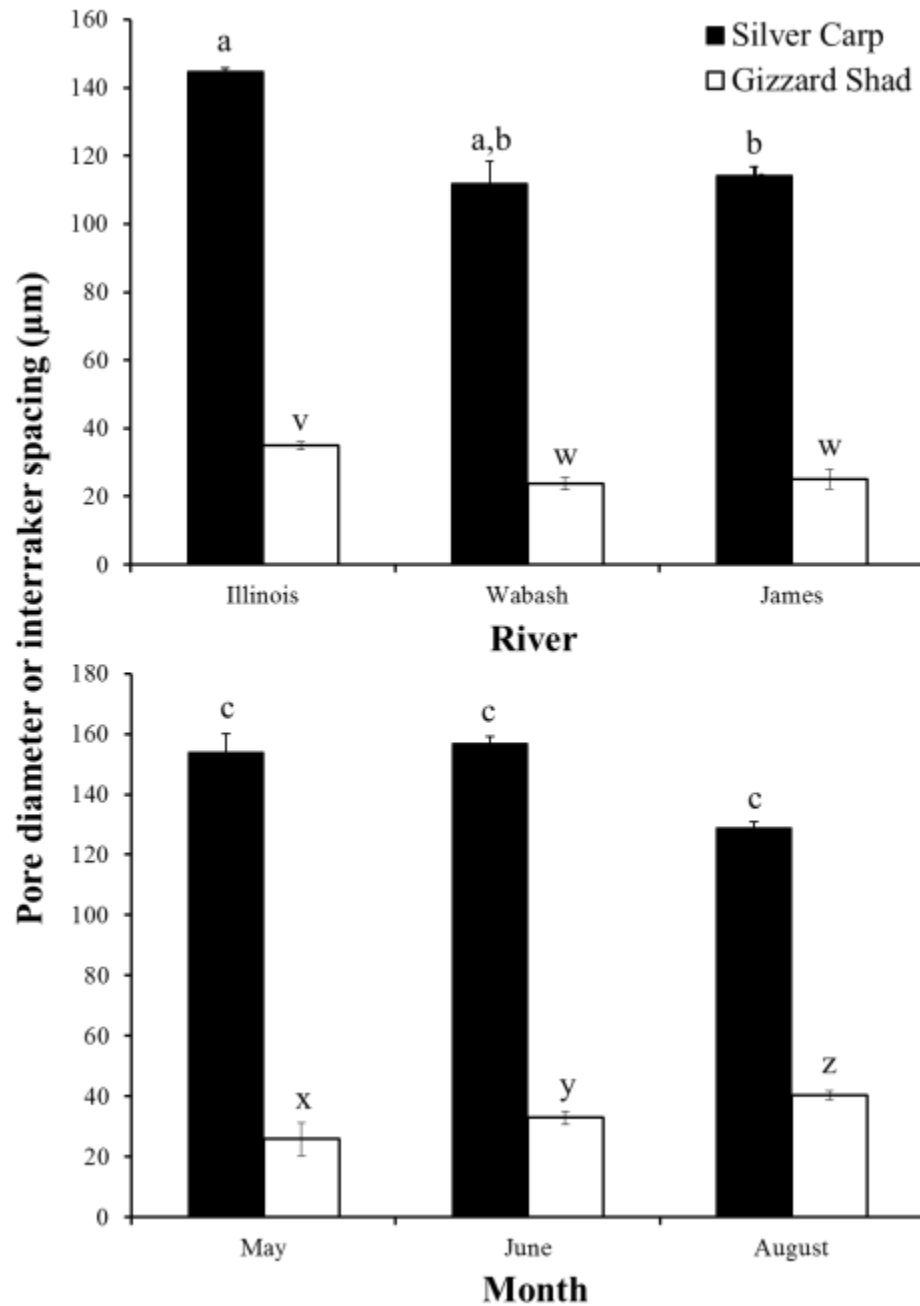


Fig. 5. Mean (+SE) pore diameter of gill rakers from silver carp and interraker spacing of gill rakers from gizzard shad among sites and months. Presence or absence of significant difference was indicated by letters (a-c) in silver carp and letters (v-z) in gizzard shad. Pairs that share a common letter are not significantly different.

Table 1. Characteristics of silver carp and gizzard shad collected May through November 2011 in the Illinois, Wabash and James Rivers. The 95% confidence interval (CI) for gill raker space is based on spacing between gill rakers in gizzard shad and pore diameter in gill rakers of silver carp.

Site	Month	Mean $\pm$ SE fork length (mm)	Mean $\pm$ SE weight (g)	N	95% CI gill raker spacing (μ m)
<u>Silver carp</u>					
Illinois	May	480.00 $\pm$ 30.95	1752.58 $\pm$ 340.89	8	(133.73, 185.75)
	June	482.65 $\pm$ 17.61	1677.69 $\pm$ 186.71	20	(144.73, 178.05)
	August	426.65 $\pm$ 10.65	1126.79 $\pm$ 60.83	20	(143.96, 177.80)
	All	458.88 $\pm$ 10.53	1460.63 $\pm$ 105.30	48	(146.88, 168.50)
Wabash	June	473.60 $\pm$ 55.91	1702.12 $\pm$ 516.04	5	(80.69, 147.71)
James	November	389.43 $\pm$ 4.89	926.29 $\pm$ 27.99	7	(126.71, 185.36)
<u>Gizzard shad</u>					
Illinois	May	240.63 $\pm$ 27.52	280.99 $\pm$ 73.34	8	(19.86, 31.79)
	June	170.90 $\pm$ 12.39	95.82 $\pm$ 20.11	20	(33.59, 41.07)
	August	203.65 $\pm$ 8.40	138.23 $\pm$ 20.60	20	(40.10, 47.36)
	All	196.17 $\pm$ 28.31	144.35 $\pm$ 18.99	48	(35.46, 41.91)*
Wabash	June	228.50 $\pm$ 9.89	177.33 $\pm$ 21.66	20	(20.65, 30.43)*
James	November	289.50 $\pm$ 10.57	372.95 $\pm$ 51.35	8	(16.72, 33.25)*

*Fork length was not a significant factor affecting spacing between gill rakers.

DISCUSSION

The decision to analyze only the first gill arch after initial analysis of all four gill arches was consistent with results from previous research (Drenner 1977; Wright et al. 1983; Northcott and Beveridge 1988; MacNeill and Brandt 1990). The results of this study suggested that fish size may relate to the size of particle which some filter-feeding fish retain in their gill rakers, though not always. This association was indicated in silver carp, with a positive relationship between fork length and diameter of gill raker pores. There was no such relationship observed in gizzard shad, where fork length did not relate to the spacing between gill rakers.

Consequently, the size of particles retained by silver carp at any location is likely determined by the size-frequency distribution of the population. However, because of profuse mucous production, silver carp may retain particles much smaller than the size estimated based on spacing between gill rakers (Adamek and Spittler 1984). Further research is required to determine the minimum size of particles retained in the gill rakers and gut, and if both parameters are related to fork length of the body. To date, there remain inconclusive results detailing the relationship between size of gill raker pores to the size of particles found in the gut (e.g. Wilamowski 1972; Cremer and Smitherman 1980; Xie 1999), as well the influence of pharyngeal teeth (Xie 1999) and suprabranchial organ (Kolar et al., 2007) on this relationship.

In this study, after accounting for fork length, it was observed that variation of pore size in silver carp only occurred among sampling sites. This may suggest that there are

site-specific differences influencing the size of particle retained in the mucous network of the gill rakers of silver carp. It is hypothesized that this could be due to varying structures of gill rakers themselves, or the mucus which covers the gill rakers. Mucus variation could be related to differences in histological or biochemical composition (Asakawa 1970; Cremer and Smitherman 1980; Hunt 1970), pH (Bitterlich 1985; Xie 1999), “stickiness” (Northcott and Beveridge 1988; Sanderson et al. 1991), or other characteristics.

Regarding gizzard shad, after accounting for fork length, the spacing of gill rakers was observed to differ among sampling sites and months of collection. Though these patterns could be artifacts of small sample sizes, it is possible that environmental or genetic variables are driving these patterns. Differences in food availability among sites may be the cause of variation in spacing between gill rakers, rather than ontogenetic development. All three sampling sites were geographically isolated and populations of the same species probably exhibit different abilities to filter food based on significantly different interraker spacing. Thus, if the spacing between gill rakers of gizzard shad were plastic in response to the size of available food resources, they may modify the spacing within this filtering organ to best adapt to food availability (Xie 1999). In this study, the lack of variation among sampling months in addition to sampling sites suggests this modification could occur at a single site along a temporal gradient. Alternatively, if genetic diversity was driving these patterns of variation among populations, this may warrant further study to investigate distinct filtering abilities of separate lineages.

The finding that the spacing between gill rakers of gizzard shad was not related to length of the fish was contrary to results of Mummert and Drenner (1986), who noted

interraker spacing in gizzard shad was growth-dependent. For silver carp, however, the findings of this study relating gill raker spacing to length of fish were supported by previous work. Jirasek *et al.* (1981) and Hampl *et al.* (1983) found that the overall filtering portion of the gill arch and the length of individual gill rakers increased with length of silver carp. Though, they did not find that the size of pores in the gill rakers increased with fish length. We speculate this disagreement may originate from differences in preparatory methods used prior to microscopic examination. In Jirasek *et al.* (1981) and Hampl *et al.* (1983) the mucous network was chemically dissolved off the gill arch prior to measuring the distances between gill rakers. In our study we left the mucous network intact to simulate natural conditions of a live fish, in which it is assumed the prolific cover of mucous network over the gill arch may highly regulate particle retention.

Some studies have quantified gill rakers of silver carp by considering the pores or “mesh size” in order to leave the mucous network intact. Voropaev (1968) and Spataru *et al.* (1983) reported pore sizes ranging 20-25 μm and 33-37 μm in diameter; which was much smaller than pore sizes in silver carp we collected from the Illinois, Wabash, and James River. Though, Spataru *et al.* (1983), and possibly Voropaev (1968), examined substantially smaller fish than those reported in this study. Additionally, their studies used stocked fish in Russia and Israel decades prior to this study. We hypothesize those fish may not reflect the same feeding characteristics as fish sampled in our study due to differences in food availability, captive versus wild samples or genetic variation.

Overall, this work contributes to the growing body of work to better understand the feeding ecology of one native filter-feeding fish and an invading Asian carp species.

This research could be used for developing mathematical models of filtration ability in silver carp based on pore size and possibly improving those already established for gizzard shad based on interraker spacing (Drenner et al. 1984, Mummert and Drenner 1986). Moreover, variations noted in feeding abilities of gizzard shad among multiple locations and time points could indicate how relative exposure to the ever-increasing biomass of silver carp feeding on similar-sized particles could reduce the amount of high quality foods available to gizzard shad and cause them to shift the structure of their gill rakers to take advantage of more abundant, but less desirable foods.

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FIGURE CAPTIONS

Figure 1. Approximate size of particles (μm) retained in gill rakers of silver carp, bighead carp, gizzard shad, bigmouth buffalo, paddlefish, mussels, filter-feeding insects, and zooplankton: (A) complete scale of retention 0-3000 μm , (B) same as (A) but with focus on particles sizes less than 500 μm . Line indicates range of literature values and box indicates estimated mean of most cited values. Literature cites silver carp with retention of 3.2 μm - 300 μm , bighead carp 5 μm -3000 μm , gizzard shad 10 μm - 200 μm , bigmouth buffalo 200 μm - 2000 μm , paddlefish 100 μm - 1300 μm , freshwater mussels 1 μm - 25 μm , filter-feeding insects 0.01 μm - 1000 μm , and zooplankton 0.5 μm - 100 μm . Number below organism name indicates the sources of data: 1. Baker and Levinton (2003), 2. Cremer and Smitherman (1980), 3. Cummins (1974), 4. Dabrowsky and Bartega (1984), 5. Dong and Li (1996), 6. Drenner et al. (1984), 7. Drenner et al. (1986), 8. Edler and Georgian (2004), 9. Helfrich et al. (1995), 10. Herodek et al. (1989), 11. Irons et al. (2007), 12. Jennings (1988), 13. Jorgensen et al. (1984), 14. Jorgensen (1996), 15. Kajak et al. (1977), 16. Kaul and Rishi (1993) (as cited in Kolar et al. 2007), 17. Kolar et al. (2005), 18. Kolar et al. (2007), 19. Kucklantz (1985), 20. Leventer and Teltsch (1990), 21. Ma et al. (2009), 22. Merritt and Cummins (1996), 23. Merritt and Wallace (1981), 24. Omarov (1970), 25. Opuszyński (1981), 26. Opuszyński et al. (1991), 27. Paterson (1986) (as cited in Mueller et al. 2004), 28. Rosen and Hales (1981), 29. Ross and Craig (1980) (as cited in Edler and Georgian 2004), 30. Sampson et al. (2009), 31. Sanderson et al. (1994), 32. Sieburth et al. (1978), 33. Smith (1989), 34.

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Figure 2. Gill arch one in (A) silver carp and (B) gizzard shad. Gill rakers are on the concave margin of the gill arch and gill filaments are on the convex margin. Single-headed arrows point to the top (T), middle (M), and bottom (B) portions of gill raker sampled to determine intra- and interraker variation in gill raker morphology. Double-headed arrows designate measurements for gill arch length and raker length.

Figure 3. Method of measuring gill rakers for silver carp and gizzard shad. (A) For silver carp the area (white circle) and the maximum width (white arrow) of pores were measured. (B) For gizzard shad the interraker distances (white arrow) were measured.

Figure 4. Relation between fork length (mm) and pore diameter (μm) of gill rakers in silver carp.

Figure 5. Mean (+SE) pore diameter of gill rakers from silver carp and interraker spacing of gill rakers from gizzard shad among sites and months. Presence or absence of significant difference was indicated by letters (a-c) in silver carp and letters (v-z) in gizzard shad. Pairs that share a common letter are not significantly different.

CONCLUSION

This research contributes to the management of populations of Asian carp in North America, and particularly in the upper Mississippi River basin. A method was developed to examine completely intact gill rakers of filter-feeding Asian carp and native fishes with confocal microscopy. This methodology improves upon traditional microscopic techniques through preservation of structure and condition of the gill rakers. This microscopy method provided the means to describe unique morphologies of gill rakers in four different fish species. This included a new scientific description of gill rakers for a species previously undocumented, bigmouth buffalo.

Additionally, this work developed a protocol for assessing the shape, size, and spacing of gill rakers in a way which quantified parameters most significantly related to filter-feeding abilities of two species. The protocol is intended to contribute toward clarifying the previous lack of established methodology in assessing feeding abilities of suspension-feeding fishes. Results from this study documented a unique pattern in variation of spacing in gill rakers between one native filter-feeding fish and an invasive species with which it competes.

Because spacing of gill rakers in silver carp correlated with fish length and did not generally differ among different time points of a single site, this suggests application of a biological control agent to target silver carp would be most effective with particle diameters corresponding to the spacing of gill rakers within a particular size class of silver carp. In contrast, the spacing of gill rakers in gizzard shad was not correlated with

fish length. This suggests that designing a size-specific control agent to target silver carp at a particular site needs to cautiously evaluate the size of particle retention for gizzard shad on a time- and site-specific basis before application. Additional research is required to support hypotheses asserted in this work and, most importantly, test them empirically before applying any control agent to wild populations.