Food Webs 16 (2018) e00095

Contents lists available at ScienceDirect

Food Webs

journal homepage: www.journals.elsevier.com/food-webs

Evaluating potential effects of bigheaded carps on fatty acid profiles of multiple trophic levels in large rivers of the Midwest, USA

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ARTICLE INFO

Article history:
Received 3 May 2018
Received in revised form 19 June 2018
Accepted 2 August 2018
Available online xxxx

Keywords:
Asian carp
Freshwater mussel
Hydropsychid
Bluegill
Seston
Zooplankton

ABSTRACT

Recent work indicates that the establishment of bigheaded carps (*Hypophthalmichthys* spp.) in the United States has led to a reduction in condition of native planktivores and may detrimentally affect other trophic levels by altering the base of aquatic food webs. We used fatty acids to evaluate potential effects of bigheaded carps on taxa from multiple trophic levels in the Upper Mississippi, Illinois, and St. Croix rivers. Seston fatty acid concentrations were highest in the Illinois River lotic sites and connected backwaters and were positively associated with omega-3 highly unsaturated fatty acids, indicating that these locations had abundant, high-quality basal food resources despite hosting the greatest bigheaded carp densities. Fatty acid profiles of threeridge freshwater mussels tracked the fatty acid values in the seston and were not influenced by bigheaded carp abundances. Hydropsychid caddisflies and bluegill did not differ significantly in total fatty acids or percent lipid among spatial locations, indicating that omnivorous species may be relatively unaffected by bigheaded carps. Gizzard shad, however, exhibited the lowest fatty acid concentrations in the locations with the highest relative bigheaded carp densities, and multivariate models identified bigheaded carp densities as the predictive factor that explained the greatest amount of variability. Zooplankton abundance has been greatly reduced after bigheaded carps' establishment in the Illinois River, which may explain the disconnect between the gizzard shad fatty acids and the plentiful, high-quality phytoplankton in that river. Our data provide additional evidence that bigheaded carps are negatively affecting native planktivores such as gizzard shad.

1. Introduction

The rate at which species are becoming established outside of their native range has escalated rapidly as our planet moves into a period of increasing global connectivity (Ricciardi, 2007). In the Laurentian Great Lakes, the introductions of non-native species were documented at an average rate of circa two per year from 1960 to 2006 (Ricciardi, 2006), and exotic species introductions into North American waters have averaged circa six per year since 2000 (U.S. Geological Survey, 2018). Not all species that are moved outside of their native range will become deleterious (Ricciardi et al., 2013), but there is a high degree of uncertainty regarding risks posed by non-native introductions, and particularly those that occur in aquatic habitats (Leprieur et al., 2009). Invasions provide a unique opportunity for unplanned, large scale experiments that can help to illuminate biotic and abiotic conditions that may regulate community assemblages (Sax et al., 2007). A recent meta-analysis of the effects of invaders in aquatic systems documented that invasive filter feeders led to reduced abundances of zooplankton and phytoplankton, but an increase in benthic invertebrates (Gallardo et al., 2016). These cases exemplify why an improved understanding of how exotic species may alter native food webs is necessary to improve the management and mitigation of invasions.

Bigheaded carps (i.e. silver carp *Hypophthalmichthys molitrix*, bighead carp *Hypophthalmichthys nobilis*) are non-native species with a rapidly expanding range in North America. During the early stages of their invasion in the Mississippi River basin (i.e. 1990s), a number of researchers raised concerns that bigheaded carps could be detrimental to North American native planktivorous fishes and native filter feeding mussels (Chick and Pegg, 2001; Laird and Page, 1996; Pflieger, 1997; Tucker et al., 1996). These concerns existed because of the diet overlap of bigheaded carps with native planktivores (e.g. Sampson et al., 2009),

Abbreviations: VSS, volatile suspended solids; TSS, total suspended solids; FAME, fatty acid methyl ester; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; DHA, docosahexaenoic acid, 22:6n3; EPA, eicosapentaenoic acid, 20:5n3; CAP, canonical analysis of principal coordinates; DistLM, distance-based linear model; dbRDA, distance-based redundancy analysis.

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https://doi.org/10.1016/j.fooweb.2018.e00095
2352-2496/Published by Elsevier Inc.

as well as the sheer quantity of food that these invaders could ingest (i.e. 6–11% of their body weight per day (Jennings, 1988; Opuszynski and Shireman, 1993)). These voracious planktivores can consume both phytoplankton and zooplankton (Domaizon and Devaux, 1999; Pongruktham et al., 2010; Spataru and Gophen, 1985; Williamson and Garvey, 2005), and they have specialized gill rakers that allow them to consume very small plankton (i.e. 10 μm) (Bitterlich and Gnaiger, 1984; Smith, 1989; Vörös et al., 1997). These feeding strategies have the potential to detrimentally affect multiple trophic levels by fundamentally altering the base of the aquatic food web.

Over the past decade, numerous publications have reported deleterious effects that bigheaded carps have had on native fishes. Multiple studies have documented a reduction in condition, catch-per-unit-effort (CPUE), and abundance of native planktivores (i.e. gizzard shad and bigmouth buffalo) in the Illinois and Mississippi rivers (Irons et al., 2007; Pendleton et al., 2017; Phelps et al., 2017). Laboratory studies have also documented a reduction in gizzard shad survival and reduction in bigmouth buffalo biomass with interspecies competition with silver carp (Phelps et al., 2017). These studies have used more traditional metrics for evaluating the effects of bigheaded carps, but there is a need to evaluate the underlying physiological mechanisms that may be leading to these reductions in native planktivore fitness (Horodysky et al., 2015).

Fatty acid quantification is a biochemical measure that can be used as a complimentary tool for evaluating the condition and health of aquatic taxa (Arts and Kohler, 2009). Fatty acids are the predominant source of metabolic energy for both growth and reproduction in aquatic organisms (Henderson et al., 1984; Sargent et al., 2002). Fatty acids are also an integral component of cell-membrane bilayers and assist in regulating fluidity and cold tolerance (Brett and Muller-Navarra, 1997). Polar phospholipids serve as the building blocks of cell membranes and nonpolar triacylglycerols serve as a primary storage product (Guschina and Harwood, 2009). Highly unsaturated fatty acids (HUFA), including docosahexaenoic acid (DHA), are essential components of neural tissues and deficiencies in HUFA may reduce prey capture efficiency by larval fishes (Sargent et al., 1993). Many fatty acids are obtained through an organism's diet and originate in primary producers, such as phytoplankton (Guschina and Harwood, 2009). If ecosystem dynamics are altered, such as by the introduction of an exotic invader, there is potential for cascading effects through the ecosystem. However, this is an area of research that has been proposed but not actively investigated to date (Arts and Kohler, 2009; Gladyshev et al., 2017).

The bigheaded carps' invasion may affect the transfer of fatty acids through the food web and ultimately affect the underlying health of native organisms (Arts and Kohler, 2009). Based upon other notable invasions that had cascading effects through the entire food web (e.g. filter feeding *Dreissena* mussel invasion in the Great Lakes (Rennie et al., 2009; Roberts, 1990)), one might expect filter feeding bigheaded carps to impact all levels of the food web because of the direct effects on phytoplankton and zooplankton, which are essential for all aquatic organisms at various stages in their ontogeny.

Our objectives were to use fatty acid concentrations as a sensitive biochemical measure to evaluate the potential impact that bigheaded carps may have on multiple levels of the native aquatic food web (i.e. seston, freshwater mussels, net-spinning caddisflies, bluegill *Lepomis macrochirus*, and gizzard shad *Dorosoma cepedianum*). To better understand which factors may be shaping fatty acid profiles among our taxa of interest, we used multivariate modeling to evaluate the relationship between fatty acid concentrations and various environmental variables, including the relative density of bigheaded carps.

2. Methods

2.1. Study sites and design

Our study was conducted in 2013 and 2014 in four main regions of the Upper Mississippi River system that encompass a gradient of bigheaded carp densities (Fig. 1). The La Grange Pool of the Illinois River near Havana, Illinois has a high relative abundance of bigheaded carps (Sass et al., 2010), Mississippi River Navigation Pool 19 near Keokuk, Iowa has a low relative abundance of bigheaded carps (Maher, 2016), and Mississippi River Navigation Pool 2 near St. Paul, Minnesota and St. Croix River downstream of St. Croix Falls, Wisconsin have no bigheaded carp populations established as of the writing of this paper, but have reported a small number of pioneer captures (Minnesota Department of Natural Resources, 2017). We sampled three lotic main channel and three connected lentic sites in each of these four regions; connected lentic sites in the Illinois and Mississippi rivers were backwaters located adjacent to the main channel sites, and lentic sites in the St. Croix River were located in the downstream riverine lake. In the Illinois River location, we also sampled two additional isolated backwaters (i.e. Emiquon and Banner Marsh) that were immediately adjacent to the Illinois River, but had no connection with the river and did not contain any bigheaded carps.

We collected samples from five taxa that encompass multiple trophic levels: seston (<63 μm filtered fraction), threeridge mussels (*Amblema plicata*), hydropsychid caddisflies (Hydropsychidae), young-of-year bluegill, and young-of-year gizzard shad. Seston and gizzard shad were collected at lotic and lentic sites. Bluegill were sampled exclusively at lentic sites, whereas threeridge mussels and hydropsychid caddisflies were sampled exclusively at lotic sites. Seston, threeridge mussels, and hydropsychid caddisfly samples were collected in late July and fish samples were collected in late August–September. Seston, hydropsychid, and mussel samples were collected within 11 calendar days for all sites in 2013, and 10 calendar days for 2014. Fish samples were collected within 15 calendar days for all sites in 2013, and 10 calendar days for 2014. Sampling sites within each region were located at least 3 km apart to provide some degree of independence among sites.

We collected three samples of one size fraction of seston (<63 μm) at each site by pre-filtering site water through a 63-μm Nitex filter, which was then vacuum filtered onto a 47-mm diameter glass-fiber filter, Type A/E with a 1.0 μm nominal pore size. The <63-μm fraction of seston generally excludes macro-algae and macrozooplankton and is considered more ingestible than larger size classes of seston by some invertebrate planktivores (e.g., herbivorous zooplankton; Kainz et al., 2009). (During 2014, a single sample of seston was collected from each site in the La Grange reach and Pool 19 because of time and budget constraints.) Foot tissue (~2 cm × 0.5 cm) was excised from five adult threeridge mussels from each lotic site. We were able to obtain threeridge mussels from only one Illinois River site in 2013, but were then unable to obtain mussels at that same site in 2014. Mussels were successfully collected from the other two Illinois River sites in 2014. Three hydropsychid caddisfly samples (each a composite of five individuals for sufficient tissue mass) were collected from wood substrates. Five age-0 bluegill (lentic sites only) and five age-0 gizzard shad were collected adjacent to shorelines at each site with pulsed-DC electrofishing equipment or hoop nets in general accordance with methods presented in Ratcliff et al. (2014). Captured fish and mussels were measured and handled in accordance with the Upper Midwest Environmental Sciences Center (UMESC) Animal Care and Use procedures. Fish were euthanized by a sharp blow to the head and dissected in the field or nearby laboratory to obtain anterior dorsal muscle tissue (~2 cm × 0.5 cm). Tissue samples from all biota were immediately frozen in liquid nitrogen and taken to UMESC for storage at −80 °C (Rudy et al., 2016).

Water samples were also collected from each site to calculate total suspended solids (TSS; mg/L), volatile suspended solids (VSS; mg/L), and chlorophyll-*a* (μg/L) in both whole water and the <63-μm size fraction of the water column. The <63-μm fraction VSS data were used to convert seston fatty acid content from μg/L to μg/mg VSS. Additional environmental data, including dissolved oxygen (mg/L), water temperature (°C), and current velocity (m/s), were also collected at each location.

To evaluate phytoplankton community assemblages, two depth-integrated phytoplankton samples (<63-μm fraction) were collected at each site. Phytoplankton composition as both cell density (cells/mL)