

# Assessing Consumption of Bioactive Micro-Particles by Filter-Feeding Asian Carp

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## Abstract

Silver carp *Hypophthalmichthys molitrix* (SVC) and bighead carp *H. nobilis* (BHC) have impacted waters in the US since their escape. Current chemical controls for aquatic nuisance species are non-selective. Development of a bioactive micro-particle that exploits filter-feeding habits of SVC or BHC could result in a new control tool. It is not fully understood if SVC or BHC will consume bioactive micro-particles. Two discrete trials were performed to: 1) evaluate if SVC and BHC consume the candidate micro-particle formulation; 2) determine what size they consume; 3) establish methods to evaluate consumption of filter-feeders for future experiments. Both SVC and BHC were exposed to small (50-100  $\mu\text{m}$ ) and large (150-200  $\mu\text{m}$ ) micro-particles in two 24-h trials. Particles in water were counted electronically and manually (microscopy). Particles on gill rakers were counted manually and intestinal tracts inspected for the presence of micro-particles. In Trial 1, both manual and electronic count data confirmed reductions of both size particles; SVC appeared to remove more small particles than large; more BHC consumed particles; SVC had fewer overall particles in their gill rakers than BHC. In Trial 2, electronic counts confirmed reductions of both size particles; both SVC and BHC consumed particles, yet more SVC consumed micro-particles compared to BHC. Of the fish that ate micro-particles, SVC consumed more than BHC. It is recommended to use multiple metrics to assess consumption of candidate micro-particles by filter-feeders when attempting to distinguish differential particle consumption. This study has implications for developing micro-particles for species-specific delivery of bioactive controls to help fisheries, provides some methods for further experiments with bioactive micro-particles, and may also have applications in aquaculture.

**Keywords:** Micro-particles; Filter-feeding; Asian carp; Oral delivery

## Introduction

Silver carp (SVC) *Hypophthalmichthys molitrix* and Bighead carp (BHC) *H. nobilis* are native to Asia, were imported into the US, and since their escape into the Mississippi River are found throughout the Mississippi River basin [1]. These species have successfully established themselves in many areas within this basin, and there exists a risk of invasion into the Great Lakes through the Illinois River and other pathways [2,3]. Since invading the Illinois River, decreases in body condition of native gizzard shad *Dorosoma cepedianum* and, to a lesser extent, bigmouth buffalo *Ictiobus cyprinellus* and paddlefish *Polyodon spathula* have occurred [4,5]. It is known that SVC and BHC can have serious ecological, economic and human safety related impacts; and their success in the Mississippi River basin is driving scientists and managers to seek out methods to control them [2,5]. Some current controls include harvest, behavioral deterrents, physical obstacles, and piscicidal compounds (i.e. rotenone and antimycin-A) [2,6]. The latter control has been practiced for nearly a century and is very effective means of controlling fish populations [2,6]; however, rotenone and antimycin are considered general fish toxicants with little specificity and their effectiveness may vary depending on application variables [7].

Control tools specifically targeting SVC and BHC are desired by fisheries managers, and new technologies may make this possible. Both SVC and BHC filter-feed [4,5,8-12] and this feeding strategy may be a trait that can be exploited for delivering bioactive micro-particle compounds containing piscicides. Similar micro-particle stabilization technologies already exist for the delivery of nutrients to larval fishes and mollusks [13] and can be made in a variety of sizes, which may increase specificity. By determining particle sizes SVC and BHC will consume, it is hypothesized that delivery may be increased to these species and

reduced to non-target species [14]. The potential applications in aquaculture may enhance the utility of this technology as a fish control tool or to deliver beneficial compounds (e.g. therapeutic agents). However, scientific evaluations of any new technology are required before its potential may be realized and development of reliable methods to evaluate the technology is required before proceeding onto more in-depth evaluations.

Two discrete trials were conducted exposing SVC and BHC to a candidate micro-particle formulation. The goals of these trials were to verify that SVC and BHC would consume the micro-particle formulation and identify reliable methods to quantify consumption. The specific objectives were to: 1) determine if filter-feeding Asian carps will consume the candidate micro-particles; 2) determine if there may be a preference of SVC and BHC for small (50-100  $\mu\text{m}$ ) or large (150-200  $\mu\text{m}$ ) size particles; 3) identify reliable methods that can be used in future studies designed to examine consumption of micro-particles by filter-feeding aquatic organisms. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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**Received** February 07, 2012; **Accepted** March 21, 2012; **Published** March 28, 2012

**Citation:** Jensen NR, Amberg JJ, Luoma JA, Walleser LR, Gaikowski MP (2012) Assessing Consumption of Bioactive Micro-Particles by Filter-Feeding Asian Carp. J Aquac Res Development 3:126 doi:10.4172/2155-9546.1000126

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## Materials and Methods

### Test animals

Age-0 SVC (average 141 mm, 35.9 g and 143 mm, 36.6 g, Trial 1 and Trial 2 respectively) and BHC (average 150 mm, 47.1 g; and 151 mm, 48.4 g, Trial 1 and Trial 2 respectively) were captured from the Kansas River; sorted based on morphological attributes (e.g. presence/absence of abdominal keel, coloration of body and fins) and held in a recirculating aquaculture system (RAS) consisting of six 1,500L tanks, 0.2 m<sup>3</sup> floating bead biofilter and UV sterilization. Each species were reared separately (19°C), fed by hand one time daily a granulated salmon/trout fry starter diet. Fish were held a minimum of six months before experimentation. Fish were taken off starter diet three days before each trial to limit the amount of fecal particles expelled into the test tanks and to increase the likelihood of consumption [15]. Six fish were randomly stocked into each test tank on the third day and allowed to acclimate to test tank conditions for 24-h, (total four days without feed) before being exposed to micro-particles.

### Test system

The test system consisted of 329L flat bottom, cylindrical, polypropylene tanks arranged in linear blocks. Each tank was plumbed for both single-pass and independent RAS operation. Each tank contained approximately 100 L of water. Tanks were provided fresh water (100 mL min<sup>-1</sup>) during the 24-h acclimation period then converted to RAS operation immediately before adding the micro-particles. Photoperiods were maintained constant at approximately 200 lux. Water temperature (°C) and dissolved oxygen (mg L<sup>-1</sup>) were recorded after each sampling time from three random tanks. The water quality means ± SD were 18.4 ± 0.1°C and 8.57 ± 0.2 mg L<sup>-1</sup> respectively in Trial1, and in Trial 2, 20.0 ± 0.1°C and 7.10 ± 0.7 mg L<sup>-1</sup>.

### Test material

Micro-particles consisted of a commercial micro bound micro-particle (Advanced Bio Nutrition Corporation, Columbia, MD) that contained astaxanthin (0.1 percent dry weight); except the small particles used in Trial 1 did not contain astaxanthin. Particles are also designed to be neutrally buoyant and stable in water. Prior to each trial, micro-particles from the manufacturer were further sieved into specific size

Micro-particle size range (µm)	Mass (g)	Particle counts	Particles per g	Mean particles per g	Small: Large particle ratio	g of particles added to tanks	Particles added to tanks
Trial 1							
150-200	0.00014	63	450,000	459,595	4.71	4.35	1,999,238
150-200	0.00011	49	445,454				
150-200	0.00018	87	483,333				
50-100	0.00025	552	2,208,000	2,165,166		0.92	1,991,952
50-100	0.00015	330	2,200,000				
50-100	0.00016	334	2,087,500				
Trial 2							
150-200	0.00016	75	468,750	434,979	5.61	4.60	2,000,903
150-200	0.00015	64	426,666				
150-200	0.00042	172	409,523				
50-100	0.00030	727	2,423,333	2,443,789		0.82	2,003,906
50-100	0.00014	380	2,714,285				
50-100	0.00016	351	2,193,750				

**Table 1:** Enumeration of micro-particles to determine quantity of food particles added to test tanks for Trial 1 (top) and Trial 2 (bottom).

Trial 1					
SVC MM (4 tanks)	BHC MM (4 tanks)	SVC No MM (1 tank)	BHC No MM (1 tank)	SVC No MM Trout diet (1 tank)	BHC No MM Trout diet (1 tank)
Trial 2					
SVC MM (4 tanks)	BHC MM (4 tanks)	SVC No MM (1 tank)	BHC No MM (1 tank)		

SVC-Silver carp  
 BHC-Bighead carp  
 MM-Micro-particles added to tanks  
 No MM-Micro-particles not added to tanks  
 Trout diet-Granulated salmon/trout fry starter diet

**Table 2:** Schematic of treatment assignments to 12 tanks for Trial 1 and 10 tanks for Trial 2.

ranges, small (50-100 µm) and large (150-200 µm). The mean number of particles for each size group was determined by counting particles in three 0.01-0.04 mg samples using light microscopy. Particle counts were used to estimate the mass of particles required to supply 40 particles mL<sup>-1</sup> including equal proportions of both micro-particle size ranges to add to each test tank (Table 1).

### Experimental trials

Two trials were conducted. A total of 12 tanks were used in Trial 1: four treatment tanks per species, one trout starter diet control tank per species, and one unfed control tank per species. A total of 10 tanks were used in Trial 2: four treatment tanks per species, and one unfed control tank per species (Table 2). In each trial, six fish were stocked into each tank with each species held separately. Due to fish sloughing particles such as skin and particles present within the test system from sources such as the water, unfed control tanks were used to measure the particle sizes associated with these inadvertent sources and to eliminate them from analysis. The two tanks fed trout starter diet in Trial 1 were included to verify fish would eat following handling conditions of the experiment. All fish were euthanized by cranial concussion. Fork length (mm) and wet weight (g) were recorded for each fish at the end of each Trial.

In Trial 1, the small micro-particles (50-100 µm) did not contain astaxanthin while the large (150-200 µm) micro-particles did contain astaxanthin. The two colors allowed distinguishing the two different size classes during manual particle counts, gill raker counts, and calculation of ratios of small: large particles. The ratios were used to indicate particle removal of a particular size range. Gill rakers were examined to determine if fish were concentrating micro-particles in their branchial regions.

Electronic count sampling included triplicate water samples (approximately 110 mL) collected from mid-depth at 1, 6, 12 and 24-h. Particles were immediately electronically counted using a Multisizer 4™ Coulter Counter® (Beckman Coulter Incorporated, Brea, CA) fitted with a 400mm aperture tube following sample preparations needed by the counter. A cuvette containing the sample was placed in the coulter counter, which drew in 35 mL of the 100 mL sample aliquot. The remaining water samples were discarded.

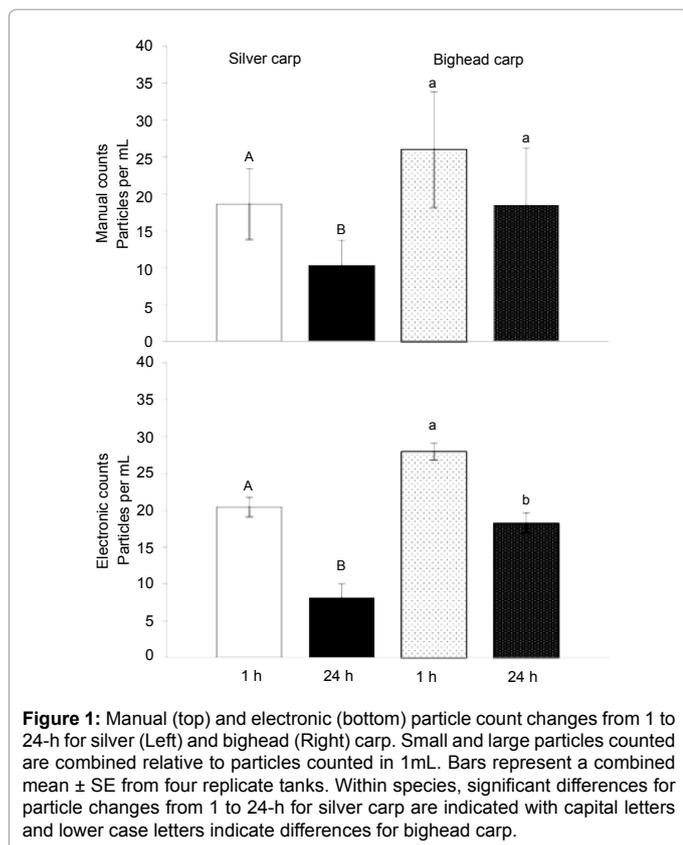
Immediately prior to collecting samples for electronic counts at 1 and 24-h, mid-depth water grab samples (50 mL) were collected for manual counts. These grab samples were stored approximately 24-h at 4°C until the particles could be counted. For each manual count, all particles were counted in triplicate 3mL aliquots from each grab sample. The gastrointestinal tracts (GI) of 10 fish were examined per spe-

cies (minimum two fish per tank) and from all fish in tanks offered the trout starter diet to assess the presence or absence of GI contents. Of the remaining fish, gill rakers (four rakers from one side) were taken from three fish at random for each species. Particles in gill rakers were distinguished based on color and counted under light microscopy.

Following the results from Trial 1, a second trial (Trial 2) was conducted focused on the total number of fish consuming micro-particles and the approximate quantity consumed. The procedures used in Trial 2 were the same as those in Trial 1 except that: 1) all particles contained astaxanthin; 2) offering some tanks trout starter diet was discontinued; 3) manual particle counts were discontinued; 4) water samples for electronic counting were taken at 1, 12, 24-h; and 5) the GI contents of all fish were examined after euthanasia. A scale of 0-3 was applied during GI content examinations to rank gut fullness as previous research has shown this to be a useful tool [9]. Each rank value was converted to a percentage for fullness comparisons where: 0=GI empty (0 percent full); 1=GI 1-33 percent full; 2=GI 34-66 percent full; 3=GI 67-99 percent full.

### Data analysis

Tanks were considered the experimental units in each trial. Each treatment was randomly assigned to tanks using a random number generator. Comparisons were not made between trials as each trial was considered discretely. The electronic particle counts were corrected prior to analysis by subtracting the number of particles measured in tanks with fish unexposed to micro-particles. Repeated measures and one-way Analysis of Variance (ANOVA) with multiple comparisons (Tukey) were used to determine particle changes over time, differences among individual species tanks, and differences between species following correction and square root transformation. For repeated measu-



**Figure 1:** Manual (top) and electronic (bottom) particle count changes from 1 to 24-h for silver (Left) and bighead (Right) carp. Small and large particles counted are combined relative to particles counted in 1mL. Bars represent a combined mean  $\pm$  SE from four replicate tanks. Within species, significant differences for particle changes from 1 to 24-h for silver carp are indicated with capital letters and lower case letters indicate differences for bighead carp.

Particle size ( $\mu$ m)	Sample time	BHC tank 1	BHC tank 2	BHC tank 3	BHC tank 4	p-value
50-100	1-h	825 $\pm$ 73 <sup>a</sup>	688 $\pm$ 54 <sup>a</sup>	699 $\pm$ 102 <sup>a</sup>	780 $\pm$ 31 <sup>a</sup>	0.12
	6-h	301 $\pm$ 146 <sup>a</sup>	635 $\pm$ 149 <sup>ab</sup>	666 $\pm$ 22 <sup>ab</sup>	777 $\pm$ 269 <sup>a</sup>	0.04
	12-h	155 $\pm$ 56 <sup>a</sup>	622 $\pm$ 45 <sup>b</sup>	675 $\pm$ 90 <sup>b</sup>	605 $\pm$ 136 <sup>b</sup>	<0.01
	24-h	439 $\pm$ 57 <sup>a</sup>	637 $\pm$ 89 <sup>b</sup>	522 $\pm$ 63 <sup>ab</sup>	582 $\pm$ 25 <sup>ab</sup>	0.03
150-200	1-h	72 $\pm$ 15 <sup>a</sup>	51 $\pm$ 31 <sup>a</sup>	55 $\pm$ 23 <sup>a</sup>	81 $\pm$ 12 <sup>a</sup>	0.36
	6-h	34 $\pm$ 25 <sup>a</sup>	50 $\pm$ 32 <sup>a</sup>	47 $\pm$ 10 <sup>a</sup>	66 $\pm$ 21 <sup>a</sup>	0.48
	12-h	14 $\pm$ 14 <sup>a</sup>	71 $\pm$ 24 <sup>b</sup>	43 $\pm$ 16 <sup>ab</sup>	58 $\pm$ 25 <sup>ab</sup>	0.04
	24-h	11 $\pm$ 10 <sup>a</sup>	16 $\pm$ 7 <sup>a</sup>	18 $\pm$ 6 <sup>a</sup>	61 $\pm$ 18 <sup>b</sup>	<0.01
		SVC tank 1	SVC tank 2	SVC tank 3	SVC tank 4	p-value
50-100	1-h	556 $\pm$ 49 <sup>a</sup>	493 $\pm$ 81 <sup>a</sup>	586 $\pm$ 80 <sup>a</sup>	403 $\pm$ 181 <sup>a</sup>	0.19
	6-h	106 $\pm$ 31 <sup>ac</sup>	769 $\pm$ 138 <sup>b</sup>	520 $\pm$ 74 <sup>d</sup>	312 $\pm$ 101 <sup>cd</sup>	<0.01
	12-h	131 $\pm$ 40 <sup>a</sup>	431 $\pm$ 56 <sup>b</sup>	254 $\pm$ 48 <sup>ab</sup>	470 $\pm$ 158 <sup>b</sup>	<0.01
	24-h	179 $\pm$ 17 <sup>a</sup>	383 $\pm$ 9 <sup>b</sup>	188 $\pm$ 52 <sup>a</sup>	154 $\pm$ 29 <sup>a</sup>	<0.01
150-200	1-h	54 $\pm$ 25 <sup>a</sup>	63 $\pm$ 29 <sup>a</sup>	45 $\pm$ 26 <sup>a</sup>	22 $\pm$ 2 <sup>a</sup>	0.26
	6-h	13 $\pm$ 1 <sup>a</sup>	88 $\pm$ 22 <sup>b</sup>	40 $\pm$ 13 <sup>a</sup>	19 $\pm$ 10 <sup>ac</sup>	<0.01
	12-h	4 $\pm$ 3 <sup>a</sup>	45 $\pm$ 17 <sup>b</sup>	27 $\pm$ 3 <sup>ab</sup>	37 $\pm$ 16 <sup>b</sup>	0.02
	24-h	0 $\pm$ 0 <sup>a</sup>	34 $\pm$ 18 <sup>b</sup>	1 $\pm$ 1 <sup>a</sup>	1 $\pm$ 0 <sup>a</sup>	0.03
Combined means						
			BHC	SVC	p-value	
50-100	1-h		748 $\pm$ 32	509 $\pm$ 40	<0.01	
	6-h		595 $\pm$ 102	427 $\pm$ 141	0.37	
	12-h		514 $\pm$ 120	321 $\pm$ 78	0.23	
	24-h		545 $\pm$ 42	226 $\pm$ 52	<0.01	
150-200	1-h		64 $\pm$ 7	46 $\pm$ 8	0.15	
	6-h		49 $\pm$ 6	40 $\pm$ 17	0.64	
	12-h		47 $\pm$ 12	28 $\pm$ 8	0.27	
	24-h		26 $\pm$ 11	9 $\pm$ 8	0.20	

**Table 3:** Trial 1 electronic particle counts at 1, 6, 12, 24-h. Mean ( $n=3\pm$ SD) counts for bighead (BHC; top rows) and silver carp (SVC; middle rows) are compared across rows relative to particle size, sample time. Combined means ( $n=4\pm$ SE) for each particle size and sample time between species are also compared (bottom). Significant differences are indicated with different letter superscripts across rows.

res, mean particle counts were the dependent variable and rearing tanks were independent variables. Manual counts of particles in water and on gill rakers were normalized by square root transformation before comparisons by one-way ANOVA. The mean proportions and binomial proportions of fish with GI contents were normalized by arcsine and square root transformation before comparisons by one-way ANOVA. Statistical Analysis Software (SAS 9.1; SAS Institute Inc., Cary, NC, USA) was used for all comparisons. Significance level was declared at  $\alpha \leq 0.05$ .

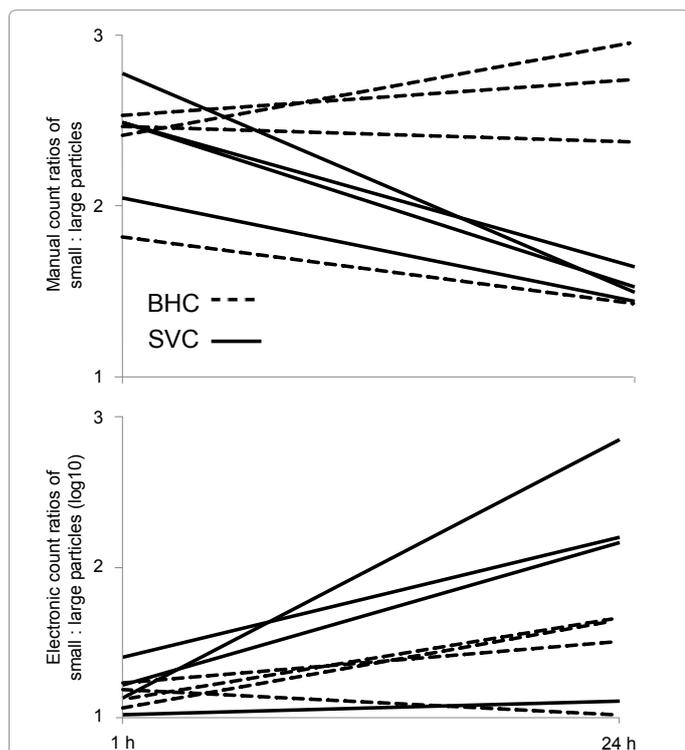
## Results

### Trial 1

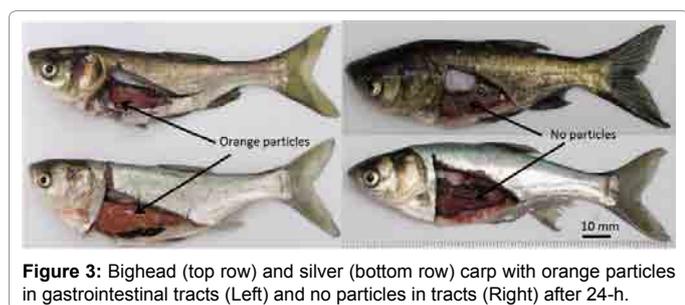
Decreasing trends were observed in both electronic and manual counts (Figure 1). At the first sampling period (1-h), the mean number of small and large particles did not differ among individual species tanks. However, mean numbers of both small and large particles differed among tanks at later sampling times for both species (Table 3). Numbers of small particles were different among tanks at 6, 12 and 24-h and large particles at 12 and 24-h for both species (Table 3). Tanks holding SVC had fewer small particles at 1 and 24-h than those holding BHC ( $p \leq 0.01$ ) and were numerically, but not statistically, lower at 6 or 12-h (Table 3). Manual counts of all particles (50-200  $\mu$ m) in tanks containing BHC did not decrease between 1 and 24-h, but did decrease in those containing SVC ( $p<0.01$ ; Figure 1). The mean  $\pm$  SD total number of particles per mL counted manually decreased from 1 to 24-h in SVC

(18 ± 5 to 10 ± 5) and BHC (26 ± 5 to 18 ± 6) tanks (Figure 1). Variability appeared higher with the manual count data compared to electronic count data. The ratio of small: large particles decreased in all SVC tanks and decreased in two of four BHC tanks. These trends were not observed with electronic count ratios (Figure 2). Manual count mean small: large particle ratios decreased from 1 to 24-h in tanks containing SVC; whereas the ratios were more similar between 1 and 24-h in those holding BHC. Contrasting this with electronic count data, mean small: large particle ratios increased from 1 to 24-h in SVC tanks and BHC tanks over the same time periods.

Fewer SVC were observed to consume micro-particles than BHC; 6 of 10 SVC and 8 of 10 BHC respectively. Both species had various amounts of the particles in their GI ranging from empty to full (Figure 3). All fish fed trout starter diet were full. Consumption comparisons using binomial proportional analysis showed no significant difference between species consumption levels (p=0.07). The number of small particles counted on the gill rakers did not differ between SVC and BHC (p=0.41), yet BHC had significantly more large particles in gill rakers (p<0.01) compared to SVC (Table 4).



**Figure 2:** Ratios of small: large particles from each treatment tank of silver (SVC) and bighead (BHC) carp from 1 to 24-h.



**Figure 3:** Bighead (top row) and silver (bottom row) carp with orange particles in gastrointestinal tracts (Left) and no particles in tracts (Right) after 24-h.

	Mean number of small particles	Mean number of large particles	Mean number of total particles
Silver carp	0.67 <sup>a</sup>	1.67 <sup>a</sup>	2.34 <sup>a</sup>
Bighead carp	4.33 <sup>a</sup>	22.67 <sup>b</sup>	27.00 <sup>b</sup>
p-value	0.21	0.03	0.01

**Table 4:** Mean number of small, large and total micro-particles in gill rakers removed from three silver carp and three bighead carp following 24-h particle exposure. Within columns, superscripts with different letters indicate significant differences.

Particle size (µm)	Sample time	BHC tank 1	BHC tank 2	BHC tank 3	BHC tank 4	p-value
50-100	1-h	423±168 <sup>a</sup>	389±105 <sup>a</sup>	798±105 <sup>b</sup>	519±90 <sup>ab</sup>	0.01
	12-h	162±151 <sup>a</sup>	200±111 <sup>a</sup>	448±11 <sup>a</sup>	258±198 <sup>a</sup>	0.13
	24-h	478±82 <sup>a</sup>	213±29 <sup>b</sup>	536±61 <sup>a</sup>	577±56 <sup>a</sup>	<0.01
150-200	1-h	43±30 <sup>a</sup>	25±16 <sup>a</sup>	75±18 <sup>a</sup>	45±6 <sup>a</sup>	0.08
	12-h	20±13 <sup>a</sup>	10±7 <sup>a</sup>	44±8 <sup>b</sup>	70±4 <sup>c</sup>	<0.01
	24-h	39±9 <sup>a</sup>	14±1 <sup>a</sup>	37±12 <sup>a</sup>	82±14 <sup>b</sup>	<0.01
		SVC tank 1	SVC tank 2	SVC tank 3	SVC tank 4	p-value
50-100	1-h	524±93 <sup>a</sup>	416±21 <sup>a</sup>	552±83 <sup>a</sup>	392±58 <sup>a</sup>	0.06
	12-h	402±38 <sup>a</sup>	354±106 <sup>a</sup>	196±89 <sup>a</sup>	219±94 <sup>a</sup>	0.05
	24-h	480±9 <sup>a</sup>	435±141	159±37 <sup>b</sup>	159±16 <sup>b</sup>	0.04
150-200	1-h	87±12 <sup>a</sup>	32±6 <sup>b</sup>	55±12 <sup>b</sup>	30±8 <sup>b</sup>	<0.01
	12-h	75±6 <sup>a</sup>	46±24 <sup>a</sup>	41±15 <sup>a</sup>	3±4 <sup>b</sup>	<0.01
	24-h	39±5 <sup>a</sup>	48±15 <sup>a</sup>	16±5 <sup>b</sup>	1±1 <sup>c</sup>	0.02
		Combined means	BHC	SVC	p-value	
50-100	1-h		532±92	471±39	0.57	
	12-h		267±63	293±50	0.76	
	24-h		451±81	308±86	0.28	
150-200	1-h		47±10	51±13	0.81	
	12-h		36±13	41±14	0.79	
	24-h		43±14	26±10	0.37	

**Table 5:** Trial 2 electronic particle counts at 1, 12, 24-h. Mean (n=3±SD) counts for bighead (BHC; top rows) and silver carp (SVC; middle rows) are compared across rows relative to particle size, sample time. Combined means (n=4±SE) for each particle size and sample time between species are also compared (bottom). Significant differences are indicated with different letter superscripts across rows.

### Trial 2

Decreasing trends were observed with electronic count data. Electronic counts at 1-h showed micro-particle variability among the tanks (Table 5). Small particles were significantly different among BHC tanks (p=0.01) but SVC tanks were not statistically different (p = 0.06); however, by 12-h, SVC were different (p<0.01). Differences in the number of large particles were found for tanks holding BHC after 12-h (p<0.01) and those holding SVC by 1-h (p<0.01). Repeated measures analysis indicated statistical differences with no interactions between species and among sample times. More total particles were found in tanks with BHC compared to those holding SVC (p=0.01) and the total number of particles decreased significantly over time (p<0.01).

A total of 10 of 24 SVC and 6 of 24 BHC had micro-particles in their GI. Binomial proportional analysis indicated a significant difference in consumption between SVC and BHC (p=0.02). Both species had various amounts in their GI, but GI fullness indicated when SVC consumed micro-particles, they consumed more than BHC (87.9 percent versus 38.9 percent full; p = 0.01).

### Discussion

This study applied methods to evaluate consumption of micro-particles by filter-feeding SVC and BHC and provides an example of the importance of validating methodology. Although not all fish con-

sumed micro-particles in both trials, identification of micro-particles in the GI tracts (41 percent or 60 percent for SVC and 25 percent and 80 percent for BHC in Trial 1 and Trial 2, respectively) shows that consumption of this micro-particle formulation occurred. Trial 1 included particle removal evaluations involving two different colored particles to distinguish small and large particles. This method was useful when examining gill rakers for particles, viewing particles in the GI and ratio analysis to suggest potential particle preferences. These findings are similar to previous research indicating SVC consume particles 5-75  $\mu\text{m}$  [9] and also filter particles larger than 100  $\mu\text{m}$  [5]. Defining these ranges is important for sizing particles to help decrease consumption by non-target organisms, like native mussels which may prefer particles less than 25  $\mu\text{m}$  [16]. Interestingly, it has been shown that SVC and BHC carp may have less dietary overlap than other similar species [17]. In this study both SVC and BHC consumed small and large particles, indicating overlap, and suggests a narrower size range within those tested herein may be of interest for further study. This study also showed both species consumed a formulated micro-particle diet and a commercial trout/salmon diet which is contrary to a previous report that they may or may not ingest artificial diets [8].

The variation in micro-particle consumption observed in this study may be partially explained by factors affecting filter-feeding efficiency, and preferences of SVC and BHC because fish gustatory-system mediated feeding is both oral and extra-oral [11,18]. Age or size, metabolic influences and intrinsic behaviors affect feeding [10,14]. In fish, particle retention by filter-feeding planktivores is primarily based on gill raker morphology, but mucus, water flow dynamics and particle size and shape also influence particle retention [14,19]. Gill rakers of SVC are spaced differently than those of BHC (12-41  $\mu\text{m}$  versus 85  $\mu\text{m}$ ) thus it is conceivable that different sized particles may be differentially ingested [8,12,19]. However, some filter-feeding fishes feed continuously, indirectly ingesting detritus and low nutritional particles simultaneously [12], suggesting filtering is more a passive and mechanical [9] rather than a complex particle selection process. Although SVC are considered typical pump-feeders, there is evidence that they may triturate food to enhance digestion in their stomach less GI [12]. In addition, some chemicals may stimulate SVC feeding [9] and both SVC and BHC select for more nutritious food when abundant [19]. SVC may select for blue green algae [10] as fry, yet reject some types of blue green algae at a size comparable to the fish used in this study [20]. BHC also show sensitivity to different food types, which may be due to higher densities of filtering apparatus taste buds [21]. Pharyngeal water flow dynamics and branchial sieving enables common carp *Cyprinus carpio* to separate organic from inorganic materials in conjunction with palatal chemosensory [22]. Although common carp are not considered filter feeders, it is conceivable that similar chemo-selective micro-particle consumption selection may occur with SVC and BHC. Thus, it may be possible to improve consumption of the micro-particles tested in this study by incorporating palatability enhancers or chemo-attractants.

One objective of this study was to establish methods to analyze these specific micro-particles in water for future studies. The particle density to test was of primary concern, since differential particle retention may be related to particle density [10]. This study targeted 40 particles  $\text{mL}^{-1}$ . This concentration was approximately 52-54  $\text{mg L}^{-1}$ , or 2.4 percent and 1.8 percent body weight of the SVC and BHC; respectively, used in this study. This concentration was also chosen because the threshold concentration of algae to stimulate feeding and growth in SVC is 12-22  $\text{mg L}^{-1}$  [10] however no positive correlation has been shown to exist between amount of food available and amount eaten by triploid BHC [11]. Regardless, this particle density was sufficient for

consumption to occur and at a quantity where changes in the number of particles in water could be quantified using the methods in this study. Using binomial proportions to compare consumption between species was effective and when coupled with colored GI contents, consumption was clearly observed. The GI fullness index applied in Trial 2 was simplistic and provided valuable information on consumption quantities so comparisons could be made between species.

Both manual counts and electronic counts indicated particle decreases between 1 and 24-h in all tanks in both Trials. Although some settling was observed in all tanks in both trials, and a small amount of particles were removed for sample counts (estimated to be 0.5-0.6 percent), both counting methods were effective at measuring particle reductions at sequential time periods. However, when applying ratio changes the results were different. Manual counts of different color particles with subsequent ratio change calculations indicated small particles may have been favored by SVC, yet electronic counts indicated large particles may be favored. The disagreement between methods remains in question. The sample volumes were greater with the electronic counter, and subsequent particle counts showed less variability. However, the different colored particles allowed visual confirmation of particles during manual counts whereas the electronic counter was unable to distinguish colors. These particles were designed to be stable in water, but these conflicting results may indicate a geometry change (e.g. particle break-down, swelling, etc.) may have occurred during the mechanical water sampling processes using the electronic counter. For future trials, it is suggested the electronic counter may be more effective if only one size range is evaluated at a time to alleviate the possibility of micro-particle shearing affecting results. Additionally, manually counting different colored particles produced valid results but may be improved if sample sizes are increased.

In summary, the methods employed in this study suggest that both SVC and BHC will consume bioactive micro-particles in the 50-200  $\mu\text{m}$  size range and commercial trout/salmon diet. Since many factors may influence consumption by filter-feeding Asian carp [8-12,14,18,19,22], modifications to the micro-particle formulation and additional studies are warranted. On-going research will include methods used in this study to define the relative amount of micro-particles these fish consume, which can then be used to calculate loading concentrations of various compounds (e.g. biocides, therapeutic agents, nutrients, etc.). The development of these technologies may be incorporated into an integrated pest management control tool for use in fisheries management, but also may be applied to deliver beneficial compounds (e.g. therapeutics, nutrients) in aquaculture.

#### Acknowledgements

Thanks to Theresa Schreier, Todd Severson and Lily Moua for calibration of the electronic counter and James (Mac) Makay and Aaron Cupp for constructing the test system. Thanks to the Kansas Department of Fish and Game for finding the fish used in these trials. Thanks to Diane Sweeney for constructive statistical consultation. Funding for this study was provided by the Great Lakes Restoration Initiative. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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