Abundance and distribution of early life stages of Asian carp in the Ohio River:

Geographic Location: Ohio River Basin

Participating Agencies: Indiana Department of Natural Resources (INDNR) Kentucky Department of Fish and Wildlife Resources (KDFWR), West Virginia University (WVU), United States Fish and Wildlife Service (USFWS), West Virginia Division of Natural Resources (WVDNR)

Statement of Need:

The negative effects of Silver (*Hypophthalmichthys molitrix)* and Bighead Carp (*H. Nobilis)*, also known as Asian carp, have been widely documented throughout their introduced range. These effects are numerous and varied in nature, some with direct implications to native biota (Irons et al. 2007, Sampson et al. 2009). Others may be indirect and difficult to quantify, such as economic loss and negative social perception. Research investigating factors that lead to Asian carp range expansion is critical for the control of these invasive fishes, and mitigation of the deleterious effects they can cause.

As of late, extensive research efforts have been directed towards Asian carp reproduction in terms of timing, location, and environmental conditions. Asian carp exhibit a boom and bust pattern of reproduction, with strong year classes usually linked with large sustained flooding and critical temperature ranges (DeGrandchamp et al. 2007). Although some understanding of their reproductive requirements exist, recent evidence suggests that spawning of these species is possible over wider environmental ranges (Coulter et al. 2013), and in more habitats (i.e. tributaries) than previously thought (Kocovsky et al. 2012). In addition, factors leading to successful recruitment of these species are difficult to identify because juveniles are extremely mobile, and effective sampling methods have not been extensively examined. Identifying factors promoting reproduction and recruitment of these invasive fishes is critical in suppressing their spread into novel environments.

Confirmed Asian carp spawning events have been reported in tributaries (i.e. Wabash River) and as far upstream as JT Myers Locks and Dam and signs of spawning (i.e. spawning patches) have been observed as far up river as the Markland Pool. Successful reproduction of *Hypophthalmichthys* spp. was detected at river mile (RM) 560 (McAlpine Pool) in 2015, and further upstream at river mile 405.7 (Meldahl Pool) in 2016 (EA engineering, personal communication). This defined the leading edge of spawning in the Ohio River (EA Engineering, personal communication). To support Basin Framework objectives (ORFMT 2014) this project was initiated in 2016 in an effort to improve capabilities to detect early stages of invasion and spawning populations of Asian carp (Strategy 2.7) and also monitor upstream range expansion and changes in distribution and abundance (Strategy 2.3). Results of sampling prior to 2018 determined the extent of recruitment as below Cannelton Lock and Dam, with the majority of young-of-year (YOY) and Juvenile detections below Newburgh Lock and Dam in J.T. Myers Pool (Jansen and Stump 2017, Roth 2018). In addition to the Basin Framework, this project directly supports the National Plan (Conover et al. 2007) by assisting in the forecast and detection of Asian carp range expansions (Strategy 3.2.4), determining life history characteristics (Strategy 3.3.1), and assembling information about the distribution, biology, life history, and population dynamics of Bighead and Silver Carps (Strategy 3.6.2). Additionally, the results of this project will help managers make informed decisions during future planning efforts regarding resource allocation for Asian carp deterrent and control strategies.

Project objectives:

1. Determine the extent of Asian carp spawning activity in the Ohio River via sampling for Asian carp eggs, embryos, and larvae.
2. Determine the extent of Asian carp recruitment in the Ohio River via targeted sampling for juvenile Asian carp.
3. Identify characteristics of potential Asian carp nursery areas when juvenile Asian carp are encountered.

Project Highlights:

- As of 2016, Asian carp (*Hypophthalmichthys* spp.) larvae were collected at river mile 405.7 (Meldahl Pool).
- Six sites from RM 546 to RM 773 were sampled via ichthyoplankton tows weekly from late-May through July; Asian carp "type" larvae were collected at all six sites.
- Six sites from RM 497 to RM 260 were not able to be sampled via ichthyoplankton tows in 2018.
- Targeted electrofishing efforts were reduced and refined from previous years to target a few suspected recruitment areas in each pool.
- Sampling in 2018 detected the most upstream juvenile Silver Carp in Newburgh Pool (RM 750).
- Majority of recruitment remains in J.T. Myers Pool, although previous sampling suggests limited recruitment in Cannelton and Newburgh Pools.
- 197 Asian carp were collected for a total of 591 pounds of fish removed.

Methods:

For analysis purposes and for the remainder of this report, both "YOY" and "immature" are collectively referring to "juvenile" Asian carp; "YOY" will be defined as fish less than 200 mm, and "immature" will define fish between 200 to 400 mm (likely 1 to 2 years old) which have undeveloped gonads and are not capable of spawning. Adult Asian carp are defined as fish greater than 400 mm with mature, identifiable gonads.

Ichthyoplankton tows:

Ichthyoplankton sampling was more fully incorporated during the 2018 sampling season to provide an updated delineation of the spawning extent from what EA engineering documented in 2015 and 2016. Ichthyoplankton sampling was conducted weekly at six sites within Newburgh (N=2), Cannelton (N=2), and McAlpine (N=2) Pools from late-May through July. A fine-mesh conical ichthyoplankton net (0.76m, 500 µm mesh) fitted with a General Oceanics Flowmeter to estimate volume of water filtered was used for sampling. Four tows were conducted during each sampling event at a site; one tow either at the intake structure or within the tributary, and one tow at each the right-descending, middle, and left-descending portions of the Ohio River. Sample contents were rinsed into collection jars, preserved in 95% ethanol, and sent to WVU for processing and identification.

Surface trawl:

Experimental surface trawling was conducted at seven sites within J.T. Myers (N = 2), Newburgh (N = 1), Cannelton (N = 2), and McAlpine (N = 2) Pools from June to August, 2018. The surface trawl was 3.7 m wide, 0.6 m tall, and 5.2 m deep with 16 mm bar mesh. The last eight feet of the purse had an additional layer of 4.8 mm mesh bag attached internally to improve capture of small fishes. Additional foam floats were added to the top line of the trawl to provide extra buoyancy. Otter boards were 30.5 cm tall, 61.0 cm

long, and each had a 12.7 cm diameter, 27.9 cm long "buoy style" PVC float attached to the top of the board allowing them to float. The trawl was deployed off of the front of the boat and attached with 24.4 m ropes. The boat was motored in reverse for 5 minutes before retrieving the net. Fish captured were identified to species and all Asian carp were processed as described below in electrofishing methods.

Electrofishing:

Electrofishing was conducted in J.T. Myers, Newburgh, Cannelton and McAlpine Pools of the Ohio River from July 23rd to August 22nd, 2018. Flooded creek mouths, tributaries, side channels, and other backwater areas large enough for entrance with an electrofishing boat were selected in each pool to be sampled; to allocate more effort towards larval sampling, the number of sites was reduced from 2017 to only include those locations with the greatest likelihood of producing juvenile Asian carp. To account for temporal variability in abundance and environmental conditions, all sites were sampled twice, at least two weeks apart, depending on accessibility.

Electrofishing effort consisted of 15-minute transects at each sampling location, unless otherwise impeded. At the biologist's discretion, more sampling time or multiple runs were conducted at sites where either coverage was limited or juvenile Asian carp were suspected. In some cases, only transects shorter than 15 minutes were possible. Specific electrofishing settings were 80 pulses per second at 40% duty cycle, and volts were adjusted based on water conductivity to achieve standard power goals and maximize Asian carp collection. Dippers specifically targeted all fish resembling Asian carp. All Asian carp were then identified to species, measured to total length, weighed, and sexed when possible. Lapilli otoliths were removed from fish under 600 mm for age estimation. Young-of-year Asian carp were frozen whole for potential additional analyses.

Environmental variables:

A suite of habitat variables were collected at each electrofishing site including: water temperature, Secchi disk visibility, conductivity, pH, dissolved oxygen, maximum depth, average depth, tributary width, and presence/absence of woody debris and aquatic vegetation. To increase sample size and statistical power, juvenile occurrences and associated habitat variables were pooled from 2016 to 2018 data. These variables were used to describe the possible habitat preferences of juvenile Asian carp. Using an alpha level of 0.05, two-sample student's t-Tests (assuming unequal variances) were performed individually on each numerical habitat variable to compare mean measurements between locations with juvenile Asian carp present (N = 21) to those locations without (N = 347). Chi-square test statistic was used to determine whether juvenile Asian carp exhibited a preference for a range of water colors, presence of woody debris, and presence of aquatic vegetation.

Results:

Ichthyoplankton tows:

A total of 120, three-minute ichthyoplankton tows were conducted by KDFWR at Mill Creek Plant, Clifty Creek Plant, and Kentucky River sites. Additionally, 108 tows were conducted by INDNR at FB Cully Plant, Anderson River, and Clover Creek sites (Figure 1). A total of 3,008 larval fish were collected, the majority of which were unidentified Asian carp (N = 1407; Silver Carp, Bighead Carp, or Grass Carp), Freshwater Drum (N = 771), Gizzard Shad (N = 386), and unidentified cyprinids (N = 227). In addition, eight unidentified eggs were collected. Asian carp were unable to be identified to species. The total number of larvae and Asian carp "type" larvae generally decreased farther upstream, however the proportion of Asian carp "type" larvae was highest in McAlpine Pool (Table 1). In addition, spikes in

discharge were typically noticed around the same time as increases in Asian carp "type" larvae in the weekly samples (Figure 2).

Surface trawl:

A total of 24 trawl runs were conducted across seven sites in 2018, totaling 1.93 hours of sampling effort. Catch included 213 YOY *Hypophthalmichthys* spp., all of which were captured in either Hovey Lake (N = 70) or Hovey Lake Drain (142) except for one YOY Silver Carp captured in a borrow pit within Newburgh Pool. The majority of YOY fish were captured in late-June during trial runs of the gear at Hovey Lake Drain; individuals during this time ranged from 14 to 36 mm total length and could only be confidently identified to genus.

Electrofishing:

Electrofishing was conducted at 15 sites; 4 sites were sampled in J.T. Myers Pool, 5 in Newburgh Pool, 3 in Cannelton Pool, and 3 in McAlpine Pool for a total of 2.25, 2.50, 2.50, and 2.08 hours of electrofishing per pool, respectively (Figure 1). A total of 9.33 hours of electrofishing effort were expended. All but one site was sampled twice with at least two weeks between sampling dates; six sites were large enough for multiple transects (left bank/right bank, upper/lower).

Young-of-year Silver Carp were only captured in the lower portion of J.T. Myers Pool at Hovey Lake; eight were captured, ranging in length from 47 to 56 mm. Mean YOY CPUE (fish/hour ± SE) in Hovey Lake was 10.6 ± 3.6. Immature Silver Carp were not captured at any location. A total of 118 adult Silver Carp were collected; one Grass Carp and zero Bighead Carp were captured. Overall catch rates of adult fish were highest in Cannelton Pool (18.0 fish/hour) followed by McAlpine (12.5 fish/hour), Newburgh (12 fish/hour), and JT Myers Pools (7.6 fish/hour).

Habitat Parameters:

Significant differences in mean habitat parameters existed between sites where juvenile Asian carp were present to those where they were not. Mean water temperature was greater in sites with juvenile Asian carp (84.0°F) than those without (79.8°F); $t(22) = 3.77$, $p < 0.001$. Secchi visibility was significantly lower in sites where Asian carp were captured (13.5 in) than those without (17.7 in); $t(24) = -2.58$, $p = 0.008$. Similarly, conductivity was lower in sites with Asian carp (382.1 µS) than those without (464.3 µS), $t(27) = -2.73$, $p = 0.006$. Depths were lower in sites with juvenile Asian carp (max depth: 8.6 ft, avg. depth: 5.2 ft) than sites without (max depth: 12.8 ft ± 0.4, avg. depth: 7.8 ft). Finally, pH, dissolved oxygen, and tributary width were similar between habitats containing juvenile carp and those without. Chi-square tests showed no significant differences in juvenile Asian carp occurrences between water colors $\chi^2(6, N = 364) = 6.47$, $p = 0.373$, presence of woody debris $\chi^2(1, N = 367) = 0.433$, $p = 0.101$, or presence of aquatic vegetation $\chi^2(1, N = 363) = 0.566$, $p = 0.452$.

Discussion:

Results of the third year of the Abundance and Distribution of Asian Carp Early Life Stages in the Ohio River project offer the most up to date information on the extent of Asian carp spawning and recruitment in the Ohio River. The collective efforts of targeted electrofishing, surface trawls, and ichthyoplankton tows directly addressed Basin Framework Strategy 2.7 by improving capabilities to detect early stages of invasion and spawning populations of Asian carp. This project continues to provide data to describe our current understanding of the distribution of Asian carp recruitment for the Water Resources Reform and Development Act (WRRDA) reporting. Moreover, knowledge acquired from this project directly informs planning efforts for future Asian carp deterrent, control, and other management strategies.

In 2015, the most upstream location where verified Asian carp eggs and larvae were detected was river mile 560 in McAlpine Pool, and extended to river mile 405.7 in Meldahl Pool the following year (EA Engineering, personal communication). These eggs and larvae were identified as *Hypophthalmichthys* spp., so it is unclear whether Bighead and/or Silver Carp have spawned in these pools in the past. Spawning of Silver Carp has been confirmed in Cannelton Pool with the collection of yolk-sac larvae at river mile 625.8 by EA Engineering in 2015 and 2016 as well. With the incorporation of a more thorough ichthyoplankton sampling design to this project in 2018, we hoped to provide the most up-to-date delineation of the extent of Asian carp spawning within the Ohio River. However, due to unforeseen circumstances, the six most upstream sites were unable to be sampled via ichthyoplankton tows. Based off of previous larval data, the upper extent of spawning was presumed to occur somewhere across the gradient of these upstream sampling sites (from RM 497 to RM 260). Therefore it is paramount that these sites be sampled in the near future to accurately define the extent of Asian carp spawning in the Ohio River to inform ongoing projects and prospective management strategies.

Between 37.8% to 69.4% of all larvae captured at six sites in 2018 were identified as Asian carp "type" larvae. The fish were only able to be confidently identified to the family level, meaning that there is a possibility that they could be either Grass Carp, Bighead Carp, or Silver Carp (Black Carp would also be in the same family but hasn't been found in those pools of the Ohio River). We suspect that many of these Asian carp "type" larvae are in fact Silver or Bighead Carp due to these sites being downstream of the areas where *Hypophthalmichthys* spp. were collected in 2015 and 2016. Interestingly, rising water levels were correlated with peaks in Asian carp larvae collected, highlighting the prolonged spawning season of these fish. Throughout the extent of sampling, water temperatures ranged between 68.6 and 85.1 for the Ohio River, which suggests larvae will hatch approximately 20 to 35 hours after a spawning event (Murphy and Jackson 2013). Using this knowledge, managers should target larval sampling during increased flows following a rain event to more efficiently capture Asian carp and further pinpoint specific spawning locations.

As recommended in the 2017 technical report and to address Strategy 2.3 of the basin framework, 2018 sampling was conducted to monitor the extent of Asian carp spawning and recruitment across years and environmental conditions. Results of 2018 sampling largely support the extent of recruitment we defined in 2016 and 2017, with the majority of juvenile carp again collected in the lower portion of J.T. Myers Pool. This pattern of recruitment in J.T. Myers Pool has been consistent annually, and highlights the need for more-extensive larval sampling to identify timing and location(s) of spawning. The collection of several juvenile Asian carp (269-399mm TL) in Cannelton Pool during multiple 2017 Basin Framework projects (Early Life Stages, Monitoring, Removal) suggests the extent of recruitment to be above Cannelton Lock and Dam. Although no juvenile Asian carp were captured in or upstream of Cannelton Pool during 2018 Ohio River Basin Framework projects, it appears that Cannelton Pool provides several areas suitable for Asian carp recruitment and therefore should still be considered the upstream extent of recruitment. There has not been a strong spawning event or year-class since this project was initiated in 2016. Based on the apparent Asian carp spawning in McAlpine Pool, as highlighted by ichthyoplankton data, a highly successful spawning event could quickly shift the current known extent of recruitment to pools farther upstream. Therefore, the spatial and temporal variation in Asian carp recruitment in the Ohio River emphasizes the need for continued long-term monitoring with this project as well as others within the basin.

The additional utilization of a surface trawl demonstrated its value as an efficient tool for sampling juvenile Asian carp. The surface trawl effectively samples smaller size Asian carp (as small as 14 mm) which allows managers to begin targeted sampling earlier in the season. The small size of trawl allows maneuverability in many of the smaller tributaries of the Ohio River. A multi-gear approach will provide a more accurate picture of Asian carp spawning and recruitment in the Ohio River.

As in previous years, the evaluation of abiotic habitat parameters showed juvenile carp were found in habitats with significantly greater water temperature, lower depth, lower secchi visibility, and lower conductivity. This suggests shallow, turbid, and potentially more productive habitats promote survival and recruitment of Asian carp. Additionally, we observed no significant effects of water color, presence of woody debris, or presence of aquatic vegetation. Future sampling may benefit by sampling these variables quantitatively to reduce subjectivity. Although we were limited by a small sample size and suitable analyses for this dataset, this information will be used to help guide future sampling and management efforts.

Efforts in this project provide valuable insight into factors that promote the reproduction and recruitment of Asian carp, and ultimately range expansion. Results support several Basin Framework and National Plan strategies and will be used by biologists to mitigate the spread of these invasive fishes. In addition to this project, INDNR biologists aided KDFWR with the "Monitoring and Response to Asian carp in the Ohio River", and "Control and Removal of Asian carp in the Ohio River" projects.

Recommendation:

While the extent of Asian carp recruitment has been relatively stable, we suspect recruitment within Cannelton Pool might be occurring more often than our limited sampling is detecting. Therefore, we suggest electrofishing and surface trawling efforts should be consolidated to focus on determining the recruitment potential of Cannelton Pool. A more thorough, multi-gear approach in Cannelton Pool sites where juveniles have been captured or where abiotic factors may promote recruitment is needed. This will allow us to continue to monitor recruitment, by not spending so much time downstream in areas that recruitment appears to be consistent and stable (JT Myers Pool).

As our ichthyoplankton sampling was limited in 2018, there is still a lack of information of the true extent of spawning in the Ohio River. We recommend completing weekly ichthyoplankton tows in 2019 at the six upstream sites that were missed in 2018 to determine the extent of spawning. Also, we recommend conducting targeted ichthyoplankton tows following rain events (and subsequent river rises) in tributaries of Newburgh, Cannelton, and McAlpine Pools where Asian carp spawning is suspected to begin identifying specific spawning locations. In addition to these effort, we highly recommend that techniques are utilized to confidently identify larval fish to at least the genus level to rule out the possibility of Grass Carp falsely inflating our estimated extent of spawning *Hypophthalmichthys* species. We recommend working with Whitney Genetics Lab to utilize eDNA and other techniques to confirm the presence of *Hypophthalmichthys* spp. larvae in ichthyoplankton samples. In addition, we suggest field staff from various agencies be trained on larval fish identification to aid in the processing of samples.

Other ongoing projects in the Ohio River basin are gathering data on presence of spawning patches on Asian carp; combining these data with information gathered through this project will help managers identify spatiotemporal patterns of Asian carp reproduction in the Ohio River. This information, along with recruitment patterns we have documented previously, can ultimately be used to identify sources of Asian carp population expansion throughout the basin, and help guide other ORFMT efforts such as deterrents and targeted removals.

Literature Cited:

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Table 1. Number of total larvae and Asian carp "type" larvae collected at each Ohio River sampling site from 5/21/18 to 7/23/18.

Sample Site	River Mile	Larvae Sampled (N)	AC-type Larvae (N)	% AC-type
FB Cully Plant	773	884	334	37.8%
Anderson River	731	560	258	46.1%
Clover Creek	711	522	266	51.0%
Mill Creek Plant	626	684	311	45.5%
Clifty Creek Plant	560	193	134	69.4%
Kentucky River	546	165	104	63.0%

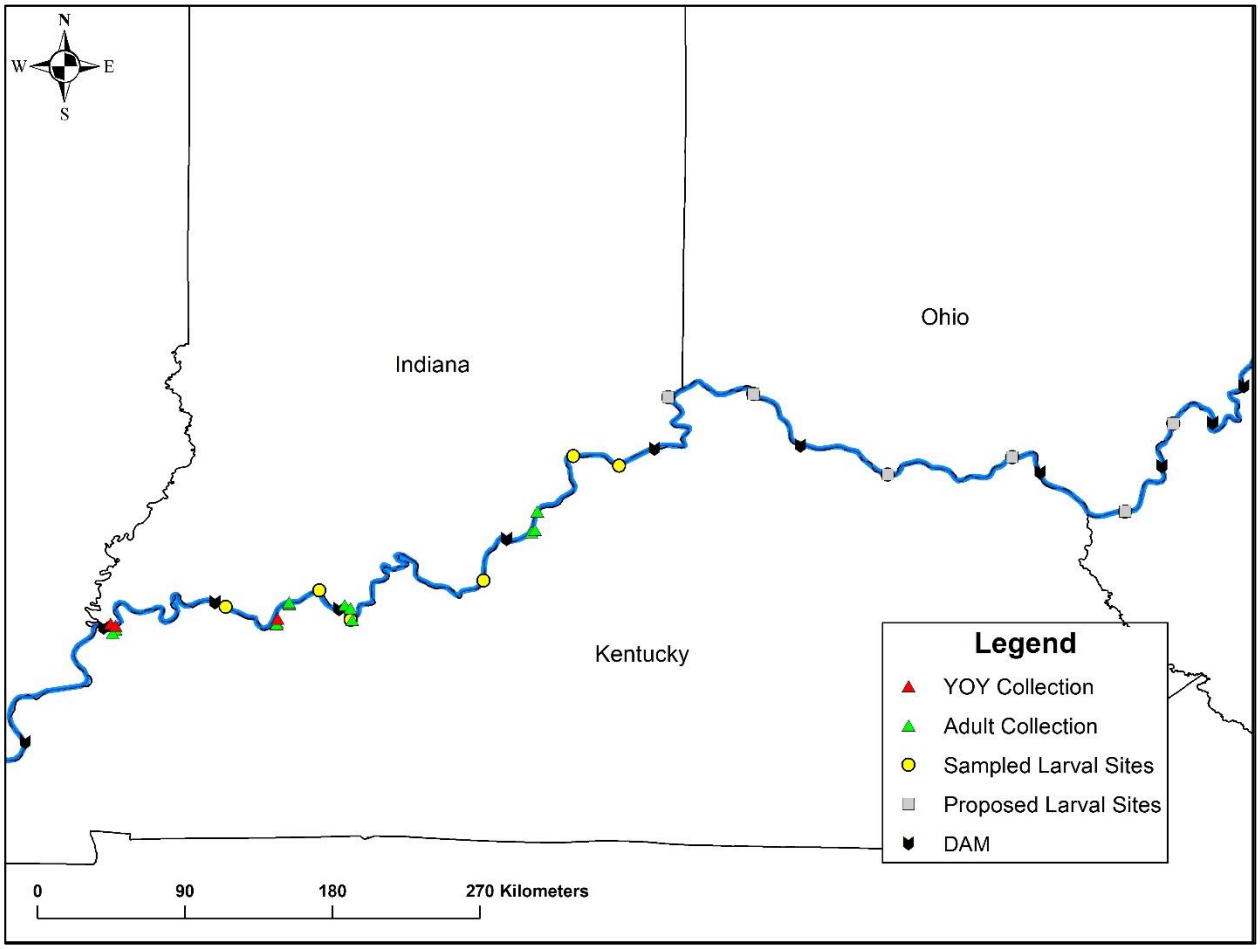

Figure 1. Map of electrofishing, trawling, and larval sites among eight pools of the Ohio River (J.T. Myers, Newburgh, Cannelton, McAlpine, Markland, Meldahl, Greenup, RC Byrd). Red triangles = young-of-year Asian carp collection sites, green triangles = adult only Asian carp collection sites, yellow circles = larval sites that were sampled, grey squares = proposed larval sites that were unable to be sampled.

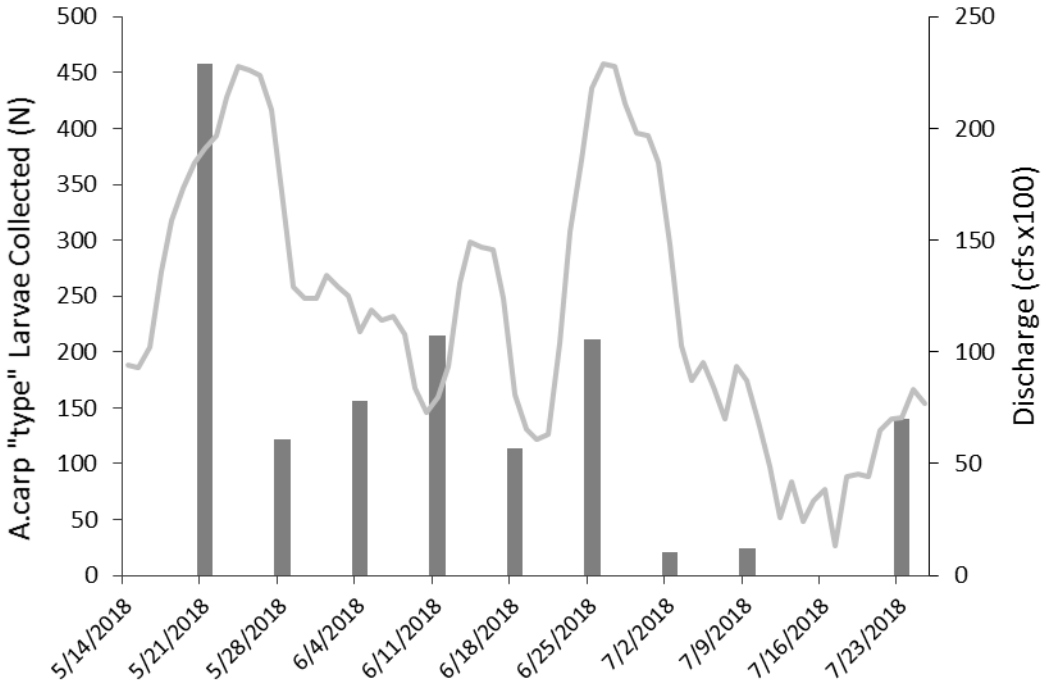

Figure 2. Total number of Asian carp "type" larvae collected weekly at six downstream sites (RM 546 to RM 773) overlaid with mean daily discharge at Cannelton L&D during the sampling timeframe.