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Veligers of the invasive Asian clam *Corbicula fluminea* in the Columbia River Basin: broadscale distribution, abundance, and ecological associations

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**ABSTRACT**


The invasive Asian clam *Corbicula fluminea* was introduced to North America in the 1930s and now inhabits most regions of the conterminous United States; however, the distribution and ecology of *C. fluminea* in the Columbia River Basin is poorly understood. During 2013 and 2014, 5 Columbia-Snake River reservoirs were sampled monthly from May through September, along with 23 additional lakes and reservoirs sampled once each summer. Associations among *C. fluminea* veligers, other components of the plankton, and environmental variables were analyzed using non-metric multidimensional scaling and canonical correspondence analysis. *Corbicula fluminea* veligers were found in high abundances in all mainstem Columbia-Snake River reservoirs, with an annual mean abundance of 71.2 individuals per cubic meter (inds./m\(^3\)). Only 3 of 23 lakes and (non-mainstem) reservoirs contained *C. fluminea*, with abundances considerably lower (maximum = 21.2 inds./m\(^3\)) than in the mainstem reservoirs. A diatom-dominated community preceded the spawning of *C. fluminea* in early summer at all sites. *Corbicula fluminea* veligers characterized the plankton community in late summer and were associated with cyanobacteria and high water temperatures. A third community, characterized by cyanobacteria, was apparent in non-mainstem sites in July and August. Our analyses describe the relationship of *C. fluminea* to the plankton community and environment, which contributes to our understanding of the possible effects of *C. fluminea* infestations and which waterbodies in the Columbia River Basin are at risk for infestation. Understanding the effects and environmental determinants of invasive mollusks will be increasingly important in the future with the possible arrival of zebra (*Dreissenia polymorpha*) or quagga (*D. bugensis*) mussels to the region.

**KEYWORDS**

*Corbicula fluminea*, FlowCam; invasive species; nonindigenous bivalves; planktonic larvae

Biological invaders are one of the most influential drivers in the loss of biodiversity across the globe (Sodhi et al. 2009). This invasion is cause for concern in freshwater environments where current and future extinction rates estimates are 5 times that of terrestrial environments (Ricciardi and Rasmussen 1999). In freshwater, mollusks are disproportionally successful as aquatic invaders (Karatayev et al. 2009, Strayer 2010), largely affecting ecosystems through their consumption of primary producers and consequently disrupting broader food web dynamics and nutrient cycling (Strayer 2009, 2010). Although much attention has been given to the zebra (*Dreissenia polymorpha*) and quagga (*D. bugensis*) mussel invasions that have transformed freshwater ecosystems in the Laurentian Great Lakes region (Higgins and Zanden 2010), the Asian clam (*Corbicula fluminea*) is another highly successful freshwater invader. *C. fluminea* has altered the structure and function of freshwater ecosystems across much of the United States (Karatayev et al. 2007, Sousa et al. 2008a) and has resulted in high economic costs for anti-fouling efforts (Pimentel et al. 2005).

Native to southeast Asia, Australia, and Africa, the first documented occurrence of *C. fluminea* in North America was in the Columbia River in 1938 (Counts 1981). *C. fluminea* now seems to be well-established in the Columbia River Basin (Haertel and Osterberg 1967, McCabe et al. 1993, Dexter et al. 2015) with a low likelihood of eradication. Within 40 years, *C. fluminea* had extended its range to the Atlantic coast of the United...
States. (Sousa et al. 2008a). *C. fluminea* is characteristic of bivalves found in Asia and Africa in that they are particularly successful filter-feeders capable of attaining high population densities (Karatayev et al. 2007). Along with other filter-feeders (i.e., zebra and quagga mussels), *C. fluminea* has successfully spread to North America through anthropogenic activity (e.g., ship ballast water, international trade; Sousa et al. 2014). The introduction of *C. fluminea* to aquatic ecosystems in North America has resulted in novel communities of mollusk species (Karatayev et al. 2003).

*C. fluminea*’s life history traits, including early maturity, high growth rate, and fecundity (Sousa et al. 2008b, Kamburska et al. 2013), in combination with human-mediated dispersal have contributed to its invasive success on a global scale. Many species of corbiculids, including *C. fluminea*, have a rare reproductive mode allowing them to self-fertilize, or reproduce asexually (Ishibashi et al. 2003, Pigneure et al. 2012). Known as androgenesis, this ability has potentially facilitated their invasive success shown by the presence of androgenetic lineages of *Corbicula* spp. globally, whereas sexual lineages have remained limited to their native ranges (Pigneure et al. 2012).

Ecologically and economically, *C. fluminea* is regarded as a serious threat to freshwater ecosystems and manmade infrastructure. Numerous studies have shown that *C. fluminea* dominates the benthic communities it invades (McMahon 1999, Karatayev et al. 2003). Most notably, *C. fluminea*’s high filtering rates reduce phytoplankton abundance (Cohen et al. 1984, Vaughn and Hakenkamp 2001, Kamburska et al. 2013), ultimately transforming food webs and nutrient cycling within the ecosystem (Vaughn and Hakenkamp 2001, Sousa et al. 2008b). Economically, *C. fluminea* is a major biofouler of underwater infrastructure, infiltrating power stations, irrigation pipelines, channels, and canals. Planktonic larvae (veligers) and juveniles are carried into water intake pipes, where they settle, mature, and reproduce *in situ*, reaching densities of >20,000 inds./m³ (McMahon 1999, Lucy et al. 2012).

Despite their initial introduction to the West Coast of the United States, the extent of *C. fluminea*’s range in the Pacific Northwest region is poorly documented in the literature. The Columbia River is the largest river within the Pacific Northwest and has exceptional economic and ecological value, providing 77% of the electricity demand for the basin through hydropower, along with irrigation, navigation, flood control, recreation, and fish and wildlife habitat (Hamlet et al. 2002). The Columbia River is heavily managed through a series of dams, which has resulted in the conversion of a high flow environment to a series of controlled flow reservoir impoundments. These managed reservoirs can serve as invasion hubs, facilitating the spread of freshwater invasive species (Johnson et al. 2008, Rahel and Olden 2008). This increase in standing water can serve as an environment conducive to the reproduction of invasive bivalves, potentially dispersing and sustaining populations downstream (Allen and Ramcharan 2001, Havel et al. 2005). To date, high abundances of *C. fluminea* have been noted in the Columbia River Estuary, including in fish diet studies and in a study of the benthic community in navigation channels of the lower Columbia River, which found *C. fluminea* to be one of the 3 most common invertebrates. Although several studies have provided some documentation of *C. fluminea*’s distribution in the lower Columbia River (Haertel and Osterberg 1967, McCabe et al. 1993, 1997, Bottom et al. 2005, Haskell et al. 2013, Dexter et al. 2015), a comprehensive analysis has yet to be undertaken to characterize the current range of veligers of *C. fluminea* in the Columbia River Basin more broadly.

This study had 3 main objectives: (1) characterize the distribution and abundance of *C. fluminea* veligers within selected lakes and reservoirs in the Columbia River Basin; (2) identify potential ecological associations of *C. fluminea* veligers within the plankton community; and (3) identify abiotic environmental variables that are associated with populations of *C. fluminea* veligers.

**Materials and methods**

The study area spanned the western half of the Columbia River Basin (Fig. 1), a hydroelectrically developed and heavily managed river system located in the Pacific Northwest region of the United States. Sample collection occurred at multiple sites within 5 major reservoirs in the Columbia-Snake River system (Bonneville, The Dalles, John Day, McNary, and Ice Harbor reservoirs; Table 1), here referred to as “mainstem” sites, and 23 additional lakes and reservoirs throughout Oregon and Washington states, here referred to as “non-mainstem” sites (Fig. 1). All sampling locations (mainstem and non-mainstem)
were selected as part of an early detection monitoring program (presence/absence) for zebra and quagga mussels, and these samples were subsequently used for the current analysis of *C. fluminea*. Furthermore, all non-mainstem lakes and reservoirs were identified as being at high risk for the introduction or establishment of quagga and zebra mussels (T. Counihan, US Geological Survey, Research Fishery Biologist, Dec. 2015, unpubl.) and were not previously monitored for the presence of these invasive mussel species. The 5 mainstem sites were intensively sampled monthly from late spring to early fall, whereas non-mainstem sites were sampled once annually during the summer.

**Mainstem sample collection**

In 2013 and 2014, samples were collected monthly from May through September, a period when *C. fluminea* veliger density is at its annual maximum in the Columbia River Estuary (Dexter et al. 2015) and the likelihood of detection is greatest. All mainstem reservoir sites were located 5 to 42 km upstream of their respective dams: Bonneville and John Day reservoir sites were located 42 and 41 km upstream, respectively; The Dalles and McNary reservoir sites were located 20 km upstream; and Ice Harbor reservoir sites were located 5 km upstream of each dam. A minimum of 2 shallow (<3 m) and 2 deep water (>3 m) sites were sampled within each reservoir. Shallow water sites were located within or close to tributaries, inlets, docks, marinas, and boat ramps. Deep water sites were located along a transect perpendicular to shore, crossing the main channel. We collected 113 samples (94 mainstem, 19 non-mainstem) in 2013 and 126 samples (103 mainstem, 23 non-mainstem) in 2014.

**Table 1.** Characteristics of mainstem Columbia–Snake River system reservoirs. Capacity was retrieved from the National Inventory of Dams, and length and distance from the Columbia River mouth were retrieved from the US Army Corps of Engineers. Retention time was calculated using data from the Columbia Basin Fish Passage Center and the US Army Corps of Engineers.

<table>
<thead>
<tr>
<th>Reservoir River</th>
<th>Bonneville Columbia</th>
<th>The Dalles Columbia</th>
<th>John Day Columbia</th>
<th>McNary Columbia</th>
<th>Ice Harbor Snake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance upstream from Columbia River mouth (km)</td>
<td>233</td>
<td>305</td>
<td>347</td>
<td>469</td>
<td>763</td>
</tr>
<tr>
<td>Capacity (km³)</td>
<td>0.662</td>
<td>0.407</td>
<td>3.12</td>
<td>1.67</td>
<td>0.501</td>
</tr>
<tr>
<td>Length (km)</td>
<td>77</td>
<td>39</td>
<td>122</td>
<td>102</td>
<td>51</td>
</tr>
<tr>
<td>Retention Time (d)</td>
<td>1.6</td>
<td>1</td>
<td>7.7</td>
<td>4.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Mean <em>C. fluminea</em> veliger density (inds./m³ ± SE)</td>
<td>40.9 ± 0.9</td>
<td>246 ± 46</td>
<td>6.61 ± 0.61</td>
<td>63.6 ± 3.6</td>
<td>1.62 ± 0.62</td>
</tr>
</tbody>
</table>
Vertical plankton tows were collected from a 7 m boat using a 0.3 m diameter, 64 µm mesh conical plankton net from 1 m off the reservoir bottom to the surface to avoid collection of benthic organisms and sediment. All samples were preserved in the field in 70% ethanol buffered with sodium bicarbonate (0.2 grams sodium bicarbonate per 100 mL of sample) and transported to the Washington State University Aquatic Ecology Laboratory in Vancouver, Washington, for analysis. Environmental variables (temperature, dissolved oxygen, depth, and conductivity) were measured using a YSI 6920 multiparameter sonde. Daily river discharge data for each reservoir were obtained from the Fish Passage Center (http://www.fpc.org/river_home.html). Total river discharge was calculated as the mean monthly discharge corresponding to the sample collection month. Retention time for each reservoir was estimated by dividing total-reservoir capacity by daily total-river discharge.

**Non-mainstem sample collection**

Samples from 19 non-mainstem lakes and reservoirs were collected once during maximum *C. fluminea* reproductive times (Jul or Aug) in 2013 and 2014 (Fig. 1). In 2014, samples were collected from 4 additional non-mainstem lakes and reservoirs: Lake Billy Chinook, Ochoco Reservoir, Paulina Lake, and East Lake. Horizontal surface plankton tows were collected from piers and docks using a 0.3 m diameter, 64 µm mesh conical plankton net. All samples were preserved in the field in 70% ethanol buffered with sodium bicarbonate (0.2 grams sodium bicarbonate per 100 mL of sample) and transported to the Washington State University Aquatic Ecology Laboratory for analysis. Environmental variables (temperature, dissolved oxygen, depth, and conductivity) were measured using a YSI 6920 multiparameter sonde.

**Corbicula fluminea veliger abundance**

All field samples from 2013 and 2014 were analyzed for *C. fluminea* veliger abundance using microscopy, a 1 mL subsample was removed with a Stempel pipette and analyzed for other components of the plankton using a Bench Top VS model FlowCam (Fluid Imaging Technologies, Scarborough, ME) fitted with a 4× objective lens and a 300 µm flow cell. The FlowCam uses a combination of a flow cytometer and microscope to magnify and capture an image of particles (e.g., planktonic organism) in moving fluid. Images can be sorted and classified using Visual Spreadsheet software (Fluid Imaging Technologies). Useful applications of this instrument in plankton studies have included measuring plankton density and size structure (See et al. 2005, Buskey and Hyatt 2006, Álvarez et al. 2012, Le Bourgeois et al. 2015, Wang et al. 2015), and the FlowCam’s high image rate can result in greater sample processing efficiency than traditional microscopy (Wang et al. 2015). The FlowCam was operated in AutoImage mode, which captures images of the fluid sample at regular intervals. To ensure particles (plankton, suspended sediment and debris) would not clog the 300 µm flow cell, samples were filtered through a 280 µm mesh filter before processing. To manage high particle densities, all mainstem samples were diluted to 20 mL with deionized water (DI), and a 1 mL subsample was extracted. Each 1 mL subsample (mainstem and non-mainstem) was then diluted with 5 mL DI water, and 2 mL of glycerin was added to aid in particle separation. Diluted samples were introduced to the FlowCam pipette, and analysis was stopped when the sample had completely passed through the flow cell.

Based on the resolution capacities of the FlowCam, 18 different plankton groups were created to sort the plankton into the lowest possible order, ranging from genus to phylum level. Using Visual Spreadsheet software, statistical object recognition filters were then developed from a library of images collected from Columbia River plankton. These filters, when applied to the resulting image files, sorted the images into the 18 different plankton groups. The filters successfully classified images in approximately half of the cases. All of the automated classifications were counted by the subsample volume and multiplying by the plankton tow volume.

**Plankton community analysis**

After all the 2014 field samples (mainstem and non-mainstem) were analyzed for *C. fluminea* abundance using microscopy, a 1 mL subsample was removed with a Stempel pipette and analyzed for other components of the plankton using a Bench Top VS model FlowCam (Fluid Imaging Technologies, Scarborough, ME) fitted with a 4× objective lens and a 300 µm flow cell. The FlowCam uses a combination of a flow cytometer and microscope to magnify and capture an image of particles (e.g., planktonic organism) in moving fluid. Images can be sorted and classified using Visual Spreadsheet software (Fluid Imaging Technologies). Useful applications of this instrument in plankton studies have included measuring plankton density and size structure (See et al. 2005, Buskey and Hyatt 2006, Álvarez et al. 2012, Le Bourgeois et al. 2015, Wang et al. 2015), and the FlowCam’s high image rate can result in greater sample processing efficiency than traditional microscopy (Wang et al. 2015). The FlowCam was operated in AutoImage mode, which captures images of the fluid sample at regular intervals. To ensure particles (plankton, suspended sediment and debris) would not clog the 300 µm flow cell, samples were filtered through a 280 µm mesh filter before processing. To manage high particle densities, all mainstem samples were diluted to 20 mL with deionized water (DI), and a 1 mL subsample was extracted. Each 1 mL subsample (mainstem and non-mainstem) was then diluted with 5 mL DI water, and 2 mL of glycerin was added to aid in particle separation. Diluted samples were introduced to the FlowCam pipette, and analysis was stopped when the sample had completely passed through the flow cell.

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manually reviewed for accuracy and reclassified if necessary using Smith (2001) and Wehr and Sheath (2003). The remaining images not recognized by the filters were manually sorted and classified. Because the FlowCam images any object that passes the field of view, colony-forming taxa were enumerated by colony rather than by individual. Taxa enumerated by colony included diatoms (Fragilaria, Asterionella, Synedra, and “other diatoms”), cyanobacteria (Anabaena [now Dolichospermum] and “other cyanobacteria”), Dino-

bryon, chlorophytes, Pediasstrum, and Spirogyra. Taxa enumerated by individual included Cyclorella diatoms, dinoflagellates, nauplii, ciliates, gastropods, cladocer-

ans, Keratella, and “other rotifers.”

Statistical analyses

Differences in C. fluminea veliger abundance among the 5 mainstem reservoirs during the 2013–2014 sample period were evaluated using Kruskal–Wallis analysis. A Dunn’s multiple comparison post-hoc analysis was conducted on significant Kruskal–Wallis results. Differences in veliger abundance between shallow and deep water sites in mainstem reservoirs during the 2013–2014 sample period were evaluated using a Mann–Whitney U test. Nonparametric tests were chosen based on the data not meeting the assumption of homogeneity of variances required of parametric tests. Finally, to evaluate if veliger abundance was possibly a function of reservoir retention time within the mainstem reservoirs, correlation between retention time and veliger abundance was evaluated in mainstem sites during the 2013–2014 sample period using linear regression analysis. We predicted a positive relationship between residence time and C. fluminea veliger abundance because reservoirs are thought to facilitate invasive species generally (Havel et al. 2005), and high residence times can act as chemostats or batch reactors to allow planktonic organisms to accumulate. All univariate tests were performed in SigmaStat 3.5 (Systat Software Inc., San Jose, CA).

Non-metric multidimensional scaling (NMS; Kruskal and Wish 1978, McCune and Grace 2002) was used to determine associations of C. fluminea veligers, environmental variables, and plankton community groups for all 2014 field samples. NMS was chosen over other ordination methods as a means to identify broad patterns because limited knowledge is available on the types of relationships that could exist between C. fluminea veligers and the biotic and abiotic environment. Once these broad patterns were identified, an additional ordination technique, canonical correspondence analysis (CCA; Braak 1986), was used to quantitatively evaluate the strength of significant associations between environmental variables and plankton community structure for all 2014 field samples. Environmental variables above the vector cutoff value of $r^2 = 0.3$ were included in the NMS and CCA ordination plots.

Before running the NMS and CCA ordinations, rare species (present in <5% of the samples) were removed, and species data were log-transformed because of a high degree of variation among plankton abundances (McCune and Grace 2002). To identify natural groupings within the plankton community, community groups were determined through hierarchical clustering using a relative Euclidean distance measure. The strength of these groupings was evaluated using multi-

response permutation procedures, a nonparametric method used to determine differences between groups (Biondini et al. 1985). Species that best described each community grouping were identified using indicator species analysis, and significant association values for each species were calculated using a Monte Carlo test of significance (Dufrene and Legendre 1997). The NMS ordination used the relative Euclidean distance measure, and after multiple iterations, the ordination with the lowest stress value was chosen. All multivariate analyses were performed in PCORD 5.10 (MjM Software Design; Glenden Beach, OR).

Results

Broadscale distribution and abundance of C. fluminea veligers

C. fluminea veligers were found in all mainstem reservoirs in 2013 and 2014 with maximum densities reaching 1780 inds./m$^3$ (Fig. 2). Veligers were either absent or in low abundance in spring (May–Jun) and peaked in all reservoirs in July. Lower abundances were observed in August and September, suggesting a single reproductive period from May to September (Fig. 3). Significant differences in veliger abundance among reservoirs were observed during the sampling period. During the 2013–2014 sampling period, the Dalles, Bonneville, and McNary reservoirs had significantly higher veliger abundances than Ice Harbor Reservoir (Kruskall–Wallis, df = 4, P-value < 0.05). Mean veliger abundances plus or minus one standard
Figure 2. Spatial distribution of *C. fluminea* veligers during months of maximum abundance in July/August of 2013 (top panel) and 2014 (bottom panel).

Error (SE) during the 2013–2014 sampling period of *C. fluminea* in each mainstem reservoir were as follows: The Dalles had the highest mean abundance of *C. fluminea* veligers (246 ± 138 ind.s./m$^3$), followed by McNary (63.6 ± 36.2 ind.s./m$^3$), Bonneville (40.9 ± 29.0 ind.s./m$^3$), John Day (6.61 ± 4.24 ind.s./m$^3$), and Ice Harbor reservoirs (1.62 ± 1.17 ind.s./m$^3$; Table 1). In mainstem sites where plankton samples were collected at both shallow and deep water stations, *C. fluminea* veligers were more abundant in deep water stations (Mann–Whitney, $U = 7249$, $P$-value < 0.001). Mean *C. fluminea* veliger abundances in these deep water stations during the 2013–2014 sampling period were 120.3 ± 29.1 ind.s./m$^3$, versus 2.52 ± 0.63 ind.s./m$^3$ for shallow water stations. Mainstem reservoir retention time during the 2013–2014 sampling period was not significantly correlated with *C. fluminea* abundance ($P$-value = 0.57, $F$-ratio = 3.88).

In non-mainstem sites from 2013 to 2014, *C. fluminea* veligers were detected in 3 of the 22 lakes and reservoirs sampled, with abundances considerably lower (mean = 0.966 ind.s./m$^3$) than in the mainstem reservoirs (Fig. 2). In 2013, non-mainstem sites containing *C. fluminea* veligers were Fern Ridge (max = 16.8 ind.s./m$^3$) and Wannacot (max = 21.2 ind.s./m$^3$) reservoirs. In 2014, however, veligers were not found in Fern Ridge or Wannacot reservoirs. The only non-mainstem reservoir that contained *C. fluminea* veligers in 2014 was Lake Billy Chinook (max = 3.1 ind.s./m$^3$), but this lake was not sampled in 2013.

**Plankton classifications**

The plankton in all field samples collected in 2014 were classified into 18 different taxonomic groups, sorted to the lowest taxonomic group possible given the
resolution limitations of the FlowCam (Table 2). The most abundant taxonomic group was “other diatoms,” which consisted primarily of centric diatoms and comprised 67.2% of the total plankton identified (Table 2). Fragilaria and Asterionella, also diatom genera, comprised 14.3% and 12.0% of the total plankton, respectively. The cyanobacteria genus Anabaena was the fourth most abundant group, comprising 2.6% of the total plankton abundance (Table 2.)

Cluster and indicator species analyses

For all sites combined in 2014, 3 distinct community groupings were identified, here referred to as Clusters A, B, and C (Fig. 4). Indicator species analysis revealed Cluster A was characterized primarily by diatoms (Asterionella, Synedra, Fragilaria, Cyclotella, and “other diatoms”; Table 3). Cluster B was strongly characterized by C. fluminea veligers, which were present exclusively in this group and Pediastrum (Table 3). Cluster C was dominated by cyanobacteria (Anabaena and “other cyanobacteria”; Table 3). Additional significant indicators of Cluster C included gastropod larvae and the chlorophyte Spirogyra.

When looking exclusively at samples from mainstem sites in 2014 (n = 103), hierarchical cluster analysis identified 2 community groupings, hereafter referred to as Clusters 1 and 2 (Fig. 5). Indicator species analysis revealed Cluster 1 was best described by diatoms, which included Asterionella, Synedra, Fragilaria, Cyclotella, and other diatoms. Chrysophytes (Dinobryon) and ciliates were also significant indicators of this cluster (Table 4). Cluster 2 was characterized by C. fluminea veligers, which occurred in this cluster exclusively and were the strongest indicator of this community. Anabaena (cyanobacteria)

Table 2. List of plankton classifications, ranked by relative abundance for all sites combined in 2014. Taxa found in <5% of sites were omitted from statistical analyses.

<table>
<thead>
<tr>
<th>Plankton group</th>
<th>Mean abundance/mL</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other diatoms</td>
<td>12.24</td>
<td>67.17</td>
</tr>
<tr>
<td>Fragilaria</td>
<td>2.601</td>
<td>14.28</td>
</tr>
<tr>
<td>Asterionella</td>
<td>2.178</td>
<td>11.96</td>
</tr>
<tr>
<td>Anabaena</td>
<td>0.482</td>
<td>2.64</td>
</tr>
<tr>
<td>Synedra</td>
<td>0.142</td>
<td>0.78</td>
</tr>
<tr>
<td>Dinobryon</td>
<td>0.126</td>
<td>0.69</td>
</tr>
<tr>
<td>Cyclotella</td>
<td>0.097</td>
<td>0.53</td>
</tr>
<tr>
<td>“Other” Rotifers</td>
<td>0.073</td>
<td>0.40</td>
</tr>
<tr>
<td>Chlorophytes</td>
<td>0.069</td>
<td>0.38</td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>0.055</td>
<td>0.30</td>
</tr>
<tr>
<td>Keratella</td>
<td>0.050</td>
<td>0.27</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>0.040</td>
<td>0.22</td>
</tr>
<tr>
<td>Pediastrum</td>
<td>0.025</td>
<td>0.14</td>
</tr>
<tr>
<td>Nauplii</td>
<td>0.019</td>
<td>0.10</td>
</tr>
<tr>
<td>Spirogyra</td>
<td>0.006</td>
<td>0.03</td>
</tr>
<tr>
<td>Ciliate</td>
<td>0.006</td>
<td>0.03</td>
</tr>
<tr>
<td>Gastropod</td>
<td>0.003</td>
<td>0.02</td>
</tr>
<tr>
<td>Cladocerans</td>
<td>0.002</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 3. Indicator values for significant taxa, associated cluster, and Monte Carlo P-values for all sites combined (mainstem and non-mainstem) in 2014.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Plankton group</th>
<th>Indicator value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Other diatoms</td>
<td>40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fragilaria</td>
<td>42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Asterionella</td>
<td>43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Synedra</td>
<td>38</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Dinobryon</td>
<td>28</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Cyclotella</td>
<td>40</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Ciliates</td>
<td>15</td>
<td>0.014</td>
</tr>
<tr>
<td>B</td>
<td>C. fluminea</td>
<td>100</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pediastrum</td>
<td>32</td>
<td>0.012</td>
</tr>
<tr>
<td>C</td>
<td>Anabaena</td>
<td>65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Chlorophytes</td>
<td>42</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Cyanobacteria</td>
<td>46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Nauplii</td>
<td>25</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>Spirogyra</td>
<td>31</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Gastropods</td>
<td>39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Cladocerans</td>
<td>19</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Note. Strong indicators are represented in bold (indicator values 5 times that of any other cluster).
Figure 4. Non-metric multidimensional scaling (NMS) ordination of plankton samples from all samples collected in 2014 (mainstem and non-mainstem sites), coded by community group (above) and month of year (below). Cluster A was characterized by diatoms, Cluster B was characterized by *C. fluminea* veligers, and Cluster C was dominated by cyanobacteria. Vector cutoff is $r^2 = 0.3$. Stress = 12.95. The 3-axis solution explains 89% of the variation between samples.

was the only other significant indicator of Cluster 2. This *C. fluminea*-associated community typically occurred during periods of higher temperature and conductivity.

Figure 5. NMS ordination of plankton samples from mainstem sites in 2014, coded by community group (above) and month of year (below). Cluster 1 was characterized by diatoms and Cluster 2 was characterized by *C. fluminea* veligers. Vector cutoff is $r^2 = 0.3$. Stress = 17.38. The 2-axis solution explains 87.3% of the variation between samples.

**Multidimensional analysis of plankton communities and environmental variables**

For all sites combined in 2014, a 3-axis NMS ordination was produced with a final stress value of 12.95 (Fig. 4). According to McCune and Grace (2002), stress values in the 10–20 range can be considered acceptable and are commonly encountered in ecological data.
Table 4. Indicator values for significant taxa, associated cluster, and Monte Carlo P-values for mainstem sites only in 2014.

<table>
<thead>
<tr>
<th>Mainstem sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Note. Strong indicators are represented in bold (indicator values 5 times that of any other cluster).

When samples were grouped by month, seasonal variation was apparent between clusters (Fig. 4). Cluster A, dominated by diatoms, occurred most frequently from May through July (Fig. 4); Cluster B, dominated by C. fluminea veligers, typically occurred from July through September (Fig. 4); and Cluster C, dominated by cyanobacteria, also occurred in July and August. Note, however, that all sites in Cluster C consisted of non-mainstem sites and were sampled once per year in July or August (Fig. 4).

When considering mainstem sites only in 2014, a 2-axis NMS ordination was produced with a final stress value of 17.38 (Fig. 5). Cluster 1, characterized by diatoms, typically occurred from May through July (Fig. 5), and Cluster 2, characterized by C. fluminea, was most observed from July through September. For the evaluation of correlations between plankton community structure and abiotic environmental variables, CCA ordination of mainstem sites revealed that 2 canonical variables significantly correlated with Axis 1: water temperature and total river discharge (Fig. 6). These variables shared a negative relationship; temperature was positively associated with Axis 1, whereas total-river discharge was negatively associated with Axis 1. C. fluminea and the cyanobacteria genus Anabaena, both significant indicators of the C. fluminea-associated cluster (2), occurred high on this axis and were positively correlated with temperature. These canonical variables, however, explained only 10.7% of the variation in plankton community structure in mainstem sites.

Discussion

**Broadscale distribution and abundance of C. fluminea veligers**

The lower mainstem Columbia-Snake River system reservoirs are heavily invaded with C. fluminea veligers, which follow a consistent pattern of seasonal abundance. One reproductive maximum occurred in July of each year. Immediately prior to each maximum abundance, veligers were nearly absent in all reservoirs. Although sampling occurred only from May to September, Dexter et al. (2015) also reported annual maximum abundances of C. fluminea veligers in July and August based on monthly, year-round sampling of one site in the lower Columbia River. Both our data and those of Dexter et al. (2015) suggest that a single C. fluminea spawning period occurs in late summer in the Columbia-Snake River system.

To date only one study has investigated the planktonic veliger stage of C. fluminea in the Columbia River Basin. As part of an 8.5-year study, Dexter et al. (2015) found maximum abundances of C. fluminea larvae occurred during periods of high water temperature and dominated the autumn zooplankton community along with another invasive zooplankter, Pseudodiaptomus forbesi. Studies of veligers from other systems show that they are released into the water column after a brief brooding period, where they remain for ~48 hours before settling (King et al. 1986). After settling, juveniles can be re-suspended through currents, tides, wind, and other forms of turbulence (McMahon 1999, Hoyer et al. 2015).

Temperature has been described as the main driver of spawning (Denton et al. 2012). A single annual spawning period for C. fluminea has been reported in a central Arizona canal (Marsh 1985), a large river delta in Argentina (Cataldo and Boltovskoy 1998), and a population in southern California (Heinsohn 1958). Most other studies, however, have reported 2 annual spawning periods, one as temperatures increase in early summer and one as temperatures decrease in autumn (Heinsohn 1958, Aldridge and McMahon 1978, Eng 1979). In one instance, however, 3 annual spawning periods were reported in a large river system in Virginia, one of which occurred mid-summer when temperatures were relatively steady, suggesting possible other contributing factors (Doherty et al. 1987). Our results showed an association between C. fluminea veligers and high water temperatures once a waterbody was invaded, but other factors not evaluated in our study could be related to spawning, including influences from upstream or tributary sources. Additionally, no correlation between mainstem reservoir retention time and C. fluminea veliger abundance was found in mainstem reservoirs.
Temperature can also affect reproductive phenology. Our study area is near the northern extent of the range of *C. fluminea* in North America, which is likely constrained by low winter temperatures (Crespo et al. 2015). *C. fluminea* veligers were present in Columbia–Snake River system reservoirs when temperatures ranged from 16 to 25 °C, although one detection occurred at 12.8 °C in Ice Harbor Reservoir in May of 2014. This finding largely agrees with the literature from other regions, where a 16–18 °C minimum threshold for spawning has been reported (McMahon 1999, Denton et al. 2012). Temperature ranges of 15–26 °C and 16–24 °C for spawning events have been reported by Doherty et al. (1987) and Cataldo and Boltovskoy (1998), respectively.

Although no differences were found in mean *C. fluminea* veliger abundance among Columbia River system reservoirs (Bonneville, The Dalles, McNary, and John Day), abundances were highly variable. This variability could be explained by the patchy distribution of *C. fluminea* veligers within the Columbia and Snake river reservoirs (Fig. 2). When high abundances of veligers were detected in deep water or main channel stations, veligers would frequently be absent or in low abundances at shallow water stations, resulting in high variances in reservoir-wide monthly mean abundances. Although high natural variability is expected for most planktonic organisms in general, the variability in spatial distribution between shallow and deep sites could be due to the interaction of the hydrology of the system and the limited amount of time veligers are usually suspended in the water column. After spawning occurs, planktonic corbiculid veligers can settle out of the water column within 2 days (Sinclair 1971, King et al. 1986) and can be resuspended by currents, winds, and tidal influence (McMahon 1999, Hoyer et al. 2015). In the Columbia-Snake River system, veligers in the main channel would have a greater likelihood of resuspension because of higher flow and would therefore have a greater chance of detection by vertical plankton tows. Total river discharge was also evaluated and does not seem to be a significant predictor of *C. fluminea* veliger abundance in mainstem reservoirs.
The presence of *C. fluminea* veligers in non-mainstem reservoirs was much less apparent than in mainstem reservoirs. In 2013, *C. fluminea* veligers were detected at 2 non-mainstem sites, Wannacot Lake and Fern Ridge Reservoir. These reservoirs were sampled on the same date in 2014 but no veligers were detected. Given the low abundances present in both reservoirs and natural spatiotemporal variability, this absence is not unexpected. Adult *C. fluminea* are reported to thrive in environments with flowing water and when found in lentic environments such as lakes are limited to well-oxygenated areas near to shore (Matthews and McMahon 1999). In Lake Tahoe, California, the highest abundances of *C. fluminea* veligers were found at shallow water (5 m) sites, even though adult *C. fluminea* abundances were greatest at deep water (20 m) sites (Denton et al. 2012). Sampling at all of our non-mainstem sites was conducted near the shore and occurred only once per year in July or August. Given that veligers quickly settle after spawning and are only resuspended by turbulence, the likelihood of detection by a plankton net could be reduced in a lentic system. The use of horizontal plankton tows in non-mainstem sites versus vertical tows in mainstem sites could also have introduced a source of variability in observed abundances. Also, considering our low sampling frequency of non-mainstem sites (once annually), *C. fluminea* could possibly be present in these waterbodies even though they were undetected by our sampling.

**Plankton communities and environmental associations – mainstem sites**

Associations among plankton communities, temperature, and cyanobacteria were revealed by the NMS analysis when applied to mainstem sites. A *C. fluminea*-associated plankton community, associated with high temperatures, was apparent when looking exclusively at mainstem sites (Fig. 5). Dexter et al. (2015) also found maximum abundances of *C. fluminea* veligers at one site in the lower Columbia River during periods of high temperatures, typically in late summer/early autumn. *Anabaena* (now *Dolichospermum*), a form of potentially toxic cyanobacteria, was the only other significant indicator of the *C. fluminea*-dominated community in mainstem sites (Table 4). *Anabaena* is known to thrive at warmer temperatures (Paerl et al. 2001, Paerl and Huisman 2008), and blooms have been observed in Vancouver Lake in the Columbia River Basin (Rollwagen-Bollens et al. 2013, Lee et al. 2015a, 2015b; Rose 2016). Other invasive bivalves, primarily dreissenid species (zebra and quagga mussels), have been positively associated with cyanobacteria blooms (Vanderploeg et al. 2001, Knoll et al. 2008, Sarnelle et al. 2010) and can promote potentially harmful cyanobacteria blooms through selective feeding on non-cyanobacteria (Vanderploeg et al. 2001). Although not specifically investigated in this study, based on the presence of cyanobacteria in mainstem and non-mainstem sites, the association between *C. fluminea* veligers and cyanobacteria is likely due to a preference for similar environmental conditions.

CCA revealed significant associations with the plankton community structure and 2 environmental variables, temperature and total river discharge. *C. fluminea* and *Anabaena*, the 2 significant indicators of Cluster 2, positively correlated with higher temperatures. These environmental variables, however, explained only 10.7% of variation in the plankton community structure, offering low predictive power. Analysis could have been confounded by a high degree of correlation among various environmental variables (e.g., temperature, conductivity, dissolved oxygen, and total river discharge).

**Plankton communities and environmental associations – all sites combined**

A third plankton community, Cluster C, was apparent with the addition of non-mainstem sites to the ordination, which was comprised entirely of non-mainstem samples and characterized by *Anabaena* and other cyanobacteria taxa (Fig. 4 and 5). The dominance of cyanobacteria in these non-mainstem systems is likely due, at least in part, to the time of year the sampling occurred. All non-mainstem sites were sampled in July or August when temperatures are warm and cyanobacteria blooms are more likely (Paerl and Huisman 2008). Dinoflagellates (primarily *Ceratium*) were a significant indicator of this community, which can also produce nuisance blooms (Paerl et al. 2001).

Given the high abundances of veligers in mainstem reservoirs, the effects of *C. fluminea* on plankton communities, particularly phytoplankton, are potentially high. In similar large freshwater and tidally influenced river systems such as the Potomac River in Maryland...
(Cohen et al. 1984), the Paraná River in Argentina (Boltovsckoy et al. 1995), and the River Meuse in northern Europe (Pigneur et al. 2014), adult C. fluminea have been shown to reduce phytoplankton biomass through high rates of filter-feeding. This phenomenon does not seem to be limited to large river systems; a similar reduction in phytoplankton biomass has been seen in a low-order sandy-bottom stream (Hakenkamp and Palmer 1999). Although quantifying the effects of C. fluminea as grazers on plankton was beyond the scope of our study, plankton communities in the Columbia–Snake River system are possibly influenced by the presence of C. fluminea, and this relationship should be quantified in future studies.

Aquatic invasive species, particularly bivalves, are of great concern in the Pacific Northwest of the United States. The Columbia–Snake River system is a heavily managed series of reservoirs that can act as invasion hubs conducive to sustaining populations of invasive bivalves (Allen and Ramcharan 2001, Havel et al. 2005). Furthermore, the Columbia–Snake River system is predicted to be at high risk of zebra and quagga mussel invasion (Bossenbroek et al. 2007). C. fluminea can increase invasion risk for zebra and quagga mussels by increasing the calcium concentrations in the water column (Hoyer et al. 2015). C. fluminea often undergoes mass mortality events under fluctuating environmental conditions, such as low winter temperatures, low water flow, flood events, and heat waves (Werner and Rothhaupt 2007, Ilarri et al. 2011, Bódis et al. 2014). The resultant shells not only provide a rough substrate ideal for dreissenids, they also locally increase calcium, often a limiting nutrient for bivalves, through leaching (Hoyer et al. 2015). Lastly, the Columbia–Snake River system has recently been invaded by several species of planktonic copepods from Asia (Cordell et al. 2008, Bollens et al. 2012, Breckenridge et al. 2015, Dexter et al. 2015, Emerson et al. 2015), suggesting that this system is likely susceptible to additional invasions in the future.

Sousa et al. (2008b) stated that it is essential to minimize transport of C. fluminea to freshwater systems not yet invaded because of the economic costs and potential effects to other biota. By determining the current distribution and ecology of C. fluminea in the Columbia River Basin, additional approaches can be developed to minimize the transport and spread of C. fluminea to currently unininvaded waterbodies. These results could also provide insight into the control and management of other aquatic invasive bivalves (e.g., zebra and quagga mussels) that have not been found in the region but are spreading rapidly to states west of the Rocky Mountains (Wong et al. 2010).

**Summary**

This study is the first documentation of the broadscale distribution and abundance of veligers of the invasive Asian clam C. fluminea in the Columbia River Basin. Given its widespread distribution and high abundances, C. fluminea is well-established in the Columbia River system reservoirs to the degree that eradication is likely infeasible. C. fluminea has spread to 3 of 23 non-mainstem lakes and reservoirs sampled, but given the low abundance of veligers and low frequency of occurrence, management to reduce the spread of this invasive species is still possible. Veligers of C. fluminea characteristic of the plankton community in late summer are associated with cyanobacteria (Anabaena) and increasing water temperatures and seem to have a single annual spawning event in the Columbia–Snake River system. A diatom-dominated community preceded the spawning of C. fluminea in early summer at all sites, and a third plankton community characterized by cyanobacteria was most apparent in non-mainstem sites sampled in July and August. This study improves understanding of the relationship of C. fluminea to the plankton community and the abiotic environment, which in turn contributes to a better overall understanding of the potential effects of C. fluminea infestations and identifying waterbodies in the Columbia River Basin at risk of infestation. This research will be increasingly important given the global spread of aquatic invasive species generally and the likely arrival of other aquatic invasive molluscs to the Pacific Northwest region specifically.

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References


