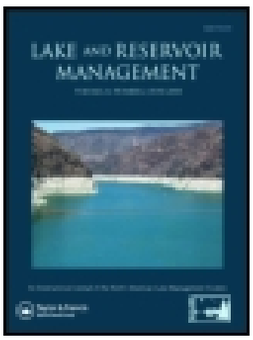

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An experimental evaluation of the efficacy of imaging flow cytometry (FlowCam) for detecting invasive Dreissened and Corbiculid bivalve veligers

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ABSTRACT

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Zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels, first introduced from central Asia into the Great Lakes of North America in the late 1980s, have crossed the continental divide and more recently spread across western North America. At the same time, several new technologies have been developed for the early detection of dreissenids, including the FlowCam, a digital imaging-in-flow instrument, intended to detect dreissenid planktonic larvae (veligers). However, the efficacy of this technology has rarely been tested. We experimentally evaluated the FlowCam's ability to capture identifiable images of quagga mussel veligers under 2 different types of conditions: (i) deionized water, and (ii) Columbia River Basin water (CRBW), including natural sediment and native plankton. We further evaluated the FlowCam's ability to distinguish between dreissenid veligers and corbiculid veligers (Asian clam, *Corbicula fluminea*). We interpret our results to indicate that the FlowCam can consistently detect dreissenid veligers across a range of veliger densities. Moreover, the presence of other plankton and detritus only slightly affected dreissenid detection by the FlowCam. However, the orientation of individual bivalve veligers as they were imaged by the FlowCam precluded specific identification of a substantial proportion (24.8%) of veligers as either dreissenid or corbiculid. We suggest that the FlowCam is an important detection tool best utilized as part of a multifaceted approach, including traditional microscopy and possibly environmental DNA.

KEYWORDS

Aquatic invasive species; bivalve veligers; Columbia River Basin; *Corbicula fluminea*; *Dreissena bugensis*; *Dreissena polymorpha*; early detection monitoring; FlowCam

Zebra and quagga mussels (*Dreissena polymorpha* and *D. bugensis*) have had substantial ecological, economic, and recreational effects on freshwater systems across North America (Pimentel et al. 2005, Strayer 2009, Higgins and Vander Zanden 2010). Ecologically, zebra and quagga mussels (collectively referred to as dreissenids) have drastically shifted food web structure and nutrient flow (Higgins and Vander Zanden 2010), and are causing the decline of already jeopardized populations of native bivalves (Ricciardi et al. 1998). Zebra and quagga mussels are thought to be "ecosystem engineers" in that they physically alter habitat and reduce food resources in the areas they invade, transforming entire plankton communities (Sousa et al. 2009, 2014). The economic effects of dreissenid mussels are far-reaching and include impacts on water intake pipes and electricity generation facilities in North America totaling an estimated $267 million from 1989 (the year of their introduction) to 2004 (Connelly et al. 2007). The expansion of zebra and quagga mussels across North America is expected to continue until their entire potential range is occupied (Strayer 2009). This is cause for concern for all uninvaded waterways in North America, and particularly the Pacific Northwest (PNW) region of the United States, one of the most hydroelectrically developed areas in the

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world and home to several endangered or at-risk species of salmonid fishes (Hamlet et al. 2002).

Zebra and quagga mussels have not yet been detected in the PNW; however, the Columbia River (CR) is predicted to be at high risk of dreissenid invasion (Bossenbroek et al. 2007). Indeed, several other planktonic invaders, including several species of Asian copepods and the Asian clam *Corbicula fluminea* (a corbiculid bivalve), have successfully invaded the CR (Cordell et al. 2008, Bollens et al. 2012, Breckenridge et al. 2015, Emerson et al. 2015). The ability to detect zebra and quagga mussels at an early stage of invasion allows for more effective mitigation measures and aids in preventing the spread of invasive mussels to other waterways (Counihan and Bollens 2017). For instance, early detection could provide facilities approximately 3 to 5 years to implement control measures to curb the colonization and subsequent clogging of water intake structures (Hosler 2011).

The most effective method for early detection of zebra and quagga mussels is through monitoring of planktonic larvae, particularly the veliger stage, which typically are 70–280 µm in size (Hosler 2011). After introduction to a new waterbody, planktonic veligers are often (but not always) the first stage to be observed, and serve as a proxy for the presence of adult zebra and quagga mussels (Johnson 1995). Veligers are also more uniformly distributed throughout the water column (Johnson 1995), potentially facilitating detection when adult infestations are not apparent. Cross-polarized light microscopy is the prevailing early detection method for dreissenid veligers (Johnson 1995), in which veligers are identified by a characteristic cross-pattern on the shell when exposed to polarized light (i.e., birefringence). With the development of image analysis systems, however, new tools for automated detection have become available. The FlowCam, a combination of a flow cytometer and microscope, has been proposed as a viable method for dreissenid veliger detection (Fluid Imaging Technologies 2011). The FlowCam's ability to digitally image particles (planktonic organisms) in moving fluid at a high flow rate has resulted in greater sample processing efficiency than in traditional microscopy (Wang et al. 2015). Stored images either can be sorted using image analysis software (based on defined image criteria, such as circularity) or can be manually sorted by the user. The FlowCam has proven to be an effective tool for measuring plankton density and size structure in both freshwater (Wang et al. 2015, Graham et al. 2018, Stanislawczyk et al. 2018, Whitten et al. 2018, Hrycik et al. 2019) and marine systems (See et al. 2005, Álvarez et al. 2014, Le Bourg et al. 2015, Camoying and Yñiguez 2016).

To date, however, very little has been published on the effectiveness of the FlowCam's ability to detect dreissenid veligers. The developers of the FlowCam (Fluid Imaging, Inc., now known as Yokogawa Fluid Imaging Technologies) have reported its use as an efficient tool for veliger detection (Buskey and Hyatt 2006, Spaulding 2009, Spaulding et al. 2009). Yet in the peer-reviewed literature, only 2 studies have been published on the use of the FlowCam for veliger detection. Frischer et al. (2012) compared several techniques for dreissenid veliger detection and found cross-polarized light (CPLM) microscopy to be the most reliable method when compared to the FlowCam and conventional polymerase chain reactions (cPCR) techniques, although the number of participating laboratories in their study was limited. Frischer et al. (2012) also found the FlowCam was prone to underestimating veliger abundance, but they suggested that in the future the FlowCam might attain the accuracy of microscopy. More recently, Johansson et al. (2020) compared 3 techniques for detecting zebra mussel veliger larvae—(i) CPLM, (ii) FlowCam, and (iii) cPCR analysis of environmental DNA (eDNA)—and found the prevalence of veligers (percentage of samples with at least one veliger) was highest for CPLM, although all 3 methods were viewed as complementary and perhaps best employed in concert.

Our study had 2 research questions. First, what is the FlowCam's ability to capture identifiable images of dreissenid veligers under ideal conditions (deionized water) vs. in natural conditions (water containing natural sediment and native plankton) in the Columbia River Basin? Second, can the FlowCam capture images of specimens that allow for distinguishing between dreissenid

and corbiculid veligers? Asian clams (*Corbicula fluminea*) are highly abundant in the lower Columbia River (Dexter et al. 2015, Bolam et al. 2019, Dexter et al. 2020a, 2020b; Henricksen and Bollens unpubl. data), can overlap in geographic range with dreissenids (Karatayev et al. 2007), and are commonly misidentified with dreissenid species in their larval form (Nichols and Black 1994). By considering both dreissenid and corbiculid species in our study, we were able to assess the ability to differentiate between these 2 families of bivalve veligers using FlowCam technology. Thus, our experimental results provide the basis for evaluating the efficacy of the FlowCam to detect dreissenid and corbiculid veligers, which should help guide early detection and monitoring methods for invasive bivalves more broadly.

Methods

Collection of dreissenid and corbiculid veligers

Preserved quagga mussel (*D. bugensis*) veligers were obtained from the US Bureau of Reclamation laboratory in Denver, Colorado. Samples were collected from plankton net tows (0.5 m diameter, 64 µm mesh) on the lower Colorado River in May 2014, and preserved in 20% ethanol buffered with sodium bicarbonate (0.2 grams sodium bicarbonate per 100 mL of sample). Asian clam (*C. fluminea*) veligers were collected from plankton tows (0.3 m diameter, 64 µm mesh) from the Dalles Reservoir (45°38'32.9"N 120°55'25.1"W) on the Columbia River in August 2014, and were preserved in 70% buffered ethanol.

Collection of dreissenid-free and corbiculid-free CRBW

Concentrated Columbia River Basin water (CRBW), including natural debris and plankton, was collected via vertical plankton net tows (0.3 m diameter, 64 µm mesh) from 25.5 m to the surface (1.82 m^3 volume filtered) in Ice Harbor Reservoir (46°15'10.7"N 118°51'35.9"W), located on the Snake River, Washington, in May 2014. For the purpose of excluding corbiculid veligers, we used samples collected in early spring, prior to typical spawning events for corbiculid species in the region (Dexter et al. 2015, Dexter et al. 2020b). Based on previous plankton sampling in this reservoir (Emerson et al. 2015), dreissenids are thought to be absent. All CRBW samples were transported to the laboratory in Vancouver, Washington, and were verified to be free of dreissenid and corbiculid veligers using cross-polarized light microscopy.

Experimental design and setup

To assess the efficacy of the FlowCam to capture identifiable images of dreissenid veligers in optimal conditions (i.e., unimpeded by particulate matter or nontarget coexisting organisms), 10 mL samples of deionized water were spiked with a known number of preserved quagga mussel veligers. To prepare the samples, 10 mL of deionized water was measured into each of nine 50 mL glass beakers. Veligers were identified from the samples described above using cross-polarized light microscopy and individually transferred via pipette to the beaker containing the deionized water. Three of the 9 beakers were spiked with 10 veligers each, 3 beakers with 50 veligers each, and the remaining 3 beakers with 100 veligers each. These 3 spiking densities were consistent with the range observed in CR plankton samples by Dexter et al. (2015) and Hassett et al. (2017). After transfer, the contents of each beaker were verified to contain the correct number of dreissenid veligers using cross-polarized light microscopy.

To evaluate the FlowCam's ability to capture identifiable images of dreissenid veligers in CRBW, we used concentrated CRBW instead of deionized water. CRBW was filtered through a 280 µm nylon (mesh) filter to ensure that particles in the filtrate were small enough to pass through the 300 µm flow cell of the FlowCam. Aliquots of 2 mL of CRBW (0.8% of the plankton net sample) were pipetted into each of nine 50 mL beakers. Three of the 9 beakers were spiked with 10 veligers each, 3 beakers with 50 veligers each, and 3 beakers with 100 veligers each. After transfer, the contents of each beaker were diluted to 10 mL with deionized water and verified to contain the correct number of

dreissenid veligers using cross-polarized light microscopy.

To evaluate the FlowCam's ability to capture images that allow for distinguishing between dreissenid and corbiculid veligers in CRBW, "mixed" samples consisting of CRBW spiked with a known number of both dreissenid and corbiculid veligers were established. CRBW was filtered through a 280 µm mesh filter prior to spiking. Aliquots of 2 mL of CRBW (0.8% of the plankton net sample) were pipetted into each of nine 50 mL beakers. Three of the 9 beakers were spiked with 10 veligers each (5 corbiculid and 5 dreissenid veligers), 3 beakers with 50 veligers each (25 corbiculid and 25 dreissenid veligers), and 3 beakers with 100 veligers each (50 corbiculid and 50 dreissenid veligers). After transfer, the contents of each beaker were diluted to 10 mL with deionized water and verified to contain the correct number of dreissenid veligers using cross-polarized light microscopy.

For corbiculids, we have very good historical data from the CR on veliger densities (Dexter et al. 2015, Hassett et al. 2017, Dexter et al. 2020a). Specifically, Dexter et al. (2015) observed an average of 28.2 *C. fluminea* veligers per plankton sample (average volume = 1.88 m^3), with lesser (10) or greater (100) numbers of veligers sometimes observed. Hassett et al. (2017) used slightly different collection methods in the CR but observed similar numbers of veligers per plankton sample. Thus, our spiking densities of corbiculids compare very favorably to real-world samples in the CR, which is currently experiencing a late-stage (nearly a century long; Counts 1981) invasion.

For dreissenids, typical veliger densities in nature can range from 0.5 to 61 individuals/L (Bowen et al. 2018), and can be as high as 260 individuals/L (Barnard et al. 2003). In our case, as already described, we filtered 1.82 m^3 of CR water into a concentrated plankton sample and then subsequently placed 0.8% of the plankton sample (comparable to 14.6 L of filtered CR water) into each experimental container before adding veligers. Thus, our spiking densities of 5, 25, and 50 dreissenid veligers/14.6 L of filtered CR water translate into densities of 0.34, 1.7, and 3.4 veligers/L. These experimental stocking densities compare very favorably to natural or real-world densities of dreissenid veligers (referenced in the preceding), although our densities were on the lower end of natural densities, and thereby are likely more representative of an early- to mid-stage invasion.

FlowCam parameter settings and operation

Analyses were performed on a Bench Top VS1 model FlowCam (Yokogawa Fluid Imaging Technologies) equipped with a single Sony XCD-SX90 black and white camera, red LED light source, cross-polarizing components, a 4 objective lens, and a standard 300 µm flow cell (FC300). The FlowCam was operated in AutoImage mode in standard light, which takes images of the sample flow at regular intervals, and is optimal for samples with high particle concentrations. It is important to note that according to the manufacturer, when using a standard flow cell (as in this study) a portion of the sample on either side of the flow cell will pass outside the view of the camera (Fluid Imaging Technologies 2017), resulting in a reduced detection rate (of 36.0% in the case of our instrument; Nelson H, Yokogawa Fluid Imaging Technologies, Aug 2020, pers. comm.).

A flow rate of 0.875 mL/min and an AutoImage rate of 18 frames per second (on a scale of 1–22) were chosen to produce a high percent efficiency, appropriate for detection of rare species. A higher efficiency ensures that there is a lower likelihood that particles (specimens) are missed. These settings were not optimized per se as part of this study, but rather were determined after preliminary experimentation. Four mL of glycerin was added to each 10 mL sample to increase viscosity and aid in particle separation immediately before processing through the FlowCam (Nelson et al. 2015). After pouring each sample (14 mL) into the FlowCam pipette, 5 mL of deionized rinse water was used to dislodge any remaining organisms from the 50 mL sample beaker into the FlowCam pipette. Transfer vessels (50 mL beakers) were subsequently inspected using cross-polarized light microscopy to verify that all veligers had been removed from the beaker and introduced into the FlowCam pipette. FlowCam analysis was stopped when the complete sample and rinse water had passed through the flow cell.

Veliger classification and statistical analysis

All images were analyzed using Visual Spreadsheet software (Yokogawa Fluid Imaging Technologies 2017). The FlowCam collects images of particles that pass through the field of view of the camera and stores them in a comprehensive "list file" for each sample processed. All "list file" images (i.e., unsorted by the software) were first sorted using the software's particle property of "Circularity (Hu)" to sort all particles from most circular to least circular, and then images were manually (visually) examined by the user to isolate veliger images and identify each veliger as either dreissenid or corbiculid using criteria from Nichols and Black (1994). However, depending on the orientation of the veliger as it passes through the flow cell, distinguishing characteristics necessary for identification (e.g., hinge shape or presence of an umbonal hump) were not always visible in the FlowCam images. Thus, in samples containing both corbiculid and dreissenid veligers, any veliger lacking discernable characteristics was classified as "indeterminate." Example images of veliger classifications are shown in Figure 1.

We calculated the proportion of veligers detected by the FlowCam in each sample. Because analysis of variance is not recommended for discrete (noncontinuous) data such as proportions (Cribari-Neto and Zeileis 2010), we modeled the

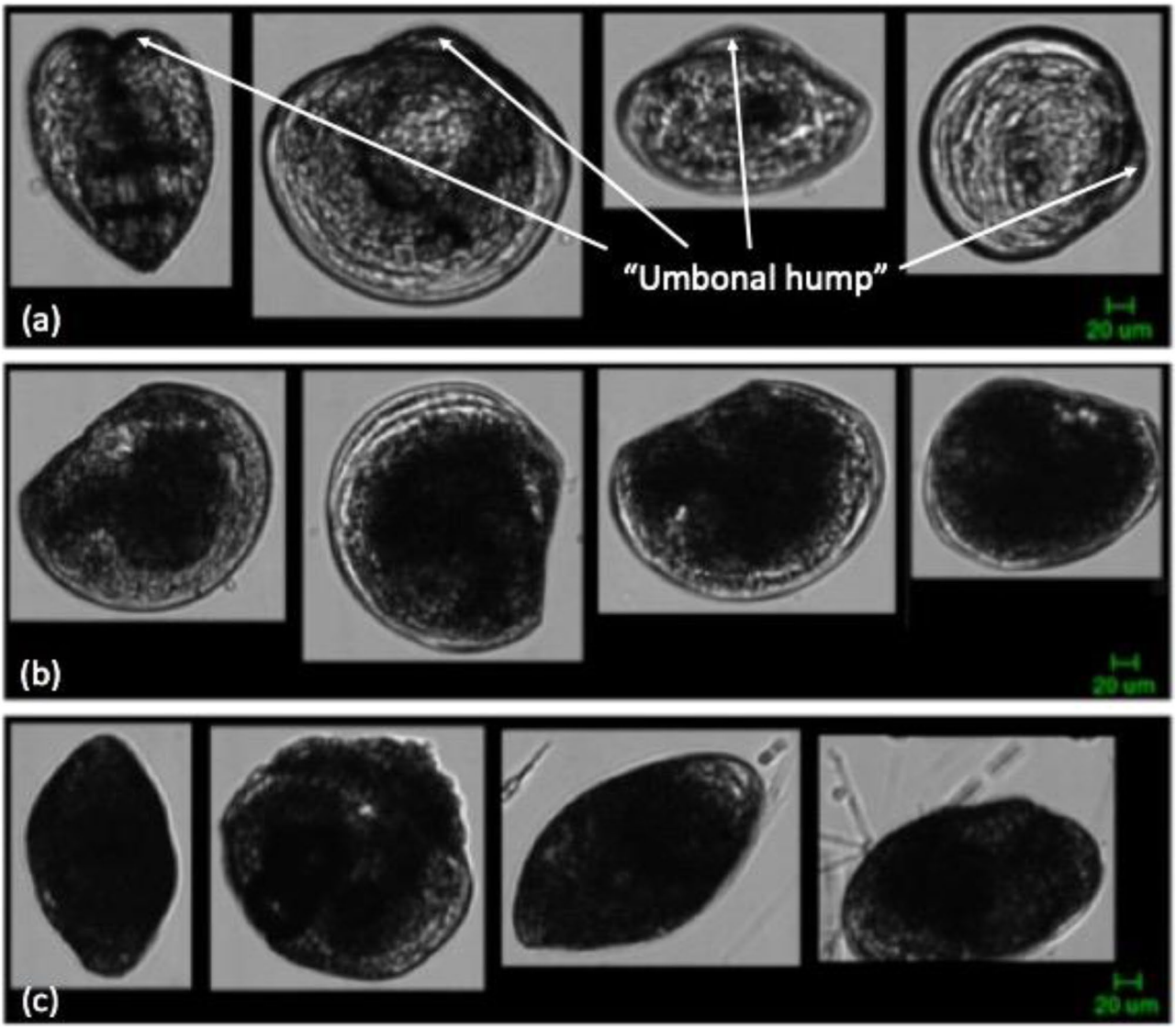

Figure 1. Digital images of bivalve veligers produced by the FlowCam and manually classified as either (a) dreissenid, (b) corbiculid, or (c) indeterminate. Note presence of umbonal hump indicative of dreissenid veligers.

relationship of the proportion of veligers detected to the treatment (class variable) and spiking densities (continuous variable) using beta regression (Ferrari and Cribari-Neto 2004) as implemented by the "betareg" package in R (Cribari-Neto and Zeileis 2010). We used an information-theoretic approach (e.g., Burnham and Anderson 2002, Fisher et al. 2018) to assess the parsimony of several models that included combinations of potential explanatory variables, that is, 2 treatment conditions (deionized water and CRBW) and 3 spiking densities (10, 50, and 100 veligers). Model parsimony was evaluated using Akaike information criteria (AIC), ΔAIC ($\Delta AIC_i = AIC_i - AIC_{min}$), Akaike weights ($w_i$), and evidence ratios (Burnham et al. 2011). Additionally, a pseudo-R^2 was calculated for each model, as a complement to the information-theoretic approach.

Results

To address our first research question—what is the FlowCam's ability to detect dreissenid veligers under ideal conditions (deionized water) vs. natural conditions (including natural sediment and native plankton) in the Columbia River Basin?—our most parsimonious model describing the proportion of veligers detected had 3 parameters: "Treatment" (deionized water vs. Columbia River Basin water [CRBW]), "Spike" (10, 50, and 100 veligers), and the interaction term "Treatment * Spike" (Table 1); however, the pseudo-R^2 was 0.35, suggesting that a relatively low portion of the variation in the proportion of veligers detected was explained by the model given the data. Our 3 other models all performed less well, based on all 5 of our performance metrics, and the pseudo-R^2 values of these other 3 models were all <0.1 (Table 1). Thus, there is not strong evidence to support the hypothesis that the 2 treatment conditions (deionized water and CRBW) and 3 spiking densities (10, 50, and 100 veligers) affected detections of veligers by the FlowCam.

When using a standard flow cell, a reduced detection rate of 30–50% (36.0% in the case of our instrument) is expected because a portion of any given sample will lie outside the field of view of the camera and will not be imaged; this obviously needs to be taken into account when calculating the percent of veligers imaged. The overall percent of dreissenid veligers imaged by the FlowCam, as configured (i.e., available to the camera), across all 27 of our experimentally manipulated samples, was 90.4%, with some variability between treatments and replicates (Figures 2 and 3). These results indicate that the FlowCam is capable of assessing the presence of veligers in diverse water types.

To address our second research question—can the FlowCam capture images that allow for distinguishing between dreissenid and corbiculid veligers?—we examined the proportions of both types of veligers in the "mixed" samples (i.e., those containing equal numbers of both taxa). In samples spiked with 10 veligers (5 dreissenid and 5 corbiculid), 19.5% were classified as dreissenids, 35.1% as corbiculids, 20.4% as indeterminate, and 25.0% were not detected (Figure 3). In samples spiked with 50 veligers (25 dreissenid and 25 corbiculid), 44.8% were classified as dreissenids, 26.2% as corbiculids, 23.8% as indeterminate, and 5.2% were not detected (Figure 3). In samples spiked with 100 veligers (50 dreissenid and 50 corbiculid), 43.4% were classified as dreissenids, 36.3% as corbiculids, 20.4% as indeterminate, and 0% were not detected (Figure 3). Overall, the majority (75.2%) of veliger images in these "mixed" samples provided sufficient detail to allow for

Table 1. Akaike information criteria (AIC), ΔAIC ($\Delta AIC_i = AIC_i - AIC_{min}$), Akaike weights ($w_i$), and evidence ratios associated with 4 models that relate proportions of dead dreissenid veligers detected by the FlowCam in 2 water types (Treatment: deionized water vs. Columbia River Basin water [CRBW]) and 3 different spiking densities (Spike: 10, 50, and 100 veligers).

Model variable(s)	AIC	ΔAIC	w_i	Evidence R^2 ratio
Treatment + Spike + Treatment * Spike	−20.17	0	0.71	1 0.354
Spike	−16.71	3.45	0.13	5.61 0.015
Treatment	−16.59	3.57	0.12	5.96 0.009
Treatment + Spike	−14.86	5.30	0.05	14.14 0.024

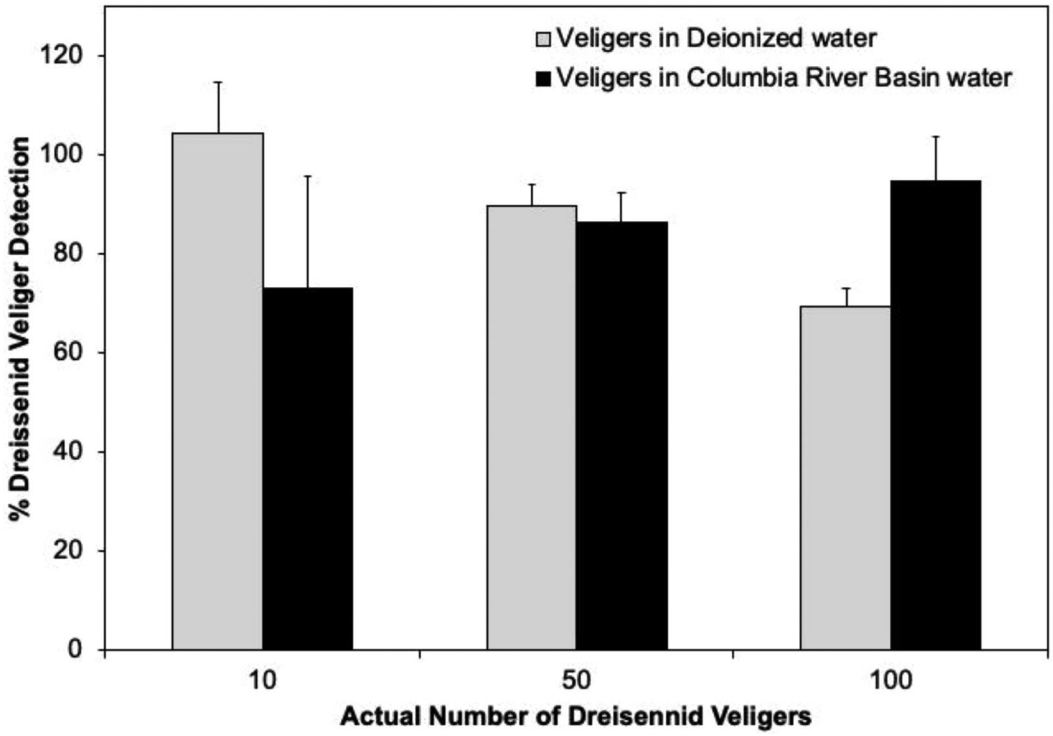

Figure 2. Comparison of percent of dreisennid veligers detected by the FlowCam in samples spiked with 3 different numbers of dreissenid veligers (10, 50, and 100) and 2 different water treatments (deionized water, Columbia River Basin water). Error bars represent one standard error of the mean.

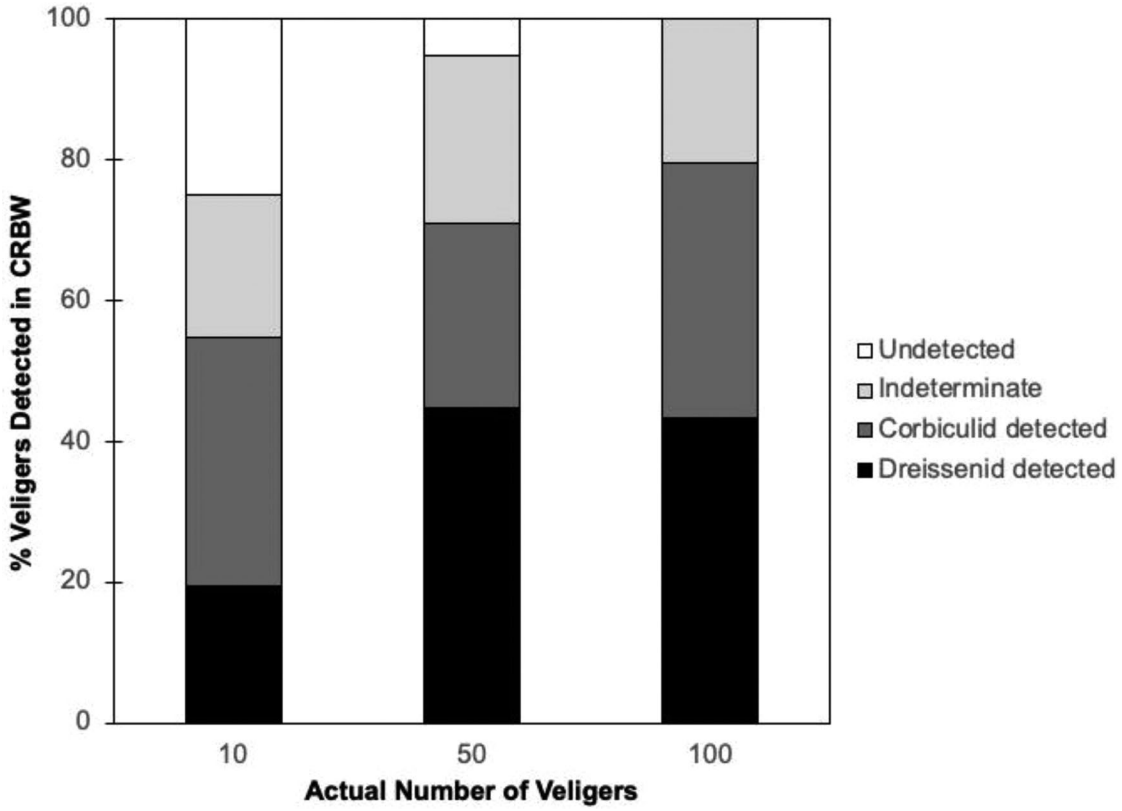

Figure 3. Comparison of percent of veligers detected by the FlowCam in Columbia River Basin water (CRBW) samples containing both dreissenid and corbiculid veligers: 10 veligers (5 dreissenid and 5 corbiculid), 50 veligers (25 dreissenid and 25 corbiculid), and 100 veligers (50 dreissenid and 50 corbiculid).

identification as either dreissenid or corbiculid (Figures 1 and 3). The ability to differentiate was reduced when veligers were imaged at orientations obscuring defining characteristics (Figure 1c), or when there was obstruction by other plankton and detritus. Overall, 24.8% of veligers imaged from these "mixed" samples were classified as indeterminate.

Discussion

We interpret the results from our experiments to indicate that the FlowCam can consistently capture identifiable images of dreissenid veligers within the manufacturer's stated limitations of the field of view of the camera when using the standard flow cell. The presence of other plankton, detritus, and corbiculid veligers did not substantially affect imaging of dreissenids by the FlowCam, and the percent of veligers detected varied only slightly with veliger density.

More specifically, the effect of "Treatment" is straightforward to interpret, as the mean percent of veligers detected in deionized water (87.7%) was slightly higher than that in CRBW (84.7%) (Figure 2), with the slight difference (3.0%) likely being due to sediment and/or native plankton in the CRBW occasionally obscuring the view of a veliger. Although we made only qualitative observations of co-occurring planktonic organisms present in the CRBW, we observed the presence of diatoms, dinoflagellates, cyanobacteria, ciliates, rotifers, and small crustaceans (copepod nauplii and cladocerans) in the 70 – 280 µm size range.

Likewise, the effect of "Spike" (10, 50, and 100 veligers) is straightforward to interpret, as the mean detection rates of veligers at these 3 spiking densities were 85.1%, 92.7%, and 93.4%, respectively (Figure 2), with the slight differences (0.7 – 7.6%) likely being due to one veliger occasionally obscuring the view of another veliger. However, the interaction term "Treatment * Spike" is more difficult to interpret. In particular it is hard to interpret the results from the 100 veliger spiking, in which the percent of veligers detected seemingly increased in the CRBW treatment relative to the deionized water treatment. Moreover, the sometimes large variance between replicates, especially at lower spiking levels (e.g., the 10 veligers with CRBW; Figure 2), further complicates interpretation of results. For instance, the larger error in our low density treatment may suggest caution in using the FlowCam as a detection tool in very early-stage (low veliger density) invasions, although the instrument was still able to detect a sizable proportion (75%) of veligers even at these low densities (Figure 3). And again, the results of our statistical modeling (described earlier) indicate that there was little overall effect of veliger density on FlowCam performance. Thus, we conclude that there is not strong evidence for thinking the proportion of veligers detected by the FlowCam was influenced by treatments and/or spiking densities, suggesting that the FlowCam performs similarly across a range of conditions. However, we acknowledge several limitations of the current study and we recommend that these be addressed in future studies, including the use of a broader range of veliger spiking densities, the use of different types of natural waters, and investigating the role of ostracods (which have a shape similar to that of veligers) in possibly affecting detection efficiency.

For dreissenid veliger detection, numerous advantages and disadvantages of the FlowCam in relation to cross-polarized light microscopy became apparent throughout the course of our experiments. Advantages of the FlowCam include semi-automated sample processing and a permanent, retrievable record of particle images (i.e., of veligers and other organisms). In general, processing samples on a FlowCam is less demanding than traditional microscopy, generates images of large numbers of organisms, and requires only basic technician training for instrument operation. However, we have found that to be assured of definitive identification and to distinguish between dreissenids, corbiculids, and other bivalves, postprocessing of the images by a skilled taxonomist is required. Images can be reduced to a manageable number by using the software (Visual Spreadsheet) to sort by one or more characteristics (e.g., circularity), and then manually reviewing the resulting presorted images for identification of dreissenid veligers (or other taxa).

In short, the FlowCam consistently captured identifiable images of dreissenid veligers across all of our experimental conditions (including

variable veliger densities). Our findings are in general agreement with 2 other recent studies. Frischer et al. (2012) found the reliability of the FlowCam to detect the presence/absence of *Dreissena* spp. larvae was 91.7% (with CPLM being more reliable, at 96.3% accuracy, and PCR being less reliable, at 75.8% accuracy). Johansson et al. (2020) also found detection prevalence for zebra mussel veligers was highest for CPLM, and lower for FlowCam and cPCR, but that all 3 methods had a high probability of veliger detection if samples were sufficiently large.

One notable disadvantage to using the standard flow cell for detection of rare species is the reduced field of view of the objective lens relative to the flow field, resulting in some particles going undetected (i.e., those particles passing outside the field of view). For our instrument, the manufacturer stated that 36.0% of the flow field is not imaged (Nelson H, Yokogawa Fluid Imaging Technologies, Aug 2015, pers. comm.). This could be improved by the use of a field-of-view (FOV) flow cell, also manufactured by Yokogawa Fluid Imaging Technologies, Inc., which allows the entire introduced sample to be viewed and imaged. However, FOV flow cells are not currently offered as an option with cross-polarization filters (commonly used for dreissenid veliger sample processing) and they come at a substantially higher cost, which may not be economically viable for some monitoring projects with limited budgets.

The inability to manipulate the orientation of organisms of interest within the FlowCam is another significant limitation. Particles suspended in the fluid medium are imaged as they pass in front of the FlowCam camera, and thus appear in a variety of orientations, which may or may not allow for definitive identification, depending on the attributes of the particles. For instance, for differentiating between dreissenid and corbiculid veligers in the 70 – 280 µm size range, the presence of an umbonal hump (dreissenid) vs. a flat hinge (corbiculid) was the most useful feature for veliger identification (Figure 1). Overall, 24.8% of veligers detected in our experiments were deemed indeterminate, due to the orientation of the veliger obscuring the presence/absence of the umbonal hump or, more rarely, due to obstruction by other particles. This latter limitation can be mitigated by diluting the sample prior to introduction into the FlowCam, but there remains a limitation in the FlowCam's ability to capture images that allow for distinguishing between dreissenid veligers and corbiculid veligers due to the orientation of veligers as they pass through the camera's field of view. However, for any given sample, determining the relative abundance of both dreissenid veligers and corbiculid veligers in the 75.2% of determinate cases would allow a user to apportion the remaining 24.8% of indeterminate veligers into one or the other type, allowing for a quantitative estimate of both types of veligers.

Quagga and zebra mussels pose an enormous threat to both the economy and ecology of the aquatic ecosystems which they invade (Ricciardi et al. 1998, Pimentel et al. 2005, Connelly et al. 2007, Sousa et al. 2009, 2014, Strayer 2009, Higgins and Vander Zanden 2010). Thus, the ability to accurately and quantitatively evaluate plankton samples for the presence and absence of dreissenid veligers is a key component of many early detection and monitoring programs. This is particularly true of the Columbia River Basin within the Pacific Northwest of the continental United States, where dreissenids have not yet been found, but which is nevertheless threatened by high recreational boat traffic and propagule pressure from other adjacent areas where dreissenids have recently become established (Hickey 2010, Wong et al. 2010).

Conclusions

We interpret our experimental results to indicate that the FlowCam consistently captured identifiable images of dreissenid veligers across a range of veliger densities. Moreover, the presence of other plankton, detritus, and corbiculid veligers from the Columbia River only slightly affected dreissenid imaging by the FlowCam. However, the orientation of individual veligers as they were imaged by the FlowCam precluded identification of a substantial proportion (24.8%) of veligers as either dreissenid or corbiculid. Nevertheless, the FlowCam can detect both dreissenid and corbiculid veligers across a range of

water conditions, although the larger error in our low-density treatment may suggest caution in using the FlowCam as a detection tool in very early-stage (low veliger density) invasions. Thus, while our results suggest that the FlowCam can be an important tool in the detection of invasive bivalve veligers in water samples, we recommend that this method be utilized in conjunction with other methods, for example, cross-polarized light microscopy, and, pending further research and development, new early detection and monitoring techniques such as environmental DNA (Rees et al. 2014, Gingera et al. 2017, Cowart et al. 2018, Xia et al. 2018, Amberg et al. 2019).

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