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PRIMARY RESEARCH PAPER

Evaluating the use of side-scan sonar for detecting freshwater mussel beds in turbid river environments

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Abstract Side-scan sonar is a valuable tool for mapping habitat features in many aquatic systems suggesting it may also be useful for locating sedentary biota. The objective of this study was to determine if side-scan sonar could be used to identify freshwater mussel (unionid) beds and the required environmental conditions. We used side-scan sonar to develop a series of mussel-bed reference images by placing mussel shells within homogenous areas of fine and coarse substrates. We then used side-scan sonar to map a 32-km river reach during spring and summer. Using our mussel-bed reference images, several river locations were identified where mussel beds appeared to exist in the scanned images and we chose a subset of sites ($n = 17$) for field validation. The validation confirmed that $\sim$60% of the sites had mussel beds and $\sim$80% had some mussels or shells present. Water depth was significantly related to our ability to predict mussel-bed locations: predictive ability was greatest at depths of 1–2 m, but decreased in water >2-m deep. We determined side-scan sonar is an effective tool for preliminary assessments of mussel presence during times when they are located at or above the substrate surface and in relatively fine substrates excluding fine silt.

Keywords Sonar images · Mussel habitat · Distribution · Detection

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Introduction

Freshwater mussels are an ecologically important component of lotic ecosystems. In many aquatic ecosystems, mussels make up a large portion of biomass and provide important ecosystem functions (Lopes-Lima et al., 2014). Freshwater mussels influence ecosystem processes through particle processing (i.e., filter feeding), release of nutrients from captured suspended matter in the form of pseudofeces, and oxygenation of sediments via burrowing (Vaughn & Hakenkamp, 2001; Vaughn et al., 2004; Howard & Cuffey, 2006). Vaughn et al. (2004) found a linear response between mussel biomass and particle processing and nutrient release from pseudofeces. Freshwater mussels are a valuable food source to many terrestrial (Toweill, 1974; Tyrrell & Hornbach, 1998; Sousa et al., 2012; Bódis et al., 2014) and aquatic species (Tiemann et al., 2011). Further, freshwater mussels possess several characteristics that make them sensitive indicators of aquatic ecosystems: they are long lived, relatively sessile, and sensitive to changing water quality, habitat, and fish communities (Neves, 1993; Naimo, 1995; Strayer, 2008; Haag, 2012). Of the nearly 300 species found in the U.S., 70% are in some state of decline (Williams et al., 1993; Master et al., 2000).

A major impediment to the conservation and management of freshwater mussel populations is a general lack of knowledge of their distributions (National Native Mussel Conservation Committee, 1998). Locating mussel populations is an important first step in identifying mechanisms that influence distributions, monitoring populations, and protecting these areas when necessary. Identifying the location of freshwater mussel beds is challenging and resource intensive, usually involving tactically searching of the stream bottom. In clear-water streams, visual searches can be completed using either snorkeling or diving (Miller & Payne, 1993; Beasley & Roberts, 1996), but these techniques are somewhat limited when rivers are deep and turbid.

Side-scan sonar is a useful technology for examining features of aquatic systems without the requirement of direct observation and could aid mapping freshwater mussel locations. Side-scan sonar works by emitting conical acoustic signals toward the bottom and across a wide angle, perpendicular to the path of the sensor. These acoustic signals are then reflected back to the transducer and are relayed to the head unit where it stitches the information from the signal to produce a high-resolution two-dimensional image of the underwater landscape (Fish & Carr, 1990).

Uses of side-scan sonar have evolved over time due to technological advances. Side-scan sonar was developed in the 1960s; however, early use was primarily limited to oceans and large bodies of water because it required a big vessel to pull a very large towfish (transducer) through the water (Newton & Stefanon, 1975; Fish & Carr, 1990, 2001; Edsall et al., 1993) to chart navigational channels and identify debris along the bottom (Newton & Stefanon, 1975; Hobbs, 1985). In the last decade, side-scan sonar technology has advanced, leading to the development of smaller, relatively inexpensive units ($\sim$USD \$2000). These new side-scan sonar units operate at high frequencies (455 or 800 kHz) and produce high-resolution images (<10-cm pixel). With the recent decrease in size and cost, side-scan sonar technology has become more readily available and applicable to inland aquatic systems. Moreover, it has become useful in relatively shallow-water (<10 m) systems including rivers and streams. Recent applications include in-channel substrate and woody-debris mapping (Kaeser & Litts, 2008; Kaeser & Litts, 2010; Kaeser et al., 2012), and suggest the technology may have other applications in river and turbid aquatic systems (i.e., locating freshwater mussels). The objectives of this study were to (1) develop a series of reference images of freshwater mussels clustered in different substrates, and (2) assess the usefulness of side-scan sonar for locating freshwater mussels under different stream-habitat conditions.

Materials and methods

Study area

Side-scan sonar images were captured on portions of Lake McMurty and over a 32-km reach of the Muddy Boggy River (Fig. 1). Lake McMurtry is a 1,155-acre eutrophic reservoir located in Noble County, Oklahoma, USA. Lake McMurtry was impounded for flood control, and is used for water supply and recreation. Average turbidity of the reservoir is 20 NTU (OWRB, http://www.owrb.ok.gov/quality/monitoring/bump/pdf_bump/Current/Lakes/McMurtry.pdf, Accessed March 31, 2014). The Muddy Boggy River is a major tributary of the Red River. The catchment drains 6,291 km^2 including rugged terrain in the headwaters that transition to gentle hills with a wide valley in the lower catchment (Pigg, 1977). The Muddy Boggy River meanders through three major ecoregions but the study reach was located in the South Central Plains ecoregion where dominant soils are calcareous sands, clays, and gravels. The Muddy Boggy River has a dendritic drainage pattern and a gradient that ranges from 7.9 to 26.4 m/km (Pigg, 1977). The study reach was selected because it is known to currently support freshwater mussel beds (Powers, Unpublished data) and includes several deep pools (>2 m), separated by run and riffle complexes. Dominant substrate varies from coarse (e.g., cobble) to fine (e.g., clay) materials. This reach of the Muddy Boggy River was ideally suited for this study because its physicochemical characteristics make traditional freshwater mussel

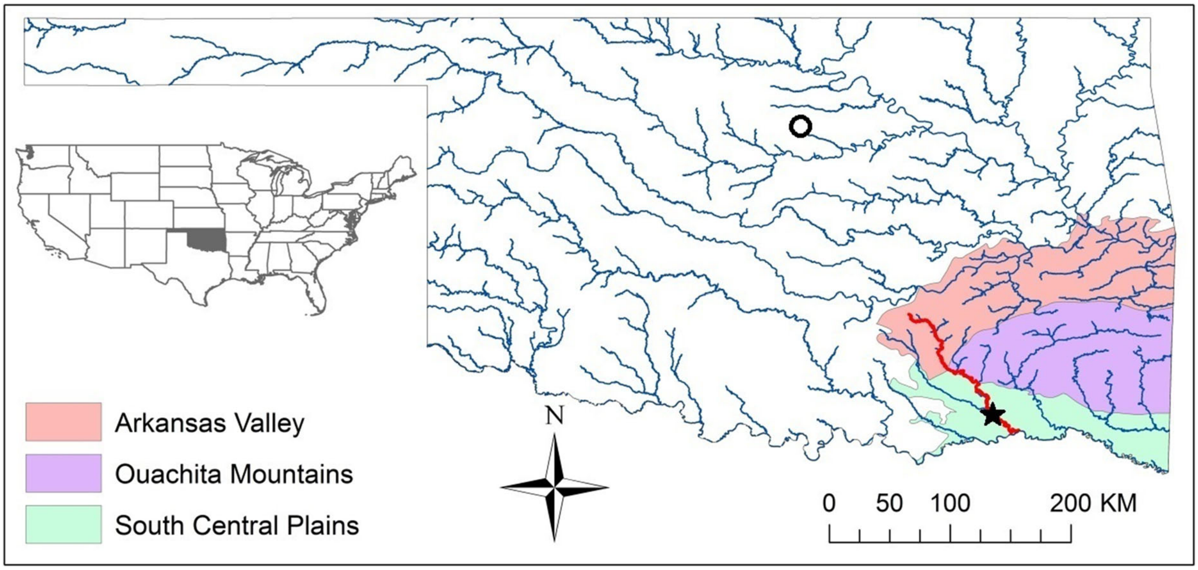

Fig. 1 Lake McMurtry (*open circle*) where reference images of placed mussel shells were developed using side-scan sonar and the Muddy Boggy River where the 32-km mussel-bed survey was conducted with side-scan sonar

sampling difficult. The river carries high suspended sediment loads even during base-flow conditions and has an abundance of instream woody debris.

Development of reference images

We developed a series of reference images using a side-scan sonar system (Humminbird® 1198c SI system, Eufaula, AL, USA) by scanning areas of a reservoir with and without freshwater mussel shells (Lake McMurtry, Stillwater, Oklahoma, USA, Fig. 1). We selected several 9-m^2 areas dominated by (estimated visually, the mean percentage and particle diameter in parentheses): silt (90%, <0.1 mm), sand (90%, 0.1–2 mm), gravel (85%, 2–50 mm), and cobble (85%, 50–250 mm), and scanned each area multiple times to capture images with and without mussel shells. Water depths within the 9-m^2 area ranged 0.8–1.2 m. Multiple scanning passes were made directly over the area and at varying distances (5 and 15 m) from the outside edge. We placed 50 mussel shells (matching right and left valve were bound together but did not contain living tissue) of multiple species and sizes throughout the selected 9-m^2 area (Table 1). All shells were used for each reference scan and were buried 2/3 to 3/4 in the substrate leaving the posterior portion of the shell protruding to reflect how a mussel would be positioned naturally (Allen & Vaughn, 2009). We examined the characteristics of the reflected properties at these known mussel-bed locations looking for commonalities in the images to apply to unknown areas.

Side-scan sonar mapping and processing

Side-scan sonar was used during base-flow conditions in July 2012 and elevated discharge in May 2013 to capture images of potential mussel beds. The surveys coincided with the freshwater mussel reproductive period (April through July) when mussels were more likely to be at the substrate surface (Galbraith & Vaughn, 2009). Side-scan surveys were completed in 1–2 days so discharge conditions would be relatively constant on each scanning day.

The side-scan sonar unit was set up to reduce image distortion and capture as much detail as possible in the images. Side-scan surveys were conducted with the transducer mounted on the front of a canoe to prevent the wake from causing image distortion (Kaeser & Litts, 2010). A 3.5 hp outboard motor was used to power the canoe at a relatively constant speed of approximately 6.5 kph to capture consistent sonar imagery. Prior to imagery capture, we compared multiple scanning frequencies: low (83 kHz-down-facing beam and 455 kHz-side-scan beam), high (200 kHz-down-facing beam and 800 kHz-side-scan

Table 1 Freshwater mussel species and mean length and width (mm, range in parentheses) of shells used for reference images

Species		Length	Width
Amblema plicata	Threeridge	194.89 (120.66–225.55)	71.04 (55.40–76.89)
Fusconaia flava	Wabash pigtoe	77.14 (74.35–79.93)	41.81 (41.49–42.14)
Lampsilis cardium	Plain pocketbook	89.19 (78.82–99.43)	48.10 (43.01–56.31)
Lampsilis teres	Yellow sandshell	96.62 (83.00–110.19)	38.47 (32.62–43.65)
Leptodea fragilis	Fragile papershell	107.24 (90.84–121.59)	36.82 (29.28–42.5)
Megalonaias nervosa	Washboard	169.24 (122.68–204.91)	58.06 (47.01–66.89)
Obliquaria reflexa	Threehorn wartyback	64.39 (63.84–65.43)	38.41 (37.03–40.33)
Potamilis purpuratus	Bleufer	130.72 (95.17–149.84)	56.42 (43.06–66.02)
Quadrula quadrula	Mapleleaf	70.40 (70.40)	38.34 (38.34)
Tritogonia verrucosa	Pistolgrip	98.92 (98.92)	27.77 (98.92)

beam), and a combination of the two frequencies (83 kHz-down-facing beam and 800 kHz-side-scan beam, and 200 kHz-down-facing beam and 455 kHz-side-scan beam). The optimal scanning frequency is a balance between capturing the entire bottom of the stream channel and obtaining high-quality image resolution. For our purposes, which required locating small mussels, we used high-frequency scans (down-facing beam-200 kHz, and side-scan beam-800 kHz) to evaluate the ability of side-scan sonar to identify mussel bed locations (Fig. 2). During side-scan sonar surveys, all images were captured from approximately a mid-channel position. Captured side-scan images were recorded as video files and the corresponding Global Positioning System (GPS) coordinates were recorded to a secure digital high capacity (SDHC) memory card in the side-scan head unit for post-processing.

Side-scan video images were imported into Dr. Depth® software (DrDepth, Göteborg, Sweden) and processed into a complete static, geo-referenced image mosaic. Mosaic settings for the internal map size were set to 3.125 cm pixel size to maximize resolution, converted to a map image, and saved as a keyhole markup language (.kml) file. Map images were imported into ArcMap 10 (Environmental Systems Research Institute, Redlands, CA, USA), georeferenced to aerial photographs and converted to a grid file for map-image evaluation in ArcMap (Hook, 2011).

Field validation

Using the reference images as a guide, we determined putative mussel-bed locations in the Muddy Boggy River from the side-scan imagery. Each of the 94 identified locations was assigned to one of the three categories (high, intermediate, and low) as a potential mussel bed based on how closely the location images matched our reference images. We haphazardly chose a subset of these potential sites ($n = 17$) for field validation. Validation sites were located using GPS coordinates of the upstream and downstream locations of the possible bed location. A 5-m buffer was added to the perimeter of the site to account for GPS error and ensure complete sampling. Field validation used two approaches: divers using self-contained underwater breathing apparatus (SCUBA) in deep water (>1 m) and tactile snorkeling in shallow water (<1 m). Three to four individuals were approximately evenly spaced across the deep portion of the river channel. Divers searched the river bed using tactile searches as visibility was extremely limited (<10 cm). In addition, tactile searches via snorkeling were performed in shallow-water sections (≤1 m, often the inside bend of the river) by three or four additional individuals to ensure adequate coverage of each site. We recorded the presence of any mussel shells, species, and approximate density within the area sampled. We defined a mussel bed as an area with a minimum of one mussel per m^2.

Habitat parameters

Habitat characteristics were measured at each of the 17 field validation sites. We haphazardly measured depth (1.0 cm) at 3–6 points and recorded temperature (°C) at each site. Dominate substrate type was determined at each site using a modified Wentworth scale (gravel 2–15 mm, pebble 16–63 mm, cobble 64–256 mm,

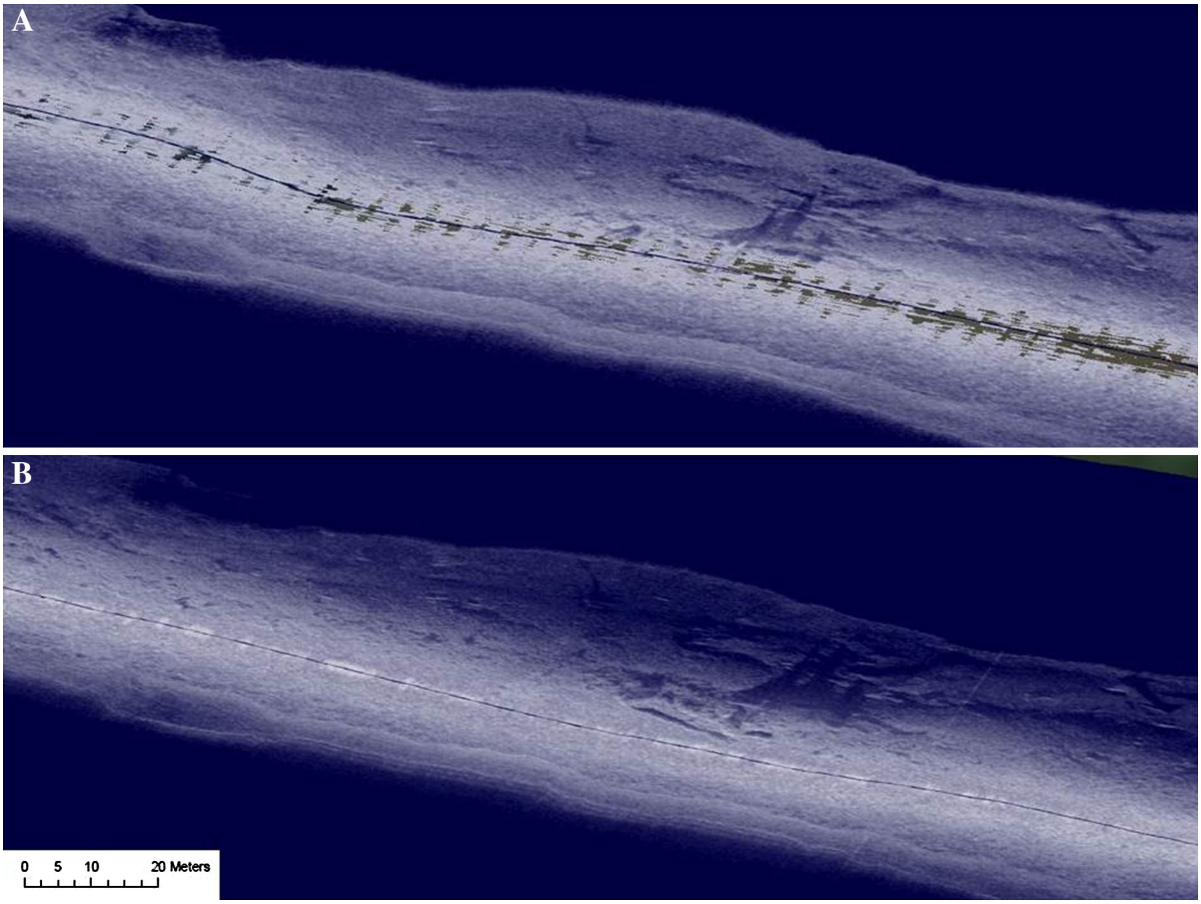

Fig. 2 Side-scan images of a selected area using two different frequencies for image capture. **A** image captured at 455 kHz frequency and **B** image captured at 800 kHz frequency

boulder >256 mm, and bedrock; Bovee & Cochnauer, 1977). We measured average water-column velocity at 0.6 from the water's surface (if water depth <0.8 m) or averaged measurements from 0.2 to 0.8 from the surface (when water depth $\geq$0.8 m) using an electromagnetic flow meter (Marsh McBirney, Loveland, CO, USA). Mean depth and velocity and the coefficient of variation were calculated from subsamples taken at each site. Bankfull width (0.10 m) and bankfull depth (0.10 m) were measured one time at each site following methods of Gordon (2004).

Statistical analyses

We developed a logistic regression model to determine what habitat factors related to positive detection of mussel beds via evaluation of side-scan sonar images. Analyses were conducted using Statistical Analysis Systems (SAS Institute, Carey, NC, USA). Explanatory variables were first evaluated for multicollinearity using Spearman's rank correlation coefficient procedure to exclude highly correlated variables ($r \geq 0.30$, Graham, 2003) from the final model. To prevent bias when examining multicollinear variables, we selected a subset of correlated variables for model building that we hypothesized would have the most influence on mussel-bed locations. Additionally, we excluded variables if there was very little variation in the measurements across study sites. The final set of variables was used to create a logistic regression model using forced entry (forced logistic regression, Colombet et al., 2001). If the model was significant, standardized coefficients were calculated to determine the importance of the explanatory variables in the model. The interaction between depth and sinuosity was fit to an additional model to assess if the influence of depth might depend on stream

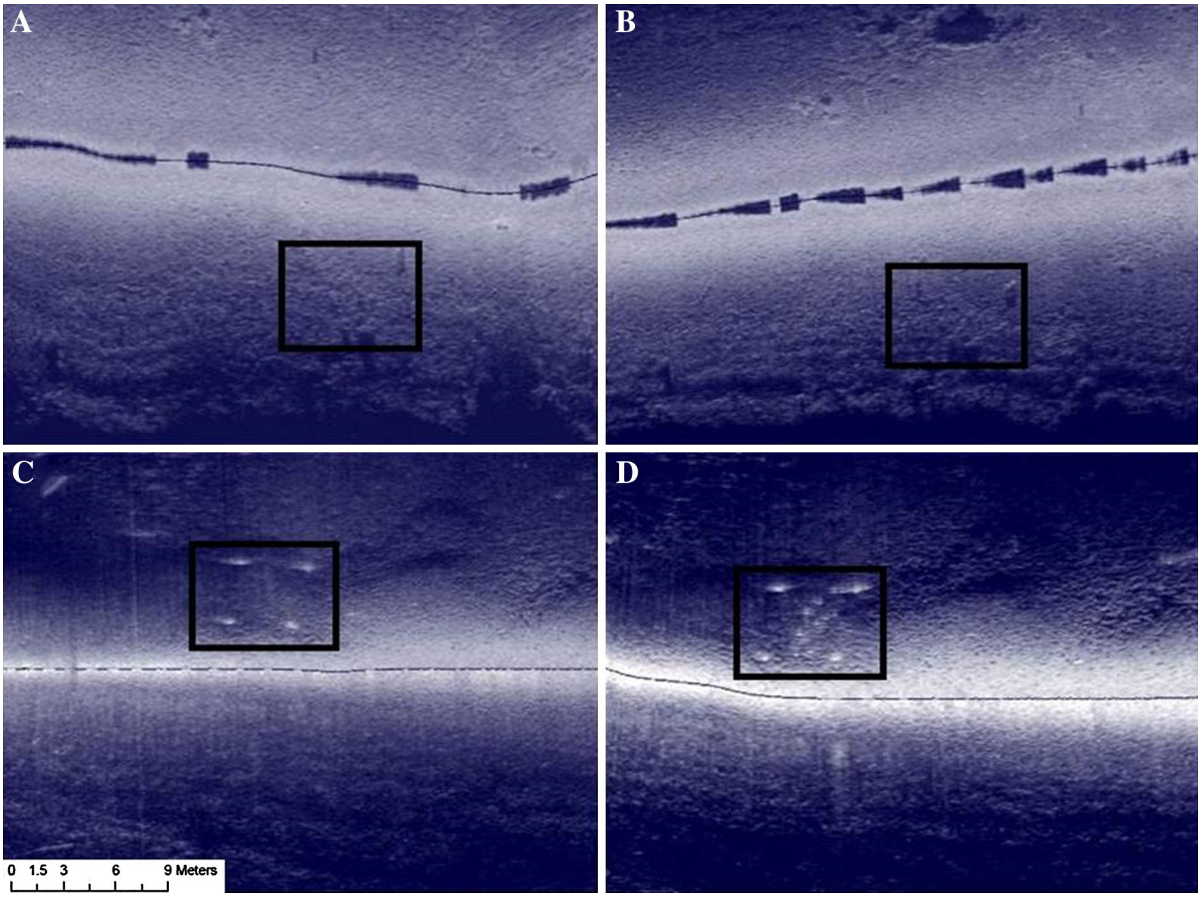

Fig. 3 Side-scan images including 9-m^2 areas of Lake McMurtry, Oklahoma containing: **A** coarse substrate with no mussel shells, **B** coarse substrate with mussel shells, **C** fine substrate with no mussel shells, and **D** fine substrate with mussel shells. The four *white images* in **C** and **D** are reflectance from T bars outlining the sample area

sinuosity. We completed diagnostic procedures using residual plots (Pearson and Deviance) to identify observations not well explained by the model. We also examined influence statistics (DFBETA, DIFDEV, and DIFCHISQ) to measure changes in the coefficients if an observation was deleted (Allison, 1999). These statistics allow the influence of individual observations on the model outcome to be examined to prevent undue influence from limited observations. The Hosmer–Lemeshow test is often used to evaluate model fit via logistic regression but is not appropriate for very large or small data sets. Therefore, we evaluated model fit using the *c*-statistic, values range from 0.5 to 1.0 where values near 0.5 suggest poor model fit and values near 1.0 indicates the model classifies cases very well (Field & Miles, 2010).

Results

Reference imagery

Using the captured images of mussel shells within varying substrate types, we were able to create reference images based on the reflectance characteristics (signal reflected off objects at varying strengths apparent in the image captured) of the shells. Mussel shells placed in coarse substrates (i.e., pebble and cobble) and also fine silt were nearly impossible to identify from the surrounding substrates (Fig. 3); however, we were able to easily distinguish mussel shells placed within sand and clay. Mussel shells were clearly visible as a cluster of white dots scattered within the fine substrate (Fig. 3d).

Side-scan sonar mapping

Captured side-scan sonar survey imagery of the two sampling periods (July 2012 and May 2013) revealed that images captured at elevated discharges were more complete and provided more image detail than images captured at base-flows. Images captured at base-flow conditions often lacked complete bank to bank coverage and had gaps in image capture (e.g., riffles and runs with extreme low flow). Survey images captured at elevated discharges provided a more complete picture of the stream bed and allowed for better identification of potential mussel beds. Both survey images were used to identify potential mussel beds, however, the images recorded during May were more likely to contain areas that had similar reflectance properties to that of our mussel-bed reference images.

Field validation

Overall, field validations proved to be effective for locating mussel beds, but were not improved based on our potential mussel-bed classification (i.e., high, medium, low). Field validations revealed approximately 60% (10 of 17) of sites were confirmed to be mussel beds. However, four additional locations (14 of 17) had living mussels, mussel shells, or both present but did not fit our definition of a mussel bed. Our qualitative classification of likelihood of finding potential mussel beds proved to be ineffective: low 56% (5 of 9 sites confirmed as a mussel bed), medium 75% (3 of 4 sites confirmed as a mussel bed), and high 50% (2 of 4 sites confirmed as a mussel bed). A mussel bed was as likely to be found in an area ranked as low potential as one ranked as high potential.

Habitat associated with mussel-bed presence

Spearman's rank correlation coefficients indicated several habitat variables (58% of all possibilities) were multicollinear ($r \geq 0.30$, Table 2). Bankfull width and depth, and substrate were highly correlated and therefore not included in the final model. Width:depth ratio (W:D) was not highly correlated with substrate so it was chosen to represent bankfull characteristics. Although velocity and temperature were not highly correlated with the remaining variables, they were excluded from the final model due to limited variation across sites (i.e., velocity range: 0.01–0.03 m/s, temperature range: 28–31°C). Other retained variables were depth and sinuosity. These variables were chosen because we anticipated they were more likely to influence mussel-bed presence (e.g., reach scale factors are better predictors than microhabitat factors, McRae et al., 2004; Strayer, 2008).

Residual plots and influence statistics indicated one observation with a major influence on the regression parameters (deviance value was 6.95), so we removed this observation and fit an additional logistic regression model. However, the new model did not change in significance or improve fit. The likelihood ratio test for depth and sinuosity interaction was not significant ($P = 0.11$), and not included in the final model.

Our final logistic regression model indicated only depth was significantly related to our ability to detect mussel beds using side-scan sonar (Table 3). Our ability to accurately identify potential mussel beds was greatest at water depths of approximately 1 to 2 m (83%, 10 out of 12 sites confirmed as mussel beds), whereas our ability to accurately identify potential mussel beds decreased in the deepest areas sampled (2 to 3.4 m, 45%, 5 out of 11 sites confirmed as mussel beds). Model fit was considered to be very good (c-statistic $= 0.91$).

Discussion

We have shown that side-scan sonar can be a useful tool for assessing potential freshwater mussel beds over a broad area and under environmental conditions where traditional sampling may be difficult or impossible. This is one of the first studies that we are aware of that used an inexpensive side-scan sonar system in a river to locate freshwater mussel beds. Our results are similar to a study that used a large and expensive side-scan sonar unit with towfish to accurately map ($\sim$80%) zebra mussel *Dreissena polymorpha* coverage on substrate in Lake Erie (Haltuch et al., 2000). However, some refinement to the methodology presented in this paper would be helpful to improve detection. For example, our ability to accurately identify mussel beds diminished at water depths greater than 2 m. We hypothesize this may be caused by how the side-scan sonar sound signal is reflected from the mussel shells due to incident angle. In shallow-water habitat, the signal is more likely to be reflected at a horizontal path, whereas in deeper water the signal would travel a more oblique path such

Table 2 Matrix of *r*-values for Spearman's rank correlation coefficient of mussel bed habitat variables

	Depth	BFD	BFW	Sinuosity	W:D	SS
Substrate	−0.10	−0.02	−0.32*	0.65*	−0.06	−0.02
Depth		0.26	0.21	−0.04	−0.10	0.26
BFD			0.01	0.53*	−0.84*	1.00*
BFW				−0.33*	0.44*	0.01
Sinuosity					−0.49*	0.53*
W:D						−0.84*

Values of 0.30 or more are considered multicollinear and indicated by asterisks

BFD bank full depth, *BFW* bank full width, *W:D* width to depth ratio, *SS* shear stress

Table 3 Model output values of beta, standard error, odds ratio, and confidence intervals for model relating habitat conditions with the presence of mussel beds as observed by side-scan sonar samples

	B	SE	95% CI for odds ratio		
			Lower	Odds ratio	Upper
Intercept	14.89	10.14			
Depth*	−5.97	3.52	<0.001	0.003	2.54
W:D	−0.08	0.28	0.53	0.92	1.61
Sinuosity	−0.69	1.55	0.02	0.50	10.47

Significant variables are indicated by asterisks

that much of the reflected energy is directed away from the transducer. Several of the potential mussel bed areas identified during field validation were deep pools with silt substrates. Silt sediments can degrade image quality due to a loss in energy of backscatter (Degraer et al., 2003; Dartnell & Gardner, 2004; Collier & Brown, 2005), and this was an issue we also encountered when creating our reference images in silt substrates (~90%). Additionally, deeper pools typically have homogeneous substrates; however, isolated amounts of coarse substrates may appear as mussel reflectance increasing false-positive results. Our reference images developed under relatively homogenous conditions in a reservoir suggest that substrate is a major factor to detecting mussels; however, W:D ratio (highly correlated with dominant substrate) was not a significant factor related to our ability to detect mussels in the scanned images. Increased heterogeneity within the river channel is a probable reason why riverine factors were more difficult to determine with our logistic regression model. We suggest more intense habitat mapping (e.g., substrate at each 1-m area scanned rather than dominant substrate across a channel unit) would provide more insight. Other physical factors that we did not measure may also be important determinants of useful side-scan sonar images (e.g., woody debris, microhabitat substrate mapping, and suspended sediment).

Side-scan sonar can help managers safely locate freshwater mussels over extensive areas that may be too difficult or dangerous to sample using traditional techniques. Traditional sampling for freshwater mussels involves intensive visual and tactical searches of an aquatic system (Miller & Payne, 1993; Beasley & Roberts, 1996; Hastie & Cosgrove, 2002). In some cases, only certain habitat areas are sampled in an attempt to target habitats perceived to be suitable for mussels (Metcalfe-Smith et al., 2000). Additionally, some areas are targeted because of ease of sampling over other habitats (Smith et al., 2003). Traditional mussel sampling can be difficult if not impossible in systems that are deep and turbid (Isom & Gooch, 1986). Visual searches cannot be performed in very turbid water and instead, the investigator must rely on tactile searches to locate mussels. In deep-water systems, SCUBA may be required and multiple divers needed to ensure safety (Isom & Gooch, 1986; Metcalfe-Smith et al., 2000). Side-scan sonar could be a helpful tool to allow a cursory examination of hazardous areas without needing to spend much time in the water. Follow-up sampling can then be used to target locations where mussels are likely to occur to gain information on assemblage structure and population dynamics.

Using a tool to target intensive sampling locations can be useful when directing limited resources. In our study, a two-person team could survey a 32-km reach with side-scan sonar in approximately 5 h (~6.5 km per hour), whereas labor-intensive field sampling of an area of similar size (34 km) can take 47 person days (0.09 km per hour) to complete (Christian & Harris, 2005). Although time spent in the field using side-scan sonar is substantially less when compared to traditional sampling, processing the sonar data took an additional 40 to 60 h ($\sim$1.5 h per km); however, user experience can substantially decrease this time. These times vary depending on habitat conditions and the speed traveled when sonar data are collected. In addition, side-scan sonar can be used to gain a general idea about substrate size and location of major underwater structure within a reach (Kaeser & Litts, 2008; Kaeser et al., 2012) that may be helpful when evaluating mussel-bed distributions. Quickly identifying underwater habitats associated with mussel beds allows less time in the field and more insight into potential environmental influences.

Side-scan sonar provides an inexpensive and effective method for locating freshwater mussels, though its application is limited. The side-scan sonar unit we used in this study cost approximately US \$2000, substantially less when compared to other side-scan units used for benthic mapping (Klein 595, $\sim$US \$20,000, www.l-3mps.com, Hewitt et al., 2004; CM 800, $\sim$US \$26,000, www.cmaxsonar.com, Hartstein, 2005; EdgeTech 4100, $\sim$US \$40,000, www.edgetech.com, Teixeira et al., 2013; Accessed March 31, 2014). We were able to successfully identify mussel-bed locations, but we were not able to distinguish species. We currently do not foresee an ability to identify mussels to species with this technology due to resolution constraints; however, if a particular species is known to occur in certain habitats or possesses characteristics much different than sympatric species, then surveys could target these locations or examine differences in reflectivity of the shells. Our ability to identify freshwater mussel beds using side-scan sonar was promising but also limited to moderate depths (1–2 m). We could improve our ability to detect mussels in deeper water by incorporating a towfish. There is readily available information about how the transducer can be modified into a towfish (e.g., http://forums.sideimagingsoft.com, http://bb.sideimageforums.com). Additionally, adding the transducer to a longer pole may allow for better image quality by reducing water depth between the transducer and the benthos. Further, times of year and discharge conditions during sampling are additional limitations. Many freshwater mussels remain beneath the substrate surface during winter months (Allen & Vaughn, 2009) making this period ineffective for locating mussel beds. Sampling during the reproductive cycle when adults are exposed above the substrate surface provides the best opportunity to capture sonar images of a mussel bed. Sampling during elevated-discharge conditions during the early tachytictic reproductive period (late spring, early summer; Graf & Foighil, 2000; Galbraith & Vaughn, 2009) would enable image capture of the entire channel in a single survey during ideal navigation conditions (Kaeser & Litts, 2010; Kaeser et al., 2012). Side-scan sonar surveys during low-flow periods of the bradytictic reproductive cycle (late summer; Graf & Foighil, 2000; Galbraith & Vaughn, 2009) would result in difficult and increased image distortion in shallow water.

Taking the proper steps to refine sonar image capture quality will improve the clarity and reliability of side-scan sonar images while improving the probability of mussel-bed detection. First, frequency settings may need to be adjusted for different bodies of water. A high frequency of 800 kHz provides for the greatest resolution for image capture, but can limit stream width captured by a single image ($\sim$35 m for the current study). Wider streams may require a lower frequency to capture bank to bank images but the resolution of the data would be reduced. Kaeser et al. (2012) reported that a frequency of 455 kHz allowed for image capture of a stream up to 98 m wide (49 m on each side of the transducer). Sampling wider streams, while maintaining adequate image detail, would likely require two complete passes to adequately capture images of each bank. Multiple side-scan sonar surveys would also allow for cross comparison among recorded sonar images. Comparisons among multiple side-scan images can help validate potential mussel-bed locations if the same mussel bed is present in multiple images even when habitat conditions have changed.

We provided initial reference images for other investigators; however, more images would be helpful under controlled environmental conditions. In particular, we suggest developing a series of reference images to distinguish shell characteristics in more heterogeneous habitats. We found we could clearly identify mussel shells in homogenous fine substrates (excluding fine sediment), which agrees with Haltuch et al. (2000), but our commission errors likely resulted from some coarse substrates at misidentified sites. One possible way to improve detections would be to conduct multiple scans during winter when mussels are beneath the substrates and then re-scan when mussels emerge for reproduction and assess images for discrepancies. This might provide a helpful approach as long as major floods have not reworked the alluvium between scans. Additionally, multiple side-scan sonar surveys of a study area over a short period of time would likely improve detection accuracy. We anticipate the refinements made by sampling multiple passes over multiple seasons will increase the accuracy of detecting mussels in turbid environments making side-scan sonar more broadly applicable to freshwater environments. However, the reference images provided in the current study can be used to examine mussel beds in other aquatic environments if species have similar shells and the riverbed is dominated by similar substrate conditions. Additional reference images from rivers with differing morphologies or containing a different assemblage would also be beneficial.

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