DETECTABILITY AFFECTS THE PERFORMANCE OF SURVEY METHODS - A COMPARISON OF SAMPLING METHODS OF FRESHWATER MUSSELS IN CENTRAL TEXAS

by

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ABSTRACT

Designing effective surveys for freshwater mussels (Unionidae) is a challenge, because they are spatially clustered and often found in low densities. The objective of this study was to examine how the effectiveness of three different survey methods (timed searches, transect method, and adaptive cluster method) varied between different habitats at six sites in the San Saba, Guadalupe, and San Antonio Rivers in Central Texas. Species richness, the total number of mussels per search effort, species composition and size distribution obtained with different survey methods were compared between sites. Timed searches were generally the most effective method in detecting species especially when densities were low (≤ 0.2 individuals per m^2) or mussels were highly clustered. The adaptive cluster method, however, was as effective as timed searches in detecting species when densities were moderate or higher (>2 ind. per m^2) and detected more species than timed searches at a site at which habitat conditions hindered searches. The performance of adaptive cluster in respect to number of mussels found per unit search effort seemed to be enhanced by sandy substrate facilitating the detection of mussels, and timed searches were less effective at sites at which habitat conditions hindered the detectability of mussels. Differences in detectability of mussels was not only associated with habitat conditions, but also with the size of mussels, their behavior and morphology. Timed searches detected a larger proportion of larger mussels that tended to be less burrowed and that had shells with more sculpturing compared to quantitative methods. In addition, surveyors with more search experience detected a larger number of mussels. Our results

suggest that to design effective surveys variation in detectability of mussels must be considered which depends on local habitat conditions, experience of surveyor, behavior, size and morphology of mussels.

I. INTRODUCTION

Freshwater mussels can play an important role in the functioning of freshwater ecosystems affecting water clarity and chemistry by filtering water, providing physical habitat for other organisms, and enhancing benthic algae and macroinvertebrates (Vaugh et al. 2008). However, populations of freshwater mussels have globally declined (Lopes-Lima et al. 2014). Globally, the highest diversity of Unionida exists in North America, where they are one of the most imperiled group of organisms (Haag 2012); Texas alone is home to approximately 50 native unionid species (Burlakova et al. 2011). Currently, Texas Parks and Wildlife Department (TPWD) has listed 15 of these species as threatened and one species (*Popenaias popei*) has been listed as endangered under the Endangered Species Act (Texas Register 35 2010). Declines of freshwater mussels in Texas and elsewhere in North America have been attributed to habitat loss and degradation, pollution, dewatering (groundwater pumping), and to impact of dams (Burlakova et al. 2011, Haag 2012, Inoue et al. 2014, Randklev et al. 2015).

A critical part of the successful conservation of mussel communities is a reliable account of their distribution and abundance. Mussels have a highly patchy distributions and rare species often occur at low densities (Strayer 1999, Pooler and Smith 2005, Strayer and Smith 2003, Dickson 2000). Designing an efficient sampling scheme for rare and clustered populations is challenging in general (Salehi and Smith 2005), and the patchy nature of mussel populations in particular presents substantial challenges to field sampling for population enumeration and species detection, and the costs associated with these efforts in terms of person-time and effort can be expensive. Currently, there is no standardized and accepted protocol for sampling mussels in Texas. There are, however, a number of studies which have described and compared different sampling methods for uninoids (e.g., Hornbach and Deneka 1996, Vaughn et al. 1997, Obermeyer 1998, Mecalfe-Smith et al. 2000, Smith et al. 2001, Villella and Smith 2005). Timed searches are semi-quantitative, less expensive and less time consuming than quadrat sampling and provide quick exploration of larger areas and a variety of habitats (e.g., Metcalfe-Smith 2000). Several studies have found that timed searches tend to have higher rates of detection of rare species compared to quantitative searches (Hornbach and Deneka 1996, Vaughn et al. 1997, Strayer et al. 1997, Obermeyer 1998, Smith et al. 2001), but also larger individuals and species (Hornbach and Deneka 1996, Vaughn et al. 1997, Obermeyer 1998) and mussels with sculptured shells (Miller and Payne 1993, Vaughn et al. 1997, Obermeyer 1998). It is crucial to set an adequate search time to obtain reliable estimates of mussel community composition (e.g., Metcalfe-Smith 2000), which can differ substantially between different habitat types and conditions (Smith et al. 2000).

To obtain density or demographic data quantitative methods need to be used (Vaughn et al. 1997, Dickson 2000), but these methods may underestimate unionid species richness with the exception of very small mussel beds (Vaughn et al. 1997, Hornbach and Deneka 1996). Because the patchy distribution of mussels, a potentially large number of sampling units (e.g., quadrats) are needed to obtain relatively acceptable levels of precision e.g., for mussel density (Dickson 2000). Thus, random quadrat searches are often considered inferior to transect methods for estimation of some population-level parameters (Dickson 2000), which allow for quicker and more efficient searches than quadrats (Strayer and Smith 2003). In addition, excavation of materials in each quadrat is often necessary to obtain precise density estimates (Strayer and Smith

2003) because a considerable proportion of mussels may be completely burrowed (Schwalb and Pusch 2007). Adaptive cluster sampling is a different quantitative method, which allows investigators to concentrate their efforts where mussels occur, which is useful for populations that are rare and clustered (Salehi and Smith 2005, Smith et al. 2009). For instance, if one or more mussels is found in a quadrat, the four adjacent units quadrat areas of that quadrat are then searched. If mussels are found in any of those adjacent quadrats then their adjacent quadrats are search allowing the direction of searches to focus where mussels are.

Not all methods will work under different habitat conditions and surveys may not accurately measure mussel richness or miss rare endangered species (Strayer 2008). Thus, different habitat types may require different survey methods (Burlakova et al. 2011). The objective of this overall thesis was to evaluate the relative effort and effectiveness of three different unionid mussel survey methods (timed searches, transect method, and adaptive cluster method) and to examine how their effectiveness vary in different habitats in Texas rivers. Based on a review of the literature, we developed the following predictions: (1) Timed searches will be more effective compared to quantitative methods in detecting species presence (particularly rare species) and in finding a larger number of mussels (per unit search effort) especially when density is low and/or distribution is highly clustered. (2) Adaptive cluster will be more effective than transect method when patchiness is high, and density is low. (3) Adaptive cluster and Transect methods will detect smaller individuals and smaller species than timed searches. (4) Precision in density estimates will increase as the number of quadrats increases.

II. METHODS

Sites

Field studies were conducted at six riverine sites in the central Texas region: Guadalupe River (two sites), San Antonio River, Llano River, and the San Saba (two sites) River between Fall of 2016 and Summer 2017 (Fig. 1; Table 1). These sites covered three different ecoregions: Edwards Plateau (Llano and San Saba Rivers,), South Texas Plains (San Antonio River), and the Western Gulf Coast Plains (Guadalupe River). Study sites varied in size so and the same area of stream (50 m stream length and 10 m width) was used to perform the following sampling methods on the six sites:

A. Timed search Method

B. Transect Method

C. Adaptive Cluster Method

Sites were visited weekly or biweekly to allow the mussels to settle back into their habitat after surveys, and to ensure similar seasonal conditions. There were two exceptions to this sampling schedule caused by high water levels preventing access to the field sites. At the Llano River the third sampling was only possible two months after the second sampling, and in the San Antonio River the second sampling was performed five months after the first sampling (Table 1). A different order of methods was applied to each site to avoid potential bias (Table 1). All unionids contained within the area were collected, identified, enumerated and then returned to the riverbed to the approximate spot in which they were found.

Timed Search Sampling Method

Three surveyors initiated sampling of the downstream boundary of each study reach and moved together upstream covering as much habitat as possible within the entire site. Sites were searched using waders, wet suits, snorkels, underwater viewers, diving and weight belts were used in deeper waters to snorkel at the bottom, and mussels were collected in mesh bags. After each person-hour (p-H, number of people multiplied by time) it was determined whether new species were found or not. Timed searches were continued until no new species were found for three consecutive 1-p-H. In addition to these data, on one date (May 3, 2017) in the San Antonio site I recorded how many mussels were found by each surveyor (n = 3 surveyors) with varying experience to examine if the number of mussels found by a surveyor varied with the amount of previous experience searching for mussels (previous experience ranged from 3 months to 2 years).

Transect Method

To conduct a transect search method, nine 10m transects were set-up perpendicular to the flow at 5m intervals along 50 m stream length at each study site. At each transect five quadrat samples were taken. A 50cm x 50 cm quadrat was used for all sites except for the Llano and Guadalupe 2 site, for which 1m x 1m and 25cm x 25cm quadrats was used respectively (due to lower density at the former and higher densities at the latter site). During searches, substrate in each quadrat was excavated to a depth of up to 10 cm.

Adaptive Cluster Method

To conduct this search methodology, the entire search area of each site was divided into equal non-overlapping quadrat locations. The quadrat size used for each site was the same as the transect method (see above). Initially, three quadrat locations were chosen randomly and searched for mussels. If one or more mussels was found in a quadrat, the four adjacent quadrat areas were then searched and this was repeated until a total of n = 45 quadrats transect method). On two occasions, no mussels were detected in the initial 3 quadrats (Guadalupe 1 and the Llano site), and 22 random initial quadrats were added. Again, substrate in each searched quadrat was excavated to a depth of up to 10 cm. The dominant substrate type at each site was observed and average velocity at 60% of stream depth in the middle of the stream was measured.

Data analysis

Densities (mean number of mussels per m^2) were determined for each site based on the results of the transect method and was calculated for the adaptive cluster method for comparison. The Clumping Index (Cressie 1993) was used to examine differences in patchiness between sites, which is calculated as the variance/mean ratio -1 (of density estimates). To examine whether adaptive cluster method would result in significantly higher density estimates, a paired t-test was used. To determine the effect of using 4, 3, 2, and 1 quadrat per transect instead of 5, we used a bootstrapping approach, in which the dataset was re-sampled while restricting the number of sampled quadrats and repeated 1000 times. The coefficient of variation was calculated for each scenario.

III. RESULTS

Habitat Conditions

Substrate, depth and flow conditions varied considerably between sites (Table 2). Site depth ranged from 0.45-1.7m, with the deepest sites being Guadalupe 1 and San Saba 1. Among sites, substrate conditions ranged from predominantly bedrock (Llano) with gravel filled divots scattered throughout the site, to substrates composed of sand and cobble mixtures (San Saba 2 and San Antonio). At the San Saba 1 site, a large woody debris from previous flooding in the deeper middle (~1m depth) section of the river hindered searches, but quadrat searches were still possible because flow was relatively slow. Water velocity was fastest at Guadalupe 1, and substrate was predominately cobble. Guadalupe 2 had slow flow and substrate was predominately sand (Table 2).

The Llano and Guadalupe 1 sites had the lowest density (i.e. average density ≤ 0.2 individuals per m^2 as determined by transect method). Moderate densities were found at the San Saba sites and the San Antonio River (1.3 to 2.1 ind. per m^2, transect method), whereas the Guadalupe 2 site had the highest mussel density (7.1 ind. per m^2, Table 2). In terms of the patchiness of the mussel populations within a site, the San Antonio had the highest patchiness (clumping index = 1.1), and all other sites had clumping indices <0.5. Density estimates obtained with adaptive cluster were significantly higher compared to the transect method ($T_5 = 2.2$, $p = 0.04$). The biggest difference was found at the Llano site (20 times higher; Table 3), but is should be noted that considerably more than 3 random starts were used a the two sites with the lowest density (Llano and Guadalupe 1). As no mussels were found at Guadalupe 1 after 25 random starts, search with the adaptive cluster was discontinued. Densities estimated with the adaptive cluster method were about 4 times higher than the transect method at the San Antonio and San Saba 2 sites, about 3 times higher at Guadalupe 2, and 2 times higher at San Saba 1 (Table 3).

Species Richness

As predicted, timed searches detected a higher number of species than the transect method; however, timed searches did not necessarily detect more species than the adaptive cluster method (Fig. 2). The greatest number of species detected with timed searches compared to the transect method was most pronounced at the two sites with the lowest density (<0.2 mussels/m^2). For example, 4 species were found at the Guadalupe 1 site with the timed search method, whereas only 1 species were found with the transect method (no mussels were found with the adaptive cluster method, Fig. 2A). Timed searches were equally effective compared to transect method in detecting species at the site San Saba 2, but only two species were found at this site. Similarly, timed searches detected more species than the adaptive cluster method at the two sites with lowest density, and also at the site with the highest patchiness (Fig. 2B). In contrast, more species were found with the adaptive cluster method than the transect method at San Saba 1, where wooden logs and branches occurred in the middle of the river.

Species Composition

Differences between search methods were not only found for the number of species, but also their relative abundance. This was most obvious at sites where all methods found a similar number of species (i.e., Guadalupe 2, San Antonio, and San Saba sites). In general, timed searches found a higher proportion of larger-sized species. For example, a considerably higher proportion of *Tritogonia verrucosa*, (usually larger sized, average length=89 mm) was found with timed searches (50%, n = 134) at the San

Antonio site compared to adaptive cluster (37%, n = 97) and transect method (29%, n = 24). This was also the case at San Saba 2, where 74% of the mussels detected with the transect methods were *T. verrucosa*, whereas 55% of the mussels detected with the other two methods were *T. verrucosa*. Similarly, 15% of all mussels detected with the transect method at the Guadalupe 2 site were *Crytonaias tampicoensis* (also, larger sized ~82 mm), while the other two methods detected a lower proportion of that species (~5%). In contrast, a higher proportion of smaller species, e.g., *Cyclonaias aurea* (average length: 53 mm), were found with the adaptive cluster method (29%) compared to timed searches (5%) at the San Antonio site.

Number of mussels per unit search effort

In general, the adaptive cluster method tended to have a similar search effort compared to timed searches (mean 3.8 p-H; range =2.8-4.2 p-H) with the exception of the Guadalupe 2 site, where the adaptive cluster method took considerably less time ~2.8 p-H. In contrast, the transect method had the lowest search effort (mean of ~2.5 p-H; range = 1.5-3.7 p-H). In accordance with prediction 2, the adaptive cluster method was more effective at detecting a higher number of mussels per unit search effort than the transect method, especially at sites where patchiness was high (i.e., the San Antonio site) or density was high (i.e., the Guadalupe 2 site (Fig. 3 A), but also at the site with moderate density (San Saba 2, Fig. 3A) These three sites were also the only sites with sandy substrate. Timed searches were more effective than quantitative methods at most sites in respect to number of mussels per unit search effort (Fig. 3 B, C), but not at the sites with the gravel and cobble substrate (San Saba 1 and Guadalupe 1).

A comparison of the number of mussels found by surveyors with different levels of experience (ranging from >3 months to 2 years) showed that the number of mussels found increased with experience level (Fig. 4).

The bootstrapping analysis showed that the coefficient of variation (CV of density estimates) increased as the number of quadrats per transect was decreased from 5 to 1 (Fig.5). This was most pronounced for the site with the lowest density, the Llano site (CV up to ~250% with 9 compared to 45 quadrats, Fig. 5), followed by the site with second lowest density (Guadalupe 1, CV up to ~120%, Fig. 5). For the other four sites with moderate to high densities, CV ranged between 20-30% when the total number of quadrats were decreased from 45 to 27 and between 40-60% with 9 quadrats.

Mussel size

As predicted, there were indications that timed searches tended to be biased towards finding larger individuals and those that were less burrowed. Timed searches found a higher proportion (94%) of larger mussels (i.e., those with >60mm shell length) compared to the other two methods at the San Antonio (72%), and both San Saba sites. Only the transect and adaptive cluster method found smaller individuals (<48 mm) of the following species, *A. plicata, T. verrucosa*, and *C. houstonensis* at the San Saba sites. At the San Antonio site only the transect method found smaller species of *A. plicata* (<55mm), but all methods found smaller individuals of *T. verrucosa.* In contrast, at 2 of the 6 sites there was no difference in the size frequency of mussels detected with different methods (Llano and Guadalupe 2), and timed searches also detected smaller sized mussels at these sites.

IV. DISCUSSION

This is the first study in Texas that examined differences in the effectiveness of three different unionid survey methods across multiple sites in four different rivers. Our findings were generally in accordance with our predictions and the results from previous studies which compared qualitative and quantitative surveys (e.g., Hornbah and Deneka 1996, Vaughn et al. 1997, Obermeyer 1998). However, there were some notable exceptions that were associated with special local habitat conditions. Firstly, while timed searches clearly outperformed quantitative methods at most sites in respect to number of mussels per unit search effort, this was not the case at sites where searching for mussels was considered more difficult (i.e., rough gravel or cobbles hindering tactile searches) and therefore the detectability of mussels. Secondly, the adaptive cluster method was only more effective compared to the transect methods at sites where local habitat conditions facilitated the detectability of mussels (i.e., sandy substrate). This is in accordance with model simulations by Smith et al. (2010), which found that performance of adaptive cluster degraded as detectability declined. Thirdly, the adaptive cluster method only detected a larger number of species compared to timed searches at the site, where surveyors avoided an area with wooden logs in the middle of the river (which was difficult to search), but in which the additional species (*Cyclonaias apiculata* and *Amblema plicata)* were found with the adaptive cluster method. Thus, habitat conditions affect the performance of survey methods by facilitating or hindering the detectability of mussels.

It has been shown previously that the detection of mussels can vary with habitat conditions such as depth, water velocity/turbulent flow, and substrate (Meador 2008, Smith and Mayer 2010, Shea et al. 2013, Wisniewski et al. 2013), but this is the first study that shows how it can affect the relative performance of different survey methods. Differences in detectability of mussels cannot only be associated with habitat conditions, but also with the size of mussels, their behavior and morphology. This study found that timed searches tended to detect a higher proportion of larger species, such as *T. verrucosa* and a smaller proportion of smaller species such as *C. aurea.* These species do not only differ in their size, but also in their burrowing behavior and morphology. *C. aurea* burrows more deeply, whereas *T. verrucosa* tends to be less burrowed and can be sometimes found laying at the surface, e.g., a survey in April 2017 at the San Antonio site found that 47% of *T. verrucosa* were completely at the surface, 30% of *A. plicata,* but only 15% of *C. aurea* (Hernandez 2016, Zachary Mitchell, Texas State University, unpublished data). This study also found that timed searches found a larger proportion of larger species with sculptured shells such as *A. plicata*, which makes it easier to find the mussel within cobble and gravel with tactile searches. In contrast, quantitative methods found more burrowed, small, and species with smooth shells, which is consistent with findings by other studies (Hornbach and Deneka 1996, Vaughn et al. 1997).

Detection also depended on surveyor experience in this study, which has been previously shown by other studies (e.g., Wisniewski et al. 2013, Reid 2016). A study in the Flint River in Georgia, found that searchers with more experience tended to better recognize mussels from substrate, to be less affected by sampling fatigue, and better able to negotiate challenging sampling conditions (Reid 2016). Thus, in order to avoid incomplete detection and potential biases, training of field staff for the rigors of searcher fatigue and their ability to discriminate mussels in same size or larger sized substrate will be necessary.

When designing mussel surveys, it should be considered that the relative effectiveness of different survey methods varies between sites and rivers with different habitat conditions. The ideal search method for a given study and at specific sites will depend on the search goal (i.e., is the purpose to find as many number of mussels, find as many species, or find a certain species, or get an idea of the species composition of an area). Timed searches are especially useful when densities are low (e.g., Llano and Guadalupe 1) and when distribution is extremely, but predictably clustered. For example, timed searches allowed to focus on the pockets of gravel on bedrock at the Llano site and to avoid the deeper and faster areas where not mussels were found with the quantitative methods at the Guadalupe 1 site. It should be noted that our assumption was that densities would not change between sampling dates, but this may not have been the case at Guadalupe 1, where anglers were observed to use mussels as bait, and pile of shells of dead mussels were present at the shore.

Transect methods can be effective in obtaining density estimates, but the accuracy and precision will depend on the search effort, including the number of quadrats. The increase in the coefficient of variation for density estimates when the number of quadrats were reduced in the bootstrapping analyses indicated that there is a high likelihood that surveys in the same area would over-or underestimate mussel densities. This was especially pronounced at sites with lower densities. Although it was possible to calculate how the CV may change with a lower number of quadrats, a modelling approach would be necessary to predict how many quadrats may be needed to obtain reliable density estimates of mussels. For example, a modelling study of a mussel bed in the Upper Mississippi River showed that a low CV< 0.25 was achieved with samples sizes of >500 quadrats for populations with density ≥ mussels $0.2m^{-2}$ (Smith et al. 2009). For even lower density populations, sample size would have to increase even further to achieve similar precision, which may not be feasible. Thus, density estimates of mussel species that occur in very low densities should be considered with caution.

Findings from the adaptive cluster method cannot be easily translated into a density estimate for the search area, but it has the advantage to guide searches towards areas where mussels occur, including those avoided by searchers during timed searches (e.g., when obstacles hinder searches). As previously suggested (Villella and Smith 2005, Smith et al. 2011) a combination of timed searches and quantitative methods should suffice for most survey needs. To improve the performance of timed searches, searches should also be done in areas usually avoided by searchers, where searching is hindered by habitat conditions. In summary, our results suggest that to design effective surveys variation in detectability of mussels must be considered which depends on local habitat conditions, experience of surveyor, behavior and morphology of mussels, as well as size of mussels. Future studies should examine how the effectiveness of survey methods vary in rivers with a higher species richness and mussel densities and further examine the role of detectability for the performance of survey methods.

TABLES

Table 1. List of survey sampling days and methods applied.

Site	Visit 1	Date	Visit 2	Date	Visit 3	Date
Llano	Timed search	06/30/2017	Adaptive cluster	07/14/2016	Transect	09/12/2016
San Saba 1	Timed search	03/06/2017	Transect	03/15/2017	Adaptive cluster	03/22/2017
San Saba 2	Transect	03/06/2017	Adaptive cluster	03/15/2017	Timed search	03/22/2017
San Antonio	Adaptive cluster	12/01/2016	Timed search	05/03/2017	Transect	05/11/2017
Guadalupe 1	Timed search	07/07/2017	Adaptive cluster	07/14/2017	Transect	07/28/2017
Guadalupe 2	Transect	07/07/2017	Timed search	07/14/2017	Adaptive cluster	07/28/2017

Table 2 List of sites and their habitat characteristics (substrate, depth, and average velocity), and mussel density determined by the transect method.

Site	Mussel density (mean±SE) [Number of ind. /m^2]	Substrate	Depth	Average Velocity
Llano	0.02±0.02	Bedrock/gravel divots	0.5m	0.24 m/s
San Antonio	2.1±0.2	Sand/corbicula shells	0.45m	0.18 m/s
San Saba 1	1.3±0.1	Gravel/cobble/silt	1m	0.29 m/s
San Saba 2	2.0±0.5	Cobble/sand	0.45m	0.61 m/s
Guadalupe 1	0.2±0.03	Gravel/Sand	1.7m	1.1 m/s
Guadalupe 2	7.1±0.1	Sand	1.5m	0.03 m/s

Table 3 Species and density estimates for Adaptive cluster and Transect method.

Site	**Method**	**Species**	Density [mean ± SE]
Llano	Timed search	3 (*Lampsilis bracteata, Strophitus undulatus, Cyclonaias petrina)*	
	Adaptive Cluster	2 (*Lampsilis bracteata and Cyclonaias petrina)*	0.4±0.2
	Transect	1 (*Strophitus undulatus)*	0.02±0.02
San Antonio	Timed search	5 (*Amblema plicata, Lampsilis teres, Cyclonaias aurea, Cyclonaias petrina, Tritogonia verrucosa)*	
	Adaptive Cluster	4 *(Amblema plicata, Lampsilis teres, Cyclonaias petrina, Tritogonia verrucosa)*	9.3±0.5
	Transect	4*(Amblema plicata, Lampsilis teres, Cyclonaias aurea, Tritogonia verrucosa)*	2.1±.0.2
San Saba 1	Timed search	*4(Leptodea fragilis, Cyclonaias houstenensis, Cyclonaias petrina, Tritogonia verrucosa)*	
	Adaptive Cluster	5 (*Amblema plicata, Leptodea fragilis, Cyclonaias apiculata, Cyclonaias houstenensis, Tritogonia verrucosa)*	2.9±0.1
	Transect	3 (*Cyclonaias houstenensis, Cyclonaias petrina, Tritogonia verrucosa)*	1.3±0.1
San Saba 2	Timed search	2 *(Cyclonaias petrina, Tritogonia verrucosa)*	
	Adaptive Cluster	2 *(Cyclonaias petrina, Tritogonia verrucosa)*	7.6±0.5
	Transect	2 *(Cyclonaias petrina, Tritogonia verrucosa)*	2.0±0.1
Guadalupe 1	Timed search	4 (*Amblema plicata, Cyrtonaias tampicoensis, Lampsilis teres, Cyclonaias aurea*)	
	Adaptive Cluster	*Unable to apply method	
	Transect	1 (*Amblema plicata*)	0.2±0.03
Guadalupe 2	Timed search	3(*Amblema plicata, Cyrtonaias tampicoensis*, and *Cyclonaias aurea*)	
	Adaptive Cluster	3(*Amblema plicata, Cyrtonaias tampicoensis*, and *Cyclonaias aurea*)	20.9±0.2

Table 3 Continued

	Transect	3(*Amblema plicata, Cyrtonaias tampicoensis*, and *Cyclonaias aurea*)	7.1±0.1

FIGURES

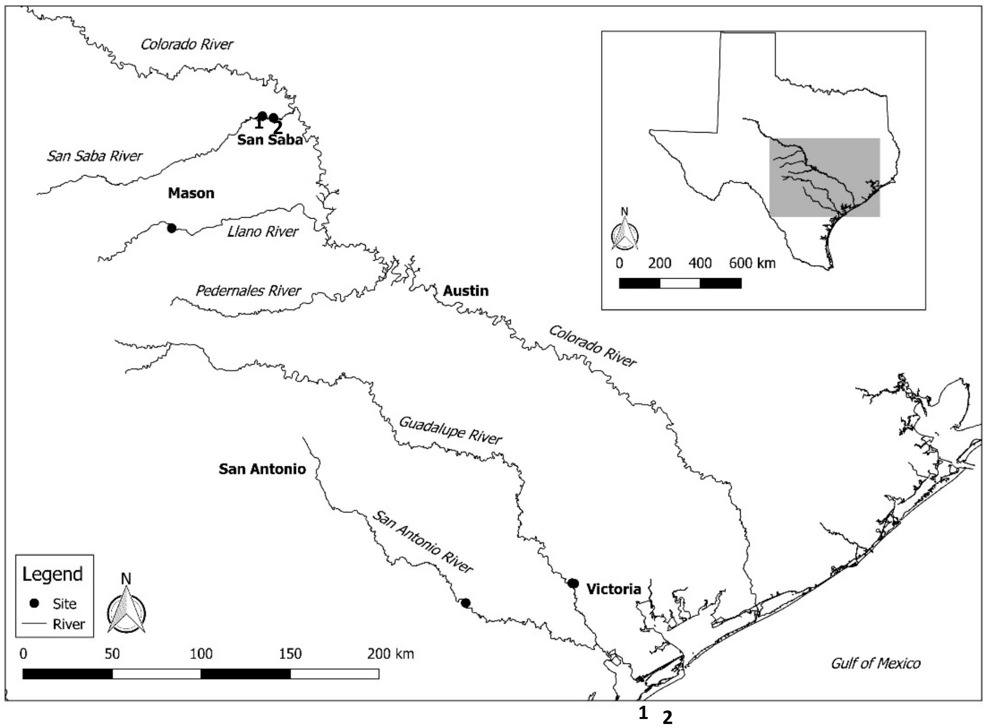

Fig. 1 Map of study sites in the San Saba River (2 sites), Llano River, San Antonio River, and Guadalupe River (2 sites)

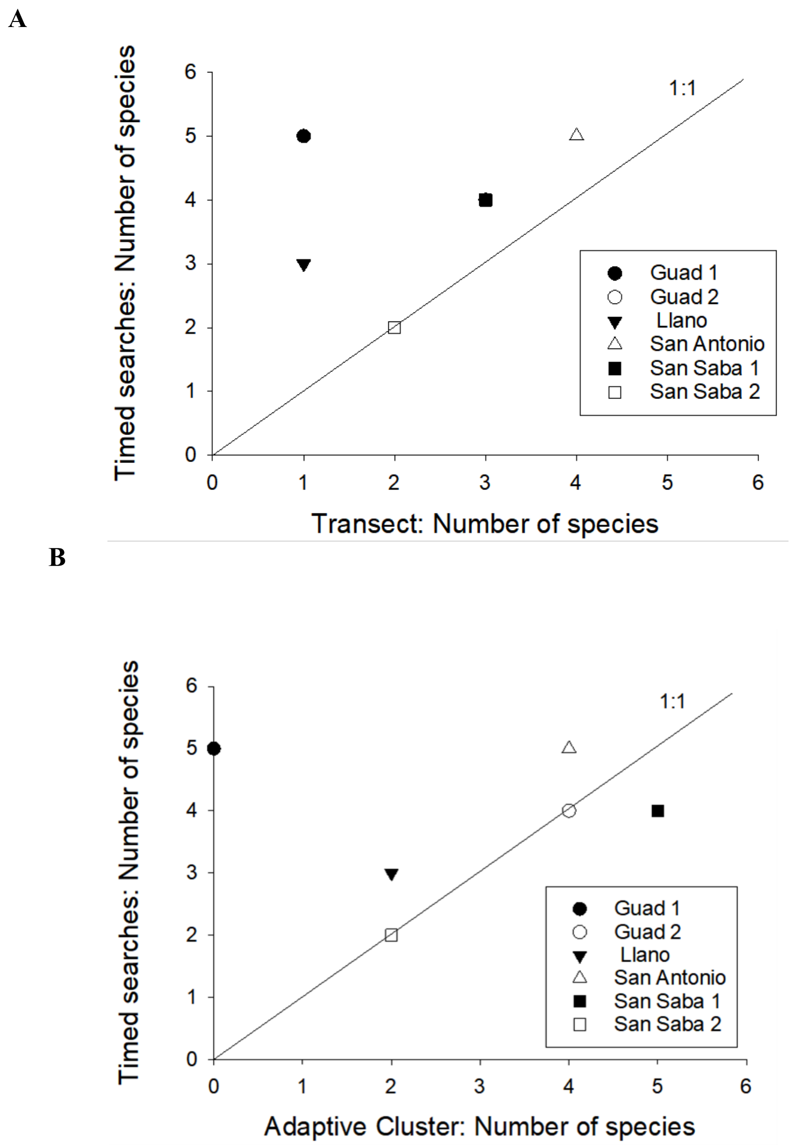

Fig. 2.Number of species found. A) Comparison between the transect method and timed searches and B) Comparison between the adaptive cluster method and timed searches. Different symbols indicate different sites (Guadalupe 2 is “below” San Saba site 1 in panel A).

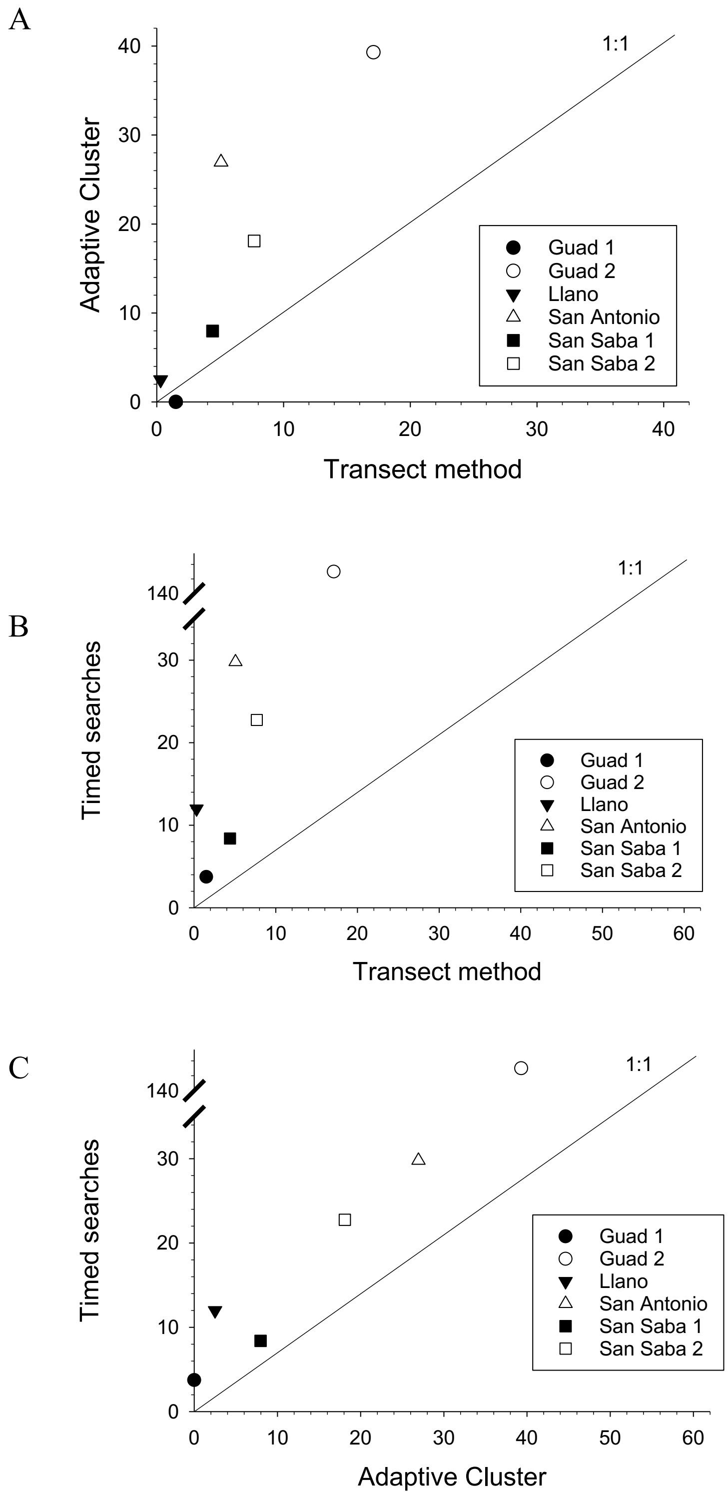

Fig. 3 Number of mussels found per unit search effort (p-H). A) Adaptive cluster vs. transect method, B) timed searches transect method, C) timed searches vs. adaptive cluster methods. Different symbols indicate different sites.

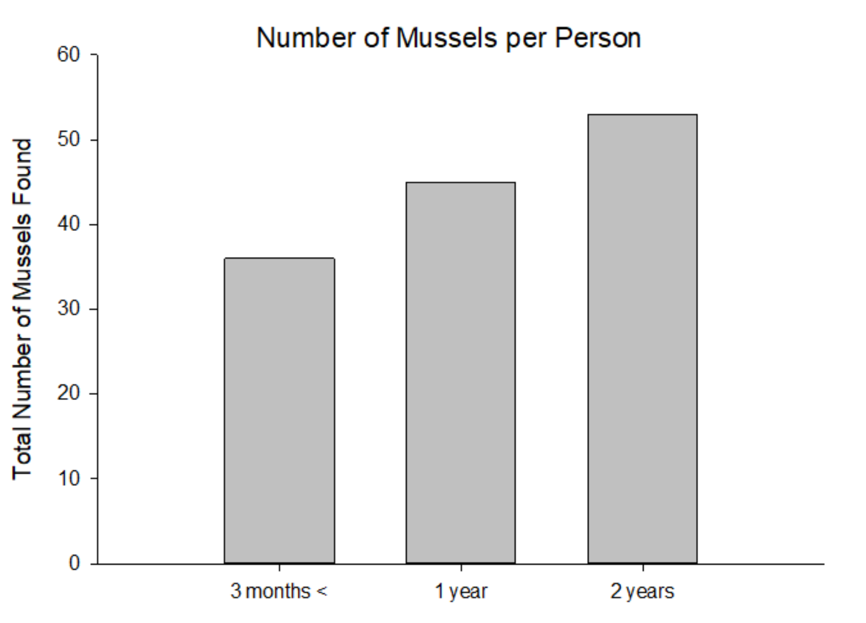

Fig. 4 Surveyor experience: Number of mussels per person during a timed search performed at the San Antonio River on May 03, 2017.

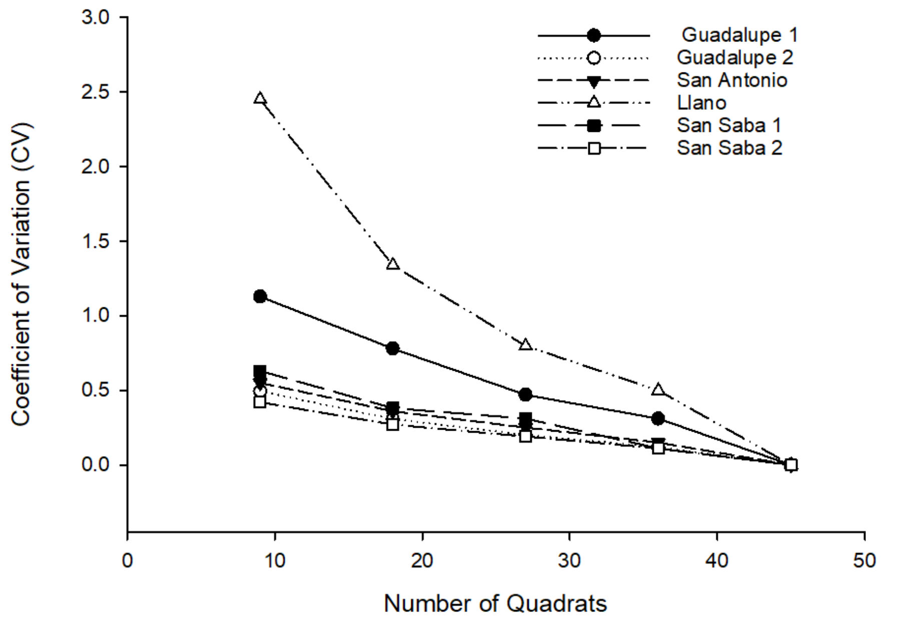

Fig. 5 Coefficient of variation for different numbers of quadrats (i.e. 1, 2, 3, 4, and 5 quadrats for each of the 9 transects). The data obtained with the transect method was re-sampled with a boot-strapping method.

APPENDIX SECTION

Table A1 Summary of findings of research comparing semi-quantitative timed searches and more quantitative quadrat methods.

Timed search Method				**Quadrat Method**
Obermeyer, 1998	**Method:** 9 sites 1.5 hr timed search	**Findings:** Rare species. Relative abundance and species composition similar, less effort and time than quadrat.	**Method:** 9 sites 40 1m^2 quadrat searches	**Findings:** Twice as many species found. Detection of smaller species.
Vaughn et al., 1997	**Method:** 31 sites 1hr timed search	**Findings:** More species found in timed search at 25 (80%) of the 31 sites.	**Method:** 31 sites 15 quadrats	**Findings:** Sampling with quadrats alone will underestimate species richness, unless large # quadrats searched. Finds more juveniles (smaller).
Hornbach and Deneka, 1996	**Method:** 4 sites 2 hr timed search	**Findings:** Detected more species. Capable of detecting even sparse populations than quadrat. Less expensive.	**Method:** **4 sites** 30-100 0.25m^2 quadrats	**Findings:** Detected fewer species and less efficient at detecting rare species. Gives precise estimated of density, but takes more effort and time.
Dickson, 2000	**Method:** 8 sites 10-segments (transects, width of the river)	**Findings:** Lower CV, lower percent error than quadrat searches.Less samples needed to achieve 20% error.Cover large area of stream and encompass different stream conditions.	**Method:** 3 sites 20-40 quadrats	**Findings:** Takes less time than segment method but more samples to achieve 20% error, and does not encompass all stream conditions.

Table A1 Continued

Strayer et al., 1997	Method: **1-9 study sites on 13 streams (53 sites)** **2hr timed search**	Findings: **Detected more species. Capable of detecting even sparse populations than quadrat. Less expensive.**		
Metcalfe-Smith, 2000	**Method:** 37 sites 4.5 hr	**Findings:** Less expensive/ time consuming compared to quadrat searches. Many different habitats can be explored. Sampling time is critical when dealing with rare clustered species. 4.5hr was adequate.		
Smith et al., 2001	**Method: Phase-1** 2 site (100-m upstream from bridge and 200-m downstream from potential bridge) 4 hr Divided reaches into cells, each cell searched for about 15 minutes.	**Findings:** The timed search is generally efficient (less costly) at detecting the presence of rare species.	**Method: Phase-2** Used a double sampling design with 0.25-m^2 quadrats, systematically placed(~100). Excavation of a random subset of the quadrats (size of the subsample depends on the expected proportion of mussels on the substrate surface).	**Findings:** The combination of surface counts and excavation in the double sampling design allows increased spatial coverage while estimating mussel densities free from the biases of detect-ability, which affect qualitative methods.
Combination of qualitative and quantitative sampling approaches in this protocol because neither method alone is sufficient to meet all objectives. Not all mussels can be observed on the substrate surface, so we included excavation in the sampling protocol. Use of a double sampling design reduces the amount of excavation, and therefore cost, required to achieve precise estimates.				

Table A1 Continued

Villella and Smith, 2005	**Method:** Phase-1 31 sites 1 hr (e.g. 1 person for 1 hr, 2 people for 30 min or 4 people for 15 min) using view buckets and snorkel	**Findings:** Timed search is generally efficient (less costly) and more effective (more species/level of effort) and detects the presence of rare mussel species.	**Method:** Phase-2 Used the number of live mussels counted during timed search to categorize the site as low (≤30/h) or high density (>30/h). Depending on density systematic (low density) or adaptive sampling (high density) was used for quantitative. $0.25m^2$ quadrats used for both. Snorkeling was used to search each site (did not excavate).	**Findings:** Adaptive-cluster sampling did increase the number of individual mussels found (concentrating efforts on where mussels exist).
Together the two (2-phase sampling design) is flexible and can be implemented at multiple scales to select sample sites within a river. The cost of sampling should be lower because not all sites must be revisited during the 2nd phase of sampling. It also enables us to learn how mussel-habitat-relationships and to identify areas of high abundance through the river.				

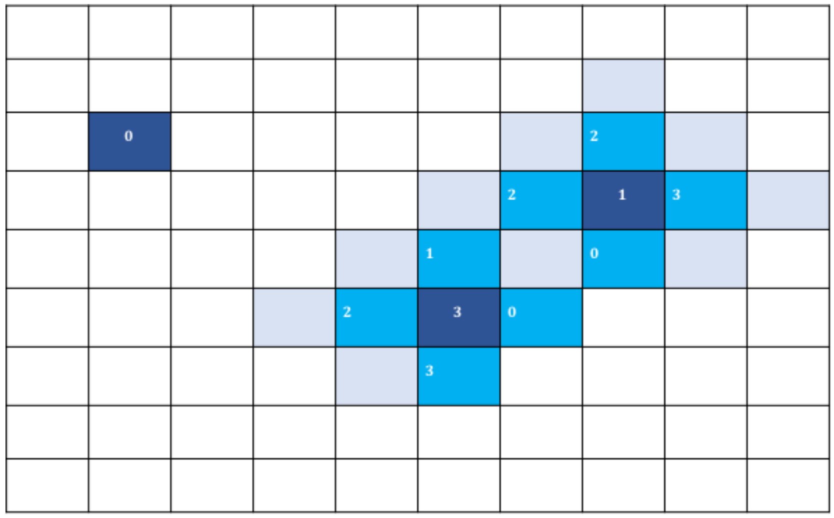

Figure A1 Illustration of the adaptive cluster method with three random starts and serach of adjacent quadrats that contain mussels.

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