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Habitat Requirements of the Golden Orb (*Quadrula aurea*)

Final Report for Texas Water Development Board and Texas Parks and Wildlife Department

TWDB Contract No. 0904830970

TPWD Contract No. 404304

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31 May 2012

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Department of Biological Sciences

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Introduction

Introduction to freshwater mussel ecology

Freshwater mussels belong to the class Bivalvia, with the majority of species in North America belonging to the family Unionidae. They typically live in large, multispecies aggregations, called beds, in flowing waters with stable substrate. As filter feeders, mussels play an important role in the ecosystem. They collect fine particulate organic matter, including phytoplankton, bacteria, and detritus, from the water column (McMahon & Bogan 2001); this improves water quality and makes energy available to higher trophic levels. Mussels also exhibit a unique life cycle; their larval forms, called glochidia, are ectoparasites on fish gills, fins and scales (McMahon & Bogan 2001). Some mussel species have been shown to only parasitize one or a few species of fish, while others utilize a number of different fish hosts (Strayer *et al.* 2004). They are also very long-lived, with some populations having a median age of about 50 years (Strayer *et al.* 2004). Their long life span, slow growth, and limited mobility make mussels susceptible to a variety of both natural and anthropogenic changes to their environment.

Freshwater mussel conservation

Freshwater mussels in the superfamily Unionacea, have experienced widespread extinctions and population declines, stimulating discussion and research aimed at understanding their ecology and conservation needs. Of the 288 total mussel species evaluated, 70% were classified as vulnerable, imperiled, critically imperiled or extinct (Figure 1) based on their Global Conservation Status Rank (Master *et al.* 2000). There are a number of factors attributed to the cause of these declines; degradation of water quality, habitat destruction/alteration, impoundments, exotic species introductions, changes to flow regime, human exploitation, failed recruitment efforts, watershed or riparian alterations, and predation were all important parameters found in a review of the literature (Strayer *et al.* 2004). While water quality issues, habitat alteration and impoundments were the most frequently proposed causes (Strayer *et al.* 2004), all of the factors are interrelated. For example, a dam can alter the flow regime (Poff *et al.* 1997), cause changes to habitat (Vaughn & Taylor 1999) and exclude fish hosts, thereby limiting upstream colonization by mussels (Watters 1996). However, these impacts from impoundments have been shown to dissipate with distance downstream of the dam (Vaughn & Taylor 1999); this is of particular importance for rivers that have a series of dams with little free-flowing river between them. This type of arrangement may inhibit mussel populations from becoming reestablished. Another issue important to mussel conservation is drought. In southwestern Georgia, mussel populations declined significantly in regions where flows ceased during drought conditions; of particular interest were the few endangered or special-concern species that had declines in non-flowing segments of the river and no changes in the flowing segments (Golladay *et al.* 2009). This would indicate that species that are already imperiled are at an even greater risk during drought conditions. Maintaining flow during droughts,

particularly in river segments with known populations of imperiled mussels, will be critically important to the conservation of these at risk species. To better inform conservation practices, it is important to evaluate factors that are currently influencing mussel distributions, especially for imperiled and critically imperiled species.

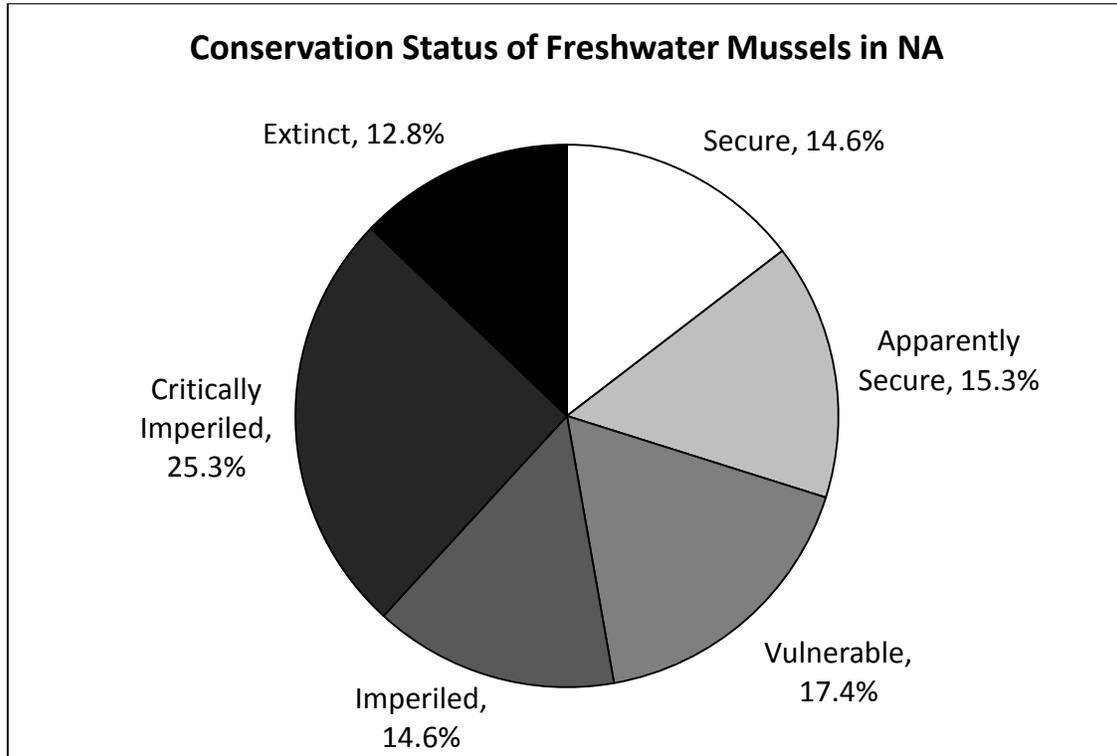


Figure 1. Conservation status of freshwater mussels in North America, percentage of total species (288) in each status group from Master et al. (2000).

Mussel Habitat Research

Substrate

Substrate composition, or granulometry, is one of the most commonly measured microhabitat variables for evaluating distribution patterns in freshwater mussels. This along with other substrate analyses are often performed in habitat assessments because mussels, particularly their juveniles, bury themselves in the sediment so sediment composition may have a great impact on their distribution (McMahon & Bogan 2001). Most studies have shown that relationships exist between substrate analyses and mussel presence or density (Holland-Bartels 1990; Strayer & Ralley 1993; Layzer & Madison 1995; Johnson & Brown 2000; Brim Box et al. 2002; Mcrae et al. 2004). While granulometry is the most common measure, other studies have shown that porosity and degree of sediment sorting (Brim Box *et al.* 2002) or sediment

compaction (Johnson & Brown 2000) may also have some effects on mussel presence or abundance. Some researchers are critical of these relationships as they have limited capacity for predicting overall mussel distribution (Holland-Bartels 1990; Strayer & Ralley 1993); these studies found that mussel communities tended to be found in a variety of substrates and that there was limited statistical power associating substrate granulometry with mussel community metrics.

Alternatively, some researchers support the use of these metrics. The viability of sediment analyses used in predictive modeling has had some support recently (Mcrae *et al.* 2004). It also appears that certain species may be more selective of the sediment composition than others. In Louisiana, a study of a threatened species, *Margaritifera hembeli* (Conrad 1838) (Unionoida: Margaritiferidae), found that there was a positive relationship between particle size and mussel density (Johnson & Brown 2000); the authors reasoned that this relationship was not coincidental based on the limited availability of the preferred gravel-cobble substrates in the studied streams. A study based in Kentucky found that *Villosa iris* (I. Lea 1829) have the strongest associations with boulder and cobble sediment, while most other species were found more often in gravel substrate (Layzer & Madison 1995). While there may be mixed evidence regarding the usefulness of substrate analyses, they can potentially be an important factor that influences the distribution of a single species, and should be considered in an analysis of species habitat requirements.

Hydrology

Simple hydrological variables, such as current velocity, are often used to evaluate the distribution of freshwater organisms, including mussels. Like substrate granulometry, current velocity measurements have been shown to have little predictive power in establishing patterns of distribution for mussel communities (Holland-Bartels 1990; Strayer & Ralley 1993). It has been reasoned that instantaneous current velocity measurements have little impact on the adult distribution patterns of mussels because the hydrologic conditions during the settlement period for juveniles as they release from fish host are more critical for revealing patterns of distribution (Holland-Bartels 1990). Additionally, patterns found with variables such as velocity, are flow conditional, changing in response to discharge (Layzer & Madison 1995), so low flow measurements are not related to those forces acting upon the mussel beds at high discharges (Strayer 1999).

While velocity measurement has not been an effective tool, a variety of more complex hydraulic variables have become commonly used in habitat analyses. Parameters such as Froude number, Reynolds number, and shear stress are frequently used to evaluate hydrologic conditions at microhabitat scales. Froude number and Reynolds number were found to have positive correlations with mussel densities, though only at low discharges, while shear stress

had a negative relationship with mussel density in a fourth order Kentucky stream (Layzer & Madison 1995). Alternatively, Froude number was found to be positively correlated with mussel density in three other Kentucky rivers (Hardison & Layzer 2001), while other measures such as shear stress had more consistent relationships.

Shear forces at the substrate-water interface are thus an important parameter to determine when conducting habitat analyses. The majority of studies have used a value for shear stress calculated from simple hydraulic measures including depth, current velocity, and substrate roughness (Layzer & Madison 1995; Hardison & Layzer 2001; Morales *et al.* 2006) derived from (Statzner *et al.* 1988). It has also been shown that a combination of complex hydraulic variables, such as shear stress, along with substrate variables is useful for predicting both mussel abundance and species richness of the mussel beds (Allen & Vaughn 2010). One measure that combines hydraulic variables with substrate characteristics is the relative substrate stability or RSS value described by Morales *et al.* (2006). This RSS measure could be an important parameter when evaluating the habitat requirements of particular species.

Fliesswasserstammtisch (FST) hemispheres can also be used to estimate the shear stress through a relationship between the density of a hemisphere and the water velocity it takes to move the hemisphere (Statzner *et al.* 1988). This method has had some successes in establishing patterns for benthic organisms (Merigoux & Doledec 2004; Doledec *et al.* 2007), as well as mussels (Hardison & Layzer 2001; Gangloff & Feminella 2007). One study that used this method for evaluating shear stress found a significant relationship between mussel densities and hemisphere density, as well as a strong relationship between hemisphere data and other complex hydraulic parameters (Hardison & Layzer 2001). Therefore, this is a valid tool for evaluating shear forces at the channel bottom, and it has the potential to be an important factor when analyzing patterns of mussel distribution.

Introduction to the Biology of the Golden Orb Mussel (*Quadrula aurea*)

The golden orb, *Quadrula aurea* (I. Lea 1859), is a species of freshwater mussel that is endemic to Texas, with historic distributions throughout the Guadalupe-San Antonio and the Nueces-Frio river basins (Figure 2; (Howells 2002). Populations in the Nueces-Frio drainage have become restricted to the lower reaches of the Nueces River within the Lake Corpus Christi reservoir (Howells 2006). As recently as 2005, live individuals were found in the upper reaches of the Guadalupe River in Kerr County (Howells 2006); however the largest, most stable populations in the Guadalupe have been found in the lower reaches, near Lake Gonzales and Lake Wood in Gonzales County (Howells 2006; Karatayev & Burlakova 2008). The lower reaches of the San Marcos River, a major tributary of the Guadalupe, near Palmetto State Park support a relatively large population of *Q. aurea* (Howells 2006). In the San Antonio River, the largest known population of *Q. aurea* were found in Goliad County, near Goliad State park (Karatayev &

Burlakova 2008). Reports indicate that numbers of sub-populations within these drainages have decreased over time (Howells 2006).

Based on the declines in the range of the *Q. aurea* populations, Texas Parks and Wildlife has designated this species as threatened and it is currently a candidate for placement on the federal Endangered Species list ("Rare, Threatened, and Endangered Species of Texas" 2010).

Study Objectives

This study is focused on the determination of habitat parameters influencing distributions of Golden Orb (*Q. aurea*) mussels in the Guadalupe-San Antonio river basin. The objectives of this study can be summarized by the following tasks:

1. Identify four sample sites with sufficient populations of *Q. aurea* to evaluate habitat parameters.
2. Evaluate the distribution of *Q. aurea* at selected sample sites in relation to habitat parameters and monitor flow conditions at these locations.
3. Calculate the shear stress ratio (RSS) for multiple flow conditions and the relationship with mussel density at selected sites.
4. Compile and summarize life history information for *Q. aurea* and related species.

Conservation of *Q. aurea* will depend on gaining an understanding of their habitat and flow requirements, so that conditions may be maintained for existing populations. Also, it will be important to know what conditions *Q. aurea* need to grow and reproduce if drought conditions persist and populations become exposed, so that protection measures can be taken as necessary.

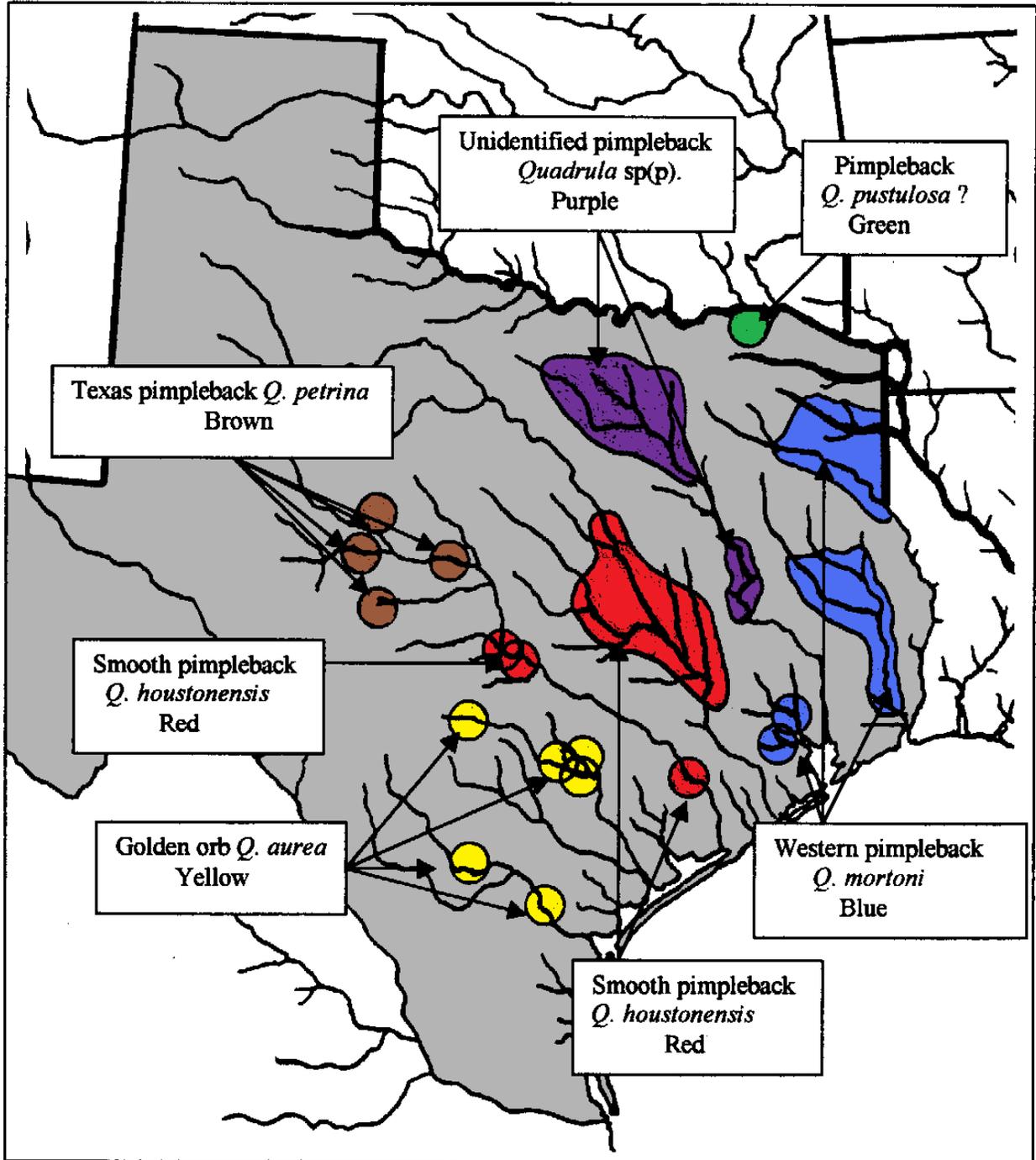


Figure 2. Locations where living or recently dead pimpleback species (*Quadrula* spp.) have been collected in Texas 1990 - 2002 (Howells 2002)

Methodology

Field methods

In order to determine the habitat requirements of the Golden Orb (*Q. aurea*), first it was necessary to establish locations of *Q. aurea* populations. Based on previous reports, there were known populations in the San Antonio River near Goliad State Park, one site within the park boundaries and another site downstream of the park, accessed through private property (Karatayev & Burlakova 2008). According to Howells (2006), other possible locations to find *Q. aurea* populations include Palmetto State Park on the San Marcos River as well as Lake Gonzales and downstream of the dam at Lake Wood on the Guadalupe River. Each of these locations, as well as others along the Guadalupe and San Antonio rivers were surveyed by performing timed-searches in a preliminary study of the area. Sites were surveyed for at least one man-hour to determine presence of *Q. aurea*; more time was spent to determine the extent of the population if *Q. aurea* were found. Upon completion of these reconnaissance surveys, four sites were selected for full quadrat surveys, based on size and stability of the populations of *Q. aurea*. At each of the four selected sites, mussel bed size was determined based on the extent of *Q. aurea* populations present as well as the ability of researchers to sample based on the following methodology.

All quadrat surveys took place during very low discharges during the summer of 2011. For three of the sites, there was a series of six transect lines set across the mussel bed, set perpendicular to the flow of water. Transects were placed equidistant from each other throughout the length of the mussel bed and width of each transect was determined by width of the mussel bed. For each transect, six randomly selected quadrats were selected for sampling. At the site on the San Antonio downstream from Goliad state park, mussels were only found in narrow bands near the banks of the stream during reconnaissance surveys. To survey the bed, transects were placed parallel to the shoreline at a given increment. Transect A was set 1m from the bank and 12 quadrats were randomly selected along the length of the transect, determined by the upstream and downstream extent of where mussels were found. Upstream from transect A, another small near shore bed was found, which extended somewhat further from the banks. Two transects were placed parallel to the bank with one 2m out and one 3m out. For each of these transects, 12 quadrats were randomly selected along the length of the bed.

The first measurement taken in each quadrat was current velocity. Current velocity was measured with a Flo-Mate™ 2000 Marsh-McBirney flow meter and a 2m wading rod. Measurements were taken at 60% of depth and at 5cm above the channel bottom. Near-bed velocity measurements (5cm) were taken in a similar manner at a higher discharge during

March 2012; a modified rod (~3m) was used for measurements taken from a boat when depths were deemed to be not wadeable or velocities were too high to wade safely.

Fliesswasserstammtisch (FST) hemispheres were used as an alternative method for measuring near-bed flow velocity. The concept behind FST hemispheres involves designing a set of identically shaped objects (in this case hemispheres) having different densities and known physical properties when exposed to a flow field. Hemispheres were built at the University of North Texas using acrylic plastic and lead weights and calibrated to known flow velocities using a research flume at Texas Tech University. Calibration procedures followed the standard method for utilizing the hemispheres in the field (Statzner and Muller 1989) (detailed below) and were performed on the same substrate that was used for field measurements. Various flow velocities were created in the flume and tested against individual hemispheres. Stream velocity was measured using a Sontek/YSI Acoustic Doppler Velocimeter positioned to measure flow velocity at the midline of the hemispheres (3 cm above the flume bed). Incidental movement related to placing the hemisphere on the plane (Statzner et al. 1991) was discounted and the heaviest hemisphere moved in the current was recorded. The final calibration values for each hemisphere were created using a least squares regression model that best fit the distribution of the data. The final calibration function was a power function: $Y=5.9407x^{1.26882}$ with an $R^2 = .84$ (Figure A13).

Use of the hemispheres in the field requires placing a level flat plane on the stream bottom (Statzner and Muller 1989). Individual hemispheres are then placed with the forward edge touching the plane and released from a height of approximately 1cm to the surface of the plane. The calibrated velocity value for the heaviest hemisphere that moves in the flow was taken as an estimate of the near-bed velocity.

Sediments were also sampled from each quadrat. For clay to sand dominated sediments, a 2.5cm diameter sediment coring tube was used. The coring tube was inserted to a depth of 10cm. A flat surface was placed under the end of the tube to prevent loss of sediment as the tube was removed. For sediment that was dominated by gravel to cobble sized particles, a large sediment sampler was used. This apparatus is a 15.24cm diameter plumbing T-junction, with a PVC tube attached. The sampler was inserted to a depth of 10cm into the sediment within the quadrat and all sediment was removed into the PVC tube. All sediments were closely examined for the presence of live mussels, particularly juveniles, before being transferred to sample containers.

Each quadrat was then excavated to a depth of 10cm. Any live mussels or spent valves were removed. Live mussels were identified to species in the field, measured with calipers, and replaced in the vicinity of the quadrat from which they were sampled. Pictures were taken of each species found. Digital images and reference collections of shells collected during this

study are archived in the UNT Elm Fork Natural Heritage Museum Joseph Britton Mussel Collection.

Laboratory Analyses

For each sediment sample, grain size analysis was performed in the following manner. First, sediments were dried in an American Scientific Product DX-68 drying oven at 110°C for at least 4 hours, until all moisture was removed. Then, samples were separated with Fisher Scientific Company USA Standard Test Sieves into size fractions (4mm, 2mm, 1mm, 500 µm, 250 µm, 125 µm, and 62.5µm) based on the Wentworth scale using a W.S. Tyler, Inc. RX-24 Portable Sieve Shaker. For particles greater than 4mm, a Wildlife Supply Company 14-D40 gravelometer was used to separate into pebble classes (8mm, 16mm, 32mm). Any sediment that was collected that was greater than 64mm was excluded in order to prevent bias toward the course grained sediments for calculations of median particle size. The mass of each size fraction was measured using a Sartorius M-prove top-loading balance. For each sample, percent by mass of each size class was calculated and median particle size (d_{50}) was determined as the particle size for which 50% of the sample is less than that diameter.

Relative substrate stability (RSS) was calculated for each quadrat based on the ratio of observed shear stress (τ) to critical shear stress (τ_c) from Morales et al. (2006). Observed shear stress was calculated based on the formula: $\tau = \rho U_*^2$, where ρ is the density of water, and

$$U_* = \frac{U}{5.75 \log_{10} \frac{12d}{k_v}},$$
 where U is current velocity, d is water depth, and k_v is a measure of bed

roughness calculated by $k_v = \frac{(5C_1+3C_2+C_3)}{9}$ where C_{1-3} are the most dominant size classes of sediments and C is 1 for sediment <3mm, 2 for sediment between 3mm and 30mm and 3 for sediment between 30mm and 300mm (Statzner et al. 1988). Critical shear stress was calculated based on the formula: $\tau_c = \theta_c g d_{50} (\rho_s - \rho)$ where θ_c is the dimensionless critical shear stress, 0.065 and ρ_s is the substrate density, 2.65g/cm³ (Allen & Vaughn 2010). Values for RSS were calculated for both low and high flow measurements.

Statistical Analyses

All statistics were performed using SAS (version 9.3). Linear regressions were performed to evaluate relationships between continuous habitat variables and mussel population densities. As the population data was not normally distributed, a log transformation was performed prior to regression analysis. Spearman rank correlation tests were also used to test the relationship between habitat parameters, such as hemisphere density, and mussel population densities. Non-parametric tests, such as the Spearman correlation, work well for data that is not normally distributed and non-linear in nature. Preliminary results indicated sediment grain size was significantly associated with mussel density (see below). To further assess this relationship the

sample sites were categorized in terms of median particle size (course or fine) and compared with a two sample t-test. Course sites included Goliad State Park canoe dock and Palmetto State Park while the fine sites were Mueller Ranch and the site 5km below Lake Wood (Table 1). Initial analysis indicated the variance in median particle size was significantly different between categories ($F = 483.6$, $p < .0001$) therefore Satterthwaite's approximate t-test, which accounts for unequal variances, was utilized (Armitage et al. 2002). Two null hypotheses were tested with Satterthwaite's t-test: 1) There is no significant difference in median particle size between sites categorized as course or fine, and 2) there is no significant difference in mussel densities between sites categorized as course or fine.

Literature Review for Quadrula Life History

A survey of the literature using the Zoological Record database with the search terms "Quadrula aurea" yielded no records. When the search parameters were changed to "Quadrula" and "reproduction", only 6 records related to reproduction and life history of species within this genus were found. A survey of the literature using the Biosis Previews database with the search terms "Quadrula" and "reproduction" or "Quadrula" and "fish host" yielded 6 additional records. Findings from these papers were analyzed for information that could be relevant to the life history of *Q. aurea*.

Results

Task 1: Identify four sample sites with sufficient populations of *Q. aurea* to evaluate habitat parameters.

A reconnaissance survey was conducted from May 13-17 and June 8-11 with the goal of locating *Q. aurea* populations of sufficient size to allow for the quantification of their habitat requirements in relation to stream flow conditions. Surveys targeted suitable habitat in publicly accessible locations and combined visual searches for spent valves along banks with in-stream snorkeling searches for live mussels. Survey lengths were dependent on finding evidence of *Q. aurea* presence or live mussels. Surveys were extended when either of these factors occurred in order to verify the presence of live *Q. aurea* and to determine the spatial extent of any beds that were discovered.

A total of 22 sites were searched during reconnaissance surveys, and live mussels were located at 14 sites – live specimens of *Q. aurea* were found at all sites with live mussels (Table 1; Appendix Figure A1 and A2). Spent valves of long dead *Q. aurea* were found at two locations on the Guadalupe along with other spent valves, but no live mussels were located.

A large mussel bed was located at the site below Lake Wood Dam on the Guadalupe River, but only 5 *Q. aurea* were found at this site. Although this site does not appear to harbor sufficient

Q. aurea to warrant habitat assessment work it is a substantial bed that appears to extend for some distance down river. We expended 9 man hours searching this site and were still finding live mussels when we decided to end our search for *Q. aurea*. Live mussels found at this site included 54 *Amblema plicata* (Say 1817), 11 *Potamilus purpuratus* (Lamarck 1819), 3 *Megaloniais nervosa* (Rafinesque 1820), 2 *Lampsilis teres* (Rafinesque 1820), 5 *Q. aurea*, and 1 *Toxolasma texasiensis* (I. Lea 1857).

Table 1. Reconnaissance sites for *Q. aurea* in May and June 2011

Date	River	Site name	Man Hours	Mussels found	Condition	Live <i>Q. aurea</i>
5/13/2011	Guadalupe	Below Lake Wood	9	Yes	Live	5
5/13/2011	San Marcos	Palmetto State Park*	6	Yes	Live	#
5/16/2011	Guadalupe	downstream FM 447	3	Yes	Live	15
5/16/2011	Guadalupe	Riverside Park, Victoria	1.5	No		
5/17/2011	Guadalupe	Nichol's Landing	4.5	Yes	Live	4
5/17/2011	Guadalupe	downstream FM 466	3	Yes	Spent valves	
5/17/2011	Guadalupe	Guadalupe River State Park	3	Yes	Spent valves	
5/17/2011	Guadalupe	HWY 46	3	No		
5/17/2011	Guadalupe	HWY 281	3	No		
5/17/2011	Guadalupe	Gruene Rd	1.5	No		
5/17/2011	San Marcos	John J. Stokes Park	1.5	No		
5/17/2011	San Marcos	Ramon Lucio Park	1.5	No		
6/8/2011	San Antonio	Mueller Ranch*	4	Yes	Live	#
6/10/2011	San Antonio	Goliad SP, downstream*	2	Yes	Live	100
6/10/2011	San Antonio	Goliad SP, upstream	1	Yes	Live	23
6/11/2011	Guadalupe	downstream FM 447	2	Yes	Live	3
6/11/2011	Guadalupe	State Hwy 72 – upstream A	1	Yes	Live	2
6/11/2011	Guadalupe	State Hwy 72 – upstream B	1	Yes	Live	5
6/11/2011	Guadalupe	State Hwy 72 – upstream C	1	Yes	Live	20
6/11/2011	Guadalupe	State Hwy 72 – upstream D	1	Yes	Live	7
6/11/2011	Guadalupe	State Hwy 72 – upstream E	1	Yes	Live	4
6/11/2011	Guadalupe	downstream Lake Wood Dam*	2	Yes	Live	27

* Denotes final sample sites where population and environmental parameters were collected

Denotes sites where reconnaissance data was not recorded because a full survey was performed

A mussel bed was located on the Guadalupe River below FM 447 that appeared to harbor sufficient *Q. aurea* for habitat assessment. This site produced 15 live *Q. aurea* in 3 man hours of searching. Other live mussels present at this location included *A. plicata* and *P. purpuratus*. The

site was surveyed again on June 11 to determine the extent of the bed; however, even with SCUBA surveys in the deeper portions of the river, minimal numbers of live mussels were found on this survey. Researchers agreed that the decrease in mussel numbers from May indicated an unstable or depositional population and decided to travel upstream to see if a more stable population could be located.

Nichols landing on the Guadalupe River (at FM 311 crossing) produced four live *Q. aurea* in 4.5 man hours of searching, but no other live mussels and few spent valves were found. While it appears that *Q. aurea* may persist in this area the numbers are not sufficient for habitat assessment purposes. The survey of the San Marcos River at Palmetto State Park produced numerous live *Q. aurea* in a bed upstream of the low water footbridge. Mussel numbers here were not quantified as it was decided to return the next day and perform a full quadrat survey at this location.

Researchers were able to contact a local land owner and gain access to the San Antonio River approximately 4km downstream of Goliad State Park. This site had been previously accessed by other researchers, and a large population of *Q. aurea* was documented. While performing reconnaissance, substantial numbers of live *Q. aurea* were found and researchers determined that this site should be surveyed while access to the river through the private property was being made available.

Two mussel beds were found within Goliad State Park, one between the US 183 bridge and the canoe dock and one upstream of the river access from the Vaquero campground. The first bed was fairly extensive and approximately 100 mussels were found within 2 man hours of tactile searching – the upper and lower extent of the bed was determined by GPS and was designated for quadrat survey. The upstream bed was less extensive, but yielded 23 *Q. aurea*, 16 *Tritogonia verrucosa* (Rafinesque 1820), 4 *A. plicata*, and 1 *L. teres* in one man hour of searching.

Several sites in the Guadalupe River were surveyed upstream of Texas State Highway 72, northeast of Cuero, TX on June 11; sites were traveled to by motorized boat. The first large gravel bar upstream of where the boat was put in was surveyed upon visualization of spent valves on shore; across from the gravel bar, just below a riffle area, there were a large number of mussels stranded in a shallow pool created by a woody debris dam. Of those found, there were 42 *A. plicata*, 2 *Q. aurea*, and 2 *M. nervosa*. Just upstream of this site, above the riffle, a small bed of mussels was found in a 1 man hour timed search, including: 5 *Q. aurea*, 5 *A. plicata*, 1 *L. teres*, 1 *Lampsilis hydiana*, and 1 *M. nervosa*.

Continuing upstream from there was a gravel bar surrounded by a small side flow toward the right bank and the main flow toward the left bank. In approximately 1 man hour of searching,

20 *Q. aurea* and 4 *A. plicata* were found, primarily in the right bank offshoot from the main flow. Many of these mussels were found in the shallows or even stranded on shore just out of the flow of water. This was considered by the researchers to be an unstable population. Just upstream of this gravel bar, the sediment was comprised primarily of silt and fine sands and a large population of mussels was discovered on the right bank side; the stream bed was steeply sloped for about 5 meters out from the bank. In approximately 1 man hour, over 100 *A. plicata*, 20 *Cyrtoneias tampicoensis*, 20 *L. teres*, and 7 *Q. aurea* were found in this bed. Another similar bed was found further upstream, but only 4 *Q. aurea* were present. There were unidentified juvenile mussels present in both of these silt beds.

The final site evaluated on June 11 was located downstream of the dam for Lake Wood, approximately 5km. Similar to the other mussel beds found in the Guadalupe River, the mussels were found in the silty slope of the stream bed. This bed was also dominated by *A. plicata*, with numerous *L. teres* and *C. tampicoensis*. The researchers discovered 27 *Q. aurea* in a 2 man hour timed search, and so this location was determined suitable for quadrat survey.

Tasks 2 & 3: Evaluate the distribution of *Q. aurea* at selected sample sites in relation to habitat parameters and monitor flow conditions at these locations; calculate the shear stress ratio (RSS) for multiple flow conditions and the relationship with mussel density at selected sites.

Basic habitat parameters were measured or calculated for each quadrat and then compared with the mussel population density, which was log transformed to normalize data. The clumped nature of the mussel distributions led to the occurrence of many zero results in the density data. As a result regression analyses that attempted to associate mussel density measures to environmental variables explained little of the variation in the data. Several examples of regression results are given in the Appendix (Figures A3, A4, and A5). Therefore, Spearman rank correlation and Satterthwaite's t-test were utilized to evaluate the general relationships between mussel density and environmental variables.

Spearman correlation results indicated a weakly positive relationship between near-bed velocity at higher flows and *Q. aurea* densities, but no significant relationship was found with total mussel density (Table 2). FST hemisphere density was also related to mussel density through Spearman correlation, finding a weakly positive correlation between hemisphere density and total mussel density and a moderately positive relationship with *Q. aurea* densities (Table 2) (Figure A6).

For sediment analysis, the median particle size (d_{50}) in each quadrat was calculated and compared to quadrat mussel density results. Spearman correlation indicated a moderate, positive relationship between median grain size and *Q. aurea* densities (Table 2) (Figure A7), while a somewhat weaker, yet still significant, relationship was found with total mussel density

(Table 2). However, Satterthwaite's t-test on median particle size strongly rejected the null hypothesis and indicated a significant difference between coarse and fine sites ($t = 14.3$, $p < .0001$) (Figure A8). Furthermore, Satterthwaite t-tests on total mussel density (Figure A9) and *Q. aurea* density (Figure A10) were both highly significant ($t = 4.6$, $p < .0001$ and $t = 7.57$, $p < .0001$ respectively).

The relative substrate stability (RSS) parameter combines basic hydrologic measures with sediment composition. RSS values were calculated for both moderate and low flow conditions. An attempt to relate these parameters to mussel density through linear regression found that there was not a linear relationship between these factors (Figure A3). The non-normal distribution of the density data lends itself better to a correlation analysis, so a Spearman correlation was performed for both moderate and low flow RSS values. Only moderate flow RSS values were found to have a significant, negative relationship with density, both *Q. aurea* densities as well as total mussel densities (Table 2) (Figures A11 and A12 respectively).

Table 2. Summary of Spearman correlation values evaluating relationships between mussel density and habitat parameters.

Habitat Parameter/Density	R Statistic	p Value
RSS – low flow Total Mussel Density (#/m ²)	-0.13700	0.1028
RSS – low flow <i>Q. aurea</i> Density (#/m ²)	0.04446	0.5980
RSS – moderate flow Total Mussel Density (#/m ²)	-0.42394	<.0001
RSS – moderate flow <i>Q. aurea</i> Density (#/m ²)	-0.37017	<.0001
Velocity (U) – moderate flow (cm/s) Total Mussel Density (#/m ²)	-0.01081	0.8981
Velocity (U) – moderate flow (cm/s) <i>Q. aurea</i> Density (#/m ²)	0.23045	0.0056
FST Hemisphere density Total Mussel Density (#/m ²)	0.26062	0.0017
FST Hemisphere density <i>Q. aurea</i> Density (#/m ²)	0.43516	<.0001
Median particle size (d ₅₀) Total Mussel Density (#/m ²)	0.36301	<.0001
Median particle size (d ₅₀) <i>Q. aurea</i> Density (#/m ²)	0.50322	<.0001

Task 4: Compile and summarize life history information for *Q. aurea* and related species.

As the primary focus of research on *Q. aurea* is related to population distribution, little is known about the reproductive biology of the species (Howells 2002). The genus *Quadrula*, in general, is composed of species that are short term brooders holding fertilized eggs and glochidia for only 3 to 6 weeks before release (Gorden and Layzer 1989). Gravid females have been reported from May through August (Howells 2000) suggesting reproduction in *Quadrula* is limited to the summer months. While there has been no research to determine host fish species for *Q. aurea*, some research has been performed with the more common Pimpleback, *Quadrula pustulosa* (I. Lea 1831), which was shown to be the most genetically similar to *Q. aurea* of the tested *Quadrula* species, based on an analysis of mitochondrial ND1 sequences (Serb et al. 2003). One study found that *Q. pustulosa* glochidia exclusively infected channel catfish, *Ictalurus punctatus* (Rafinesque 1818), in Kentucky (Weiss & Layzer 1995). Other sources have an expanded list of host fish, including: shovelnose sturgeon, black bullhead, brown bullhead, flathead catfish, and white crappie (Howells et al. 1996) in addition to the channel catfish. Based on this information, researchers could narrow down from this list those species that are common in the areas where *Q. aurea* are known to be present, or species that are closely related to those listed above, and these fish could be monitored for glochidia to evaluate which fish species are acting as viable hosts. This is an area of much needed attention as fish host availability is an essential aspect to distribution of mussel species.

Discussion

Analytical results suggest a relationship between *Q. aurea* densities and the physical habitat of the stream. Both instantaneous current velocity at moderate flows and FST hemisphere density were positively related to their density (Table 2). While hemisphere density has been indicated as an estimate of shear stress (Statzner et al. 1988), it offers a different way of measuring the near bed velocity – there is a positive, linear relationship between hemisphere density and the velocity required to move the disc (Figure A13). This indicates that *Q. aurea* are likely to be found in areas where the near bed current velocities are generally moderate. This was not the case, however with total mussel density. There was no significant relationship between mussel density and instantaneous current velocity in moderate flow conditions and only a weakly positive relationship with FST hemisphere density (Table 2). *Q. aurea* may thus be better adapted to living in these higher velocities, which gives them an advantage; mussels are filter feeders, so being able to tolerate moderate flows allows the *Q. aurea* to have access to more particulate organic matter that is suspended in the water column.

Substrate composition also demonstrated a positive relationship to *Q. aurea* densities. In sites with larger median grain size (d_{50}), *Q. aurea* densities were much higher. While there was a

positive relationship with total mussel density, the relationship was stronger for *Q. aurea* independently (Table 2), suggesting that this is an important habitat parameter for this species. The importance of the larger particle sizes – these sites were generally dominated by large pebbles with cobble and larger sized substrate also commonly present at the site but not included in the composition analysis – is associated with greater stability in the faster flows also characteristic of these sites. Therefore it is critically important to look at velocity and substrate together. It is also interesting to note that the sites with larger substrate tended to show a bimodal pattern in substrate composition, with medium to fine grain sands being secondarily most abundant (Table A1, Figure A14). The bimodal distribution in this context relates to a substrate composed of finer particles, suitable for burrowing, protected by a covering of larger, less mobile particles that stabilize the bed.

In order to evaluate the combined influence of the velocity and the substrate, relative substrate stability (RSS) values were calculated at both low and moderate flow conditions. While neither RSS value had a clear linear relationship to mussel density, RSS values for moderate flows demonstrated a significant, negative relationship to mussel density in general as well as for *Q. aurea* specifically (Table 2). Smaller RSS values indicate a stable environment, where the sediment is not likely to get dislodged (Morales et al. 2006). All RSS values calculated were well below the critical threshold, even at the more moderate flows, which indicates a very stable environment. This is likely why the mussel beds are able to persist in these more stable environments (Strayer 1999). The *Q. aurea* densities are highest at sites where the larger substrate provides stability to the medium to fine sands that lie underneath, even in the higher flows that they prefer. This combination of sediment and flow parameters appears to be very important to the distribution of this species.

It is important to note that based on the limited reconnaissance surveys and this information, that there could be undiscovered populations of *Q. aurea* in these drainages, which are likely to be most dense in the courser substrate where velocities are higher. Systematic surveys of the river basin, focusing on areas with this combination of habitat variables, could be helpful in determining the true extent of *Q. aurea* populations. This information can also help inform conservation efforts, so that segments of river with this combination of habitat variables can be preferentially preserved in order to provide the best possible environmental conditions for the *Q. aurea*. Research also needs to be done to determine which fish species are acting as hosts for the *Q. aurea* glochidia, so that factors affecting the fish distributions can be incorporated into plans for conservation.

Acknowledgements

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Appendix A

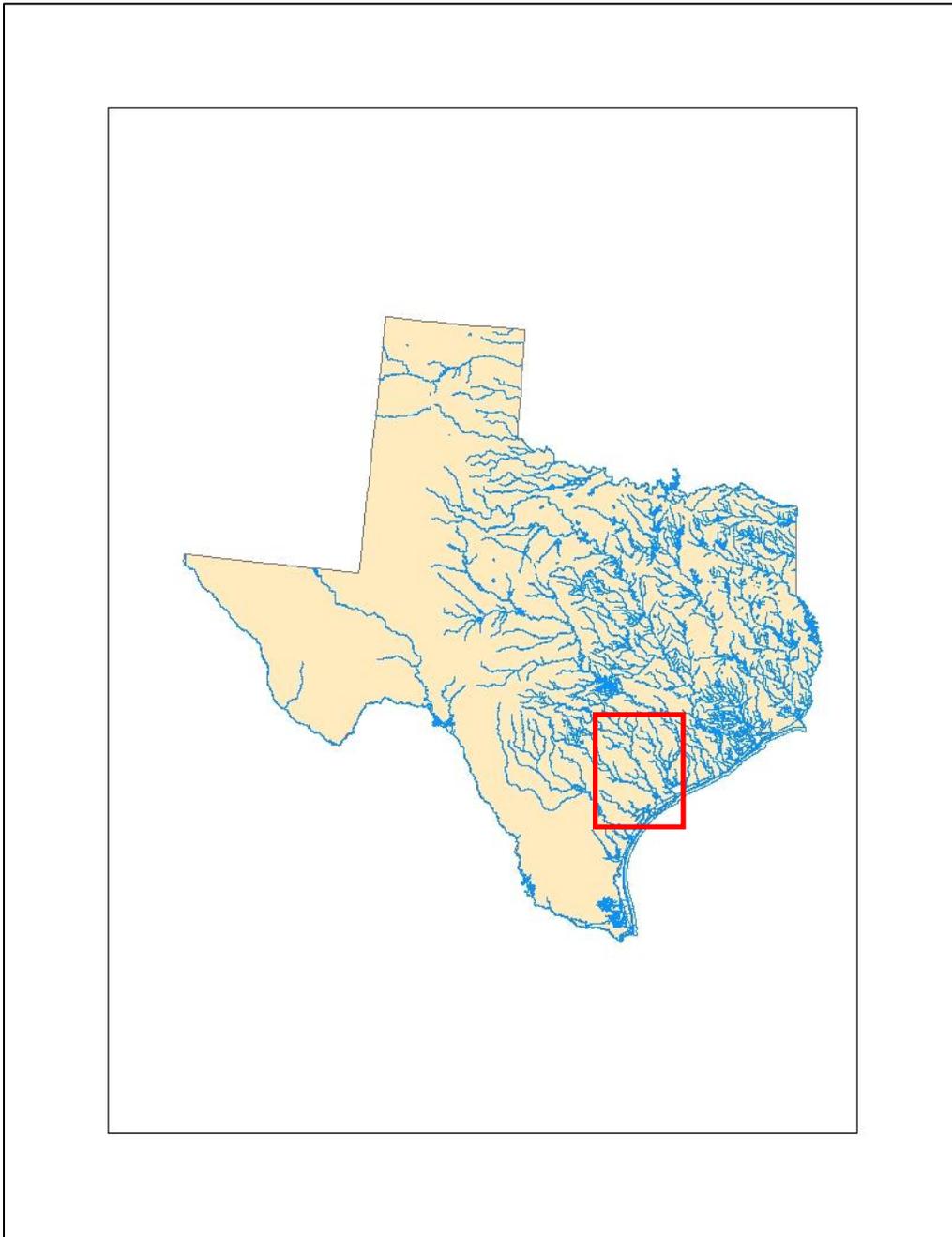


Figure A1. Map of Texas with major stream segments; study area outlined in red.

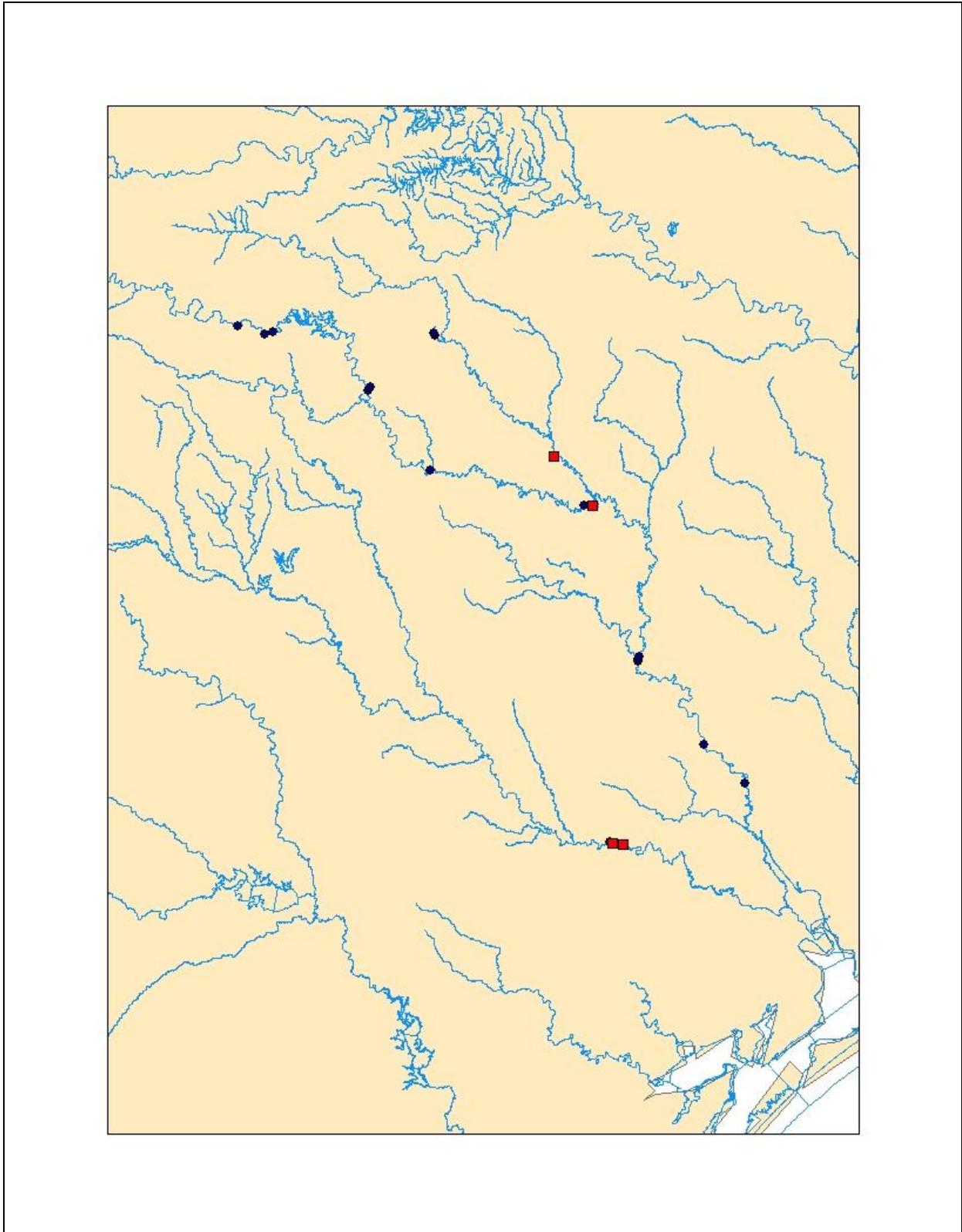


Figure A2. Map of study area where black circles represent sites that were surveyed during reconnaissance and red circles represent sites which were monitored.

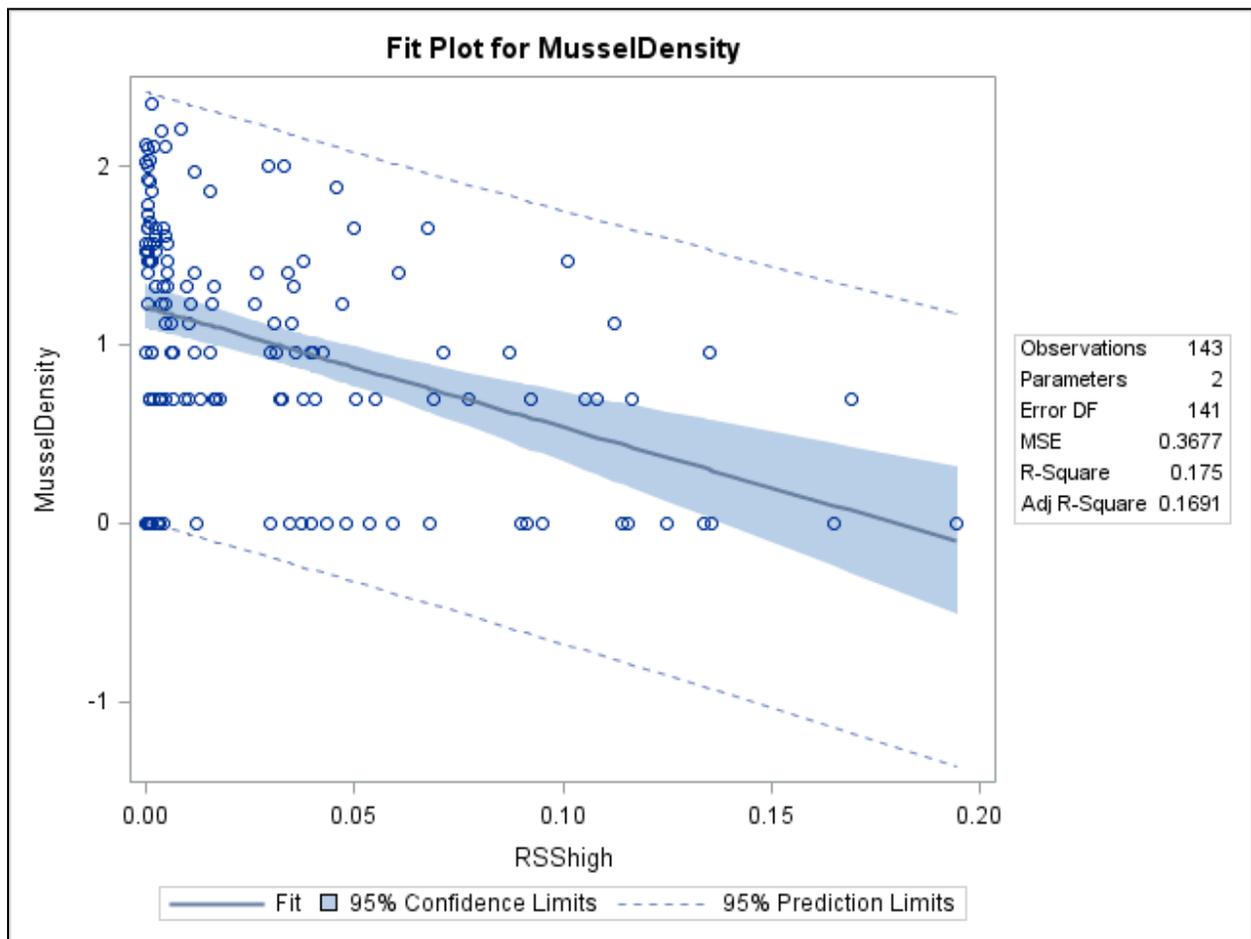


Figure A3. Best fit regression line for total mussel density in relation to RSS values at moderate discharge.

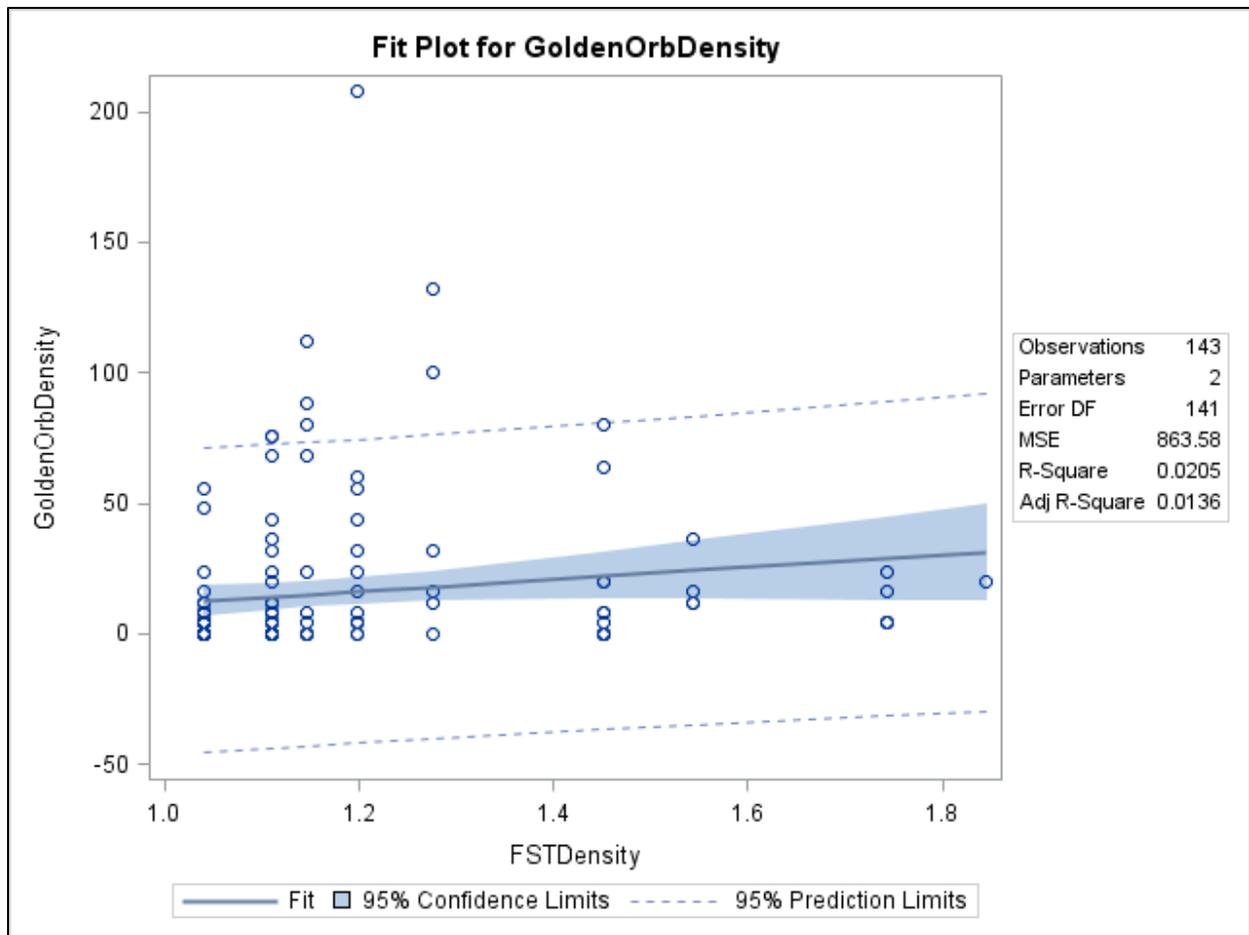


Figure A4. Best fit regression line for Golden Orb density (*Q. aurea*) in relation to FST density (g/cm³).

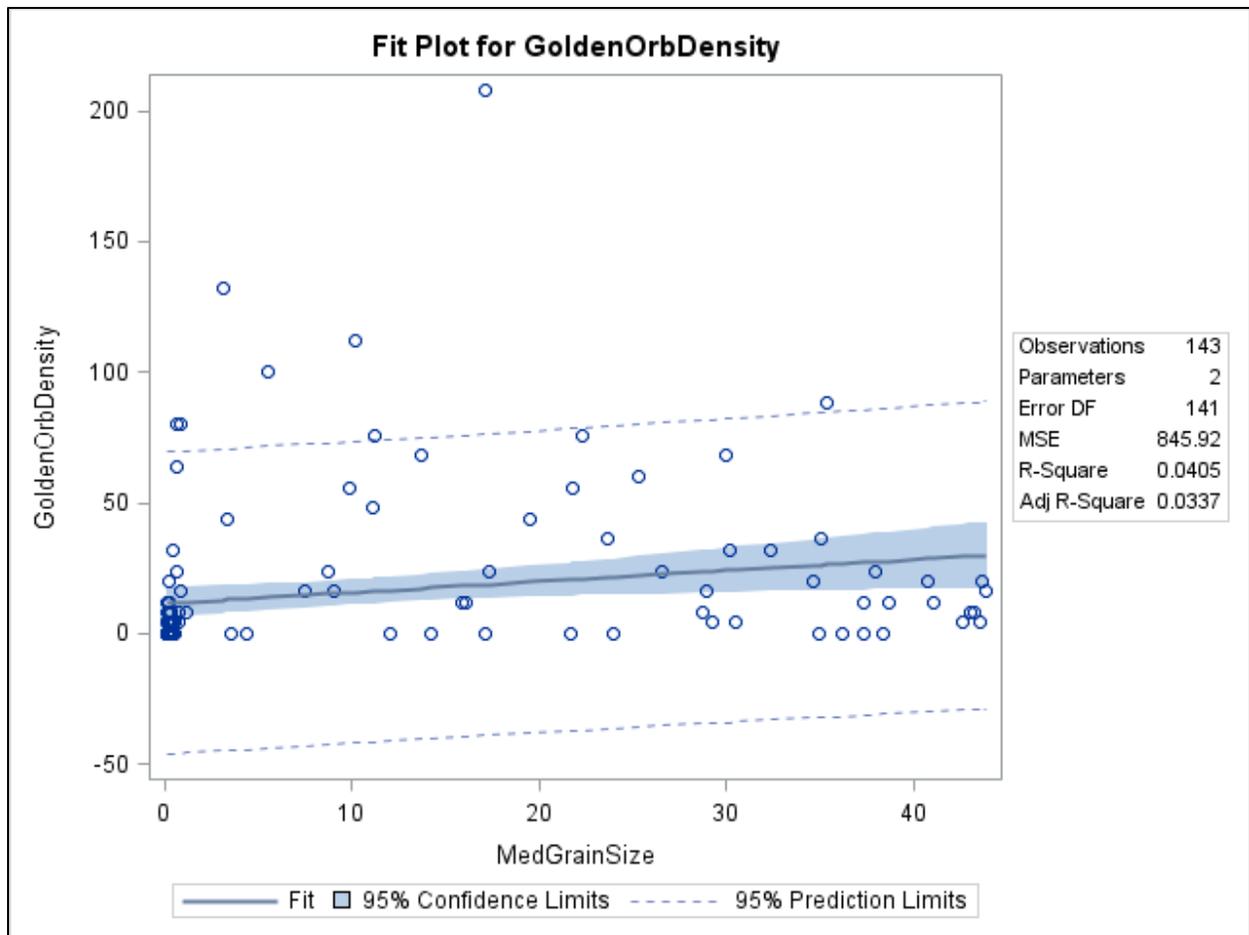


Figure A5. Best fit regression line for Golden Orb (*Q. aurea*) density in relation to the median particle size (d50).

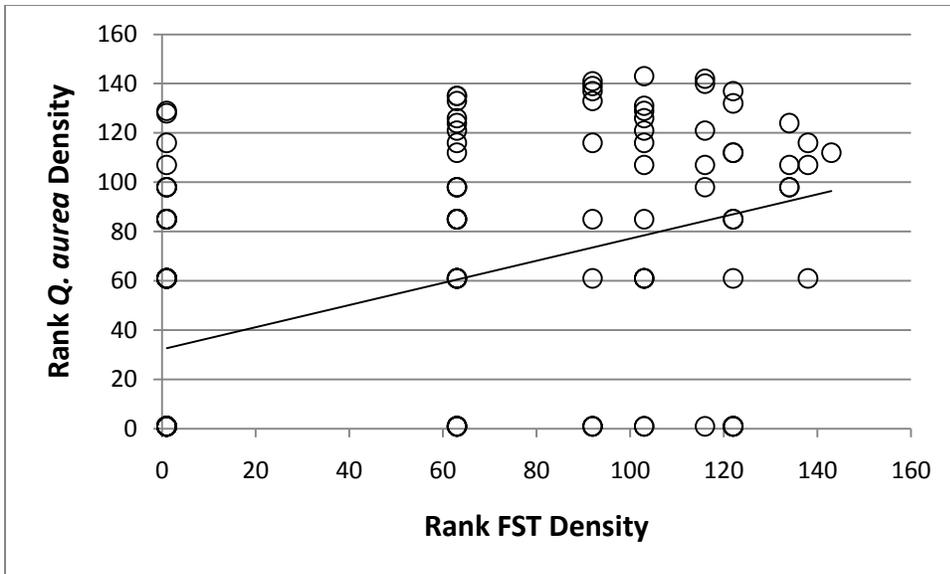


Figure A6. Plot of Spearman correlation between ranked data for FST hemisphere density and *Q. aurea* density ($R = .44$, $p < .0001$). The trend line is presented to demonstrate the general trend in the data.

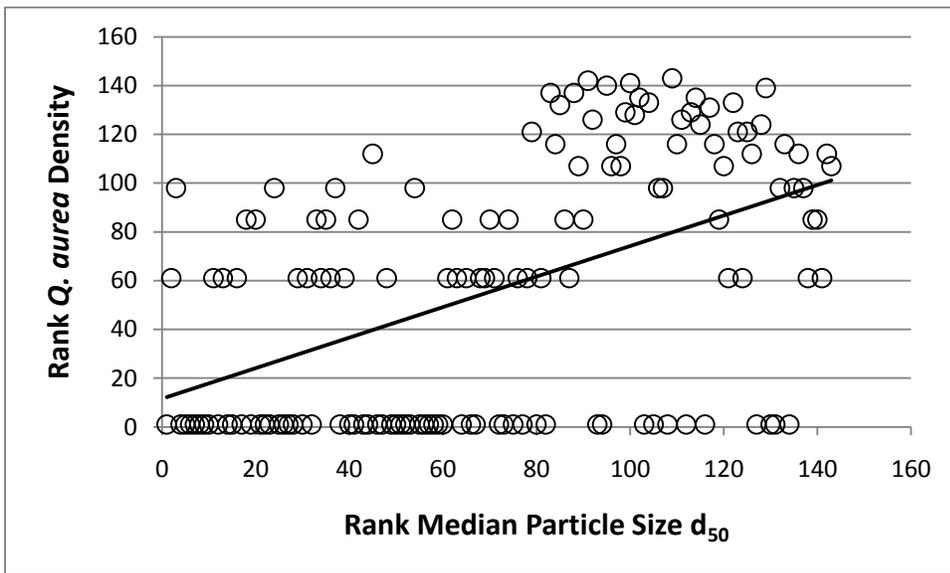


Figure A7. Plot of Spearman correlation between ranked data for median particle size and *Q. aurea* density ($R = .50$, $p < .0001$). The trend line is presented to demonstrate the general trend in the data.

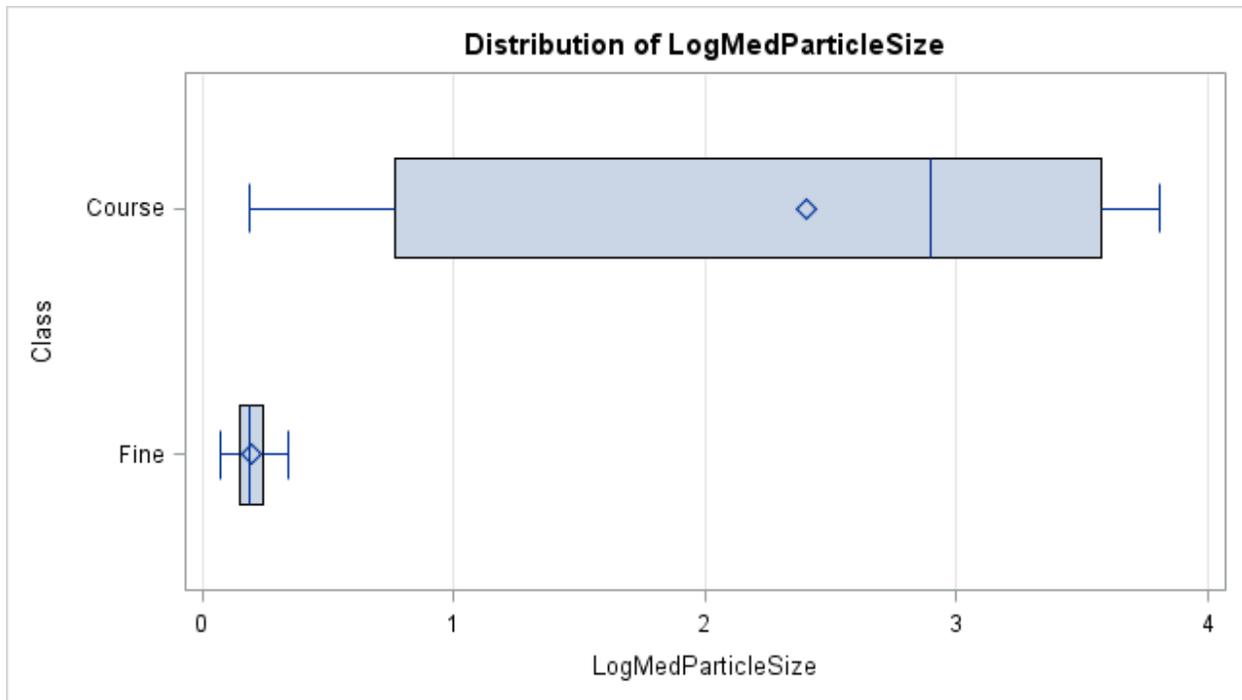


Figure A8. Median particle size distribution (log scale) for sites categorized as course or fine. Satterthwaite's t-test indicated difference between categories was highly significant ($t = 14.3$, $p < .0001$).

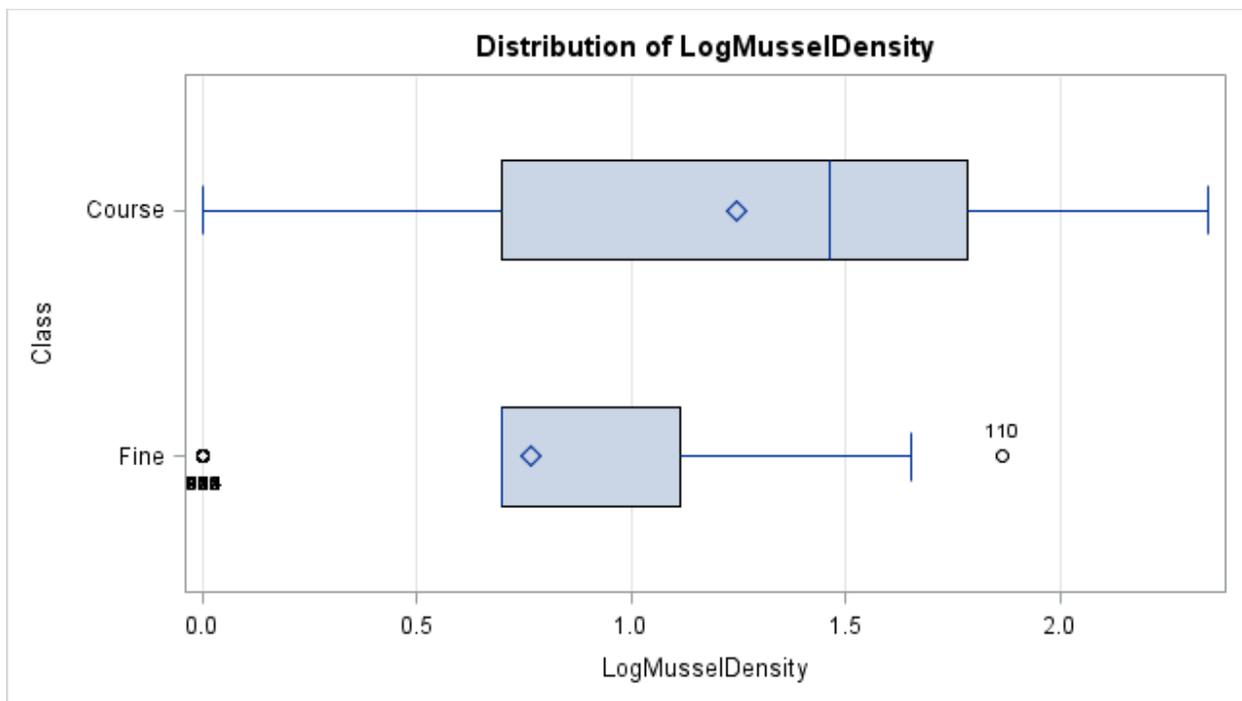


Figure A9. Total mussel density (log scale) for sites categorized as course or fine. Satterthwaite's t-test indicated difference between categories was highly significant ($t = 4.6$, $p < .0001$).

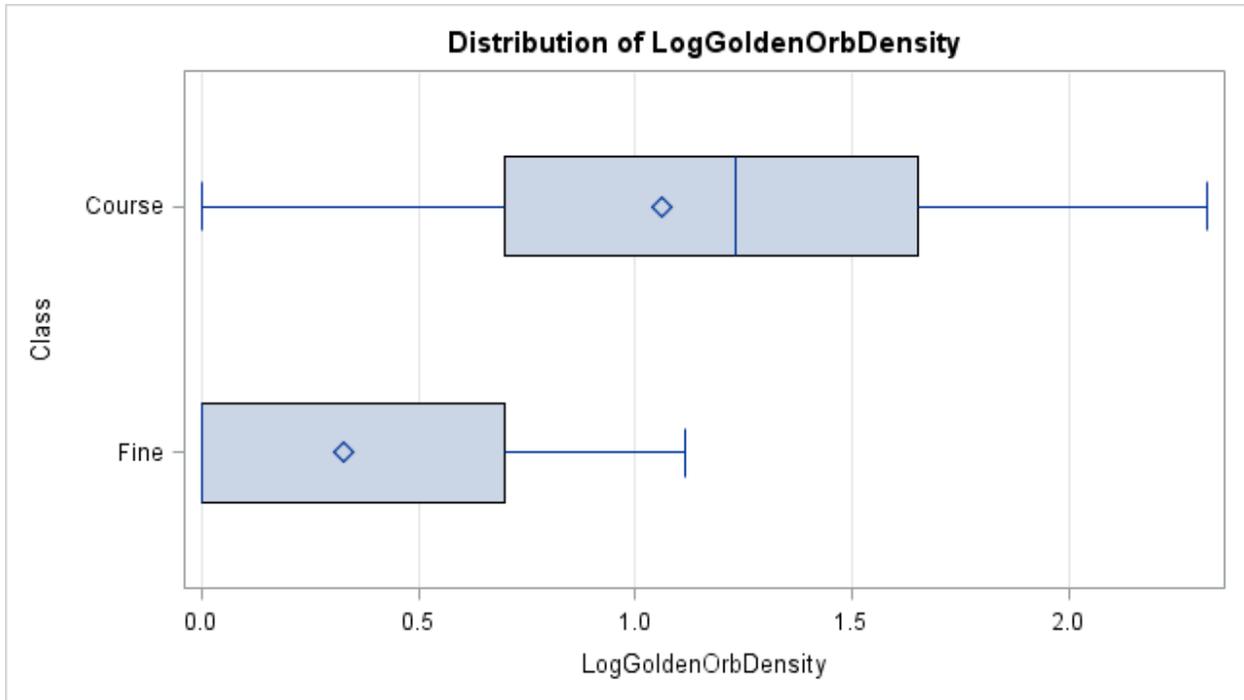


Figure A10. Golden Orb (*Q. aurea*) density (log scale) for sites categorized as course or fine. Satterthwaite's t-test indicated difference between categories was highly significant ($t = 7.57$, $p < .0001$).

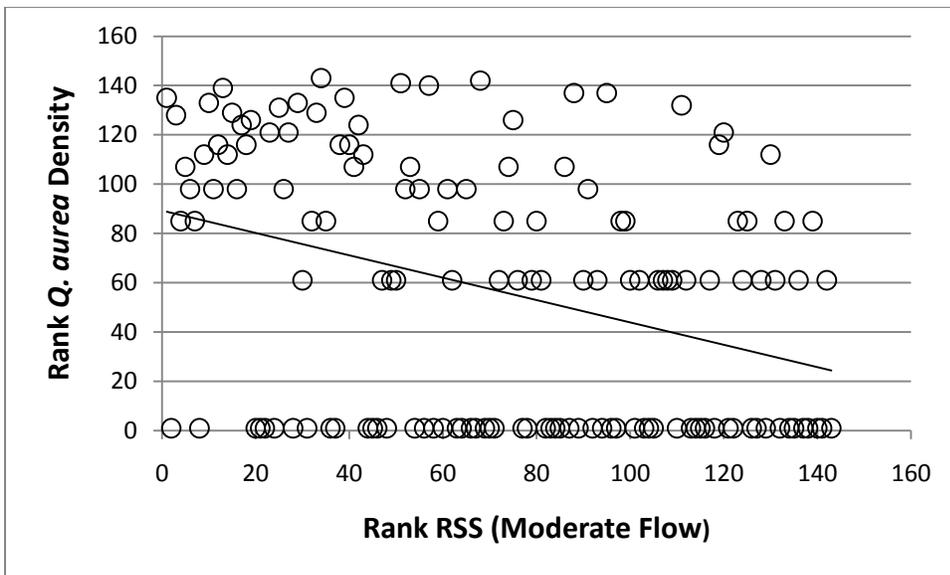


Figure A11. Plot of Spearman correlation between ranked data for RSS under moderate flow conditions and *Q. aurea* density ($R = -.37$, $p < .0001$). The trend line is presented to demonstrate the general trend in the data.

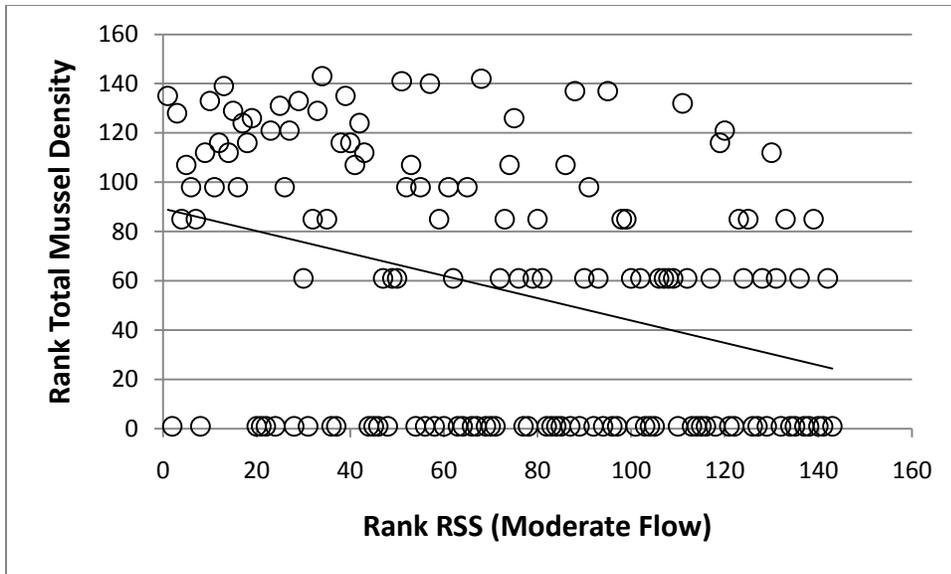


Figure A12. Plot of Spearman correlation between ranked data for RSS under moderate flow conditions and total mussel density ($R = -.42$, $p < .0001$). The trend line is presented to demonstrate the general trend in the data.

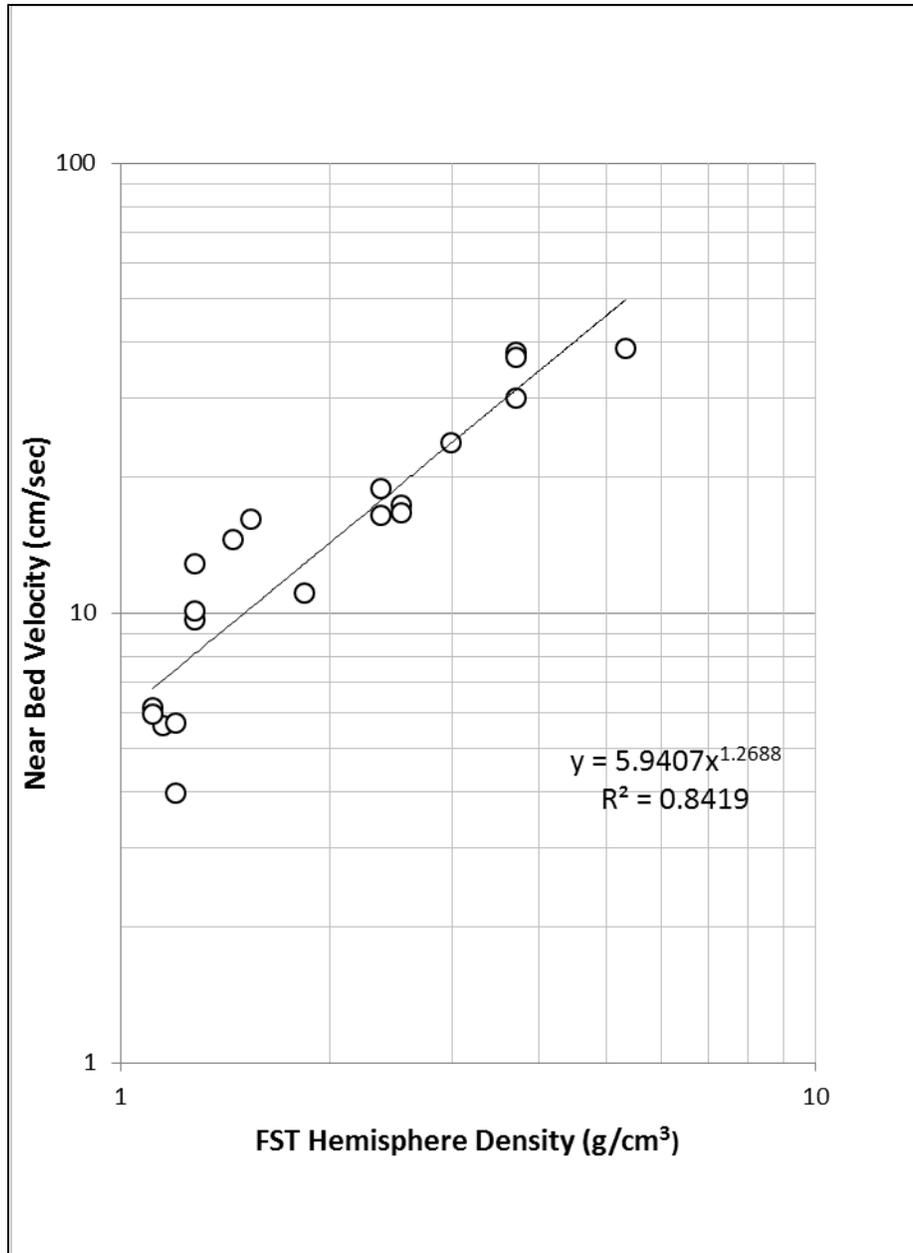


Figure A13. Best fit regression for relationship of hemisphere density to near bed velocities required to move the hemisphere in a flume.

Table A1: Average sediment composition, percent by mass, for each site.

	Goliad	Mueller	Palmetto	Lake Wood
%32mm	32.49%	0.00%	40.93%	0.00%
%16mm	14.17%	0.00%	15.54%	0.00%
%8mm	6.56%	0.00%	10.36%	0.00%
%4mm	2.75%	0.12%	4.58%	0.80%
%2mm	1.38%	0.08%	2.28%	0.17%
%1mm	1.96%	0.27%	2.02%	0.58%
%500μ	11.59%	5.84%	3.10%	6.35%
%250μ	21.08%	37.56%	7.48%	27.19%
%125μ	5.65%	39.24%	11.03%	34.35%
%63μ	1.53%	10.07%	1.86%	19.93%
%fines	0.84%	6.81%	0.82%	10.63%

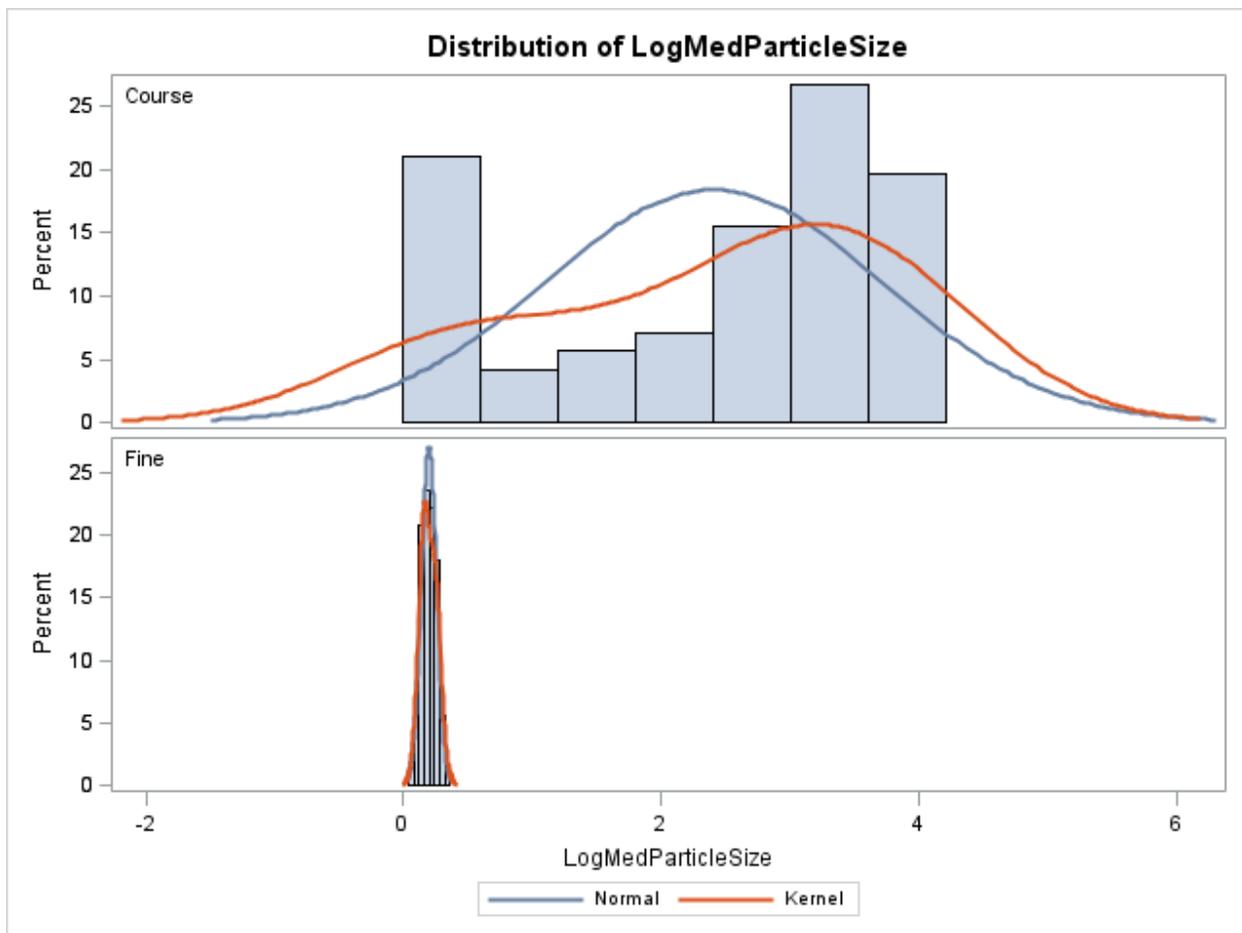


Figure A14. Histogram of median particle size distribution (log scale) for sites categorized as course or fine.

**Exhibit A
Scope of Work**

**Habitat Requirements for *Quadrula aurea* (Golden Orb) in the lower San Antonio
and Guadalupe River drainages.**

INTRODUCTION

Freshwater mussels or unionids (Bivalvie: Unionidae) have experienced a dramatic decline in both numbers and distribution throughout the United States. In fact, it has been estimated that of the 297 species known to occur in North America, 12 % are thought to be extinct and 23 % are considered threatened or endangered (references in Galbraith et al. 2008). Freshwater mussels possess a suite of biological characteristics that render them susceptible to range reductions and extirpations (Vaughn and Taylor 1999). Unionids are long-lived, sedentary organisms that spend a portion of their lives as ectoparasites on fish (Galbraith et al. 2008; Vaughn and Taylor 1999). As a result, anthropogenic impacts such as overharvesting, urban sprawl, impoundments, poor agriculture practices, introduction of alien species, and apathetic land-management policies have reduce or eliminate many unionid populations (Bogan 1993; Lydeard et al. 2004; Neck 1982; Strayer 1999a; Vaughn and Taylor 1999).

Despite a general knowledge of unionid ecology, little is known regarding the physical habitat necessary to maintain mussel populations. Freshwater mussels are patchy in distribution, existing in multispecies aggregates called mussel beds (Strayer 1999b). Suitable habitat is considered the initial limiting factor for these populations which may explain the patchy distribution of unionids within lotic systems (Strayer 2008). At reach and catchment scales, unionid occurrence is correlated with regional factors such as landuse and geology (Arbuckle and Downing 2002; McRae et al. 2004; Strayer 1983, 1993; Vaughn 1997). However, at local-scales, similar descriptors of habitat have been largely unsuccessful for predicting unionid occurrence (e.g. Brim Box et al. 2002; Holland-Bartels 1990; Strayer and Ralley 1993). One explanation for this is that traditional habitat descriptors are often vague, tend to be based on flow conditional measurements, or fail to address the underlying factors responsible for mussel occurrence (Layzer and Madison 1995; Morales et al. 2006; Strayer 1999b, 2008). Moreover, habitat preferences for many species are based on observations of adults rather than juveniles. Consequently, traditional measurements of habitat may be completely irrelevant to understanding habitat requirements needed to maintain existing mussel beds (Layzer and Madison 1995).

Because mussels are long-lived and relatively sessile they require stable substrate to burrow and anchor. Recent studies have observed that low mussel abundance occurs in portions of a stream with high shear stress (Layzer and Madison 1995). High shear stress is problematic for benthic organisms because during episodes of high river discharge the drag force of water (i.e. shear stress) may exceed the weight force of gravity holding bed particles in place. For mussels, entrainment of the substratum may result in bed movement, which in turn may lead to burial, scouring, crushing or dislodgment of juvenile and adult unionids (Hastie et al. 2001; Johnson and Brown 2000; Lorang and Hauer 2003; Strayer 1993,1999b). Thus, mussel distribution within lotic systems is thought to reflect portions of a stream that remain stable during periods of high flow. Several studies support this hypothesis (Hastie et al. 2001; Johnson and Brown 2000; Layzer and Madison 1995; Morales et al. 2006; Strayer 1999b). Because it is clear that substrate stability plays a role in mussel occurrence, recognizing stable mussel habitats and identifying the degree of stability for known mussel beds is important for maintaining viable unionid populations. The purpose of this study will be to examine the effects of hydrology and substrate on Golden Orb (*Quadrula aurea*) populations in the lower San Antonio and Guadalupe River drainages. This information is important because *Q. aurea* is currently being petitioned for protection under the Endangered Species Act.

Task 1: Identify four sample sites with sufficient numbers of live *Quadrula aurea* to assess substrate stability.

With cooperation between Texas Parks and Wildlife (TPWD) and the University of North Texas (UNT) a maximum of four sample sites with sufficient numbers of live individuals of *Q. aurea* will be selected in the lower San Antonio and Guadalupe River drainages. Because limited information exists regarding the demography and current status of known populations of *Q. aurea*, a site reconnaissance survey will be performed. The reconnaissance survey will examine sites in the lower San Antonio and Guadalupe River drainages where live individuals of *Q. aurea* have been collected (see Table 1). During the reconnaissance survey UNT personnel will use timed searches to determine the abundance and extent of *Q. aurea* distribution within these sites. Sampling will follow qualitative mussel sampling methods outlined in Strayer and Smith (2003). Search times may vary by site, but in general will be approximately two to three person-hours per site. For live individuals collected shell length (defined as the greatest distance between the anterior and posterior end of each mussel) will be measured to gauge recruitment.

Task 2: Monitor flow conditions and the distribution of *Quadrula aurea* at sites on the lower San Antonio and Guadalupe River drainages.

Data from the reconnaissance survey will be used to choose a maximum of four sample sites in the lower San Antonio and Guadalupe River drainages. A GPS will be employed to mark sample site coordinates; photographs will also be captured. Initially, systematic sampling will be used to estimate mussel densities. A stratified-block design will be used to examine the effects of hydrology and substrate on the abundance and distribution of *Q. aurea*. Specifically, 6 equidistant transects with a minimum of 6, 0.25 m² quadrats will be deployed at each site. Transects and quadrats will be marked with a GPS and distance markers so they can be located during high flows. Within each quadrat sediment will be excavated to 15 cm, this will ensure that both adult and juvenile mussels are collected. Shell length will be measured for these individuals. A subsample of *Q. aurea* will be retained for biomass determination to assess whether mussels located in portions of a stream with high shear stress have lower biomass compared to those inhabiting areas with low shear stress. At each quadrat substrate composition, water depth, water velocity and percentage of filamentous green algae, diatoms, cyanobacteria and detritus will be measured and/or estimated. Substrate characterization will follow Randklev et al. (2010) using a modified Wentworth scale. These parameters with exception of water depth and water velocity will be measured only during low flow. For both high and low flow sampling, water temperature, dissolved oxygen, pH and conductivity will be recorded from the midpoint of the river channel. Flow conditions will be monitored several times per year during different rates of discharge. Specific flow ranges will be agreed upon in advance with TPWD once sample sites are selected. However, these ranges should include low, medium and high flows. For both medium and high flows water depth will be measured using a digital depth sounder and velocity will be measured using a flow meter fixed to a Columbus-type sounding weight. Collection of instream flow data will occur at the beginning, middle and end of the study period.

Task 3: Calculate the shear stress ratio and its relationship with mussel density on selected sites in the lower San Antonio and Guadalupe River drainages.

Enumeration of this ratio will follow Randklev et al. (2010); see Appendix A for equations that will be used in this study. The results from this will be used to help develop instream flow requirements needed to maintain existing populations of *Q. aurea*.

Task 4: Compile and summarize life history information for *Quadrula aurea*.

Existing literature and other information on *Q. aurea* life history will be compiled and summarized. The reproductive life history information that will be compiled will consist of, but not limited to, spawning season and glochidia-fish host information. Life history information of *Q. aurea* sister taxa will also be assessed as a surrogate for reproductive life history information if the sister taxa information is deemed similar for *Q. aurea* in order to fill, in the interim, information gaps that may exist. A strategy for filling those gaps will be developed.

Task 5: Prepare progress and final reports

Progress reports will be provided quarterly and include summarized survey results and hydrological conditions. The final product will include raw data files, a GIS with appropriate data layers created using Arc GIS Version 9.3.1. A final report will convey survey results, analysis of flow requirements for *Q. aurea*, data collection methods and other valuable information will be provided in a PDF format. A draft of the final report will be provided for comments prior to final submission. The final report will be provided to the study sponsor within eight weeks of the study finish. All deliverables will be submitted in a digital format on CD.

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Table 1. List of *Q. aurea* mussel populations in the lower San Antonio, San Marcos and Guadalupe River drainages (courtesy of Robert G. Howells).

Locations for known populations of *Q. aurea*

Guadalupe River, Kerrville

Guadalupe River, below Lake Gonzales

Guadalupe River, below Lake Wood

San Marcos River, Palmetto State Park

San Antonio River, FM 2506, Goliad Co.

San Antonio River, Goliad State Park, Goliad Co.

Appendix A.

Shear stress equations are used to estimate the force exerted by flow on the stream bottom, whereas critical shear stress equations are used to estimate the shear stress required for incipient motion. For this study shear stress (τ_o) will be calculated as:

$$\tau_o = gSD\rho_w$$

where g = acceleration due to gravity (m/s^2), S = slope of water surface (dimensionless), D = depth of water (m), and ρ_w = density of water (kg/m^3). Although τ_o applies theoretically to uniform flow conditions it is considered useful for estimating shear stress at a specific locations relative to depth and flow (Lorang and Hauer, 2003).

The Shields (1936) entrainment function (τ_c), estimates the shear stress needed to initiate particle entrainment. Because, the Shields entrainment function was originally tested in flumes variance arises when this equation is applied to data collected from natural systems. To improve estimates of τ_c two derivations of Shield's equation will be used to take into account particle size and angles of repose. Critical shear stress will be enumerated using the following formulas:

$$\tau_c = \tau_*c d_*^{-0.6} g(\rho_s - \rho_w)d \tan \Phi \quad \text{:For silts and sands}$$

$$\text{Where; } d_* = d_{50}[(G-1)g/\eta^2]^{1/3}$$

$$\tau_c = \tau_*c g(\rho_s - \rho_w)d_{50} \tan \Phi \quad \text{:For gravels and cobbles}$$

where ρ_s is the density of the substrate particle ($2.65 kg/m^3$), ρ_w is the density of water (kg/m^3), g is acceleration due to gravity (m/s^2), d_{50} is the median sediment size (m), τ_*c is dimensionless critical sheer stress (0.25 for silts/sands and 0.06 for gravels and cobbles), Φ is the angle of repose of the particle (angles are given by Julien, 1995), η is the kinematic viscosity of water (m^2/s), and G is the specific gravity of sediment.

Sediment entrainment potential or relative substrate stability (RSS) for a given discharge and substratum profile can be evaluated by comparing τ_o and τ_c (Morales et al. 2006) and will be calculated as:

$$RSS = \tau_o/\tau_c$$

where τ_o is shear stress (defined above) and τ_c is critical shear stress (see derivation listed above). The ratio between τ_o and τ_c integrates water depth, energy gradient or water-surface slope, median sediment-particle size, and critical shear stress. Unlike other hydraulic measures that are flow conditional (e.g., depth, water velocity, shear stress), RSS normalizes shear stress so that entrainment potential can be compared between sample sites and during different flow regimes. Morales et al. (2006) suggest that mussel densities will be greatest at sites where entrainment threshold is less than one and lowest in portions of a stream where RSS values are greater than one. However, critical shear stress is considered, at best, a minimum estimate of sediment entrainment potential therefore we will use two entrainment thresholds following Elliot (2002):

Partial entrainment threshold (limited movement of d_{50} particles) - $\tau_o = \tau_c$

Complete entrainment threshold (complete movement of d_{50} particles) - $\tau_o = 2\tau_c$

Appendix C – Review Comments and Response

All required and suggested changes have been addressed.

Attachment 1

Habitat Requirements of the Golden Orb (*Quadrula aurea*) Draft-final report to the Texas Water Development Board

Contract Number 0904830970

The researchers appear to have accomplished all of the objectives of this project. In addition to the final report, please provide all data collected for this project in electronic format (e.g. quadrant data including velocity, depth, substrate, etc. and mussel occurrence data). Staff review generated the following comments:

REQUIRED CHANGES

1. There appears to be a mismatch between what is labeled as “high” flow in Table 2 on page 16 and what is described as “moderate” flow in the text on pages 16 and 17. In Table 2, only the adjectives “low” and “high” are used to describe flow conditions. On page 16, 1st paragraph, 2nd sentence, the text states that “RSS values were calculated for both moderate and low discharge values.” The 5th sentence of the same paragraph “Only moderate flow RSS values were found to have a significant, negative relationship with density....” Please adopt a consistent means of referring to flow conditions. I suggest changing “high” to “moderate” in Table 2.
2. From Table 2 on page 16, it appears that there are several relationships between habitat parameters and mussel density that are worth investigating. One is the relationship between RSS at moderate/high flow. Figure A3 on page 24 provides a very useful plot of the data related to this relationship. Please provide similar plots of the data for the other relationships shown in Table 2 (specifically Golden Orb density versus FST hemisphere density and Golden Orb density versus median particle size). These plots could be inserted in an appendix to the report.
3. Table 1 on page 12 provides fairly precise location data that could provide unscrupulous readers of a public document with the location of individuals from a species whose population is state listed as threatened. Please have the subcontractor consult with Texas Parks and Wildlife Department staff regarding appropriate level of location data to provide with the report in order to comply with that agency’s policies regarding the publication of the location of threatened populations.
4. The axis of the chart in Figure A4 on page 25 are not labeled. Please provide appropriate labels (including units) for this figure. In addition, it is unclear if the figure represents data that was collected for this project or is cited from another source. If the former, the data and the way that it was collected should be described in the report. If the latter, a citation should be provided.

SUGGESTED CHANGES

5. Table 1 on page 12 appears to have a misleading title, “Site locations where *Q. aurea* were found during reconnaissance in May and June 2011.” The table shows locations where *Q. aurea* were found, as well as locations where they were not found. Consider re-titling this table as “Reconnaissance sites for *Q. aurea* in May and June 2011.”
6. “The sediment-water interface” is referenced in several places in the document (e.g. page 9, 3rd paragraph, 3rd sentence). We suggest that this term should be replaced with the more readily understood phrase “the channel bottom.”