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Literature Review to Evaluate Mussel-Flow Ecology in the Lower Guadalupe River Basin

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Final Report

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Project Goals and Objectives

In 2007, the passage of Senate Bill 3 (SB3) of the 80th Texas Legislature amended the Texas water code (Section 11.0235) and established a stakeholder-driven process for identifying and quantifying flow regimes needed to maintain sound ecological environments in Texas rivers and estuaries. Environmental flow recommendations for the lower Guadalupe River were made in 2011 and used to develop environmental flow standards by the Texas Commission on Environmental Quality (TCEQ) in 2012 (TCEQ 2012). The SB3 process includes an adaptive management component wherein a Basin and Bay Area Stakeholder Committee (BBASC) can recommend changes as new data and information become available within their areas. Since 2011, several studies related to freshwater mussels (order Unionoida) and fish have been conducted that could help guide refinement of flow standards for the lower Guadalupe River.

Freshwater mussels are one of the most imperiled aquatic groups in North America due in part to human impacts to the natural flow regime of river systems (Williams et al., 1993; Strayer et al., 2004; Haag, 2012; Randklev et al., 2018). As a group, mussels are particularly susceptible to modified flow regimes and subsequent changes in water quality (e.g., temperature, sediment, and pollution) due to their biology and unique life history (Randklev et al., 2018; Khan et al., 2019). Specifically, mussels are sessile, filter-feeding, ectotherms that require host fish (in most cases) to complete reproduction and for dispersal (Haag, 2012). Because of these traits, mussels are often unable to respond to changes in habitat such as stream dewatering or scouring.

The Guadalupe River basin, located in southcentral Texas, is a managed river system with variable hydrology, which has raised questions about whether current mussel flow needs are being met. The river is sustained through a combination of spring-fed tributaries, groundwater inputs, dam releases, and surface runoff (Perkin and Bonner, 2011; Khan et al., 2020). Additionally, the Guadalupe River (~644 km [i.e., ~400 mi] in length) is modified by several mainstream impoundments, including Canyon Lake Reservoir (Canyon Lake, Comal County, TX, USA) which accounts for approximately 20% of the

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river's flow into Guadalupe Bay (Phillips, 2012). The Guadalupe basin harbors a unique mussel fauna,

including three mussel species (*Cyclonaias necki* [Guadalupe orb], *Fusconaia mitchelli* [False spike], and

Lampsilis bergmanni [Guadalupe fatmucket]) under review for protection under the U.S. Endangered

Species Act (ESA; USFWS, 2009, 2011; TPWD, 2020). Human impacts and climate change have altered

the natural flow regime, which has been suggested as a contributing factor to the decline of these species

(Khan et al., 2019, 2020).

Since 2011, there has been an increased effort to understand mussel-flow relationships in the

basin. These results have yet to be summarized or placed in the context of environmental flow

management. Both are important for developing flow standards for mussels based on the best available

science. To begin addressing these knowledge gaps, we reviewed and summarized available mussel and

fish flow literature, using data within the Guadalupe River basin, when possible, in an attempt to identify

thresholds that can be used to determine flow needs for mussels in the Guadalupe basin. From this

information, a research plan was created to help guide future research focused on developing mussel

protective environmental flow standards.

Summary of Findings

Task 1: Review and summarize mussel research in the basin.

Impact of changes in flow on reproduction

Freshwater mussels are gonochoristic and reproduce through a process that begins with spawning. Males release spermatozeugmata directly into the water column, which females then filter out to fertilize their eggs (McMahon & Bogan, 2001). Females brood the glochidia in the interbranchial chambers of their gills (marsupia) until the glochidia are mature (Kat, 1984; Richard et al., 1991). Timing and duration of spawning and brooding can vary by species (Haag, 2012). Generally, mussels are categorized as either short- or long-term brooders. Short-term brooders spawn in the late winter and early spring and brood their glochidia for a short period following fertilization (2 – 8 weeks). Long-term brooders spawn in the late summer and early fall and brood their glochidia through the winter until spring (Garner et al., 1999; Haag, 2012). Following brooding, unionid female mussels either actively, through the use of mantle lures or conglutinates (i.e., packets containing glochidia), or passively release their larvae into the water column. The released larvae typically attach to the fins or gill filaments of their host fish and metamorphosize several weeks later into free-living juveniles (McMahon & Bogan, 2001; Haag, 2012).

Gametogenesis

Changes to the natural flow regime can potentially affect the mussel life cycle in several ways. Freshwater mussel sperm/egg production and function are rooted in accumulated degree days (a measure of the total heat an organism has experienced over time; Dudding et al., 2020) and photoperiod, and because of this, changes in water temperature due to increases or decreases in flow can decrease gamete viability (Galbraith & Vaughn, 2009; Gascho Landis et al., 2012). For example, Galbraith and Vaughn (2009) examined environmental factors that influence the successful reproduction of three freshwater mussels (*Quadrula cylindrica* [Rabbitsfoot], *Cyclonaias pustulosa* [Pimpleback], and *Quadrula quadrula* [Mapleleaf]) in the Little River (OK, USA). Authors found a significant relationship between ovum

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105 diameter and the number of accumulated degree days in all three species and between sperm

106 concentration and number of accumulated degree days in two of the three species (i.e., *Q. cylindrica* and

107 *Q. pustulosa*). Peak ovum size was observed when the number of accumulated degree days was less than

108 1,000 days with the sharpest decline seen in *C. cylindrica* followed by *C. pustulosa* and *Q. quadrula*,

109 respectively. Peak sperm concentration was observed when the number of accumulated degree days was

110 just over 1,000 days with the sharpest decline observed in *Q. cylindrica* (Galbraith & Vaughn, 2009).

111 Dudding et al. (2020), investigating environmental relationships to spawning/brooding of three freshwater

112 mussel species (*Cyclonaias necki* [Guadalupe orb], *Fusconaia mitchelli* [False spike], and *C. pustulosa*)

113 in the Guadalupe River (TX, USA), observed similar results. Authors found accumulated degree days to

114 be a good determinant of gamete production with egg diameter and sperm concentration both being

115 maximized during a low number of degree days. Peak egg diameter was observed when the number of

116 accumulated degree days was approximately 1,000 days, with the sharpest decline seen in *F. mitchelli*

117 followed by *C. pustulosa* and *C. necki*, respectively. Peak sperm concentration was observed during a low

118 number of accumulated degree days in all three species. In fact, for *C. necki*, *C. pustulosa* and *F.*

119 *mitchelli*, sperm concentration began to decrease as degree days increased, indicating additional

120 accumulated temperature beyond 1,000 days constrained gametogenesis (Dudding et al., 2020).

121 *Spawning and Brooding*

122   Alterations to natural flow regimes may further impact the mussel life cycle by disrupting

123 spawning cues and limiting fertilization success. Sperm release within male mussels is hypothesized to be

124 cued by a combination of temperature, photoperiod, and food availability (Borcherding, 1995). Flow

125 alterations may impact these factors during high and low flows, which may result in biological shifts for

126 freshwater mussels outside of their optimal range. Specifically, higher water temperature and decreased

127 food availability are associated with low flow while decreased water temperature and decreased food

128 availability are associated with high flow (Booker & Whitehead, 2021). Galbraith (2009) examined how

129 sperm viability is impacted by water temperature in *C. pustulosa* (Little River [OK, USA]) by exposing

130   sperm to temperatures of 5, 15, 25, and 35˚C and estimating the percentage of motile sperm after 0, 2, 4,

131   8, 24, and 48 hours. Authors found sperm to be most motile/viable when water temperatures were

132   between 15˚C (9.16% motile sperm) and 25˚C (11.02% motile sperm) with highest motility occurring at

133   the latter temperature. No significant differences in percent motile sperm were observed between 8, 24,

134   and 48 hours (Galbraith, 2009). Because low motility and viability can have profound impacts on the

135   success of sperm reaching eggs, changes to water temperature via flow alteration could inhibit successful

136   mussel reproduction (Mojares et al., 1995; Ciereszko et al., 2001).

137   The distance motile sperm travel depends on stream discharge (Galbraith, 2009) and so changes

138   to flow could prevent fertilization. For example, during high flow events, sperm may be washed quickly

139   downstream (Galbraith, 2009) or have their concentrations diluted, both of which could decrease the

140   chances of successful fertilization (Haggerty et al., 1995). In contrast, during low flow events sperm

141   dispersal may be restricted to perennial pools due to stream intermittency, which could limit fertilization.

142   Galbraith (2009), estimating how far downstream viable *C. pustulosa* sperm could travel under four

143   different flow regimes (0.51, 8.34, 18.89, 58.26 m^3s^{-1} [i.e., 18.01, 294.52, 667.09, 2057.43 ft^3s^{-1}]) of the

144   Little River (OK, USA) during the summer, found as discharge increased so did sperm settling distance

145   (i.e., flow rate + sperm settling rate). Authors concluded successful mussel reproduction requires adequate

146   flow to deliver sperm to females of the same species and altered flow has the potential to disrupt the

147   delivery of sperm to females.

148   The development of embryos to glochidia and their subsequent release is linked to environmental

149   factors such as water temperature, photoperiod, and food availability, which are regulated by streamflow

150   (Gascho Landis et al., 2012; Schneider et al., 2018; Zając & Zając, 2021). Of these factors, water

151   temperature appears to play a proximate role. Zając and Zając (2021), investigating seasonal patterns in

152   the developmental rate of glochidia in *Unio crassus* (Thick shelled river mussel) in southern Poland,

153   found brooding period shortened significantly from 35 days during early spring (4 – 13.5°C) to 9 days

154   during the summer (19 – 25°C) due to thermal conditions. This decrease in brooding period with

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155   increased water temperatures indicates developmental rates are linked to water temperature (Zajac &

156   Zajac, 2021). Spawning and brooding are energetically costly (e.g., deplete Ca^{2+} reserves) and so

157   decreases in brooding period could exhaust individuals' resource and energy reserves due to increases in

158   metabolic demand to support faster developmental rates (Silverman et al., 1985). Changes to how an

159   organism manages its metabolism can have cascading impacts to growth, survivorship, and reproduction,

160   which can affect long-term viability.

161   Similarly, glochidial release is thought to be triggered by environmental cues such as water

162   temperature and/or river level (Hastie & Young, 2003). Hastie and Young (2003), studying the timing of

163   spats (i.e., glochidial release) in a *Margaritifera margaritifera* (Freshwater pearl mussel) population in

164   Scotland, found spats occurred when water temperature increased >2°C and/or river level increased >0.1

165   m (i.e., >0.3 ft). Schneider et al. (2018), evaluating the effect of temperature on the reproduction success

166   of *U. crassus* (River Tommarpsan [Sweden]), found the timing of glochidial release was delayed at low

167   (i.e., <10°C) and high temperatures (i.e., 10-20°C [increased 1°C day^{-1}]). Schneider et al. (2018) further

168   noted increased mussel activity moving from cold (<10°C) to natural temperatures (10 – 15°C), which

169   resulted in gravid females releasing their glochidia soon after, indicating a temperature threshold for

170   glochidial release. Given the relationship between mussel reproduction and the hydrological cycle, it is

171   likely natural or human-mediated changes that alter the timing, magnitude and variability of the natural

172   flow and thermal regimes could negatively impact reproductive success.

173   *Host Fish Interactions*

174   Altered flow regimes can also interfere with interactions between gravid females and their host

175   fish. While this hypothesis is reasonable, studies directly investigating this relationship are non-existent.

176   Most stream fish have a distinct range of habitat preferences that are shaped by abiotic characteristics

177   (e.g., depth, velocity, temperature, DO; Hutchinson, 1957). As discharge decreases, changes to water

178   quality could exceed host fish thresholds and isolate or force host fish to seek refuge in areas that do not

179   overlap with mussels (Avery-Gomm et al., 2014). Similarly, as discharge increases, host fish may seek

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180 refugia to avoid sediment plumes and high flows (Carlson et al., 2001; Goldsmith et al., 2020). For

181 example, Gelwick (1990), examining riffle and pool fish assemblages in Battle Branch (northeastern OK,

182 USA), found species occupying riffle habitats often seek pool habitat as refuge during droughts and

183 floods. Results suggest flow related stress during reproductive windows may limit glochidia and host fish

184 interactions, especially for riffle species (Gelwick, 1990). This idea is further corroborated by DeAngelis

185 et al. (1997), who modeled fish dynamics in a hydrologically pulsed ecosystem (i.e., Everglades / Big

186 Cypress region of southern Florida [USA]). Specifically, authors found small fish abundance declined

187 during longer hydroperiod simulations (i.e., year-around flooding; 5 to 15 fish/m^2) compared to historical

188 fish population data (15.5 to 17.1 fish/m^2 [1977 – 1978)] and 30.2 to 34.5 fish/m^2 [1983 – 1984]),

189 indicating host fish likely leave their habitat in search of refugia during high pulse events. Also

190 confirming Gelwick's (1990) ideas, Davey and Kelly (2007), investigating fish community responses to

191 drying disturbances in an intermittent stream (Selwyn River, New Zealand), found *Galaxias vulgaris*

192 (canterbury galaxias), *Gobiomorphus breviceps* (upland bullies), and *Salmo trutta* (brown trout) migrated

193 upstream to permanent water as the stream began to dry. As drought dynamics change, it is likely fish

194 assemblages will shift in abundance and dominance (Zeug et al. 2005; Lennox et al., 2019), which overall

195 may result in reduced interactions between gravid females and host fish due to mussels' limited ability to

196 move.

197   Alterations to natural flow regimes can also negatively impact settlement of juveniles after

198 excystment (i.e., post-metamorphic stage in which juveniles escape from cysts on the host fish's gills

199 and/or gill filaments; Haley et al., 2007). However, information demonstrating this idea is limited, with

200 the exception of Daraio et al. (2012). Authors simulated juvenile dispersal distance of *Amblema plicata*

201 (Threeridge) from the Upper Mississippi River (USA) under six excystment scenarios (*A1* = <1.0 m [0.03

202 m^{3}s^{-1} {i.e., 1.06 ft^{3}s^{-1}}]; *A2* = <1.5 m [0.02 m^{3}s^{-1}{i.e., 0.71 ft^{3}s^{-1}}]; *A3* = <2.0 m [0.019 m^{3}s^{-1}{i.e., 0.67 ft^{3}s$^-$

203 ^{1}}]; *A4* = >2.0 m [0.018 m^{3}s^{-1}{i.e., 0.64 ft^{3}s^{-1}}]; *A5* = excysted in locations based on USGS host fish

204 coordinate data [0.049 m^{3}s^{-1}{i.e., 1.73 ft^{3}s^{-1}}]; and *A6* = excysted in every cell within the domain [0.019

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205   m^3s^{-1} {i.e., 0.67 ft^3s^{-1}}]) and found more than 50% of juveniles settled within 500 meters (i.e., 1,640 ft) of

206   excystment, except *A4*, regardless of water column depth (Daraio et al., 2012). Results suggest juvenile

207   dispersal distance is more closely regulated by velocity than excystement height with higher dispersal

208   distances observed when flow is elevated (Daraio et al., 2012). Thus, during low flows juvenile dispersal

209   may be severely limited and during high flows juveniles may be deposited in unsuitable habitats.

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211   Impact of changes in flow on feeding, growth, respiration, and metabolism

212   Freshwater mussels utilize filter feeding not only for food acquisition, but also for oxygen uptake,

213   waste excretion, and gamete dispersal and procurement (Haag, 2012). The process of filter feeding occurs

214   when water is drawn in through the inhalant aperture via ciliary action and then passed through the gills.

215   Material captured on the gills are then moved via ciliary action to the labial palps, which sort non-food vs.

216   food items. Material deemed food items are then moved to the mouth for ingestion, while non-food items

217   are bound in mucus agglomerations known as pseudofeces (Nichols et al., 2005; Cummings & Graf,

218   2010). Pseudofeces accumulate at the base of the inhalant aperture and are periodically expelled through

219   rapid valve closure (McMahon & Bogan, 2001).

220   *Feeding*

221   Flow regime alteration can directly interfere with filter feeding by physically impeding the

222   amount and type of food an individual can filter. Flow related stressors may impact species and

223   populations differently as filtration rates are a function of gill-surface morphology, algal flux (i.e., food

224   concentration x velocity), and valve gap (Riisgård & Larsen, 2010; vanden Byllaardt & Ackerman, 2014;

225   Mistry & Ackerman, 2018). vanden Byllaardt and Ackerman (2014), evaluating how hydrodynamic

226   habitat (i.e., lentic and lotic) influences the clearance rates (i.e., suspension feeding ability) of four

227   freshwater mussels (*Elliptio complanata* [Eastern elliptio], *Elliptio dilatata* [Spike], *Fusconaia flava*

228   [Wabash pigtoe], and *Strophitus undulatus* [Creeper]) in northeastern Ontario (Canada), found clearance

229 rates significantly varied with velocity. On average, clearance rates of all four mussels were 20 times

230 greater in flowing water than static, no-flow conditions, regardless of habitat origin (lentic or lotic).

231 However, comparing lotic versus lentic species, authors found the clearance rate of *E. dilatata*, a lotic

232 species, from the Grand River was about four times greater than lentic and other lotic species including its

233 conspecific (i.e., other *E. dilata* population) within the Ausable River. Authors suggest differences may

234 be due to *E. dilatata's* specialized gill structure, which appears to be adapted for removing small particles.

235 Based on these results, authors suggest differences in clearance rates among and within species may be

236 due to differences in algal flux (i.e., food concentration x velocity) among habitats, indicating mussel

237 species are likely specialized to different hydrodynamic conditions (vanden Byllaardt & Ackerman,

238 2014).

239 Given the role streamflow plays in shaping mussel feeding, substantive changes, human or

240 natural, could have a negative impact (Pusch et al., 2001). Widdows et al. (2002), investigating the effects

241 of current velocity on mussel feeding rate in southwest England, found clearance rates of *Mytilus edulis*

242 (Blue mussel; a marine mussel) were impacted at both low and high current velocity. Specifically, authors

243 observed increased algal cell depletion zones (i.e., reduced algae available within water surrounding

244 mussels) with declining currents below 0.05 ms^{-1} [i.e., 0.16 fts^{-1}]. Additionally, when velocity was above

245 0.8 ms^{-1} [i.e., 0.26 fts^{-1}], clearance rates declined from 3.3 to 1.8 L h^{-1}individual^{-1}. Results indicate

246 discharge rates that are too low or high may negatively impact species feeding rate (Widdows et al.,

247 2002). Ćmiel et al. (2019), evaluating the response of freshwater mussel recruitment in five species (*Unio*

248 *tumidus* [Swollen river mussel], *Unio pictorum* [Painter's mussel], *Anodonta anatina* [Duck mussel],

249 *Anodonta cygnea* [Swan mussel], and *Pseudanodonta complanata* [Depressed river mussel]) to

250 hydrological changes in a eutrophic floodplain lake (Poland), found a slight increase in discharge

251 positively influenced recruitment in three of the species examined. Specifically, authors found a change in

252 flow velocity between 2013 (0.0009 ms^{-1} [i.e., 0.003 fts^{-1}]) and 2015 (0.0052 ms^{-1} [i.e., 0.017 fts^{-1}])

253 strongly, positively influenced the occurrence of juveniles in all mussels analyzed. Authors hypothesize

254   before the construction of the new outflow channel, velocity may have been slow enough to create an

255   algal depletion zone which may have prevented filter feeding in juveniles (Ćmiel et al., 2019).

256   Research suggests freshwater mussels can discriminate among food particles based on size and

257   shape (Dionisio Pires et al., 2004), nutritional value (Atkinson et al., 2011), and cell surface properties

258   (Jing et al., 2011). Altering natural flow regimes can change the algal taxa available to mussels, which

259   could impact mussels if preferred algal taxa are eliminated or reduced in number. Mistry and Ackerman

260   (2018), researching the impact of algal flux on the clearance rates of four freshwater mussels (*Lampsilis*

261   *siliquoidea* [Fatmucket], *Lampsilis fasciola* [Wavyrayed lampmussel], *Ligumia nasuta* [Eastern

262   pondmussel], and *Villosa iris* [Rainbow shell]) from the Thames River (ON, Canada), found the clearance

263   rate of all species increased linearly with flow chamber velocity, with *L. siliquoidea* (0, 2, 10, 15, 20, 25

264   m^3s^{-1} [i.e., 0, 0.07, 0.33, 0.49, 0.66, 0.82 fts^{-1}]; rate of change = 0.413) increasing at the fastest rate,

265   followed by *L. fasciola* (0, 2, 10, 15, 20, 25 m^3s^{-1} [i.e., 0, 0.07, 0.33, 0.49, 0.66, 0.82 fts^{-1}]; rate of change

266   = 0.363), *L. nasuta* (0, 2, 25 m^3s^{-1} [i.e., 0, 0.07, 0.82 fts^{-1}]; rate of change = 0.013), and *V. iris* (0, 2, 25,

267   35, 45 m^3s^{-1} [i.e., 0, 0.07, 0.82, 1.15, 1.48 fts^{-1}]; rate of change = 0.0073). However, the clearance rate of

268   both *Lampsilis* mussels declined with algal flux. Specifically, the clearance rate of *L. siliquoidea*

269   decreased nonlinearly with algal flux for *Chloromonas* sp. (rate of change = -1.28 ± 0.24), while the

270   clearance rate of *L. Fasciola* declined linearly with algal flux for *Stephanodiscus* sp. (rate of change = -

271   0.3 ± 0.11), *Chlorella* sp. (rate of change = -0.14 ± 0.05), and pennate diatom (rate of change = -0.08 ±

272   0.03). These declines in algal flux indicate a reduced ability to discriminate among algae at higher flux

273   (Mistry & Ackerman, 2018). Therefore, modifications to natural flow regimes due to climate change and

274   anthropogenic activities may limit or alter feeding activities, negatively impacting mussel growth and

275   survival.

276   *Growth*

277   Generally, freshwater mussels are considered long-lived, slow-growing animals; however, growth

278   and longevity are dependent on environmental conditions (Black et al., 2010). Despite this knowledge, the

279   effects of natural and anthropogenic disturbances to flow on freshwater mussel growth remain largely

280   unstudied. An exception is Dycus et al. (2015), a study focused on the effects of flow and stream

281   characteristics on variation in freshwater mussel growth in three species (*Villosa vibex* [Southern

282   rainbow], *Villosa lienosa* [Little spectaclecase], and *Elliptio crassidens* [Elephantear]) within the lower

283   Flint River basin (GA, USA). Authors found the most significant factor influencing variations in mussel

284   growth was spring 10-day high discharge (0.071 m^3s^{-1} [i.e., 2.51 ft^3s^{-1}]), which displayed a strong,

285   quadratic relationship. Authors suggest the processes that led to this relationship may be due to stream

286   transport and total suspended solids (TSS). Specifically, stream transport is dependent on water velocity

287   and under low flow conditions, particulate matter may settle within the water column becoming

288   unattainable to suspension-feeding mussels. Further, increased TSS associated with high flow conditions

289   may increase sorting time and thus energetic costs leading to reduced mussel growth (Black et al., 2010;

290   Dycus et al., 2015). Therefore, it is likely altered flow that results in higher or lower than normal

291   discharge rates will negatively impact mussel growth and survival.

292   *Respiration*

293   Freshwater mussels respire through the use of their gills and oxygen consumption rate can vary

294   with environmental conditions (e.g., water temperature and dissolved oxygen [DO] levels). Thus, changes

295   to the flow regime that impact oxygen concentrations can be detrimental to mussels. For example, Pusch

296   et al. (2001), examining the respiration rate of two unionid freshwater mussels (*A. anatina* and *U.*

297   *tumidus*) from the River Spree (Berlin) in response to increasing water temperatures (8 - 26˚C), found

298   respiration rates significantly increased as ambient temperatures increased. Specifically, respiration rates

299   ranged from 0.14 to 0.63 $mgO_2g^{-1}h^{-1}$ for *A. anatina* and between 0.23 to 0.5 $mgO_2g^{-1}h^{-1}$ for *U. tumidus*,

300   with *U. tumidus* experiencing lower respiration rates after temperatures exceeded 14˚C. Alterations to the

301   natural flow regime (e.g., water withdrawal and/or diversion) that reduce instream flow can lead to

302   increases in water temperature, which could decrease DO concentration (Pusch et al., 2001). Reductions

303   in DO can increase respiration rates, which could lead to shifts in metabolism that impact mussel survival,

304   growth, and reproduction. Kiibus and Kautsky (1996), investigating the respiration rates of tropical

305   freshwater mussels (*Aspatharia wahlbergi* and *Corbicula africana*) in Zimbabwe, found increases in

306   water temperature resulted in increases in respiration until reaching a threshold (29.2˚C in *C. africana* and

307   34.0˚C in *A. wahlbergi*). For both mussels, respiration declined after reaching their threshold, resulting in

308   mussels becoming severely stressed and alternating between active periods where authors noted

309   individuals were coughing and moving around and longer inactive periods of valve closure and inactivity.

310   Additionally, the water became turbid as the stressed individuals produced higher levels of mucus (Kiibus

311   & Kautsky, 1996). These results indicate changes in flow that decrease DO concentration within the water

312   column will likely negatively impact mussel's ability to respire and ultimately persist.

313   *Metabolism*

314   Declines in feeding and respiration may result in shifts in energetic pathways that can negatively

315   affect freshwater mussel growth. When oxygen consumption is not maintained, species may activate their

316   anaerobic metabolism, which is inefficient with respect to energy production (Gade & Grieshaber, 1986).

317   This is problematic as species with indeterminant growth (e.g., freshwater mussels) experience life history

318   tradeoffs when allocating energy (i.e., ATP [adenosine triphosphate]). During optimal conditions,

319   individuals' supply of ATP is enough to cover growth, reproduction, and basal maintenance costs.

320   However, during stressful conditions, individual's supply of ATP may only be sufficient to cover basal

321   metabolic costs. Water temperature plays a significant role in metabolic regulation and so changes to flow

322   that alter the thermal regime may negatively impact mussel metabolism. Despite this knowledge, little

323   information is available on how flows directly impact mussel metabolic rates. Ganser et al. (2015),

324   investigating the impacts of elevated water temperature (20, 25, 30, and 35˚C) on the physiological

325   response of four adult freshwater mussels (*A. plicata, E. complanata, F. flava,* and *Lampsilis cardium*

326   [Plain pocketbook]) which occur in the Upper Mississippi River (USA), found O:N ratio (an index of the

327   relative utilization of proteins during metabolism; Widdows 1978) to be directly affected by temperature

328   in *A. plicata* (between 35˚C and all other treatments), *E. complanata* (between 30˚C and 20˚C / 25˚C),

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329   and *F. flava* (between 20°C and 30°C / 35°C; and between 25°C and 35°C). Specifically, O:N ratio was

330   lower in the 20°C treatment compared to the 35°C treatment in all species, with *E. complanata* and *A.*

331   *plicata* decreasing 20% and 60%, respectively (Ganser et al., 2015). While individuals may survive for a

332   brief time, this is concerning because overtime individuals may die and/or recruitment rates may decrease,

333   increasing the species' chance of extinction.

334

335   Impact of changes in flow on survival

336   The natural flow of a river varies with temporal and spatial fluctuations and limits the distribution

337   and abundance of riverine species (Resh et al., 1988; Power et al., 1995). Over the years, stream

338   biologists have attempted to quantify the effect of flow on biotic communities. In doing so, researchers

339   found physical, chemical, and biological characteristics of a river are shaped by variability in the flow

340   regime (i.e., magnitude, frequency, duration, timing, and rate of change; Poff et al., 2007), and species life

341   history traits are strongly influenced by hydrological conditions (i.e., the habitat template; Olden &

342   Kennard, 2010; Mims & Olden, 2012). Thus, alterations to natural flow regimes and subsequent

343   degradation of habitat quality (e.g., water quantity and quality) within various habitats will likely lead to

344   impacts to population and community structure (Poff et al., 2007; Mittal et al., 2014).

345   *Water Quantity*

346   Flow regime alterations due to climate change and anthropogenic impacts can affect the quantity

347   of water within a river, which could result in increased mussel mortality (Layzer & Madison, 1995;

348   Randklev et al., 2019). For example, increased flow can decrease bed stability, increasing the chances of

349   individuals being entrained within the water column and deposited in unsuitable habitat. Layzer and

350   Madison (1995), examining instream flow needs of freshwater mussels in Horse Lick Creek (KY, USA),

351   found water depth and velocity were significant factors in limiting the distribution of mussels during base

352   flows. Randklev et al. (2019), investigating the hydraulic requirements of freshwater mussels in the

353   Brazos and Trinity River basins (Central TX, USA), found low relative shear stress (RSS) values (i.e., a

354   measure of substrate stability) were associated with high mussel density and species richness.

355   Specifically, authors found diversity was maximized when RSS values were 1 or less. Additionally,

356   Randklev et al. (2019) noted some genera (*Potamilus* and *Lampsilis*) were observed to persist at higher

357   RSS values, when bed mobility is greater, than others (*Amblema*, *Cyclonaias*, and *Quadrula*).

358   Changes to bed stability are problematic for mussels, which are largely sessile, because

359   individuals can be swept downstream and stranded in areas with little to no flow, which can be lethal

360   depending on the time of the year. Bartsch et al. (2000), determining emersion and thermal tolerances of

361   three unionid species (*L. cardium*, *C. pustulosa*, and *E. dilatata*) from Wolf River (WI, USA), found

362   individuals exposed to air temperatures between -10 and 20˚C closed their valves, while those exposed to

363   air temperatures greater than 25˚C demonstrated sublethal behavioral responses (e.g., shell gaping, foot

364   extension, and mucus production). The authors' findings suggest mussels stranded during periods of

365   elevated temperatures are more likely to be negatively impacted (Bartsch et al., 2000).

366   *Water Quality*

367   Decreases in streamflow can affect water quality, which in turn can impact species' long-term

368   viability. Khan et al. (2020), evaluating the upper thermal limits (LT05 [i.e., lethal temperature resulting

369   in 5% mortality] and LT50 [i.e., lethal temperature resulting in 50% mortality]) of three freshwater

370   mussels (*A. plicata, C. necki,* and *F. mitchelli*) from the Guadalupe River (TX, USA), found the LT50 in

371   acute 96 h trials averaged 36.4˚C (ranged from 33.7 to 37.5˚C), while the LT50 in chronic 10 d trials

372   averaged 35.9˚C (ranged from 32.4 to 37.5˚C). Although these averages were not exceeded within the

373   Guadalupe River, comparison of *F. mitchelli*'s LT05 (both acute 96 h [30.5˚C] and chronic 10 d [28.4˚C]

374   values) to continuous water temperature data revealed exceedances. Further, Khan et al. (2019),

375   investigating the upper thermal limits (LT05 and LT50) of eight larvae (glochidia) freshwater mussel

376   species (*A. plicata, C. necki, F. mitchelli, Lampsilis bergmanni* [Guadalupe fatmucket]*, Lampsilis*

377   *hydiana* [Lousiana fatmucket], *Lampsilis satura* [Sandbank pocketbook], *Lampsilis teres* [Yellow

378 sandshell], and *Obovaria arkansensis* [Ouachita creekshell]) from four different basins (Neches,

379 Guadalupe, San Antonio, and Colorado; TX, USA), found LT50 among glochidia averaged 32.4°C

380 (ranged from 26.9 to 36.4°C). Specifically, within the Guadalupe, authors found summer LT50s ranged

381 from 26.9 to 34.1°C (*A. plicata* [28.3°C], *L. hydiana* [34.1°C], and *C. necki* [26.9°C]), while spring

382 LT50s ranged from 33.1 to 36.4°C (*L. bergmanni* [33.1°C] and *C. necki* [36.4°C]). While data was not

383 tied back to discharge, results indicate tolerance likely varies by species and season (Khan et al. 2019).

384 Goldsmith et al. (2021), examining the upper thermal limits (LT05 and LT50) of juvenile and glochidia

385 *Lampsilis bracteata* (Texas fatmucket) in the North Llano and San Saba rivers (TX, USA), found the

386 LT05 was exceeded for both juveniles (30.8˚C) and glochidia (27.9˚C) in the San Saba River when

387 compared to continuous water temperature data ranging from July 2017 to November 2019. In the Llano

388 River, both the juvenile and glochidia LT05 (31.1˚C and 28.2˚C, respectively) and LT50 (32.4˚C and

389 31.8˚C, respectively) were exceeded when compared to water temperature samples reported by the Texas

390 Commission on Environmental Quality from 1968 to 2020. These findings indicate alterations to natural

391 flow regimes that result in low flows and elevated water temperature may be contributing to the decline of

392 the species (Goldsmith et al., 2021).

393 During periods of extreme high and low flow, water quality may degrade as contaminants

394 become more concentrated (Augspurger et al., 2003; Spooner & Vaughn, 2008). Ammonia, which is a

395 common pollutant to streams and rivers, can increase in concentration during drought conditions when

396 streamflow is low, after rapid melting events, and/or after an intense rain event shortly after fertilizer

397 surface application (Sawyer, 2008). Ammonia occurs in both ionized and unionized forms with

398 temperature and pH determining the dominant form (Thurston et al., 1979). During periods of low flow,

399 characterized by elevated water temperature, unionized ammonia becomes more dominant within the

400 water column (Cherry et al., 2005). Unionized ammonia is lipid soluble and readily passes through

401 mussels' gill membrane causing toxic effects (e.g., decreased siphoning ability, depleted energy stores,

402 mortality; Cherry et al., 2005). Newton et al. (2003), investigating the effects of ammonia on juvenile

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403   freshwater mussels (*L. cardium*) in the St. Croix River (MN, USA), found concentrations as low as 127µg

404   NH_3L^{-1} were lethal to 50% of the mussels after four days, while concentrations as low as 93µg NH_3L^{-1}

405   killed 50% of the mussels after 10 days of exposure. Additionally, authors noted growth rate was

406   substantially reduced at concentrations as low as 31µg NH^3L^{-1} (Newton et al., 2003). Augspurger et al.

407   (2003), examining water quality guidance for the protection of freshwater mussels (genera *Amblema,*

408   *Utterbackia, Cyrtonaias,* and *Toxolasma*) from ammonia exposure, found genus mean acute values

409   ranged from 2.56 to 8.97 mg L^{-1} total ammonia nitrogen. Additionally, authors found concentrations as

410   low as 0.7 ppm total ammonia nitrogen to be lethal to juveniles, while concentrations as low as 2.4 ppm

411   total ammonia nitrogen were lethal to glochidia. Flow alterations that degrade water quality will likely

412   negatively impact mussel's ability to persist.

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425   Literature Cited

426   Atkinson, C., First, M., Covich, A., Opsahl, S., & Golladay, S. (2011). Suspended material availability

427   and filtration-biodeposition processes performed by a native and invasive bivalve species in

428   streams. *Hydrobiologia*, 667:191-204. https://doi.org/10.1007/s10750-011-0640-5

429   Augspurger T., Keller A.E., Black M.C., Cope W.G., & Dwyer F.J. (2003). Water quality guidance for

430   protection of freshwater mussels (Unionidae) from ammonia exposure. *Environmental*

431   *Toxicology and Chemistry*. 22(11):2569-2575. https://doi.org/10.1897/02-339.

432   Bartsch, M.R., Waller, D.L., Cope, W.G., & Gutreuter, S. (2000). Emersion and thermal tolerances of

433   three species of unionid mussels: Survival and behavioral effects. *Journal of Shellfish Research*.

434   19(1):233-240.

435   Black B.A., Dunham J.B., Blundon B.W., Raggon M.F. & Zima D. (2010) Spatial variability in growth-

436   increment chronologies of long-lived freshwater mussels: implications for climate impacts and

437   reconstructions. *Ecoscience*, 17: 240–250. https://doi.org/10.2980/17-3-3353

438   Booker, D. & Whitehead, A. (2021). River water temperatures are higher during low flows after

439   accounting for meteorological variability. River Research and Applications, 38:3-22.

440   https://doi.org/10.1002/rra.3870

441   Borcherding, J., 1995. Laboratory experiments on the influence of food availability, temperature and

442   photoperiod on gonad development in the freshwater mussel *Dreissenia polymorpha*.

443   *Malacologia*, 36: 15–27.

444   Carlson, T.J., Ploskey, G.R., Johnson, R.L., Mueller, R.P., Weiland, M.A., & Johnson, P.N. (2001).

445   Observations of the behavior and distribution of fish in relation to the Columbia River navigation

446   channel and channel maintenance activities. U.S. Army Corps of Engineers. Report number:

447   PNNL-13595-400403209

448   Cherry, D., Scheller, J., Cooper, N., & Bidwell, J. (2005). Potential effects of Asian clam (*Corbicula*

449   *fluminea*) die-offs on native freshwater mussels (Unionidae) I: water-column ammonia levels and

450   ammonia toxicity. Journal of the North American Benthological Society, 24(2): 369-380.

451   http://dx.doi.org/10.1899/04-073.1

452   Ciereszko, A., K. Dabrowski, B. Piros, M. Kwasnik, & J. Glogowski. (2001). Characterization of zebra

453   mussel (*Dreissena polymorpha*) sperm motility: duration of movement, effects of cations, pH and

454   gossypol. *Hydrobiologia*, 452:225-232.

455   Ćmiel, A., Struzynski, A., Wyrebek, M., Lipinska, A., Zajac, K., & Zajac, T. (2019). Response of

456   freshwater mussel recruitment to hydrological changes in a eutrophic floodplain lake. Science of

457   the Total Environment, 703: 1-10. https://doi.org/10.1016/j.scitotenv.2019.135467

458   Cummings, K.S., & Graf, D.L. (2010). Mollusca: Bivalvia. In: J.H. Thorp and A.P. Covich (Eds.)

459   *Ecology and Classificiation of North American Freshwater Invertebrates*. London, UK:

460   Academic Press, pp. 309–384.

461   Daraio, J., Weber, L., Zigler, S., Newton, T., & Nestler, J. (2012). Simulated effects of host fish

462   distribution on juvenile unionid mussel dispersal in a large river. *River Research and*

463   *Applications*, 28:594-608. https://doi.org/10.1002/rra.1469

464   Davey, A.J.H., & Kelly, D.J. (2007). Fish community responses to drying disturbances in an

465   intermittent stream: a landscape perspective. *Freshwater Biology*, 52(9):1719-1733.

466   https://doi.org/10.1111/j.1365-2427.2007.01800.x.

467   DeAngelis, D.L., Loftus, W.F., Trexler, J.C., & Ulanowicz, R.E. (1997). Modeling fish dynamics and

468   effects of stress in a hydrologically pulsed ecosystem. *Journal of Aquatic Ecosystem Stress and*

469   *Recovery*, 6:1-13.

470   Dionisio Pires, M., Donk, E., & Laanbroek, H. (2004). Selective grazing by adults and larvae of the

471   zebra mussel (*Dreissena polymorpha*): Application of flow cytometry to natural seston.

472   *Freshwater Biology*, 49(1):116-126. https://doi.org/10.1046/j.1365-2426.2003.01173.x

473   DuBose, T., Atkinson, C., Vaughn, C., & Golladay, S. (2019). Drought-induced, punctuated loss of

474   freshwater mussels alters ecosystem function across temporal scales. Frontiers in Ecology and

475   Evolution, 7: 1-13. https://doi.org/10.3389/fevo.2019.00274

476   Dudding, J., Hart, M., Khan, J., Robertson, C.R., Lopez, R., & Randklev, C.R. (2020). Reproductive

477   life history of 2 imperiled and 1 widely distributed freshwater mussel species from the

478   southwestern United States. Freshwater Science, 39(1):156-168. http://doi.org/10.1086/707774

479   Dycus, J. C., Wisniewski, J. M. & Peterson, J. T. (2015). The effects of flow and stream characteristics on

480   the variation in freshwater mussel growth in a Southeast US river basin. *Freshwater Biology*,

481   60:395-409. https://doi.org/10.1111/fwb.12504

482   Gade, G., & Grieshaber, M. (1986). Pyruvate reductions catabolize the formation of lactate and opines

483   in anaerobic invertebrates. *Comparative Biochemistry and Physiology*, 83B:255-272.

484   Galbraith, H.S., Spooner, D.E. & Vaughn, C.C. (2008) Status of rare and endangered freshwater mussels

485   in southeastern Oklahoma. The Southwestern Naturalist, 53: 45-50. https://doi.org/10.1894/0038-

486   4909(2008)53[45:SORAEF]2.0.CO;2

487   Galbraith, H. & Vaughn, C. (2009). Temperature and food interact to influence gamete development in

488   freshwater mussels. Hydrobiologia, 636(1):35-47. http://dx.doi.org/10.1007/s10750-009-9933-3

489   Galbraith, H. (2009). Reproduction in a changing environment: mussels, impoundments, and

490   conservation. Dissertation, University of Oklahoma. Norman, OK.

491   Ganser, A.M., Newton, T.J., & Haro, R.J. (2015). Effects of elevated water temperature on physiological

492   responses in adult freshwater mussels. *Freshwater Biology*, 60(8): 1705- 1716.

493   https://doi.org/10.1111/fwb.12603

494   Garner, J.T., Haggerty, T.M., & Modlin, R.F. (1999). Reproductive cycle of *Quadrula metanerva*

495   (Bivalvia: Unionidae) in the Pickwick Dam Tailwater of the Tennessee River. *The American*

496   *Midland Naturalist*, 141(2), 277-283. http://doi.org/10.1674/0003-

497   0031(1999)141[0277:RCOQMB]2.0.CO;2

498   Gascho Landis, A., Mosley, T., Haag, W., & Stoeckel, J. (2012). Effects of temperature and photoperiod

499   on lure display and glochidial release in a freshwater mussel. *Freshwater Science*, 31(3): 775-

500   786. http://doi.org/10.1899/11-082.1.

501   Gelwick, F.P. (1990). Longitudinal and temporal comparisons of riffle and pool fish assemblages in a

502   northeastern Oklahoma Ozark stream. Copeia, 1990(4):1072-1082.

503   https://doi.org/10.2307/1446491

504   Goldsmith, A.M., Jaber, F.H., Ahmari, H., & Randklev, C.R. (2020). Clearing up cloudy waters: a

505   review of sediment impacts to unionid freshwater mussels. *Environmental Reviews*, **29**(1): 100-

506   108. https://doi.org/10.1139/er-2020-0080.

507   Goldsmith, A., Khan, J.M., Robertson, C.R., Lopez, R. & Randklev, C.R. (2021). Using upper thermal

508   limits of *Lampsilis bracteata* (Texas fatmucket) from the North Llano and San Saba rivers, Texas

509   to inform water management practices in the Edwards Plateau. *Aquatic Conservation: Marine*

510   *and Freshwater Ecosystems*, 32(1): 85– 97. https://doi.org/10.1002/aqc.3749

511   Haag, W.R. (2012). *North American Freshwater Mussels: Natural History, Ecology, and Conservation*.

512   New York, USA: Cambridge University Press.

513   Haggerty, T. M., J. R. Garner, G. H. Patterson, & L. C. J. Jones. 1995. A quantitative assessment of the

514   reproductive biology of *Cyclonaias tuberculata* (Bivalvia:Unionidae). *Canadian Journal of*

515   *Zoology*, 73:83-88.

516   Haley, L., M. Ellis & J. Cook. 2007. Reproductive timing of freshwater mussels and potential impact of

517   pulsed flows on reproductive success. PEIR Final Project Report. California Energy Commission,

518   Sacramento, California. Contract # 500-01-044.

519   Harvey, B.C., Nakamoto, R.J., & White, J.L. (2006). Reduced streamflow lowers dry-season growth of

520   rainbow trout in a small stream. Transactions of the American Fisheries Society, 135(4):998-

521   1005. http://dx.doi.org/10.1577/T05-233.1

522   Hastie, L. & Young, M. (2003). Timing of spawning and glochidial release in Scottish freshwater pearl

523   mussel (*Margaritifera margaritifera*) populations. Freshwater Biology, 48(12):2107-2117.

524   http://dx.doi.org/10.1046/j.1365-2427.2003.01153.x

525   Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology

526   22:415–427.

527   Jing, X., Pales Espinosa, E., Perrigault, M., & Allam, B. (2011). Identification, molecular

528   characterization and expression analysis of a mucosal C-type lectin in the eastern oyster,

529   *Crassostrea virginica*. *Fish & Shellfish Immunology*,30(3): 851–858.

530   https://doi.org/10.1016/j.fsi.2011.01.007

531   Kat, P.W. (1984). Parasitism and the Unionacea (Bivalvia). *Biological Reviews*, 59(2), 189–207.

532   http://doi.org/10.1111/j.1469-185X.1984.tb00407.x.

533

534

535 Khan, J.M., Hart, M., Dudding, J., Robertson, C.R., Lopez, R., & Randklev, C.R. (2019). Evaluating the

536   upper thermal limits of glochidia for selected freshwater mussel species (Bivalvia: Unionidae) in

537   central and east Texas, and the implications for their conservation. *Aquatic Conservation: Marine*

538   *and Freshwater Ecosystems*, 29(8): 1202- 1215. https://doi.org/10.1002/aqc.3136

539 Khan, J., Dudding, J., Hart, M., Robertson, C., Lopez, R., & Randklev, C. (2020). Linking flow and upper

540   thermal limits of freshwater mussels to inform environmental flow benchmarks. *Freshwater*

541   *Biology*, 65(12): 2037-2052. https://doi.org/10.1111/fwb.13598

542 Kiibus, M., & Kautsky, N. (1996). Respiration, nutrient excretion and filtration rate of tropical

543   freshwater mussels and their contribution to production and energy flow in Lake Kariba,

544   Zimbabwe. *Hydrobiologia*, 331(1):25-32. http://dx.doi.org/10.1007/BF00025404

545 Layzer, J. & Madison, L. (1995). Microhabitat use by freshwater mussels and recommendations for

546   determining their instream flow needs. *Regulated Rivers: Research and Management*, 10:329-

547   345. https://doi.org/10.1002/rrr.3450100225

548 Lennox, R.J., Crook, D.A., Moyle, P.B., Struthers, D.P., & Cooke, S.J. (2019). Toward a better

549   understanding of freshwater fish responses to an increasingly drought-stricken world. *Reviews in*

550   *Fish Biology and Fisheries*, 29:71-92. https://doi.org/10.1007/s11160-018-09545-9

551 McMahon, R., & Bogan, A. (2001). Mollusca: BIvalvia. In: J.H. Thorp and A.P. Covich (Eds.) *Ecology*

552   *and classification of North American freshwater invertebrates*. *San Diego, California:*Academic

553   Press, pp. 321-429.

554 Mims, M. C., & J. D. Olden. 2012. Life history theory predicts fish assemblage response to hydrologic

555   regimes. *Ecology*, 93:35–45. https://doi.org/10.1890/11-0370.1.

556 Mistry, R., & Ackerman, J. D. (2018). Flow, flux, and feeding in freshwater mussels. *Water Resources*

557   *Research*, 54:7619– 7630. https://doi.org/10.1029/2018WR023112

558 Mittal, N., Mishra, A., Singh, R., Bhave, A., & van der Valk, M. (2014). Flow regime alteration due to

559   anthropogenic and climatic changes in the Kangsabati River, India. *Ecohydrology and*

560   *Hydrobiology*, 14(3):182-191. https://doi.org/10.1016/j.ecohyd.2014.06.002

561 Mojares, J., Stachecki, J., Kyozuka, k., Armant, D., & Ram, J. (1995). Characterization of zebra mussel

562   (*Dreissena polymorpha*) sperm morphology and their motility prior to and after spawning. The

563   *Journal of Experimental Zoology*, 273:257-263.

564 Newton, T. (2003). The effects of ammonia on freshwater unionid mussels. *Environmental Toxicology*

565   *and Chemistry*, 22(11):2543-4. https://doi.org.10.1897/03-84

566 Nichols, S.J., Silverman, H., Dietz, T.H., Lynn, J.W., & Garling, D.L. (2005). Pathways of food uptake in

567   native (Unionidae) and introduced (Corbiculidae and Dreissenidae) freshwater bivalves. *Journal*

568   *of Great Lakes Research*, 31(1), 87–96. http://doi.org/10.1016/S0380-1330(05)70240-9.

569 Olden, Julian & Kennard, Mark. (2010). Intercontinental comparison of fish life history strategies along a

570   gradient of hydrologic variability. Community ecology of stream fishes: concepts, approaches,

571   and techniques. *American Fish Society*. 73.

572 Perkin, J.S. & Bonner, T.H. (2011), Long-term changes in flow regime and fish assemblage

573   composition in the Guadalupe and San Marcos Rivers of Texas. *River Research and Applications*,

574   27: 566-579. https://doi.org/10.1002/rra.1373

575 Phillips, J. D. (2012). Geomorphic responses to changes in flow regimes in Texas rivers. Project Report

576   for the Texas Water Development Board and Texas Instream Flow Program, TWDB contract

577   number 1104831147.

578 Poff, N. L., Olden, J. D., Merritt, D. M., & Pepin, D. M. 2007. Homogenization of regional river

579   dynamics by dams and global biodiversity implications. Proceedings of the National Academy of

580   Sciences of the United States of America 104:5732-5737.

24

581   Power, M., Sun, A., Parker, M., Dietrich, W., & Wootton, J. (1995). Hydraulic food-chain models: an

582   approach to the study of food-web dynamics in large rivers. *BioScience,* 45:159–167.

583   https://doi.org/10.2307/1312555

584   Pusch, M., Siefert, J., & Walz, N. (2001). Filtration and Respiration Rates of Two Unionid Species and

585   Their Impact on the Water Quality of a Lowland River. *In*: Bauer, G., Wächtler, K. (eds) Ecology

586   and Evolution of the Freshwater Mussels Unionoida. Ecological Studies, vol 145. Springer,

587   Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-56869-5_17

588   Randklev, C.R., Hart, M.A., Khan, J.M., Tsakiris, E.T., & Robertson, C.R. (2019). Hydraulic

589   requirements of freshwater mussels (Unionidae) and a conceptual framework for how they

590   respond to high flows. *Ecosphere*, 10(12), 1–19. http://doi.org/10.1002/ecs2.2975

591   Randklev, C.R., Tsakris, E.T., Johnson, M.S., Popejoy, T., Hart, M.A., Khan, J., & Robertson, C.R.

592   (2018). The effect of dewatering on fresh-water mussel (Unionidae) community structure and the

593   implications for conservation and water policy: A case study from a spring-fed stream in the

594   southwestern United States. *Global Ecology and Conservation*, 16, 1-15.

595   https://doi.org/10.1016/j.gecco.2018.e00456

596   Resh, V., Brown, A., Covich, A., Gurtz, M., Li, H., Minshall, G., Reice, S., Sheldon, A., Wallace, J., &

597   Wissmar, R. (1988). The role of disturbance in stream ecology. *Journal of the North American*

598   *Benthological Society,* 7: 433–455. https://doi.org/10.2307/1467300

599   Richard, P., Dietz, T., & Silverman, H. (1991). Structure of the gill during reproduction in the Unionids

600   *Anodonta grandis*, *Ligumia subrostrata*, and *Carunculina parva texasensis*. *Canadian Journal of*

601   *Zoology*, 69(7), 1744–1754. http://doi.org/10.1139/z91-243

602   Riisgård, H. U., & Larsen, P. S. (2010). Particle capture mechanisms in suspension-feeding

603   invertebrates. *Marine Ecology Progress Series*, 418:255–293. https://doi.org/10.3354/meps08755

604   Sawyer, J. (2008). Surface Waters: Ammonium is not ammonia-Part 3. Iowa State Extension <

605   https://crops.extension.iastate.edu/cropnews/2008/05/surface-waters-ammonium-not-ammonia-

606   %E2%80%93-part-3> Accessed 15 June 2022.

607   Schneider, L.D., Nilsson, P. & Österling, E. (2018). Evaluating temperature- and host-dependent

608   reproduction in the parasitic freshwater mussel *Unio crassus*. *Hydrobiologia,* 810**:** 283–293.

609   https://doi.org/10.1007/s10750-017-3217-0

610   Silverman, H., Steffens, W.L., & Dietz, T.H. (1985). Calcium from extracellular concretions in the gills

611   of freshwater unionid mussels is mobilized during reproduction. *Journal of Experimental*

612   *Zoology,* 236: 137–147. https://doi.org/10.1002/jez.1402360204

613   Spooner, D.E., & Vaughn, C.C. (2008). A trait-based approach to species' roles in stream ecosystems:

614   Climate change, community structure, and material cycling. *Oecologia*, 158(2), 307-317.

615   https://doi.org/10.1007/s00442-008-1132-9

616   Strayer, D.L., Downing, J.A., Haag, W.R., King, T.L., Layzer, J.B., Newton,T.J. et al. (2004). Changing

617   perspectives on pearly mussels, North American's most imperiled animals. *BioScience*, 54(5),

618   429-439. https://doi.org/10.1641/0006- 3568(2004)054[0429:CPOPMN]2.0.CO;2

619   Texas Parks and Wildlife Department [TPWD]. (2020). Threatened and endangered nongame species.

620   Texas Register 45. Chapter 65. Wildlife subchapter G. §65.175. Adopted Rules. Austin, TX:

621   Texas Secretary of State

622   Thurston, R., Russo, R., & Emerson, K. (1979). Aqueous ammonia equilibrium – Tabulation of percent

623   un-ionized ammonia. Environmental Protection Agency [EPA], Report #: EPA-600/3-79-091.

624   U.S. Fish and Wildlife Service [USFWS]. (2009). Endangered and threatened wildlife and plants: 90-day

625   finding on petitions to list nine species of mussels from Texas as threatened or endangered with

626   critical habitat. Federal Register. Report number: E9- 29698

627   U.S. Fish and Wildlife Service [USFWS]. (2011). Endangered and threatened wildlife and plants; 12-

628   month finding on a petition to list Texas fatmucket, golden orb, smooth pimpleback, Texas

629   pimpleback, and Texas fawnsfoot as threatened or endangered. Federal Register. Report number:

630   2011-25471

631   vanden Byllaardt, J., & Ackerman, J. D. (2014). Hydrodynamic habitat influences benthic suspension

632   feeding in freshwater ecosystems. *Freshwater Biology*, 59(6): 1187–1196.

633   https://doi.org/10.1111/fwb.12339

634   Widdows, J. (1978). Physiological indices of stress in *Mytilus edulis*. *Journal of the Marine Biological*

635   *Association of the Unite Kingdom*. 58:125-142. https://doi.org/10.1017/S0025315400024450

636   Widdows, J., Donkin, P., Staff, F., Mattiessen, P., Law, R., Allen, Y., Thain, J., Allchin, C. & Jones, B.

637   (2002). Measurement of stress effects (scope for growth) and contaminant levels in mussels

638   (*Mytilus edulis*) collected from the Irish Sea. *Marine Environmental Research*, 53(4):327-356.

639   http://dx.doi.org/10.1016/S0141-1136(01)00120-9

640   Williams, J.D., Warren, M.L., Cummings, K.S., Harris, J.L., & Neves, R.J. (1993). Conservation status of

641   freshwater mussels of the United States and Canada. *Fisheries*, 18(9), 6-22.

642   https://doi.org/10.1577/1548-8446(1993)018<0006:CSOFMO>2.0.CO;2

643   Zając, K. & Zając, T. (2021). Seasonal patterns in the developmental rate of glochidia in the endangered

644   thick-shelled river mussel, *Unio crassus* Philipsson, 1788. *Hydrobiologia*, 848:3077-3091.

645   https://doi.org/10.1007/s10750-020-04240-y

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651   Task 2: Develop mussel research plan.

652   Based on our literature review of mussel flow relationships (Task 1; Appendix 2A), we outlined

653   knowledge gaps and research opportunities to better inform environmental flow standards within the

654   Guadalupe River basin. In August 2012, environmental flow standards were developed by the Texas

655   Commission of Environmental Quality (TCEQ) for selected gage stations within Texas, including sites

656   along the Guadalupe River (TCEQ, 2012; TCEQ, 2022). Using historical hydrology data, flow standards

657   for the basin were developed to outline seasonal base and subsistence flows and high flow pulses, that

658   when implemented would maintain the Guadalupe River and its associated tributaries. Currently, within

659   the Guadalupe River (~644 km in length [i.e., ~400 mi]), the development of environmental flow

660   standards has been limited to nine gages, only five of which are along the mainstem (USGS, 2022;

661   Appendix B). Further, mussel and host fish environmental flow data has yet to be fully considered within

662   this framework. Therefore, it is likely current environmental flow standards, especially during subsistence

663   flows (i.e., minimum flow needed to maintain tolerable water quality conditions and habitat for the

664   survival of aquatic organisms), are not protective of freshwater mussels or their host fish. In fact, other

665   research within the state, including within the Guadalupe River basin, has already shown the need for

666   current flow standards to be updated (Khan et al., 2020; Goldsmith et al., 2021).

667   In Texas, multiple studies have used thermal tolerance data to demonstrate current flows are not

668   protective of mussels (Khan et al., 2020; Goldsmith et al., 2021). These same studies have then used

669   thermal exceedance information to make flow recommendations. For example, Khan et al. (2020),

670   examining the upper thermal limits of three freshwater mussel species (*Amblema plicata* [Threeridge]*,*

671   *Cyclonaias necki* [Guadalupe orb]*,* and *Fusconaia mitchelli* [False spike]) within the lower Guadalupe

672   River (TX, USA), found current summer subsistence flows for two gages (08173900 near Gonzales, TX

673   [5.95 m^3s^{-1} (i.e., 210 ft^3s^{-1})] and 08175800 near Cuero, TX [3.68 m^3s^{-1} (i.e., 130 ft^3s^{-1})]) may not be

674   protective (15.3 m^3s^{-1} [i.e., 540.31 ft^3s^{-1}] – mean discharge estimated to result in catastrophic impacts).

675   Similarly, Goldsmith et al. (2021), determining the upper thermal limits of one species (*Lampsilis*

676 *bracteata* [Texas fatmucket]) at two sites along the San Saba River (TX, USA), found environmental flow

677 standards for summer base flows and dry hydrological periods were also not protective of glochidia or

678 juveniles (Site 1 [0.3 – 0.4 m^{3}s^{-1} {i.e., 10.6 – 14.13 ft^{3}s^{-1}}] and Site 2 [0.7 – 1.0 m^{3}s^{-1} {i.e., 24.72 – 35.31

679 ft^{3}s^{-1}}]). The findings of these studies indicate current flow standards should be reevaluated to ensure

680 mussels are protected.

681   The methods presented in Khan et al. (2020) and Goldsmith et al. (2021) provide a clear and

682 reproducible means for integrating river water temperature, discharge, and experimentally derived

683 thermal tolerance data to ensure water quantity and flow are supportive of freshwater mussel presence

684 (i.e., a keystone species within the environment). While other methods have been developed (see Maloney

685 et al., 2012; Gates et al., 2015), the uniform continuous above temperature (UCAT) approach provides a

686 direct, reproducible method that can be utilized, including for other species, to inform best water

687 management practices. Additionally, while Khan et al. (2020) and Goldsmith et al. (2021) used thermal

688 tolerance data (LT05 and LT50) to identify periods of exceedance, the UCAT approach proposed by

689 Castelli et al. (2012) does not require the use of mussel thermal performance data. Castelli et al. (2012).

690 did not have thermal performance data for their target species, *Alasmidonta heterodon* (Dwarf

691 wedgemussel), and so the authors determined the species' thermal optima using historical time series

692 gage data. Because the authors did not use experimentally derived thermal tolerance data, it is likely their

693 estimates for *A. heterodon* mussels are less accurate than the approach taken by Khan et al. (2020) and

694 Goldsmith et al. (2021). However, if thermal tolerance data is limited, which is often the case for many

695 mussel species, then Castelli et al.'s (2012) approach provides a method for determining flow restoration

696 targets. These recommendations, in turn, can be later refined using thermal performance data. Although

697 Texas has USGS gages across the state, temperature records are limited to a few gage stations, none of

698 which are along the Guadalupe River. The installation of temperature loggers at each USGS gage station

699 would allow water managers and conservationists to monitor temperature in nearby mussel habitats both

700   upstream and downstream of gage locations. This information can then be used to better identify flow

701   management needs for mussels.

702   Even though alterations to natural flow regimes are often cited as a main contributor to freshwater

703   mussel decline, studies demonstrating the impact of flow on the population performance (i.e.,

704   reproduction, growth, and survivorship) of freshwater mussels are limited, especially within the

705   Guadalupe River basin. This is problematic as the Guadalupe River basin contains 20 mussel species,

706   including three state threatened species that are currently candidates for federal protection under the

707   Endangered Species Act (ESA; *C. necki*, *F. mitchelli*, and *Lampsilis bergmanni* [Guadalupe fatmucket];

708   USFWS 2009, 2011; TPWD 2020; Appendix C). Further, the three state threatened species represent

709   three tribes (Quadrulini, Pleurobemini, and Lampsilini), which likely have different evolutionary and

710   physiological adaptions to flow related stressors (e.g., increased water temperature; Khan et al., 2020).

711   While mussel-flow relationship data is lacking within the Guadalupe River, water managers and

712   researchers can use general biological thresholds identified in Task 1 to understand mussels' hydraulic

713   needs within the river (Appendix 2A). However, methods from these studies should then be used and/or

714   refined to confirm results and generate mussel-flow data specific to the Guadalupe River (Appendix D &

715   E).

716   Generally, research shows mussel reproduction patterns are regulated by streamflow and changes

717   to the natural flow regime impact species' reproductive potential and recruitment rate. Specifically,

718   research shows gamete viability is negatively impacted when flows are inadequate to support optimal

719   water temperature (Galbraith & Vaughn, 2009; Dudding et al., 2020). This relationship was explored by

720   Dudding et al. (2020) for rare and common species within the lower Guadalupe River. The authors found

721   gametogenesis (peak sperm concentration and ovum diameter) was significantly constrained when

722   accumulated degree days exceed 1,000 days. Spatial and temporal patterns in water temperature are

723   tightly linked to flow patterns, yet information presented in Dudding et al. (2020) was not tied back to

724   discharge. Future research efforts should focus on identifying the range of flows species gamete

725   development is optimized to ensure reproductive success (Appendix D & E). Further, future efforts

726   should include populations and species throughout the length of the Guadalupe River to determine how

727   stream location impacts reproductive success. Studies linking gametogenesis and flow can aid water

728   managers in prescribing flows that not only ensure survival but also support reproductive success.

729   Hydrological flows have also been shown to impact mussel reproduction by disrupting spawning

730   cues and limiting fertilization success (Galbraith et al., 2009; Zajac & Zajac, 2021; Hastie & Young,

731   2003). However, studies investigating these relationships within the Guadalupe River do not exist.

732   Therefore, information generated in studies outside of Texas may provide water managers with important

733   insight as to how flow impacts mussel reproduction. For example, a study in Oklahoma revealed sperm is

734   most viable and motile between a set range of water temperatures (Galbraith, 2009). Additionally, a study

735   conducted in southern Poland found brooding period was significantly shortened as seasonal water

736   temperatures rose (Zajac & Zajac, 2021), while another study in Scotland revealed glochidial release

737   often coincides with sudden thermal or hydrological events (Hastie & Young, 2003). These studies are

738   important for making broad inferences, but specific findings were not related back to discharge. Future

739   efforts could build off these studies by using gage data (Appendix D & E). In the Guadalupe, researchers

740   could monitor known mussel populations to determine when gamete viability peaks and glochidia are

741   released. Data produced could then be tied back to discharge data from nearby USGS gage stations to

742   determine discharge needs during spawning and fertilization periods. This data could then be utilized by

743   water managers to ensure flow is adequate during reproductive windows to deliver sperm to female

744   mussels of the same species as well as ensure glochidia are viable and released during optimal conditions.

745   Due to mussel's unique life history, it is essential prescribed flows are also protective of host fish.

746   Current studies suggest alterations to the natural flow regime may limit invertebrate drift and alter host

747   fish behavior (Gelwick, 1990; Harvey et al., 2006). Therefore, it is likely flows that are too high or low

748   may prevent glochidia from encountering their associated host fish. However, studies investigating how

749 flow impacts mussel-host fish interactions are non-existent and so more research is needed on this topic

750 (Appendix E).

751   Research shows mussel growth is regulated by hydrological flow and impacts to the natural flow

752 regime of a river may limit species' growth rates by interfering with individuals' ability to feed and

753 respire (Pusch et al., 2001; Dycus et al., 2015; vanden Byllaardt & Ackerman, 2014). Despite this

754 knowledge, research investigating the impact flow has on species filtration and respiration rate is limited

755 to species outside of Texas, which could help managers in Texas draw inferences. For example, a study

756 conducted in northeastern Ontario (Canada) found mussel filtration rate varied significantly with velocity

757 (between 0.008 and 0.20 ms^{-1} [i.e., 0.03 and 0.66 fts^{-1}]) and between lentic and lotic species (vanden

758 Byllaardt & Ackerman, 2014). Additionally, a study in Zimbabwe found mussels' oxygen uptake rate was

759 optimized between a set range of water temperatures (16.5 to 34.0°C [*Aspatharia wahlbergi*] and 18.6 to

760 29.2°C [*Corbicula Africana*]; Kiibus & Kautsky, 1996). Data generated in studies linking

761 filtration/respiration rate and flow, can aid water managers in prescribing flows that support mussel

762 growth. However, it is important to note, data generated from these studies may not directly translate to

763 the southwestern United States. Further research is needed to confirm results and generate Guadalupe

764 mussel-flow data (Appendix D & E).

765   Generally, hydrodynamic conditions shape mussel distribution, abundance, and survival (Resh et

766 al., 1988; Power et al., 1995). Yet, studies investigating the influence streamflow has on species

767 abundance and distribution are unavailable within the Guadalupe River. This is problematic as the

768 Guadalupe River is modified by several mainstream impoundments, including Canyon Lake reservoir,

769 that have resulted in flows becoming more homogenized (Phillips, 2012; Perkin & Bonner, 2011; Khan et

770 al., 2020). While mussel-flow relationships are not well known in the Guadalupe, a study conducted by

771 Randklev et al. (2019) along the Brazos and Trinity rivers, both modified by several impoundments,

772 provides insight as to how flow impacts mussel density and richness. Generally, results indicated high

773 mussel density and species richness were associated with low relative shear stress (RSS), a measure of

774   stream bed stability. Some of the species included within their study are found within the Guadalupe

775   River, and so water managers could use findings for those same species reported in Randklev et al. (2019)

776   to understand their hydraulic needs in the Guadalupe River. However, future research should focus on

777   generating similar data for species in the Guadalupe not evaluated in Randklev et al. (2019), particularly

778   for those that are state protected or under consideration for ESA listing (Appendix D & E).

779   Several studies have shown altered flow that results in lower-than-normal discharge may result in

780   accumulation of environmental contaminants (Augspurger et al., 2003; Spooner & Vaughn, 2008). This is

781   troubling as ammonia is a common pollutant in streams and has been shown to reach toxic levels during

782   periods of low to no flow (Newton et al., 2003). Yet, studies investigating this idea are unavailable within

783   the Guadalupe River. Thus, studies are needed within the basin to determine the sublethal and lethal

784   concentration of ammonia for rare and common species and how those concentrations are affected by

785   changes in streamflow and water temperature (Appendix D & E).

786   The general distribution of unionids in the Guadalupe is known, but more contemporaneous

787   information is needed. The last comprehensive surveys in the basin were conducted in 2011 and between

788   2014 and 2016 (Randklev et al., 2011; Tsakiris & Randklev, 2016). Future surveys in the basin should

789   focus on areas where information exists to evaluate if community composition and abundance have

790   changed since 2011 (Appendix D & E). Observed changes, or lack thereof, are helpful for generating

791   hypotheses and/or evaluating the efficacy of monitoring. Future surveys could also focus on areas where

792   survey information is lacking to determine if mussels are present. Both sets of information are critical for

793   evaluating how mussels are impacted by environmental change. New survey data also provides the

794   opportunity to refine landscape modeling efforts, identify locations for studies focused on increasing

795   understanding of mussel life history (e.g., timing of spawning/brooding, host fish use), and designate

796   areas for long-term monitoring (e.g., mark-recapture sites). The latter can provide the necessary

797   information to understand how changes in flow directly impact mussel population performance. The

798   resulting data could then be used to refine future flow recommendations.

799   Overall, general trends within the literature demonstrate stream flow significantly influences the

800   distribution, abundance, and long-term persistence of unionid species. However, our review also revealed

801   mussel flow data within the Guadalupe River is heavily lacking (i.e., restricted to 2 articles [7.41% of

802   total articles reviewed]). Thus, to infer how Guadalupe species may be impacted, water managers and

803   researchers should use general biological thresholds identified in Task 1 to understand mussel flow needs

804   within the river (Appendix 2A). More specifically, the most useful data to water managers includes same

805   species data from other basins/states as well as congener data (i.e., data from a member of the same

806   taxonomic genus; Appendix C, D, and E). For example, while no information linking spawning/brooding

807   to flow is available for species within the Guadalupe River, data collected in Oklahoma (USA) is

808   available for *C. pustulosa*, a species also found within the Guadalupe River. Data from this study can be

809   used to directly infer how *C. pustulosa's* sperm viability/motility within the Guadalupe River may be

810   impacted. While useful, it is important to note data generated in such studies may not directly translate to

811   the southwestern United States. Therefore, methods from these studies should then be used and/or refined

812   to confirm results and generate mussel-flow data specific to the Guadalupe River. Based on information

813   outlined in Task 1 and 2, we have put together a mussel flow research plan outlining order of tasks,

814   location, which species to test, cost of project, and timeline (Appendix E). Additionally, abiotic needs to

815   perform research with identified methods has also been outlined (Appendix D). Data generated from these

816   tasks can aid in refining future flow recommendations within the Guadalupe as well as help inform

817   recommendations in other basins. Further, methods generated can be used to demonstrate how to conduct

818   similar studies within other parts of the state.

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823   Literature Cited

824   Augspurger T., Keller A.E., Black M.C., Cope W.G., & Dwyer F.J. (2003). Water quality guidance for

825   protection of freshwater mussels (Unionidae) from ammonia exposure. *Environmental*

826   *Toxicology and Chemistry*. **22**(11):2569-2575. https://doi.org/10.1897/02-339.

827   Castelli, E., Parasiewicz, P., & Rogers, J.N. (2012). Use of frequency and duration analysis for

828   the determination of thermal habitat thresholds: Application for the conservation of

829   *Alasmidonta heterodon* in the Delaware River. *Journal of Environmental Engineering*, 138(8),

830   886-892. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000520

831   Dudding, J., Hart, M., Khan, J., Robertson, C.R., Lopez, R., & Randklev, C.R. (2020). Reproductive life

832   history of 2 imperiled and 1 widely distributed freshwater mussel species from the southwestern

833   United States. Freshwater Science, 39(1):156-168. http://doi.org/10.1086/707774

834   Dycus, J. C., Wisniewski, J. M. & Peterson, J. T. (2015). The effects of flow and stream characteristics

835   on the variation in freshwater mussel growth in a Southeast US river basin. *Freshwater Biology*,

836   60:395-409. https://doi.org/10.1111/fwb.12504

837   Galbraith, H. & Vaughn, C. (2009). Temperature and food interact to influence gamete development in

838   freshwater mussels. Hydrobiologia, 636(1):35-47. http://dx.doi.org/10.1007/s10750-009-9933-3

839   Galbraith, H. (2009). Reproduction in a changing environment: mussels, impoundments, and

840   conservation. Dissertation, University of Oklahoma. Norman, OK.

841   Ganser, A.M., Newton, T.J., & Haro, R.J. (2015). Effects of elevated water temperature on physiological

842   responses in adult freshwater mussels. *Freshwater Biology*, 60(8): 1705- 1716.

843   https://doi.org/10.1111/fwb.12603

844   Gates, K.K., Vaughn, C.C., & Julian, J.P. (2015). Developing environmental flow recommendations for

845   freshwater mussels using the biological traits of species guilds. *Freshwater Biology*, 60(4), 620-

846   635. https://doi.org/10.1111/fwb.12528

847   Goldsmith, A., Khan, J.M., Robertson, C.R., Lopez, R. & Randklev, C.R. (2021). Using upper thermal

848   limits of *Lampsilis bracteata* (Texas fatmucket) from the North Llano and San Saba rivers, Texas

849   to inform water management practices in the Edwards Plateau. *Aquatic Conservation: Marine*

850   *and Freshwater Ecosystems*, 32(1): 85– 97. https://doi.org/10.1002/aqc.3749

851   Harvey, B.C., Nakamoto, R.J., & White, J.L. (2006). Reduced streamflow lowers dry-season growth of

852   rainbow trout in a small stream. Transactions of the American Fisheries Society, 135(4):998-

853   1005. http://dx.doi.org/10.1577/T05-233.1

854   Hastie, L. & Young, M. (2003). Timing of spawning and glochidial release in Scottish freshwater pearl

855   mussel (*Margaritifera margaritifera*) populations. Freshwater Biology, 48(12):2107-2117.

856   http://dx.doi.org/10.1046/j.1365-2427.2003.01153.x

857   Khan, J., Dudding, J., Hart, M., Robertson, C., Lopez, R., & Randklev, C. (2020). Linking flow and upper

858   thermal limits of freshwater mussels to inform environmental flow benchmarks. *Freshwater*

859   *Biology*, 65(12): 2037-2052. https://doi.org/10.1111/fwb.13598

860   Kiibus, M., & Kautsky, N. (1996). Respiration, nutrient excretion and filtration rate of tropical

861   freshwater mussels and their contribution to production and energy flow in Lake Kariba,

862   Zimbabwe. *Hydrobiologia*, 331(1):25-32. http://dx.doi.org/10.1007/BF00025404

863   Maloney, K. O., Lellis, W. A., Bennett, R. M., & Waddle, T. J. (2012). Habitat persistence for sedentary

864   organisms in managed rivers: The case for the federally endangered dwarf wedgemussel

865   (*Alasmidonta heterodon*) in the Delaware River. *Freshwater Biology*, *57*, 1315–1327.

866   https://doi.org/10.1111/j.1365-2427.2012.02788.x

867   Newton, T. (2003). The effects of ammonia on freshwater unionid mussels. *Environmental Toxicology*

868   *and Chemistry*, 22(11):2543-4. https://doi.org.10.1897/03-84

869   Perkin, J.S. & Bonner, T.H. (2011), Long-term changes in flow regime and fish assemblage

870   composition in the Guadalupe and San Marcos Rivers of Texas. *River Research and Applications*,

871   27: 566-579. https://doi.org/10.1002/rra.1373

872   Phillips, J. D. (2012). Geomorphic responses to changes in flow regimes in Texas rivers. Project Report

873   for the Texas Water Development Board and Texas Instream Flow Program, TWDB contract

874   number 1104831147.

875   Power, M., Sun, A., Parker, M., Dietrich, W., & Wootton, J. (1995). Hydraulic food-chain models: an

876   approach to the study of food-web dynamics in large rivers. *BioScience,* 45:159–167.

877   https://doi.org/10.2307/1312555

878   Pusch, M., Siefert, J., & Walz, N. (2001). Filtration and Respiration Rates of Two Unionid Species and

879   Their Impact on the Water Quality of a Lowland River. *In*: Bauer, G., Wächtler, K. (eds) Ecology

880   and Evolution of the Freshwater Mussels Unionoida. Ecological Studies, vol 145. Springer,

881   Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-56869-5_17

882   Randklev, C. R., M. S. Johnson, E. T. Tsakiris, S. Rogers Oetker, K. J. Roe, J. L. Harris, S. E. McMurray,

883   C. Robertson, J. Groce, & N. Wilkins, 2012. False spike, *Quadrula mitchelli* (Bivalvia:

884   Unionidae), is not extinct: first account of a live population in over 30 years. *American*

885   *Malacological Bulletin* 30: 327–328.

886   Randklev, C.R., Hart, M.A., Khan, J.M., Tsakiris, E.T., & Robertson, C.R. (2019). Hydraulic

887   requirements of freshwater mussels (Unionidae) and a conceptual framework for how they

888   respond to high flows. *Ecosphere*, 10(12), 1–19. http://doi.org/10.1002/ecs2.2975

889 Resh, V., Brown, A., Covich, A., Gurtz, M., Li, H., Minshall, G., Reice, S., Sheldon, A., Wallace, J., &

890   Wissmar, R. (1988). The role of disturbance in stream ecology. *Journal of the North American*

891   *Benthological Society,* 7: 433–455. https://doi.org/10.2307/1467300

892 Spooner, D.E., & Vaughn, C.C. (2008). A trait-based approach to species' roles in stream ecosystems:

893   Climate change, community structure, and material cycling. *Oecologia*, 158(2), 307-317.

894   https://doi.org/10.1007/s00442-008-1132-9

895 Texas Commission on Environmental Quality [TCEQ]. (2012). <

896   https://www.tceq.texas.gov/waterquality/assessment/12twqi> Accessed 20 July 2022.

897 Texas Commission on Environmental Quality [TCEQ]. (2022). <https://www.tceq.texas.gov/> Accessed

898   5 May 2022.

899 Randklev, C.R. (2016). Assessing the conservation status of rare endemic mussel species (Family:

900   Unionidae) in the Lower Guadalupe River, Texas. Texas Parks and Wildlife, Report # TX E-156-

901   R

902 Texas Parks and Wildlife Department [TPWD]. (2020). Threatened and endangered nongame species.

903   Texas Register 45. Chapter 65. Wildlife subchapter G. §65.175. Adopted Rules. Austin, TX:

904   Texas Secretary of State.

905 U.S. Fish and Wildlife Service [USFWS]. (2009). Endangered and threatened wildlife and plants: 90-day

906   finding on petitions to list nine species of mussels from Texas as threatened or endangered with

907   critical habitat. Federal Register. Report number: E9- 29698

908 U.S. Fish and Wildlife Service [USFWS]. (2011). Endangered and threatened wildlife and plants; 12-

909   month finding on a petition to list Texas fatmucket, golden orb, smooth pimpleback, Texas

910   pimpleback, and Texas fawnsfoot as threatened or endangered. Federal Register. Report number:

911   2011-25471

912   United States Geological Survey [USGS]. (2022). <https://www.usgs.gov/> Accessed 10 June 2022.

913   vanden Byllaardt, J., & Ackerman, J. D. (2014). Hydrodynamic habitat influences benthic suspension

914   feeding in freshwater ecosystems. *Freshwater Biology*, 59(6): 1187–1196.

915   https://doi.org/10.1111/fwb.12339

916   Zając, K. & Zając, T. (2021). Seasonal patterns in the developmental rate of glochidia in the endangered

917   thick-shelled river mussel, *Unio crassus* Philipsson, 1788. *Hydrobiologia*, 848:3077-3091.

918   https://doi.org/10.1007/s10750-020-04240-y

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933 Task 3: Using existing distribution data along with output from two-dimensional hydrodynamic
934 models to quantify suitable mussel habitat based on measures of bed stability and temperature
935 over a range of flows
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937   Freshwater mussels (Bivalvia: Unionida) while globally distributed are among the most imperiled

938 aquatic fauna worldwide (Lydeard et al., 2004). In the United States where they reach their greatest

939 diversity it is estimated that 65% of the approximately 300 described species have some conservation

940 designation (Williams et al., 1993, 2008; Lydeard et al., 2004; Strayer et al., 2004). These declines signal

941 a state of imperilment for many aquatic ecosystems primarily due to poor water management practices

942 (Randklev et al., 2018). The impact of altered flows and degraded water quality are particularly severe to

943 mussels because they are largely sessile and reliant on fish for dispersal. This means the ability of mussels

944 to cope with environmental impacts, such as altered flow regimes, is primarily limited to behavioral,

945 physiological, and life history traits (Haag, 2012; Randklev et al., 2015; Sansom et al., 2018; Randklev et

946 al., 2019).

947   To better understand the risk posed by changes to the historical and natural flow regime on

948 mussels, scientists have focused on evaluating mussel habitat at high and low flows. The reason for

949 examining these two endpoints is based on empirical studies showing mussels are most likely to occur in

950 areas of the stream bottom that remain stable during high flow events and are wetted and thermally

951 buffered during periods of low flow (Randklev et al., 2019). To date, recent efforts measuring the impact

952 of high flows on mussels has primarily focused on shear stress, which is the measure of frictional force

953 from flow resistance along the stream bottom. These studies have shown that mussel species richness and

954 abundance is typically maximized in areas where shear stress is low and is reduced in areas where shear

955 stress is high (Morales et al., 2006; Gangloff and Feminella, 2007; Allen and Vaughn, 2010). In Texas,

956 Randklev et al. (2019) noted low values of relative shear stress (RSS), a measure of substrate stability,

957 were associated with high mussel species richness and density. Change point analysis using threshold

958 indicator taxa analysis (TITAN) indicated species-specific preferences for varying levels of bed stability.

959   Furthermore, these preferences were best explained by life-history strategy and shell morphology based

960   on the results of a principal component analysis.

961   Given the role that bed stability plays in shaping mussel communities, occurrence, and

962   abundance, the goal of this task was to explore whether existing two-dimensional hydrodynamic models

963   in the Guadalupe River basin could be used to quantify mussel habitat based on RSS. Determining

964   whether this approach could be useful is important for better understanding how extreme high flow events

965   structure habitat and therefore mussel occurrence in the lower Guadalupe River. This effort will also be

966   helpful for making environmental flow recommendations and identifying additional modeling or

967   monitoring efforts that may be necessary to fully characterize the suitability of habitat conditions for

968   mussels in the lower Guadalupe River.

969   **Methods**

970   *Study area*

971   The Guadalupe River originates in Kerr County, Texas and runs approximately 644 km (i.e.,

972   ~400 mi) southeast until reaching the Gulf of Mexico (Huser, 2000). This spring fed river drains 15,539

973   km² of land (i.e., ~6,000 mi^2; Huser, 2000). Ten major impoundments for purposes ranging from water

974   supply to hydropower generation occur on the main stem of the Guadalupe River. Canyon Lake is the

975   largest reservoir in the Guadalupe River Basin with a combined conservation and flood control storage of

976   more than 700,00 acre-feet (i.e., 0.86 km^3; TWDB 2023). No other reservoir on the mainstem Guadalupe

977   River has a volume of greater than 7,000 acre-feet (i.e., 8.6 million m^3; TWDB 2023). Land use in the

978   Guadalupe watershed is characterized by livestock grazing, concentrated animal feeding operations,

979   pecan orchards, and oil and gas development. Urban areas (San Marcos and San Antonio) are situated in

980   the upper portion of the watershed along the Interstate 35 corridor. Smaller cities consist of Gonzales,

981   Cuero, and Victoria located along the lower reaches of the river. This study took place in a reach of the

982   Guadalupe River near Victoria, Texas, that has been the focus of environmental flow studies by the Texas

983   Instream Flow Program which is administered by the Texas Commission on Environmental Quality,

984   Texas Parks and Wildlife Department, and Texas Water Development Board.

985   *Data acquisition*

986   Modeled estimates for shear stress (SS), critical shear stress (CSS), and relative shear stress

987   (RSS) were obtained from the Texas Water Development Board (TPWD) for two reaches in the

988   Guadalupe River near Hochheim, Tx (~2.8 km [i.e., ~1.7 mi], starting 29.325 N, -97.304 W; ending 29.32

989   N, -97.304 W) and Victoria, Tx (~3.1 km [i.e., ~1.9 mi], starting 28.822 N, -97.017 W; ending 28.813 N,

990   -97.025 W). Obtained data included an approximately 8.5 m hexagonal array of nodes where SS, CRS,

991   and RSS were calculated based on substrate type, water velocity, and river channel morphology at various

992   flow conditions from low flow conditions (120 ft^3s^{-1}) to historical flood levels (3200 ft^3s^{-1}). The node

993   array and associated values were imported into ArcMap 10.7 where a 150 m^2 (i.e., ~1,615 ft^2; 15 x 10m)

994   north-south grid of uniquely identified cells were overlaid (Figure 1 and 2).

995   *Randomized site selection*

996   To assess the impact of shear stress on freshwater mussel distribution, abundance, and species

997   richness within the Guadalupe River, we used a randomized sample design to determine survey locations.

998   In a study on the hydraulic requirements of freshwater mussels, Randklev et al. (2019) found that RSS

999   values under high flow conditions were predictive of both mussel species richness and density. Based on

1000   this study, we grouped RSS values at the highest flow (3200 ft^3s^{-1}) into five categories: 0-1, 1-2, 2-3, 3-4,

1001   4+. Where possible, the grid cells with homogenous node values (i.e., where all nodes were of the same

1002   RSS category) were assigned a rank 1 to 5 based on the respective RSS category. In grid cells with

1003   heterogeneous node values (i.e., a range of RSS categories) the mode of the node categories was used to

1004   assign cell rank. Ten cells of each rank category were then randomly selected using a random number

1005   generator as survey sites (Figure 3 and 4). Selected sites were located in the field using the cell centroid

1006   point coordinates using a handheld GPS (Garmin 66sr). The 150 m^2 (i.e., ~1,615 ft^2) grid cells were then

1007　　recreated in the field by measuring 15m (i.e., ~49 ft) on the north-south gradient, and 10 m (i.e., ~33 ft)

1008　　on a west-east gradient using a meter tape.

1009　　*Mussel sampling*

1010　　　　We used timed searches in a subset of the randomly selected RSS categories, see previous

1011　　section, to locate mussels. The timed search method was chosen because it provides a more effective

1012　　means of detecting rare species than quantitative sampling methodologies (Vaughn et al., 1997). At each

1013　　site, we confined the search boundaries to the 150 m^2 (i.e., ~1,615 ft^2) grid cell designated by survey

1014　　poles. Each site was then surveyed tactilely and visually for a total of 4 person-hours (p-h). Surveyors

1015　　were spread out in the search area and every effort was made to search all available microhabitats. At the

1016　　end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept

1017　　submerged in water until completion of the survey. Following completion of the survey, all live mussels

1018　　from each search period were identified to species, counted, measured and then returned back to the river

1019　　into the appropriate habitat.

1020　　*Data analysis*

1021　　　　To document changes in the mussel assemblage across RSS values, we assessed patterns in

1022　　observed species richness and assemblage composition. We used survey data to estimate species richness

1023　　(total number of species) and estimated catch-per-unit-effort (CPUE; number of mussels/p-h). For

1024　　analysis of assemblage composition, we used non-metric multidimensional scaling (NMDS) and fuzzy set

1025　　ordination (FSO) to compare mussel assemblage composition between RSS categories. NMDS was used

1026　　for unconstrained ordination of spatial locations of mussel communities, while FSO was used as

1027　　constrained ordination to visualize the associations between site membership (i.e., RSS value) and

1028　　similarity in community composition relative to other sites. Bray-Curtis distance matrix with and without

1029　　abundance data was used in the NMDS and FSO ordinations. All statistical analyses were performed in

1030　　the R statistical language and environment (Version 4.2.3, R Core Team, 2023); the vegan package was

1031   used to compute NMDS (Oksanen et al., 2018) and the fso and LabDSV packages were used to compute

1032   FSO (Roberts, 2016, 2018).

1033   **Results/Discussion**

1034   A total of 12 sample sites were surveyed across the 4 of the 5 RSS categories to explore whether

1035   existing two-dimensional hydrodynamic models in the Guadalupe River basin could be used to quantify

1036   mussel habitat based on substrate stability. We were unable to sample sites with an RSS value of 3 due to

1037   elevated flow conditions at those locations at time of sampling. We observed a total of 12 species and

1038   1,109 live mussels during this effort, with *Amblema plicata* (threeridge) and *Cyrtonaias tampicoensis*

1039   (Tampico pearlymussel) being the most abundant, 506 and 393 individuals, respectively, and the most

1040   prevalent species, occurring at all 12 sites for both species. *Tritigonia verrucosa* (pistolgrip) and

1041   *Megalonaias nervosa* (washboard) were the least abundant with 1 individual per species, and the least

1042   prevalent occurring only at one site each (Table 1). We collected a total of 27 individuals across 9 sites of

1043   *Cyclonaias necki* (Guadalupe Orb), which has been proposed for listing under the U.S. Endangered

1044   Species Act (USFWS 2019). We did not observe *Fusconaia mitchelli* (false spike), or *Lampsilis*

1045   *bergmanni* (Guadalupe fatmucket), which occur in the basin and have also been proposed for ESA listing

1046   (USFWS 2019).

1047   Species richness varied from 3 to 10 species and there was no observable pattern related to RSS

1048   ($r^2 = 0.19$, $p = 0.23$; Figure 3). Specifically, mean richness for low RSS values ranged from 5 to 7 and for

1049   high RSS values 5 to 4 (Table 2). In contrast, abundance, i.e., CPUE, decreased significantly with

1050   increase in RSS ($r^2 = 0.30$, $p = 0.01$; Figure 3) such that mean CPUE was around 30 mussels/p-h for RSS

1051   values of 1 and 2, and CPUE was 12 and 8 mussels/p-h for RSS values of 4 and 5, respectively (Table 2).

1052   These findings suggest that increases in streambed instability reduces abundance, which has been reported

1053   for other rivers in Texas (Randklev et al. 2019).

1054   Comparing community structure across RSS values, the fuzzy ordination found RSS was not a

1055   significant predictor of mussel community composition using the Bray-Curtis distance matrix without

1056   abundance ($r = 0.08$, $p = 0.80$; Figure 4). This finding underscores our results that change in RSS had

1057   little effect on species richness. In contrast, RSS was a significant predictor of mussel community

1058   composition using the Bray-Curtis distance matrix with abundance ($r = 0.70$, $p = 0.01$; Figure 4),

1059   corroborating our finding that increases in RSS result in decreases in mussel abundance. Taken together,

1060   our findings indicate species composition can be similar across sites with different RSS values, but the

1061   abundance of species shared will be significantly lower at high RSS sites. This is not unexpected given

1062   that mobilization of the streambed during high flows can entrain and disperse mussels downstream

1063   (Randklev et al. 2019).

1064   To the best of our knowledge, this is the first time that a 2D hydraulic model has been used to

1065   quantify mussel habitat using RSS and then ground-truthed using qualitative sampling methods for

1066   mussels in a randomized sampling grid. The model results indicate our approach could be useful for

1067   environmental flow assessments, because RSS values were determined based on 3200 ft^3s^{-1}, which was

1068   used as maximum discharge in prior instream flow studies. Model estimates could be improved by

1069   sampling more sites, which would help further test whether RSS shapes species richness as well as

1070   abundance, which has been documented in other rivers in Texas (Randklev et al. 2019). It would also

1071   provide more data to identify specific RSS-mussel relationships, which are useful for predicting how

1072   mussels will respond to changes in high flows. Finally, incorporation of water quality information, such

1073   as temperature, could be fruitful because mussels are sensitive to temperature (Khan et al., 2020;

1074   Goldsmith et al., 2022), which would allow managers to quantify mussel habitat during lows flow when

1075   water temperature can become limiting.

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1078 **Table 1**. Raw data collected from qualitative timed-search surveys near Victoria, Texas. Relative shear
1079 stress (RSS) is the ratio of observed shear stress (force of friction on the substrate) to critical shear stress
1080 (shear stress required to initiate substrate motion). Higher values of RSS indicate greater potential for
1081 mussel entrainment. Total denotes total number of individuals collected at a site, species richness is the
1082 total number of species at a site, and CPUE is relative abundance, which is calculated by dividing the total
1083 number of mussels at a site by 4, which is the total amount of time spent searching for mussels.

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Site	101	104	106	506	203	204	207	503	401	406	502	509
RSS	1	1	1	1	2	2	2	2	4	4	5	5
Amblema plicata	99	123	3	14	24	98	12	48	28	19	9	29
Arcidens confragosus	2	0	0	0	2	2	0	1	0	0	0	0
Cyclonaias necki	4	9	1	0	2	1	4	0	1	2	0	3
Cyclonaias pustulosa	6	20	0	0	5	1	2	6	3	3	2	4
Cyrtonaias tampicoensis	71	16	14	59	70	49	28	42	4	29	4	7
Fusconaia mitchelli	0	0	0	0	0	0	0	0	0	0	0	0
Lampsilis bergmanni	0	0	0	0	0	0	0	0	0	0	0	0
Lampsilis hydiana	0	0	0	1	0	6	0	0	0	0	0	0
Lampsilis teres	15	0	3	20	10	12	13	14	2	4	0	3
Megalonaias nervosa	1	0	0	0	0	0	0	0	0	0	0	0
Pyganodon grandis	0	0	0	2	1	1	0	0	0	0	0	0
Toxolasma parvum	0	0	0	0	0	1	0	0	0	1	0	0
Toxolasma texasense	0	0	0	0	2	10	0	1	0	0	0	0
Tritigonia verrucosa	1	0	0	0	0	0	0	0	0	0	0	0
Total	199	168	21	96	116	181	59	112	38	58	15	46
Species richness	8	4	4	5	8	10	5	6	5	6	3	5
CPUE	49.8	42.0	5.3	24.0	29.0	45.3	14.8	28.0	9.5	14.5	3.8	11.5

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1093   **Table 2**. Mean, standard deviation, standard error and sample size of mussel species richness and
1094   abundance by RSS value. Relative shear stress (RSS) is the ratio of observed shear stress (force of friction
1095   on the substrate) to critical shear stress (shear stress required to initiate substrate motion). Higher values
1096   of RSS indicate greater potential for mussel entrainment.
1097

Richness (number of species)

RSS	Mean	Standard deviation	Standard error	Sample size
1	30.3	19.9	9.9	4
2	29.3	12.5	6.2	4
4	12.0	3.5	2.5	2
5	7.6	5.5	3.9	2

Abundance (mussels/p-h)

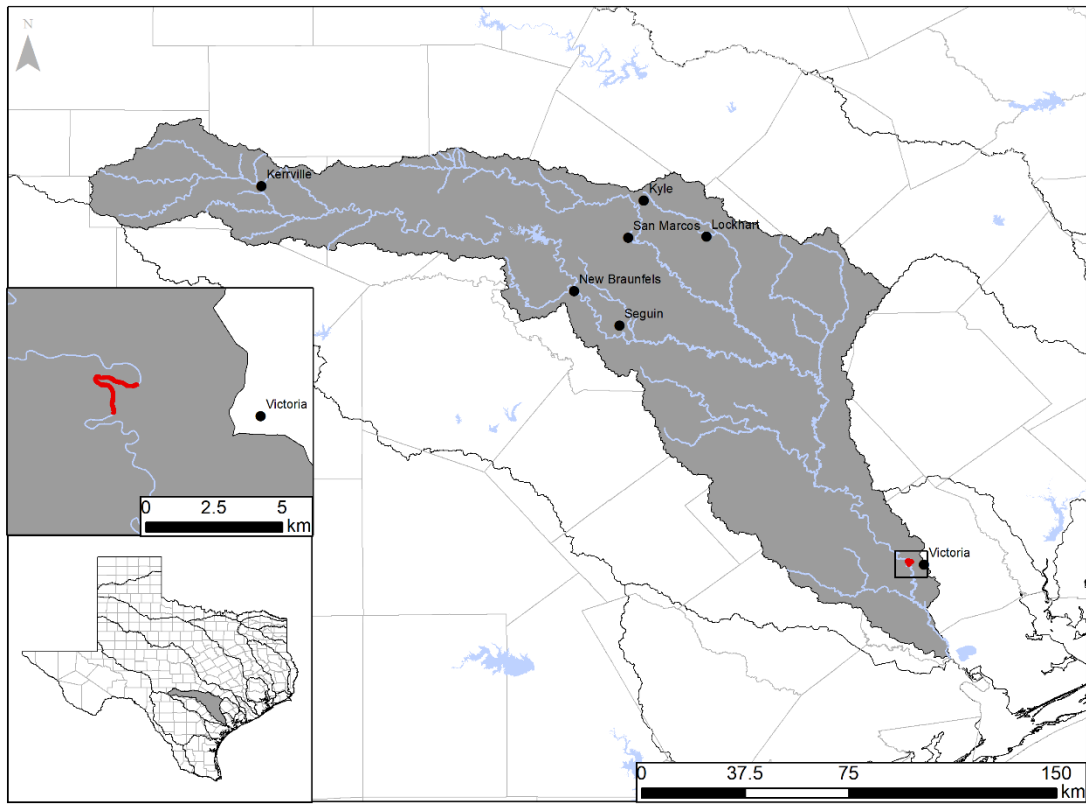| RSS	Mean	Standard deviation	Standard error	Sample size
1	5.3	1.9	0.9	4
2	7.3	2.2	1.1	4
4	5.5	0.7	0.5	2
5	4.0	1.4	1.0	2

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1105   **Figure 1.** Map of study area showing study reach near Victoria, Texas.
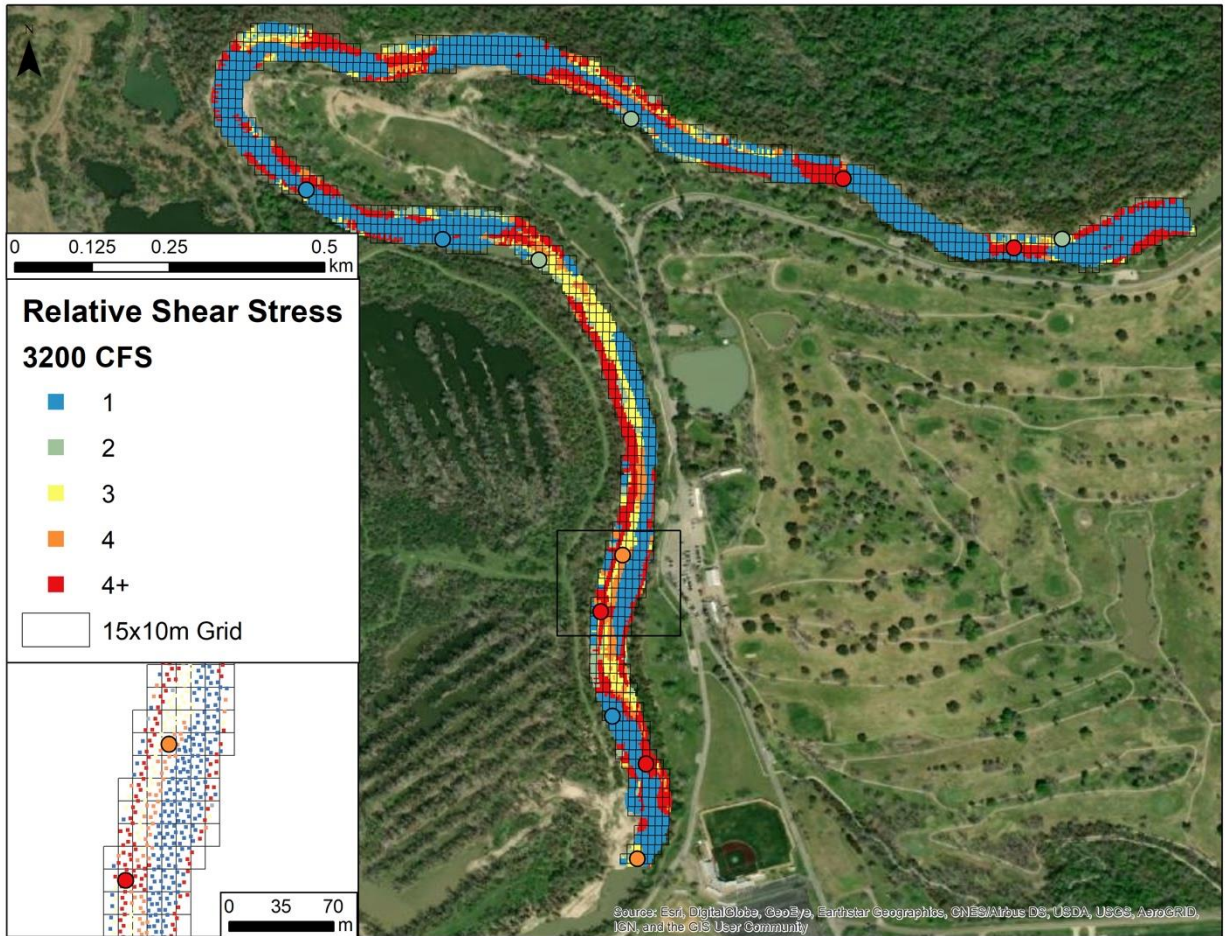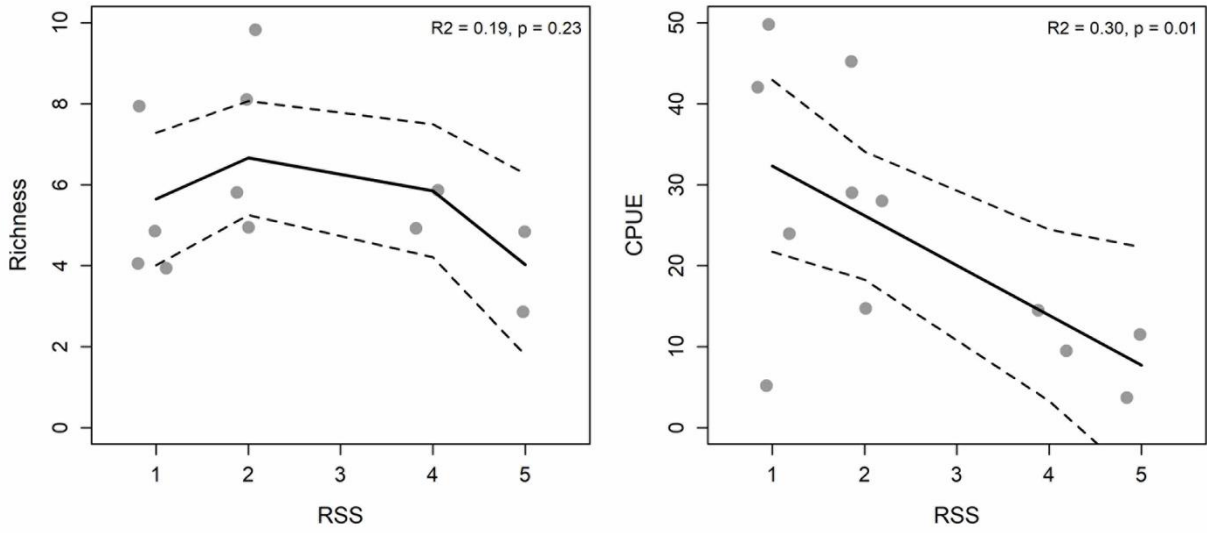
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Figure 2. Map of study reach showing RSS cells values at 3200 ft^{3}s^{-1}. RSS is the ratio of observed shear stress (force of friction on the substrate) to critical shear stress (shear stress required to initiate substrate motion). RSS values are denoted by color – blue (0 to 1), green (1 to 2), yellow (2 to 3), orange (3 to 4), and red (>4). Sample sites are denoted by circles shaded based on their corresponding RSS value.

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Figure 3. General additive models for species richness (left) and mussel abundance, (mussels/p-h), (right) by RSS value. Fitted models (solid lines) and 95% confidence intervals (dashed lines) are plotted on sites (gray circles) and coefficient of determination and their significance are given.
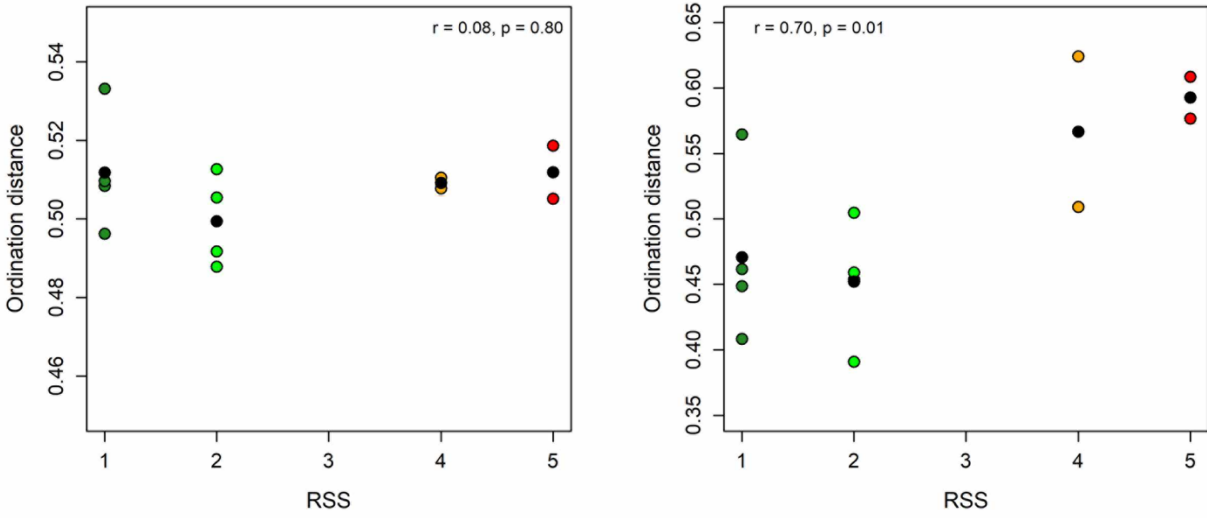
1132

1133 **Figure 4.** Fuzzy set ordination showing the relationship between sites based on the similarity of

1134 community structure calculated using presence/absence (left) or abundance, (mussels/p-h), (right) and

1135 RSS value. Colored circles are sites, black circles denote mean ordination distance for a given RSS value,

1136 and correlation coefficients and their significance are given.

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Literature Cited

Allen, D.C., and C.C. Vaughn. 2010. Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. Journal of the North American Benthological Society 29: 383–394.

Gangloff, M.M., and J.W. Feminella. 2007. Stream channel geomorphology influences mussel abundance in southern Appalachian streams, USA. Freshwater Biology 52:64–74.

Goldsmith, A.M., J.M. Khan, C.R. Robertson, R. Lopez., and C.R. Randklev. 2022. Using upper thermal limits of *Lampsilis bracteata* (Texas fatmucket) from the North Llano and San Saba Rivers, Texas to inform water management practices in the Edwards Plateau. Aquatic Conservation: Marine and Freshwater Ecosystems 32: 85-97.

Haag, W.R. 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press, Cambridge, Ma.

Huser, V., 2000. Rivers of Texas. Texas A&M University Press, College Station.

Khan, J., J. Dudding, M. Hart, E. Tsakiris, and C.R. Randklev. 2020. Linking life history strategies and historical baseline information shows effects of altered flow regimes and impoundments on freshwater mussel assemblages. Freshwater Biology 65: 1950–1961.

Lydeard, C., R.H. Cowie, W.F. Ponder, A.E. Bogan, P. Bouchet, S.A. Clark, K.S. Cummings, T.J. Frest, O. Gargominy, D.G. Herbert, and R. Hershler. 2004. The global decline of nonmarine mollusks. BioScience 54: 321–330.

Morales, Y., L.J. Weber, A.E. Mynett, and T. J. Newton. 2006. Effects of substrate and hydrodynamic conditions on the formation of mussel beds in a large river. Journal of the North American Benthological Society 25: 664–676.

1171 Oksanen, J., F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, … H. Wagner. 2018.

1172   vegan: community ecology package. R package version 2.5–3. Retrieved from

1173   490 https://cran.r-project.org/package=vegan

1174 Randklev, C.R., H.H. Wang, J.E. Groce, W.E. Grant, S. Robertson, N. Wilkins. 2015. Land use

1175   relationships for a rare freshwater mussel species endemic to central Texas. Journal of Fish and

1176   Wildlife Management 6: 327–337.

1177 Randklev, C.R., E.T. Tsakris, M.S. Johnson, T. Popejoy, M.A. Hart, J. Khan, D. Geeslin, and C.R.

1178   Robertson. 2018. The effect of dewatering on freshwater mussel (Unionidae) community

1179   structure and the implications for conservation and water policy: a case study from a spring-fed

1180   stream in the southwestern United States. Global Ecology and Conservation, 16, e00456.

1181 Randklev, C.R., M.A. Hart, J.M. Khan, E.T. Tsakiris, and C.R. Robertson. 2019. Hydraulic requirements

1182   of freshwater mussels (Unionidae) and a conceptual framework for how they respond to high

1183   flows. Ecosphere 10: 1–19.

1184 Roberts, D.W. 2008. Statistical analysis of multidimensional fuzzy set ordinations. Ecology,

1185   526: 1246–1260.

1186 Sansom, B.J., S.J. Bennett, J.F. Atkinson, and C.C. Vaughn. 2018. Long-term persistence of freshwater

1187   mussel beds in labile river channels. Freshwater Biology 63: 1469–1481.

1188 Strayer, D.L., J.A. Downing, W.R. Haag, T.L. King, J.B. Layzer, T.J. Newton, and J.S. Nichols. 2004.

1189   Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience

1190   54: 429–439.

1191 Texas Water Development Board [TWDB]. (2023).

1192   <https://www.twdb.texas.gov/surfacewater/rivers/reservoirs/> Accessed 10 July 2023.

1193   U.S. Fish and Wildlife Service (USFWS). 2019. Species Status Assessment Report for the Central Texas

1194   Mussels, Central Texas Mussels SSA Report.

1195   Vaughn, C.C. C.M. Taylor, and K.J. Eberhard. 1997. A comparison of the effectiveness of timed searches

1196   vs. quadrat sampling in mussel surveys. Proceedings of an Upper Mississippi River Conservation

1197   Committee Symposium. Conservation and Management of Freshwater Mussels II: Initiatives for

1198   the Future. Rock Island, Illinois,157–162.

1199   Williams, J., M. Warren Jr., K.S. Cummings, J. Harris, and R. Neves. 1993. Conservation status of

1200   freshwater mussels of the United States and Canada. Fisheries 18: 6–22.

1201   Williams, J.D., A.E. Bogan, and J.T Garner. 2008. Freshwater mussels of Alabama and the Mobile Basin

1202   in Georgia, Mississippi and Tennessee. The University of Alabama Press, Tuscaloosa.

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1214	Appendix 1A
1215	Summary of Responses to TWDB Comments to Draft Report
1216	Contract No. 2100012464
1217	Texas A&M Natural Resources Institute
1218	"LITERATURE REVIEW TO EVALUATE MUSSEL-FLOW ECOLOGY IN THE LOWER GUADALUPE RIVER BASIN"
1219	

1220 Overall, authors have diligently worked to complete the scope of work and address all edits. This paper
1221 will increase the understanding of mussel-flow relationships within the Guadalupe River Basin (TX, USA)
1222 and aid in developing protective environmental flow standards.

1223

1224 Specific Comments

1225 1. Title Page. Please update the date of the final report which is currently listed as "September
1226 2022."
1227
1228 Updated to "July 2023".
1229
1230 2. Please provide a more accurate estimate of the length of the Guadalupe River. Throughout the
1231 document (*e.g.* Page 2, Line 54), the length of the river is underestimated as ~230 miles (370
1232 kilometers).
1233
1234 Updated throughout paper to ~644 km (i.e., ~400 miles).
1235
1236 3. Please refer to environmental flow standards by the exact values provided in the Texas
1237 Administrative Code. For example, on Page 28, Lines 673, subsistence flows should be listed as
1238 210 and 130 ft^{3}s^{-1} (rather than 210.12 and 129.96 ft^{3}s^{-1}).
1239
1240 Edited subsistence flow values to reflect exact flow standard values provided within the Texas
1241 Administrative Code.
1242
1243 4. On Page 29, Lines 698 and 699, the authors recommend installation of temperature loggers at
1244 USGS gage locations to better identify the flow needs of mussels. Please clarify if it is well
1245 established that temperatures recorded at gage locations are reflective of temperatures in mussel
1246 habitats located upstream or downstream of gage locations or if additional temperature data
1247 collected within the mussel habitats themselves would be required to establish how temperature
1248 conditions at gage locations compare to temperatures in mussel habitats.
1249
1250 Provided clarification to highlight temperature logger data can be used to monitor water
1251 temperature in nearby mussel habitats both upstream and downstream.
1252
1253 5. On Page 32, Line 755, there appears to be a typo or miscalculation in the conversion between
1254 meters per second and feet per second. 0.008 to 0.20 meters per second is equivalent to 0.03 to
1255 0.66 feet per second (not 0.03 to 6.56 feet per second). Please correct.
1256

1257	Edit addressed.
1258	
1259	6. On Page 41, Line 974, the drainage area of the combined Guadalupe and San Antonio River
1260	basins is underestimated as 15,539 km^2 (6,000 mi^2). A more accurate estimate would be 26,200
1261	km^2 (10,100 mi^2) (see http://www.twdb.texas.gov/surfacewater/rivers/river_basins/index.asp). If
1262	the Guadalupe River Basin is considered separate from the San Antonio River Basin (which is
1263	typically the case in Texas and would be consistent with Figure 1 on Page 49), the estimated area
1264	of the Guadalupe River would be approximately 15,539 km^2 (6,000 mi^2), as stated in the draft
1265	report. Please remove the San Antonio River Basin from the description of the area or increase
1266	the estimate of the drainage area and adjust Figure 1 accordingly.
1267	
1268	The inclusion of the San Antonio River Basin was removed from the description of the area.
1269	
1270	7. On Page 41, Lines 978 and 979, the statement that Lake McQueeney is the next largest reservoir
1271	on the main stem of the Guadalupe River behind Canyon Lake is inaccurate. Please correct this
1272	statement. Canyon Lake has storage of 733,517 acre-feet at the top of the flood control pool
1273	(https://www.twdb.texas.gov/surfacewater/rivers/reservoirs/canyon/index.asp). Lake Gonzales is
1274	the next largest reservoir on the main stem Guadalupe River with approximate storage of 6,500
1275	acre-feet (https://www.twdb.texas.gov/surfacewater/rivers/reservoirs/gonzales/index.asp). Lake
1276	McQueeney has storage of 5,000 acre-feet
1277	(https://www.twdb.texas.gov/surfacewater/rivers/reservoirs/mcqueeney/index.asp). Rather than
1278	ranking the reservoirs, it may be more informative to the reader to know how the volume of
1279	Canyon Lake compares to those of the other main stem reservoirs. Suggest a statement such as:
1280	"Canyon Lake is the largest reservoir in the Guadalupe River Basin with combined conservation
1281	and flood control storage of more than 700,000 acre-feet (0.86 km^3). No other reservoir on the
1282	mainstem Guadalupe River has a volume of greater than 7,000 acre-feet (8.6 million m^3)."
1283	
1284	Statement was revised as suggested.
1285	
1286	8. Please revise the sentence describing studies by the state agencies on Page 42, Lines 983-985 to
1287	accurately describe the participants. Something such as the following should suffice: "This study
1288	took place in a reach of the Guadalupe River near Victoria, Texas, that has been the focus of
1289	environmental flow studies by the Texas Instream Flow Program which is administered by the
1290	Texas Commission on Environmental Quality, Texas Parks and Wildlife Department, and Texas
1291	Water Development Board."
1292	
1293	Statement was revised as suggested.
1294	
1295	9. Figure 2, Page 50, please clarify what is displayed in this figure. Do the colors in this figure
1296	correspond to exact values of Relative Shear Stress (RSS), ranges of RSS value, or rankings of
1297	RSS? The legend for Figure 2 lists divisions of "1, 2, 3, 4, and 4+." In the text on Page 42, Line
1298	1002, five categories are listed (0-1, 1-2, 2-3, 3-4, and 4+). On Page 43, Line 1003, five ranks are
1299	listed (1, 2, 3, 4, and 5). It is unclear if the color blue on Figure 2 designates areas with RSS value
1300	between 0 and 1; areas with RSS value of exactly 1; or a cell that has RSS rank 1.
1301	
1302	Revised figure caption to clarify data within Figure 2. The colors within the figure correspond to
1303	a range of RSS values rather than an exact value. For example, blue represents areas where RSS
1304	values range from zero to one.
1305	

Suggestions for the Conceptual Model Report:

10. Throughout the document, please consider providing lengths, areas, velocities, and flow rates in both imperial units (feet, miles, square feet, feet per second, cubic feet per second, *etc.*) as well as scientific units (meters, kilometers, square meters, meters per second, cubic meters per second, *etc.*). The authors do so in several locations in the report (*e.g.* flow rates on Page 29, Line 678 and 679), but not in others (*e.g.* length of the Guadalupe River on Page 2, Line 54).

Revised throughout paper to provide both imperial and scientific units when referring to length, area, velocity, and/or flow rate.

11. Please use a consistent abbreviation for the units of cubic feet per second throughout the document, either "ft^{3}s^{-1}" (as on Page 6, Line 143), "CFS" (as on Page 42, Line 993), or the more common "cfs."

Edit addressed. Cubic feet per second is now referred to as ft^{3}s^{-1} throughout the paper.

12. Suggest spelling out "person-hours (p-h)" the first time this abbreviation is used in the document on Page 43, Line 1016 as this unit and abbreviation may not be familiar to all readers.

Edit addressed. Person-hours is now spelled out the first time the abbreviation (p-h) is used.