



Results, Discussion, and Interpretation of Mussel Translocations

Michael A. Hart (NRI)
Mark Fisher (TxDOT)
Charles R. Randklev (NRI)

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TEXAS A&M NATURAL RESOURCES INSTITUTE
578 JOHN KIMBROUGH BLVD.
2260 TAMU
COLLEGE STATION, TX 77843

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16. Abstract 1. Translocation is used to conserve mussels, yet there remains a debate about its merit due to poor understating of its effects on transported mussels. 2. This study evaluated survivorship, body condition, and total glycogen and lipids for one common and widely distributed species (<i>Cyclonaias pustulosa</i>), two rare species (<i>Cyclonaias petrina</i> ; <i>Lampsilis bracteata</i>), and one species complex (<i>Fusconaia sp.</i> – <i>Fusconaia chunii</i> and <i>Fusconaia flava</i>) from the East Fork of the Trinity River and the Llano River of Texas. 3. Survivorship estimates for <i>C. pustulosa</i> and <i>Fusconaia sp.</i> using the Kaplan-Meier estimator were high in the East Fork. Body condition, glycogen, and total lipids varied for <i>C. pustulosa</i> and <i>Fusconaia sp.</i> , which may have indicated a short-term impact. For the Llano, survivorship of <i>C. petrina</i> and <i>L. bracteata</i> was high for the resident treatments but significantly reduced for the translocation treatments. The decline in survivorship for <i>C. petrina</i> was mirrored by decreases in the body condition, which may indicate inability to acclimate to novel environments. For <i>L. bracteata</i> , declines in survivorship were due to predation by <i>Procyon lotor</i> , racoon. A large flood of 3,766 m ³ s ⁻¹ at the end of the study eliminated both study sites. 4. The findings of this study indicate translocating mussels can be successful; however, sublethal effects and mortality may still occur. These effects are rooted in species-specific differences, which is not unexpected because mussel species vary in how they cope with environmental change based on their life-history traits. However, these traits are rarely considered when translocating mussels. To complicate matters, most mussel species have yet to be evaluated on how they respond to translocation, and for species where such information is available, adults are the primary focus. Addressing these knowledge gaps is critical for determining the appropriateness of translocation and improving its efficacy.			
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Michael A. Hart (NRI)

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Texas A&M Natural Resources Institute
578 John Kimbrough Blvd.
2260 TAMU
College Station, TX 77843

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1 | INTRODUCTION

Freshwater mussels of the family Unionidae (hereafter mussels) historically dominated the benthic biomass of many rivers in eastern North America (Parmalee & Bogan, 1998) but are now among the most imperiled groups in the world due to human impacts (Bogan, 1993). The United States is a biodiversity hotspot for freshwater mussels, with approximately three hundred species currently recognized (Haag & Williams, 2014). However, twenty-nine of these species have become extinct within the past one hundred years, and an additional 65% are considered endangered, threatened, vulnerable or otherwise imperiled (Williams et al., 1993). These declines will have long-term negative consequences for freshwater ecosystems because mussels influence primary and secondary production through filter feeding, waste excretion, and burrowing activities (Vaughn & Hakenkamp, 2001; Spooner et al., 2011). Their physical presence can also stabilize and expand habitat for themselves and other benthic macroinvertebrates (Gutiérrez et al., 2003; Spooner & Vaughn, 2008). Additionally, mussels are an important food source for birds, mammals and fish (Zimmerman & de Szalay, 2007; Haag, 2012).

To address threats to freshwater mussels, resource agencies and conservationists are working to develop strategies to mitigate threats and restore extirpated or diminishing populations, mostly through stocking programs (Cosgrove & Hastie, 2001; Mackie, Morris, & Ming, 2008; Luzier & Miller, 2009). However, these activities alone are unlikely to curb further declines, particularly in cases where: 1) factors responsible for declines are unknown; 2) there is little regulatory recourse or political appetite to address underlying issues; or 3) impacts are occurring at rates that outpace the ability to mitigate environmental change. In such cases, translocating mussels from these impacted areas may be an important conservation tool. Translocation consists of moving populations of animals from one location to another similar location where they have the potential to perform similarly to or better than in their original location (International Union for Conservation of Nature [IUCN Species Survival Commission], 2013). For mussels, translocation has been widely used for several decades as a means for removing populations from danger, to increase numbers or genetic diversity, and recolonize areas where mussels have been extirpated (Villemela, King, & Starliper, 1998; Grabarkiewicz, 2008; Klunzinger et al., 2012).

Despite the wide use of translocation, its efficacy remains in question and is not widely accepted in the conservation community (Fischer & Lindenmayer, 2000). Haag & Williams (2014) in their review of conservation strategies for mussels in North America argued that translocation is intuitively appealing to reduce mussel mortality but is costly and of dubious mitigation value if individuals are moved to another location within the same river. Their reasoning for this was that neither the total population size nor the number of populations for a species are likely to be increased. We argue that translocating local populations, especially under scenarios where extirpation may occur (e.g., dewatering of a stream reach or construction activities that results in loss of habitat), will likely have a net conservation benefit for the species being translocated. This is because mussels are patchily distributed within riverine systems (Strayer, 1999), and local mussel populations likely interact with each other such that some (i.e., sources) serve as exporters of individuals due to positive population growth, whereas others (i.e., sinks) are unable to support

themselves due to declining population growth and therefore rely on dispersal from sources (Dias, Verheyen, & Raymond, 1996; Gundersen et al., 2001; Dauphinais et al., 2018; Seward et al., 2019). Source and sink populations are important for ensuring overall population persistence at a landscape scale (Furrer & Pasinelli, 2016; Seward et al., 2019). In the case of rare mussels, by definition, they are limited in population size, and it is often unknown whether a local population that is being considered for translocation is a source or sink. Thus, translocating mussels out of danger should have a positive mitigation value in cases where extirpation of a local population has the potential to jeopardize overall population persistence.

Although translocation is potentially an important tool for reducing extirpation/extinction risk, there remain practical questions about the effects to transported mussels. For example, it is well known that relocating mussels to suitable habitat using appropriate handling techniques can improve survival of relocated populations (e.g., Chen, Heath, & Neves, 2001; Cope et al., 2003; Hamilton, Brim Box, & Dorazio, 1997; Tsakiris et al., 2017). However, there are cases in which habitat is selected but low survivorship still occurs (e.g., Dunn, Sietman, & Kelner, 1999; Tsakiris, 2016; Stodola, Stodola, & Tiemann, 2017). This indicates that other factors can negatively affect performance of a relocated population. For example, Dunn, Sietman, & Kelner (1999) observed higher mortality rates when mussels were relocated during cooler months, which they hypothesized was due to the inability of individuals to reburrow into compact substrate combined with rapidly declining water temperatures. Stodola, Stodola, & Tiemann (2017) observed that translocated mussels in the Salt and Middle Forks of the Vermilion River, Illinois, were less likely to survive after floods than after periods with no floods. Sheehan, Neves, & Kitchel (1989) made a similar observation in the North Fork of the Holston River, Virginia, noting that flood events were likely responsible for loss of translocated individuals. The effect of low flows on translocated mussels is not as well documented but should be equally detrimental as mussels have thermal optima that when exceeded can result in lethal and sublethal impacts (Pandolfo, Cope, & Arellano, 2009; Archambault, Cope, & Kwak, 2014; Khan et al., 2019). The extent to which changes in environmental conditions, such as water quality or quantity impact mussels tends to be species-specific and related to suites of coevolved traits that enable a given species to cope with a range of ecological problems (Stearns 1992). For example, Tsakiris (2016) evaluating the effects of translocation on *Cyclonaias petrina*, Texas pimpleback, from the San Saba River, in central Texas, observed this species was unable to acclimate to novel environments, which the author hypothesized was due to trade-offs among basic demographic patterns of survival, fecundity, and reproduction.

In Texas, translocations are routinely used to move threatened mussels away from hazards during construction projects. It is also considered as a recovery action within conservation frameworks such as Habitat Conservation Plans [HCPs] and Candidate Conservation Agreements [CCAs]. Because translocation is being widely used for threatened mussel species coupled with uncertainties regarding its efficacy, the overall goal of this project was to evaluate survivorship and sublethal stress for a suite of species across two different river systems. The specific objectives of this study were the following: 1) evaluate survivorship and recovery for one common, widely

distributed species (*Cyclonaias pustulosa*, pimpleback) and one species complex (*Fusconaia* sp. – *Fusconaia chunii*, Trinity pigtoe, which is endemic to the Trinity River basin, and *Fusconaia flava*, Wabash pigtoe, which is widely distributed) from two sites in the East Fork of the Trinity River of northcentral Texas; 2) evaluate survivorship and recovery for two threatened species (*C. petrina* and *Lampsilis bracteata*, Texas fatmucket) from two sites in the Llano River of central Texas; 3) evaluate sublethal effects by assessing changes in body condition for all four species and glycogen and lipid concentrations for two species in the East Fork of the Trinity River; and 4) compare the resulting information between the two study locations and discuss the implications for management and conservation.

2 | METHODS

2.1 | Site and species selection

This study took place in the East Fork of the Trinity River (Trinity River drainage) and Llano River (Colorado River drainage) in northern and central Texas, respectively (Figure 1). Within each river, two sites were selected based on the presence of desired study animals and ease of access.

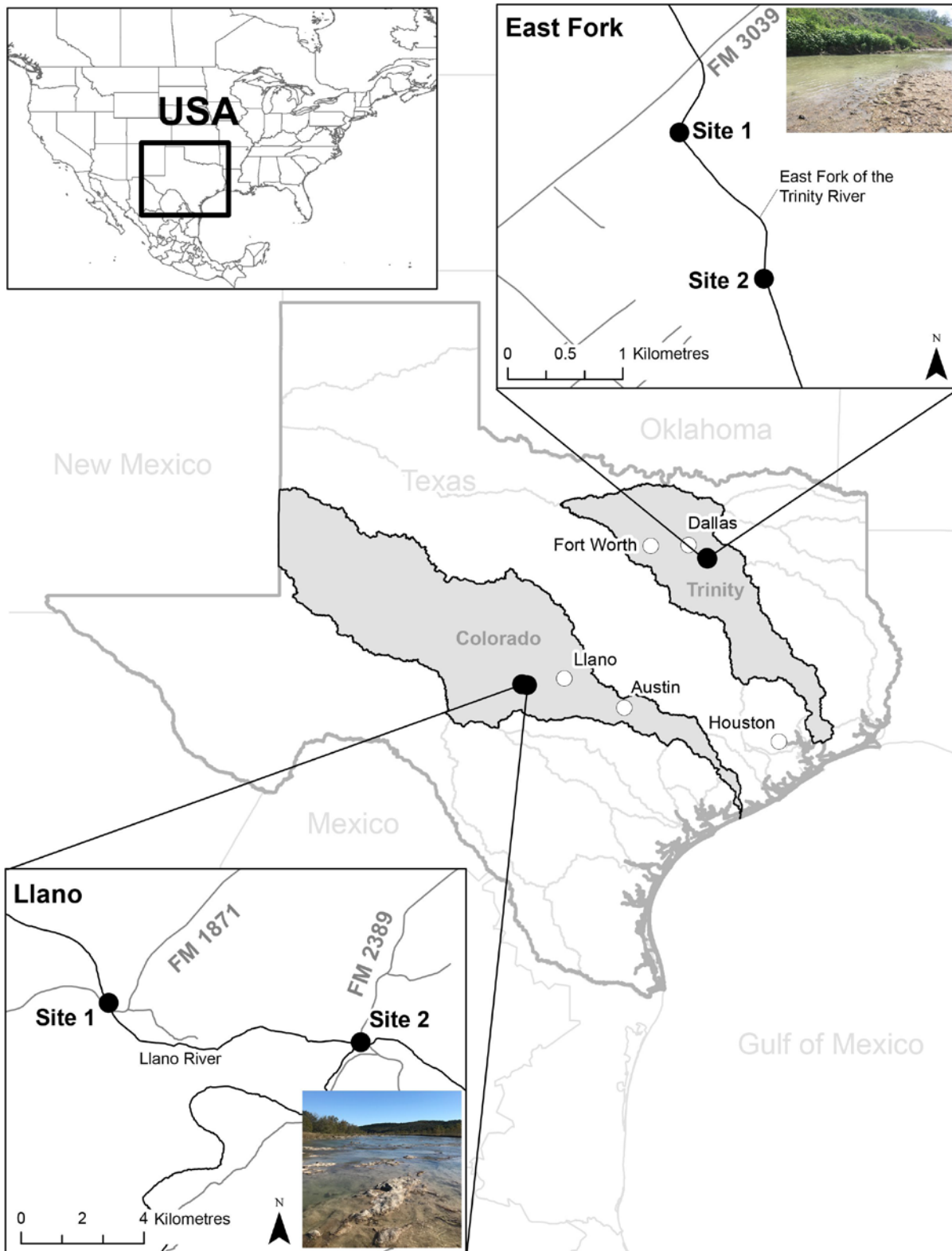


Figure 1. Map of study area showing locations of study sites in the East Fork of the Trinity River and the Llano River.

East Fork of the Trinity River

The East Fork of the Trinity River originates in Cooke County and flows south through two impoundments (Lake Ray Hubbard, and Lavon Lake) before joining the mainstem Trinity River in Kaufman County (Texas Water Commission [TWC], 1988). This river was channelized in the late 1970s (U.S. Army Corps of Engineers [USACE], 1976) to help with flood control. Flow in the river is prone to rapid changes due to impoundment releases and wastewater discharge from upstream wastewater treatment plants. Mean discharge near our sample sites (USGS gauging station 08062000, located ~ 5.4 km and 6.9 km upstream from Sites 1 and 2) during the study was $34.07 \text{ m}^3 \text{ s}^{-1}$ ($\text{SE} \pm 4.91$) and ranged from a minimum of $0.80 \text{ m}^3 \text{ s}^{-1}$ to a maximum of $405 \text{ m}^3 \text{ s}^{-1}$. The adjacent land use within the basin is a mix of urban centers with outlying agricultural areas and near both sites land use is a mixture of riparian forest and row crop agriculture. The climate within the basin is considered humid subtropical which is characteristic of the southern Plains (Griffith et al., 2007).

Llano River

The Llano River is located in the Edwards Plateau of central Texas and is composed of two spring fed branches; the North and South Llano Rivers. The Llano River mainstem flows approximately ~161 km through three counties (Mason, Llano, and Kimble) before reaching its confluence with the Colorado River. Flow within the Llano River is typically constant but is punctuated by extreme low and high flow events. Mean discharge near our sample sites (USGS gauging station 08150700, located ~ 23.9 km and 16.4 km downstream from Sites 1 and 2) during the study was $16.57 \text{ m}^3 \text{ s}^{-1}$ ($\text{SE} \pm 6.46$) and ranged from a minimum of $0.29 \text{ m}^3 \text{ s}^{-1}$ to a maximum of $3,766 \text{ m}^3 \text{ s}^{-1}$. Adjacent land use comprises of rolling terrain with brush over limestone formations (Broad et al., 2016), and near both our sites land use is brush with a mosaic of woody cover. Climate in the Edwards Plateau is meso-thermal, and semiarid to arid (Thorntwaite, 1931; Griffith et al., 2007).

Study species

East Fork Trinity

Fusconaia sp. includes both *F. chunii* and *F. flava*, which co-occur and cannot be distinguished using external morphology (Pieri et al., 2018). *Fusconaia chunii* is endemic to the Trinity River drainage of north central Texas while, *F. flava* is more widely distributed, occurring from Texas to Louisiana and to the north throughout the entire Mississippi River drainage to the Great Lakes drainage basins (Williams, Bogan, & Garner, 2008). *Cyclonaias pustulosa* is also a widely distributed species that occurs from Texas to Louisiana and north throughout much of the Mississippi basin to Canada (Johnson et al., 2018).

Llano River

Lampsilis bracteata, Texas Fatmucket, occurs in tributaries of the Colorado River drainage within Central Texas (Inoue et al., 2019) and is listed as a state threatened species by Texas Parks

and Wildlife (Texas Parks and Wildlife [TPWD], 2010; TPWD, 2020) and a candidate for listing under the US Endangered Species Act (U.S. Fish & Wildlife Service [USFWS], 2009; USFWS, 2011). *Cyclonaias petrina* occurs throughout much of the Colorado River drainage (Johnson et al., 2018) and is listed as state threatened (Texas Parks and Wildlife [TPWD], 2010; TPWD, 2020) and a candidate for listing under the ESA (U.S. Fish & Wildlife Service [USFWS], 2009; TPWD 2010).

2.2 | Mussel sampling

East Fork Trinity

Two sites (Site 1 – upstream and Site 2 – downstream), located 1.5 km apart, were selected based on similar densities for both focal species. At each site, a 10 x 5-meter plot was demarcated using a Trimble GeoXT 6 GPS device (Figure 1). At Site 1, mean water depth and velocity during sampling was 0.21 (SE \pm 0.03) m and 0.53 (\pm 0.03) m s⁻¹ and at Site 2 was 0.22 (\pm 0.02) m and 0.26 (0.03) m s⁻¹. In October of 2017, mussels (i.e., individuals \geq 30 mm) of similar size were collected using a multiple pass depletion method, wherein surveyors lined up shoulder to shoulder to cover the entire area and multiple passes were made until the number of mussels found was less than 20% of the previous pass count. Collected individuals of both focal species were then double tagged with hall print vinyl tags using super glue gel and 12.5-mm passive integrated transponder (PIT) tags using epoxy putty. Pit tags were used to increase the likelihood of recapturing the tagged mussels (Kurth et al., 2007). After tagging, individuals were measured, weighed, and then assigned to either the resident, translocated or sublethal assay treatments.

Resident treatments were used as controls to compare survivorship, recovery, body condition, and total glycogen and lipids with the translocated treatments. Translocated treatment groups consisted of mussels transported from upstream to downstream, or downstream to upstream. Treatment groups consisted of 51 individuals each of *C. pustulosa* and *Fusconaia* sp. that were moved from upstream (Site 1) to downstream (Site 2), 51 of *C. pustulosa* and *Fusconaia* sp. that were from upstream (Site 1) but not translocated, 41 *C. pustulosa* that were moved from downstream (Site 2) to upstream (Site 1), and 41 *C. pustulosa* that were from downstream (Site 2) but not translocated. Sample sizes for the resident/translocated treatments varied due to animal availability. An additional 48 individuals of *C. pustulosa* and 24 of *Fusconaia* sp. were collected for glycogen and lipids assays, which were tagged during setup and then a subsample of 4 individuals per species and treatment were collected during each monitoring period.

Llano

Sites were selected using similar criteria to those in East Fork (i.e., ease of access and presence of target species) but were spaced further apart (7.5 km) due to lack of public access to this river (Figure 1). At each site, a 3.5 x 6.5 m plot was demarcated using the same methods as the East Fork sites. The plots used in the Llano River were smaller than those in the East Fork to accommodate the unique geomorphology of this river, which comprised of pockets of flowing water interspersed among exposed stream bottom (Figure 1). At Site 1, mean water depth and

velocity during sampling was 0.35 ($SE \pm 0.03$) m and 0.06 ($SE \pm 0.01$) $m\ s^{-1}$ and at Site 2 was 0.28 ($SE \pm 0.04$) m and 0.05 ($SE \pm 0.01$) $m\ s^{-1}$. In October of 2017, mussels (i.e., individuals ≥ 30 mm) were tagged and weighed using the same methods employed in the East Fork of the Trinity. Due to concerns regarding oversampling of threatened species, mussels were translocated in only one direction (i.e., from Site 1 to Site 2). The total glycogen and lipids treatments were also omitted. Treatment groups consisted of 35 *C. petrina* and 23 *L. bracteata* that were moved from upstream (Site 1) to downstream (Site 2; translocated) and 33 *C. petrina* and 23 *L. bracteata* that remained at the upstream (Site 1) site to serve as a resident control.

2.3 | Post translocation monitoring

Survivorship and recovery

Sites were initially monitored monthly for two months and then were monitored quarterly, but the exact timing varied due to high flows in both rivers (Figure 2). Mussels were located using a PIT tag reader with a sweeping antenna receiver. Once a PIT tag was detected, visual and/or tactile searching was used to locate the individual mussel. Each site was searched extensively until all mussels both live and dead were found and/or until no more PIT tags were detected.

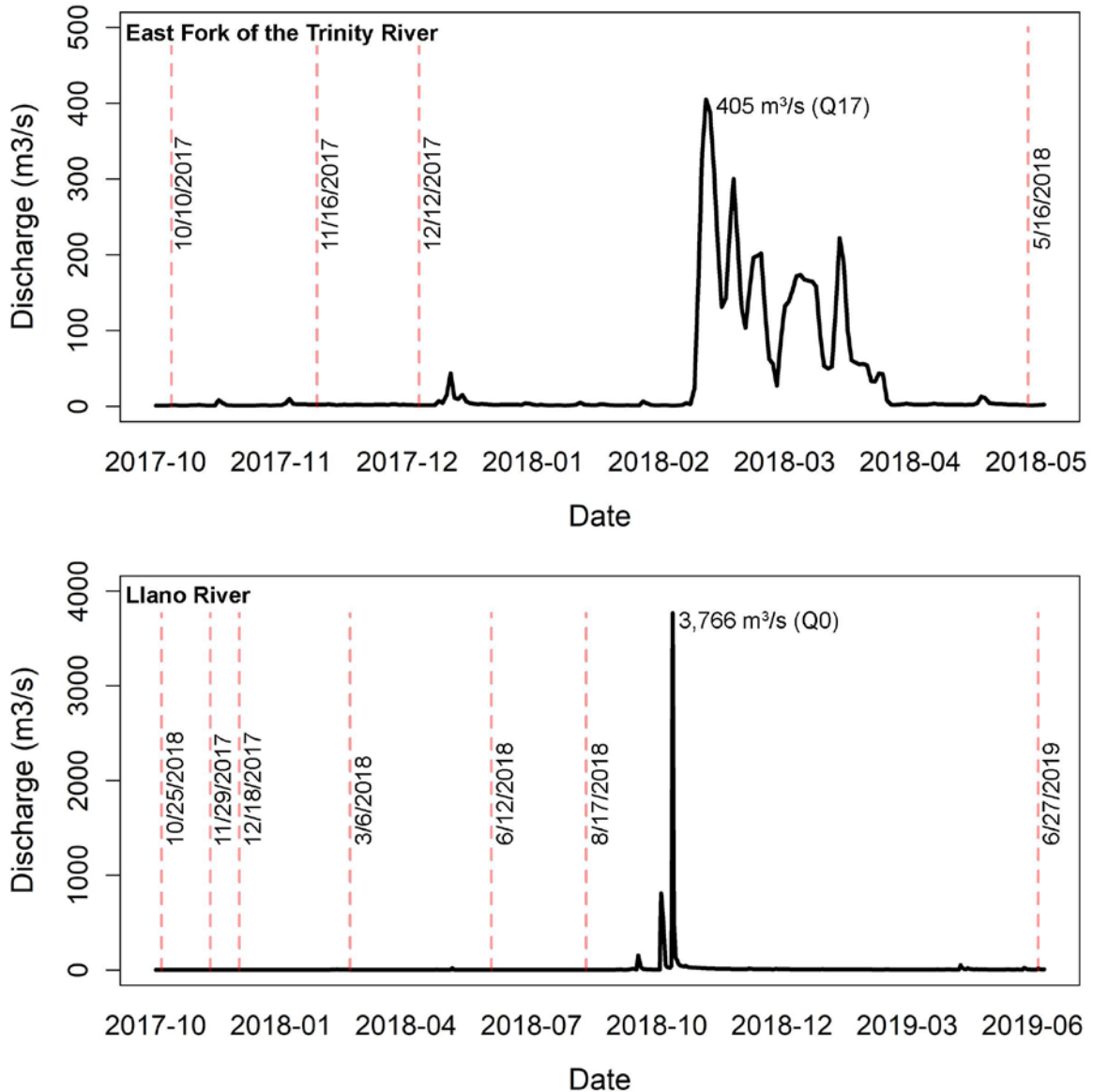


Figure 2. Discharge in the East Fork of the Trinity River (A) and Llano River (B) during the study. Vertical red dashed lines denote sampling periods. Maximum discharge ($m^3 s^{-1}$), and its exceedance value, are shown for the largest flood events for each river.

Fulton's K condition index

Measurements of shell length, width, and height (mm) as well as wetted weight (g) were recorded for all mussels found alive during each monitoring event. These data were used to calculate Fulton's K body condition factor using the following formula following Tsakiris et al., (2017):

$$Fulton's\ K = \frac{wetted\ weight}{shell\ length^3} * 10x^6$$

Total glycogen and lipids

Individuals assessed for total glycogen and lipids were collected during each sampling event, placed on dry ice to flash freeze, and then transported back to the lab. The tissue of each animal was homogenized because glycogen and lipid concentrations can vary by tissue type and non-lethal biopsies may cause decreases in survivorship (Naimo et al., 1998; Naimo & Monroe, 1999; Fritts et al., 2015; Vodáková & Douda, 2019). The homogenized tissue was then weighed to 10.0 ± 0.3 mg in triplicate for each assay. To quantify glycogen concentrations, the phenol-sulfuric acid assay method was used (Montgomery, 1957; Naimo et al., 1998) and for lipids the sulpho-phospho-vanillin assay method (Van Handel, 1985). These assays were performed for months 1 (2017-10), 2 (2017-12), and 7 (2018-05).

Statistical analysis

The Kaplan-Meier estimator was used to create survivorship curves for resident and translocated treatments for the East Fork and Llano study sites. Curves were generated using the *survival* package in program R version 3.4.3 (Therneau, 2020; R Core Team, 2018). Kaplan-Meier estimator is a nonparametric statistic used to estimate survival or recovery over a period of time (Kaplan & Meier, 1958). To compare Kaplan-Meier curves between treatments, the log-rank test was used, which tests the null hypothesis that there is no difference between curves, using the *survival* package in R and considered results with $P \leq 0.05$ significant. Recovery was calculated as the cumulative probability of recovering marked individuals relative to the at-risk population in a given sampling period. Recovery curves were then compared using 95% confidence intervals, and results where confidence intervals did not overlap were considered to be significant.

To compare changes in Fulton's K body condition index over time the 95% confidence intervals were compared by treatment and sampling period. In cases where 95% confidence intervals did not overlap, a Mann-Whitney U test was used in R to determine differences across treatments and considered results with $P \leq 0.05$ significant. Mann-Whitney test is a rank-based nonparametric test that is used when assumptions for an unpaired t-test are not met. For glycogen and lipids assays, sample sizes were small for each treatment (4 individuals per species) therefore differences between treatments were evaluated using 95% confidence intervals.

3 | RESULTS

3.1 | Survivorship and Recovery

East Fork of the Trinity River

The probability of survival for the East Fork Trinity sites remained high, ranging from 93–98% at the end of the study, for *C. pustulosa* and *Fusconaia* sp. resident and translocated treatments (Figure 3). The log-rank test revealed that the probability of survival for *C. pustulosa* and *Fusconaia* sp. did not differ between resident and translocated treatments (R = resident and T = translocated; *C. pustulosa*: R1 vs. T (1→2), $\chi_1^2 = 1.00$, $P = 0.32$ and R2 vs. T (2→1), $\chi_1^2 = 1.04$, $P = 0.31$); *Fusconaia* sp.: R1 vs. T (1→2), $\chi_1^2 = 0.35$, $P = 0.55$) and 95% confidence intervals overlapped across treatments (Figure 3). Comparison of probabilities of survival between sampling periods for both species by treatment revealed overlapping 95% confidence intervals, which indicated that survival did not vary based on time of year of sampling (Figure 3).

The probability of recovery remained high for *C. pustulosa* and *Fusconaia* sp., ranging from 85–92% at the end of the study (Figure 3). However, it did decline (up to 16%) between 2017-12 and 2018-5 sampling events, which coincided with floods during this period (Figure 2A). Comparison of 95% confidence intervals by treatment revealed that the probability of recovery did not differ for either species (Figure 3). Comparison of probability of recovery between sampling periods for both species by treatment revealed overlapping 95% confidence intervals, except for 2018-5 sampling period, which occurred after a series of floods (Figure 2A).

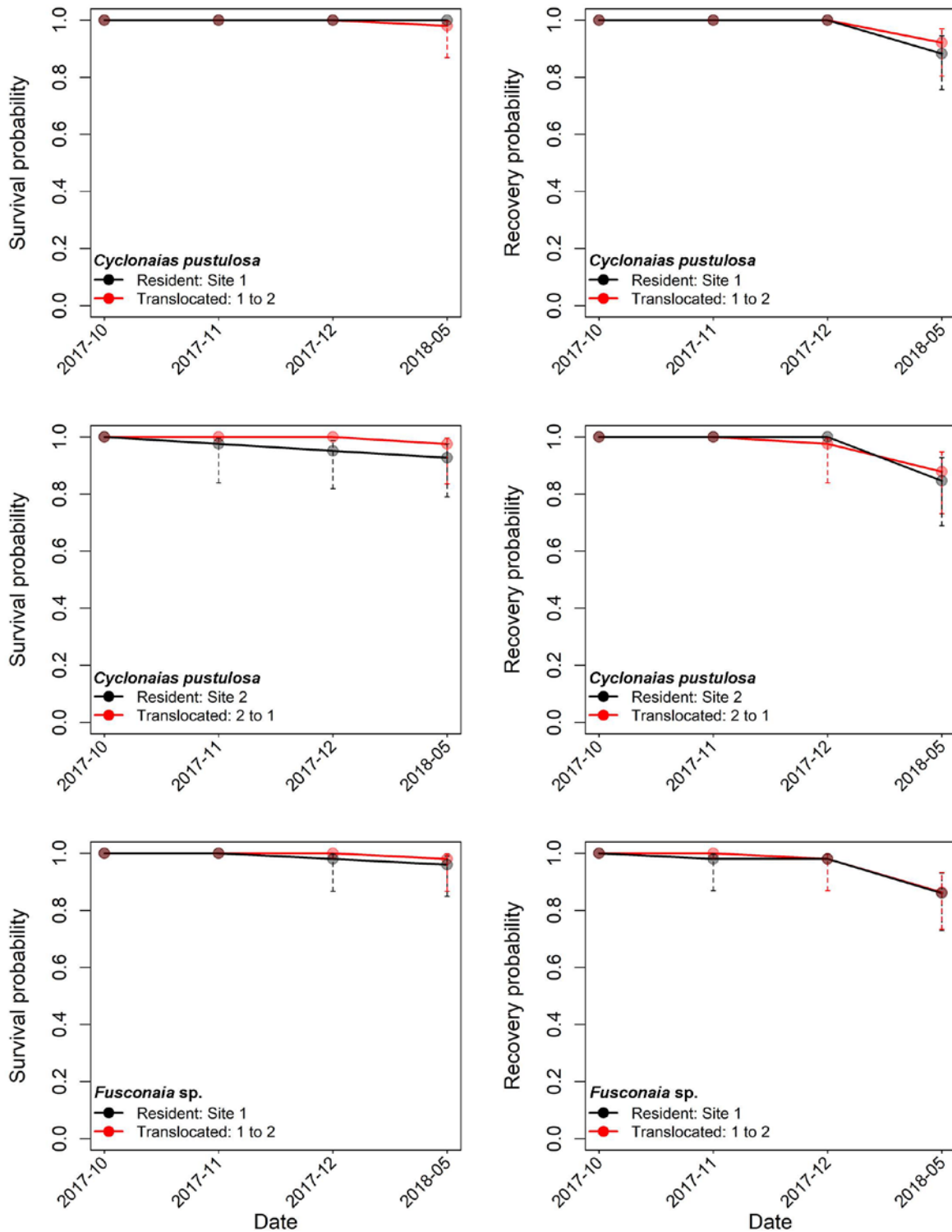


Figure 3. Kaplan-Meier estimate of survival and recovery probabilities for *Cyclonaias pustulosa* (pimpleback) and *Fusconaia* sp. (which includes *Fusconaia chunii* and *Fusconaia flava*, which co-occur and cannot be differentiated using external morphology, Pieri et al. 2018) in the East Fork of the Trinity. Solid black and red lines denote resident and translocated treatments, respectively. Error bars correspond to 95% confidence intervals.

Llano River

The probability of survival for translocated treatments for both species declined over time relative to the resident treatments (Figure 4). For *C. petrina*, the probability of survival for resident and translocated treatments was 100% and 83%, respectively, at the end of the study, and the two treatments were significantly different based on the log-rank test (R1 vs. T (1→2) $\chi_1^2 = 6.04$, $P = 0.01$). 95% confidence intervals between the two treatments did not overlap for most of the sampling periods, except for the 2017-11 and 2017-12 sampling dates (Figure 4). Comparison of probability of survival between sampling periods by treatment revealed overlapping 95% confidence intervals, which indicated that survival did not vary based on time of year of sampling (Figure 4). For *L. bracteata*, the probability of survival for resident and translocated treatments was 91% and 22% at the end of the study, respectively, and the two treatments were significantly different based on the log-rank test (R1 vs. T (1→2) $\chi_1^2 = 19.59$, $P < 0.001$). 95% confidence intervals between the two treatments did not overlap except for those from the 2017-11 sampling period (Figure 4). The decline in survivorship for *L. bracteata* coincided with predation by *Procyon lotor* (raccoon), as most of the dead individuals that were recovered showed marks from scratches, bites, and loss of shell, primarily at the posterior end. *Procyon lotor* was observed actively foraging in the study plot (Figure 5). Comparison of probability of survival between sampling periods by treatment revealed nonoverlapping 95% confidence intervals between summer (2018-6, 2018-8, and 2019-6) and fall/winter sampling periods (2017-11 and 2017-12), which indicated that time of year may have affected survivorship for both treatments (Figure 4).

The probability of recovery remained high (100%) for resident and translocated treatments of *C. petrina* until the 2019-06 sampling period and then declined to 0% for both treatments during the 2019-06 sampling period (Figure 4). Between the 2018-08 and 2019-06 sampling periods, a flood exceeding $3,766 \text{ m}^3 \text{ s}^{-1}$ occurred (Figure 2B) completely scouring the resident site and eliminating the translocation site (Figure 5). Survivorship estimates were unaffected because the fates of individuals that were washed away is unknown. Comparison of probability of recovery of *C. petrina* between sampling periods by treatment revealed overlapping 95% confidence intervals (Figure 4). For *L. bracteata*, the probabilities of recovery for resident and translocated treatments was 100% and 83% prior to the flood, respectively, and the two treatments were significantly different based on nonoverlapping 95% confidence intervals (Figure 4). Following the flood, the probability of recovery was reduced to 0% for both treatments. Comparison of probability of recovery of *L. bracteata* between sampling periods by treatment revealed non-overlapping 95% confidence intervals between winter (2017-11 and 2017-12) and summer months (2018-6 and 2018-08) (Figure 4).

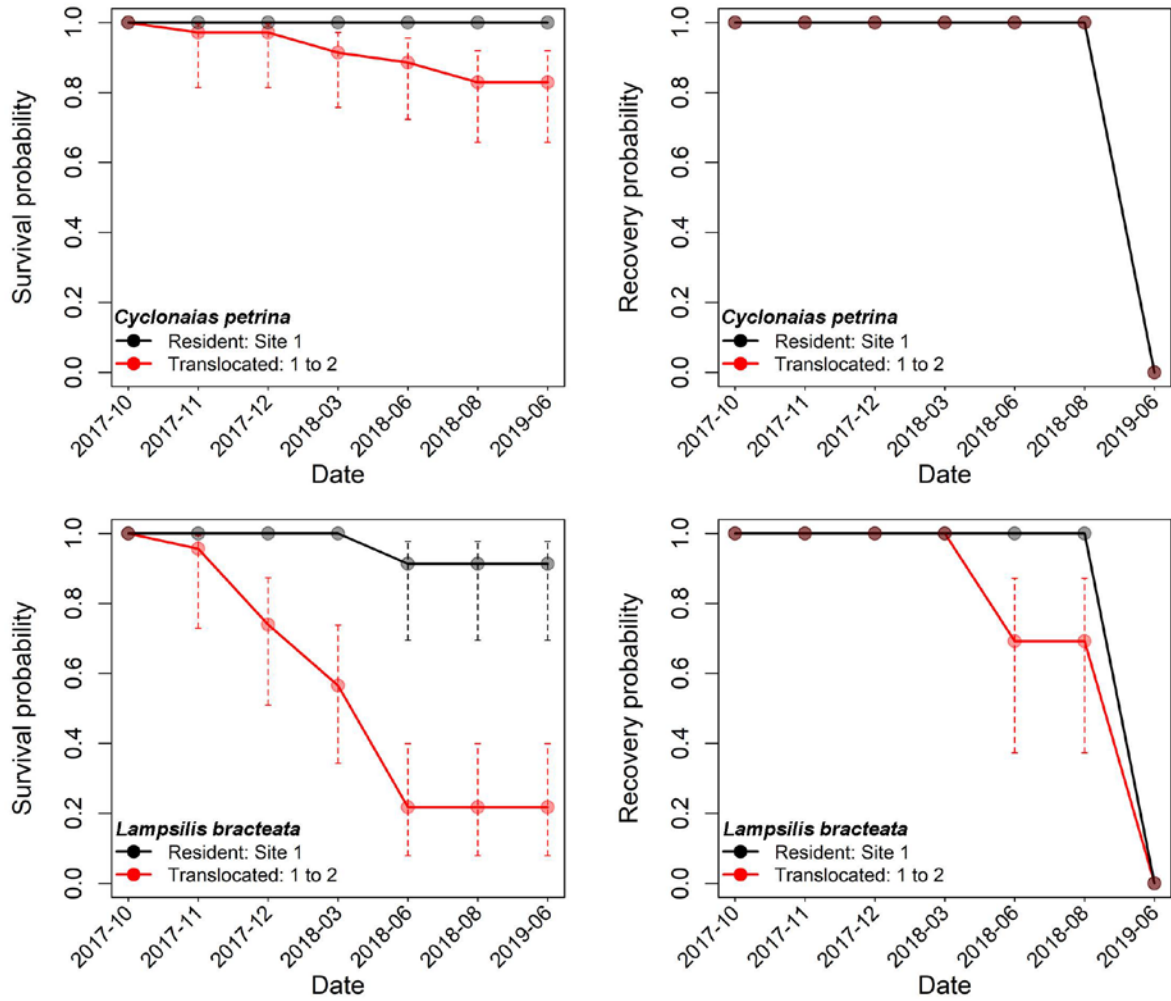


Figure 4. Kaplan-Meier estimate of survival and recovery probabilities for *Cyclonaias petrina* (Texas pimpleback) and *Lampsilis bracteata* (Texas fatmucket) in the Llano River. Solid black and red lines denote resident and translocated treatments, respectively. Error bars correspond to 95% confidence intervals.



*Figure 5. Game camera photograph, inset, of *Procyon lotor* (raccoon) foraging within Site 2 in the Llano River. Photograph of field crew personnel at the same site pointing to the exact location of the study plot and river channel prior to a flood of $\sim 3,766 \text{ m}^3 \text{ s}^{-1}$, which restructured the river channel and eliminated both study sites in the Llano River.*

3.2 | Fulton's K body condition index

East Fork of the Trinity River

Fulton's K body condition index decreased over time for both *C. pustulosa* resident and translocation treatments (Figure 6A, B) and were not significantly different except during the 2017-11 sampling period, during which translocated treatments had lower condition than the residents (R1 vs. T (1→2), Mdn R1 = 499.65, Mdn T = 436.98, U = 474, $P < 0.001$; and R2 vs. T (2→1), Mdn R2 = 499.65, Mdn T = 424.35, U = 130, $P < 0.001$). 95% confidence intervals did

not overlap for the 2017-11 period. For *Fusconaia* sp. the Fulton's K body condition index increased over time and did not differ between the resident and translocated treatments (Figure 6C).

Llano River

Fulton's K body condition for *C. petrina* was overall lower for the translocation treatment and significantly differed from the resident treatment during the 2018-03 and 2018-08 sampling periods based on the Mann-Whitney U test (2018-03: R1 vs. T (1→2), Mdn R1 = 227.35, Mdn T = 207.07, U = 287, $P = 0.003$; and 2018-08: R1 vs. T (1→2), Mdn R1 = 214.84, Mdn T = 187.28, U = 249, $P = 0.003$) and nonoverlapping 95% confidence intervals (Figure 6D). For *L. bracteata*, Fulton's K body condition index decreased slightly over time and was similar between treatments except for 2018-03 sampling period, which showed a significantly higher condition for the resident treatment based on the Mann-Whitney U test (2018-03: R1 vs. T (1→2), Mdn R1 = 179.92, Mdn T = 143.76, U = 18.5, $P < 0.001$) and nonoverlapping 95% confidence intervals (Figure 6E).

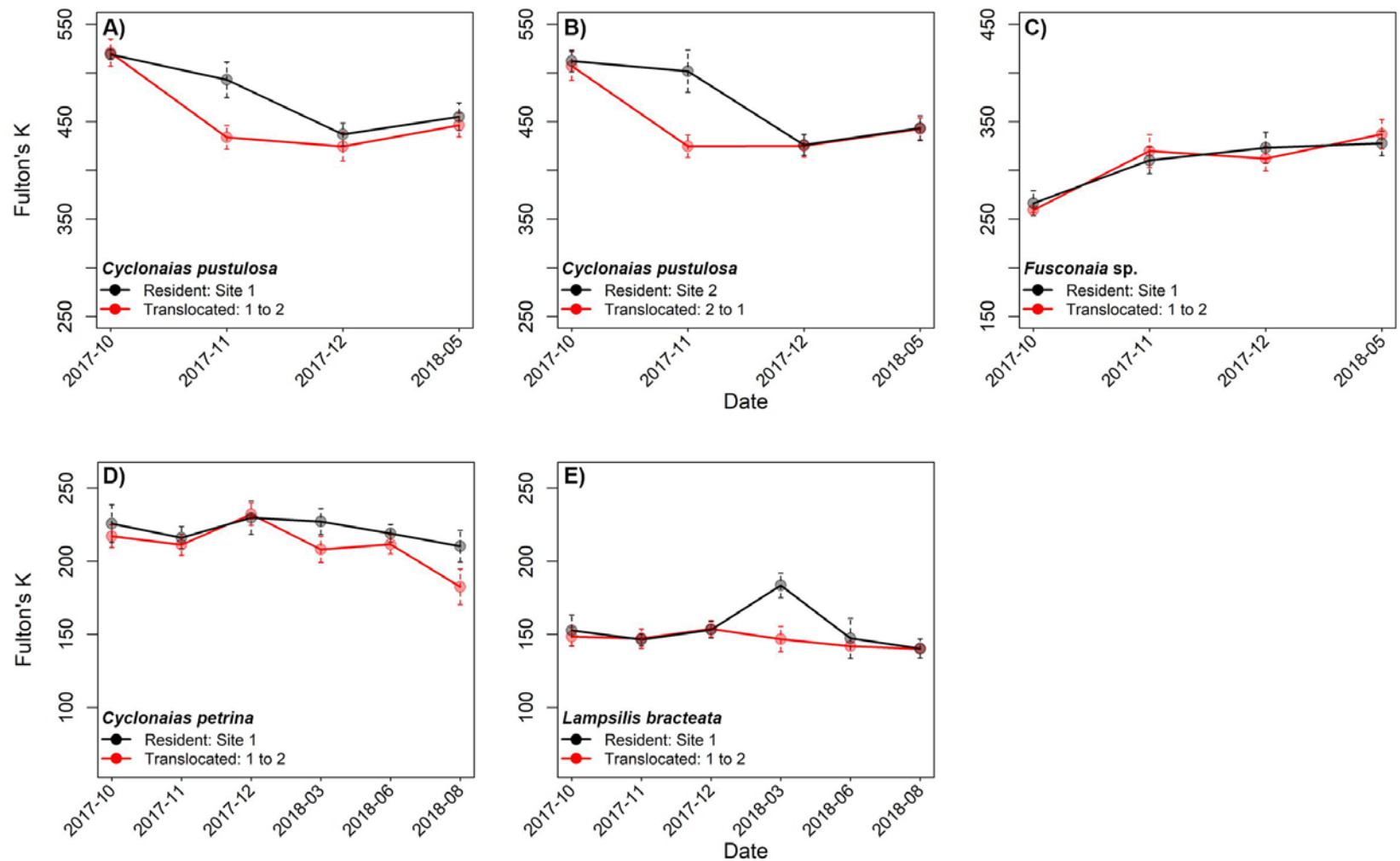


Figure 6. Mean Fulton's K body condition index for *Cyclonaias pustulosa* (pimpleback) (A–B), *Fusconaia* sp. (which includes *Fusconaia chunii* and *Fusconaia flava*, which co-occur and cannot be differentiated using external morphology, Pieri et al. 2018) (C), *Cyclonaias petrina* (Texas pimpleback) (D), and *Lampsilis bracteata* (Texas fatmucket) (E). Solid black and red lines denote resident and translocated treatments, respectively. Error bars correspond to 95% confidence intervals.

3.3 | Biochemical composition

East Fork of the Trinity River

Total glycogen concentrations for *C. pustulosa* resident and translocated treatments increased slightly during the 2017-12 sampling period but then decreased during the 2018-05 sampling period. Total glycogen values for translocated treatments were lower than those of their respective resident controls for the 2017-12 sampling period but were not significantly different, as 95% confidence intervals were overlapping (Figures 7A, B). For *Fusconaia* sp., total glycogen increased over time for resident and translocated treatments (Figure 7C). Total glycogen was greater for the translocated treatment than for the resident, and the two were significantly different during the 2017-12 sampling period based on nonoverlapping 95% confidence intervals (Figure 7C).

Total lipid concentration for *C. pustulosa* resident treatments increased over time, whereas the translocated treatments decreased during the 2017-12 sampling period but then increased during the 2018-05 sampling period. Total lipid values for translocated treatments were lower than those for their respective resident controls, and both sites differed during the 2017-12 sampling period based on nonoverlapping 95% confidence intervals (Figure 7D, E). For *Fusconaia* sp. total lipid values increased over time for both resident and translocated treatments (Figure 7F). Total lipid values were greater for the translocated treatment than for the resident, and the two were significantly different during the 2017-12 sampling period based on nonoverlapping 95% confidence intervals (Figure 7F).

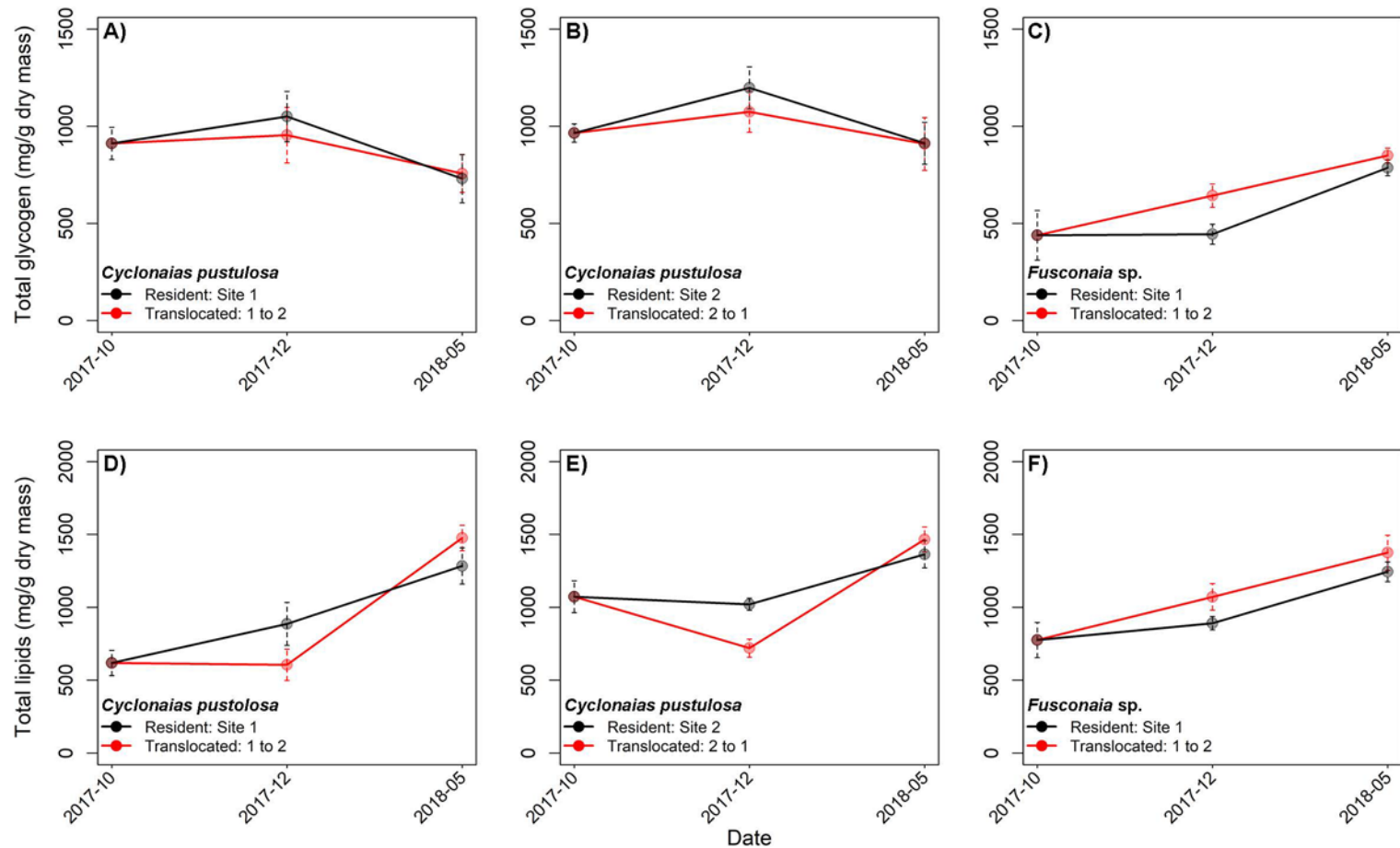


Figure 7. Total glycogen (mg g^{-1} dry mass) for *Cyclonaias pustulosa* (pimpleback) (A–B) and *Fusconaia* sp. (which includes *Fusconaia chunii* and *Fusconaia flava*, which co-occur and cannot be differentiated using external morphology, Pieri et al. 2018) (C). Total lipids (mg g^{-1} dry mass) for *C. pustulosa* (D–E) and *Fusconaia* sp. (F). Solid black and red lines denote resident and translocated treatments, respectively. Error bars correspond to 95% confidence intervals.

4 | DISCUSSION

4.1 | Survivorship and Recovery

Survivorship

Survival probability of *C. pustulosa* and *Fusconaia* sp. in the East Fork of the Trinity River was minimally affected by translocation in this study. For *C. pustulosa*, survival probability ranged from 93 to 100% across resident and translocated treatments ~ 8 months after translocation, which mirrors findings for this species in other studies. For example, Tsakiris et al., (2017) found that *C. pustulosa* survival in the San Saba River, located in central Texas, was 97% and 85% for resident and translocated treatments 2 years after translocation. Cope et al., (2003) found that *C. pustulosa* survival rates were ~80% after translocation in the St. Croix River, Minnesota. Survival probability of *Fusconaia* sp. was also high for resident and translocated treatments, 96 and 98% respectively. Dunn, Sietman, & Kelner (1999), found that *F. flava* in the upper Mississippi River had high survival rates ($\geq 94\%$) 2 years after translocation.

For the sites in the East Fork of the Trinity River, survival did not differ between sampling periods, which suggests that for *C. pustulosa* and *Fusconaia* sp. the time of year in which translocation activities occur may not impact translocation success if proper sampling and handling protocols are used. Previous researchers have suggested that time of year could be an important factor affecting mussel survival (Dunn, Sietman, & Kelner, 1999), which is based on the presumption that because mussels are ectotherms, extreme temperatures can cause sublethal and lethal impacts, such as reduced burrowing ability (Waller, Gutreuter, & Rach, 1999; Block, Gerald, & Levine, 2013) or exceedance of upper thermal limits, respectively (Pandolfo et al., 2010; Galbraith, Blakeslee, & Lellis, 2012; Sousa et al., 2018; Khan et al., 2019). During the study, the average air temperatures ranged from 10 to 17°C in the fall/winter sampling periods to 27°C in the summer sampling period, which could be lethal, but emersion time for processing mussels was limited to ~ 7 minutes. In addition, individual mussels were placed by hand back into the substrate.

The results for *C. petrina* and *L. bracteata* in the Llano River tell a much different story as survival probabilities were significantly affected by translocation for both species. For *C. petrina*, survival probability for the resident treatment was 100% ~2 years following translocation, whereas the translocation treatment was 83%. Habitat at Sites 1 and 2 in the Llano River were similar in terms of substrate, velocity, depth, and the residing mussel community structure. Tsakiris (2016) made similar observations, reporting decreased survival probability (~69%) of *C. petrina* in the San Saba River 2 years after translocation even though the resident and translocation site had similar substrate stability, water temperature, discharge, and particulate and benthic organic matter. These results and those of Tsakiris (2016) suggest that *C. petrina* does not respond well to translocation, which is likely due to its inability to acclimate to new locations (see discussion on Fulton's K).

For *L. bracteata*, survival probability for the resident treatment was high (91%) ~2 years after translocation, whereas that for the translocation treatment was low (22%). The ~9% decline

in survivorship starting in the summer for the resident treatment did not coincide with changes in body condition or predation but instead may have been related to stress from low flows and elevated temperatures combined with those from translocation activities. During the study, average air temperature ranged from 6 to 14°C in the fall/winter sampling periods to 31°C in the summer sampling periods. Flow exceedance probabilities were less during the fall/winter sampling periods (81 to 85% with a corresponding mean discharge of 2 to 2.5 m³ s⁻¹) compared to the summer sampling periods (94 and 98% and 0.8 and 1.5 m³ s⁻¹, respectively, based on 51 years of data from USGS [United States Geological Survey] stream gauge no. 08150700. The high summer air temperatures in conjunction with reduced flows resulted in marked increases to stream temperature (~31°C). Khan et al. (2019) evaluating the upper thermal limits for glochidia (larvae) of *L. bracteata* estimated an LT50 of 32 °C for the population at the upstream (Site 1) study site, which is near summer water temperatures. Because sublethal effects occur before lethality (Pörtner, 2002), it is likely that individuals of *L. bracteata* were already stressed due to elevated temperatures and low flows during summer sampling, which may partially explain mortality at the resident site during summer months.

Predation by *P. lotor* was responsible for mortality of 83% of recovered individuals of *L. bracteata* at the translocation site. Predated individuals showed marks from scratches, bites, and loss of shell, primarily near the posterior end. *Lampsilis bracteata* is thin-shelled and can be easily pried open. Based on these attributes along with the survival data, it appears that *P. lotor* was preferentially selecting *L. bracteata* compared to *C. petrina*, which is thick-shelled and closes tightly. Predation by *P. lotor* was intense during the summer sampling period as survivorship dropped by 40% during this time. Low flows during this period may have contributed to this by offering *P. lotor* better access to individuals in the study plot. Walters & Ford (2013) made a similar observation, noting high predation (73% of individuals collected) by *P. lotor* on *Potamilus amphichaenus*, Texas heelsplitter, a thin-shelled species endemic to east Texas, during a drought. Other studies have also reported predation of mussels during low flows by other terrestrial organisms. Sousa et al. (2018) observed high mortality for stranded *Margaritifera margaritifera*, pearl mussel, by *Sus scrofa*, wild boar, in several rivers in Portugal during an extreme summer drought of 2017. These examples, plus our findings, suggest that predation of freshwater mussels during low flows can be significant and could negatively affect local populations, to include those that are translocated.

Recovery

Recovery probability of *C. pustulosa* and *Fusconaia* sp. in the East Fork was high throughout the study, ranging from 85 to 92%, but did decline up to 16% following a series of floods between 2017-12 and 2018-05. The largest was ~405 m³ s⁻¹ with an exceedance probability of 17% based on 24 years of data from USGS [United States Geological Survey] stream gauge no. 08062000. Survivorship between these sampling periods remained high (>93%) across species and treatments, which is likely due to the physical structure of both sites. Generally, mussels occur in areas along the stream bottom where the potential for bed movement is low during high flows

(Gangloff & Feminella, 2007; Stoeckl & Geist 2016; Randklev et al., 2019). The study sites in the East Fork occurred at riffles characterized by sand substrate overlaid with an armoring layer of gravel and cobble. This combination of substrates likely allowed individuals to reburrow between monitoring events while simultaneously offering protection from scour and entrainment during floods.

Recovery probability of *C. petrina* and *L. bracteata* in Llano River was high for resident treatments (100%) up until a flood of $\sim 3,766 \text{ m}^3 \text{ s}^{-1}$ with an exceedance probability of 0% based on 51 years of data from USGS [United States Geological Survey] stream gauge no. 08150700 reduced it to 0%. Extreme floods like one that occurred during this study can have profound impacts on available habitat and channel form. For example, at the upstream site a complete flushing of all soft sediment occurred following the flood of $\sim 3,766 \text{ m}^3 \text{ s}^{-1}$. Prior to the flood, fine sediment occurred between bedrock crevices and interstitial spaces of large cobble and boulders, which provided substrate for mussels to burrow. At the downstream site, the flood deposited a large quantity of gravel and cobble completely burying the site and restructuring the river channel. Given these observed changes to habitat and channel form, it is likely this flood resulted in downstream dispersal and elevated mortality for mussels at these sites. Zajac et al. (2019) examining the effects of translocation on *Unio crassus*, thick-shelled river mussel, in the River Biała in Poland observed marked individuals being transported $> 100 \text{ m}$ downstream from their original location following a flood of $45.1 \text{ m}^3 \text{ s}^{-1}$. The authors did not report the exceedance probability for this flood event, so its magnitude and frequency are unknown, but the individuals swept downstream were alive when recovered. In contrast, Hastie et al. (2001) estimated that at least 50,000 individuals of *Margaritifera margaritifera*, pearl mussel, were killed in the River Kerry, Scotland, following a 100-year flood, which caused significant channel reformation. These examples, plus our findings, demonstrate that flooding can negatively impact local populations, and significant flooding can lead to loss of mussel habitat and complete extirpation of local populations through a combination of dislodgment, burial and stranding.

4.2 | Fulton's K body condition index and total glycogen and lipids

Body condition, which is a measure of stress, appeared to be responsive to translocation. For *C. pustulosa*, body condition was lower for the translocation treatment one month after being moved, indicating that the species may have experienced sublethal affects. A similar pattern was observed for *C. pustulosa* in total lipid composition. It is likely these changes are because mussels have priority rules for energy allocation (Jokela & Mutikainen, 1995) and can shift energy investment to reproduction or maintenance when stressed. For *Fusconaia* sp. body condition increased over time and remained similar between resident and translocated treatments. Total glycogen and lipids followed a similar pattern, however translocated individuals had greater glycogen and lipid concentrations than the resident treatment during this first month following translocation. The initial increase in total glycogen and lipids for *Fusconaia* sp. suggests that individuals may have been reallocating energy to reproduction (i.e., production of egg and sperm) as a stress response while they were acclimating to their new site.

Body condition of *C. petrina* was lower for the translocation treatment and declined over time compared to the resident, which mirrors the findings for survivorship. This indicates that translocated individuals were unable to acclimate to their receiving site even though it was occupied by this species and had similar environmental conditions to the resident site. Jokela & Mutikainen (1995) observed that translocated mussels often shifted energy from growth and reproduction to maintenance during periods of elevated mortality, which mirrors the findings from this study. Tsakiris (2016) made a similar observation for *C. petrina* from the San Saba River, an adjacent river system to the Llano. For *L. bracteata*, body condition was similar between translocated and resident treatments except for during the 2018-03 sampling period, wherein the resident treatment had higher condition.

4.3 | Implications for conservation

Tsakiris et al. (2017) in reviewing factors that contribute to translocation success argued that site selection, handling protocols (e.g., collecting, processing, and transporting) and potentially time of year of translocation activities are important considerations that could affect translocation success. The results from this study confirm these factors are important, and that species-specific differences, time of year, large stochastic events such as extreme floods, and occurrence of predators should be considered when planning translocations. Stodola, Stodola, & Tiemann (2017) evaluating translocation success of *Pleurobema clava*, clubshell, and *Epioblasma rangiana*, northern riffleshell, in the Salt Fork and Middle Fork Vermilion rivers came to a similar conclusion and further argued that some species are inherently difficult to translocate due to life history differences. The authors suggested for these species that larger numbers of individuals or repeated translocations may be needed to overcome high mortality. The findings for *C. petrina* and those of Tsakiris (2016) indicate such an approach could have negative consequences because life history traits have evolved in response to specific constraints over long time scales. Thus, stocking more individuals of species whose life history traits (e.g., acclimation) are incompatible with translocation is likely to lead to more mortality not less. In addition to these limitations there is also very little information on how juveniles or subadults respond to translocation. This is problematic, because under scenarios where mussels are moved to avoid acute or chronic impacts, one would presume that multiple sizes classes will be translocated. Thus, lack of survivorship information for smaller individuals in general will likely undermine such efforts. Lastly, translocations, at least in the United States, continue to be guided by local or regional protocols, that vary in level of detail and guidance, which makes it difficult to compare studies or to identify best management practices and protocols. The IUCN (International Union for Conservation of Nature, 2013) developed guidelines to address many of these issues. This document sets forth a conceptual framework to help with determining when translocation is appropriate, and if so, processes that should be considered before implementing a translocation. The IUCN framework can be easily tailored to individual needs while at the same time providing standardization that is needed in order to make meaningful comparisons across studies. We predict translocation will continue to be used in species management, and it should in cases where extirpation or extinction

is likely, but the findings of this study and those of others (e.g., Tsakiris 2016; Tsakiris et al., 2017) also demonstrate that managers should be cautious and use appropriate planning made on a case-by-case basis.

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