A Comparative Study of the Population Dynamics of Zebra Mussels (*Dreissena polymorpha*) in Two Closely Adjacent Central Texas Water Bodies

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Prepared by Jason L. Locklin, Department of Biology, Temple College, Temple, Texas, for the Texas Parks and Wildlife Department

Executive Summary

The population dynamics and physiological conditions of zebra mussels (*Dreissena polymorpha*) were studied in two closely adjacent central Texas lakes, Belton Lake (BL) and Stillhouse Hollow Lake (SHL). The lake outlets are 12.2 miles apart and overlay Edwards Formation Cretaceous limestone. Mussels were sampled monthly from settlement plates deployed at 2 and 8 m depth at a marina on each lake from 03/05/2021 to 09/02/2022 as well as bimonthly from 2 m depth at 4 buoy sites in each lake. Physical-chemical parameters were recorded at each marina from 1 m to 11 meters depth at 1 meter intervals and 2 m at buoy sites. Measured parameters included water temperature, conductivity, pH, % of air O_2 saturation, Ca^{2+} and chlorophyll *a* concentrations and Secchi depths. Mussel shell lengths and densities were determined monthly at 2 and 8 m depths at the marinas and every two months at buoy sites. Sampling across 1-11 m depths at marina sites indicated no significant differences in lake mean temperature and % of air O_2 saturation while conductivity and pH were significantly elevated in SHL and chlorophyll *a* concentrations at BL. All measured physical-chemical characteristics fell within limits required for sustainable mussel populations.

At marina sites, monthly determinations of mussel shell length (SL) distributions indicated presence of Spring- and Fall-2021 Cohorts in the initial 2 and 8 m samples at BL and a combined

Spring- and Fall-2021 Cohort at both depths in SHL. In early June 2022, a recently settled Spring-2022 Cohort was detected at both depths in each lake. By September 2022, the LB Spring- and Fall- 2021 Cohorts no longer occurred in samples at 2 m depth while only the Fall-2021 Cohort persisted at a very low density in 8 m. At 2 m depth in SHL, the combined Spring- and Fall-2021 Cohort persisted in very low density in the final September 2022 sample and was not present at 8 m. These results suggested that the life span of spring and fall mussel cohorts was approximately one year or less at both depths in both lakes as is reported to occur in other infested Texas water bodies. Densities of mussels at marina and buoy sites tended to remain elevated during early summer months due to settlement of Spring-2022 juvenile mussels, but declined rapidly from July through September 2022, suggesting that high summer water temperatures negatively impact mussel survival rates, particularly among older, larger individuals subject to high temperature induced starvation.

The shell growth rates of Spring-2022 Cohorts were similar across lakes, being 84.5 and 50.8 µm/day and 88.9 and 60.01 µm/day in BL and SHL at 2 and 8 m depths, respectfully. The 2 m depth shell growth rates were similar to those estimated for mussels infesting other Texas water bodies and among the highest recorded for *Dreissena polymorpha* populations in Europe and North America. The decline in shell growth rate recorded at 8 m depth at both study sites suggests that mussel growth rate studies need to occur across a number of depths to develop a better understanding of mussel shell growth dynamics. In addition, multi-year studies as have been conducted at BL will provide an improved understanding the potential long-term impacts of mussels in Texas water bodies leading to development of efficacious, long-term strategies for mussel fouling prevention and control procedures in Texas water bodies and raw water using utilities and infrastructure.

Introduction

Zebra mussel populations in southwestern U.S. water bodies have been shown to have elevated growth rates, shortened life spans, and large-scale summer die-off events (Boeckman and Bidwell, 2014; Churchill et al., 2017, Locklin et al, 2020, Arterburn and McMahon, 2022) relative to populations at higher latitudes. Boeckman and Bidwell (2014) reported significant summer population mortality in Oologah Lake, OK, in 2006 and 2007, where surface water temperatures frequently reached or exceeded 30°C. Similar results were recorded for zebra mussel populations in Texas Lakes Belton, Ray Roberts and Texoma from 2011-2017 by Arterburn and McMahon (2022). However, these studies indicated that temperature was not the sole contributor to summer die-offs. Boeckman and Bidwell (2014) suggested that a confluence of factors may cause summer mussel die-offs, including high nitrate concentrations, low food availability, and low oxygen concentrations rather than temperature alone. Morse (2009) found that zebra mussels in Winfield City Lake in southern Kansas to have a higher chronic thermal tolerance than those from Hedges Lake, NY, indicative of mussels in warm southwestern water bodies having evolved elevated thermal tolerance levels. He also found that the tissue dry weights of larger, adult mussels substantially declined during warm summer months suggesting that indirect variables associated with increased water temperatures may be associated with summer adult die-offs.

A primary indirect effect of exposure to increased temperatures is increased metabolic demand. With temperature increasing from 20 to 32°C, zebra mussel oxygen consumption rates can quadruple and metabolic rates increase by as much as 265% (Aldridge et al., 1995). Feeding efficiency also declines with increasing temperatures. Studies of filtration rates of zebra mussels in Lake Huron found maximum filtration rates to occur at 20°C (Fanslow et al., 1995). Walz (1978) found that adult zebra mussel metabolic demands exceeded their energy absorption rates regardless of available food at ≥20°C. Based on a growth model incorporating weight and temperature considerations, Schneider (1992) suggested that adult mussel assimilation rates could not support metabolic demands above 24°C regardless of food availability, leading to a negative scope for growth. Under these conditions, Nalepa et al. (1993) observed declines in zebra mussel tissue mass of up to 60% between spring and fall. Similarly, Morse (2009) found that adult zebra mussels in Winfield City Lake, Kansas, lost as much as 53% of their spring peak dry tissue mass by the subsequent fall. Additionally, this negative scope for growth at elevated temperatures appears to have a disproportionately adverse effect on larger mussels (Aldridge et al., 1995; Morse, 2009). While some studies have attributed spring-to-fall weight loss to spawning events or limited food resources, both Morse (2009) and Nalepa et al. (1993) found the summer loss of tissue mass in zebra mussels to be too large in higher temperature ranges than could be attributable to reproduction alone. Bij de Vaate (1991) and Jantz and Neumann (1998) have reported similar negative correlations between zebra mussel shell length and dry tissue weight at temperatures >25 7C.

Long-term studies have also indicated that Texas zebra mussel populations tend to decline in density and shell growth rates over time after initial infestation as has been recorded in Lakes Texoma (infested during 2009), Ray Roberts (infested during 2012) and Belton (infested in 2013) (Arterburn and McMahon, 2022). The basis of these temporal declines has not been determined. The Lake Belton population has undergone an extensive reduction in density after its initial invasion in 2013 (Arterburn and McMahon, 2022). Collecting environmental data and sampling populations throughout the year to estimate shell growth rates, densities, and corresponding physiological condition from a variety of mussel infested sites could provide a predictive tool for assessing zebra mussel invasions (Hallidayschult et al., 2021) and allow determination if a reduced physiological condition is responsible for temporal summer declines observed in Texas zebra mussel populations. Such results will specifically allow prediction of the extent to which zebra mussel infestations are likely to impact Texas water bodies and associated water using facilities in the long term.

The purpose of this study was to determine the impacts of elevated temperatures on mussel shell growth rates and body physiological condition measured as dry tissue mass relative to shell length in zebra mussel populations inhabiting two closely adjacent and similar water bodies in central Texas, Belton and Stillhouse Hollow Lakes, whose outlets are only 12.2 miles apart and which have similar geological features with both overlying resistant Cretaceous limestone of the Edwards Formation with extensive rocky shores. The two reservoirs have similar water chemistry (Brazos River Authority of Texas, 1989) and temperature regimes (Locklin, unpublished). Furthermore, this study seeks to better understand the spatial population dynamics of these two mussel populations (i.e., density distribution, growth rates, length frequency distribution, and settlement dynamics) and physiological condition associated with food availability (chlorophyll *a* concentration) and physicochemical data (temperature, depth, dissolved oxygen, pH, conductivity and Secchi depth) across time. Such information will help to inform appropriate zebra mussel management and mitigation strategies in Texas and other subtropical North American lakes in both the short and long terms.

Materials and Methods

Sampling Sites

Our study was conducted on two U.S. Corps of Engineers reservoirs in central Texas, USA, Belton (31.130394°N −97.508359°W) and Stillhouse Hollow (31.020666°N -97.525164°W) Lakes. Belton and Stillhouse Hollow Lakes are impoundments of the Leon and Lampasas Rivers, respectively, which are tributaries of the Little River which flows into the Brazos River.

Belton Lake is located approximately 8 km northwest of the City of Belton and serves as source water for local municipalities, hosts multiple recreational attractions, and is a popular site for recreational fishing and boating (Texas Water Development Board 2017). Belton Lake is considered mesotrophic with water clarity averaging 1.8 m (Tibbs and Baird 2019). Zebra mussels were first reported in the lake during 2013 (Texas Parks and Wild Life Department 2013). The five sampling sites were Frank's Marina, Arrowhead Park, Belton Lake Outdoor Recreation Area (BLORA), Temple Lake Park, and Roger's Park (Figure 1).

Stillhouse Hollow Lake is located approximately 8 km southwest of the City of Belton and serves for flood control, recreation, and municipal water supply (Tibbs and Baird 2019). Stillhouse Hollow Lake is considered an oligotrophic lake with sparse native aquatic plants but with invasive *Hydrilla* occupying about half of the reservoir in 2017/2018 (Tibbs and Baird 2019). Zebra mussels were first reported in Stillhouse Hollow Lake in 2016 (Texas Parks and Wildlife Department 2016). The five sampling sites were Stillhouse Hollow Marina, the Lake Spillway area, Dana Peak Park, Union Grove Park, and Cedar Gap Park (Figure 2).

The sampling equipment (described below) was attached to buoys near boat ramps at all sites except the marinas. At both marinas, the equipment was attached to chains that were suspended from the marina's platform to depths of interest in the water column.

Physicochemical parameters and chlorophyll a

Water temperature and dissolved oxygen (DO) were recorded hourly at each buoy site (2 m depth) and at marina sites (2 m and 8 m depths) with submersible data loggers (Onset U26-001, Onset Computer Corporation, Bourne, MA, USA). Data loggers were deployed on 27 March 2021 at the marinas and on 26 October 2021 at the buoy sites. The hourly logging of temperature and DO occurred until 1 September 2022 at all sites. The 8 m data logger at Frank's Marina was lost in February 2022 and replaced on 9 March 2022; the logger on the BLORA buoy (Belton Lake) was lost during April 2022 but not replaced.

Additionally, vertical profiles of water temperature, dissolved oxygen, conductivity, pH, and chlorophyll *a* were measured at least biweekly with a DS5X Hydrolab at 1 m intervals at both marinas, and at the depths (2 m) where zebra mussel sampler plates were located at the buoy sites. These biweekly measurements occurred from 22 November 2021 – 2 Sept 2022.

Calcium concentrations were determined from water samples taken at the marina and buoy sites in both lakes on 08/19/2022, 08/26/2022 and 09/02/2022. Calcium concentrations were determined in the laboratory with a Hach model ZHAC-DT hardness (total and calcium) test kit and digital titrator (Loveland, CO).

Secchi Disk depths were determined bimonthly at the marina sites in both lakes from 04/29/2021 to 09/02/2022 (34 samples) and from buoy sites from 10/22/2021 to 09/02/2022 (23 Samples).

Zebra mussel collection

At each site, 20X20 cm zebra mussel sampler plates attached to steel chains were used to assess zebra mussel settlement, density, and growth. Sampler plates were deployed at the marinas on 5 March 2021 and at the buoys on 2 November 2021. At each marina site, 12 plates were attached to steel chains at depths of 2 m and 8 m (total of 24 plates per chain). At each buoy site, 10 plates were suspended 2 m below the buoy on a chain. At each marina site, one plate from each set of 12 was removed from the lake monthly; at buoy sites, two plates from each set of 10 were removed bimonthly. In the field, attached zebra mussels were scraped from the plates and transported to the laboratory in 70% ethanol. In the laboratory, mussels were counted, frozen for at least 24 hours (for mussel condition analysis described below), and scanned on a flatbed scanner at 600 dpi for shell length (SL) measurement to the nearest 0.01 mm (greatest linear distance from the anterior umbo to the posterior shell margin). This procedure provided temporal cohort distributions, densities, juvenile mussel settlement dynamics, and the mean shell lengths to be used to determine shell growth rates during the study.

Mussel condition

A target sample size of 50 zebra mussels >10 mm in size from 2m and 8m depths (N=100) at both marinas sites were collected monthly to assess population body condition. Mussels of this minimum size were also removed from the buoys on 6 July 2021, 1 July 2022, and 2 Sept 2022 to compare mussel conditions spatially and temporally. The preserved mussels were scanned on a flatbed scanner for shell length determinations (as described previously) and placed in a -4C freezer for at least 24 h to facilitate soft tissue removal. To remove the soft tissue, shells were pried open using a pair of fine-point forceps and the soft tissue was extracted with forceps. Freezing the mussels prior to soft tissue extraction helped the tissue remain intact for easy removal from the shell. The extracted soft tissue of each mussel was placed in a pre-weighed aluminum pan, dried to a constant weight at 60°C for at least 48 h, and weighed to the nearest 0.10 mg. The individual dry tissue weight (DTW) was fitted to an exponential regression as the dependent variable against individual shell length the independent variable allowing the estimation of mean DTWs of individuals with standard shell lengths within the range of mussel shell lengths in each sample.

Results

Statistical Testing

For statistical testing of results a probability ≤0.05 was considered an indication of significant difference.

Lake Physical-Chemical Characteristics

Mean daily water temperatures taken at 2 and 8 meters depth at Frank's Marina on Belton Lake and Stillhouse Hollow Marina on Stillhouse Hollow Lake revealed annual water temperature variation in the two lakes to be very similar with little difference between depths indicating a lack of stratification during summer months (Figure 3). Daily mean maximum summer lake temperatures at the marinas did not attain the 32°C long-term upper lethal limit of zebra mussels in warm southwestern water bodies (Morse 2009). However, hourly recordings in each lake at various sites, including the marinas, did periodically exceed this limit, but not for long enough (29 days, Morse 2009) to induce mussel mortality (Table 1). Water temperatures at the marinas fell below the lower limit for spawning of 18°C (McMahon and Arterburn, 2022) during winter months in the Belton and Stillhouse Hollow marinas at 2 m depth from 11/27/2021 to 04/17/2022 and 11/26/2021 to 04/04/2022 (Figure 3A), respectively, and at 8 m depth from 11/27/2021 to 04/23/2022 and 11/26/2021 to 04/09/2022 (Figure 3B), respectively.

Hydrolab measurements taken every two weeks at marina sites from 11/21/2021 to 09/02/2022 of water temperature, conductivity, pH, percent O_2 of full air saturation, and chlorophyll *a* concentration recorded at depths of one 1 to 11 m in 1 m intervals (Figures 4A-E) revealed similarities and differences in the physicochemical makeup of the two lakes. Paired T-Tests assuming unequal variances were used to detect significant differences ($p < 0.05$) in the tested variables across all sampling dates. No significant differences between mean water temperatures among the two marinas at any depth were detected (Figure 4A). In contrast, Stillhouse Hollow Lake had significantly elevated conductivities compared to Belton Lake across all 11 depths. Mean conductivities ranged from 394 µS/cm at 5 m to 403 µS/cm at 8 m in Belton Lake and from 476 µS/cm at 6 m to 481 µS/cm at 10 m in Stillhouse Hollow Lake (Figure 4B). At Belton and Stillhouse Hollow Lakes, pH ranged from 8.10 at 11 m to 8.32 at 2 m and 8.25 at 11 m to 8.48 at 3 m, respectively, with Stillhouse Hollow Lake having significantly higher pH levels than Belton Lake at depths of 1, 2, 3, 5, 7, 10 and 11 m (Figure 4C). Mean percent of full air O_2 saturation was not different across depths between the lakes with the exception of being significantly greater at 8 m depth in Stillhouse Hollow Lake. Mean percent of full air O_2 saturation ranged from 78.02% at 11 m to 104.83% at 1 m in Belton Lake, and in Stillhouse Hollow Lake, from 91.87% at 11 m to 109.00% at 2 m (Figure 4D). Mean chlorophyll *a* concentrations across depths at the marina in Belton Lake marina ranged from 4.55-5.74 µg/l and were significantly higher at all depths than at Stillhouse Hollow Lake were mean chlorophyll *a* concentrations ranged from 1.49-2.14 µg/l (Fig. 4E).

Physicochemical data for water temperature, conductivity, pH, percent of full air O_2 saturation, and chlorophyll *a* concentration recorded at a depth of 2 m from four buoy sites in each lake (34 samples from each lake from 06/07/2021 to 09/02/2022) (Table 2) showed levels similar to those recorded at the two respective marina sites. Mean water temperatures and pH were similar among the two lakes while mean conductivity was greater in Stillhouse Hollow Lake and mean chlorophyll *a* concentration greater in Belton Lake reflecting similar significant differences in these values recorded at the two marina sites. Oxygen concentrations were also recorded at the buoy sites at 2 m depth. The annual mean % of full air O_2 saturation values and standard deviations for Belton Lake sites were; Arrowhead Park 102.07% ±20.15, Temple Lake Park 98.42% ±26.58, Rogers Park, 105.48% ±22.10, and Belton Lake Outdoor Recreation Area 102.17% ±16.76. For Stillhouse Hollow Lake they were; Lake Spillway 103.29% ±13.14, Dana Peak Park 106.75% ±16.49, Union Grove Park 104.04% ±15.04 and Cedar Gap Park 97.43% ±17.70. The maximum % of % of full air O_2 saturation value recorded was 117.52% at the Belton Lake Outdoor Recreation Area buoy on 08/19/2022, and the minimum of 8.40%, at the Temple Lake Park buoy on 07/01/2022. A T-test assuming unequal variances indicated no significant difference in the mean % of full air O_2 saturation values between the two lakes. Lake mean calcium concentrations (n=3) tended to be relatively similar in the two lakes ranging from a low value of 44.72 Ca^{2+}/L (±1.22) at Rogers Park in Belton Lake to 52.59 Ca^{2+}/L (±1.61) at Cedar Gap Park in Stillhouse Hollow Lake. One-way Analysis of Variance with *Post-Hoc* pair wise T-tests revealed significant differences between sites with Belton Lake sites tending to have lower calcium concentrations than Stillhouse Hollow Lake sites (Table 3). Secchi Disk depths were significantly different between the two marina sites. Mean Secchi Disk depth at Frank's Marina on Belton Lake was 1.774 m (±0.772) and at Stillhouse Hollow Marina on Stillhouse Hollow Lake, 5.363 m (±1.528). Mean Secchi Disk depths were also similar among the four buoy sites within each lake. Belton Lake ranged from 0.995 m (±0.351) at the Belton Lake Outdoor Recreation Area Site to 1.616 m (±0.412) at the Arrowhead Park site. At Stillhouse Hollow Lake, they ranged from 1.541 m

(±0.704) to 4.441 m (±1.255) at the Cedar Gap Park and Stillhouse Hollow Lake Spillway sites, respectively. A T-Test assuming unequal variances indicated that the mean Secchi Disk value (n = 4) for the Belton Lake buoy sites at 1.362 m (±0.267) was not significantly different from that of the Stillhouse Hollow Lake buoy sites at 3.145 m (±1.20) due to the greater variation in mean Secchi Depths recorded at its sites.

Mussel Cohorts and Growth Rates

Measurements of shell length (SL) distributions of mussel samples drawn monthly from settlement plates held at depths of 2 and 8 m depth at the Belton Lake Marina site revealed the presence of three distinct cohorts. At 2 m and 8 m depths, Spring-2021 and Fall-2021 cohorts were present at low densities in the initial 11/06/2021 sample. The Fall-2021 cohort attained maximum densities on 03/03/2022 and 04/07/2022, at 2 and 8 m depths, respectfully. The Spring-2021 cohort was last recorded on 06/03/2022, and the Fall-2021 cohort on 07/01/2022. The presence of a Spring-2022 cohort was initially recorded in the 06/03/2022 sample at 2 and 8 m depth and remained present until sampling ended on 09/02/2022 (Figures 5A, 6A and 7). Maximum settlement densities for Belton Lake Spring-2022 cohorts at 2 m and 8 m depth occurred at their initial settlement in 06/02/2022. Thereafter, this cohort's densities continued to decline until the last sample was taken on 09/02/2022 (Figures 5A, 6A, and 7)

Mussel SL distributions at depths of 2 and 8 m at Stillhouse Hollow Marina revealed the presence of two distinct cohorts. At 2 m and 8 m depths, separate Spring-2021 and Fall-2021 Cohorts could not be determined because their shell length distributions extensively overlapped so they were combined into a single Spring-Fall-2021 Cohort that was present in high numbers at both depths in the initial 11/06/2021 samples (Figures 5B, 6B and 7). At 2 m depth, this cohort occurred in samples throughout the sampling period (Figure 5B) while, at 8 m depth, it was last recorded on 08/06/2022 (Figure 5B). The presence of a Spring-2022 Cohort at 2 and 8 m depth was initially recorded on 06/03/2022 with the cohort still present when sampling ceased on 09/02/2022 (Figures 5B, 6B and 7). At 2m depth, the combined Spring-Fall- 2021 Cohort had peak numbers in the initial 11/06/2021 sample, while at 8 m depth maximum numbers were recorded on the following 12/02/2021 sampling period. At both depths, densities of the Spring-Fall-2021 Cohort remained high through 07/01/2022, and thereafter, declined rapidly to very low numbers by the last 09/02/2022 sampling date at 2 m depth and were not present at 8 m depth (Figures 5B, 6B and 7). At 8 m depth the density of the Spring-2022 Cohort was maximal on 08/06/2001 and greatly declined in the subsequent final Sample on 09/02/2022.

Growing season shell growth rates were determined for the Spring-2022 Cohorts at 2 m and 8 m depths at the Belton Lake and Stillhouse Hollow Lake marinas. Mussel shell growth rates were determined as the final 09/02/2022 sample mean shell length in µm divided by the 119 day interval between 05/06/2022, the last day when Spring-2022 Cohorts were not present in the samples, and their mean shell lengths in the final 09/02/2022 samples. Based on these calculations, the growing season growth rates of Belton Lake mussels were 84.5 and 50.8 µm/day at 2 m and 8 m depths, respectfully, while those for Stillhouse Hollow Lake mussels at 2 m and 8 m depths were 88.6 and 61.1 µm/day, respectfully.

Densities

At the Belton Lake marina site, mussel densities were relatively similar at 2 m and 8 m depths. Spring-2021 Cohort density at 2 m depth peaked on 03/03/2022 at 386 mussels/m^2 declining to 0/m^2 on 07/01/2022. The Fall-2021 Cohort was initially observed on 12/02/2021 at 12/m^2, peaking at 11,642/m^2 on 03/03/2022, and declining to 0/m^2 on 08/06/2022. At 2 m depth, the Spring-2022 Cohort initially settled at a peak density of 110,921/m^2, thereafter falling to 18/m^2 in the final 09/02/2022 sample (Figure 7A). At 8 m depth, Spring-2021 Cohort density on 11/06/2021 was 37 mussels/m^2, declining to 0/m^2 on 07/01/2022. The Fall-2021 Cohort was initially observed on 11/06/2021, peaking at 6,562/m^2 on 03/03/2022 and declining to 0/m^2 on 08/06/2022. At 8 m depth, the Spring-2022 Cohort initially settled at a peak density of 121,565/m^2 on 06/03/2022, thereafter declining to 18/m^2 in the final 09/02/2022 sample (Figure 7B).

At the Stillhouse Hollow Lake marina site, mussel densities were also relatively similar at 2 m and 8 m depths. Combined Spring-Fall-2021 Cohort density at 2 m depth was maximal on the initial 11/06/2021 sample at 64,457 mussels/m^2 declining to 49,259/m^2 on 06/03/2022 and to 117/m^2 by the final 09/02/2022 sample. At 2 m depth, the Spring-2022 Cohort initially settled at a peak density of 28,355/m^2 thereafter falling to 2,683/m^2 in the final 09/02/2022 sample (Figure 7C). At 8 m depth, the Combined Spring-Fall Cohort attained a peak density of 68,054/m^2 on 12/02/2021. Its density declined from 51,533/m^2 to 0/m^2 between 05/06/2022 and the last sample date of 09/02/2022 (Figure 7D). The Spring-2022 Cohort initially settled at a peak density of 13,662/m^2 on 06/03/2022, thereafter increasing to 14,682/m^2 in the final 09/02/2022 sample (Figure 7D).

Overall, density dynamics of the Belton Lake Spring 2021 and Fall 2021 Cohorts and the Stillhouse Hollow Lake Combined Spring-Fall Cohort were similar with densities remaining relatively high through 06/03/2022 followed by steep density declines so that they had been lost from the final 09/02/2022 samples (at 2 and 8 m depths for the Belton Lake Spring- and Fall-2021 Cohorts and for Stillhouse Hollow Lake Combined Spring-Fall Cohorts at 8 m depth) or declining to very low densities (Stillhouse Hollow Lake at 2 m depth) (Figures 7A-D).

Overall mussel densities measured bimonthly at the four buoy sampling sites in each lake peaked on 07/01/2022 during the initial major settlement of the Spring-2022 Cohort with densities varying extensively between sites (Figures 8A and B). Densities at all sites extensively declined by the final 09/02/2022 sample (Figures 8A and B) as also occurred in the two lakes' marina sampling sites (Figures 7A-D). The exception was Union Grove Park at SHL where settlement by the Spring-2022 Cohort caused mussel density to increase from 726/m^2 on 07/01/2022 to 23,300/m^2 in the final 09/02/2022 sample (Fig 8B).

Linear Regression Analysis revealed no significant correlation in either lake between kilometer distances of sampling sites (e.g., buoy and marina sites) from the lakes' outlets (independent variable) and peak overall mussel densities on 07/01/2022.

Mussel Physiological Condition

Dry tissue weight (mg) versus shell length (mm) data fitted to exponential regressions with shell length as the independent variable and dry tissue weight as the dependent variable for zebra mussels collected from settlement plates held at 2 and 8 m depth at the Belton Lake and Stillhouse Hollow Lake marina sites (Figures 9A-L and 10A-L) revealed a tendency for larger individuals from Belton Lake to have elevated tissue dry weights compared to mussels of the same shell length from Stillhouse Hollow Lake during spring and early summer months (i.e., 03/03/2022, 04/07/2022, and 05/06/2022). Dry tissue weights of zebra mussels from 2 and 8 m depth with standard shell lengths of 10, 15, 20, 25, and 30 mm were estimated at each sampling date from exponential regression equations versus shell length as the independent variable. This analysis indicated that Belton Lake mussels with shell lengths of 20, 25 and 30 mm at depths of 2 m and 8 m appeared to have greater dry tissue weights than those in Stillhouse Hollow Lake. In addition, mussels of 20, 25, and 30 mm shell length sampled from 2 m depth appeared to have considerably higher dry tissue weights than those sampled from 8 m depth (Figures 11A and B).

Discussion

The physicochemical parameters of the two lakes fell well with the known requirements for establishment of sustainable zebra mussel populations. Throughout the course of sampling, water temperatures at 1-11 meters generally remained well below the long-term, 28-day, upper thermal limit of zebra mussels in southwestern United States water bodies of 32°C (Morse 2009) similar to temperature regimes in other Texas water bodies with sustainable zebra mussel infestations (Arterburn and McMahon 2022, Schwalb et al 2023) including Belton Lake (Locklin et al., 2020, Arterburn and McMahon 2022, Schwalb et al. 2023). Conductivities were somewhat higher at Stillhouse Hollow Lake which correlated with the lake's higher Ca^{2+} concentrations. Mean calcium concentrations across the lakes ranged from 44.72 to 52.59 mg Ca^{2+}/L, falling well above the generally accepted lower limit of 12 mg Ca^{2+}/L for maintenance of a sustainable zebra mussel population (Jones and Riccardi, 2005). While pH tended to be significantly higher at Stillhouse Hollow Reservoir, pH in both lakes was well above the lower pH limit of 7.2 required for successful development of zebra mussel veliger larvae to settled juveniles (Sprung 1987, Claudi et al., 2012). Mean Secchi Disk depths in Stillhouse Hollow Lake were up to three times greater than those recorded in Belton Lake which was correlated with its 3-fold lower Chlorophyll *a* concentrations. Overall, the physicochemical parameters of both lakes fell well within the limits for supporting a sustainable zebra mussel population. Oxygen concentrations at marina and buoy sites were generally above the long-term lower limit of 25% of full air O_2 saturation (Johnson and McMahon 1998).

Zebra mussels at both marina sites displayed one-year life cycles with initial settlement of the Spring-2022 cohorts at 2 and 8 m depth first recorded on 06/03/2022 after which they remained present through project termination on 09/02/2022. At Belton Lake, members of the Spring-2021 and Fall-2021 cohorts and at Stillhouse Hollow Lake the combined Spring-Fall-2021 Cohort were present at depths of 2 and 8 m in the initial 11/06/2021 samples. At Lake Belton, the Spring- and Fall-2021 Cohorts no longer occurred in the 2 m depth samples on 07/01/2022 and 08/06/2022, respectively. At 8 m depth, the Spring-2021 Cohort also was no longer present by 07/01/2022 while the Fall-2021 Cohort remained present in very low numbers (n = 4) by the final

09/02/2022 sample. Similarly, the combined Spring-Fall Cohort at Stillhouse Hollow Lake no longer occurred at either depth in the final 09/02/2022 sample.

These results indicated that zebra mussels in both lakes at both depths had one-year life cycles with juveniles initially settling in June and not dying out until June-September of the following year. Similar approximately one-year life spans have been reported for zebra mussels in Lake Oologah, Oklahoma (Boeckman and Bidwell 2014) and in Texas at Texoma, Ray Roberts and Belton Lakes (Locklin et al. 2020, Arterburn and McMahon 2022). In contrast, mussel life spans have been reported to range from 3 to 5 years in British and Polish populations and to 9 years in Russian populations (Chase and Bailey 1999), while in the northern areas of the United States, mussel cohort life spans have been reported to range from 1.5 to 4 years (Mackie 1993, Chase and Bailey 1999, Garton and Johnson 2000). As has been suggested by Arterburn and McMahon (2022), summer high temperature-induced mortality in second year larger mussels in warm Texas water bodies may be a result of metabolic stress. Zebra mussel metabolic efficiency declines at >20°C (Walz 1978, Dorgelo and Kraak 1993). It has been reported that mussels exposed to temperatures increasing from 20°C to 32°C experienced a near quadrupling of oxygen consumption rates, a 265% increase in metabolic rate, and a 73% decline in feeding rate (Aldridge et al. 1995). In Winfield City Lake, KS, large 20–30-mm SL mussels during warm summer months at water temperature approaching 30°C, lost 44%–53% of their peak spring dry tissue mass. Such tissue loss is indicative of negative energy balance. In contrast, smaller 5–15-mm SL mussels lost only 0%–34% of peak spring dry tissue mass (Morse 2009). This result suggests that larger second-season mussels are more susceptible to the lethal impacts of elevated summer temperatures than smaller first season mussels. Similar, extensive summer dry tissue weight loss in occurred in larger mussels (i.e., 20, 25 and 30 mm SL) at 2 and 8 m depth in Belton and Stillhouse Hollow lakes, suggesting that elevated summer temperatures induced starvation in larger mussels leading to their extirpation from the samples from July through September 2022.

Mussel growing season shell growth rates at 2 and 8 m depths at Lake Belton were 84.5 and 50.8 µm/day, respectively, and at Stillhouse Hollow Lake, 88.6 and 61.1 µm/day, respectively. The shell growth rates recorded at 2 m depth in both lakes were similar to those reported for mussels in near surface waters in other Texas water bodies including 84 µm/day in Lake Texoma and 89 µm/day in Ray Roberts Lake (Arterburn and McMahon 2022). Churchill et al. (2017) reported a zebra mussel shell growth rate of 121 µm/day in Lake Texoma. Over a four-year study (2014-2017), Arterburn and McMahon (2022) recorded and average zebra mussel shell growth rate at 2 m depth of 87 µm/day in Belton Lake and Locklin et al. (2020), 128 µm/day. These shell growth rates are among the highest ever reported for zebra mussels (Churchill et al. 2017, Locklin et al. 2020, Arterburn and McMahon 2022). The shell growth rates of Belton Lake mussels recorded in this study were similar to those recorded by Arterburn and McMahon 2022 and lower than those reported by Locklin et al. 2020, suggesting that mussel shell growth rates in Belton Lake have remained relatively stable through time. Similarly, Arterburn and McMahon (2022) did not note any reduction in mussel shell growth rates over seven years sampling in Lake Texoma and five years in Ray Roberts Lake.

Of note was that the shell growth rates of mussels held at 8 m depth in Belton and Stillhouse Hollow Lakes were 60.2% and 69.0% that of mussels held at 2 m depth, respectively, even though water temperatures, pH, % of air O2 saturation, and conductivities were similar between 2 and 8 m depths in both lakes. Also of interest is that chlorophyll *a* concentrations remained similar across depths in both lakes, but were much greater in Belton Lake than Stillhouse Hollow Lake. It would seem the increased phytoplankton food concentrations should have enabled Belton Lake mussels to maintain higher shell growth rates than Stillhouse Hollow mussels at both depths. Instead, mussel shell growth rates were similar between the two lakes although declining with depth. While their shell growth rates were similar, larger mussels in Belton Lake had higher dry tissue weights prior to spawning suggesting that access to increased phytoplankton concentrations allowed them to maintain a better physiological condition, but not increase shell growth rates. This result suggests that physiological condition in zebra mussels may be uncoupled from shell growth rate. The reduced physiological condition of mussels at 8 m depth compared to those held a 2 m depth even though physiochemical conditions and chlorophyll a concentrations were similar at the two depths appears to indicate that other unknown factors may be impacting the shell growth rates and physiological condition of zebra mussels. Phytoplankton levels may not be the only nutritional factor impacting mussel shell growth and physiological condition especially since bacterioplankton are known to make up a large portion of zebra mussels' energy intake (Cotnor et al. 2009). As such, it would be interesting to record bacterioplankton concentrations along with phytoplankton concentrations in studies of mussel population dynamics.

Mussel densities at 2 and 8 m depth at Frank's Marina on Belton Lake showed a similar pattern through time with the densities of the Spring-2021 and Fall-2021 Cohorts remaining relatively stable through May 2022 after which they disappeared from samples by July and August 2022 respectfully. Similarly, the combined Spring-Fall 2021 Cohort at 2 m depth at the Stillhouse Hollow Marina maintained relatively stable densities until June 2022 and declined thereafter to a low density of 117 mussels/m^2. In contrast, at 8 m depth in Stillhouse Hollow Lake, the combined Spring-Fall 2021 Cohort maintained relatively stable densities through August, but was no longer present in the final September sample.

Similar declines in mussel density starting in July 2022 were recorded at 2 m depth at the buoy sampling sites in both lakes. These density declines reflected the negative impact of warm summer water temperatures on the larger previous year's cohorts eventually leading to their extirpation potentially due to lethal levels of starvation. Concurrent declines in the densities of the newly settled Spring-2022 Cohorts may be a result of competition for space and food as individuals increase in size. The die-offs of mussels in both lakes was not a result of hypoxia because their oxygen concentrations remained well above the mussel's long-term tolerated lower limit of 25% of full air O_2 saturation (Johnson and McMahon 1998) throughout the study. Similar rapid declines in the density of newly settled zebra mussels in Texas water bodies has also been reported in lakes Texoma, Ray Roberts and Belton (Locklin et al. 2020, Arterburn and McMahon 2022). Thus, the basis for the rapid summer decline in the densities of newly settled mussels in Texas water bodies is worthy of further investigation.

Mussel condition measured as dry tissue weight (DTW) relative to shell length increased at both marina sites from relatively low levels in the initial November 2021 samples, attaining peak levels by April 2022 after which DTW declined through the final September 2022 samples. Zebra mussels with shell lengths of ≥20 mm from Frank's Marina at Belton Lake had greater DTW's than mussels from Stillhouse Hollow Marina on Stillhouse Hollow Lake. This difference was most apparent from February through May 2022 before mussels initiated spawning. After May, the DTW of zebra mussels at both marina sites declined reaching minimal levels by September 2022. Morse (2009) described a similar seasonal pattern of mussel DTW variation at Winfield City Lake, Kansas, in which mussels with shell lengths ≥15 mm attained maximal DTW's in June which then declined to minimal levels by August and September. Regardless of food availability, at temperatures ≥28°C, metabolic energy demands exceed energy absorption rates in larger adult zebra mussels (Walz, 1978a). Water temperatures in BL and SHL at 2 and 8 m depth were >28°C for 3-4 months, potentially causing older, larger individuals of the 2021 Spring and Fall cohorts to have a negative energy balance, resulting in tissue mass loss and eventual mortality from July-September. Similar negative losses of mussel dry tissue weight at temperatures >25°C have been reported by Nalepa et al. (1993), Stoeckmann and Garton (1997), Jantz and Neumann (1998), and Morse (2009). While Jantz and Neumann (1998) associated tissue mass loss with spawning events, Morse (2009) reported initiation of mussel tissue weight loss at 25°C in Winfield City Lake (Kansas), well above the 15 to 17°C at which veligers were first observed. Similarly, Stoeckmann and Garton (1997) found that high temperature environmental stress induced mussel tissue loss that was in excess of reproductive output.

Conclusions

Little difference was found between the population dynamics of Belton Lake and Stillhouse Hollow Lake zebra mussel populations. The lakes were infested by mussels in 2013 and 2016, respectfully. In both lakes, juvenile settlement was initiated in June and the shell growth rates were similar being greater at 2 m than at 8 m depth. Their annual density fluctuations were similar with densities of the previous year's Spring and Fall Cohorts declining and being lost from the population during July-September periods of maximum water temperature. The only real difference recorded among the two populations was that larger Belton Lake mussels had higher dry tissue weights than Stillhouse Hollow Lake mussels potentially due to Belton Lake having higher chlorophyll *a* concentrations than Stillhouse Hollow Lake. Increased chlorophyll *a* concentrations indicating higher phytoplankton food densities at Belton Lake appeared to allow BL mussels to maintain elevated dry tissue weights relative to those in Stillhouse Hollow Lake whose lower chlorophyll *a* concentrations indicated potentially lower phytoplankton concentrations. In spite of this difference in body condition, the BL and SHL mussels shells grew at similar rates suggesting that other unknown factors like bacterioplankton concentrations may also be controlling shell growth rates in the two populations. The Belton Lake mussel shell growth rates recorded in this study were similar to those previously recorded in other studies of mussel shell growth in the lake suggesting that mussel shell growth rates have remained relatively constant over time. What was surprising was that in spite of having similar shell growth rates, adult zebra mussels from Belton Lake had elevated dry tissue weights compared individuals from Stillhouse Hollow Lake. This result suggests that zebra mussel physiological condition may be uncoupled from their shell growth rates. Thus, future comparative studies of zebra mussel population dynamics may want to include dry tissue weight, shell dry weight, shell thickness and bacterioplankton and phytoplankton densities in addition to chlorophyll *a* concentrations to develop a better understanding of the drivers of mussel physiological condition and shell growth rates. This study also suggests that multi-year studies of zebra mussel population dynamics across different water bodies will be important for understanding the long-term impacts of mussel infestation of Texas water bodies. Such long-term studies would be particularly important for the development of zebra mussel management plans for infested Texas water bodies and effective prevention and control procedures for mussel fouling of raw water using utilities and infrastructure.

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Literature Cited

Aldridge, D.W., B.S. Payne, A.C. Miller. 1995. Oxygen consumption, nitrogenous excretion, and filtration rates of *Dreissena polymorpha* at acclimation temperatures between 20 and 32°C. Canadian Journal of Fisheries and Aquatic Science. 52: 1761–1767.

Arterburn, H.M., R.F. McMahon. 2022. Population and reproductive dynamics of zebra mussels (*Dreissena polymorpha*) in warm, low-latitude North American waters. The Biological Bulletin 242(3): 207-221.

Bij de Vaate, A. 1991. Distribution and aspects of population dynamics of the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), in the Lake Ijsselmeer area (The Netherlands). Oecologia 86: 40–50.

Boeckman, C.J., J.R. Bidwell. 2014. Density, growth, and reproduction of zebra mussels (*Dreissena polymorpha*) in two Oklahoma reservoirs. Pp. 369–382 in *Quagga and Zebra Mussels*, T. F. Nalepa and D. W. Schloesser, eds. CRC Press, Boca Raton, FL.

Brazos River Authority of Texas. 1989. Report on Lakes Belton and Stillhouse Hollow Water Quality. 82p.
https://www.bing.com/search?q=Brazos+River+Authority+of+Texas.+1989.+Report+on+Lakes+Belton+and+Stillhouse+Hollow+Water+Quality+Examination&qs=n&form=QBRE&sp=-1&pq=&sc=10-0&sk=&cvid=0FC0164C67AB47A7A2EB9AE72E109958&ghsh=0&ghacc=0&ghpl=

Chase, M.E., R.C. Bailey. 1999. The ecology of the zebra mussel (*Dreissena polymorpha*) in the lower Great Lakes of North America: I. Population dynamics and growth. Journal of Great Lakes Research 25: 107–121.

Churchill, C.J., D.J. Hoeinghaus, T.W. La Point. 2017. Environmental conditions increase growth rates and mortality of zebra mussels (*Dreissena polymorpha*) along the southern invasion front in North America. Biological Invasions 19: 2355–2373.

Claudi R., A. Graves, A.C. Taraborelli, R.J. Prescoot, S.E. Mastitsky. 2012. Impact of pH on survival and settlement of dreissenid mussels. Aquatic Invasions 7: 21-28.

Cotner J.B., W.S. Gardener, J.R. Johnson, R.H. Sada, J.F. Cavaletto, Heath R.T. 2009. Effects of zebra mussel (*Dreissena polymorpha*) on bacterioplankton: evidence for both size-selective consumption and growth stimulation. Journal of Great Lakes Research 21(4): 517-528.

Dorgelo, J., M.H.S. Kraak. 1993. Seasonal variation in tissue dry biomass and its relative ash and organic carbon and nitrogen content in the freshwater mussel *Dreissena polymorpha* (Pallas). Archiv für Hydrobiologie 127: 409–421.

Garton, D.W. and L.E. Johnson. 2000. Variation in growth rates of the zebra mussel, *Dreissena polymorpha*, within Lake Wawasee. Freshwater Biology 45: 443–451.

Hallidayschult T.C., J.E. Beyer, K.D. Hambright. 2021. Spatial variation in propagule pressure and establishment of zebra mussels (*Dreissena polymorpha*) within a subtropical reservoir. Aquatic Invasions 16(1): 94-112.

Jantz, B., and D. Neumann. 1998. Growth and reproductive cycle of the zebra mussel in the River Rhine as studied in a river bypass. Oecologia 114: 213–225.

Johnson, P.D. and R.F. McMahon. 1998. Effects of temperature and chronic hypoxia on survivorship of the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). Canadian Journal of Fisheries and Aquatic Science 55: 1564–1572.

Jones, L.A. and A. Ricciardi. 2005. Influence of physicochemical factors on the distribution and biomass of invasive mussels (*Dreissena polymorpha* and *Dreissena bugensis*) in the St. Lawrence River. Canadian Journal of Fisheries and Aquatic Science 62: 1953-1962.

Locklin, J.L., D.N. Corbitt, R.F. McMahon. 2020. Settlement, density, survival and shell growth of zebra mussels, *Dreissena polymorpha*, in a recently invaded low latitude, warm water Texas Reservoir. Aquatic Invasions 15(3): 408-434.

Mackie, G.L. 1993. Biology of the zebra mussel (*Dreissena polymorpha*) and observations of mussel colonization on unionid bivalves in Lake St. Clair of the Great Lakes. Pp. 153–165 in Zebra Mussels: Biology, Impacts, and Control, T. F. Nelepa and D. W. Schloesser, eds. CRC Press, Boca Raton, FL.

Morse J.T. 2009. Thermal tolerance, physiologic condition, and population genetics of dreissenid mussels (*Dreissena polymorpha* and *D.* rostriformis bugensis) relative to their invasion of waters in the western United States. Ph.D. Dissertation. The University of Texas at Arlington. 280 pp.

Nalepa, T. F., J. F. Cavaletto, M. Ford, W. M. Gordon, M. Wimmer. 1993. Seasonal and annual variation in weight and biochemical content of the zebra mussel, *Dreissena polymorpha*, in Lake St. Clair. Journal of Great Lakes Research. 19: 541–552.

Schwalb, A.N., D. Swearingen, J.J. Robinson, J.L. Locklin, J. Moore, M. McGarrity. 2023. Living on the edge: thermal limitations of zebra mussels (*Dreissena polymorpha*) in central Texas. Biological Invasions. In press. https://doi.org/10.1007/s10530-022-02950-z.

Stoeckmann, A.M., D.W. Garton. 1997. A seasonal energy budget for zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Canadian Journal of Fisheries and Aquatic Science. 54: 2743–2751.

Texas Parks and Wildlife Department. 2013. Zebra Mussels Found in Lake Belton and Suspected in Lakes Worth and Joe Pool. https://tpwd.texas.gov/newsmedia/releases/?req=20130926a

Texas Parks and Wildlife Department. 2016. Zebra Mussels Discovered in Stillhouse Hollow Reservoir. https://tpwd.texas.gov/newsmedia/releases/?req=20160812a

Texas Water Development Board. 2017. Volumetric and sedimentation survey of Belton Lake. Prepared for Brazos River Authority. Austin, Texas, USA, 32 pp. https://www.twdb.texas.gov/hydro_survey/Belton/2015-10/Belton2015_FinalReport.pdf

Tibbs J., M.S. Baird. 2019. Belton Reservoir – 2018 Survey Report. Texas Parks and Wildlife Department. https://tpwd.texas.gov/publications/pwdpubs/lake_survey/pwd_rp_t3200_1247/

Walz, N. 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. I. Pattern of activity, feeding and assimilation efficiency. Archiv für Hydrobiologie 55: 83–105.

Figures

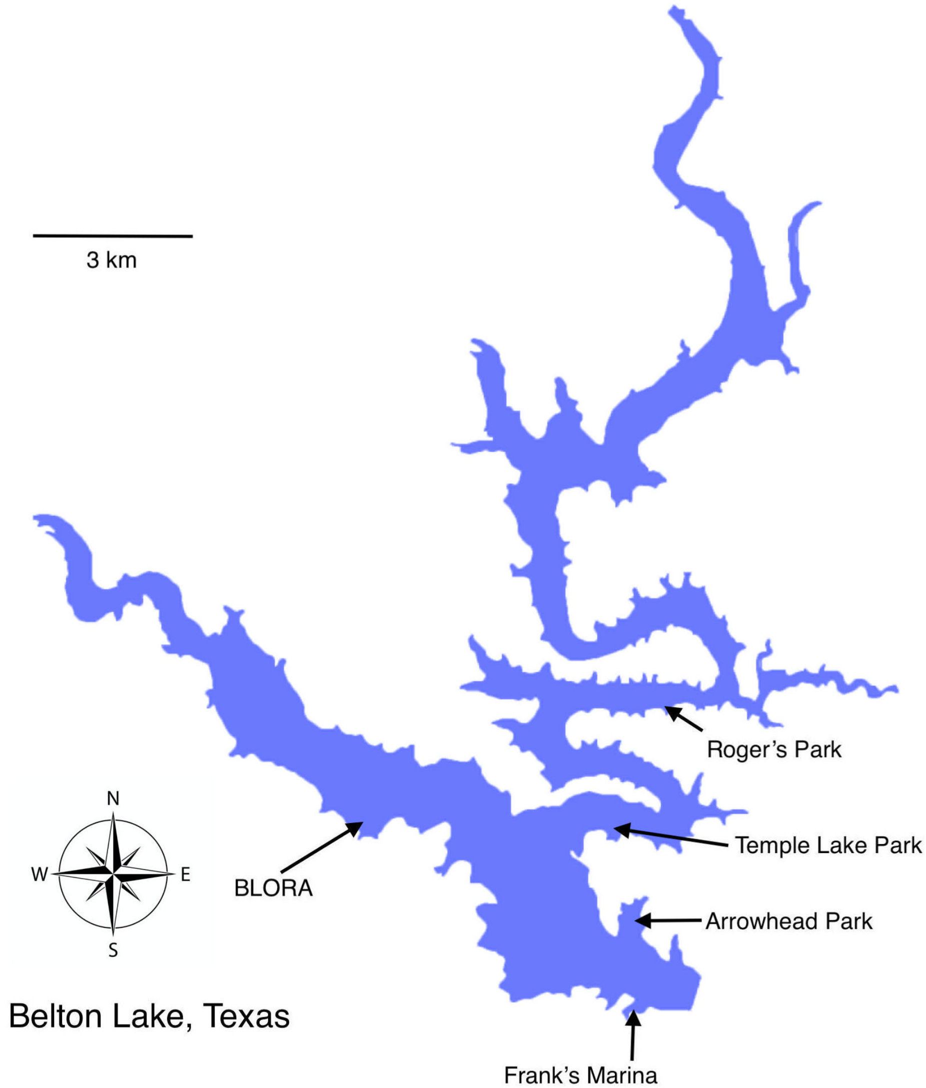

Figure 1. Map of Belton Lake showing the marina and buoy locations of sampler plates.

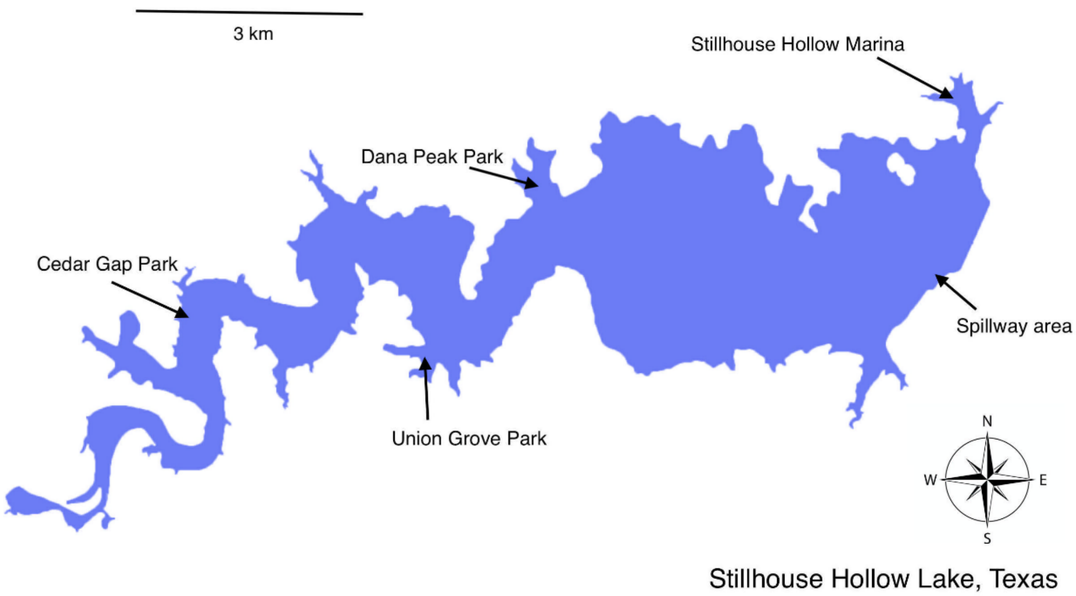

Figure 2. Map of Stillhouse Hollow Lake showing the marina and buoy locations of sampler plates.

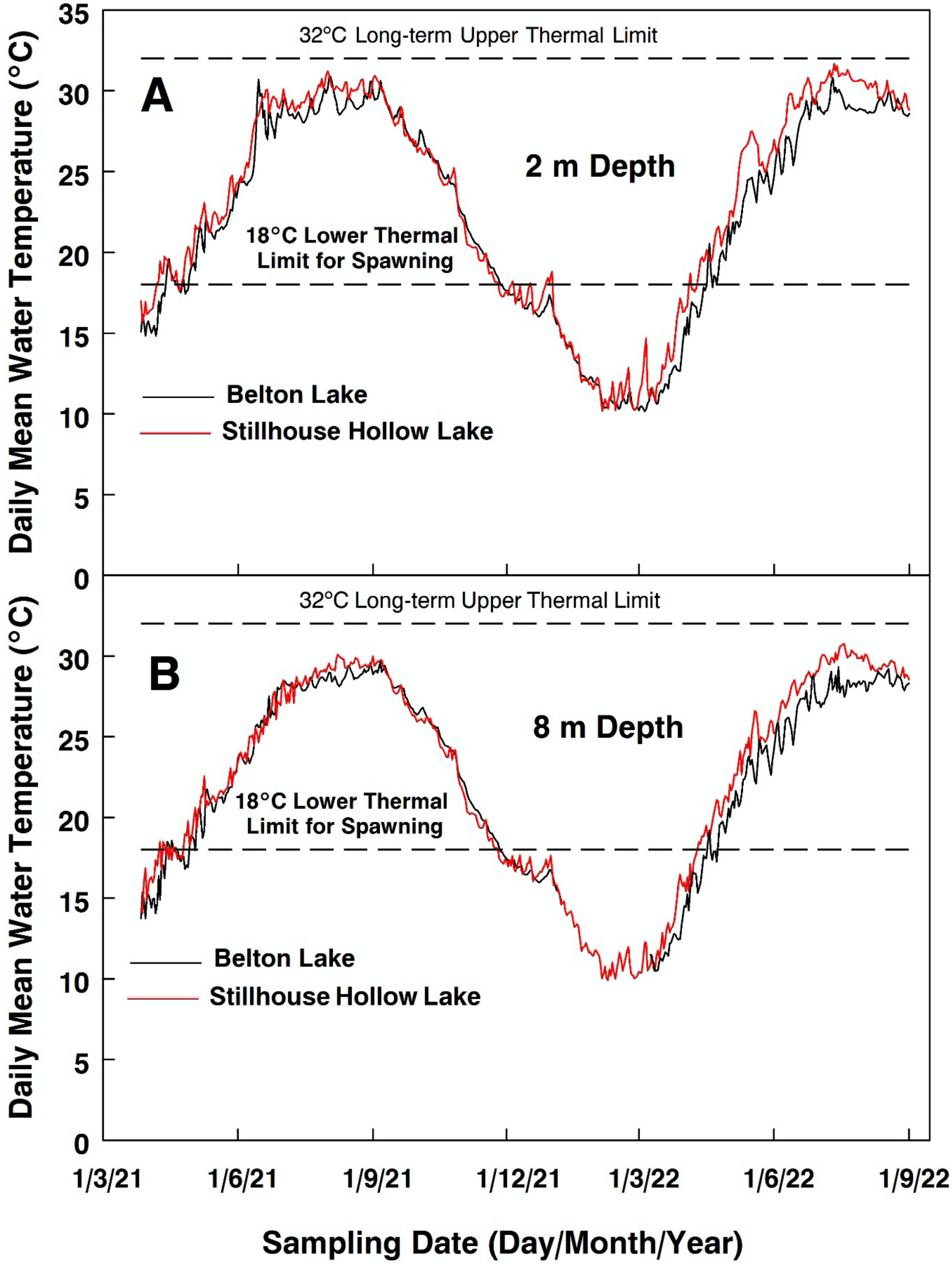

Figure 3. Daily mean water temperatures recorded from 03/27/201 to 09/01/2022 at 2 m (A) and 8 meters (B) depth at Frank's Marina on Belton Lake and Stillhouse Hollow Marina on Stillhouse Hollow Lake.

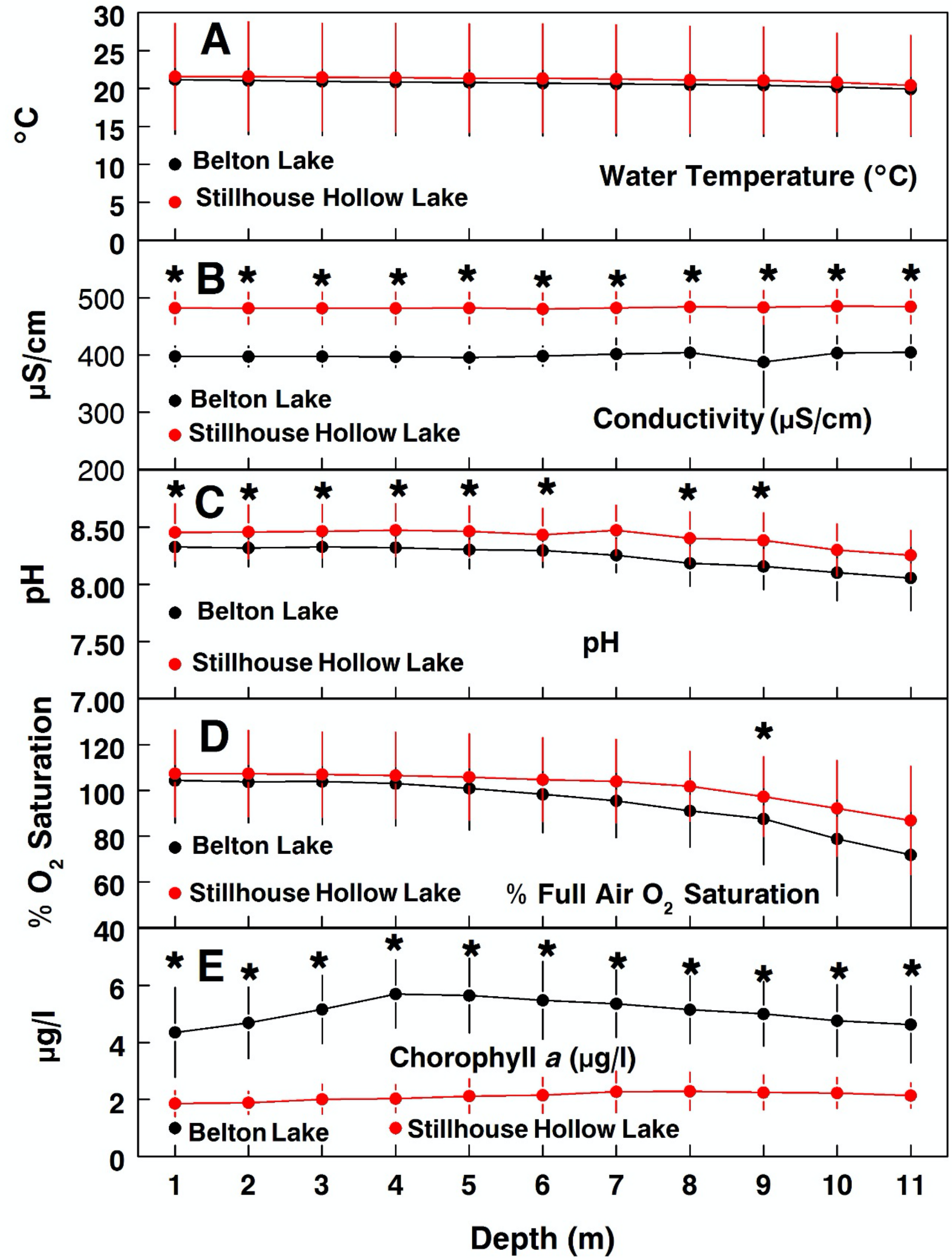

Figure 4. Mean Hydrolab recorded physical-chemical water parameters (vertical axis) recorded biweekly across depths of 1-11 m in 1-m increments from 11/21/2021 to 09/02/2022 at Franks Marina on Belton Lake (black lines and error bars) and Stillhouse Hollow Marina on Stillhouse Hollow Lake (redlines and error bars) for A) water temperature, B) Conductivity, C) pH, D) Percent of full air O_2 saturation and E) Chlorophyll *a*. Asterisks above paired values indicate a significant difference ($p < 0.05$) determined by paired T-tests assuming unequal variances.

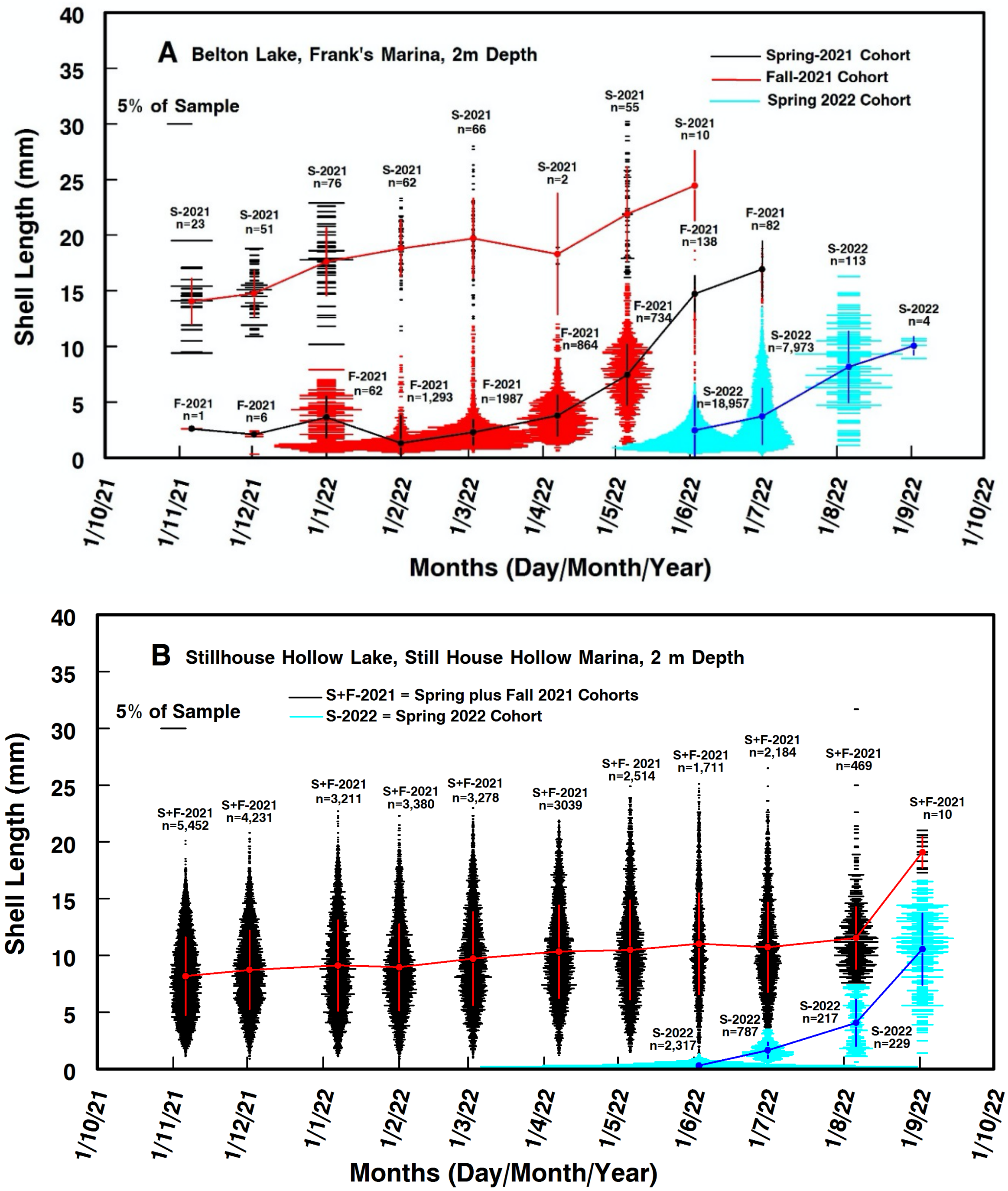

Figure 5. Shell length (SL) distributions and mean SL+SD of zebra mussel cohorts sampled monthly (horizontal axis) from 2 m depth at A) Frank's Marina, Belton Lake (BL) and B) Stillhouse Hollow Marina, Stillhouse Hollow Lake (SHL). BL had three identifiable cohorts, Spring-2021 (red points, black bars), Fall- 2021 (black points, red bars) and Spring-2022 (blue dots, sky blue bars). SHL had a combined Spring-Fall cohort (red points, black bars) and a Spring-2022 cohort (blue points and sky blue bars). Horizontal bars are % of individuals at each mm SL in a sample.

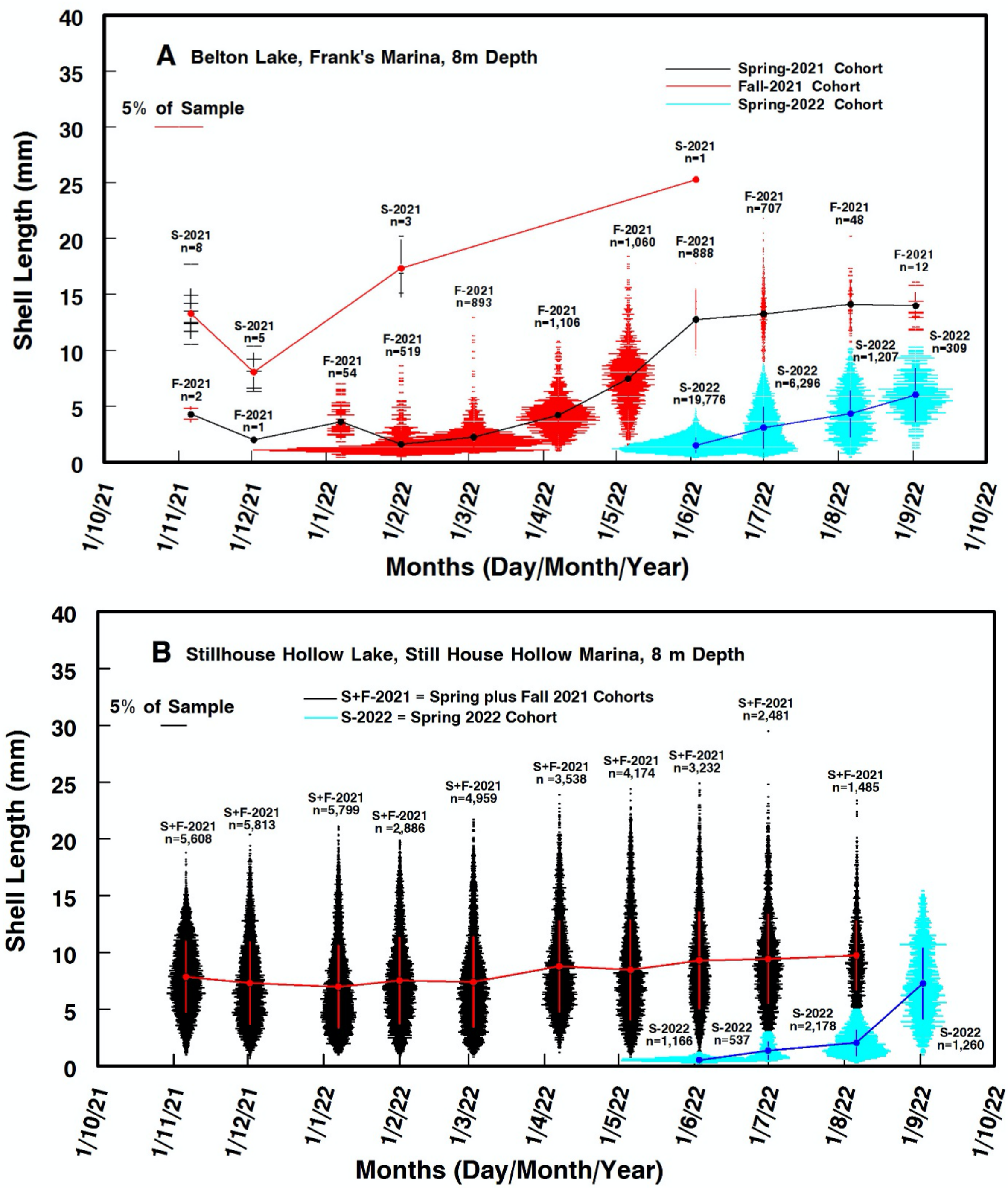

Figure 6. Shell length (SL) distributions and mean SL+SD of zebra mussel cohorts sampled monthly (horizontal axis) from 8 m depth at A) Frank's Marina, Belton Lake (BL) and B) Stillhouse Hollow Marina, Stillhouse Hollow Lake (SHL). BL had three identifiable cohorts, Spring-2021 (red points, black bars), Fall-2021 (black points, red bars) and Spring-2022 (blue dots, sky blue bars). SHL had a combined Spring-Fall cohort (red points, black bars) and a Spring-2022 cohort (blue points and sky blue bars). Horizontal bars are % of individuals at each mm SL in a sample.

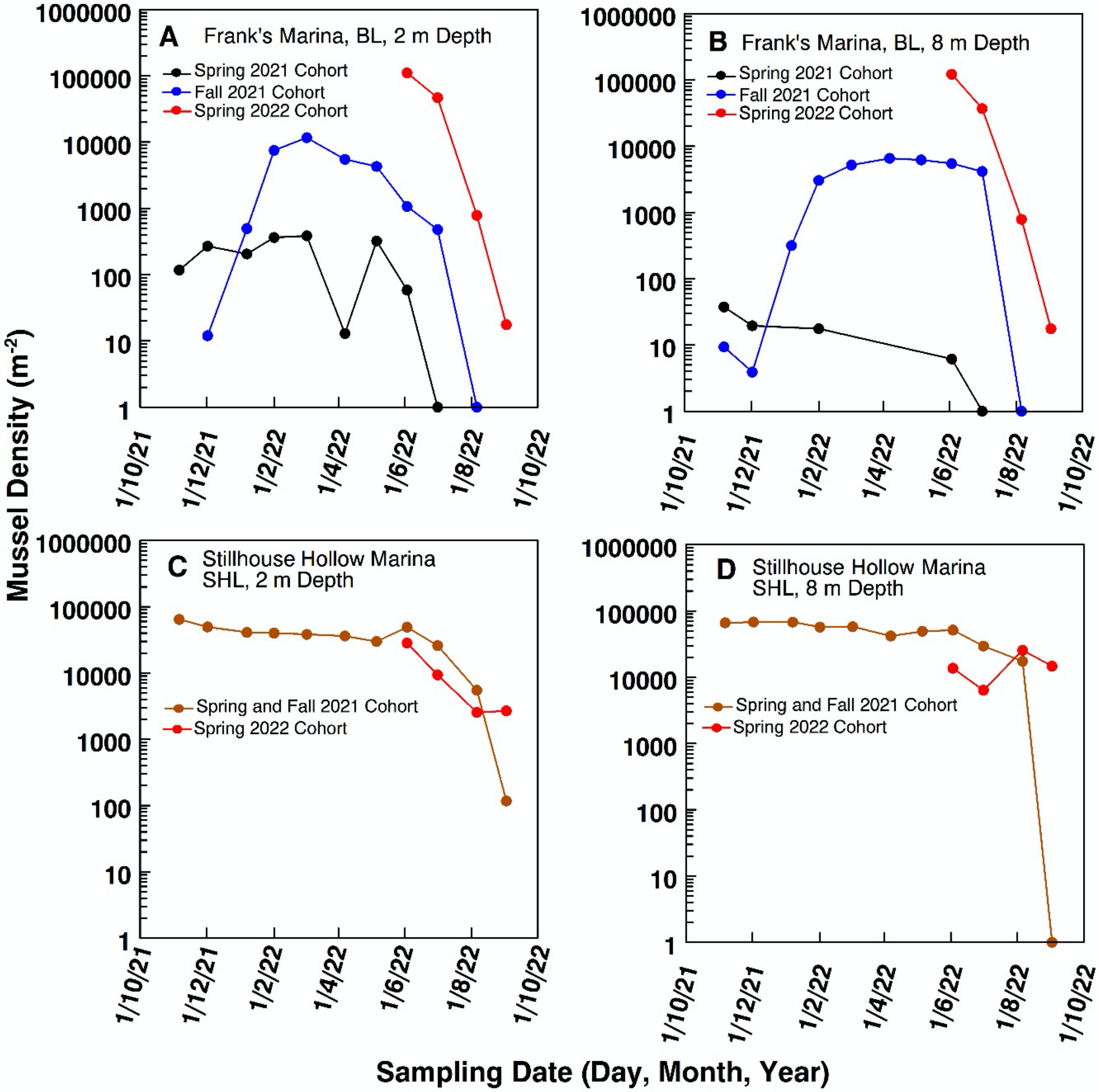

Figure 7. Densities of zebra mussel settlement cohorts (mussels/m^2) recorded monthly at marina sampling sites from 11/06/2021 – 09/02/2022 at depths of 2 and 8 m. Mussels were sampled at Frank's Marina on Belton Lake (BL) at depths of (A) 2 m and (B) 8 m and at Stillhouse Hollow Marina on Stillhouse Hollow Lake (SHL) at depths of (C) 2 m and (D) 8 m. Densities of different mussel cohorts are indicated by differently colored lines and points. For Frank's Marina, black points and lines, blue points and lines and red points and lines depict the densities of the Spring-2021, Fall-2021 and Spring-2021 settlement cohorts, respectfully. For Stillhouse Hollow Marina, brown points and lines and red points and lines depict the densities of the combined Spring- and Fall-2021 and the Spring-2022 settlement cohorts, respectfully.

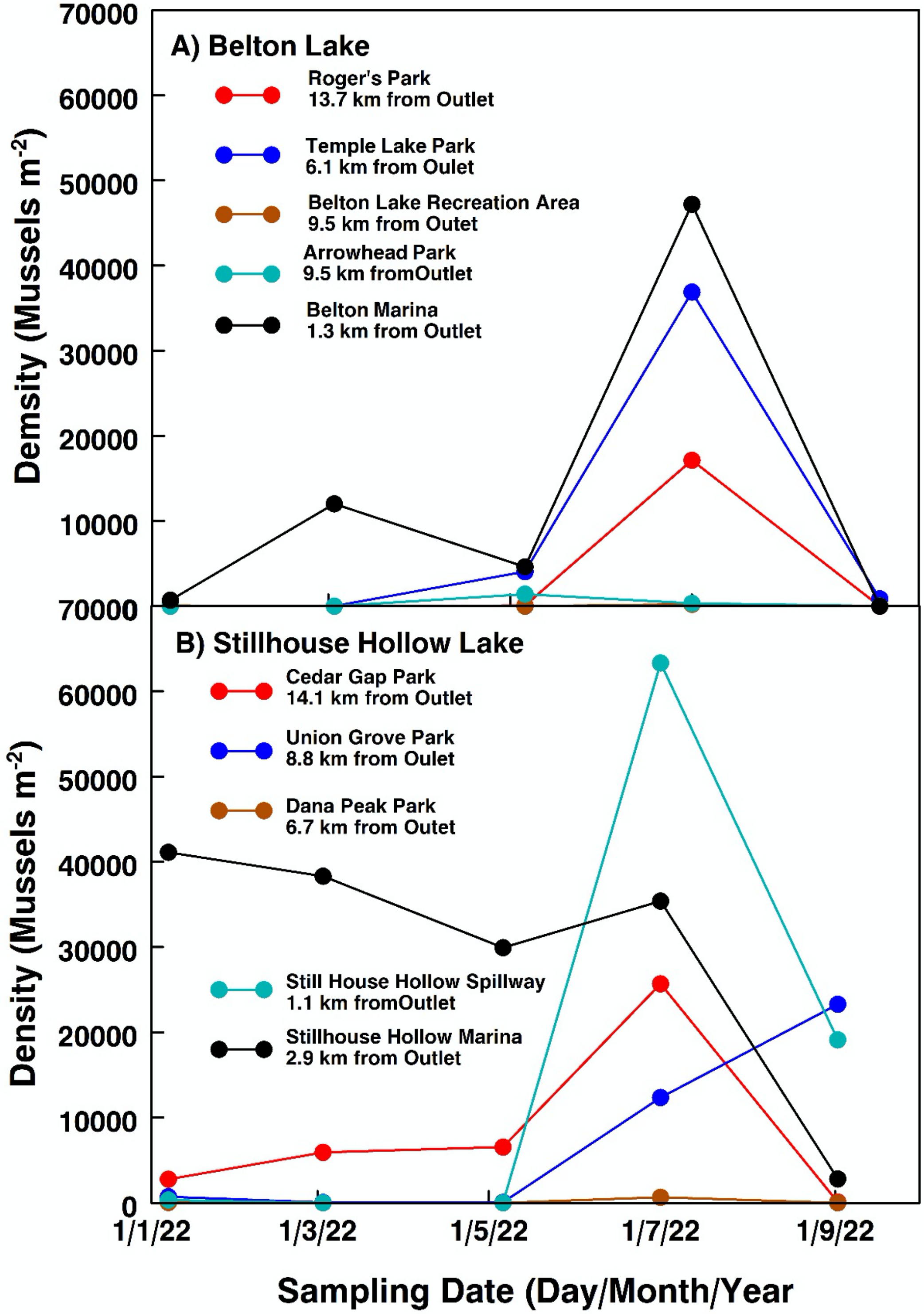

Figure 8. Densities of zebra mussels at two meters below the water's surface determined at two month-intervals for five sites at various distances from the lake outlet in A) Belton Lake and B) Stillhouse Hollow Lake. Different colored lines and points indicate densities of mussels at different sampling sites. Kilometers distance from the lakes' outlets are indicated for each sampling site. See Figure 1 for the locations of the sampling sites.

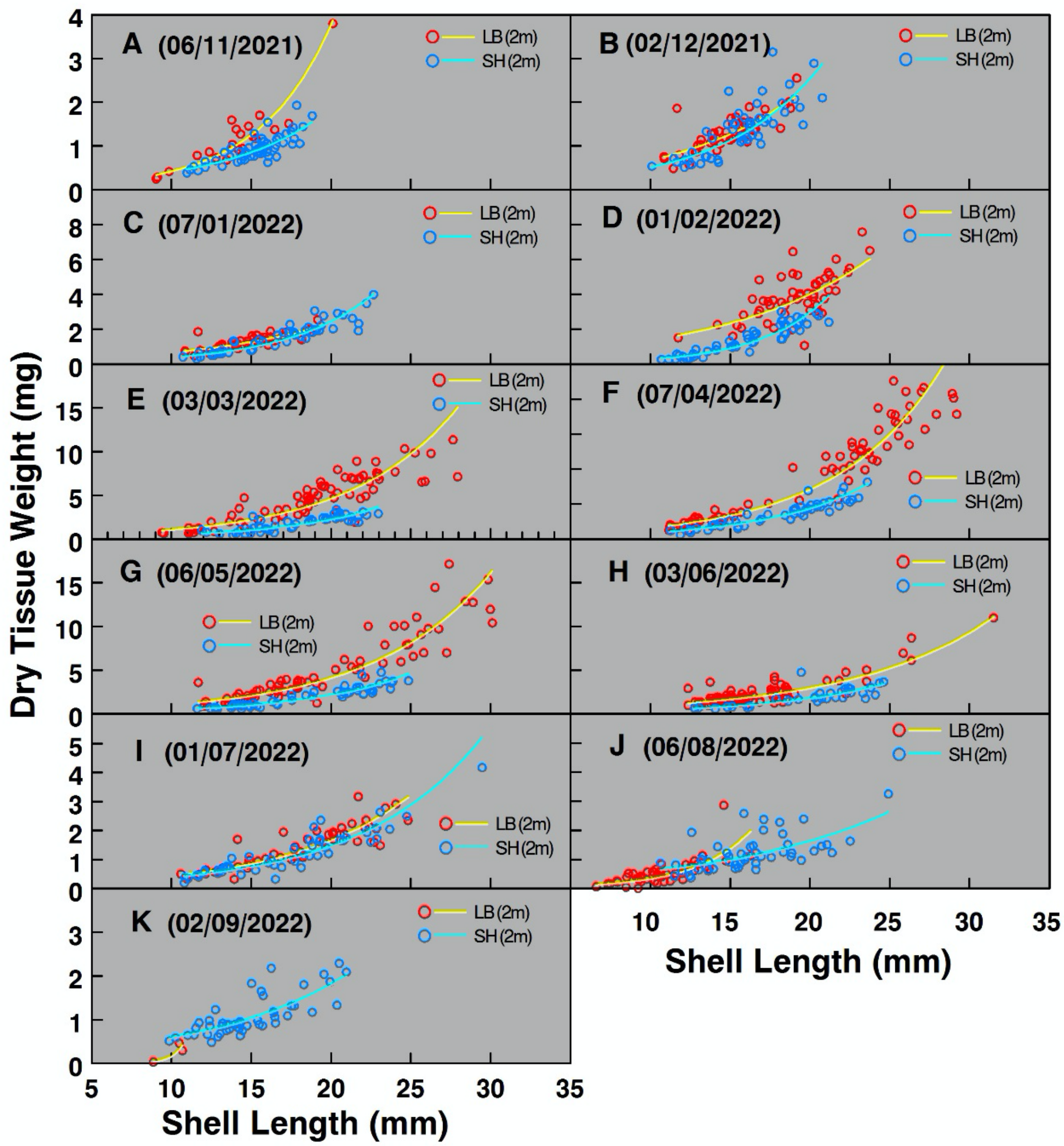

Figure 9. Exponential regressions of zebra mussel shell length (mm, horizontal axis) versus dry tissue weight (mg, vertical axis) collected from settlement plates at 2 m depth at Frank's Marina (Belton Lake) and Stillhouse Hollow Marina (Stillhouse Hollow Lake) on sampling dates (day/month/year): A (06/11/2021), B (02/12/2021), C (07/01/2022), D (01/02/2022), E (03/03/2022), F (07/04/2022), G (06/05/2022), H (03/06/2022), I (01/07/2022), J (06/08/2022) and K (02/09/2022).

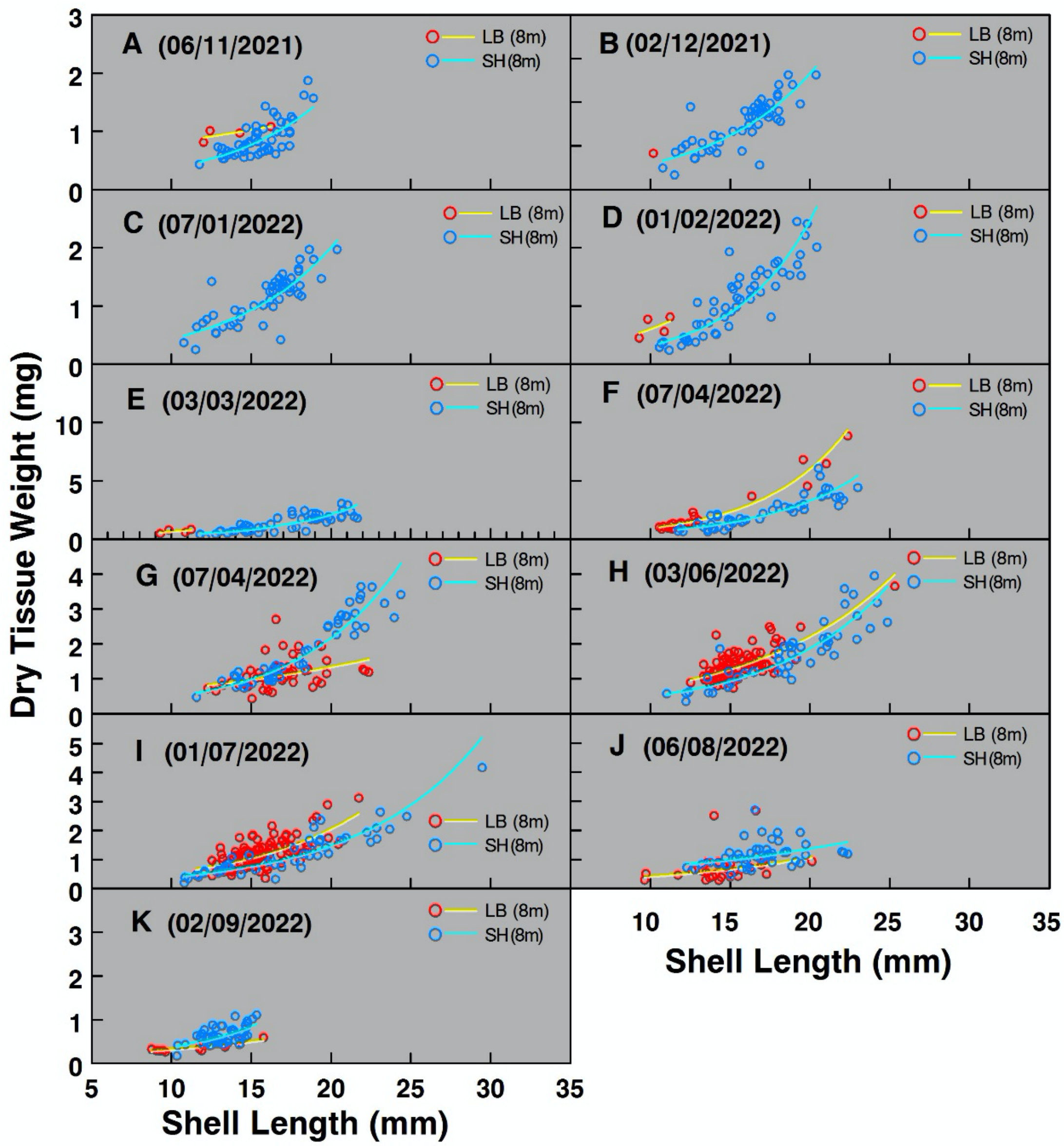

Figure 10. Exponential regressions of zebra mussel shell length (mm, horizontal axis) versus dry tissue weight (mg, vertical axis) collected from settlement plates at 8 m depth at Frank's Marina (Belton Lake) and Stillhouse Hollow Marina (Stillhouse Hollow Lake) on sampling dates (day/month/year): A (06/11/2021), B (02/12/2021), C (07/01/2022), D (01/02/2022), E (03/03/2022), F (07/04/2022), G (06/05/2022), H (03/06/2022), I (01/07/2022), J (06/08/2022) and K (02/09/2022).

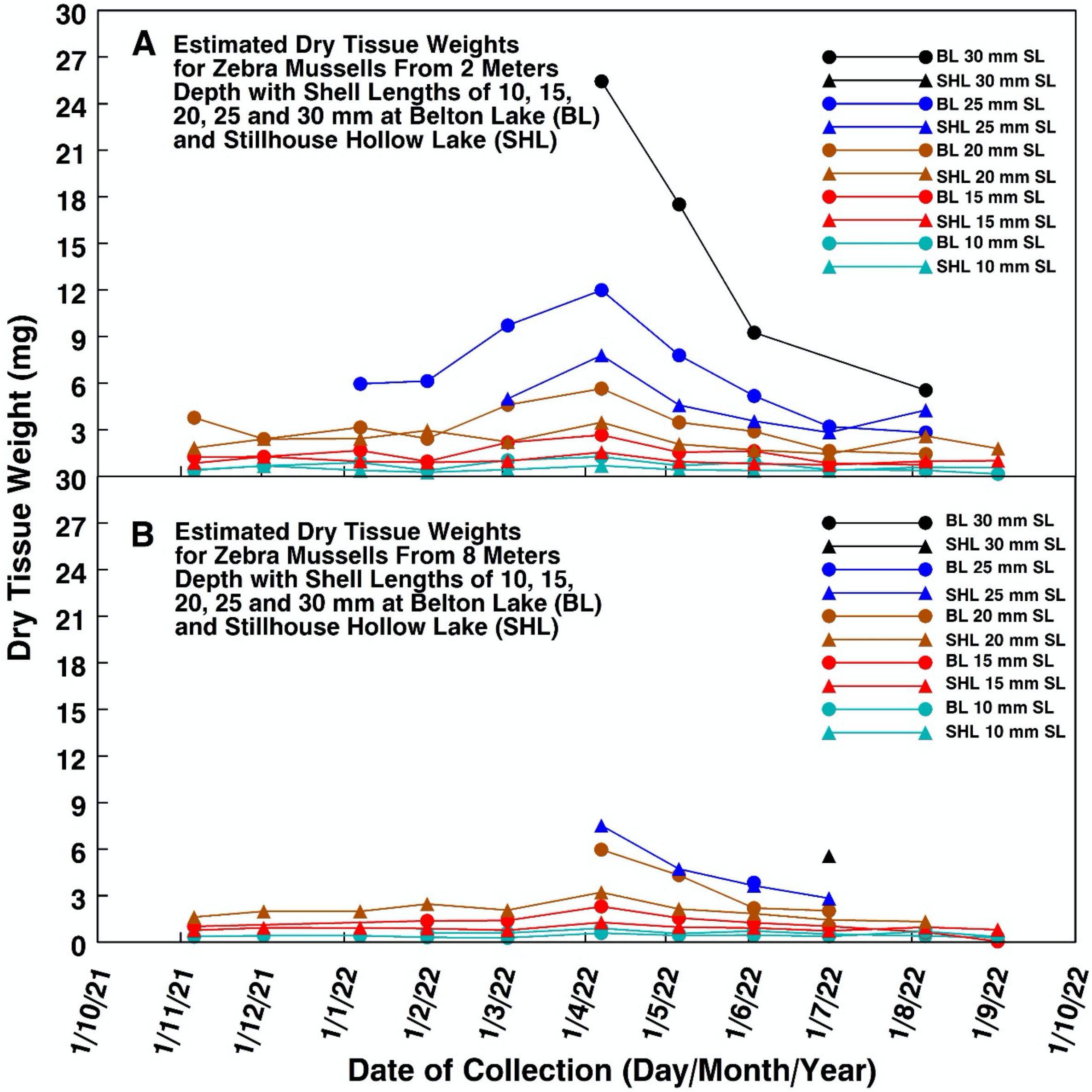

Figure 11. Dry tissue weights of zebra mussels with standard shell lengths of 10, 15, 20, 25, and 30 mm estimated by exponential regression analysis versus shell length as the independent variable (Figures 9A-L and 10A-L) of mussels sampled from settlement plates held at depths of 2 m (A) and 8 m (B) in Belton Lake (Frank's Marina) (circles) and Stillhouse Hollow Lake (Stillhouse Hollow Marina) (triangles) on day/month/year sampling dates of 06/11/2021, 02/12/2021, 07/01/2022, 01/02/2022, 03/03/2022, 07/04/2022, 06/05/2022, 03/06/2022, 01/07/2022, 06/08/2022 and 02/09/2022.

Table 1. Mean hourly temperatures (± standard deviations), maximum temperatures (with date recorded), and the number of hours exceeding 30°C, 31°C, 32°C, and 33°C at all zebra mussel sampling sites between 27 October 2021 – 1 September 2022 on Belton and Stillhouse Hollow Lakes. *BLORA data is only reported from 27 Oct 2021 – 9 March 2022 due to lost data logger.

Site	Mean Hourly Temperature (27 Oct 2021 – 1 Sept 2022)	Max Temperature (date recorded)	Number of Hours (27 Oct 2021 – 1 Sept 2022)			
			≥ 30C	≥ 31C	≥ 32C	≥ 33C
Belton Lake						
Frank's Marina, 2m	20.10 ± 6.70	31.16 (10 July 2022)	365	38	0	0
Frank's Marina, 8m	21.59 ± 5.53	29.84 (18 Aug 2022)	17	0	0	0
Arrowhead Park	20.84 ± 6.98	33.94 (11 July 2022)	1448	492	96	27
Temple Lake Park	20.84 ± 6.94	33.10 (11 July 2022)	1496	465	82	11
BLORA *	15.41 ± 3.78	24.86 (27 Oct 2021)	0	0	0	0
Roger's Park	21.16 ± 7.00	33.22 (10 July 2022)	1613	786	131	13
Stillhouse Hollow Lake						
Stillhouse Hollow Marina, 2m	21.05 ± 7.03	32.44 (11 July 2022)	1551	750	76	0
Stillhouse Hollow Marina, 8m	20.42 ± 6.84	31.04 (16 July 2022)	873	110	0	0
Spillway	20.69 ± 6.82	32.05 (13 July 2022)	1186	205	33	0
Dana Peak Park	21.22 ± 7.19	33.22 (13 July 2022)	1754	936	201	33
Union Grove Park	20.96 ± 7.14	33.38 (13 July 2022)	1587	604	149	27
Cedar Gap Park	21.22 ± 7.34	33.54 (13 July 2022)	1770	971	238	31

Table 2. Annual mean values and standard deviations (in parentheses) of physical-chemical parameters sampled biweekly at 2 m depth from buoy sites in Belton Lake (i.e., Rogers Park, Temple Park, Belton Outdoor Recreation Area, Arrowhead Park) and Stillhouse Hollow Lake (i.e., Cedar Gap Park, Union Grove Park, Dana Peak Park, and Stillhouse Hollow Spillway). Water temperature, pH conductivity and % of full air O_2 saturation were sampled from 06/07/2021 to 09/02/2022 (n = 34 samples) and Chlorophyll *a* from 11/09/2021 to 09/02/2022 (n = 22 samples).

Buoy Site	Mean (SD) Water Temperature °C	Mean (SD) Conductivity µS/m	Mean (SD) pH	Mean (SD) % of Full Air O_2 Saturation	Mean (SD) Mean Chlorophyll *a* Concentration µg/l
Rogers Park	23.302 (6.677)	395.785 (18.822)	8.359 (0.171)	104.598 (22.639)	5.638 2.672
Temple Lake Park	22.881 (6.637)	394.760 (18.445)	8.341 (0.219)	97.462 (28.608)	4.865 2.275
Belton Outdoor Recreation Area	23.034 (6.798)	394.156 (18.630)	8.398 (0.252)	101.622 (17.880)	5.935 2.581
Arrowhead Park	22.850 (6.640)	394.225 (18.998)	8.338 (0.176)	101.240 (21.633)	5.629 4.917
Cedar Gap Park	23.341 (6.863)	520.627 (48.657)	8.396 (0.161)	100.215 (16.376)	3.079 0.951
Union Grove Park	23.067 (6.587)	485.151 (32.419)	8.463 (0.163)	105.909 (15.144)	1.959 0.469
Dana Peak Park	23.020 (6.990)	479.727 (28.556)	8.465 (0.214)	108.025 (17.457)	1.836 0.446
Stillhouse Hollow Spillway	22.719 (6.423)	479.337 (29.258)	8.440 (0.171)	104.029 (14.070)	1.868 0.505

Table 3. Results of a One-way Analysis of Variance *Post-Hoc* Analysis with pair-wise T-tests for calcium concentrations determined 08/19/2022, 08/26/2022 and 09/02/2022 for Rogers Park, Temple Lake Park, Belton Lake Outdoor Recreation Area, Arrowhead Park and Frank's Marina in Belton Lake (for locations see Figure 1) and Cedar Gap Park, Union Grove Park, Dana Peak Park, Stillhouse Hollow Lake Spillway and Stillhouse Hollow Marina in Stillhouse Hollow Lake (For sampling site locations see Figure 2).

Sampling Site	Mean Ca^{2+}/l	Insignificantly Different at $p < 0.05$ (X)				
Rogers	44.72	X				
BLORA	48.13		X			
Temple Lake Park	48.33		X	X		
Franks	48.43		X	X	X	
Union Grove	49.90		X	X	X	X
Arrowhead	49.93		X	X	X	X
Stillhouse Marina	50.93			X	X	X
SH Spillway	51.12				X	X
Dana Peak	51.18					X
Cedar Gap	52.59					X