GUADALUPE RIVER HABITAT CONSERVATION PLAN

Water Quality Threshold Determinations Specific to the Freshwater Mussels Proposed for Coverage in the GRHCP

WHITE PAPER PREPARED BY:

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January 29, 2024

Introduction

Water quality impacts to GRHCP covered freshwater mussel species from wastewater discharges and water withdrawals have been identified by GBRA as a potential source of take. The primary water quality parameters associated with wastewater discharges and water withdrawals that may impact freshwater mussel species are temperature, ammonia, and dissolved oxygen (USFWS, 2021). A framework for assessing "take" from changes in these parameters was developed that utilizes thresholds related to documented species or congener tolerances developed in laboratory trials. Following is a summary of the information used to select these thresholds for use in the GRHCP take analysis.

Temperature

Detailed knowledge on lethal limits of water temperature to freshwater mussels is limited to less than 5% of the documented species (Pandolfo et al, 2010; Archambault et al, 2014; Khan et al, 2018), but it is known that thermal tolerance varies across species and within species collected from different drainages (Fogelman et al, 2023).

Thermal tolerance information has been estimated by laboratory trials for multiple life stages of all three GRHCP freshwater mussel species proposed for coverage (Table 1). Khan et al. (2019) provided acute 12-hr and/or 24-hr LT05 and LT50 values for glochidia of *Lampsilis bergmanni/bracteata, Fusconaia mitchelli,* and *Cyclonaias necki* taken from Guadalupe River populations in varying seasons. For *C. necki*, differences were noted in temperature tolerance between spring and summer collections, with summer numbers being considerably lower and having larger confidence intervals. Glochidia collected later in the brooding season (i.e., late summer) are more sensitive to toxicants, display lower initial viability, and have lower metamorphosis success as compared to glochidia collected earlier in the brooding season (i.e., spring to early summer), indicating they are not appropriate for use in toxicity tests (Bringolf et al, 2013). Therefore, spring values (which had tighter confidence intervals) are reported here. In a subsequent study, Khan et al. (2020) calculated LT05s and LT50s for adult *C. necki* and *F. mitchelli* collected from the Guadalupe River under varying acute (24-hr to 96-hr) and chronic (10-day) time intervals. A summary of acute glochidia LT05 and LT50s, and chronic adult LT05 and LT50s from these studies are reported in Table 1.

Table 1. Acute (24-hr) and chronic (10-day) lethal temperatures (LT) for adult and glochidia life stages of freshwater mussel species proposed for inclusion in the GRHCP.

Species	Glochidia LT05 (Acute)	Glochidia LT50 (Acute)	Adult LT05 (Chronic)	Adult LT50 (Chronic)
Cyclonaias necki	27.4	36.4	35.4	36.2
Fusconaia mitchelli	27.9	36.1	28.4	32.4
Lampsilis bergmanni	27.2	33.1	n/a	n/a

Relating results from laboratory studies to natural systems is one of the challenges with developing meaningful applied thresholds based on static toxicity trials. For example, glochidia viability and infectivity can impact the results of toxicity trials. As previously mentioned, the viability of glochidia begins to decrease 24-48 hours after dispersal, so holding times or acclimation periods can affect viability, which can in turn affect the results of trials (Bringolf et al, 2013). As such, the use of glochidia from late in the brooding period may result in toxicity test results that are substantially different than if glochidia were collected early in the brooding season (Goldsmith et al, 2023).

Another issue with applying results from static laboratory studies to natural systems is the difference in how the organisms experience the elevated temperatures. Static toxicity trials involve holding organisms at a constant temperature for the duration of the trial (generally 24 hours) while temperature extremes experienced in a stream only occur for a brief period (a few hours) of the day, albeit this could be on a reoccurring basis for some period of time. Additionally, natural systems may contain discrete thermal refugia from stratification in the water column/substrate, shading, or groundwater inputs (Briggs et al, 2013).

In the context of the GRHCP, thresholds are needed to estimate take of the covered species in relation to changes in specific water quality parameters that result from specific covered activities. A major challenge in quantifying take through threshold exceedance is separating impacts from the applicant's activities versus impacts from the activities of others or impacts from natural variation, such as natural flow fluctuation, biotic interactions, or climate change. This issue is only exacerbated by the fact that, in the Guadalupe Basin, the mussel species proposed to be covered are currently living near their upper thermal limits (Khan et al, 2020; Fogelman et al, 2023).

As described, many factors were considered in the selection of thresholds to assist in the quantification of take for the three mussel species included in the GRHCP, including national standards, toxicity testing limitations, the best available thermal tolerance information and the temperatures observed in the Guadalupe River system. GBRA proposes species specific thermal thresholds based on the best available adult and glochidia LT50 values reported by Khan et al (2018 and 2020) (Table 2).

Table 2. Thermal thresholds proposed for use in the GRHCP.

Species	Threshold	Life stage	Trial Duration	Reference
Cyclonaias necki	36.2	Adult	10 days	Khan (2020)
Fusconaia mitchelli	32.4	Adult	10 days	Khan (2020)
Lampsilis bergmanni	33.1	Glochidia	24-hour	Khan (2019)

The threshold proposed for *C. necki* (36.2°C) and *F. mitchelli* (32.4°C) represents the adult LT50 chronic (10-day) value. The LT05 adult chronic values were considered, but were deemed inappropriate for several reasons. First, the 95% confidence interval in adult LT05 chronic values are large (4.9° C for *F. mitchelli* and 10.9°C for *C. necki*) compared to those for LT50s (2.5°C for *F. mitchelli* and 3.4° C for *C. necki*), so there is less confidence in the accuracy of these values. Second, the adult *F. mitchelli* LT05 value (28.4°C) represents a temperature that is commonly observed in the Guadalupe River annually, are well within the range of naturally occurring temperatures, and are close to the optimal temperatures for *F. mitchelli* (27.8°C) identified by Bonner et al (2018) based on cellular respiratory enzyme effects. Lastly, the LT05 and LT50 chronic values for adult *C. necki* were not significantly different (Khan et al, 2020).

Thermal toxicity data for *L. bergmanni* is limited to acute testing on glochidia. Since adult tolerance data is not available, GBRA proposes to use the acute glochidia LT50 of 33.1°C (Khan et al, 2019). Although glochidia are generally more sensitive than adults, since no adult tolerance data is available, this glochidia LT50 will be implemented in the same fashion as the adult LT50s for other species to evaluate take. This threshold is generally similar to glochidia and juvenile thermal tolerance information available for *L. radiata* and *L. siliquoidea* (Pandolfo et al, 2010; Archambault et al, 2014b), which are close relatives of *L. bergmanni* (Inoue et al, 2019). LT50 values reported by Pandolfo et al. (2010) for *L. siliquoidea* was 32.8°C for glochidia and 34.4°C for juveniles. Similarly, Archambault et al (2014a) reported juvenile LT50s for *L. siliquoidea* ranging from 33.3°C to 36.0°C under differing acclimation temperatures and sediment exposures. Archambault et al (2014b) also reported LT50s for *L. radiata* juveniles that ranged from 29.9°C to 34.8°C. LT05 values were reported from each of the aforementioned studies, but the 95% confidence intervals associated with the values were large (7-14°C), demonstrating less confidence in these values.

As discussed above, there are a number of nuances to recognize when applying results of static laboratory tests to wild mussel populations. Most importantly, due to diel cycles in temperature in the natural environment, a constant 10-day exposure is unrealistic. Thermal extremes in natural systems occur for a shorter, albeit potentially repeated, duration when compared to laboratory trials. Choosing the 10-day chronic value for adults as a threshold and applying it to a daily maximum temperature is an attempt to account for repeated exposures. Despite the inherent uncertainty involved, the proposed temperature thresholds are based on peer-reviewed literature and provide a biologically-relevant and repeatable method to evaluate take based on water temperature.

Ammonia

The toxicity of ammonia to aquatic organisms depends on various properties of water, especially temperature and pH. In water, ammonia primarily exists in two forms, un-ionized ammonia (NH3) and ammonium ion (NH4 +). Because of the variation in ammonia relative to pH and temperature, results from toxicity trials, which are performed at 20°C and pH 8, must be adjusted to match the water quality of the waterbody of interest (USEPA, 2013). Texas Clean Rivers Program routine surface water quality monitoring on the Guadalupe River has documented that the average pH has been close to 8 S.U. with average temperatures of less than 24°C for the past two decades, as seen in Table 3.

Table 3. Summary of available TCEQ Surface Water Quality Monitoring routine grab samples collected from the Upper (1806), Middle (1804) and Lower (1803) segments of the Guadalupe River beginning in January of 2000.[1]

Upper Guadalupe River (TCEQ Segment 1806 – 17 Stations) 01/04/2000-02/14/2023				
Parameter	Temperature (°C)	pH (S.U.)	Dissolved Oxygen (mg/L)	Total Ammonia Nitrogen (mg/L)
Average	21.4	8.1	8.6	0.09
Maximum	33.0	9.7	14.9	0.52
Minimum	4.2	6.6	3.0	0.02

# of Measurements	2166	2150	2159	302
Middle Guadalupe River (TCEQ Segment 1804 – 18 Stations) 01/18/2000-11/16/2022				
Average	23.2	7.9	8.5	0.07
Maximum	33.0	9.1	15.3	0.70
Minimum	9.1	6.0	0.7	0.02
# of Measurements	2119	2115	2088	867
Lower Guadalupe River (TCEQ Segment 1803 – 5 Stations) 01/17/2000-11/01/2022				
Average	23.0	8.0	8.8	0.11
Maximum	33.4	8.6	20.1	1.27
Minimum	8.6	7.3	4.0	0.02
# of Measurements	444	441	441	309

[1] TCEQ SWQMIS public data (https://www80.tceq.texas.gov/SwqmisPublic/index.htm) accessed by GBRA on 01/10/2023.

While ammonia is known to be toxic to freshwater mussels, there was no species-specific information available for the GRHCP covered mussel species. Accordingly, GBRA collaborated with USFWS, TPWD, and Texas State University to perform acute ammonia toxicity trials on early life stages of the three GRHCP mussel species proposed for coverage. Static ammonia toxicity trials were performed according to the ASTM standards (2022) for freshwater mussels at 20°C at pH 8. A manuscript on this study is currently in progress by GBRA and USFWS staff. The results of these trials are presented in Table 4 along with the EPA Final Acute Value (FAV). The EPA FAV is based on an amalgamation of species and the only mussel species included were from the northeast United States. Because species specific data is available, GBRA proposes to use the 24-hr LC50s presented in Table 4 as TAN thresholds for use in QUAL-TX water quality modeling, which is a relevant scale given ammonia inputs are limited to wastewater treatment facility (WWTF) discharges.

Table 4. Guadalupe River Basin unionid mussel Total Ammonia Nitrogen (TAN) 24-hour exposure Lethal Concentration 50s (LC50s) adjusted to pH 8 and 30°C.

Mussel Species	24-Hour LC50	2013 EPA Final Acute Value (FAV)*
Cyclonaias necki	4.45	3.4 mg/L*
Fusconaia mitchelli	7.08 mg/L	3.4 mg/L*
Lampsilis bergmanni	7.38	3.4 mg/L*

Dissolved Oxygen

Little is known about the oxygen requirements of mussels (Haag, 2012); however, the early life stages of mussels (glochidia and juveniles) are considered less tolerant of low dissolved oxygen (DO) than adults (Sparks and Strayer 1998; Strayer 2008). McMahon and Bogan (2001) found that adult mussels do not exhibit acute stress under low DO for up to several weeks. In laboratory trials on central Texas mussels including *C. necki*, Bonner et al (2018) found that the ability of mussels to obtain oxygen from the water column remained fairly constant until DO levels fell below 2.0 mg/l. Similarly, Sparks and Strayer (1998) reported that sublethal effects are evident at 2.0 mg/l and lethal effects at 1.3 mg/l for juvenile *Elliptio complanata*.

The Texas Surface Water Quality Standards (approved by the EPA for use under the Clean Water Act as designated in Title 30 Part 1 §307.7(b)(3)(A)(i) of the Texas Administrative Code) identify 6.0 mg/l as the 24-hour mean DO concentration for streams classified in the "Exceptional" Aquatic Life Use category, such as the Guadalupe River Upstream of Canyon Lake (Segment 1806), Canyon Lake (Segment 1805) and the Guadalupe River downstream of Canyon Lake to the Comal River (Segment 1812). The Middle Guadalupe (Segment 1804) and Lower Guadalupe River (Segment 1803) are classified in the "High" Aquatic Life Use Category, which has a lower mean DO standard of 5.0 mg/L.

The concerns previously described associated with translating results from laboratory studies to natural systems also apply to identifying a DO threshold. DO concentrations in natural systems display a diurnal trend with low concentrations occurring for a short period early in the day, which is different from static levels held in a laboratory setting. Given that adult mussels are able to close valves and withstand brief low DO concentrations associated with diurnal fluctuations, the regulatory standards for 24-hour mean DO concentration are well above levels documented to adversely impact mussels. We lack data to understand the amplitude of the 24-hour diurnal variation in DO concentration in segments of interest and are therefore unable to establish a 24-hour mean DO concentration threshold under which we would assume take of mussels is likely to occur. We will use QUAL-TX water quality modeling to estimate how covered activities affect DO levels in the plan area, and we will further assess the potential impact of estimated DO changes to covered species with these modeling results. We will also explore the availability of data measuring diurnal fluctuations in DO concentration in segments of interest and gather more data this summer to inform modeling efforts.

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