Development of Habitat Suitability Criteria for Benthic Macroinvertebrates in the Lower Guadalupe River

Final Report

Submitted to:
Texas Water Development Board
Contract No. 1348311646

Prepared by:

Kevin Mayes[1], Archis Grubh[1],
Rebecca Zawalski[2], Weston Nowlin[2], Thom Hardy[2], and Astrid Schwalb[2]

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Summary

The main goal of this study was to develop habitat suitability criteria (or HSC curves) for aquatic benthic macroinvertebrates in the lower Guadalupe River by quantitatively sampling macroinvertebrates and associated habitat data at three study sites at low, medium, and high base flows. High flow conditions persisted throughout most of the study period and it was only possible to collect samples at high and medium base flows; low flow samples were not collected. HSC curves for depth and velocity were developed for:

- all benthic macroinvertebrate taxa;
- Ephemeropta (mayflies);
- elmids (riffle beetles);
- chironomids (midges);
- hydropsychids (net-spinning caddisflies);
- *Tricorythodes* sp. (mayfly);
- *Corydalus* sp. (dobsonfly); and
- *Neoperla* sp. (stonefly).

Curves were based on both non-parametric tolerance limits and probability density functions with their associated 95% confidence intervals and are presented in Appendix A. These two different approaches were compared. We recommend that a selection of the specific methodology should be carefully considered or at a minimum, application of both derived curves employed as a measure of uncertainty in the underlying representation of the depth and velocity resource functions. Texas Parks and Wildlife Department and partners will continue to collect habitat suitability data on aquatic benthic macroinvertebrates when low base flow conditions occur and gather additional similar data on benthic macroinvertebrates from other locations to augment habitat suitability criteria for the lower Guadalupe River.

Appendix B provides tables of water quality and habitat data collected from each microhabitat at each site during this study. Appendix C provides a response to the change suggested by the Texas Water Development Board. The distribution of macroinvertebrates across physiographic gradients within the Guadalupe River Basin in Central Texas was also examined. A manuscript submitted for publication is included as Appendix D.

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Introduction

Instream flow assessments quantify relationships between streamflow and ecological responses to assess proposed changes in hydrology or to determine environmental flow regime requirements for standard-setting applications (Annear et al. 2004). Utilizing the underpinnings of the Natural Flow Paradigm (Poff et al. 1997) and the review of the National Research Council (2005), the Texas Instream Flow Program (TIFP) developed a multi-disciplinary framework to quantify flow requirements for subsistence flows, base flows, high flow pulses, and overbank flows (TIFP 2008). The framework integrates five major disciplines into the assessment: hydrology and hydraulics, biology, physical processes, water quality, and connectivity. Assessments of base flows and subsistence flows rely upon, among other inputs, the development and evaluation of physical habitat models. These models consist of a hydraulic model that predicts current velocity and depth across a range of discharges for a given reach and habitat suitability criteria (HSC or HSC curves) for target taxa or guilds. The output from the habitat model typically consists of a weighted usable area relationship with discharge. Criteria have routinely been developed for fishes (Annear et al. 2004) and benthic macroinvertebrates (Gore et al. 2001). A number of analytical approaches have been used for the development of HSC curves ranging from normalization of frequency distributions (e.g. categorical data such as substrate), fitting polynomial regressions, and curve-fitting approaches such as non-parametric tolerance limits (NPTL; Sommerville 1958; Bovee 1986) and probability density functions (Som et al. 2015).

As part of the TIFP study of the lower Guadalupe River, partner agencies worked with stakeholders to identify indicators for study goals and objectives (TIFP and GBRA 2015). For the biological objectives, key species were recommended including several fishes, mussels, and benthic macroinvertebrates. To address instream flow needs for benthic macroinvertebrates, habitat suitability criteria will be required. The main goal of this study was to develop habitat suitability criteria for aquatic benthic macroinvertebrates in the lower Guadalupe River by quantitatively sampling macroinvertebrates at three study sites at low, medium, and high base flow conditions.

Methods

Study area.—The study area identified in the draft study design for the lower Guadalupe River priority instream flow study (TIFP and GBRA 2015) includes the lower Guadalupe River from Gonzales to Victoria, Texas (Figure 1). Four study sites were identified in the draft study design: 18172 in Gonzales; 18159 downstream of Gonzales; 18138 near Hochheim; and 18056 near Victoria. This study focuses on study sites at Gonzales, Hochheim and Victoria. Each of these three sites represents larger reaches which were based on geomorphic zones (Phillips 2011), tributary inputs, and Level III ecoregions (Griffith et al. 2004). Characteristics of these reaches and study site are described in TIFP and GBRA (2015).

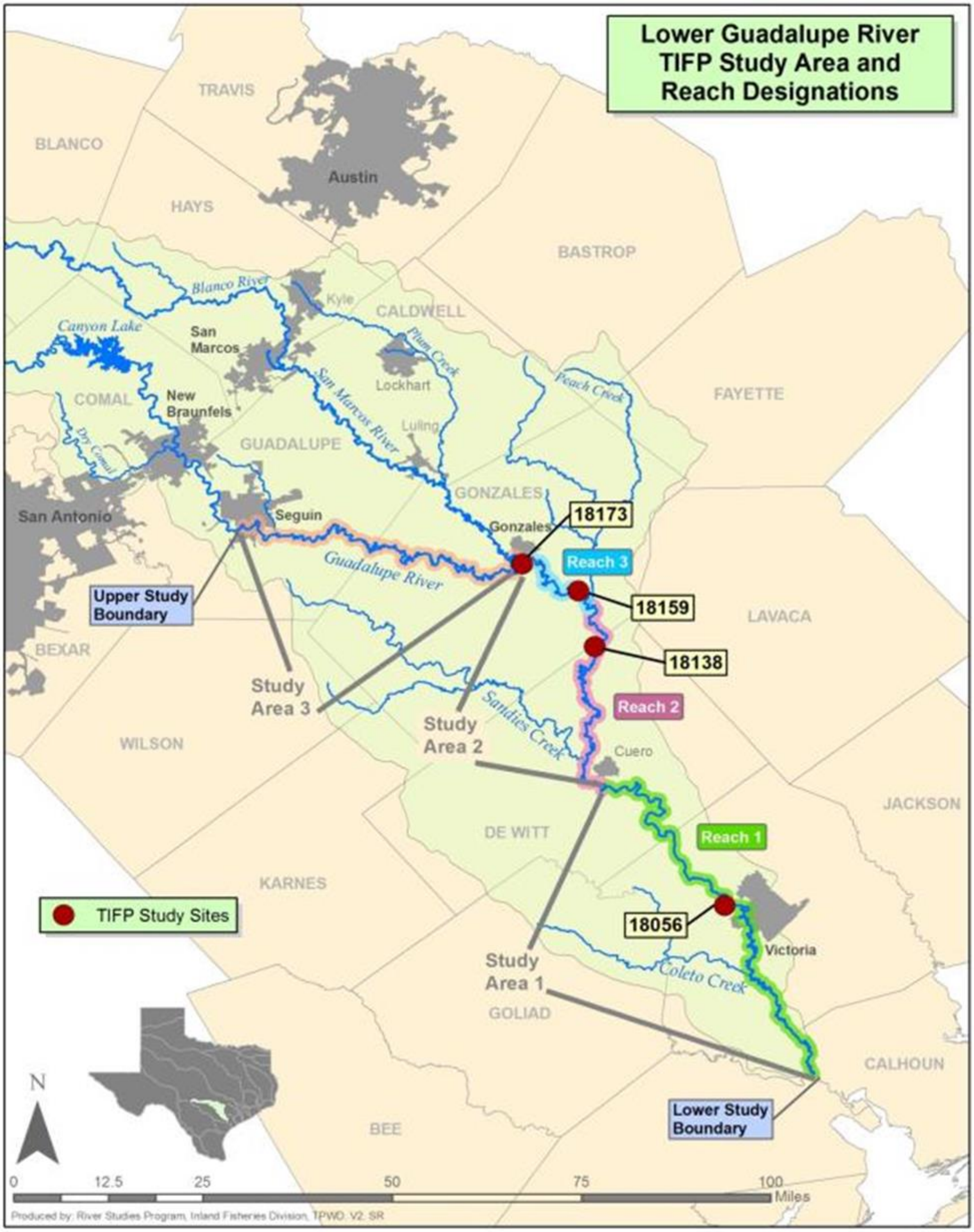

FIGURE 1.—Study area of the lower Guadalupe River Instream Flow Study. Study sites 18173, 18138, and 18056 were sampled for benthic macroinvertebrates and habitat use data.

Biotic and habitat data collection.—Aquatic benthic macroinvertebrate samples and associated habitat variables were collected in a single riffle at each of the three study sites. Four microhabitats (shallow-fast, shallow-slow, deep-fast, and deep-slow) within each riffle were identified based on visual examination. Two pairs (total of four) of macroinvertebrate samples were taken in each microhabitat using a 0.1 m^2 Hess sampler. Riffles were sampled during "high" (50^{th} percentile discharge) and "medium" base flow conditions (30^{th} percentile discharge). Due to continuously high flow conditions, samples could not be taken at "low" flow conditions (15^{th} percentile discharge) during the study period. Current velocity measurements included mean column velocity and bottom/bed velocity. Depth was measured with a top-setting (wading) rod. Substrate size was categorized visually at each site according to a modified Wentworth scale (Wentworth 1922; TIFP 2008). Water quality data were collected at each riffle using a multi-parameter water quality instrument. Table 1 includes discharge and sampling dates for each site. Macroinvertebrate samples were identified under a stereomicroscope (Nikon SMZ745T) using relevant taxonomic keys (Thorp and Covich 2001; Merritt et al. 2008; Wiggins 2009). Non-insect taxa were identified to order and all other taxa were identified to genus, except Diptera, which were identified to family (e.g., Chironomidae).

TABLE 1.—Sampling dates and discharge (cubic feet per second [cfs] and cubic meters per second [cms]) for benthic macroinvertebrate data collected at each study site and base flow condition.

Study Site	Date	Flow Condition	Discharge (cfs)	Discharge (cms)
Gonzales	9/23/2015	High	707	20.0
Hochheim	9/9/2015	High	701	19.9
Victoria	8/21/2015	High	979	27.7
Gonzales	7/20/2017	Medium	413	11.7
Hochheim	7/31/2017	Medium	544	15.4
Victoria	7/20/2017	Medium	653	18.5

Habitat suitability criteria development.—Abundance of macroinvertebrates per sample-habitat pair and associated environmental data (depth and velocity) were used to generate HSCs for depth and velocity (N=72). During the medium flow condition in 2017, only one habitat data set (velocity, depth, substrate) was collected per pair of macroinvertebrate samples as they were proximally located. For those pairs, mean abundance of the two samples was calculated which reduced the total number of samples to 24 for the 2017 dataset. Also, bottom velocity was not collected in 2017.

Two different analytical curve development approaches were undertaken. The first approach utilized non-parametric tolerance limits (NPTL; $\alpha = 0.95$; Sommerville 1958) to construct habitat suitability criteria where the tolerance limits for the central 50% of observed values were assigned a suitability value of one. The data located between the central 50% tolerance limits and the central 75% were assigned a suitability value of 0.5. The data located between the central 75% tolerance limits and the central 90% assigned a suitability value of 0.2. The data beyond the central 90% tolerance limit received a suitability of zero and are considered to represent unsuitable habitat. A second approach utilized probability density functions (PDFs) following Som et al. (2015) using

R statistical package (R Core Team 2017). Based on a review of the frequency histogram characteristics, several candidate PDFs were selected (e.g., Exponential, Rayleigh, Gamma, and Rice). We stress that we do not use histograms for the estimation of parameters or actual fitting of the HSC curves (see Som et al., 2015). Full mathematical descriptions of these PDFs can be found in Asquith (2014). The final PDF was selected based on the minimum of the computed negative log likelihood functions. We estimated the 95 percent confidence intervals for the fitted PDFs based on a bootstrap routine that estimates the PDF parameters with a resampled data set (1000 samples) and computes the HSC upper and lower bounds over the range of the data (see Som et al. 2015).

Results

Benthic macroinvertebrate communities were represented by 12 orders, 40 families, and 74 genera. Insufficient sample sizes precluded development of HSC curves specific to each study site, riffle microhabitat (i.e., deep-slow, deep-fast, etc), or flow condition; therefore, data from each site, microhabitat, and base flow condition were pooled. Utilizing the pooled hydraulic (depth and velocity) and biotic data (Table 2), we developed HSC for depth and velocity for the total macroinvertebrate community and seven taxa or guilds with sufficient observations (i.e., collected at each site and flow condition):

- all benthic macroinvertebrate taxa;
- Ephemeropta (mayflies);
- elmids (riffle beetles);
- chironomids (midges);
- hydropsychids (net-spinning caddisflies);
- *Tricorythodes* sp. (mayfly);
- *Corydalus* sp. (dobsonfly); and
- *Neoperla* sp. (stonefly).

Nonparametric tolerance limit and PDF-based HSC for all analyzed taxa and guilds are provided in Appendix A. The HSC derived from pooled data represent initial curves for the lower Guadalupe River study area to be augmented with additional samples from other sites and flow conditions.

Water quality and habitat data collected from each microhabitat at each site are provided in Appendix B.

TABLE 2.—Pooled hydraulic (depth and mean column current velocity) and macroinvertebrate data used for habitat suitability criteria development. Total benthic macroinvertebrate community = Total BMI.

Depth (m)	Velocity (m/s)	Total BMI	Ephemeroptera Guild	Elmid Guild	Chironomid Guild	Hydropsychid Guild	*Tricorythodes*	*Corydalus*	*Neoperla*
0.80	0.96	580	23	40	13	199	10	1	10
0.70	0.53	135	1	23	1	41	0	2	0
0.55	0.78	152	21	0	4	40	2	0	4
0.60	1.05	656	23	43	24	242	1	0	0
0.45	0.03	269	16	26	131	4	25	0	0
0.50	0.00	36	0	4	15	1	7	0	0
0.60	0.06	181	33	0	97	1	2	0	0
0.62	0.06	108	12	1	48	1	18	0	1
0.22	0.63	4585	873	235	149	956	101	14	9
0.30	1.00	3570	569	164	103	850	109	4	11
0.20	0.81	2530	477	87	117	525	76	12	8
0.25	0.85	666	203	40	26	54	19	2	4
0.26	0.75	535	54	10	190	13	144	0	4
0.50	0.70	104	6	7	53	0	17	0	0
0.16	0.39	924	149	57	167	80	123	0	8
0.13	0.38	96	0	0	11	0	5	0	76
0.85	0.85	336	71	28	93	0	23	1	11
0.85	0.86	258	52	41	0	0	8	0	16
0.82	0.85	497	179	36	0	1	26	0	20
0.85	0.86	168	37	23	21	2	5	0	9
0.76	0.30	110	0	3	85	0	8	0	0
0.88	0.39	153	30	6	51	0	19	0	1
0.76	0.08	253	5	5	117	0	81	0	0
0.76	0.36	351	94	18	56	7	25	0	10
0.18	0.42	470	135	10	62	8	64	2	2
0.24	0.57	1028	331	46	69	21	91	4	9

Depth (m)	Velocity (m/s)	Total BMI	Ephemeroptera Guild	Elmid Guild	Chironomid Guild	Hydropsychid Guild	*Tricorythodes*	*Corydalus*	*Neoperla*
0.18	0.67	942	329	48	73	7	43	9	22
0.27	0.77	473	172	3	29	2	25	4	17
0.43	0.30	206	40	7	33	1	40	0	2
0.46	0.32	330	58	6	54	0	112	0	6
0.37	0.01	71	7	0	42	0	8	2	1
0.40	0.01	194	5	9	85	1	66	0	0
0.60	0.46	98	5	12	5	0	49	0	1
0.57	0.52	77	3	1	5	0	1	0	7
0.52	0.99	62	19	0	0	8	2	0	1
0.60	0.94	133	37	0	1	5	3	0	21
0.60	0.03	281	12	2	23	1	6	0	0
0.73	0.07	22	2	3	2	0	2	1	0
0.49	0.04	358	1	7	92	0	74	1	2
0.60	0.15	160	39	1	10	0	54	0	3
0.21	0.75	649	68	40	122	66	49	2	71
0.27	0.89	50	11	6	0	0	2	1	10
0.03	0.36	582	42	104	162	14	42	7	42
0.03	0.00	162	14	17	68	1	13	3	5
0.09	0.00	210	30	21	39	5	25	0	7
0.12	0.00	246	18	54	3	2	61	0	7
0.15	0.19	313	2	0	113	0	132	0	3
0.21	0.00	355	29	31	82	0	97	0	4
0.50	1.45	666	211	26	4	71	2	11	4
0.50	1.28	545	135	63	2	32	13	19	1
0.50	0.48	145	23	10	31	3	16	0	1
0.51	0.52	168	43	19	9	5	7	0	1
0.30	0.72	664	156	123	0	17	23	9	2
0.31	1.20	303	118	15	0	8	1	2	2
0.31	0.21	102	6	11	37	0	9	0	0

Depth (m)	Velocity (m/s)	Total BMI	Ephemeroptera Guild	Elmid Guild	Chironomid Guild	Hydropsychid Guild	*Tricorythodes*	*Corydalus*	*Neoperla*
0.31	0.21	45	3	4	6	0	7	0	1
0.40	1.07	276	64	16	26	10	30	7	4
0.43	0.51	184	40	12	19	6	26	2	8
0.49	0.01	31	2	1	8	0	7	0	0
0.52	0.12	82	0	3	42	0	21	0	0
0.21	0.85	470	124	62	10	13	10	15	8
0.21	0.56	252	46	46	5	3	6	6	31
0.24	0.05	103	3	6	45	0	25	0	0
0.27	0.08	91	1	6	36	0	27	0	1
0.49	1.12	286	71	19	7	31	11	2	4
0.12	0.43	1074	75	103	31	300	3	4	49
0.50	0.06	118	12	20	6	10	10	0	10
0.51	0.14	32	5	1	6	4	4	0	2
0.40	0.23	302	38	16	3	80	10	0	7
0.52	0.95	473	64	56	8	83	14	4	18
0.20	0.01	56	1	4	22	1	11	0	1
0.31	0.15	10	0	0	7	0	1	0	1

Discussion

Habitat suitability criteria derived from this study broadly show similar relationships between depth and velocity reported in the literature for representative taxa (e.g., Orth and Maughan 1983; Jowett et al. 1991; Ritchie 2000; Thirion 2016). The nonparametric tolerance limit approach consistently produces a broader area of high suitability ranges (i.e., 1.0) given that the underlying mathematics is based on the rank order of the data where at least 50 percent of the data are contained within these minimum and maximum rank order values. This contrasts with the PDF-derived HSC which are generally more compressed with only a single 'optimal' suitability 1.0) value. An examination of the PDF curves in Appendix A show that the associated upper and lower confidence limits are relatively narrow and reflect the apparent higher 'N' values based on use of the abundance weighted depth and velocity values. Differences between NPTL and PDF curves suggest that NPTL curves will generally be somewhat less sensitive to small changes in depth and velocity magnitudes across the 1.0 'optimal' range which encompasses the central 50% of the data. Conversely, PDF curves will generally show a larger incremental change in suitability over changes in depth and velocity for values that encompass the central 50% of observed values. One advantage to the PDF curves is that the upper and lower confidence intervals can be utilized as a mechanism to incorporate the inherent uncertainty in deriving HSC from noisy observation data. A direct comparison between the two analytical approaches (see Figures A-1 and A-9) underscore these differences. In the case of the depth HSC for total macroinvertebrate community, the PDF curve would result in an estimated suitability of approximately 0.3 at a depth of 0.5 m while the corresponding suitability of this depth for the NPTL curve is 1.0. We stress that both the NPTL and PDF curves represent valid statistical approaches and selection of the specific methodology should be carefully considered or at a minimum, application of both derived HSC employed as a measure of uncertainty in the underlying representation of the depth and velocity resource functions. Texas Parks and Wildlife Department and partners will continue to collect habitat suitability data when low base flow conditions occur in the lower Guadalupe River and seek to gather additional HSC data from other locations that were collected with similar sampling designs to augment aquatic benthic macroinvertebrates habitat suitability criteria for the lower Guadalupe River. Once a complete dataset is obtained, statistical analyses will examine patterns in microhabitat utilization to identify key species (e.g., flow-sensitive species/taxa/guilds) for use in instream habitat modeling.

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APPENDIX A.—Habitat Suitability Curves for Aquatic Benthic Macroinvertebrates from the Lower Guadalupe River.

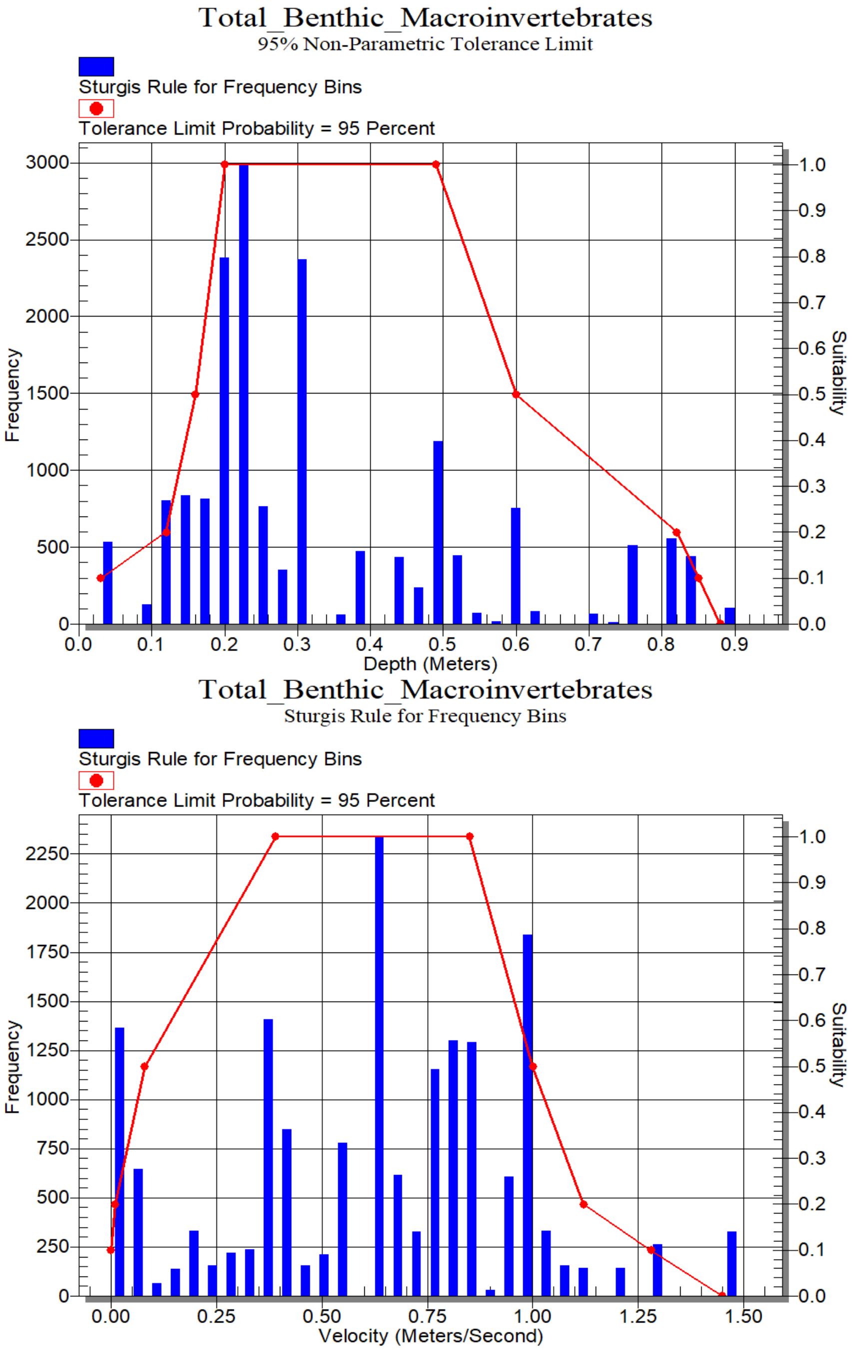


FIGURE A.1.—Nonparametric tolerance limits for depth and velocity for the total benthic macroinvertebrate community.

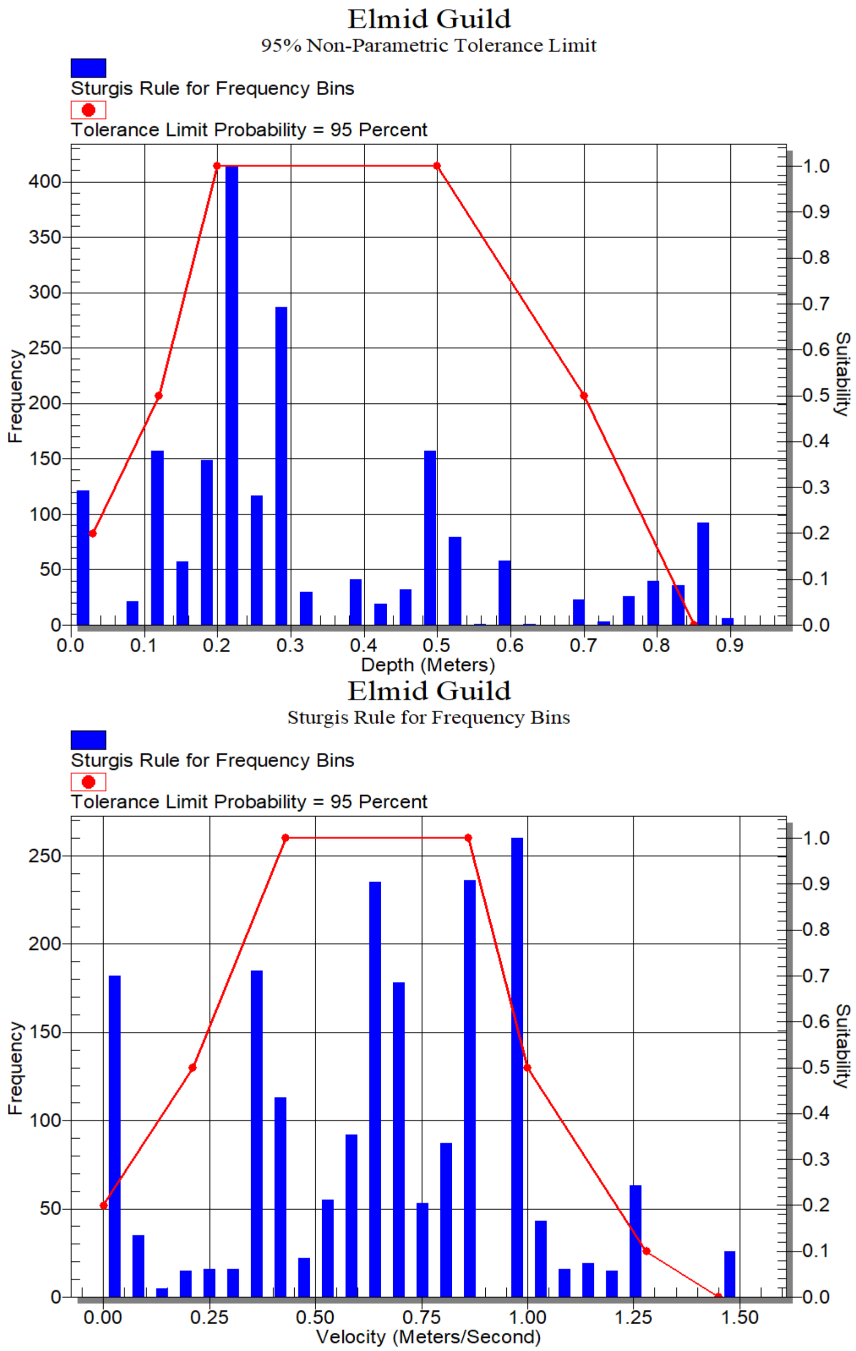


FIGURE A.2.—Nonparametric tolerance limits for depth and velocity for the Elmid Guild.

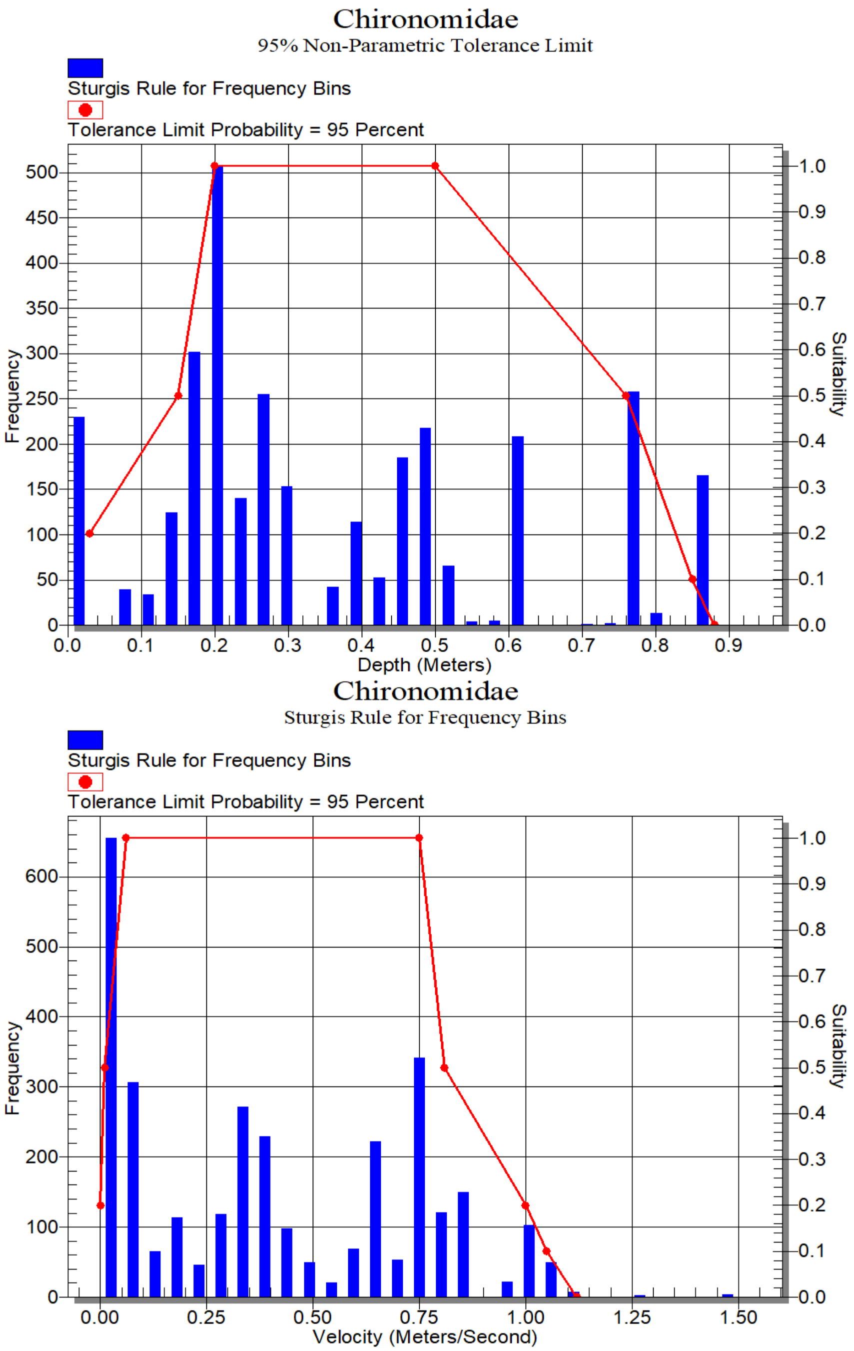


FIGURE A.3.—Nonparametric tolerance limits for depth and velocity for the Chironomid Guild.

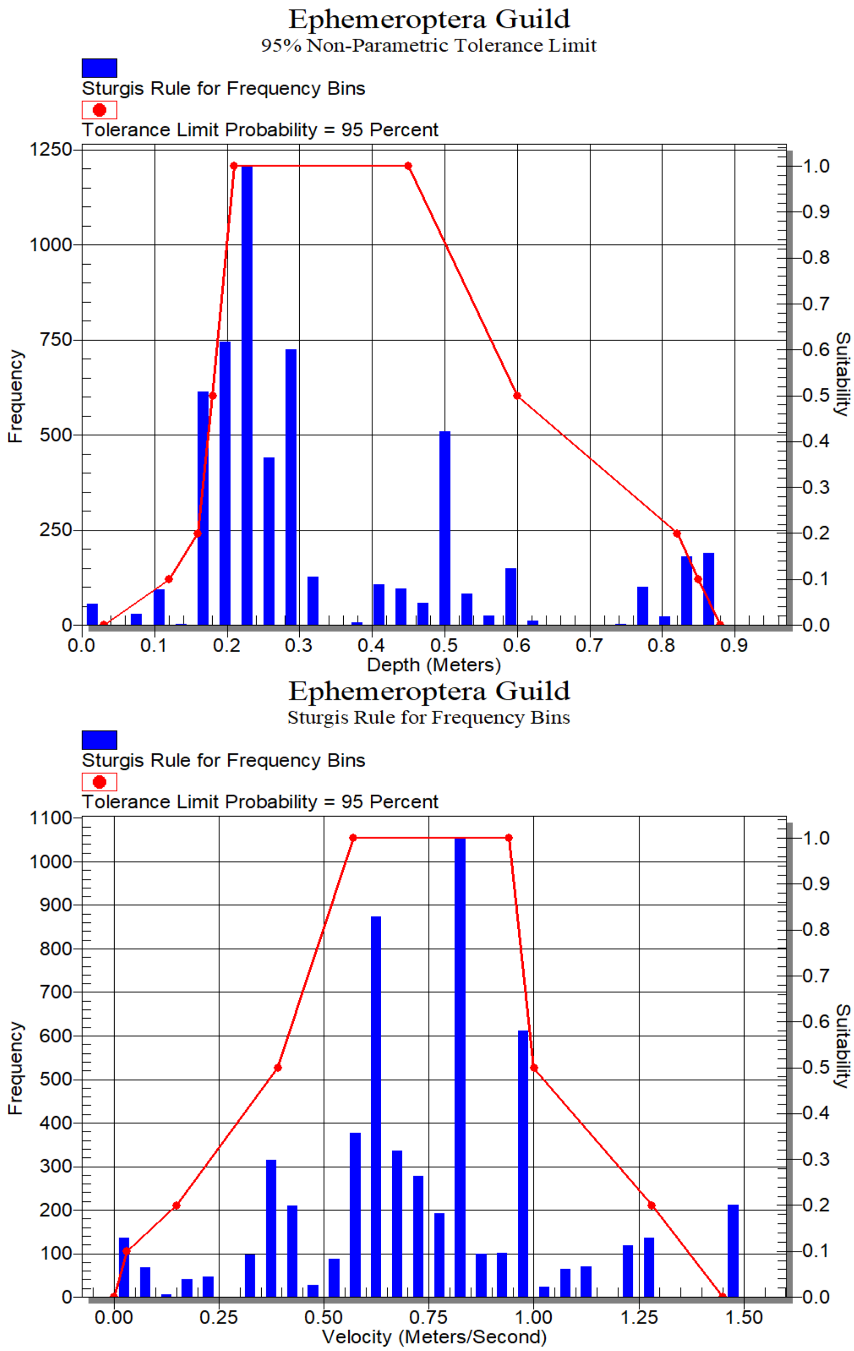


FIGURE A.4.—Nonparametric tolerance limits for depth and velocity for Ephemeroptera Guild.

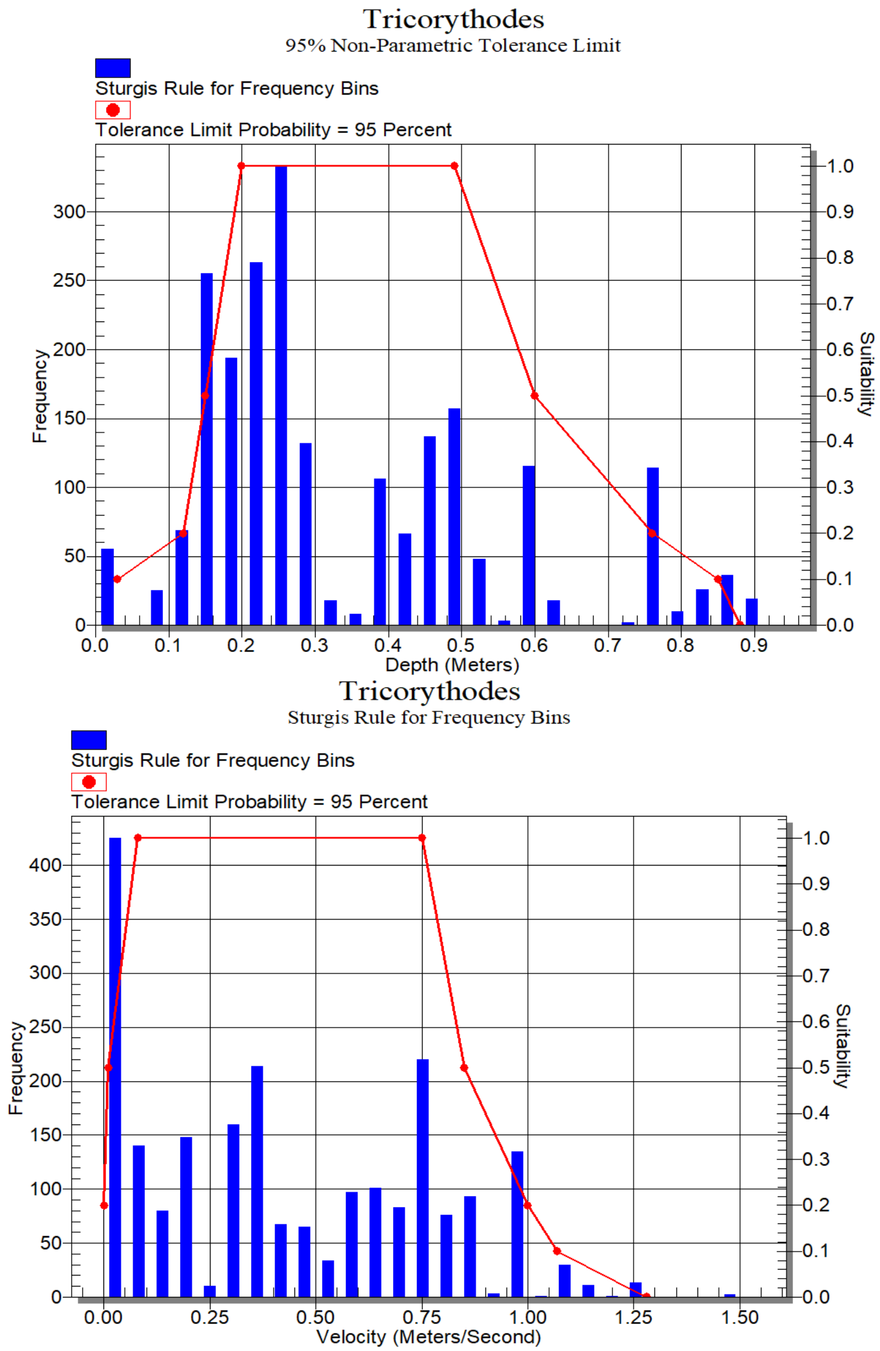


FIGURE A.5.—Nonparametric tolerance limits for depth and velocity for *Tricorythodes*.

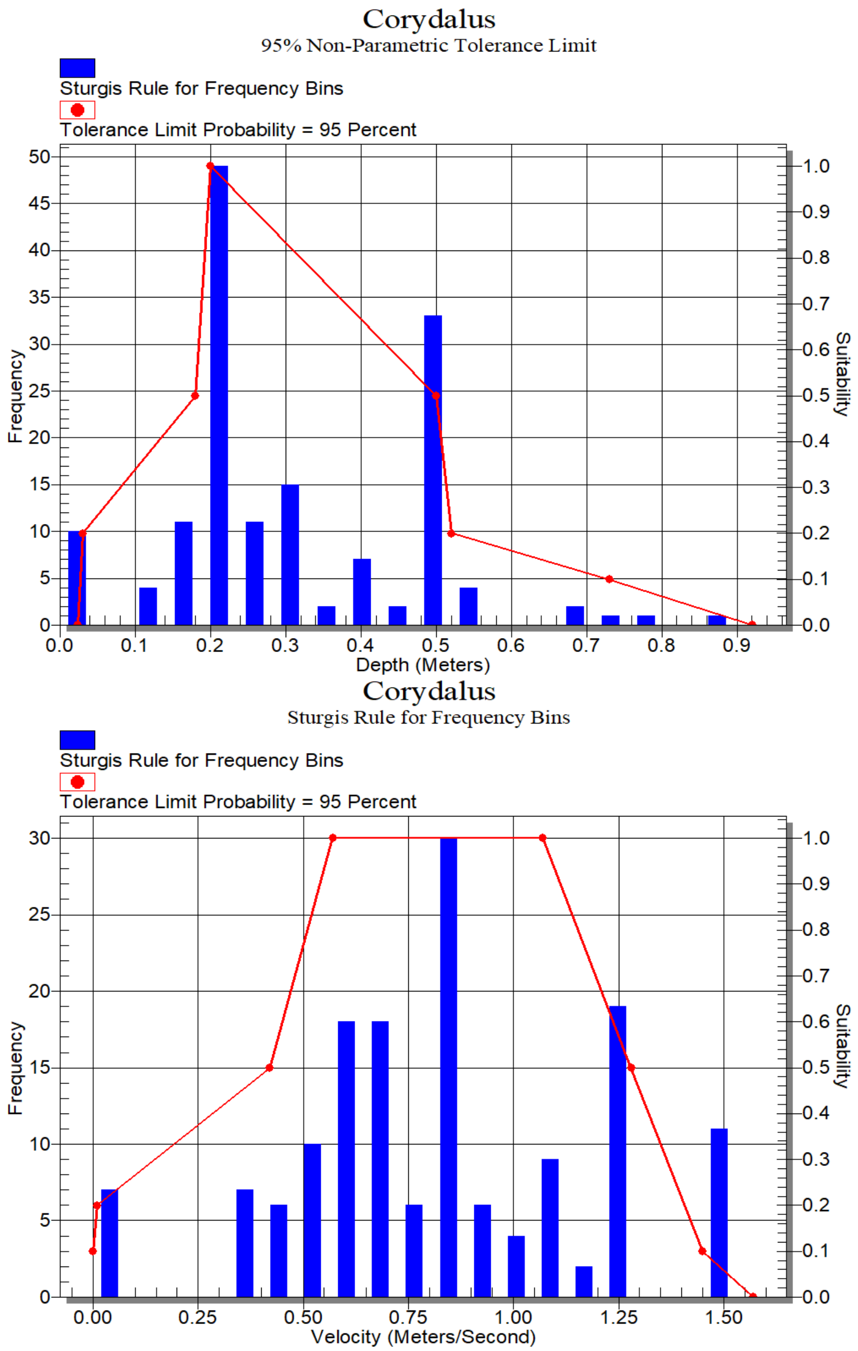


FIGURE A.6.—Nonparametric tolerance limits for depth and velocity for *Corydalus*.

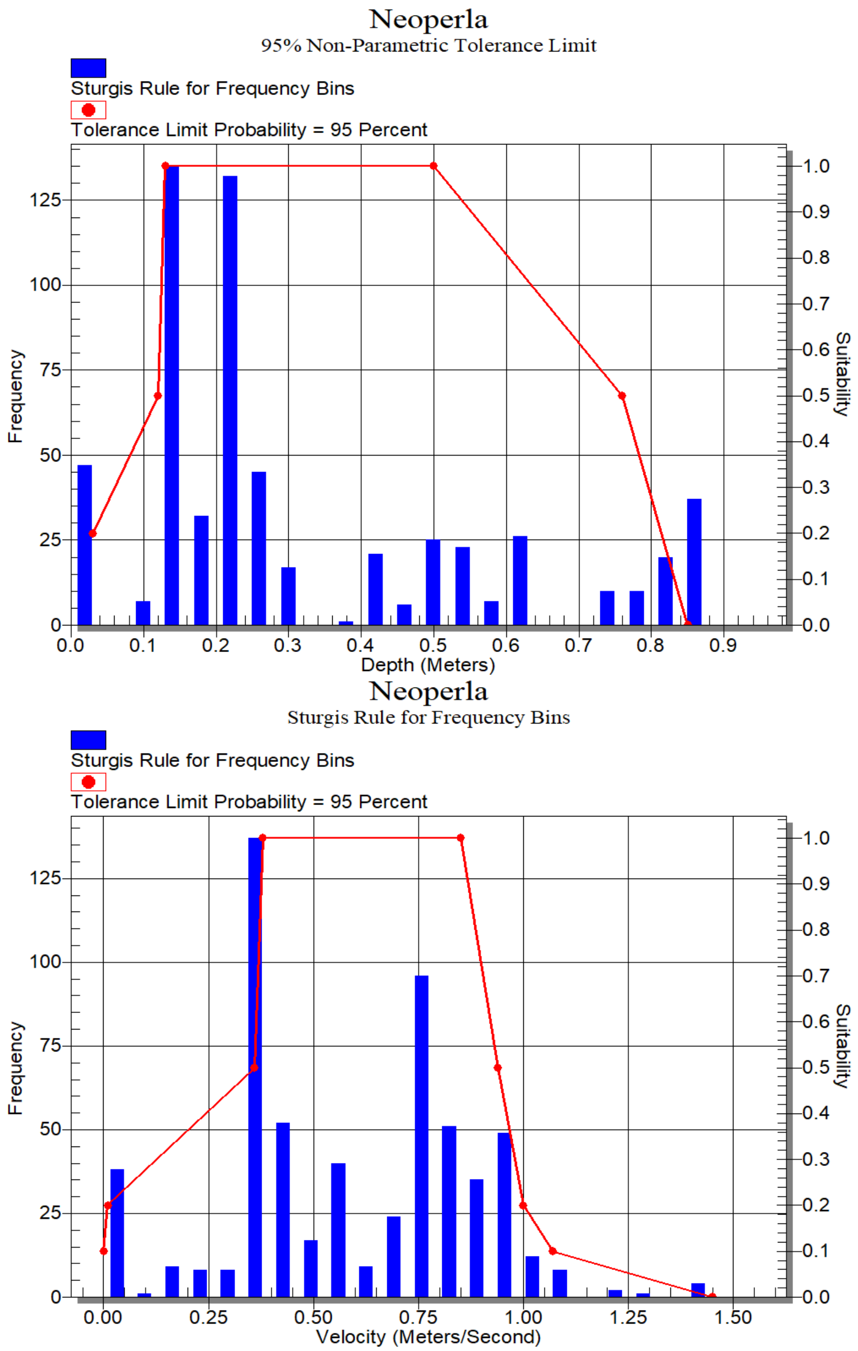


FIGURE A.7.—Nonparametric tolerance limits for depth and velocity for *Neoperla*.

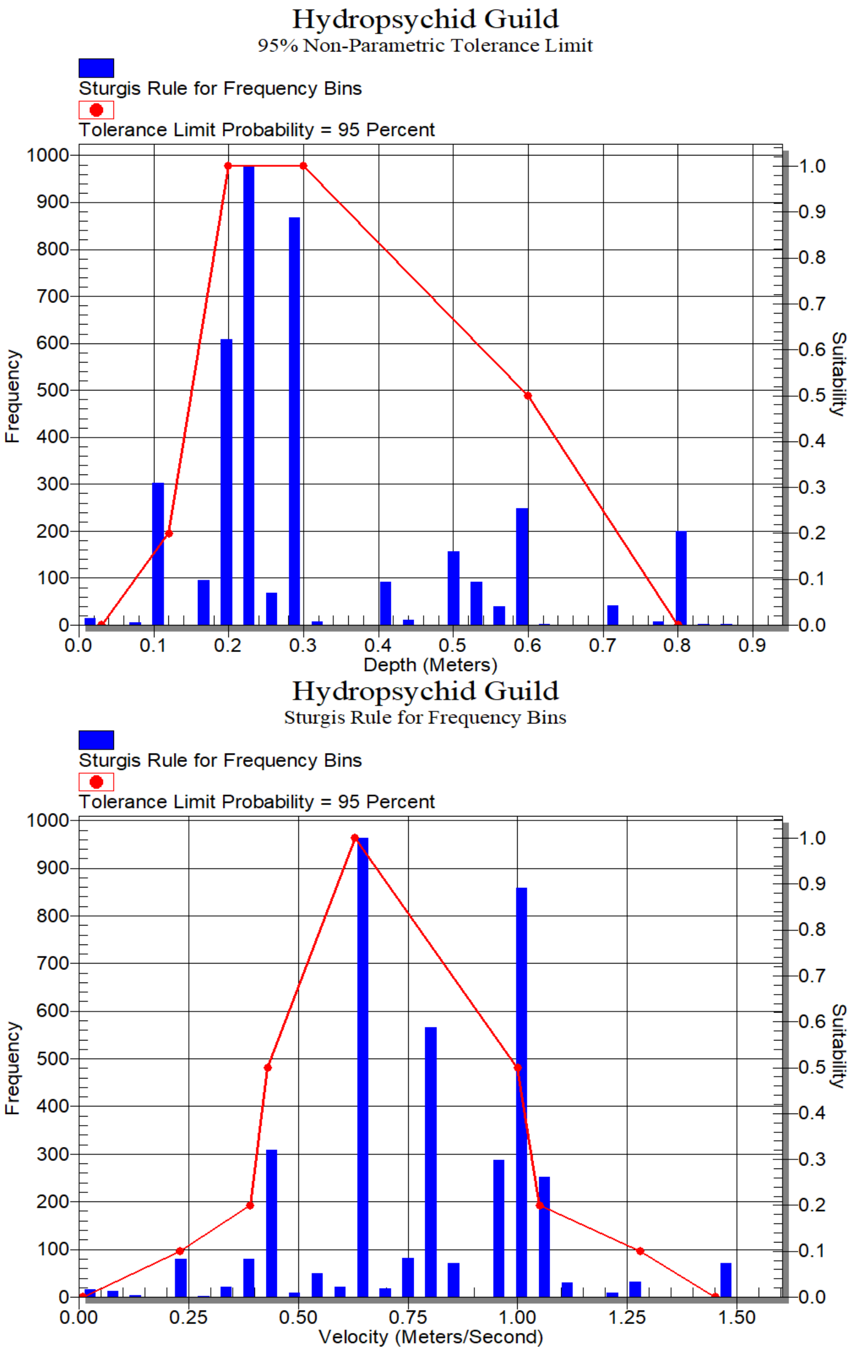
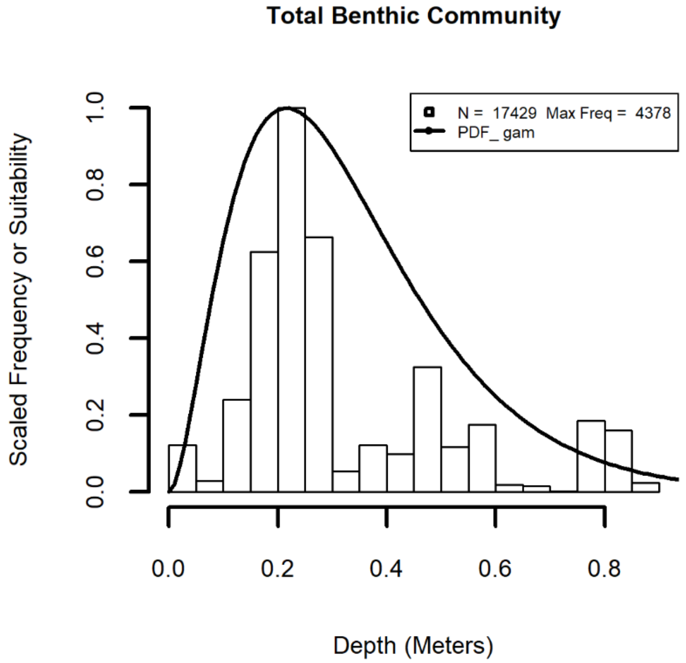


FIGURE A.8.—Nonparametric tolerance limits for depth and velocity for the Hydropsychid Guild.



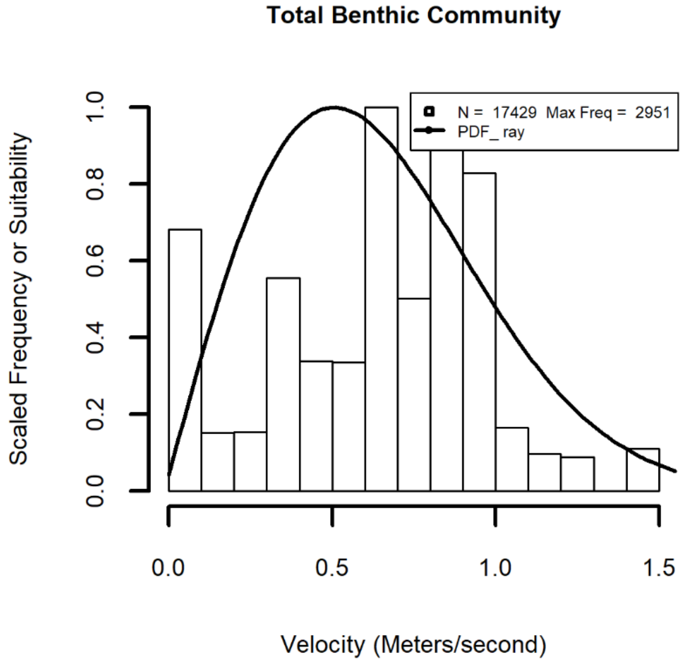
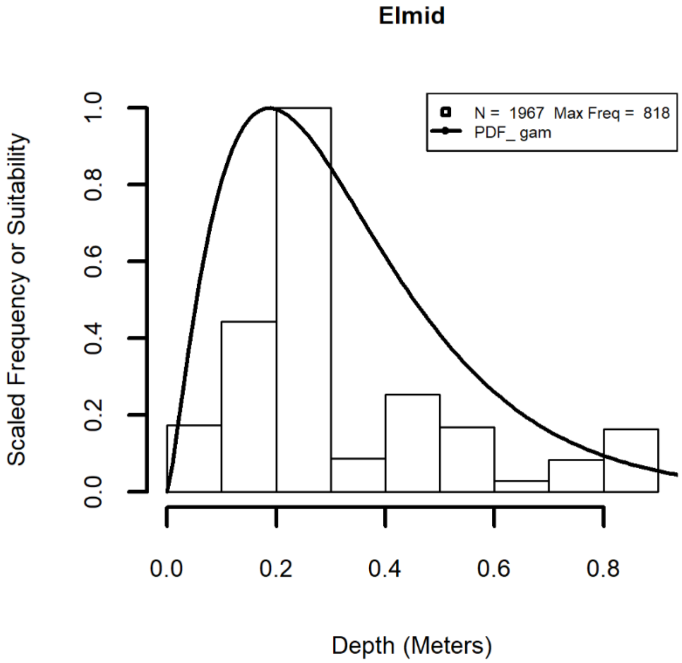


FIGURE A.9.—Probability density function HSC for depth and velocity for the total benthic macroinvertebrate community.



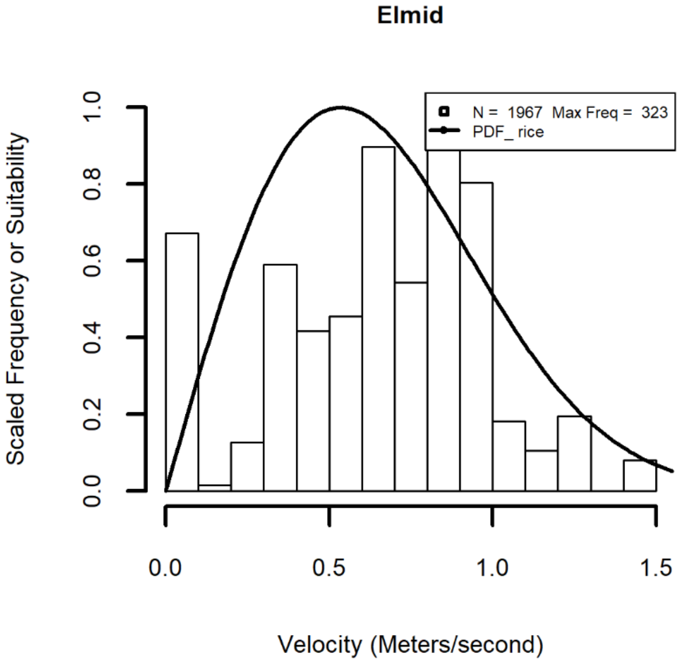


FIGURE A.10.—Probability density function HSC for depth and velocity for the Elmid Guild.

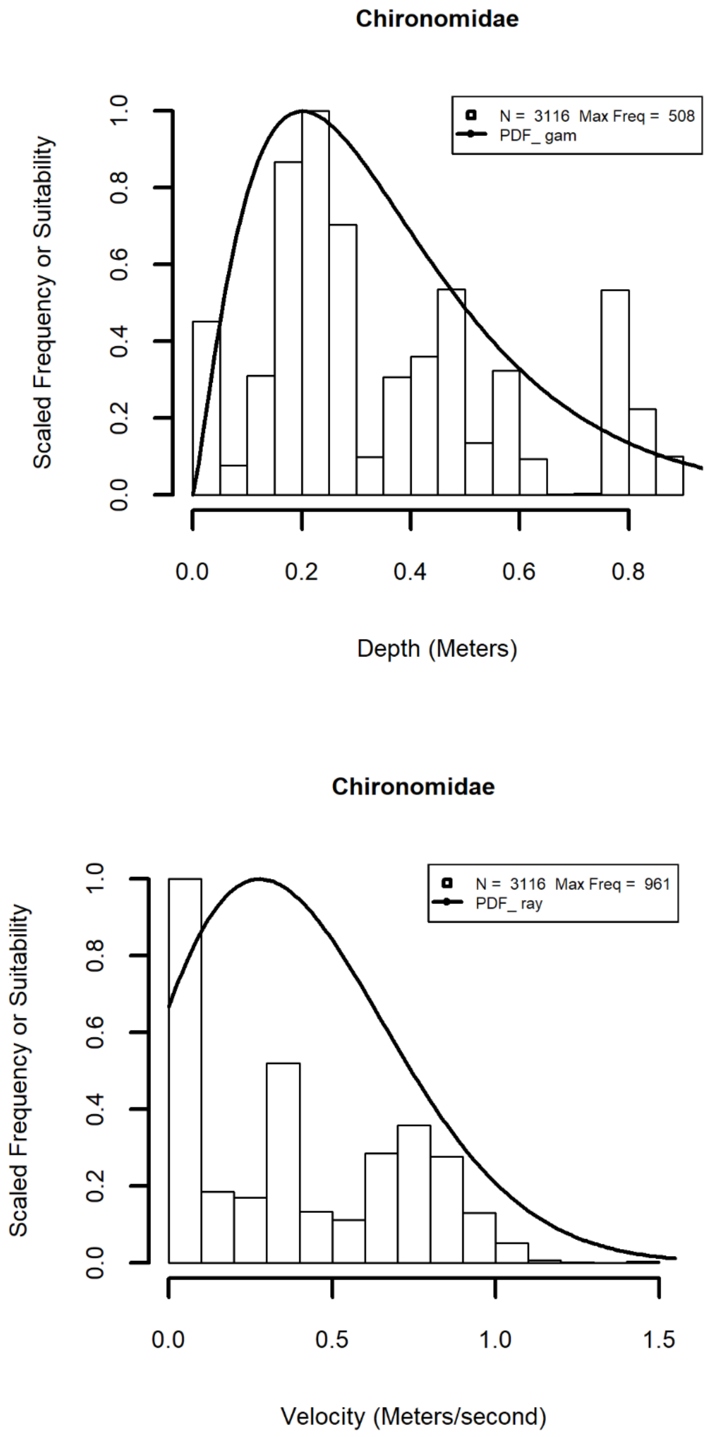
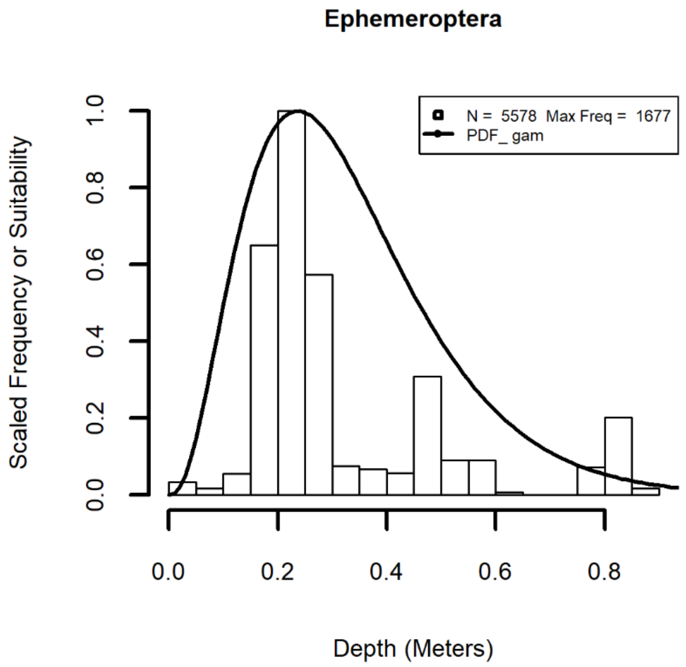


FIGURE A.11.—Probability density function HSC for depth and velocity for the Chironomid Guild.



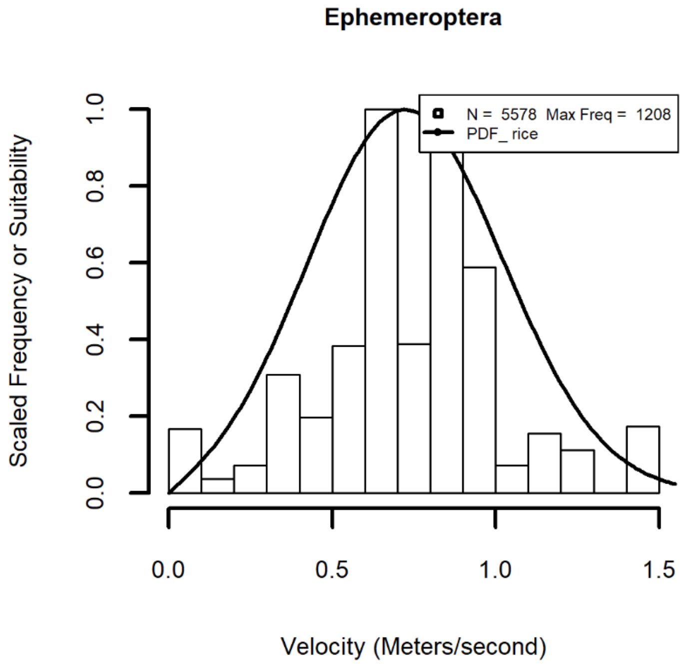


FIGURE A.12.—Probability density function HSC for depth and velocity for the Ephemeroptera Guild.

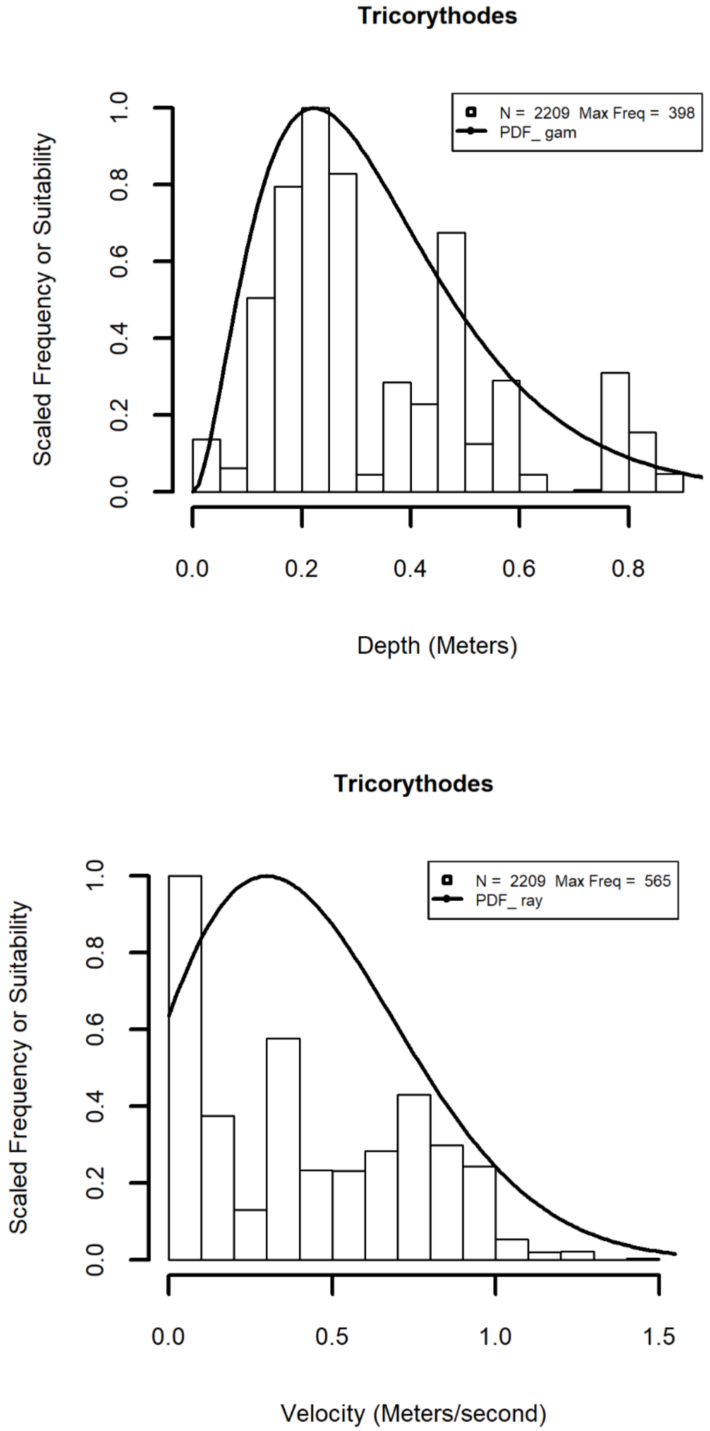
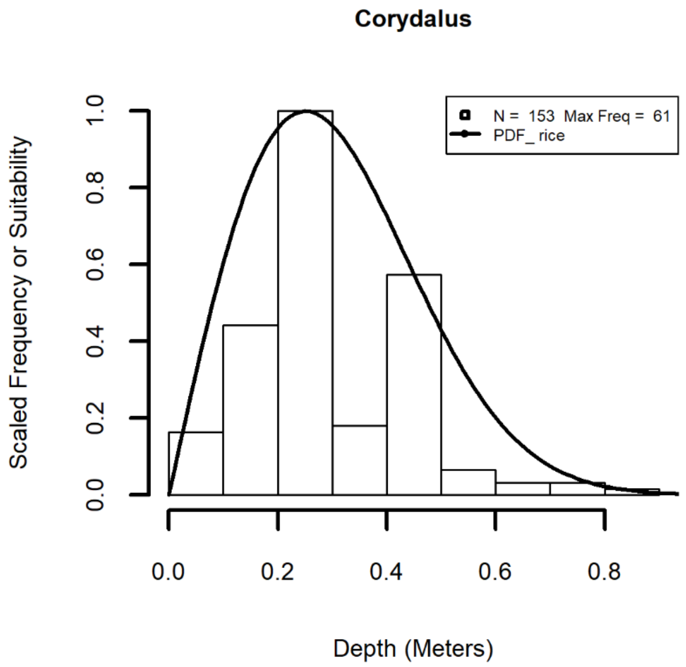


FIGURE A.13.—Probability density function HSC for depth and velocity for *Tricorythodes*.



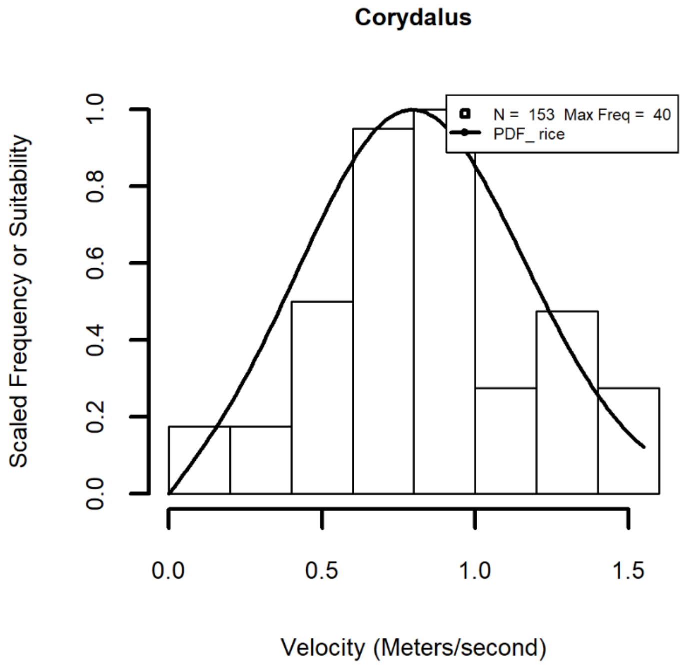
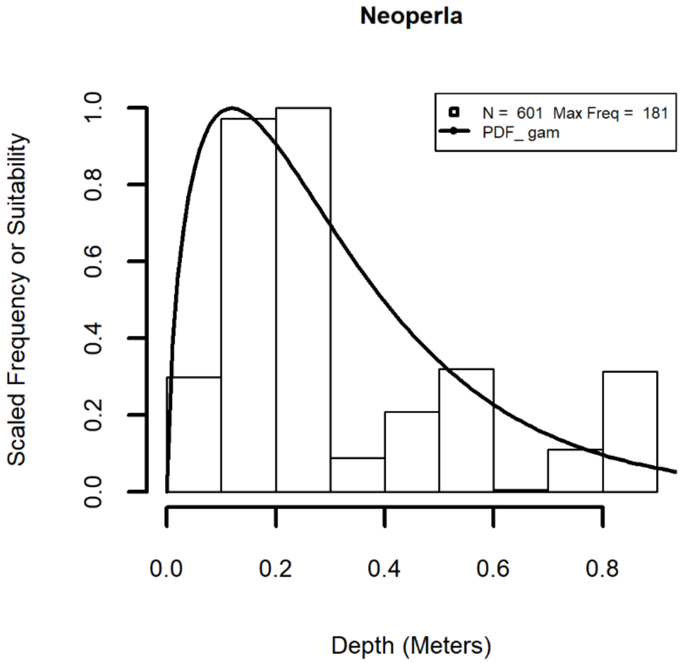


FIGURE A.14.—Probability density function HSC for depth and velocity for *Corydalus*.



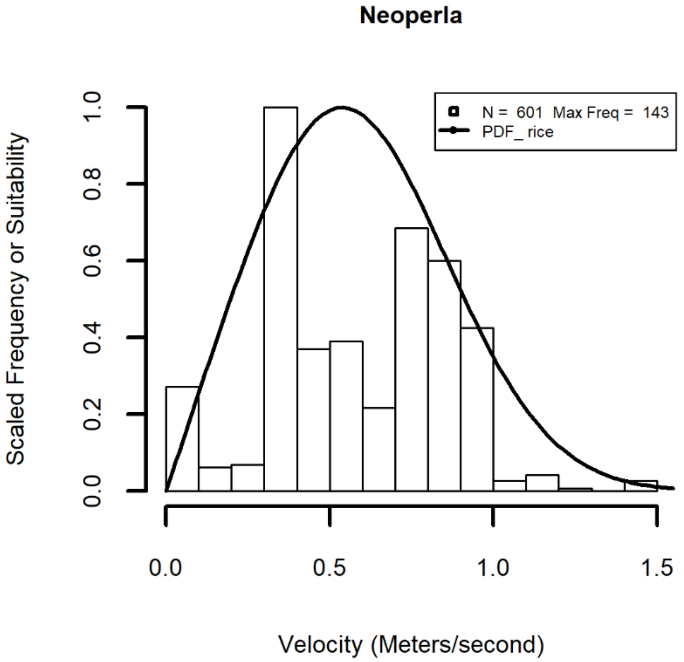


FIGURE A.15.—Probability density function HSC for depth and velocity for *Neoperla*.

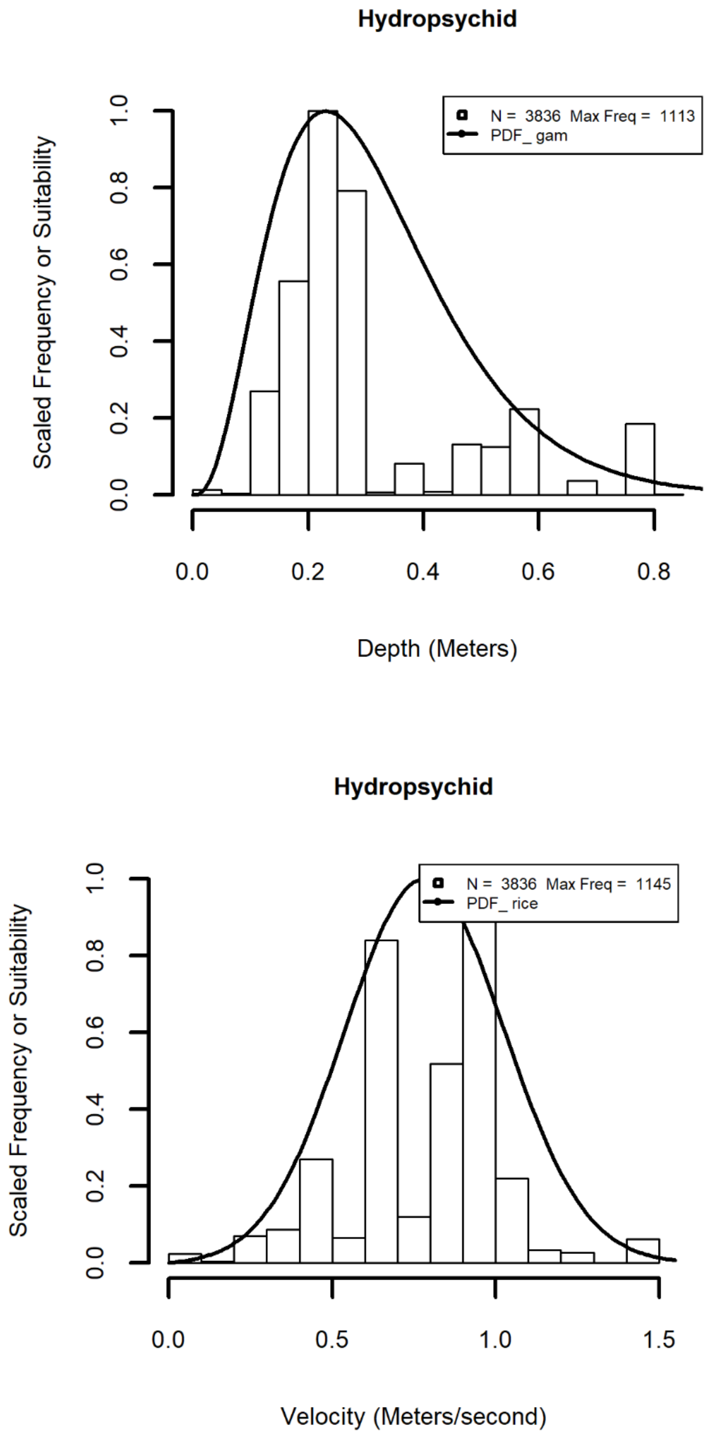


FIGURE A.16.—Probability density function HSC for depth and velocity for the Hydropsychid Guild.

APPENDIX B.—Water quality and habitat data collected for each microhabitat at each site.

TABLE B.1—Water quality, habitat, and macroinvertebrate guild data from the Gonzales study site on the Guadalupe River. Microhabitats are abbreviated as: deep-fast (D-F), deep-slow (D-S), shallow-fast (S-F), and shallow-slow (S-S). Parameters are abbreviated as: specific conductivity (Cond), water temperature (Temp), dissolved oxygen (DO), bottom/streambed current velocity (V10), mean column current velocity (V), primary substrate (Sub 1), secondary substrate (Sub 2) and percent embeddedness (Emb). Taxa and guilds are abbreviated as: total benthic macroinvertebrate community (Total BMI), elmid guild (ElmG), chironomid guild (ChiG), Ephemeroptera guild (EphG), *Tricorythodes* (Tric), *Corydalus* (Cory), *Neoperla* (Neop), and Hydropsychid guild (HydG).

Date	Micro-habitat	Rep	Cond (µS/cm)	Temp (˚C)	DO (mg/L)	pH	Depth (m)	V10 (m/s)	V (m/s)	Sub 1	Sub 2	Emb (%)	Total BMI	ElmG	ChiG	EphG	Tric	Cory	Neop	HydG
9/23/15	D-F	1A	504	31.6	4.2	8.14	0.80	0.27	0.96	N/A	N/A	N/A	580	40	13	23	10	1	10	199
9/23/15	D-F	1B	504	31.6	4.2	8.14	0.70	0.22	0.53	N/A	N/A	N/A	135	23	1	1	0	2	0	41
9/23/15	D-F	2A	501	31.6	4.1	8.16	0.55	0.38	0.78	N/A	N/A	N/A	152	0	4	21	2	0	4	40
9/23/15	D-F	2B	501	31.6	4.1	8.16	0.60	0.98	1.05	N/A	N/A	N/A	656	43	24	23	1	0	0	242
9/23/15	D-S	1A	503	31.6	2.4	8.26	0.45	0.00	0.03	N/A	N/A	N/A	269	26	131	16	25	0	0	4
9/23/15	D-S	1B	503	31.6	2.4	8.26	0.50	0.00	0.00	N/A	N/A	N/A	36	4	15	0	7	0	0	1
9/23/15	D-S	2A	500	31.6	2.4	8.30	0.60	0.00	0.06	N/A	N/A	N/A	181	0	97	33	2	0	0	1
9/23/15	D-S	2B	500	31.6	2.4	8.30	0.62	0.00	0.06	N/A	N/A	N/A	108	1	48	12	18	0	1	1
9/23/15	S-F	1A	500	31.4	2.9	8.22	0.22	N/A	0.63	N/A	N/A	N/A	4585	235	149	873	101	14	9	956
9/23/15	S-F	1B	500	31.4	2.9	8.22	0.30	N/A	1.00	N/A	N/A	N/A	3570	164	103	569	109	4	11	850
9/23/15	S-F	2A	500	31.4	2.9	8.22	0.20	N/A	0.81	N/A	N/A	N/A	2530	87	117	477	76	12	8	525
9/23/15	S-F	2B	500	31.4	2.9	8.22	0.25	N/A	0.85	N/A	N/A	N/A	666	40	26	203	19	2	4	54
9/23/15	S-S	1A	504	31.5	2.7	8.24	0.26	0.20	0.75	N/A	N/A	N/A	535	10	190	54	144	0	4	13
9/23/15	S-S	1B	504	31.5	2.7	8.24	0.50	0.68	0.70	N/A	N/A	N/A	104	7	53	6	17	0	0	0
9/23/15	S-S	2A	503	31.4	2.5	8.23	0.16	0.31	0.39	N/A	N/A	N/A	924	57	167	149	123	0	8	80
9/23/15	S-S	2B	503	31.4	2.5	8.23	0.13	0.33	0.38	N/A	N/A	N/A	96	0	11	0	5	0	76	0
7/20/17	D-F	1	524	32.0	7.0	6.95	0.50	N/A	1.45	M. Gravel	Cobble	N/A	666	26	4	211	2	11	4	71
7/20/17	D-F	2	524	32.0	7.0	6.95	0.50	N/A	1.28	Cobble	L. Gravel	N/A	545	63	2	135	13	19	1	32
7/20/17	D-S	5	524	32.0	7.0	6.95	0.50	N/A	0.48	L. Gravel	Silt	N/A	145	10	31	23	16	0	1	3
7/20/17	D-S	6	524	32.0	7.0	6.95	0.51	N/A	0.52	L. Gravel	M. Gravel	N/A	168	19	9	43	7	0	1	5
7/20/17	S-F	3	524	32.0	7.0	6.95	0.30	N/A	0.72	L. Gravel	M. Gravel	N/A	664	123	0	156	23	9	2	17
7/20/17	S-F	4	524	32.0	7.0	6.95	0.31	N/A	1.20	L. Gravel	Cobble	N/A	303	15	0	118	1	2	2	8
7/20/17	S-S	7	524	32.0	7.0	6.95	0.31	N/A	0.21	Cobble	L. Gravel	N/A	102	11	37	6	9	0	0	0
7/20/17	S-S	8	524	32.0	7.0	6.95	0.31	N/A	0.21	L. Gravel	Cobble	N/A	45	4	6	3	7	0	1	0

TABLE B.2—Water quality, habitat, and macroinvertebrate guild data from the Hochheim study site on the Guadalupe River. Microhabitats are abbreviated as: deep-fast (D-F), deep-slow (D-S), shallow-fast (S-F), and shallow-slow (S-S). Parameters are abbreviated as: specific conductivity (Cond), water temperature (Temp), dissolved oxygen (DO), bottom/streambed current velocity (V10), mean column current velocity (V), primary substrate (Sub 1), secondary substrate (Sub 2) and percent embeddedness (Emb). Taxa and guilds are abbreviated as: total benthic macroinvertebrate community (Total BMI), elmid guild (ElmG), chironomid guild (ChiG), Ephemeroptera guild (EphG), *Tricorythodes* (Tric), *Corydalus* (Cory), *Neoperla* (Neop), and Hydropsychid guild (HydG).

Date	Micro-habitat	Rep	Cond (µS/cm)	Temp (˚C)	DO (mg/L)	pH	Depth (m)	V10 (m/s)	V (m/s)	Sub 1	Sub 2	Emb (%)	Total BMI	ElmG	ChiG	EphG	Tric	Cory	Neop	HydG
9/9/15	D-F	1A	544	30.0	2.8	8.30	0.85	0.27	0.85	L. Gravel	Clay	50	336	28	93	71	23	1	11	0
9/9/15	D-F	1B	544	30.0	2.8	8.30	0.85	0.37	0.86	Clay	M. Gravel	75	258	41	0	52	8	0	16	0
9/9/15	D-F	2A	544	30.0	2.8	8.30	0.82	0.23	0.85	Rubble	L. Gravel	0	497	36	0	179	26	0	20	1
9/9/15	D-F	2B	544	30.0	2.8	8.30	0.85	0.52	0.86	Rubble	L. Gravel	0	168	23	21	37	5	0	9	2
9/9/15	D-S	1A	545	30.0	2.7	8.31	0.76	0.16	0.30	Rubble	M. Gravel	0	110	3	85	0	8	0	0	0
9/9/15	D-S	1B	545	30.0	2.7	8.31	0.88	0.11	0.39	Rubble	Cobble	0	153	6	51	30	19	0	1	0
9/9/15	D-S	2A	545	30.0	2.7	8.31	0.76	0.07	0.08	L. Gravel	Silt	25	253	5	117	5	81	0	0	0
9/9/15	D-S	2B	545	30.0	2.7	8.31	0.76	0.20	0.36	L. Gravel	Silt	25	351	18	56	94	25	0	10	7
9/9/15	S-F	1A	543	30.0	2.7	8.28	0.18	0.16	0.42	Rubble	L. Gravel	75	470	10	62	135	64	2	2	8
9/9/15	S-F	1B	543	30.0	2.7	8.28	0.24	0.41	0.57	M. Gravel	S. Gravel	75	1028	46	69	331	91	4	9	21
9/9/15	S-F	2A	543	30.0	2.7	8.28	0.18	0.41	0.67	M. Gravel	L. Gravel	25	942	48	73	329	43	9	22	7
9/9/15	S-F	2B	543	30.0	2.7	8.28	0.27	0.34	0.77	M. Gravel	S. Gravel	25	473	3	29	172	25	4	17	2
9/9/15	S-S	1A	544	30.0	2.8	8.29	0.43	0.09	0.30	M. Gravel	L. Gravel	50	206	7	33	40	40	0	2	1
9/9/15	S-S	1B	544	30.0	2.8	8.29	0.46	0.15	0.32	L. Gravel	M. Gravel	50	330	6	54	58	112	0	6	0
9/9/15	S-S	2A	544	30.0	2.8	8.29	0.37	0.01	0.01	L. Gravel	Silt	50	71	0	42	7	8	2	1	0
9/9/15	S-S	2B	544	30.0	2.8	8.29	0.40	0.00	0.01	L. Gravel	Silt	50	194	9	85	5	66	0	0	1
7/31/17	D-F	3	495	32.1	7.6	8.10	0.40	N/A	1.07	L. Gravel	Cobble	40	276	16	26	64	30	7	4	10
7/31/17	D-F	4	495	32.1	7.6	8.10	0.43	N/A	0.51	L. Gravel	Cobble	30	184	12	19	40	26	2	8	6
7/31/17	D-S	5	495	32.1	7.6	8.10	0.49	N/A	0.01	L. Gravel	Cobble	80	31	1	8	2	7	0	0	0
7/31/17	D-S	6	495	32.1	7.6	8.10	0.52	N/A	-0.12	L. Gravel	M. Gravel	70	82	3	42	0	21	0	0	0
7/31/17	S-F	1	495	32.1	7.6	8.10	0.21	N/A	0.85	M. Gravel	S. Gravel	20	470	62	10	124	10	15	8	13
7/31/17	S-F	2	495	32.1	7.6	8.10	0.21	N/A	0.56	M. Gravel	S. Gravel	40	252	46	5	46	6	6	31	3
7/31/17	S-S	7	495	32.1	7.6	8.10	0.24	N/A	-0.05	L. Gravel	M. Gravel	55	103	6	45	3	25	0	0	0
7/31/17	S-S	8	495	32.1	7.6	8.10	0.27	N/A	-0.08	L. Gravel	M. Gravel	60	91	6	36	1	27	0	1	0

TABLE B.3—Water quality, habitat, and macroinvertebrate guild data from the Victoria study site on Guadalupe River. Microhabitats are abbreviated as: deep-fast (D-F), deep-slow (D-S), shallow-fast (S-F), and shallow-slow (S-S). Parameters are abbreviated as: specific conductivity (Cond), water temperature (Temp), dissolved oxygen (DO), bottom/streambed current velocity (V10), mean column current velocity (V), primary substrate (Sub 1), secondary substrate (Sub 2) and percent embeddedness (Emb). Taxa and guilds are abbreviated as: total benthic macroinvertebrate community (Total BMI), elmid guild (ElmG), chironomid guild (ChiG), Ephemeroptera guild (EphG), *Tricorythodes* (Tric), *Corydalus* (Cory), *Neoperla* (Neop), and Hydropsychid guild (HydG).

Date	Micro-habitat	Rep	Cond (µS/cm)	Temp (˚C)	DO (mg/L)	pH	Depth (m)	V10 (m/s)	V (m/s)	Sub 1	Sub 2	Emb (%)	Total BMI	ElmG	ChiG	EphG	Tric	Cory	Neop	HydG
8/21/15	D-F	1A	542	28.6	3.57	8.31	0.60	0.09	0.46	M. Gravel	Sand	0	98	12	5	5	49	0	1	0
8/21/15	D-F	1B	542	28.6	3.57	8.31	0.57	0.27	0.52	Sand	S. Gravel	0	77	1	5	3	1	0	7	0
8/21/15	D-F	2A	542	28.6	3.59	8.29	0.52	0.60	0.99	M. Gravel	Sand	0	62	0	0	19	2	0	1	8
8/21/15	D-F	2B	542	28.6	3.59	8.29	0.60	0.37	0.94	M. Gravel	Sand	0	133	0	1	37	3	0	21	5
8/21/15	D-S	1A	541	28.6	3.54	8.33	0.60	0.00	0.03	Sand	S. Gravel	0	281	2	23	12	6	0	0	1
8/21/15	D-S	1B	541	28.6	3.54	8.33	0.73	0.00	0.07	Sand	Sand	0	22	3	2	2	2	1	0	0
8/21/15	D-S	2A	542	28.6	4.12	8.30	0.49	0.02	0.04	Sand	S. Gravel	0	358	7	92	1	74	1	2	0
8/21/15	D-S	2B	542	28.6	4.12	8.30	0.60	0.18	0.15	Sand	S. Gravel	0	160	1	10	39	54	0	3	0
8/21/15	S-F	1A	542	28.6	3.65	8.30	0.21	0.55	0.75	M. Gravel	L. Gravel	50	649	40	122	68	49	2	71	66
8/21/15	S-F	1B	542	28.6	3.65	8.30	0.27	0.07	0.89	M. Gravel	L. Gravel	50	50	6	0	11	2	1	10	0
8/21/15	S-F	2A	542	28.6	4.15	8.30	0.03	0.50	0.36	S. Gravel	M. Gravel	50	582	104	162	42	42	7	42	14
8/21/15	S-F	2B	542	28.6	4.15	8.30	0.03	0.31	0.00	S. Gravel	M. Gravel	50	162	17	68	14	13	3	5	1
8/21/15	S-S	1A	541	28.6	4.22	8.31	0.09	0.16	0.00	S. Gravel	Sand	50	210	21	39	30	25	0	7	5
8/21/15	S-S	1B	541	28.6	4.22	8.31	0.12	0.15	0.00	M. Gravel		50	246	54	3	18	61	0	7	2
8/21/15	S-S	2A	539	28.6	4.23	8.29	0.15	0.19	0.19	M. Gravel	S. Gravel	25	313	0	113	2	132	0	3	0
8/21/15	S-S	2B	539	28.6	4.23	8.29	0.21	0.15	0.00	M. Gravel	S. Gravel	25	355	31	82	29	97	0	4	0
7/20/17	D-F	3	507	31.2	5.93	7.17	0.49	N/A	1.12	L. Gravel	M. Gravel	N/A	286	19	7	71	11	2	4	31
7/20/17	S-F	4	507	31.2	5.93	7.17	0.12	N/A	0.43	S. Gravel		N/A	1074	103	31	75	3	4	49	300
7/20/17	D-S	5	507	31.2	5.93	7.17	0.50	N/A	0.06	S. Gravel	M. Gravel	N/A	118	20	6	12	10	0	10	10
7/20/17	D-S	6	507	31.2	5.93	7.17	0.51	N/A	0.14	Sand	Silt	N/A	32	1	6	5	4	0	2	4
7/20/17	S-F	1	507	31.2	5.93	7.17	0.40	N/A	0.23	L. Gravel	M. Gravel	N/A	302	16	3	38	10	0	7	80
7/20/17	D-F	2	507	31.2	5.93	7.17	0.52	N/A	0.95	L. Gravel	M. Gravel	N/A	473	56	8	64	14	4	18	83
7/20/17	S-S	7	507	31.2	5.93	7.17	0.20	N/A	0.01	M. Gravel	Sand	N/A	56	4	22	1	11	0	1	1
7/20/17	S-S	8	507	31.2	5.93	7.17	0.31	N/A	0.15	M. Gravel	Cobble	N/A	10	0	7	0	1	0	1	0

APPENDIX C.—Response to comments.

REQUIRED CHANGES TO TASK 3 REPORT

None

SUGGESTED CHANGES TO TASK 3 REPORT

1. Table 2 on pages 6 through 8 does a nice job of displaying the depth, velocity, and macroinvertebrate data. However, it would also be of interest to see where and when the samples were collected to see if there were any geographical, seasonal, or within riffle variations in the data. Also, the text mentions that substrate and water quality data were collected (1st paragraph, page 4), but none of this data is presented in the report. This data could be of interest to future studies and should be preserved. TWDB will be expecting an electronic copy of all data collected for this study along with the final report. However, please consider documenting more of the data in the report itself through additional tables, figures, or an appendix.

Response: Appendix B provides available data. TPWD will provide an electronic copy of all data available.

APPENDIX D.—Multiscale riverine network patterns should inform biomonitoring.

RH: Multiscale riverine network patterns

Multiscale riverine network patterns should inform biomonitoring

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Abstract

Macroinvertebrates are widely used as bio-indicators in streams and rivers, and it is usually assumed that their community composition is primarily controlled by local environmental conditions. We examined the distribution of macroinvertebrates within the Guadalupe River basin (3,256 km^2) in Central Texas across physiographic gradients. Variation partitioning with redundancy analysis showed that large-scale factors, which are not routinely measured in monitoring programs, i.e., riverine network patterns, climatic variation, and ecoregion explained a significant proportion (28%) of the variation in community composition within a river basin. The riverine network patterns were the most important factor, explaining 12% alone. Local environmental factors were significant, but completely confounded within these spatial patterns. Spatial analysis with variables (AEM vectors) that considers the flow direction, the connectivity and distances between sites detected distinctive communities in the lower reaches of the mainstem, in spring-influenced reaches, and in a tributary with intermittent reaches. We propose that metacommunity dynamics will vary because of the different disturbance levels found in these different parts of this subtropical riverine network. Our results suggest that biogeographic differences, the structure, and flow regime of the river network have to be considered when biomonitoring macroinvertebrates even within a river basin. We recommend spatial analysis that considers distances and connectivity within a river network as a powerful tool to recognize multiscale riverine network patterns, which can help to identify priority areas for conservation and to develop sound monitoring programs.

Keywords: metacommunity, AEM spatial variables, biomonitoring, biogeography, aquatic insects, springs, intermittent flow.

Introduction

Biological monitoring is a widely accepted survey methodology to evaluate the ecological health of rivers and streams (Barbour et al. 1999). Macroinvertebrates are often used as bioindicators, because they are relatively abundant and easy to sample, have different tolerances to changes in pollutants and water quality, and have low mobility, thus the composition and diversity of macroinvertebrates are thought to reflect local conditions (Metcalfe 1989, Cairns and Pratt 1993, Barbour et al. 1999). Macroinvertebrate community composition also reflects environmental conditions integrated over longer time periods rather than measurements of physico-chemical conditions which are more likely to be representative of shorter-term snapshots of environmental conditions (Barbour et al. 1999).

While it is well known that both local and regional factors may affect the distribution of communities, one of the principle assumptions of macroinvertebrate biomonitoring is that local communities are primarily controlled by local environmental conditions. Therefore, when high dispersal rates among spatially-connected communities (i.e., so-called mass effects) override the importance of local factors, biomonitoring may lead to inaccurate information about the environmental health of a local aquatic system (Vilmi et al. 2016). The relative importance of local environmental conditions versus dispersal for metacommunity structure may depend on the location within the river network. For example, an analysis of three river basins in Maryland, USA found that local environmental factors were most important for macroinvertebrate community structure in headwater sites, but that dispersal-driven processes were more important in riverine mainstem sites (Brown and Swan 2010, but see Schmera et al. 2018). In addition, the spatial location of sites within a riverine network influences composition and diversity across a drainage, with diversity typically being greater at the confluence points in the mainstem network and in the lower reaches of a drainage (Altermatt 2013).

Numerous studies have examined the relative importance of local environmental versus spatial factors for the structuring of metacommunities (e.g., Cottenie 2005, Logue et al. 2011). Most studies conducted in streams and rivers found that local environmental factors are generally more important than spatial factors (Heino et al. 2015b, but see Heino et al. 2015a), their relative importance, however, may vary with distances between sites and the spatial extent surveyed (Heino et al. 2015b). In contrast, dispersal processes over larger time scale such as historical colonization events are often ignored, although they may play a major role for metacommunity structure (Castillo-Escrivà et al. 2017). It is also important to differentiate between the role of dispersal processes at the spatial scale of metacommunities (local communities linked by dispersal, Leibold et al. 2004) and a biogeographical scale (e.g., along a macroclimatic gradient, Gonçalves-Souza et al. 2014). In particular, studies examining patterns in community composition and diversity over larger spatial extents, e.g., across drainage basins, should also consider biogeographic patterns which are results of long-term dispersal effects (e.g., historical colonization events, historical dispersal barriers) and large-scale environmental differences (e.g., climatic gradient; Leibold et al. 2010, Heino et al. 2015b, 2017).

The purpose of this study was to examine multiscale riverine network patterns of benthic macroinvertebrates across the Guadalupe River basin in central Texas, USA (Fig. 1). The Guadalupe River basin is relatively large (3,256 km^2) and encompasses a pronounced regional physiographic gradient, including four ecoregions (Fig. 1). Both high disturbance intensity and stable conditions occur in this basin, where flashfloods are common and where groundwater-fed tributaries with relatively consistent flow occur next to tributaries with intermittent reaches and higher variation in seasonal flow. The basin was never glaciated and also includes major springs, which are considered hotspots for endemic macroinvertebrates. The spatial extent of the basin examined by this study allowed us to also examine larger scale spatial patterns of macroinvertebrate communities that are likely the result of biogeographic processes.

An increasing number of studies have used complex spatial analyses such as Asymmetric Eigenvector Map (AEM) analysis for variation partitioning in metacommunity analyses of macroinvertebrates (e.g., Göthe et al. 2013, Zhang et al. 2014, Cauvy-Fraunié et al. 2015). However, AEM analysis can also be used to identify multiscale riverine network patterns for macroinvertebrates at a larger spatial scale to identify locations, reaches, and segments of the river with a distinct macroinvertebrate community composition. This could serve as a useful starting point to discuss and further investigate potential factors that drives these patterns, instead of attributing any differences between monitoring sites to local environmental factors, and could ultimately lead to a more effective design of monitoring programs. Therefore, we addressed the following main questions: (1) What is the spatial pattern of macroinvertebrates communities in the Guadalupe Basin? Which multiscale riverine network patterns do they show? (2) Which groups of macroinvertebrates and environmental factors are associated with these patterns? (3) What is the relative importance of climatic variation, ecoregion and larger scale riverine network patterns? (4) To what extent are climatic variation, ecoregion, and riverine network patterns correlated with local environmental conditions and land use-land cover?

Methods

Study area

The Guadalupe River, like the other Gulf coast rivers in Texas, flows from northwest to southeast, experiencing a climatic gradient with increased precipitation from west to east, and increasing temperatures from north to south (see below). The Guadalupe River basin contains portions of four US Environmental Protection Agency Level IIIs (Fig. 1): the Edwards Plateau, the Texas Blackland Prairies, the East Central Texas Plains, and the Western Gulf Coastal Plain. All four ecoregions can be characterized by homogeneity associated with both abiotic – soils, vegetation, geology, climate, and physiography (Omernik 1987, Griffith et al. 2006), and biotic factors, including algal coverage. The ecoregions do not follow a strict up to downstream pattern but 2 of the 4 ecoregions alternate in the middle and lower reaches of the Guadalupe (Fig. 1).

The Edwards Plateau is dominated by karst limestone geology and many headwaters and stream reaches are strongly spring-influenced, containing clear water with high physicochemical stability. The Blackland Prairies is dominated by clays and silty soils and contains larger fraction of cropland and urban space. The East Central Texas Plains is largely composed of savanna and mostly used for pasture. The Western Gulf Coastal Plain is a low gradient plain that ends at the Gulf of Mexico. Rainfall varies across the basin, from a minimum of 406 mm per year in the north and western portions of the basin (Edwards Plateau) to a maximum of 1473 mm per year in the southern and eastern regions (Western Gulf Coast Plain).

The tributaries, Comal and San Marcos rivers, are strongly groundwater influenced (i.e., these rivers are fed by large spring complexes in their headwaters) and thus exhibit stable physicochemical conditions and relatively more consistent seasonal flows. In contrast, the other tributary, the Blanco River and the upper portion of the Guadalupe River mainstem (sites 14 – 16 on the Guadalupe, Fig. 1) exhibit much higher variation in seasonal flows with some sections of these rivers going dry during dry years or experiencing large-scale flooding during wet periods. Indeed, the Blanco River experienced large-scale and historic flooding in early May 2015 several months before we started sampling for this study.

Field data collection

We sampled (macroinvertebrates and local environmental conditions) 28 sites across the basin between July and October 2015. Sites were located in the main tributaries of the Guadalupe River including the Comal River (2 sites), the San Marcos River (4 sites), and the Blanco River (7 sites); the remaining 15 sites were distributed along the mainstem of the Guadalupe River (Fig. 1, Table S1).

Local environmental conditions such as substrate type and composition and water velocity affect macroinvertebrate community composition within a given sampling site, (Allen 1995). We collected invertebrate samples from haphazardly-placed locations within riffles at each sampling site, which are the most ideal mesohabitat to sample when evaluating macroinvertebrates since it consistently contains higher diversity (Brown and Brussock 1991, Barbour et al. 1999) and many environmental monitoring programs focusing on macroinvertebrates from riffles (Carter and Resh 2001).

Macroinvertebrate samples were collected using a 500-µm Hess sampler (35-cm diameter). This mesh size is commonly used in biomonitoring programs, although it misses smaller benthic organisms, especially early stages of many macroinvertebrates. At each sampling site, four Hess samples were collected from within a riffle area, except for 5 of the 28 sites (3 Hess samples at sites 21, 24, 25, and 28, and 2 Hess samples at site 7). To account for differences in the number of Hess samples, macroinvertebrate densities for each taxon were expressed as number of individuals/m^2. During sampling, substrate was agitated for a 2-minute interval and samples were preserved in 90% ethanol (EtOH) for processing in the laboratory. Macroinvertebrate samples were identified under a stereomicroscope (Nikon SMZ745T) to the lowest practical taxonomic level (typically genus) using relevant several taxonomic keys (Merritt et al. 2008, Diaz 2014). A total of 59 macroinvertebrate taxa were identified, including 6 non-insect taxa (Table S2). Non-insect taxa were identified to order and all other taxa were identified to genus, except Diptera, which were identified to family.

Prior to macroinvertebrate samples at each site, we measured pH, temperature, dissolved oxygen (DO; mg/L), and conductivity (μS/cm) using a multiparameter probe (YSI 556). Water velocity immediately upstream from each sample point in a riffle was measured with a Hach flow meter (FH950). The percent sediment size composition at each sampling point was estimated using a modified Wentworth scale (Wentworth 1922) and percent algae cover was estimated using an underwater viewing window. Duplicate water samples were taken at each sampling location using 2-L brown Nalgene bottles which were rinsed three times with site water before sample collection. Water samples were placed in a cooler on ice and transported to the lab at Texas State University, where samples were filtered and preserved within 48 hours of collection.

Water samples were filtered to determine the concentration of NH_4^+, NO_3^-, soluble reactive phosphorus (SRP, assumed to be PO_4^{3-}), total suspended solids (TSS), non-volatile suspended solids (NVSS), and suspended chlorophyll-*a* (Chl*a*). Nutrients and suspended materials were determined through lab-specific standardized methods (Caston et al. 2007).

Land cover data

Land cover data was downloaded from the United States Geological Survey and overlaid on sample site locations in ArcGIS v10.4 using the National Land Cover Database (NLCD 2011 version). Land use-land cover (LULC) was determined as percent composition among 20 categories: developed open space, developed low intensity, developed medium intensity, developed high intensity, open water, perennial ice/snow, barren land (rock/sand/clay), deciduous forest, evergreen forest, mixed forest, dwarf scrub, shrub/scrub, herbaceous grassland, herbaceous sedge, lichens, moss, pasture/hay, cultivated, woody wetlands, and emergent herbaceous wetlands (NLCD 2011 Product Legend; https://www.mrlc.gov/nlcd2011.php). Three spatial scales of LULC for each sampling site were examined based on Allan (2004) and Becker et al. (2014): (1) a reach scale with land cover in a 100-m buffer on either side of the river with a 2km buffer upstream from each site; (2) a riparian scale with land cover in a 100-m buffer for total distance upstream for each site; and (3) a catchment scale with land cover for the whole watershed upstream of the site. We followed the procedure outlined in Becker et al. (2014) to combine and reduce LULC into 8 categories: urban, cultivated, evergreen forest, deciduous forest, mixed forest, rangeland, wetlands, and open water. Barren land was removed from any analyses because it made up <1% of the coverage area (Dodds and Oakes 2008, Becker et al. 2014). Ecoregions for each site were based upon USEPA Level-III Ecoregions, downloaded from the EPA (Griffith et al. 2004), and overlaid across the Guadalupe River Basin in ArcGIS. Estimates of river slope were generated using a digital elevation model (DEM), and river distances between sites were evaluated by using a river network map in ArcGIS. Mean annual precipitation data for each site was obtained from Texas Parks and Wildlife Department and reported as the annual mean during the 2000 – 2010 period.

Data analysis

Twenty taxa were excluded from analysis because they contained <5% of taxa at all sites (Zhao et al. 2017). Prior to analysis, values obtained from duplicate water samples for each analyte from each site were averaged. To avoid issues with multicollinearity in analyses, variables which were highly correlated ($r > 0.70$) were removed from the dataset. Mean, maximum, minimum, and point slope estimates for each site were highly correlated, so only site mean slope was used in further analyses. TSS and NVSS were also highly correlated, thus TSS was used in further analyses. A Pearson correlation matrix for each group of predictor variable data set revealed that the riparian and catchment scales for LULC percent coverage were highly correlated for nearly all variables and the riparian LULC scale were removed from further analyses (Becker et al. 2014).

To address question (1) and to evaluate potentially complex multiscale spatial patterns within the river network we used an Asymmetric Eigenvector Map (AEM) analysis, a spatial modelling technique that considers autocorrelation at different spatial scales. AEM analysis was developed for ecosystems such as rivers in which directional physical processes (water currents) can affect the distribution of organism (Blanchet et al. 2008a). We computed Eigenfunction-based spatial variables (eigenvectors) from a directional downstream distance matrix (Blanchet et al. 2008a), and assigned weights to the edge matrix based on watercourse distances. A forward selection procedure was used to reduce the number of spatial eigenvectors to predict the variation in community composition (Blanchet et al. 2008b). We also used Moran's eigenvector map (MEM) analysis (Dray et al., 2006) to model hydrological connections between sites without considering the direction of the flow. However, the results were very similar to the AEM-analysis and were therefore not included here.

To evaluate the association of differences in community composition with higher densities of certain groups and with environmental factors (local environmental factors, LULC, and larger-scale physiographic variables) (question 2) we used redundancy analyses (RDA). To examine the relative importance (question 3) of climatic variation (temperature, precipitation), ecoregion and large-scale riverine network patterns (AEM variables) we used variation decomposition based on redundancy analysis (RDA, Legendre and Legendre 1998, Cottenie 2005). To focus on larger-scale patterns, we only used the first 3 large-scale AEM variables selected by the forward selection (see above). The computed percentage of explained variation was adjusted for the number of explanatory variables (i.e., adjusted R^2, Peres-Neto et al. 2006). The dependent abundances of macroinvertebrates (genera or family) were Hellinger transformed to minimize the disproportional influence of rare species on the redundancy analysis (Legendre and Gallagher 2001). To determine to what extent ecoregion, climatic variation, and large-scale AEM variables were correlated (and how much variation they shared) with local environmental conditions and land use-land cover (question 4), we ran pairwise variation decomposition based on RDA (see above), i.e., ecoregion vs. local environmental conditions, and ecoregion vs. land use-land cover variables, and the same for climatic variation and large-scale AEM variables. Comparisons among more than two groups of variables (e.g., variation decomposition with AEM variables, local environmental factors and land use-land cover) were not possible, because of high correlation between the variables. All analyses were done in R (R Development Core Team, 2017, version 3.4.0) using the package vegan (Oksanen et al. 2017).

Results

Multiscale riverine network patterns

The analysis of AEM variables resulted in 9 significant variables (Fig. 2), which explained 52% ($p = 0.001$) of the variation in macroinvertebrate community composition across the Guadalupe Basin. Most of the vectors represented large-scale spatial patterns (vectors V1 to V6; Fig. 2). Overall, the analysis revealed four notable patterns which were associated with different genera and physiographic and environmental factors. First, there was a unique macroinvertebrate community composition in the lower portion of the Guadalupe River after the confluence with the San Marcos River (V1; Fig. 2), characterized by higher densities of the mayfly genus *Traverella*, the predatory stonefly *Neoperla*, and the riffle beetle *Hexacylleopus* (Fig. 3a). This spatial pattern largely corresponded with the climatic gradient in the basin, with greater precipitation (and higher temperatures, Fig. 3b) in the lower portions of the basin (Fig. 3b). These changes in community structure in the lower portion of the Guadalupe River also correlated with several local environmental factors: higher TSS and Chl*a* concentrations, slightly higher pH, and higher proportion of sand in benthic substrates (Fig. 3c). With respect to LULC patterns in the basin, these taxonomic changes were also correlated with an increase in the percent coverage of wetlands and agriculture at the catchment scale (Fig. 3b).

The second riverine network pattern was associated with the spring -influenced reaches along the Comal and San Marcos rivers (V21, Fig. 3a both spring sites have the highest negative values for second RDA axis). These sites exhibited higher densities of macroinvertebrates with lower dispersal abilities or those lacking desiccation-resistant resting stages (i.e., *Hyalella*, the water penny *Psephenus*), and the riffle beetle *Mycrocylleopus*) (Fig. 3a). In these reaches, the LULC patterns had higher percentages of urban, mixed forest, and open water (presumably associated with the headwater spring complexes).

A third distinctive community type in the basin was found along the Blanco River characterized by high abundances of the net spinning caddisfly *Chimarra* (vectors V2, V6, and partly V4; Fig. 2, 3a). Local environmental conditions at these sites tended to have greater water depths and a higher proportion of cobble in benthic substrates (Fig. 3c). Blanco sites also had slightly higher proportion of ranchland use (up to 72%, compared to up to 60% elsewhere in the basin, Fig. 3b).

The last major community type was associated with sites located within the Texas Blackland Prairies ecoregion (several sites in the lower San Marcos, the upper Guadalupe, and the lower Guadalupe rivers) (V5, Fig. 1, 2). Although there were no obvious associations with general or physiographic or environmental factors (Fig. 3), there were increased densities of the mayflies *Baetis* and *Leptohyphes* at the sites located in this region.

Other riverine network patterns that were detected included a different pattern around the confluence of the San Marcos with the Guadalupe River (V3; Fig. 2), and another smaller scale pattern with the most upstream reaches of the upper Guadalupe being different (V11; Fig. 2).

Variation partitioning

Large-scale AEM variables (the first 3 vectors) explained 12% of the variation in community composition across the Guadalupe Basin after eliminating the shared effects of the other factors climate and ecoregion (i.e., pure effects). The pure effects of climate variation and ecoregion were 4% and 3% respectively (Fig. 4). In addition, ecoregion and AEM variables shared 7% of the variation, and all variables shared 5% of the variation. Due to multicollinearity between the factors, the total amount of variation explained was 28% instead of 31%.

Pairwise variation partitioning in RDA indicated that that ecoregion shared more variation with LULC factors (15%) than with local environmental factors (7%). Similarly, climatic factors shared a small proportion of the variation with LULC factors (7%), but none with local environmental factors. Not surprisingly, both LULC factors and local environmental factors were spatially structured. Local environmental factors shared 28% (all variation) with (large-scale) AEM variables, and LULC factors 22%.

Discussion

We found that a considerable portion of variation in the community composition of macroinvertebrates in the Guadalupe River basin was explained by variation in larger-scale factors (i.e., climatic variation, ecoregion, and riverine network patterns). Such factors are not routinely included in monitoring programs, despite findings that such large scale patterns are important (e.g., Feminella 2000, Mykrä et al. 2004). Not surprisingly, part of the variation in climate and ecoregion in the Guadalupe basin were spatially structured (i.e., shared variation with large-scale AEM variables), but both climate and ecoregion also explained a small amount of the variation in community structure on its own. The pure effects of large-scale spatial variables were most important and all significant AEM variables combined explained ~50% of the variation in macroinvertebrate community structure. This result suggests that the location of a site within a river network (e.g., presence of spring influenced reaches, confluence points in the network) and historical and current connectivity were more important in determining macroinvertebrate community composition than the physiographic gradients across the basin.

The importance of local environmental factors for metacommunity structuring has been shown by many metacommunity studies in rivers (Heino et al. 2015b, but see Heino et al.

2015a), suggesting that species sorting is the prevalent dynamic in rivers. It is important, however, to consider the spatial extent encompassed by a study and the distances between sites in relation to the dispersal abilities of the study organisms as different processes may act on different scales. For example, a study on neotropical lepidopterans and spiders found that environmental effects dominated at the metacommunity scale, whereas at the biogeographical scale dispersal-based processes were more important (Gonçalves-Souza et al. 2014). The biogeographic scale was also considered in a study on aquatic organisms across drainage basins in Finland, which showed that basin identity and local environmental variables were both important for community structure, whereas the spatial effects within a basin were usually negligible (Heino et al. 2017, area of three drainage basins: 63,609 km^2.). In contrast to this, our study found that large-scale spatial patterns can also play a role within a basin (area: 3,256 km^2). Unfortunately, we could not determine the relative importance of local environmental factors, as all local environmental factors were spatially structured (local environmental factors shared all explained variation with (large-scale) AEM variables, see above). In order to avoid the local environmental factors to be completely confounded with large-scale spatial patterns, a different sampling design would have been needed with additional sites being placed closer to each other. It is also possible that local factors would explain additional variation in the distribution of macorinvertebrates, when more specific requirements of organisms were included in analyses, such as food availability and prey presence. For freshwater mussels (technically also macroinvertebrates, but usually not included in routine biomonitoring), the inclusion of a moving niche component, i.e. the presence/absence of their host fish, they need for successful recruitment, explained substantially more of their distribution across river basins in Ontario, Canada (Schwalb et al. 2013). Maybe the biggest obstacle is the scarce information available about life history of many macroinvertebrates, hence more life history studies of macroinvertebrates are needed.

The watercourse variables (AEM) revealed interesting multiscale riverine network patterns in macroinvertebrate community composition, which indicated a distinct community composition for the lower portion of the basin (lower Guadalupe mainstem), the spring-influenced reaches, the Blanco River, and the different ecoregions. The different community composition in the lower Guadalupe mainstem was associated with local environmental factors, but also larger scale climatic factors, which are usually associated with a biogeographical spatial scale. In particular, the plecopterans (mostly the genus *Neoperla*) were present in the lower mainstem Guadalupe sites, but were largely absent from sites extending up into the Edwards Plateau. The large-scale southeast-northwest spatial occurrence pattern of plecopterans in the study watershed is consistent with previous descriptions of plecopterans distributions in the region. This is likely related to precipitation patterns and its influence on stream permanence and the past biogeographic dispersal patterns of plecopterans in the region (Stewart et al. 1973, Szytko and Stewart 1977). In addition, larger-scale differences in water quality and food availability likely influence the occurrence of species in the lower Guadalupe maintstem. The lower mainstem has higher suspended Chl-a and higher TSS concentrations, indicating greater food availability for filter feeders such as *Traverella*, which also occurred in higher abundances in the lower Guadalupe.

The spring influenced reaches of the basin were characterized by macroinvertebrate communities containing species with lower dispersal abilities or an inability to tolerate periods of desiccation, such as amphipods (*Hyalella azteca*) and smaller bodied riffle beetles (*Microcylloepus pusillus*). It is also noteworthy that the proportion of shredders was considerably higher near Spring Lake in the San Marcos River and near Comal Springs compared to the rest of the basin (0-3%, see Zawalski 2017 for more details). Diversity is typically higher in lower reaches of river networks (Altermatt 2013) and that holds true for freshwater fishes and unionid mussels in Texas (Dascher et al. 2017). However, we found the highest diversity of macroinvertebrates near springs (Zawalski 2017). Spring-influenced reaches of riverine networks, especially in arid areas, play an important role as ecological and evolutionary refugia (Davis et al. 2013) in that some species found in these reaches may only be able to persist in these refugia during extended or severe drought periods. As such, protection of these segments from anthropogenic impacts, including restriction of groundwater pumping from regional aquifers provides crucial refuge and protection for these spring-associated and dispersal-limited taxa (Bowles and Arsuffi 1993) as well as maintaining locations of higher species diversity in the landscape.

Unmeasured factors such as flow permanence may be an important driver for the distinctive community in the Blanco River. Several reaches (at least 3 sites) were dry in 2014 and all of the sites in the Blanco River experienced an especially large flooding event in 2015, shortly before the sampling. The communities in this river were characterized by high abundances of *Chimarra*, which could be especially resilient to such disturbance as it was found in higher abundances in unstable substrate in the Ardèche river, France (when compared to other net-spinning caddisflies, Dolédec and Tachet 1989). A higher concentration of suspended food sources could also be a factor. Algal growth is enhanced in pools that become isolated or when flow is decreased. In addition, when intermittent reaches go dry, terrestrial organic matter can accumulate and aquatic plant material be decomposed and providing a high input of organic matter when these reaches are flooded (Williams 2006), which could be a good food source for collectors such as net building caddisflies. Indeed, the majority of collector-filterers in the Guadalupe basin can be found in the Blanco River as well as the headwaters of the Guadalupe and Comal Rivers (Zawalski 2017). The intermittent reaches may also be a good habitat for macroinvertebrates with high colonization potential. Interestingly, 99% of the genera present in the intermittent reaches in the Blanco River (i.e., dry in 2014) had winged adult stages. The average for all sites in the Blanco River was 96% compared to the rest of the basin with 82% not including the spring sites. At spring sites the percentage of winged results were the lowest (13 and 37% for San Marcos and Comal springs respectively).

It is well known that differences in regional species pools must be considered for biomonitoring and the development of biotic and multimetric indices, especially if they are based on biological attributes of species instead of functional metrics (Pont, 2006). Our data suggests that biogeographic differences can play a role not just between basins (e.g., Heino et al. 2017), but also within river basins. Furthermore, our results indicate that the distribution of macroinvertebrates may also depend on the location in the river network (near springs, lower reaches, tributary with intermittent reaches). The importance of location in the river network for the distribution of macroinvertebrates and metacommunity structuring has been previously shown, e.g., with focus on the arrangement of tributaries (Rice et al. 2001) and river network properties (Altermatt et al. 2013), and comparing headwaters and mainstem (Brown and Swan 2010). Based on our results we predict that metacommunity structure and dynamics in a subtropical river network will vary because of different disturbance levels found in different parts of the river network (Table 1). The environmental conditions are most stable close to the springs, which allows macroinvertebrates with low dispersal abilities and those unable to tolerate periods of desiccation to become abundant. As these springs are evolutionary refugia species sorting should still occur despite limited dispersal over a sufficiently large time-scale. Such a species sorting with limited dispersal (sensu Weingardner et al. 2012) assumes a trade-off between competition and dispersal, and the abundant low dispersal macroinvertebrates will co-exist with high dispersal species (Table 1). In contrast, in intermittent reaches with high disturbance levels due to drying and flash flooding, only macroinvertebrates with high dispersal abilities become abundant. The community composition is determined by colonization after disturbance events, followed by succession driven by local environmental condition and biotic interactions (i.e. species sorting with high dispersal, Table 1). However, the relative importance of dispersal vs. environmental filtering may vary with hydrological phases of dry, flowing, and non-flowing conditions in intermittent systems (Datry et al. 2016). Finally, the distinct community in the lower mainstem of the Guadalupe is subject to an intermediate disturbance level, where the community composition is mainly determined by abiotic conditions (species sorting with efficient dispersal, Table 1), but probably also by biogeographic patterns. The frequency of high vs. low dispersal groups of macroinvertebrates (e.g., percentage of winged adults) reflects the relative frequency found in the entire basin (Table 1).

It has been postulated that ecologists should also consider distances along the river network (Heino et al. 2015b), and newly developed statistical methods allow such a spatial analysis in river networks (Legendre and Legendre 2012). Watercourse spatial variables have been used increasingly in ecological studies, but have not yet gained popularity in more applied studies. We recommend spatial analyses that consider distances and connectivity as a powerful tool to recognize multiscale riverine network patterns, and which may otherwise go undetected. For instance, a survey throughout the basin will be necessary to identify distinct communities, and those differences will then need to be considered when monitoring human impact. In addition, such an analysis can help to identify priority areas for conservation and management plans (such as spring -influenced reaches, see above). Our example uses macroinvertebrates, but it could be easily applied to fish or other groups of organisms. Using multiscale spatial analysis also helps to identify the relative importance of processes at different spatial scales, and may indicate mechanisms responsible for these patterns. Thus, it would be an important step for designing an effective monitoring program, detecting human impact, and developing mitigation plans.

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Table 1 Proposed community structure and dynamics (sensu Weingardner et al. 2012), and indicator genera in relation to disturbance levels in different parts of the river network for which a distinctive community was detected in the Guadalupe basin.

River network feature	Disturbance level	Metacommunity dynamics	Macroinvertebrate community	Indicator genera
Close to springs	Stable environment	Species sorting with limited dispersal, assumes trade-off between competition and dispersal	Macroinvertebrates abundant with low dispersal abilities and/or inability to tolerate periods of desiccation, co-existing with species with high dispersal abilities (13 and 37% with winged adult stages)	Amphipod: *Hyalella*, water penny: *Psephenus*, riffle beetle: *Mycrocylleopus*
Tributary with intermittent reaches	High disturbance due to drying and flash flooding	Species sorting with high dispersal, community composition determined by colonization after disturbance, followed by succession.	Macroinvertebrates with high dispersal abilities most abundant. (99% with winged adult stages in intermittent reaches)	Net spinning caddisfly: *Chimarra*
Lower basin mainstem	Intermediate disturbance with occasional flooding but permanent flow	Species sorting with efficient dispersal, Community composition determined by abiotic conditions and biogeographic patterns	Frequency of high and low dispersal abilities reflective of entire basin. Community distinct from rest of basin	Filter feeding mayfly: *Traverella*, predatory stonefly: *Neoperla*,

Figure legends

Fig. 1 Sampling sites 1 to 28 in the Guadalupe River (green line), Texas and its tributaries, the Blanco River (pink), San Marcos River (blue), and Comal River (see insert), The four ecoregions are shown as differently colored areas.

Fig. 2 Significant AEM variables. White squares symbolize negative AEM scores, Black squares symbolize positive AEM scores, the bigger the size of the square the higher the AEM score (but differs for each panel). Sites are placed according to their geographic coordinates, and the lines are connections between sites.

Fig. 3 Biplots of redundancy analysis with sites in the upper Guadalupe (white triangles), the lower Guadaluper (black squares), the Comal River (black triangles), the Blanco (black circles) and the San Marcos River (white circles).

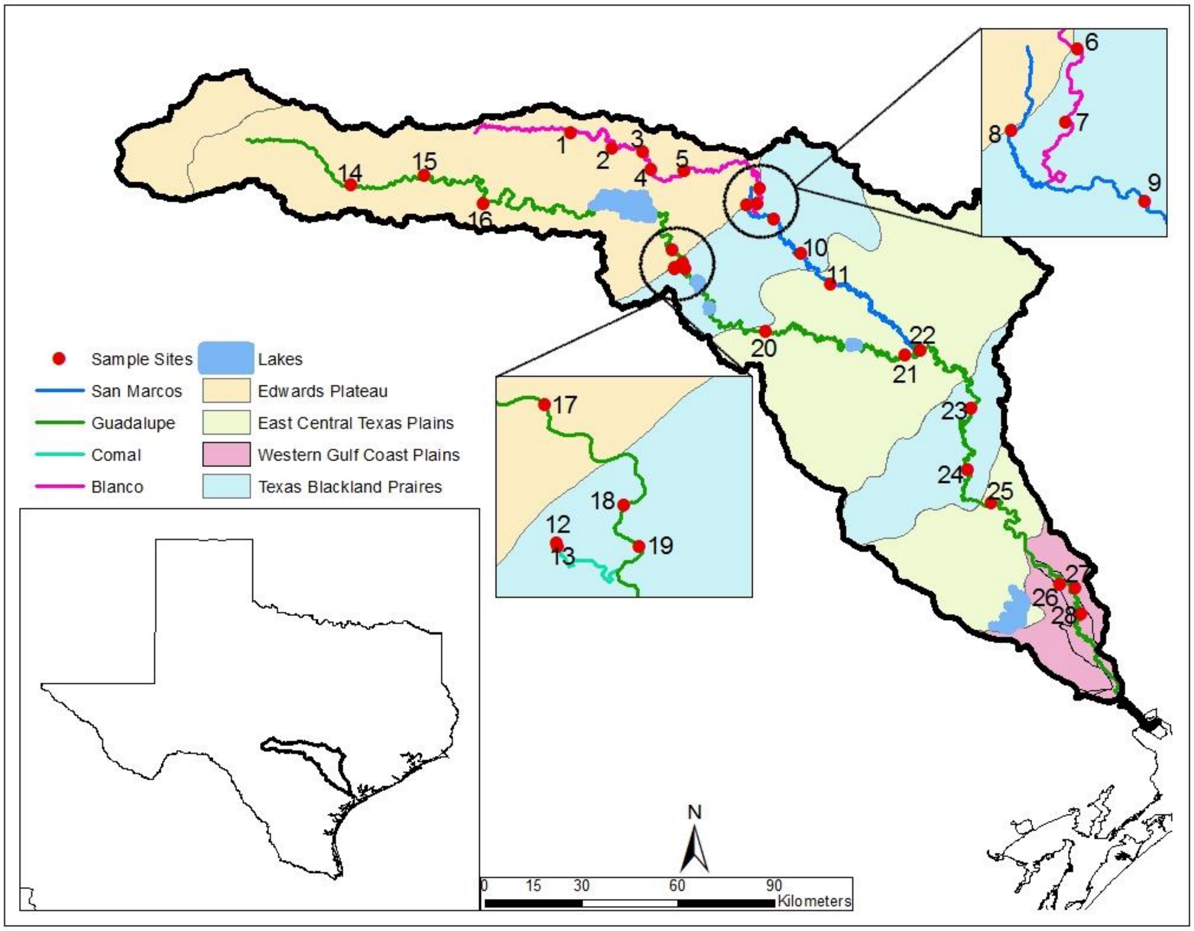
A) The arrows indicate the significant AEM variables, and the letters show genera that distinguished between different spatial patterns. Chim: *Chimarra*, Trav: *Traverella*, Hexa: *Hexacylleopus*, Neop: *Neoperla*, Hyal: *Hyallella*, Psep: *Psephenus*, Micr: *Mycrocylleopus*

B) Arrows indicate significant climatic variables and catchment land-use, land-cover variables.

C) Arrows indicate significant local environmental variables

Fig. 4 Results of the variation decomposition examining the relative importance of riverine network patterns (first 3 large-scale AEM variables), climatic variation (temperature, precipitation), and ecoregion for the distribution of macroinvertebrates in the Guadalupe basin.

Figures



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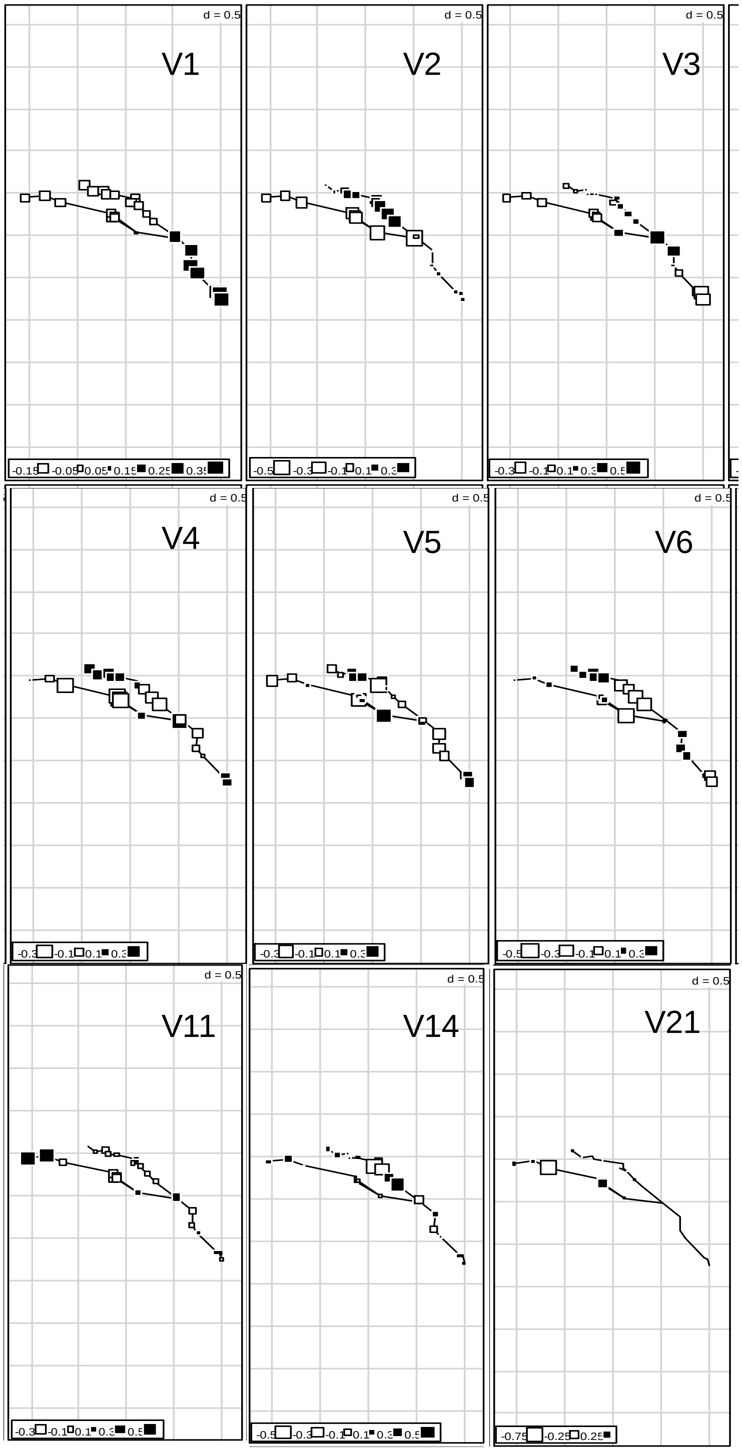

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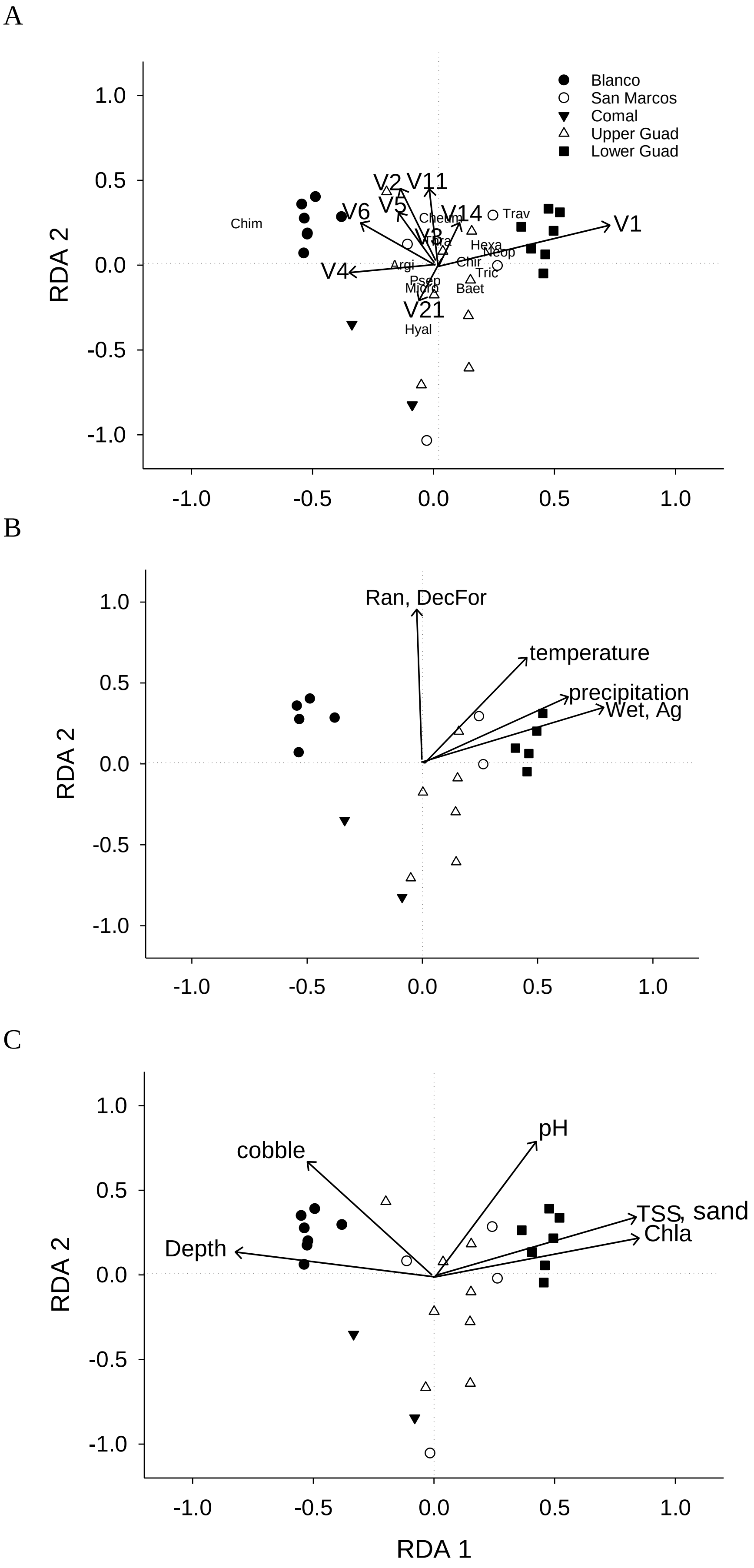A
Blanco
San Marcos
Comal
Upper Guad
Lower Guad
V2
V11
V6
V5
V14
V1
Chim
Cheum
Trav
V3
Thra
Hexa
Neop
Chir
Tric
Argi
V4
Psep
Micro
Baet
V21
Hyal
RDA 2
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
B
Ran, DecFor
temperature
precipitation
Wet, Ag
RDA 2
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
C
pH
cobble
TSS, sand
Chla
Depth
RDA 2
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
RDA 1

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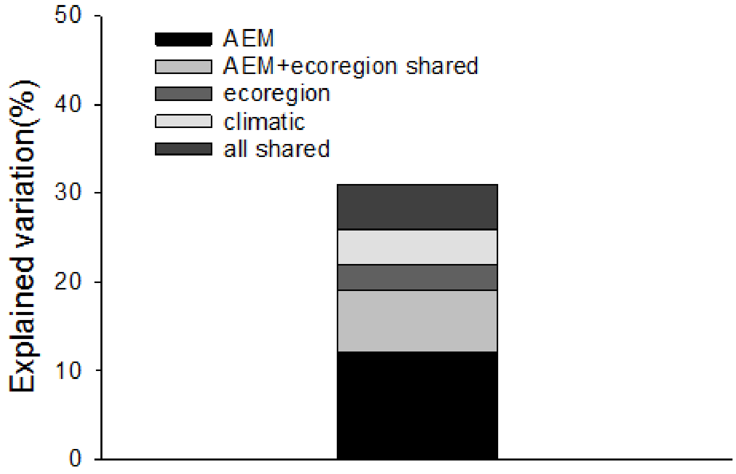

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