

Report for New Mexico Department of Game and Fish Share with Wildlife project:
“An eDNA-based survey of the distributions of Rio Grande Sucker and Rio Grande
Chub in the Upper Rio Grande and Rio Chama basins”

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An eDNA-based survey of the distributions of Rio Grande Sucker and Rio Grande Chub in the Upper Rio Grande and Rio Chama basins

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METHODS

To delineate sampling sites for this effort, we downloaded all sampling points in the Rio Chama and Upper Rio Grande basins spaced at 1km intervals from the environmental DNA (eDNA) sampling grid (<https://www.fs.usda.gov/rm/boise/AWAE/projects/eDNAtlas/edna-sampling-grid.html>). In collaboration with the New Mexico Department of Game and Fish (NMDGF), we identified streams in these basins where a lack of recent sampling has contributed to uncertainty regarding the current status of Rio Grande chub (*Gila pandora*) and Rio Grande sucker (*Pantosteus plebeius*) populations. We then trimmed the dataset to only sites on public lands below 2,805m elevation, above which these species are unlikely to occur (based on provisional analysis of location data in FishNet2, accessed 25 May 2020). Further, we refined the point set to conduct sampling at 5km intervals on the mainstem Rio Grande and Rio Chama due to reduced deposition rates and further transport of eDNA in large streams (i.e., to reduce potential for the same individual fish to be detected multiple times) and at 2km intervals in the remaining subbasins (Pont et al. 2018; Robinson et al. 2019).

The National Genomics Center for Wildlife and Fish Conservation (NGC) in Missoula, MT prepared eDNA sampling materials and field equipment. In total, we visited 60 sites in the Upper Rio Grande basin and 62 sites in the Rio Chama basin to collect eDNA samples (Tables 1 & 2, Figures 1 and 2) between 29 March 2022 and 7 April 2022 and 17 and 21 April 2022. Of the 122 sites visited, we collected eDNA samples at 107 sites while the other 15 sites were visited but eDNA samples could not be collected (stream channel dry, no public access, or inaccessible due to snow). We also share results for 76 sites sampled along the Mimbres River in April, 2022 for Chihuahua chub (*Gila nigrescens*) and Rio Grande sucker eDNA (Appendix A). For each sample collected, we pumped up to 5 L of stream water through a 1.5 µm glass filter (GE HealthCare) using a peristaltic pump (GeoTech Environmental Equipment, Inc.) following the protocol developed by Carim et al. (2016). If a filter clogged before 5L was filtered, we used an additional filter to increase the filtrate volume. Sampling stopped after 5L was filtered in total or after three filters were clogged, ending after whichever occurred first. We placed collected filters in individual plastic bags with silica desiccant and packaged those in individual envelopes labeled with field information (e.g., date and sampling location). If multiple filters were used at a site, each filter was stored in a separate bag of silica and all filters from a site were stored in a single envelope. We kept the filters cool and out of direct light until they could be mailed to the NGC.

Upon receipt of samples at the NGC, we catalogued the sampling data and stored the samples at -20°C until genetic analysis could be completed. For each sample, eDNA was extracted from half of the sample filter using the Qiagen DNEasy® Blood and Tissue Kit following a modified protocol described in Franklin et al. (2019). The other half of the filter was retained and archived at -20 °C. If more than one filter was used at a given site, DNA from all extracted filter halves used at a given site was combined during DNA extraction.

We analyzed all 107 samples from the Upper Rio Grande and Rio Chama for Rio Grande chub and

Rio Grande sucker DNA using species-specific assays designed by the NGC (Contract #: NMDGF 16-516-0000-00034). Each sample was analyzed in triplicate on a QuantStudio 3 quantitative Polymerase Chain Reaction (qPCR) System (Life Technologies). We considered a sample positive for the presence of the target species if one or more of the three qPCR reactions amplified DNA of that species.

All reactions included an internal positive control to ensure that the reactions were effective and sensitive to the presence of the target species' DNA. If the internal positive control appeared inhibited (i.e., reduced amplification of the control DNA was observed due to chemical compounds present in the sample), we treated the sample with a PCR inhibitor removal kit (Zymo Research) and re-analyzed the sample in triplicate. Removal of inhibitors may result in loss of DNA in a sample, however, with elution volumes of 100–200 µl, loss of DNA during inhibitor removal is on average less than 10% (see <http://www.zymoresearch.com> for more details). Thus, to minimize potential DNA loss, for inhibited samples, we extracted the second half of the sample filter and combined all extracted DNA from a given sample to obtain ~200 µl of extracted DNA. All laboratory experiments were conducted with negative controls to ensure there was no contamination during DNA extraction or qPCR setup.

RESULTS & CONCLUSIONS

All eDNA analyses passed internal Quality Assurance and Quality Control measures and there was no amplification of laboratory negative controls observed in this dataset, indicating that these results were not influenced by laboratory contaminations. Ninety-seven of the 107 eDNA samples from the Rio Chama and Rio Grande basins appeared inhibited and were successfully treated to remove PCR inhibition (Tables 3 & 4). Results from the Mimbres River are shown in Appendix A.

In the Rio Chama basin, we detected Rio Grande sucker DNA at four sites and Rio Grande chub DNA at 18 of the 62 sites (Table 4, Figure 4). Rio Grande sucker detections were limited to Cañones Creek and there were no sites in the basin where the two species co-occurred. Rio Grande chub DNA was detected in 18 samples across four streams (Rio Chama, Canjilon Creek, Rio Cebolla, and Rio Nutrias).

In the Upper Rio Grande basin, we detected Rio Grande sucker DNA in four samples and Rio Grande chub DNA in 24 of the 45 samples (Table 3, Figure 3). Rio Grande chub DNA co-occurred at the four sites where we detected Rio Grande sucker DNA. These four detections were limited to the downstream-most site on the Rio Pueblo de Taos and three mainstem sites on the Rio Grande downstream of the confluence with the Rio Pueblo de Taos. Rio Grande chub were detected at all 20 sites on the mainstem Rio Grande as well as three of four sites on the Rio Grande del Rancho. Twenty-one sites were negative for either species' DNA. Locations at which no eDNA of either target species was detected may have lacked that species when the sample was collected or that species may have been present in very low numbers or at too far of a distance upstream from the sampling point. The number of qPCR reactions for each sample with positive DNA detections can be used as a rough estimate of relative DNA concentration. Samples with amplification of target species DNA in all three reactions generally have more DNA than those with amplification in only one or two reactions.

All eDNA sample location data will be entered into the eDNAAtlas results page:

<https://usfs.maps.arcgis.com/apps/webappviewer/index.html?id=b496812d1a8847038687ff1328c481fa>. The eDNAAtlas is an open-access database developed through field surveys that provides precise spatial information on the occurrence locations of aquatic species in the U.S. Secondly, only a portion of the DNA extracted from each sample was needed for these analyses so the remaining

sample extracts are archived at the NGC in Missoula, MT for future analyses of these or other species should the demand arise.

eDNA INTERPRETATION GUIDELINES

Although eDNA detection is generally more sensitive than traditional fisheries methods such as visual or electrofishing surveys, it may still fail to detect an organism that is present. A variety of factors influence the abundance and detection of DNA in an environmental sample. For example, greater animal abundance and sampling proximity to target species presence may affect probability of detection. Furthermore, eDNA production rate may vary with life history stage (e.g., high production during the breeding season [NGG unpublished data, Turner et al. 2014]), suggesting a temporal influence on DNA detection depending on the time of year samples are collected. DNA detected in samples may also come from the carcass of an individual, particularly following eradication efforts (Kamoroff and Goldberg 2018), or from DNA sequestered in sediment (Merkes et al. 2014). Degradation of eDNA is influenced by factors such as water temperature and ultraviolet light (UV) exposure (Pilliod et al. 2014). Additionally, some types of chemical compounds naturally found in streams may inhibit laboratory detection of eDNA (Jane et al. 2015). Field and laboratory methods can also influence eDNA detection such as the filter pore size, amount of water filtered, extraction protocol, etc. (Renshaw et al. 2015 and Wilcox et al. 2020). Additionally, because eDNA techniques detect an organism's DNA and not the organism itself, the precise nature of what is being detected is context dependent. For example, even the temporary presence of a single individual can produce a positive detection. This, along with occurrence of false negative detections, can be resolved through repeated sampling. The probability of receiving a false negative result will decline if multiple sampling visits are completed. Furthermore, resident populations will produce repeated, positive detections in a basin, whereas individual migrants will produce local and ephemeral detections. Reaches without the target species present will continue to produce negative results.

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Table 1. Location and collection details of eDNA samples collected by the NGC in the Rio Chama basin for the delineation of Rio Grande sucker and Rio Grande chub distributions. “Y” in denotes eDNA sample was collected, “N” denotes site was visited but no eDNA could be collected. Multiple filters were used if 5L could not be filtered through a single filter. If this occurred, we used additional filters until 5L was filtered in total or three individual filters were clogged, stopping after whichever occurred first.

Site	Drainage	Stream	Sampled	Date Collected	No. Filters	Filter Volume (L)	Field Notes
100-1	Rio Chama	Polvadera Creek	Y	4/20/2022	3	a:2, b:2.1, c:2	
1071-2	Rio Chama	Rio Chama	Y	4/6/2022	3	a:0.89, b:0.8, c:0.9	
109-1	Rio Chama	Polvadera Creek	Y	4/20/2022	3	a:1.6, b:1.6, c:1.7	
1145-1	Rio Chama	Rio Cebolla	Y	4/6/2022	1	5	
1156-2	Rio Chama	Rio Cebolla	Y	4/19/2022	1	5	possible crayfish observed ~1km upstream
1160-1	Rio Chama	Rio Chama	Y	4/6/2022	3	a:0.8, b:0.8, c:0.8	
1162-1	Rio Chama	Rio Cebolla	Y	4/19/2022	1	5	Fish present ~30 M upstream, crayfish present ~270 M upstream
1167-2	Rio Chama	Rio Cebolla	Y	4/19/2022	3	a:1, b:1.5, c:2	crayfish present
1171-1	Rio Chama	Rio Cebolla	Y	4/19/2022	3	a:1.5, b:2, c:1.5	no flow
1187-3	Rio Chama	Canjilon Creek	Y	4/17/2022	3	a:0.5, b:0.5, c:0.5	
1194-2	Rio Chama	Canjilon Creek	Y	4/17/2022	3	a:0.3, b:0.3, c:0.3	
1236-1	Rio Chama	Rio Chama	Y	4/6/2022	3	a:0.8, b:0.85, c:0.85	
125-2	Rio Chama	Coyote Creek	Y	4/20/2022	1	5	
1253-3	Rio Chama	Canjilon Creek	Y	4/17/2022	3	a:1, b:0.5, c:0.5	
1268-3	Rio Chama	Rio Chama	Y	4/6/2022	3	a:0.8, b:0.8, c:0.8	
128-2	Rio Chama	Polvadera Creek	Y	4/20/2022	3	a:1.2, b:1.2, c:1.2	
1311-1	Rio Chama	Rio Chama	Y	4/6/2022	3	a:0.8, b:0.8, c:0.8	Muddy/silty
1319-1	Rio Chama	Rio Nutrias	Y	4/6/2022	3	a:1.75, b:2, c:1.75	
1319-3	Rio Chama	Rio Nutrias	Y	4/18/2022	3	a:0.01, b:0.01, c:0.01	No water moving through filters, estimated volume in tubing
1324-10	Rio Chama	Terrero Creek	Y	4/20/2022	3	a:0.5, b:0.5, c:0.5	Water stained
1324-13	Rio Chama	Terrero Creek	Y	4/20/2022	3	a:1.5, b:1.3, c:1.2	Incised channel below culvert, stained water
1352-1	Rio Chama	Rio Chama	Y	4/6/2022	3	a:1.5, b:1.4, c:1.5	
1370-1	Rio Chama	Rio Nutrias	Y	4/20/2022	3	a:0.4, b:0.4, c:0.3	
1377-1	Rio Chama	Rio Nutrias	Y	4/20/2022	3	a:0.5, b:0.5, c:0.5	Frogs in pond on intermittent tributary, bear track in snow
155-1	Rio Chama	Coyote Creek	Y	4/20/2022	3	a:1.7, b:1.9, c:1.4	
160-1	Rio Chama	Coyote Creek	Y	4/20/2022	2	a:2.5, b:2.5	
186-2	Rio Chama	Polvadera Creek	Y	4/20/2022	1	5	
192-1	Rio Chama	Canones Creek	Y	4/18/2022	2	a:2.5, b:2.5	Moved downstream 114M? For access

209-4	Rio Chama	Polvadera Creek	Y	4/18/2022	1	5	
216-1	Rio Chama	Coyote Creek	Y	4/20/2022	1	5	
225-1	Rio Chama	Canones Creek	Y	4/17/2022	3	a:2, b:2, c:1	
225-3	Rio Chama	Canones Creek	Y	4/18/2022	2	a:2.5, b:2.5	
268-2	Rio Chama	Rio Capulin - Corrected	Y	4/18/2022	3	a:0.9, b:0.75, c:0.8	
29-3	Rio Chama	Rio del Oso	Y	4/21/2022	1	5	
313-1	Rio Chama	Canones Creek	Y	4/17/2022	3	a:1.1, b:1.1, c:1	Moved upstream 300M to get on BLM land
348-1	Rio Chama	Rio Chama	Y	4/4/2022	2	a:2.5, b:2.5	
354-1	Rio Chama	Rio Chama	Y	4/4/2022	2	a:2.5, b:2.5	
372-1	Rio Chama	Rio Gallina	Y	4/18/2022	3	a:0.5, b:0.55, c:0.52	
372-3	Rio Chama	Rio Gallina	Y	4/18/2022	3	a:1, b:1, c:1	
413-2	Rio Chama	Rio Capulin	Y	4/19/2022	3	a:0.35, b:0.37, c:0.36	Site moved to road
413-4	Rio Chama	Rio Capulin	Y	4/18/2022	3	a:0.4, b:0.4, c:0.5	Snow melt/runoff. Very turbid
413-5	Rio Chama	Rio Capulin	Y	4/18/2022	3	a:0.3, b:0.3, c:0.3	
456-2	Rio Chama	Rio Gallina	Y	4/18/2022	3	a:0.5, b:0.5, c:0.5	Extremely turbid, likely clays from runoff
541-3	Rio Chama	Rio Capulin	Y	4/19/2022	3	a:0.12, b:0.13, c:0.13	
545-1	Rio Chama	Rio Gallina	Y	4/19/2022	3	a:0.1, b:0.13, c:0.1	
56-1	Rio Chama	Coyote Creek	Y	4/20/2022	1	5	
56-3	Rio Chama	Coyote Creek	Y	4/20/2022	1	5	
56-5	Rio Chama	Coyote Creek	Y	4/20/2022	1	5	
60-1	Rio Chama	Rio del Oso	Y	4/18/2022	1	5	
645-1	Rio Chama	Cañada de la Fuertes	Y	4/17/2022	3	a:0.24, b:0.24, c:0.24	
837-2	Rio Chama	Rio Chama	Y	4/17/2022	3	a:.29, b:0.28, c:0.32	
897-1	Rio Chama	Rio Gallina	Y	4/7/2022	3	a:0.1, b:0.1, c:0.1	Extremely muddy. Mink (?) in mainstream Chama upstream
958-1	Rio Chama	Rio Gallina	Y	4/19/2022	3	a:0.07, b:0.07, c:0.07	Extremely turbid
961-1	Rio Chama	Rio Chama	Y	4/7/2022	3	a:0.6, b:0.6, c:0.6	Upstream of Rio Gallina. Very low flow
961-3	Rio Chama	Rio Chama	Y	4/7/2022	3	a:1, b:1, c:1	
984-1	Rio Chama	Canjilon Creek	Y	4/17/2022	3	a:0.5, b:0.5, c:0.2	
993-3	Rio Chama	Rio Gallina	Y	4/19/2022	3	a:0.05, b:0.05, c:0.05	Extremely turbid
994-1	Rio Chama	Rio Gallina	Y	4/19/2022	3	a:0.075, b:0.075, c:0.075	Extremely silty, lots of sediment on filters
001-1	Rio Chama	Cañones Creek	Y	4/17/2022	3	a:0.53, b:0.56	
001-1	Rio Chama	Rio Chama	Y	4/17/2022	3	a:0.2, b:0.2, c:0.2	Weekend high flows, added site at big eddy takeout, all volumes between 100-200mL (estimated)
001-1	Rio Chama	Rio Chama	Y	4/20/2022	3	a:1.4, b:1.4, c:1.45	
500-1	Rio Chama	Rio Capulin	Y	4/19/2022	3	a:0.17, b:0.18, c:0.15	

Table 2. Location and collection details of eDNA samples collected by the NGC in the Upper Rio Grande basin for the delineation of Rio Grande sucker and Rio Grande chub distributions. “Y” in denotes eDNA sample was collected, “N” denotes site was visited but no eDNA could be collected. Multiple filters were used if 5L could not be filtered through a single filter. If this occurred, we used additional filters until 5L was filtered in total or three individual filters were clogged, stopping after whichever occurred first.

Site	Drainage	Stream	Sampled	Date Collected	No. Filters	Filter Volume (L)	Field Notes
1013-1	Upper Rio Grande	Rio Grande	Y	3/29/2022	3	a:1, b:1, c:1	
1013-6	Upper Rio Grande	Rio Grande	Y	4/1/2022	3	a:0.55, b:0.525, c:0.5	Moved downstream due to access
1085-2	Upper Rio Grande	Rio Grande	Y	3/29/2022	3	a:1.2, b:1.2, c:1.2	Moved downstream ~1km due to canyon wall
1166-1	Upper Rio Grande	Rio Grande	Y	4/1/2022	3	a:0.65, b:0.65, c:0.625	
1255-1	Upper Rio Grande	Rio Grande	Y	3/31/2022	3	a:0.7, b:0.7, c:0.6	
1321-5	Upper Rio Grande	Rio Grande	Y	3/31/2022	3	a:0.6, b:0.6, c:0.55	
1361-1	Upper Rio Grande	Rio Grande	Y	3/31/2022	3	a:0.45, b:1.1, c:0.5	filter "b" has two small holes that developed towards end of filtering
1390-2	Upper Rio Grande	Rio Grande	Y	3/31/2022	3	a:0.7, b:0.675, c:0.725	
1424-1	Upper Rio Grande	Rio Grande	Y	3/30/2022	3	a:0.75, b:0.7, c:0.65	~600m downstream of site
1439-1	Upper Rio Grande	Rio Grande	Y	3/30/2022	3	a:0.65, b:0.75, c:0.68	
1439-3	Upper Rio Grande	Rio Grande	Y	3/30/2022	3	a:0.65, b:0.55, c:0.55	State line
221-1	Upper Rio Grande	Rio Medio	Y	4/3/2022	2	a:3.5, b:1.5	
221-2	Upper Rio Grande	Rio Medio	Y	4/4/2022	2	a:4, b:1	
344-1	Upper Rio Grande	Rio Santa Barbara	Y	4/4/2022	1	5	
353-11	Upper Rio Grande	Rio Santa Barbara	Y	4/4/2022	1	5	
353-13	Upper Rio Grande	Rio Santa Barbara	Y	4/4/2022	1	5	
474-1	Upper Rio Grande	Rio Grande del Rancho	Y	3/29/2022	1	5	Beaver activity
502-3	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.5, b:0.5, c:0.3	
502-7	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.5, b:0.5, c:0.5	
516-9	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.6, b:0.6, c:0.7	Moved upstream due to private land
530-1	Upper Rio Grande	Rio Grande del Rancho	Y	3/29/2022	2	a:3, b:2	
533-2	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.6, b:0.625, c:0.6	
544-1	Upper Rio Grande	Rio Grande del Rancho	Y	3/29/2022	2	a:4, b:1	Beaver activity
549-3	Upper Rio Grande	Rio Grande del Rancho	Y	3/29/2022	2	a:3.75, b:1.25	
555-3	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.7, b:0.8, c:0.8	
557-1	Upper Rio Grande	Rio Chiquito	Y	3/29/2022	1	5	
559-4	Upper Rio Grande	Rio Chiquito	Y	3/29/2022	1	5	Beaver activity
559-6	Upper Rio Grande	Rio Chiquito	Y	3/29/2022	1	5	Beaver activity
565-2	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.7, b:0.85, c:0.625	

584-3	Upper Rio Grande	Rio Fernando de Taos	Y	4/1/2022	3	a:0.75, b:0.75, c:1.25	Beaver activity
587-1	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.65, b:0.65, c:0.65	Moved upstream due to access
588-2	Upper Rio Grande	Rio Fernando de Taos	Y	4/3/2022	3	a:1, b:1, c:1	
590-2	Upper Rio Grande	Rio Fernando de Taos	Y	4/1/2022	2	a:3.5, b:1.5	Beaver activity
605-4	Upper Rio Grande	Rio Fernando de Taos	Y	4/2/2022	3	a:2, b:2, c:1	No flow, fish observed
605-9	Upper Rio Grande	Rio Fernando de Taos	Y	3/29/2022	3	a:1.5, b:1, c:1.5	
645-3	Upper Rio Grande	Rio Fernando de Taos	Y	4/1/2022	1	5	
645-5	Upper Rio Grande	Rio Fernando de Taos	Y	4/1/2022	2	a:2.5, b:2.5	
645-7	Upper Rio Grande	Rio Fernando de Taos	Y	4/1/2022	1	5	
742-1	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:1, b:1, b:0.75	Angler reports "chub" in upstream reach
752-1	Upper Rio Grande	Rio Hondo	Y	3/29/2022	2	a:3, b:2	
859-1	Upper Rio Grande	Rio Grande	Y	4/3/2022	3	a:0.9, b:0.9, c:0.825	
850-3	Upper Rio Grande	San Cristobal Creek	Y	4/2/2022	1	5	Move onto stream, coordinates off
603-1	Upper Rio Grande	Rio Fernando de Taos	Y	4/3/2022	2	a:2, b:3	Shifted because private. May share coordinates with 605-11
001-1	Upper Rio Grande	Red River	Y	3/29/2022	2	a:3, b:2	Added site. Upstream of confluence with Rio Grande
001-1	Upper Rio Grande	Rio Pueblo de Taos	Y	4/3/2022	3	a:1.05, b:1.05, c:1	No access
1426-1	Upper Rio Grande	Costilla Creek	N				Dry
305-2	Upper Rio Grande	Rio Santa Barbara	N				No access, snow
474-11	Upper Rio Grande	Rio Grande del Rancho	N				No access, snow
474-3	Upper Rio Grande	Rio Grande del Rancho	N				No access, snow
474-5	Upper Rio Grande	Rio Grande del Rancho	N				No access, snow
474-7	Upper Rio Grande	Rio Grande del Rancho	N				No access, snow
474-9	Upper Rio Grande	Rio Grande del Rancho	N				No access, snow
478-2	Upper Rio Grande	Rio Grande del Rancho	N				No access, snow
478-4	Upper Rio Grande	Rio Grande del Rancho	N				No access, snow
605-11	Upper Rio Grande	Rio Fernando de Taos	N				No access
645-9	Upper Rio Grande	Rio Fernando de Taos	N				No access, snow
797-3	Upper Rio Grande	Rio Grande	N				No access
832-1	Upper Rio Grande	San Cristobal Creek	N				Dry
832-3	Upper Rio Grande	San Cristobal Creek	N				Dry
850-1	Upper Rio Grande	San Cristobal Creek	N				No access

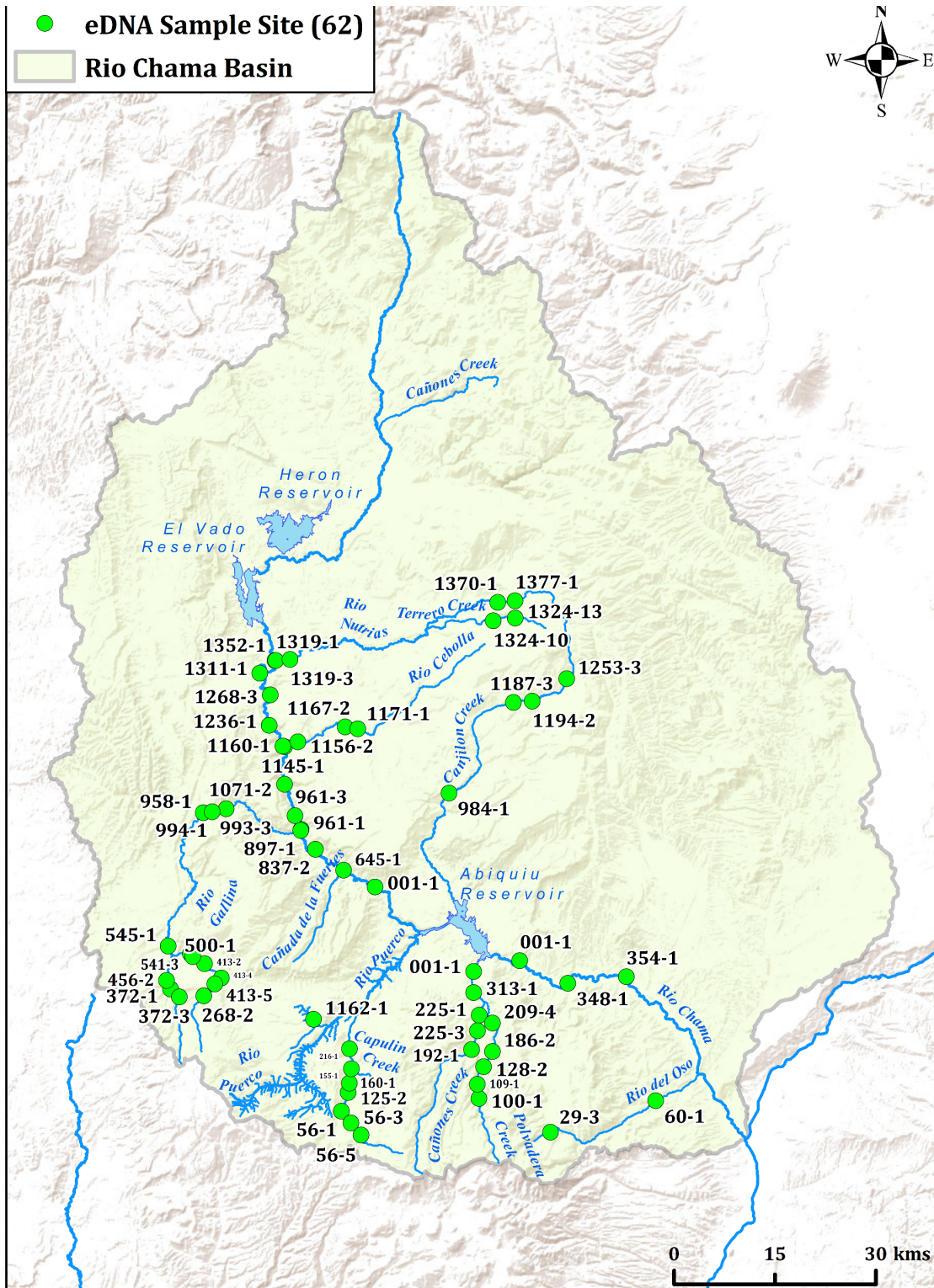


Figure 1. Locations of eDNA sites visited in the Rio Chama basin by the NGC in 2022. A green circle denotes eDNA sample collected. The transparent green background with a thick gray border represents the Rio Chama basin.

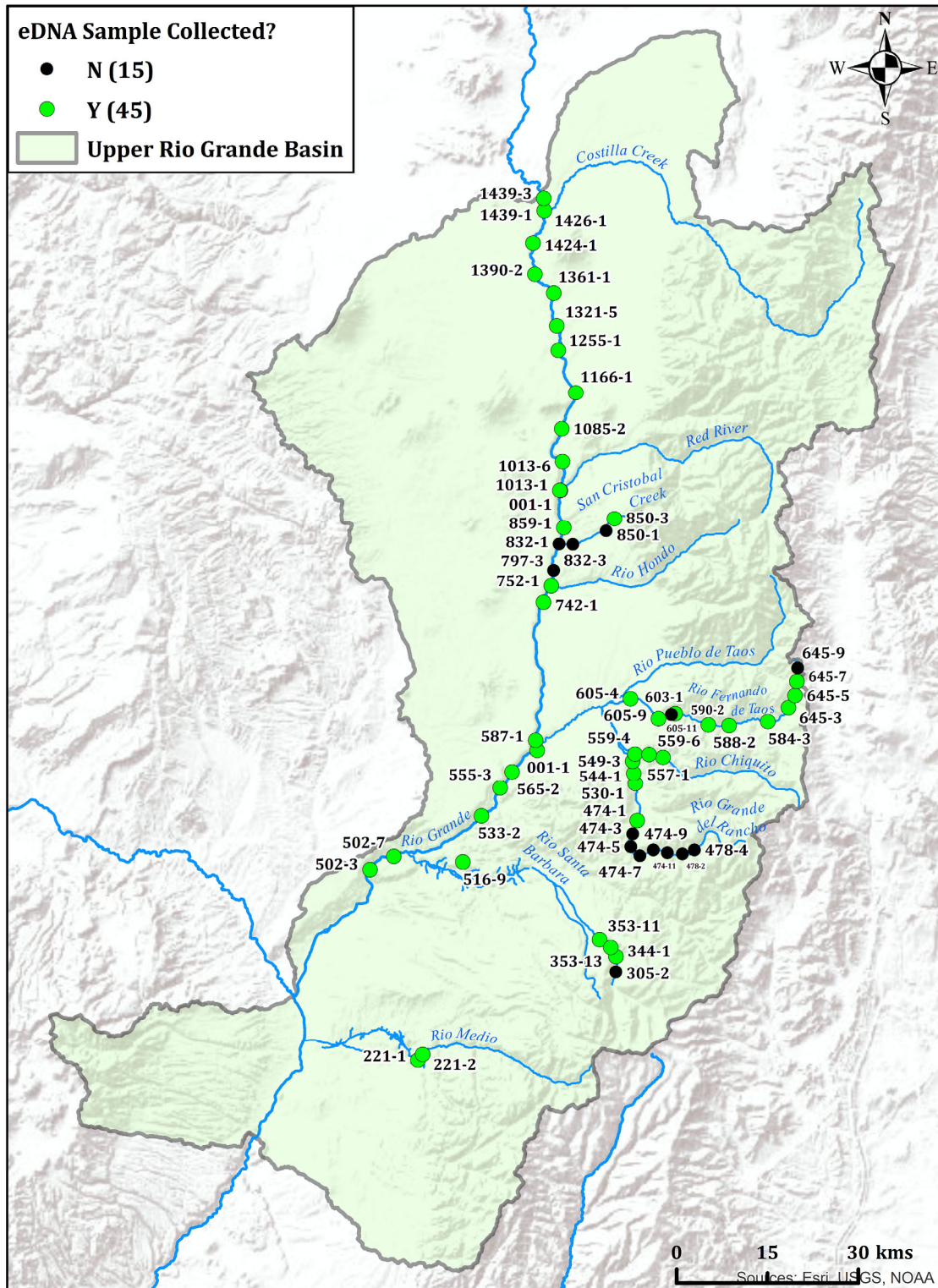


Figure 2. Locations of eDNA sites visited in the Upper Rio Grande basin by the NGC in 2022. A green circle denotes eDNA sample collected and a black circle denotes the eDNA site was visited but no sample could be collected. The transparent green background with a thick gray border represents the Upper Rio Grande basin.

Table 3. Results for eDNA samples collected by the NGC in the Rio Chama basin for the delineation of Rio Grande sucker and Rio Grande chub distributions. In DNA detected columns, “Y” in denotes DNA for the target species was detected (cells with “Y’s” are highlighted in light blue), “N” denotes target species DNA was not detected. # Positive Wells indicates the number of replicate (3 for most samples) reactions in which the species was detected. “Y” in Treated for inhibition denotes a sample was successfully treated for inhibition, and a “N” denotes a sample was not inhibited.

Site #	Feature Name	HUC Name	ID Tag	Rio Grande Sucker DNA Detected?	# Positive Wells (#/3)	Rio Grande Chub DNA Detected?	# Positive Wells (#/3)	Treated for inhibition?
1187-3	Canjilon Creek	Rio Chama	NM_041722_CANJ_27	N	0	Y	3	Y
1194-2	Canjilon Creek	Rio Chama	NM_041722_CANJ_26	N	0	Y	3	Y
1253-3	Canjilon Creek	Rio Chama	NM_041722_CANJ_25	N	0	N	0	Y
984-1	Canjilon Creek	Rio Chama	NM_041722_CANJ_24	N	0	Y	3	Y
001-1	Cañones Creek	Rio Chama	NM_041722_CANO_28	Y	3	N	0	Y
192-1	Cañones Creek	Rio Chama	NM_041822_CANO_34	N	0	N	0	Y
225-1	Cañones Creek	Rio Chama	NM_041722_CANO_29	Y	3	N	0	Y
225-3	Cañones Creek	Rio Chama	NM_041822_CANO_35	Y	2	N	0	Y
313-1	Cañones Creek	Rio Chama	NM_041722_CANO_30	Y	4/4	N	0/4	Y
056-1	Coyote Creek	Rio Chama	NM_042022_COYC_61	N	0	N	0	N
125-2	Coyote Creek	Rio Chama	NM_042022_COYC_64	N	0	N	0	Y
155-1	Coyote Creek	Rio Chama	NM_042022_COYC_65	N	0	N	0	Y
160-1	Coyote Creek	Rio Chama	NM_042022_COYC_66	N	0	N	0	Y
216-1	Coyote Creek	Rio Chama	NM_042022_COYC_67	N	0	N	0	N
56-3	Coyote Creek	Rio Chama	NM_042022_COYC_62	N	0	N	0	Y
56-5	Coyote Creek	Rio Chama	NM_042022_COYC_63	N	0	N	0	Y
209-4	Palvadera Creek	Rio Chama	NM_041822_PALC_43	N	0	N	0	N
100-1	Polvadera Creek	Rio Chama	NM_042022_POLC_48	N	0	N	0	Y
109-1	Polvadera Creek	Rio Chama	NM_042022_POLC_49	N	0	N	0	Y
128-2	Polvadera Creek	Rio Chama	NM_042022_POLC_50	N	0/9	N	0/9	Y
186-2	Polvadera Creek	Rio Chama	NM_042022_POLC_51	N	0	N	0	N
268-2	Rio Capulin	Rio Chama	NM_041822_RCAP_36	N	0	N	0	Y
413-2	Rio Capulin	Rio Chama	NM_041922_RCAP_48	N	0	N	0	N
413-4	Rio Capulin	Rio Chama	NM_041822_RCAP_37	N	0	N	0	Y
413-5	Rio Capulin	Rio Chama	NM_041822_RCAP_38	N	0/4	N	0/6	Y
500-1	Rio Capulin	Rio Chama	NM_041922_RCAP_49	N	0	N	0	Y
541-3	Rio Capulin	Rio Chama	NM_041922_RCAP_50	N	0	N	0	Y
1145-1	Rio Cebolla	Rio Chama	NM_040622_RCEB_48	N	0	N	0	Y
1156-2	Rio Cebolla	Rio Chama	NM_041922_RCEB_56	N	0	Y	3	N

1162-1	Rio Cebolla	Rio Chama	NM_041922_RCEB_57	N	0	Y	3	N
1167-2	Rio Cebolla	Rio Chama	NM_041922_RCEB_58	N	0	Y	3	N
1171-1	Rio Cebolla	Rio Chama	NM_041922_RCEB_59	N	0	Y	3	N
001-1	Rio Chama	Rio Chama	NM_041722_RCHA_31	N	0	Y	2	Y
001-1	Rio Chama	Rio Chama	NM_042022_RCHA_60	N	0	Y	3	N
1071-2	Rio Chama	Rio Chama	NM_040622_RCHA_49	N	0	N	0	Y
1160-1	Rio Chama	Rio Chama	NM_040622_RCHA_50	N	0	Y	1	Y
1236-1	Rio Chama	Rio Chama	NM_040622_RCHA_51	N	0	N	0	Y
1268-3	Rio Chama	Rio Chama	NM_040622_RCHA_52	N	0	Y	2	Y
1311-11	Rio Chama	Rio Chama	NM_040622_RCHA_53	N	0	N	0	Y
1352-11	Rio Chama	Rio Chama	NM_040622_RCHA_54	N	0	Y	3	Y
348-1	Rio Chama	Rio Chama	NM_040422_RCHA_42	N	0	Y	3	Y
354-1	Rio Chama	Rio Chama	NM_040422_RCHA_43	N	0	Y	3	Y
645-1	Rio Chama	Rio Chama	NM_041722_RCHA_32	N	0/4	Y	1/4	Y
837-2	Rio Chama	Rio Chama	NM_041722_RCHA_33	N	0	N	0	Y
961-1	Rio Chama	Rio Chama	NM_040722_RCHA_56	N	0	N	0	Y
961-3	Rio Chama	Rio Chama	NM_040722_RCHA_57	N	0	N	0	Y
29-3	Rio Del Oso	Rio Chama	NM_042122_RDOS_52	N	0	N	0	N
60-1	Rio del Oso	Rio Chama	NM_041822_ROSO_42	N	0	N	0	N
372-1	Rio Gallina	Rio Chama	NM_041822_RGAL_39	N	0/9	N	0/6	Y
372-3	Rio Gallina	Rio Chama	NM_041822_RGAL_40	N	0	N	0	Y
456-2	Rio Gallina	Rio Chama	NM_041822_RGAL_41	N	0/6	N	0/6	Y
545-1	Rio Gallina	Rio Chama	NM_041922_RGAL_44	N	0	N	0	Y
897-1	Rio Gallina	Rio Chama	NM_040722_RGAL_58	N	0	N	0	Y
958-1	Rio Gallina	Rio Chama	NM_041922_RGAL_45	N	0	N	0	Y
993-3	Rio Gallina	Rio Chama	NM_041922_RGAL_46	N	0/4	N	0	Y
994-1	Rio Gallina	Rio Chama	NM_041922_RGAL_47	N	0/4	N	0/6	Y
1319-1	Rio Nutrias	Rio Chama	NM_040622_RNUT_55	N	0	Y	2	Y
1319-3	Rio Nutrias	Rio Chama	NM_041822_RNUT_53	N	0	Y	3	Y
1370-1	Rio Nutrias	Rio Chama	NM_042022_RNUT_54	N	0	N	0	Y
1377-1	Rio Nutrias	Rio Chama	NM_042022_RNUT_55	N	0	Y	3	Y
1324-10	Terrero Creek	Rio Chama	NM_042022_TERC_51	N	0	N	0	Y
1324-13	Terrero Creek	Rio Chama	NM_042022_TERC_52	N	0	N	0	Y

Table 4. Results for eDNA samples collected by the NGC in the Upper Rio Grande basin for the delineation of Rio Grande sucker and Rio Grande chub distributions. In DNA detected columns, “Y” denotes DNA for the target species was detected (cells with “Y’s” are highlighted in light blue), “N” denotes target species’ DNA was not detected. # Positive Wells indicates the number of replicate (3 for most samples) reactions in which the species was detected. “Y” in Treated for inhibition denotes a sample was successfully treated for inhibition, and a “N” denotes a sample was not inhibited.

Site #	Feature Name	HUC Name	ID Tag	Rio Grande Sucker DNA Detected?	# Positive Wells (#/3)	Rio Grande Chub DNA Detected?	# Positive Wells (#/3)	Treated for inhibition?
001-1	Red River	Upper Rio Grande	NM_032922_REDRA_01	N	0	N	0	Y
557-1	Rio Chiquito	Upper Rio Grande	NM_032922_RCHI_02	N	0	N	0	Y
559-4	Rio Chiquito	Upper Rio Grande	NM_032922_RCHI_03	N	0	N	0	Y
559-6	Rio Chiquito	Upper Rio Grande	NM_032922_RCHI_04	N	0	N	0	Y
584-3	Rio Fernando de Taos	Upper Rio Grande	NM_040122_RFDT_20	N	0	N	0	Y
588-2	Rio Fernando de Taos	Upper Rio Grande	NM_040322_RFDT_29	N	0	N	0	Y
590-2	Rio Fernando de Taos	Upper Rio Grande	NM_040122_RFDT_21	N	0	N	0	Y
603-1	Rio Fernando de Taos	Upper Rio Grande	NM_040322_RFDT_30	N	0	N	0	Y
605-4	Rio Fernando de Taos	Upper Rio Grande	NM_040222_RFDT_27	N	0	N	0	Y
605-9	Rio Fernando de Taos	Upper Rio Grande	NM_032922_RFDT_05	N	0/4	N	0/4	Y
645-3	Rio Fernando de Taos	Upper Rio Grande	NM_040122_RFDT_22	N	0	N	0	Y
645-5	Rio Fernando de Taos	Upper Rio Grande	NM_040122_RFDT_23	N	0	N	0	Y
645-7	Rio Fernando de Taos	Upper Rio Grande	NM_040122_RFDT_24	N	0	N	0	Y
1013-1	Rio Grande	Upper Rio Grande	NM_032922_RGRA_06	N	0	Y	3	Y
1013-6	Rio Grande	Upper Rio Grande	NM_040122_RGRA_25	N	0	Y	3	Y
1085-2	Rio Grande	Upper Rio Grande	NM_032922_RGRA_07	N	0	Y	3	Y
1166-1	Rio Grande	Upper Rio Grande	NM_040122_RGRA_26	N	0	Y	3	Y
1255-1	Rio Grande	Upper Rio Grande	NM_033122_RGRA_16	N	0	Y	3	Y
1321-5	Rio Grande	Upper Rio Grande	NM_033122_RGRA_17	N	0	Y	3	Y
1361-1	Rio Grande	Upper Rio Grande	NM_033122_RGRA_18	N	0	Y	2	Y
1390-2	Rio Grande	Upper Rio Grande	NM_033122_RGRA_19	N	0	Y	2	Y
1424-1	Rio Grande	Upper Rio Grande	NM_033022_RGRA_13	N	0	Y	3	Y
1439-1	Rio Grande	Upper Rio Grande	NM_033022_RGRA_14	N	0	Y	3	Y
1439-3	Rio Grande	Upper Rio Grande	NM_033022_RGRA_15	N	0	Y	2	Y
502-3	Rio Grande	Upper Rio Grande	NM_040322_RGRA_31	Y	2	Y	3	Y
502-7	Rio Grande	Upper Rio Grande	NM_040322_RGRA_32	Y	2	Y	3	Y
516-9	Rio Grande	Upper Rio Grande	NM_040322_RGRA_33	N	0	Y	3	Y
533-2	Rio Grande	Upper Rio Grande	NM_040322_RGRA_34	N	0	Y	3	Y
555-3	Rio Grande	Upper Rio Grande	NM_040322_RGRA_35	N	0	Y	3	Y

565-2	Rio Grande	Upper Rio Grande	NM_040322_RGRA_36	Y	2	Y	3	Y
587-1	Rio Grande	Upper Rio Grande	NM_040322_RGRA_37	N	0	Y	3	Y
742-1	Rio Grande	Upper Rio Grande	NM_040322_RGRA_38	N	0	Y	3	Y
859-1	Rio Grande	Upper Rio Grande	NM_040322_RGRA_39	N	0	Y	3	Y
474-1	Rio Grande del Rancho	Upper Rio Grande	NM_032922_RGDR_08	N	0	N	0	Y
530-1	Rio Grande del Rancho	Upper Rio Grande	NM_032922_RGDR_09	N	0	Y	3	Y
544-1	Rio Grande del Rancho	Upper Rio Grande	NM_032922_RGDR_10	N	0	Y	3	Y
549-3	Rio Grande del Rancho	Upper Rio Grande	NM_032922_RGDR_11	N	0	Y	3	Y
752-1	Rio Hondo	Upper Rio Grande	NM_032922_RHON_12	N	0	N	0	Y
221-1	Rio Medio	Upper Rio Grande	NM_040322_RMED_40	N	0	N	0	Y
221-2	Rio Medio	Upper Rio Grande	NM_040422_RMED_44	N	0	N	0	Y
001-1	Rio Pueblo de Taos	Upper Rio Grande	NM_040322_RPDT_41	Y	3	Y	3	Y
344-1	Rio Santa Barbara	Upper Rio Grande	NM_040422_RSBA_45	N	0	N	0	Y
353-11	Rio Santa Barbara	Upper Rio Grande	NM_040422_RSBA_46	N	0	N	0	Y
353-13	Rio Santa Barbara	Upper Rio Grande	NM_040422_RSBA_47	N	0	N	0	N
850-3	San Cristobal Creek	Upper Rio Grande	NM_040222_SCRC_28	N	0	N	0	Y

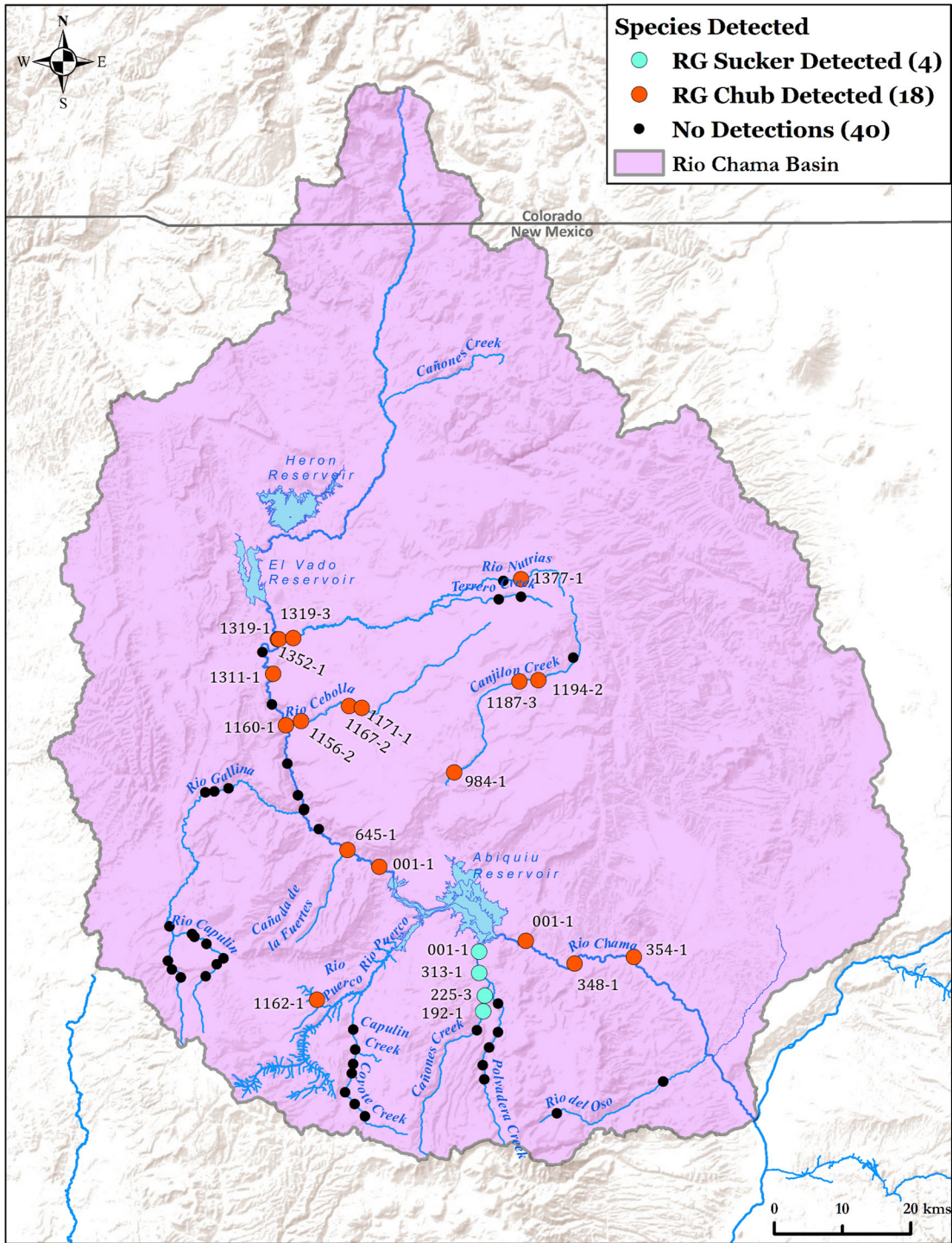


Figure 3. Results of analysis of eDNA samples collected in the Rio Chama basin by the NGC in 2022. A cyan circle denotes Rio Grande sucker DNA detected and a red circle denotes Rio Grande chub DNA detected. There were no sites where both species were detected. A black circle represents no target species DNA was detected. Site IDs are displayed for samples with a positive detection. The transparent purple background with a thick gray border represents the Rio Chama basin.

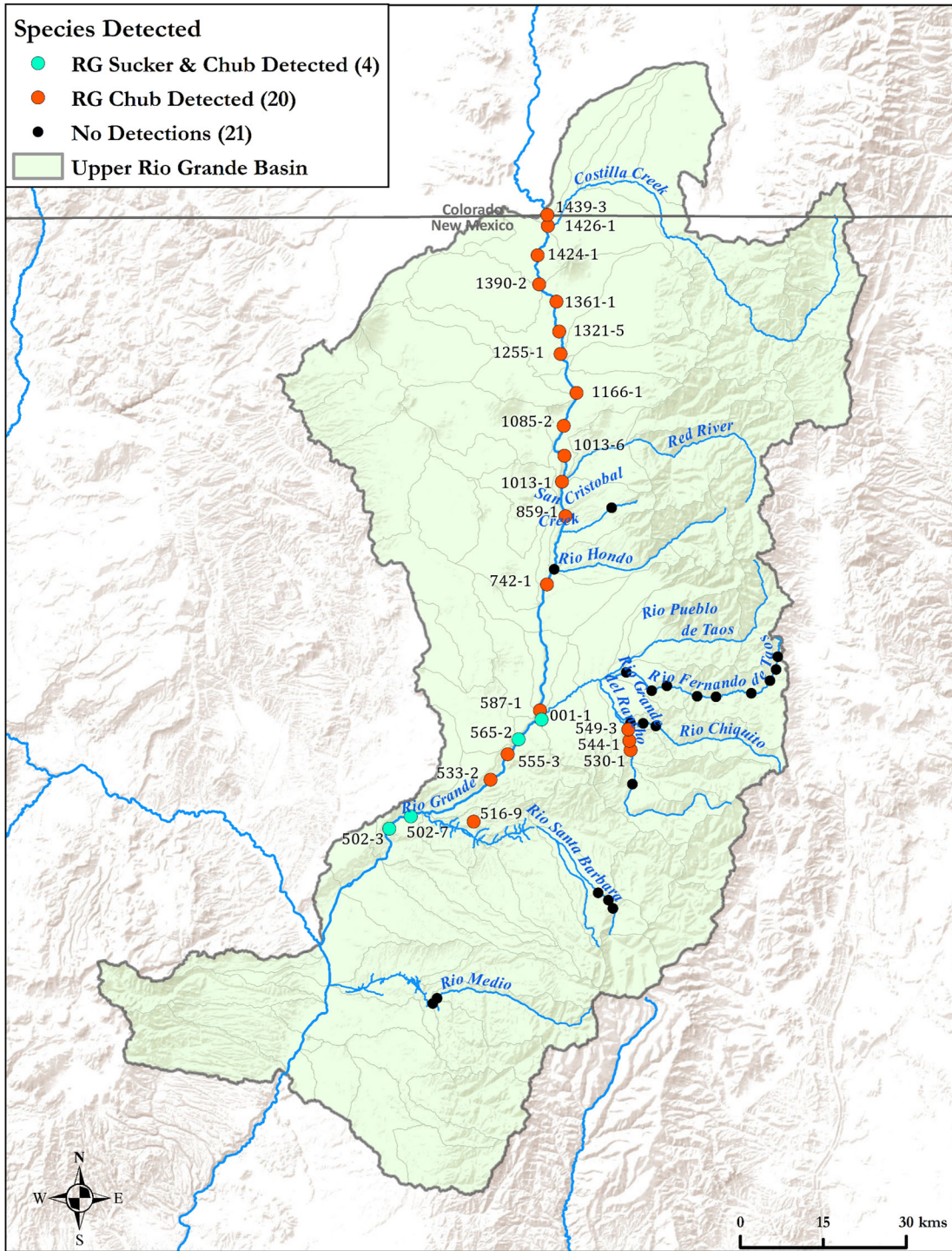


Figure 4. Results of analysis of eDNA samples collected in the Upper Rio Grande basin by the NGC in 2022. A cyan circle denotes Rio Grande sucker and Rio Grande chub DNA both detected. A red circle denotes solely Rio Grande chub DNA detected. A black circle represents no target species DNA was detected. Site IDs are displayed only for samples with a positive detection. The transparent green background with a thick gray border represents the Upper Rio Grande basin.

Appendix A

Table A-1. Results for eDNA samples collected by the NGC in the Mimbres basin for the delineation of Chihuahua chub distributions. In DNA detected columns, “Y” denotes DNA for the target species was detected (cells with “Y’s” are highlighted in light blue), “N” denotes target species’ DNA was not detected. # Positive Wells indicates the number of replicate (3 for most samples) reactions in which the species was detected. eDNA copies per liter is the number of target copies of DNA estimated in each liter of the sample.

Site ID	Site	HUC Name	Date Collected	Chihuahua Chub DNA Detected?	# Positive Wells (#/3)	eDNA copies per Liter	Field Notes
1306-1	East Canyon	Mimbres	4/10/2022	N	0		
1321-1	East Canyon	Mimbres	4/10/2022	N	0		
1321-2	East Canyon	Mimbres	4/10/2022	N	0		
1324-1	East Canyon	Mimbres	4/10/2022	N	0		
1327-1	East Canyon	Mimbres	4/10/2022	N	0		
1328-3	East Canyon	Mimbres	4/10/2022	N	0		
1328-4	East Canyon	Mimbres	4/10/2022	N	0		
1241-2	Mimbres River	Mimbres	4/10/2022	Y	3	5097.70	
1355-1	Mimbres River	Mimbres	4/10/2022	Y	3	284.74	
1358-1	Mimbres River	Mimbres	4/10/2022	Y	3	2431.40	
1190-5	Iron Creek	Mimbres	4/11/2022	N	0		
1190-6	Iron Creek	Mimbres	4/11/2022	N	0		
1220-1	Iron Creek	Mimbres	4/11/2022	N	0		
1220-2	Iron Creek	Mimbres	4/11/2022	N	0		
1220-3	Iron Creek	Mimbres	4/11/2022	N	0		
1243-1	Iron Creek	Mimbres	4/11/2022	N	0		
1234-1	Iron Creek	Mimbres	4/11/2022	N	0		
1269-1	Allie Canyon	Mimbres	4/11/2022	N	0		
1269-2	Allie Canyon	Mimbres	4/11/2022	N	0		
1273-1	Allie Canyon	Mimbres	4/11/2022	N	0		
1278-1	Allie Canyon	Mimbres	4/11/2022	N	0		
1259-1	Mimbres River	Mimbres	4/11/2022	Y	3	164.77	
1227-2	Noonday Creek	Mimbres	4/11/2022	N	0		
1227-4	Noonday Creek	Mimbres	4/11/2022	N	0		
1227-5	Noonday Creek	Mimbres	4/11/2022	N	0		
1267-1	Noonday Creek	Mimbres	4/11/2022	N	0		
1382-1	Mimbres River	Mimbres	4/12/2022	Y	3	66.22	
1389-1	Mimbres River	Mimbres	4/12/2022	Y	3	1130.20	
1389-2	Mimbres River	Mimbres	4/12/2022	Y	3	425.60	
1389-3	Mimbres River	Mimbres	4/12/2022	Y	3	142.27	
1391-1	Mimbres River	Mimbres	4/12/2022	Y	3	181.09	
1391-2	Mimbres River	Mimbres	4/12/2022	Y	3	361.45	
1395-2	Mimbres River	Mimbres	4/12/2022	Y	3	96.84	
1395-3	Mimbres River	Mimbres	4/12/2022	Y	3	102.27	
1395-4	Mimbres River	Mimbres	4/12/2022	Y	3	167.57	
1397-1	Mimbres River	Mimbres	4/12/2022	Y	3	1842.00	
1398-1	NF Mimbres	Mimbres	4/12/2022	N	0		
1407-1	NF Mimbres	Mimbres	4/12/2022	N	0		
1387-1	SF Mimbres	Mimbres	4/13/2022	N	0		
1393-1	SF Mimbres	Mimbres	4/13/2022	Y	2	10.83	

1393-2	SF Mimbres	Mimbres	4/13/2022	N	0		
1394-1	SF Mimbres	Mimbres	4/13/2022	N	0		
1402-1	NF Mimbres	Mimbres	4/13/2022	N	0		
1406-1	Tributary to NF Mimbres	Mimbres	4/13/2022	N	0		
1407-2	NF Mimbres	Mimbres	4/13/2022	N	0		
1407-3	NF Mimbres	Mimbres	4/13/2022	N	0		
1407-4	NF Mimbres	Mimbres	4/13/2022	N	0		
1347-1	East Fork Mimbres River	Mimbres	4/15/2022	Y	1	5.13	
1347-2	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1352-1	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1352-2	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1352-3	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1356-1	East Fork Mimbres River	Mimbres	4/15/2022	N	0		Sampled 50M downstream to avoid stagnant pool
1356-2	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1356-3	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1356-4	East Fork Mimbres River	Mimbres	4/15/2022	Y	1	1.58	
1356-5	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1356-6	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1356-7	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1356-8	East Fork Mimbres River	Mimbres	4/15/2022	N	0		
1363-1	Tributary to EF Mimbres River	Mimbres	4/15/2022	N	0		
1349-1	Tributary to EF Mimbres River	Mimbres	4/15/2022	N	0		wetted width ~0.5 M, no second filter
1381-2	Mimbres River	Mimbres	4/15/2022	Y	3	345.55	
1366-1	Mimbres River	Mimbres	4/15/2022	Y	3	133.52	
1366-2	Mimbres River	Mimbres	4/15/2022	Y	3	1053.69	Moved upstream 200M due to hunters
000-01	Mimbres River	Mimbres	4/16/2022	Y	2	4.37	Dog in stream
000-02	Mimbres River	Mimbres	4/16/2022	Y	3	98.01	
000-03	Mimbres River	Mimbres	4/16/2022	N	0		
959-1	Mimbres River	Mimbres	4/16/2022	N	0		
1119-4	Mimbres River	Mimbres	4/16/2022	Y	3	4695.47	
E2-U	TNC Property at East Canyon	Mimbres	4/19/2022	Y	3	169.61	Collected at Upper end of E-fishing depletion site.
E2-L	TNC Property at East Canyon	Mimbres	4/19/2022	Y	3	174.42	Collected at lower end of E-fishing depletion site.
E3-U	Mimbres River at TNC McAnaly	Mimbres	4/19/2022	Y	3	380.15	Collected at Upper end of E-fishing depletion site.

E3-L	Mimbres River at TNC McAnaly	Mimbres	4/19/2022	Y	3	318.88	Collected at lower end of E-fishing depletion site.
E1-U	Mimbres at NMDGF perm site	Mimbres	4/19/2022	Y	3	2289.31	Collected at Upper end of E-fishing depletion site.
E1-L	Mimbres at NMDGF perm site	Mimbres	4/19/2022	Y	3	6291.28	Collected at lower end of E-fishing depletion site.

Table A-2. Results for eDNA samples collected by the NGC in the Mimbres basin for the delineation of Rio Grande sucker distributions. In DNA detected columns, “Y” denotes DNA for the target species was detected (cells with “Y’s” are highlighted in light blue), “N” denotes target species’ DNA was not detected. # Positive Wells indicates the number of replicate (3 for most samples) reactions in which the species was detected. eDNA copies per liter is the number of target copies of DNA estimated in each liter of the sample.

Site ID	Site	HUC Name	Date Collected	Rio Grande Sucker DNA Detected?	# Positive Wells (#/3)	eDNA copies per Liter	Field Notes
1306-1	East Canyon	Mimbres	4/10/2022	N	0	0	
1321-1	East Canyon	Mimbres	4/10/2022	N	0	0	
1321-2	East Canyon	Mimbres	4/10/2022	N	0	0	
1324-1	East Canyon	Mimbres	4/10/2022	N	0	0	
1327-1	East Canyon	Mimbres	4/10/2022	N	0	0	
1328-3	East Canyon	Mimbres	4/10/2022	N	0	0	
1328-4	East Canyon	Mimbres	4/10/2022	N	0	0	
1241-2	Mimbres River	Mimbres	4/10/2022	Y	3	33556.28	
1355-1	Mimbres River	Mimbres	4/10/2022	Y	3	5243.03	
1358-1	Mimbres River	Mimbres	4/10/2022	Y	3	96212.58	
1190-5	Iron Creek	Mimbres	4/11/2022	N	0	0	
1190-6	Iron Creek	Mimbres	4/11/2022	N	0	0	
1220-1	Iron Creek	Mimbres	4/11/2022	N	0	0	
1220-2	Iron Creek	Mimbres	4/11/2022	N	0	0	
1220-3	Iron Creek	Mimbres	4/11/2022	N	0	0	
1243-1	Iron Creek	Mimbres	4/11/2022	N	0	0	
1234-1	Iron Creek	Mimbres	4/11/2022	N	0	0	
1269-1	Allie Canyon	Mimbres	4/11/2022	Y	1	12.96	
1269-2	Allie Canyon	Mimbres	4/11/2022	Y	3	213.40	
1273-1	Allie Canyon	Mimbres	4/11/2022	Y	3	60625.23	
1278-1	Allie Canyon	Mimbres	4/11/2022	Y	3	324.50	
1259-1	Mimbres River	Mimbres	4/11/2022	Y	3	11267.98	
1227-2	Noonday Creek	Mimbres	4/11/2022	N	0	0	
1227-4	Noonday Creek	Mimbres	4/11/2022	N	0	0	
1227-5	Noonday Creek	Mimbres	4/11/2022	N	0	0	
1267-1	Noonday Creek	Mimbres	4/11/2022	N	0	0	
1382-1	Mimbres River	Mimbres	4/12/2022	Y	3	3219.05	
1389-1	Mimbres River	Mimbres	4/12/2022	Y	3	20536.18	
1389-2	Mimbres River	Mimbres	4/12/2022	Y	3	17534.66	
1389-3	Mimbres River	Mimbres	4/12/2022	Y	3	3401.18	
1391-1	Mimbres River	Mimbres	4/12/2022	Y	3	29647.84	
1391-2	Mimbres River	Mimbres	4/12/2022	Y	3	23533.13	
1395-2	Mimbres River	Mimbres	4/12/2022	Y	3	9436.95	
1395-3	Mimbres River	Mimbres	4/12/2022	Y	3	18756.50	
1395-4	Mimbres River	Mimbres	4/12/2022	Y	3	25843.16	
1397-1	Mimbres River	Mimbres	4/12/2022	Y	3	38418.38	

1398-1	NF Mimbres	Mimbres	4/12/2022	Y	3	8166.61	
1407-1	NF Mimbres	Mimbres	4/12/2022	N	0	0	
1387-1	SF Mimbres	Mimbres	4/13/2022	N	0	0	
1393-1	SF Mimbres	Mimbres	4/13/2022	Y	3	36380.24	
1393-2	SF Mimbres	Mimbres	4/13/2022	N	0	0	
1394-1	SF Mimbres	Mimbres	4/13/2022	Y	3	3858.56	
1402-1	NF Mimbres	Mimbres	4/13/2022	N	0	0	
1406-1	Tributary to NF Mimbres	Mimbres	4/13/2022	N	0	0	
1407-2	NF Mimbres	Mimbres	4/13/2022	N	0	0	
1407-3	NF Mimbres	Mimbres	4/13/2022	N	0	0	
1407-4	NF Mimbres	Mimbres	4/13/2022	N	0	0	
1347-1	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1347-2	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1352-1	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1352-2	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1352-3	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1356-1	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	Sampled 50M downstream to avoid stagnant pool
1356-2	East Fork Mimbres River	Mimbres	4/15/2022	Y	3	65.67	
1356-3	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1356-4	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1356-5	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1356-6	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1356-7	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1356-8	East Fork Mimbres River	Mimbres	4/15/2022	N	0	0	
1363-1	Tributary to EF Mimbres River	Mimbres	4/15/2022	N	0	0	
1349-1	Tributary to EF Mimbres River	Mimbres	4/15/2022	N	0	0	wetted width ~0.5 M, no second filter
1381-2	Mimbres River	Mimbres	4/15/2022	Y	3	46975.13	
1366-1	Mimbres River	Mimbres	4/15/2022	Y	3	2300.80	
1366-2	Mimbres River	Mimbres	4/15/2022	Y	3	19145.56	Moved upstream 200M due to hunters
000-01	Mimbres River	Mimbres	4/16/2022	Y	3	2126.77	Dog in stream
000-02	Mimbres River	Mimbres	4/16/2022	Y	3	3377.33	
000-03	Mimbres River	Mimbres	4/16/2022	Y	3	33299.63	
959-1	Mimbres River	Mimbres	4/16/2022	Y	3	20189.72	
1119-4	Mimbres River	Mimbres	4/16/2022	Y	3	13385.47	
E2-U	TNC Property at East Canyon	Mimbres	4/19/2022	Y	3	13234.53	Collected at Upper end of E-fishing depletion site.
E2-L	TNC Property at East Canyon	Mimbres	4/19/2022	Y	3	20187.16	Collected at lower end of E-fishing depletion site.
E3-U	Mimbres River at TNC McAnaly	Mimbres	4/19/2022	Y	3	7928.97	Collected at Upper end of E-fishing depletion site.
E3-L	Mimbres River at TNC McAnaly	Mimbres	4/19/2022	Y	3	7594.85	Collected at lower end of E-fishing depletion site.
E1-U	Mimbres at NMDGF perm site	Mimbres	4/19/2022	Y	3	5090.91	Collected at Upper end of E-fishing depletion site.
E1-L	Mimbres at NMDGF perm site	Mimbres	4/19/2022	Y	3	12627.60	Collected at lower end of E-fishing depletion site.

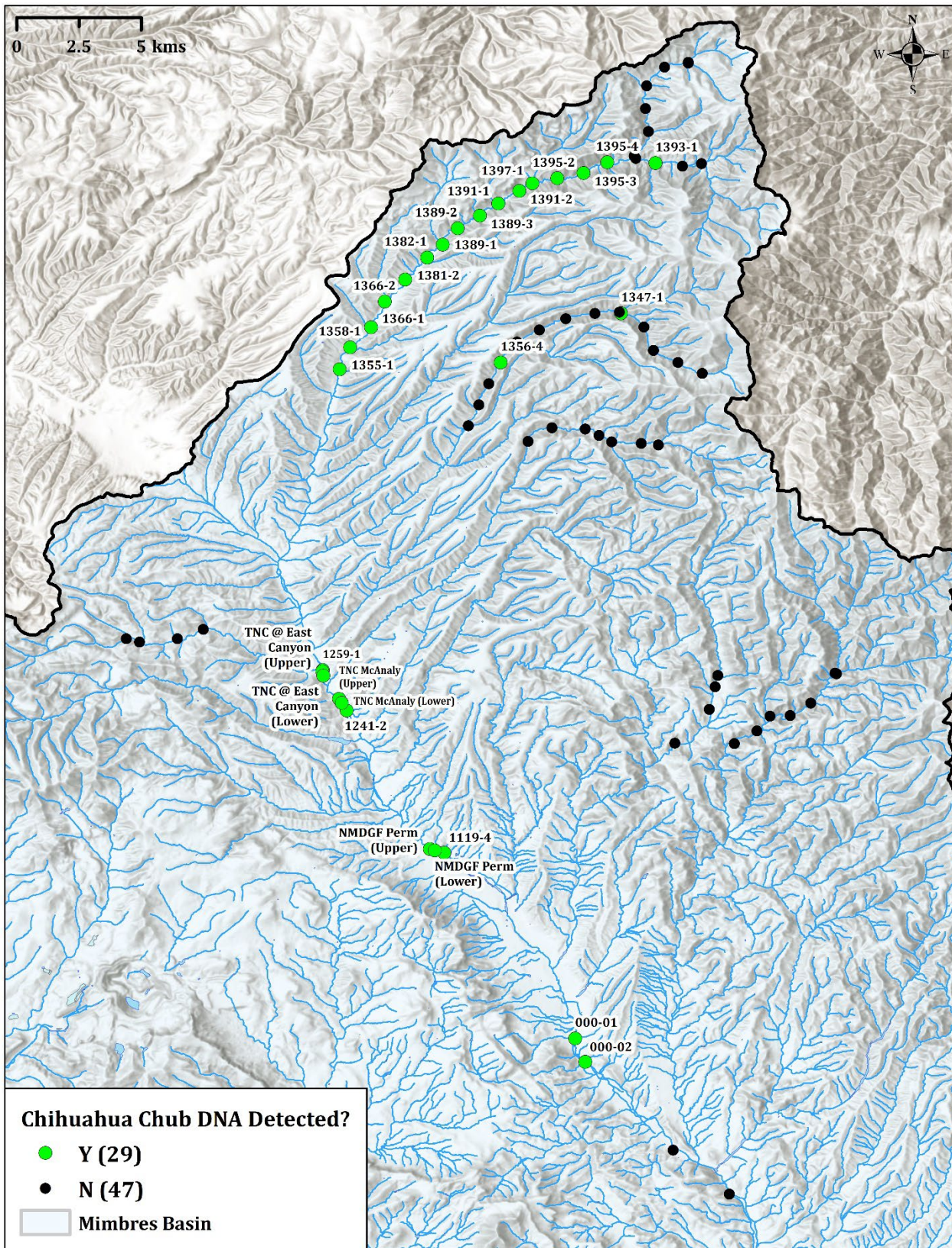


Figure A-1. Results of analysis of eDNA samples collected in the Mimbres basin by the NGC in 2022. A green circle denotes Chihuahua chub DNA detected and a black circle denotes no target DNA detected. Site IDs are displayed only for samples with a positive detection. The transparent blue background with a thick gray border represents the Mimbres basin.

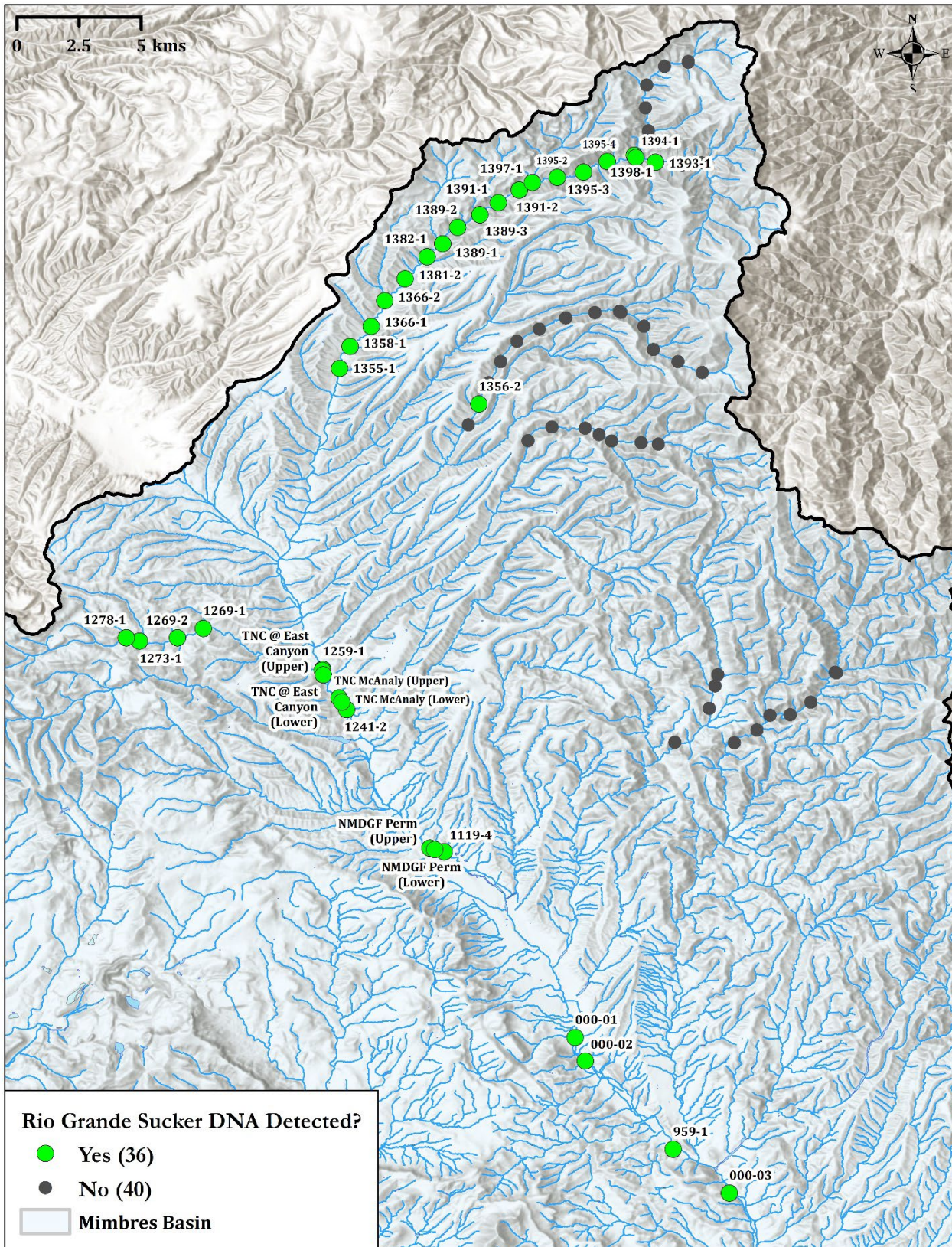


Figure A-2. Results of analysis of eDNA samples collected in the Mimbres basin by the NGC in 2022. A green circle denotes Rio Grande sucker DNA detected and a black circle denotes no target DNA detected. Site IDs are displayed only for samples with a positive detection. The transparent blue background with a thick gray border represents the Mimbres basin.