Final Report

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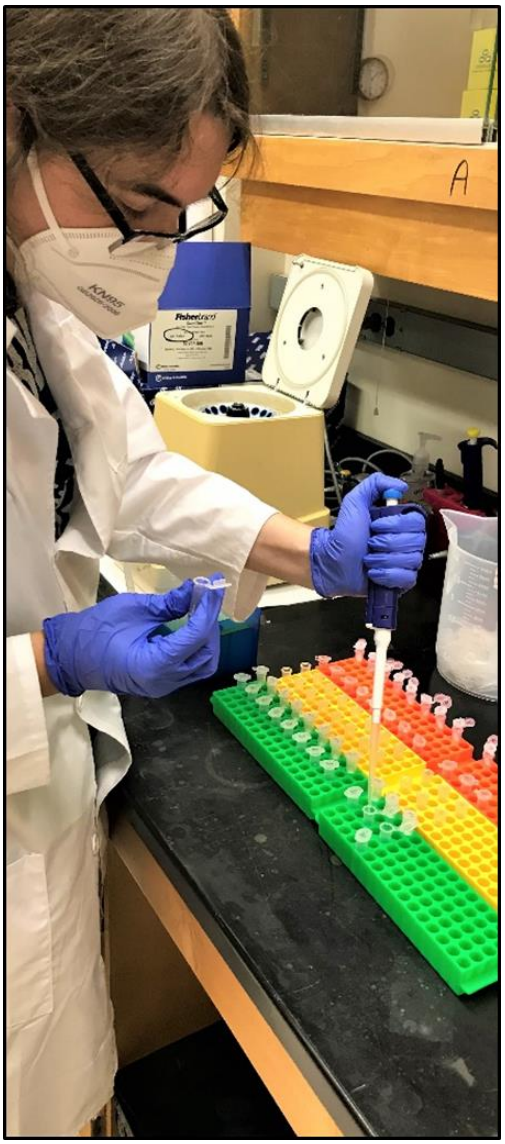
Final Report

Environmental Institute of Houston



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Abstract

The American Eel (*Anguilla rostrata*) is a catadromous panmictic species of greatest conservation need in Texas. There have been limited historical sampling and observations of juvenile stages (elver, glass eel, and leptocephali) within the Gulf coast region. Previous field efforts attempting to document juvenile eel recruitment in Texas have been unsuccessful. In response, the current study utilizes environmental DNA (eDNA) and plankton net sampling to compliment concurrent University of Houston-Clear Lake/Texas Parks and Wildlife Department eel ramp deployments along the central to upper Texas coast. Eight eel ramp sites were monitored using eDNA from July 2022 through June 2023, for a total of 50 sampling weeks. Sites selected for monitoring fit the following site characteristics: direct connectivity to the Gulf, spatial distribution, and water basins with records of American Eels. At each site, two 1-liter water samples were collected weekly, filtered, DNA extracted, and then analyzed for American Eel DNA with a dual marker quantitative polymerase chain reaction (qPCR) method. Additionally, plankton nets were deployed near coastal river mouths in an effort to detect ingressing juvenile eels. Out of the total 768 eDNA samples collected, 257 (33.5%) were positive for American eel DNA. Total percent positives for eDNA for all sites was highest between January and March, which coincided with the period of time when juvenile eels were captured in the ramp at site 119. Site 119 had the highest percent positives (84.1%) and also the highest average salinity (8 psu). No eels were collected during plankton surveys (total volume sampled 2,418.4 m^2) and no eDNA samples collected during plankton sampling were positive for American Eel DNA. A generalized additive model found that generally lower temperatures (10 – 20 ˚C), higher dissolved oxygen, higher salinities (above 1 PSU), and lower tide depth (below 1 ft) resulted in higher eDNA presence. Continued work is underway to focus eel ramp monitoring at sites that more closely resemble the conditions observed at site 119, which is fresh water flowing directly into brackish water. These efforts will be focused during the presumed recruitment window of December – April. As researchers learn more about the timing and life stages of American eels recruiting to the Texas coast, future studies can include eDNA monitoring to better determine how it may be used to monitor long-term trends in recruitment patterns along the Texas coast in a more efficient way. The completion of this study has resulted in critical baseline information for detecting the spatial and temporal recruitment of American Eel to the Texas Gulf Coast. This information can assist natural resource agencies in determining the conservation and management needs of American Eel populations in Texas.

Introduction

The American Eel (*Anguilla rostrata*) is a catadromous panmictic species that provides an important commercial fishery along the northeastern United States. The U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service reviewed the status of the American Eel in 2007 and found at that time that Endangered Species Act (ESA) protection for the American Eel was not warranted (USFWS 2007). A later petition filed by the Council for Endangered Species Act Reliability in 2010 was found to present substantial information that warranted the initiation of a more extensive status review of the species. After a second status review of the American Eel in 2015, USFWS again found that ESA protection was not warranted (Shepard, 2015; Federal Register 2015). However, within Texas, American Eel are considered a species of greatest conservation need (abundance rankings: G4 and S5, no status rankings available at this time) (TPWD 2012a).

An important aspect of American Eel life history is juvenile recruitment (leptocephali, glass eel, and elver) along the continental shelf and into the bays and estuaries of the western Atlantic Ocean, Gulf of Mexico, and the Caribbean Sea. This ingress of early life stages of catadromous fishes, especially metamorphic larvae, are likely to influence the outcome of their respective adult population success (Able et al., 2011). From 2017-2019, the Texas Parks and Wildlife Department (TPWD) in partnership with the University of Houston-Clear Lake (UHCL), Environmental Institute of Houston (EIH) and numerous citizen scientists, targeted recruiting glass and elver American Eels utilizing both eel mops and fyke nets. No juvenile American Eel were captured, despite documented occurrence of yellow eels in upstream freshwater watersheds (Oakley et al. 2021).

Traditionally, past and current sampling efforts for juvenile eels have been focused on upper estuarine and freshwater regions, especially in shallow water, because of the requirement of shallow-deployment for eel ramps, fyke nets, eel mops, and dip nets. In other regions, these methods have been the most successful for glass and elver eel capture (Harrison et al. 2014). While juvenile eels have been captured with plankton nets in large coastal areas, there has been limited success in collecting juvenile eels in great abundance using this method (Dutil et al. 2009). However, due to the lack of elevation change on the Texas Coastal Plain and the lack of major barriers (anthropogenic or natural) to upstream movement on rivers and streams, sampling with traditional methods is more difficult than on the Atlantic Coast. A UHCL/TPWD collaborative study began in 2022, deploying eel ramps along the central to upper Texas coast to continuously sample for immigrating American Eels. In this study, we utilize two novel methods for surveying American Eel in Texas; plankton nets to sample for leptocephali and glass eels as they first move into the estuary, and environmental DNA (eDNA) monitoring concurrent with eel ramp efforts.

Environmental DNA is an emerging tool for detecting single-species presence or overall biodiversity of aquatic species with the benefit of being relatively non-invasive, cost-effective, and an efficient sampling method (Lacoursiere-Roussel et al. 2016, Baillie et al. 2019, Baker et al. 2019, Port et al. 2016, Postaire et al. 2020). This methodology can be a useful method for detecting species that are not easily caught in traditional sampling gears, or are cryptic, endangered, or rare (Rees et al. 2014). Emerging research developing eDNA methodology was used to detect American Eels in the north-east United States (Chin et al. 2021), and additionally, eDNA studies on European Eels (*Anguilla anguilla*) have been used to determine seasonal trends of presence (Cardas et al. 2020, Weldon et al. 2020). The USWFS has

developed a working American Eel dual-marker quantitative eDNA assay that has been rigorously lab and field-tested (Moyer et al. 2022). This methodology has been screened in American Eels from South Carolina to Maine, is highly sensitive, and has previously detected American Eels in field samples. This research applied this assay to American Eel sampling along the Texas Gulf coast to provide additional confirmation that American Eels are present within coastal ecosystems during estimated recruitment periods.

The ability to add these additional sampling methods is important as previous field efforts were unable to detect the ingress of American Eels, and eel ramp efforts provided an opportunity to compare these two sampling methodologies (eel ramps and eDNA) for continued monitoring feasibility assessment. To assess the current status of American Eel in Texas, life history information including the recruitment timing, distribution, and abundance of leptocephalus, glass, and elver eels throughout the central and upper Texas coast was gathered using plankton nets and eDNA sampling associated with traditional sampling methods (eel ramps).

Objectives and Conservation Benefits

The objective of this research was to collect juvenile American Eels utilizing plankton nets in coastal waters along the central to upper Texas Coast, as well as collect eDNA samples in conjunction with an established eel ramp network to document the presence of American Eels in this region. The purpose of the ongoing research was to better understand the recruitment of American Eel in Texas, providing critical data required by resource management agencies to determine the conservation and management needs of American Eel populations in Texas.

Any detections of leptocephalus, glass, or elver American Eels will be key in elucidating recruitment timing, distribution, and abundance of this species of greatest conservation need. Results of this project fill critical science needs that aid in conservation of American Eel. This work supports conservation needs outlined in the Texas Conservation Action Plan particularly for investments in conservation actions (research and monitoring), priority habitats (restoration and protection of instream aquatic habitats), and priority issues (conservation planning and regulatory actions) related to the conservation of freshwater fish diversity in Texas (TPWD 2012b).

Methods

Study Sites

The study area ranged from Victoria to Orange counties along coastal Texas. A desktop evaluation was completed to identify potential eel ramp sites within the study area where a natural or man-made flow obstruction could support a gravity-fed eel ramp. A total of 121 potential study sites were identified during the desktop evaluation phase; 92 of which were visited for reconnaissance between 2020 and 2022. Out of the visited sites, 20 met the criteria for the study. A total of 12 sites were chosen for final study sites based on a combination of the following preferred criteria: direct connectivity to coastal estuarine waters, vertical relief (typically a spillway) of flowing waters with a minimum one-foot rise in elevation to provide sufficient flow for a gravity-fed eel ramp, shallow receiving waters (typically less than one foot) to support eel ramp, landowner permission for site access, and spatial distribution

throughout the study area and major watersheds. Out of the 12 final eel ramp sites, 8 were selected to be concurrently used as eDNA sites. Sites were selected based off connectivity to the Gulf, spatial distribution, and water basins with records of American Eels. (Table 1, Figure 1). Two sites were chosen for plankton sampling (Table 1, Figure 1).

Table 1. Ramp and plankton net study sites selected for eDNA sampling. Sites correspond to Figure 1. (HSC= Houston Ship Channel).

Site ID	Waterbody Name	County	Latitude	Longitude	Collecting Entity
9	Little Pine Island Bayou	Hardin	30.16224	-94.31817	EIH
40	Oyster Creek	Brazoria	29.05377	-95.46391	EIH
44	Steep Bank Creek	Fort Bend	29.52284	-95.56772	EIH
58	Tres Palacios River	Matagorda	28.94345	-96.16354	TPWD
84	Coleto Creek	Victoria	28.73196	-97.16073	TPWD
96	Carpenters Bayou	Harris	29.78128	-95.15493	EIH
104	Unnamed Ditch to Cow Bayou	Orange	30.04024	-93.81766	EIH
119	Lynn Bayou	Calhoun	28.62469	-96.62994	TPWD
120	Colorado River	Matagorda	28.97692	-96.01260	LCRA
B1	Mouth of Trinity River at Trinity Bay	Chambers	29.71825	-94.72329	EIH
B2	HSC near Morgan's Point	Harris	29.69534	-94.99452	EIH

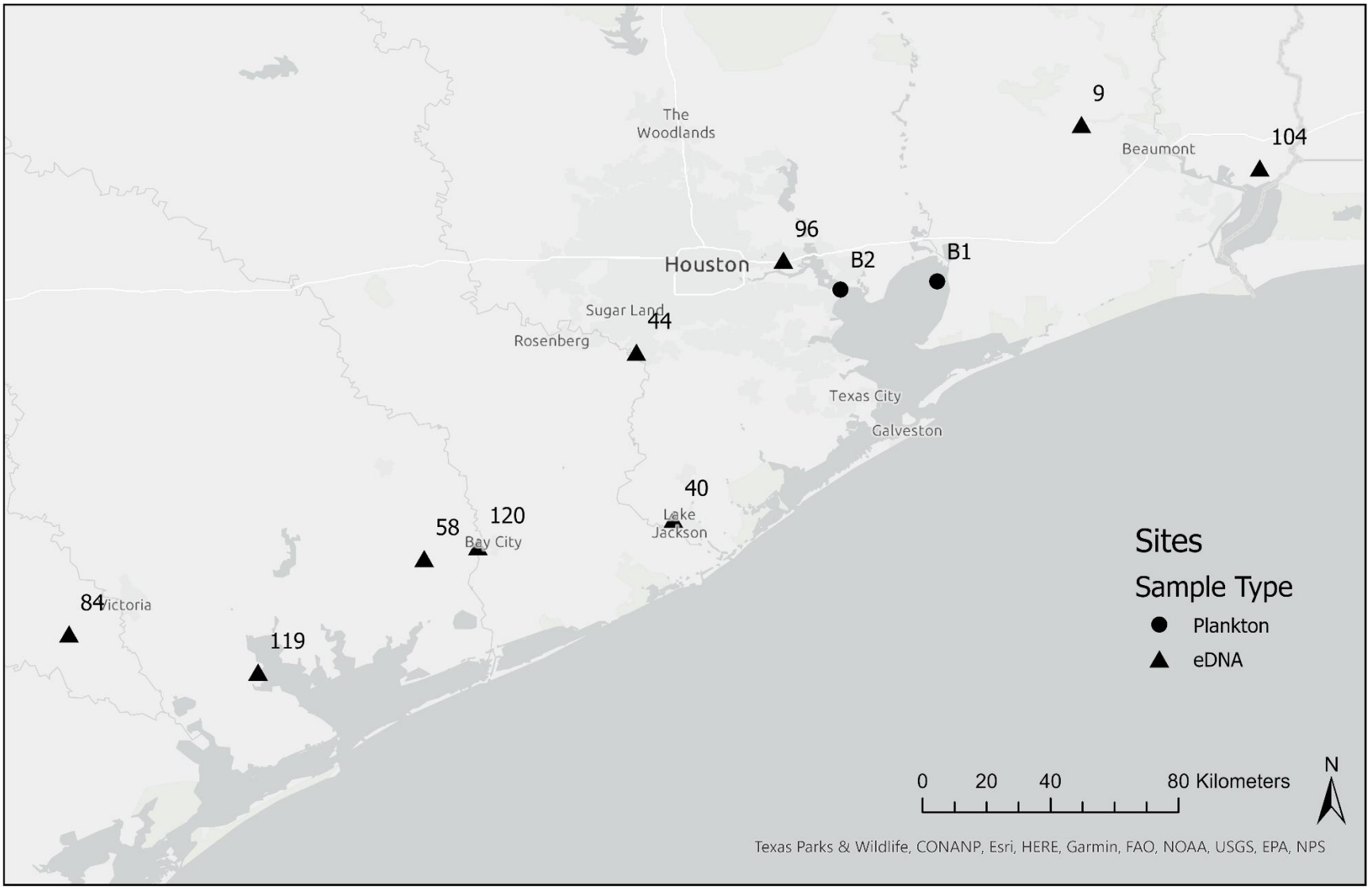

Figure 1. Map of study sites where eDNA were collected. Sites correspond with Table 1.

Plankton Net Sampling

Plankton net trawls were utilized to detect juvenile American eel, specifically leptocephali. Plankton monitoring was focused at mouths of coastal tributaries where immigrating juveniles may concentrate during ingress into the lower portions of rivers and streams. Sampling was conducted in the early spring, a similar time of year when immature American Eel have been detected in Atlantic estuaries (Able and Fahay 2010). A conical plankton net with a 1:6 mouth diameter: total length ratio, constructed of 335 µm bar mesh nitex fabric was utilized. This gear type has been widely adopted and previously used by Gulf of Mexico larval fish sampling programs (Smith and Richardson 1977; SEAMAP 2016). Before hauls were conducted, total depth and secchi were collected and water chemistry data was collected at a profile from the bottom (0.3 m from the bottom), 75%, 50%, 25%, and surface (0.3 m from the surface) of the water column. At each depth, temperature (°C), specific conductivity (uS), dissolved oxygen (DO), salinity (PSU), pH, and turbidity (NTU) were recorded. Four tow hauls were conducted at each site with a target net depth of 75% of the total depth. Depth was determined using angle and length of line. After each tow, the net contents were rinsed into a 1L Nalgene bottle and preserved with 95% ethanol. Between tow hauls, the net was rinsed with site water to remove visible debris. Average tow velocity and volume filtered was estimated using a mechanical flow meter mounted in the mouth of the net and tow time was recorded. Tow samples were examined for larval fish using a dissecting microscope.

eDNA

Sample collection for eDNA occurred simultaneously with weekly eel ramp checks. Prior to any in-water contact, 1L sample bottles, coolers, bags, and long-arm sampler were decontaminated with a 10% bleach solution, allowed to sit for a minimum of 10 minutes, then rinsed with tap water. Two water samples were taken at each site, one 10 meters downstream of the ramp (referred to as “DS”) and one directly adjacent to the ramp mouth (referred to as “R”). A long-arm sampler (Figure 2) was utilized to collect samples at all sites in order to limit cross-contamination between sites with the exception of site 58, which was only accessible from the bank opposite of the ramp. Special care was taken to disinfect waders prior to eDNA sampling at site 58. To avoid any potential for contamination, all eDNA samples were collected before any contact with the ramp or the site water occurred, and the DS sample was taken first in order to prevent contamination from upstream sampling. The 1L sample bottle was attached to the long arm sampler, inverted into the bottom half of the water column and filled with site water. The sample was placed in a sealed bag, and put in a site-specific cooler with ice. The process was repeated for the R sample. Field blanks were collected monthly – once at each site – to test for proper decontamination procedures. Field blanks consisted of a 1L bottle filled with deionized (DI) water. The bottle was placed into the longarm sampler at a field site, the lid removed and replaced after at least 1 minute to mimic a sample being taken (i.e. exposed to elements). The field blank was placed into a sealed bag and into the appropriate site cooler with the field samples.

Figure 2. Environmental DNA ramp sample collection at site 96 on Carpenter's Bayou.

After returning from the field, samples were brought to the lab and filtered the same day. Self-contained Smith-Root 1.5 µM water filter cups were used for filtering. Sample water was pulled through the filter using a peristaltic pump system until a target volume of 1L had been filtered, or until the filter became clogged. Spent filters were removed from the sample cup using sterile forceps, folded in half with the DNA-facing side of the membrane facing inside, and placed in labeled, 10 mL tubes in the freezer for preservation. The tabletops and peristaltic pump were decontaminated with a 10% bleach solution between each sample, and the hose was rinsed with DI water for one minute to ensure there was no potential for contaminated backflow. Within 48 hours of filtration, eDNA was extracted from filters and purified using the DNeasy PowerSoil Pro Kit (QIAGEN, Inc.) following the manufacturer's protocols with an inhibitor removal step using an OneStep Inhibitor Removal kit (Zymo Research Corporation) and its associated standard protocols.

Quantitative polymerase chain reaction (qPCR) assay procedures and assay primers used for this project are based on the protocols outlined in Moyer et al. (2022). A dual marker qPCR assay (assay components provided in Table 2) utilizing labeled AME1, AME2 and TaqMan MGB fluorescent probes (Applied Biosystems™) and the AME1 and AME2 forward and reverse primers, was used for detection of American Eel from eDNA samples (Moyer, et al. 2022). Dual marker detection was preferred over single marker detection because if one marker indicates a positive sample, the second marker can be used to validate positive DNA presence at an unrelated DNA locus, reducing the possibility of false positives. Quantitative PCR was carried out on all eDNA samples, using a QuantStudio 3 Real-Time PCR system (Applied Biosystems™, Waltham, MA) with the assay components from Moyer et al. (2022) and TaqMan Environmental Master Mix 2.0 (Applied Biosystems™). The two marker assays (AME1 and AME2) were run independently for each sample (no multiplexing) after initial test runs detected higher sensitivity

using singleplex PCR. Significant amplification signal detected from both the AME1 and AME2 probes were considered a positive detection of American Eel (Moyer, et al. 2022). Samples that only had amplification on one of the two probes were later run again to verify if it was a false positive or not. Any samples that had amplification on either probe during this second analysis were considered a true positive and samples with no amplification were negative.

Table 2. Primer and Probe sequences for American Eel eDNA assays.

Assay Component	**Sequence (5'-3')**
AME1 Forward Primer	TACCAGAAGTCCTACAAGGCCTA
AME1 Reverse Primer	TAGTGGTTGTTCTACTCCTGCTG
AME1 Probe	6FAM-ATGACAAAA-ZEN-ACTAGCGCCCATAGCCCT-BHQ-1
AME2 Forward Primer	ACTATGGATGATTAATTCGCAAC
AME2 Reverse Primer	CTCCAATGTTTCATGTTTCTTTG
AME2 Probe	VIC-CGTAGTAAA-ZEN-GTCCTCGGGCAATGTGAA-BHQ-1

One negative control consisting of DNAse/RNAse free water (UltraPure™ Distilled Water, Invitrogen, Waltham, MA) and one positive control, a serial dilution (1, 0.5 , $10e^{-1}$, $10e^{-2}$, $10e^{-3}$, $10e^{-4}$, $10e^{-5}$, and $10e^{-6}$) consisting of pure DNA extracted and purified from a 20 mg fin clip of an American Eel, collected in Texas. Pure eel DNA was extracted using a DNeasy Blood & Tissue Kit (QIAGEN, Inc., Hilden, Germany). Serial dilution of positive controls indicated the sensitivity of the assay at any given time to detect eel eDNA present in the environment.

Significant Deviations

Initial plans for plankton sampling included sampling at both river mouths and tidal passes, however early attempts at plankton net deployment failed, resulting in a reduced scope for the plankton sampling. On March 10, 2022 an initial attempt to conduct active plankton tows at site B2 was made but was unsuccessful. Due to the size of the plankton net (1m diameter), the water depth in the Houston Ship Channel (> 13 meters), and use of a hand-crank winch, deployment and retrieval time took too long to deploy the net at target depth and was determined to be unsafe due to ship traffic. Subsequent plankton sampling was coordinated with Texas Parks and Wildlife Department Coastal Fisheries group with the Dickinson Marine Lab on the research vessel “Trinity Bay”. The electric winch system on the research vessel was used to deploy and retrieve the plankton net. Even with the electric winch, the tows at site B2 had to occur outside of the ship channel due to tow time and ship traffic. The bottom of the ship channel was our planned target sampling location. Future efforts to conduct plankton surveys should include coordination with vessels equipped with electric winch systems capable of sufficient load capacity during an incoming tide and at night for the best chance of detecting leptocephalus.

The decontamination procedures were followed for all eDNA samples with the exception of the following site and events. During a period of sampling for site 120, Nalgene eDNA sampling bottles were not properly decontaminated according to stated protocols. Bottles were rinsed with DI water but not bleached prior to being returned to the sampling rotation. To analyze the contamination risk of improperly washed bottles on the positives detected at site 120 following the potential contamination event, a small side study was developed. In this study, it was hypothesized that though the bottles were

not bleached, they did undergo an extended drying period of five weeks minimum prior to re-use, which would potentially denature any DNA present on the bottles. To test this hypothesis, ten bottles were filled with 1000 ml DI water and purposely contaminated with 100 µl of pure eel DNA (extracted from an *A. rostrata* fin clip using a DNeasy Blood & Tissue Kit, QIAGEN, Inc., Hilden, Germany). Following the intentional "contamination", all samples were filtered according to protocol and extracted to ensure each contaminated sample was positive under the qPCR assay protocols (Table 3, Contamination qPCR Results). Following this, eight of the contaminated bottles were rinsed with DI water, and assigned a drying-time treatment (five weeks, six weeks, seven weeks, and eight weeks) with two bottles per treatment. Two contaminated bottles were cleaned following protocol, with a ten-minute 10% bleach rinse and DI rinse, and served as controls for the study. Five weeks was chosen as the shortest date a contaminated bottle could have been re-used in the field survey, and consecutive weeks were included in case longer time periods continued to produce positive contamination under the qPCR assay. Testing for contamination in consecutive weeks would allow us to isolate potential contamination events within the field data. Following the respective drying period treatments, bottles were refilled with a 1000 ml DI water sample, sample was then filtered and DNA was extracted according to protocol. Then, extracted samples were run utilizing the eel eDNA qPCR procedure. No purposely contaminated bottles produced any positives in the following qPCR (Table 3, Post-Drying qPCR Results). Therefore, all field eDNA samples, even those from site 120 that did not undergo the bleach disinfection protocol, were included in the presented results.

Table 3. Real time PCR results from contamination study. Contamination qPCR Results reflect the qPCR assay of purposely contaminated samples (contaminated with eel DNA). Post-Drying qPCR Results indicates the qPCR assay results of the second DI water samples taken post-treatment period.

Sample Name	Cleaning Treatment	Drying Treatment	Contamination qPCR Results	Post-Drying qPCR Results
Control A	Bleached Clean	Control	Positive	Negative
Control B	Bleached Clean	Control	Positive	Negative
CW1 A	Water Rinsed & Dried	5 Weeks	Positive	Negative
CW1 B	Water Rinsed & Dried	5 Weeks	Positive	Negative
CW2 A	Water Rinsed & Dried	6 Weeks	Positive	Negative
CW2 B	Water Rinsed & Dried	6 Weeks	Positive	Negative
CW3 A	Water Rinsed & Dried	7 Weeks	Positive	Negative
CW3 B	Water Rinsed & Dried	7 Weeks	Positive	Negative
CW4 A	Water Rinsed & Dried	8 Weeks	Positive	Negative
CW4 B	Water Rinsed & Dried	8 Weeks	Positive	Negative

Results

Plankton Net Sampling

On March 8th, 2023 plankton net surveys were conducted at sites B1 and B2 in collaboration with the Texas Parks and Wildlife Department, Dickinson Marine Lab. Site conditions are outlined in The tide stage at each site at the time of sampling was falling.

Table 4. Water chemistry data at site B2 was collected outside the ship channel to avoid ship traffic. Total depth at site B1 was 1.31m and 2.8m at site B2. Secchi at site B1 was 0.19m and 0.36m at site B2. Temperature at site B1 ranged from 22.8 to 26.3 °C and site B2 ranged from 22.3 to 22.7 °C. Specific conductivity at site B1 ranged from 6,579 to 7,741 uS and site B2 ranged from 20,351 to 23,319. Salinity at site B1 ranged from 3.58 to 4.29 PSU and site B2 ranged from 12.71 to 14.12 PSU. Dissolved oxygen at site B1 ranged from 7.13 to 8.24 mg/L and site B2 ranged from 0.39 to 8.90 mg/L. At site B1, pH ranged from 8.36 to 8.43 and site B2 ranged from 6.96 to 8.36. Turbidity at site B1 ranged from 11.23 to 11.90 NTU and site B2 ranged from 5.43 to 204.11 NTU. The bottom turbidity reading at site B1 was removed due to values not stabilizing. The tide stage at each site at the time of sampling was falling.

Table 4. Environmental data collected during plankton net sampling. Turbidity was not collected at B1 Bottom because of unstable readings.

Site ID	Location within Water Column	Temp (C°)	Spec. Cond. (uS)	Salinity (PSU)	DO mg/L	pH	Turbidity (NTU)
B1	Bottom	22.8	7,741	4.29	7.13	8.36	-
B1	75%	22.8	7,798	4.32	7.58	8.37	11.90
B1	Middle	23.5	7,130	3.98	8.13	8.42	11.90
B1	25%	25.9	7,065	3.81	8.25	8.42	11.52
B1	Surface	26.3	6,579	3.58	8.24	8.43	11.23
B2	Bottom	22.3	20,351	12.17	0.39	6.96	204.11
B2	75%	22.3	23,995	14.56	7.16	7.97	8.99
B2	Middle	22.5	23,780	14.36	8.58	8.22	4.91
B2	25%	22.6	23,402	14.17	8.60	8.30	6.30
B2	Surface	22.7	23,319	14.12	8.90	8.36	5.43

A total of four tow hauls were completed at each site. Net depth at site B1 ranged from 0.8 to 1.2m and site B2 ranged from 6.5 to 14.8m. During each tow the amount of line out, angle, tow speed, and time varied with each tow but a consistent depth of 75% was targeted within the water column (Table 5). No eel larvae were captured, and eDNA testing yielded no positives for American eel. Captured plankton species were still analyzed and ranked according to abundance (Table 6).

Table 5. Tow data for plankton sampling. Distance, speed, and volume towed were calculated utilizing formulas from General Oceanics, Inc. Flowmeter Operations Manual (page 5) with a Rotor Constant of 26,873 (https://www.generaloceanics.com/uploads/Files/2030%20MANUAL.pdf)

Site	Tow	Depth (m)	Distance Towed (m)	Tow speed (m/s)	Tow Time (minutes)	Volume Filtered (m^3)
B1	1	0.8	571.2	1.79	5.92	352.0
B1	2	0.9	520.5	1.12	5.87	320.8
B1	3	0.8	532.2	1.79	5.88	328.0
B1	4	1.2	498.5	1.79	6.00	307.2
B2	1	14.8	618.4	1.56	6.58	381.1
B2	2	12.0	451.8	1.56	6.58	278.4
B2	3	7.0	400.4	0.98	6.47	246.8
B2	4	6.5	331.2	0.98	6.13	204.1

Table 6. List of catch during plankton net sampling. Species were given an abundancy rank of 1-4 (1= 1-5 individuals or "rare", 2= 6-24 individuals or "occasional", 3= 25-100 individuals or "common", 4= >100 individuals or "abundant")

Common Name	Scientific Name	B1-tow 1	B1-tow 2	B1-tow 3	B1-tow 4	B2-tow 1	B2-tow 2	B2-tow 3	B2-tow 4
Gulf Menhaden	*Brevoortia patronus*	-	-	1	-	-	-	-	1
Anchovy	*Anchoa sp.*	1	1	-	3	-	-	1	-
Atlantic Croaker	*Micropogonias undulatus*	-	-	-	-	1	-	-	-
Grass Shrimp	*Palaemonetes sp.*	-	-	-	-	1	-	-	-
Porcelian Crabs	Anomura zoea	-	-	-	1	-	-	-	-
Blue Crab	*Callinectes sapidus*	-	-	-	-	1	-	-	-
Mysid Shrimp	*Taphromysis louisianae*	4	4	2	4	3	-	-	-
Isopod	Isopoda	2	-	-	-	-	1	1	2
Amphipod	Gammaridae	1	2	3	4	1	1	-	-
Copepod	Copepoda	4	4	4	4	-	-	-	4
Comb Jellyfish	Ctenophora	-	-	-	-	-	-	4	-
Cnidarian	Cnidaria	-	-	-	-	-	-	-	3
Salpid	Salpidae	-	-	-	-	1	-	-	-
Arrow Worm	Chaetognatha	-	-	-	-	-	-	-	2
Polychaete Worm	Polychaeta	1	-	-	-	1	-	-	-

eDNA

Physical and environmental conditions varied at each ramp site (*Table 7* and *Table 8*). Sites were associated with some type of obstruction or physical barrier causing a 1ft or greater vertical rise that could support a gravity feed siphon and included dams, spillways, rip/rap, and waste water treatment facility (WWTF) outfalls. The total stream width of the sites surveyed ranged from 4.3 to 65 m and the percent cover of instream habitat observed directly downstream of the ramp ranged from 11-90. The distance to the nearest pass ranged from 37.9 to 146.9 km. The average water temperature ranged from 8.1 to 34.5 °C. Specific conductivity ranged from 90.7 to 45,648 µS/cm. Site 119 is notable due to its significantly higher salinity on average (8.0 psu) compared to all other sites (<1.0 psu) which was due to its direct access to salt water (38.9km to the nearest pass). Dissolved oxygen ranged from 1.1 to 12.8 mg/L, and pH ranged from 6.3 to 8.8. Water clarity was generally poor with an average secchi of 0.04 m.

Environmental DNA water samples were collected 783 times throughout the 52-week project period. A total of 780 field-collected eDNA samples were analyzed with qPCR. Due to cross-site contamination, three samples were discarded during the extraction process. Site 104 was discontinued for eDNA sampling on February 27th, 2023 due to 34 consecutive weeks of no positive results for eel detection and was replaced with Site 119 after eels were captured there. Positive American Eel DNA detections occurred for 257 samples (*Table 9*). During some weeks, eDNA samples could not be taken due to flood events preventing access to the site(s). The site with the overall highest percent positive for eDNA samples, site 119, (84.1% of eDNA samples taken were positive) was the only site where juvenile American Eels were captured in the eel ramps.

All eels captured at site 119 were either glass or elver stage and were captured between January and April, 2023 (*Figure 3*). The eel captured at site 120 was captured with a dip net in March 2023 in proximity to the ramp. During all weeks at site 119 in which eels were captured, except for one (week 37) both the downstream and ramp eDNA samples were positive. Environmental DNA samples were not collected at week 32 and 33 site checks due to lack of eels captured via ramp, and regular eDNA sampling had yet to begin at this location. Eel catch occurred between weeks 27-40, corresponding with the time period in which the overall positive eDNA detections increased throughout all sites collectively (*Figure 4*). Data for eel captures, such as eel growth stage and length and weight, are available in the interim report Oakley et al. (2023). A polynomial order 3 trendline was calculated using the total percent positives (R^2 = 0.60) which peaked in late March/early April (*Figure 4*).

The generalized additive model (GAM) explaining the most variance in eel presence (by proxy of eel eDNA presence) included water temperature, salinity, dissolved oxygen, and tide depth (*Table 10*). All four variables were significant indicators of eel presence, though salinity and tide depth displayed the greatest significance (p-values <0.0001, and <0.0001 respectively), followed by dissolved oxygen (p-value = 0.0020) and water temperature (p-value = 0.0200) (*Table 10*). Model outputs indicated that lower temperatures (10 – 20 ˚C), higher dissolved oxygen (> 5 mg/L), higher salinities (above 1 PSU), and lower tide depth (below 1 ft) resulted in higher eDNA presence (*Figure 5*).

Table 7. Study sites with corresponding physical site characteristics.

Site	Obstruction Type	Stream Width (m)	% Cover of Instream Habitat	km to Nearest Pass
9	Dam	13.0	52	122.9
40	Spillway	23.0	23	45.7
44	Spillway	9.4	33	123.3
58	Rip/Rap	6.4	37	87.8
84	Spillway	4.5	11	146.9
96	Rip/Rap	6.6	29	72.8
104	WWTF Outfall	4.3	59	53.9
119	WWTF Outfall	20.5	90	37.9
120	Dam	65.6	65	53.2

Table 8. Minimum (min.), average (avg.), and maximum (max.) physical and environmental conditions at each ramp site where eDNA was collected. n= number of times environmental conditions were collected; some values for site data are less than listed n due to inaccuracy or inability to collect

Site	n	Min. Temp (°C)	Avg. Temp (°C)	Max. Temp (°C)	Min. Spec. Cond. (µS/cm)	Avg. Spec. Cond. (µS/cm)	Max. Spec. Cond. (µS/cm)	Min. Salinity (psu)	Avg. Salinity (psu)	Max. Salinity (psu)	Min. DO (mg/L)	Avg. DO (mg/L)	Max. DO (mg/L)	Min. pH	Avg. pH	Max. pH	Min. Secchi (m)	Avg. Secchi (m)	Max. Secchi (m)
9	48	10.3	21.9	31.9	91	366	915	0.0	0.2	0.5	4.0	6.6	9.9	6.3	7.1	8.3	0.15	0.5	1.0
40	51	11.1	23.5	32.2	248	668	1,298	0.1	0.8	23.0	2.5	5.6	9.4	7.4	7.8	8.0	0.07	0.2	0.6
44	51	12.6	23.6	30.8	365	988	1,360	0.2	0.5	0.7	5.8	7.3	9.8	7.6	7.7	7.9	0.07	0.2	0.4
58	46	10.9	23.6	31.0	105	932	1,635	0.1	0.5	0.8	3.9	7.0	10.1	7.4	7.9	8.2	0.06	0.2	0.6
84	49	10.7	23.1	32.0	111	378	567	0.1	0.2	0.7	5.5	8.9	12.8	7.4	8.1	8.6	0.23	0.5	0.9
96	52	13.7	25.2	34.5	262	697	887	0.1	0.3	0.4	3.9	9.0	12.8	7.4	7.8	8.4	0.13	0.5	1.1
104	51	17.8	24.4	29.2	402	1,350	1,695	0.2	0.7	0.9	5.7	7.1	8.5	7.0	7.3	7.6	0.12	0.5	>1.2
119	49	17.2	24.7	31.3	126	13,155	45,648	0.2	8.0	29.4	1.1	6.5	9.7	7.3	7.7	8.0	0.12	0.6	1.0
120	38	8.1	22.3	32.6	289	743	5,204	0.1	0.3	0.4	6.4	8.9	12.5	7.7	8.3	8.8	0.10	0.4	>1.2
-	**435**	**8.1**	**23.6**	**34.5**	**91**	**2,162**	**45,648**	**0.0**	**1.3**	**29.4**	**1.1**	**7.4**	**12.8**	**6.3**	**7.7**	**8.8**	**0.06**	**0.4**	**>1.2**

Table 9. Summary of eDNA samples and positives and total number of eels captured by site. Ramp and Downstream columns include total of AME1 and AME2 markers.

Site	Ramp (n)	Ramp positives	Downstream (n)	Downstream Positives	Total Positives	No. of Eels Captured
9	47	0	47	1	1	0
40	50	11	50	14	25	0
44	50	1	50	0	1	0
58	43	21	43	10	31	0
84	48	27	48	36	63	0
96	50	21	50	21	42	0
104	33	0	33	0	0	0
119	22	18	22	19	37	25
120	41	18	41	39	57	1
Total	**384**	**117**	**384**	**140**	**257**	**26**

Table 10. General Additive Model results for eel eDNA prevalence with water temperature, salinity, dissolved oxygen, and tide depth upon sample capture.

Predictor Variable	df	F-value	P-value
Water Temperature (˚C)	*3.515*	*10.117*	0.0200
Salinity (PSU)	*2.337*	*16.401*	< 0.0001
Dissolved Oxygen (mg/L)	*7.073*	*4.958*	0.0020
Tide Depth (ft)	*6.822*	*12.800*	< 0.0001

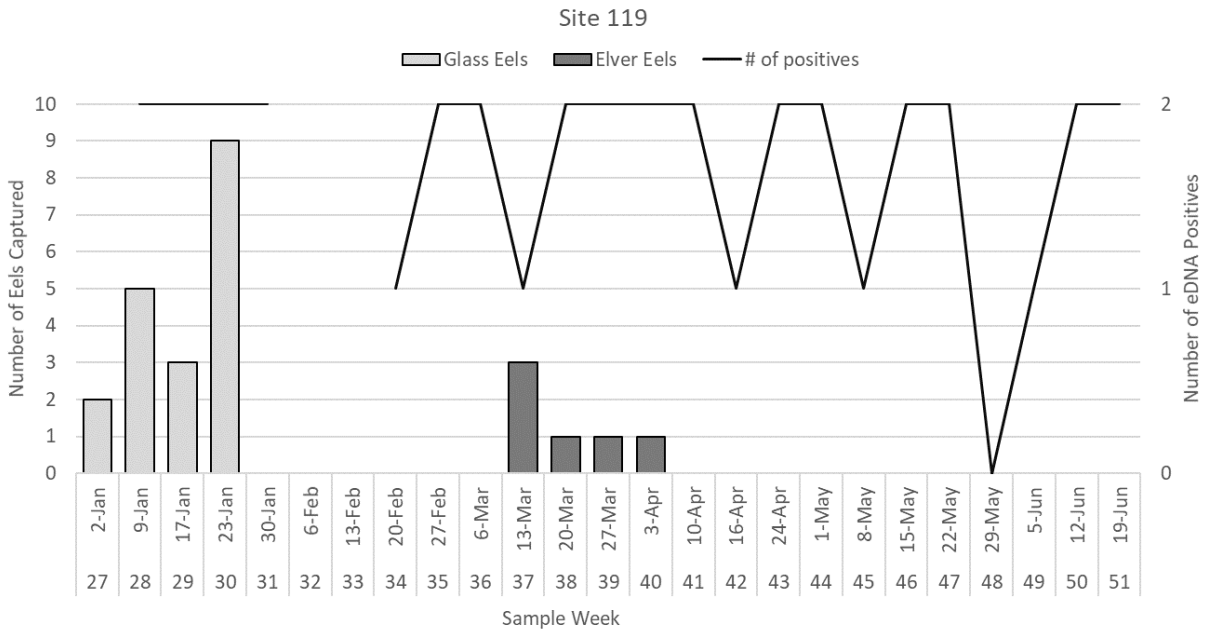

Figure 3. Weekly American eel catch and eDNA positives at site 119.

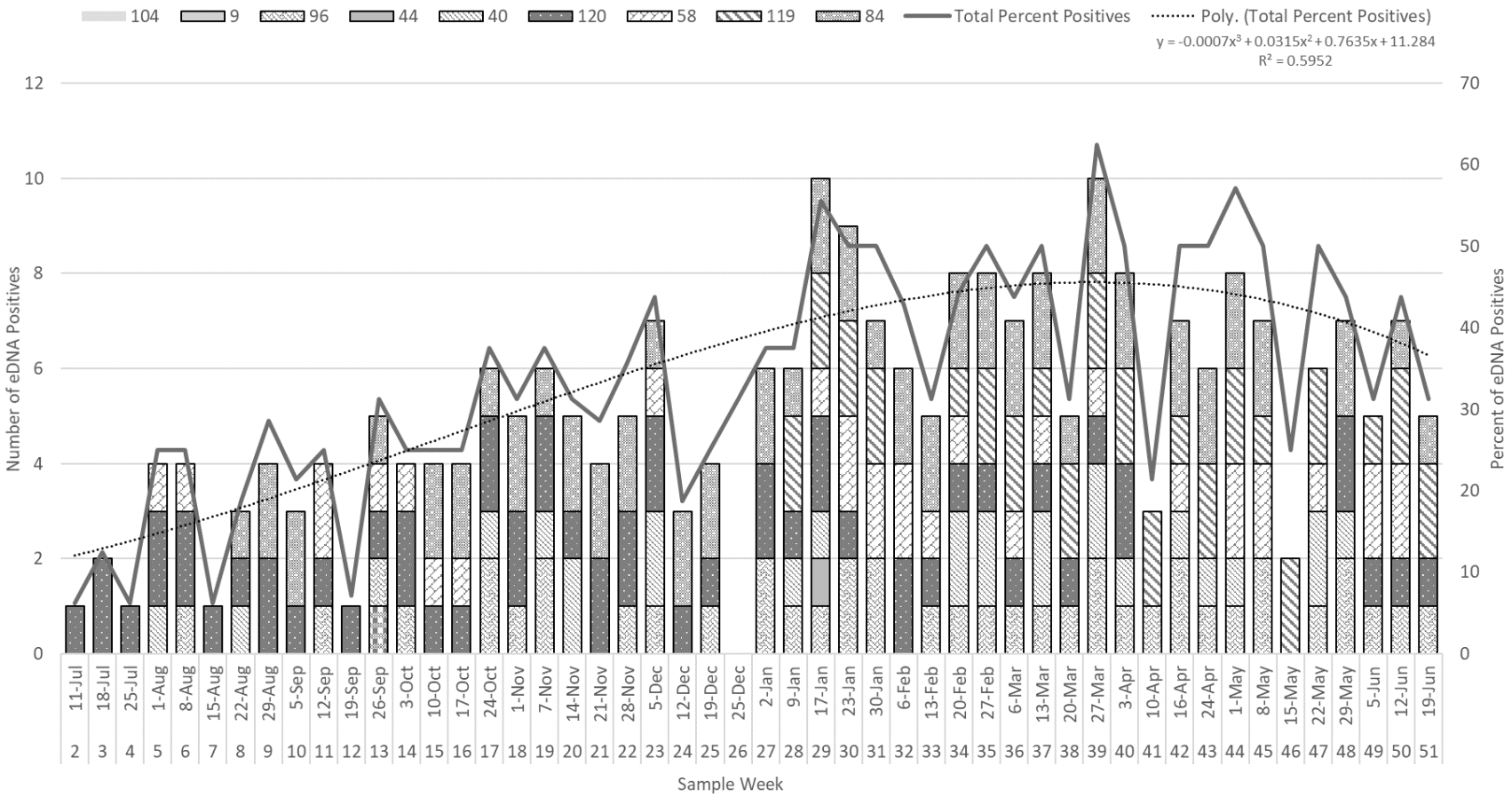

Figure 4. A breakdown of positive eDNA detections per site on a weekly basis.

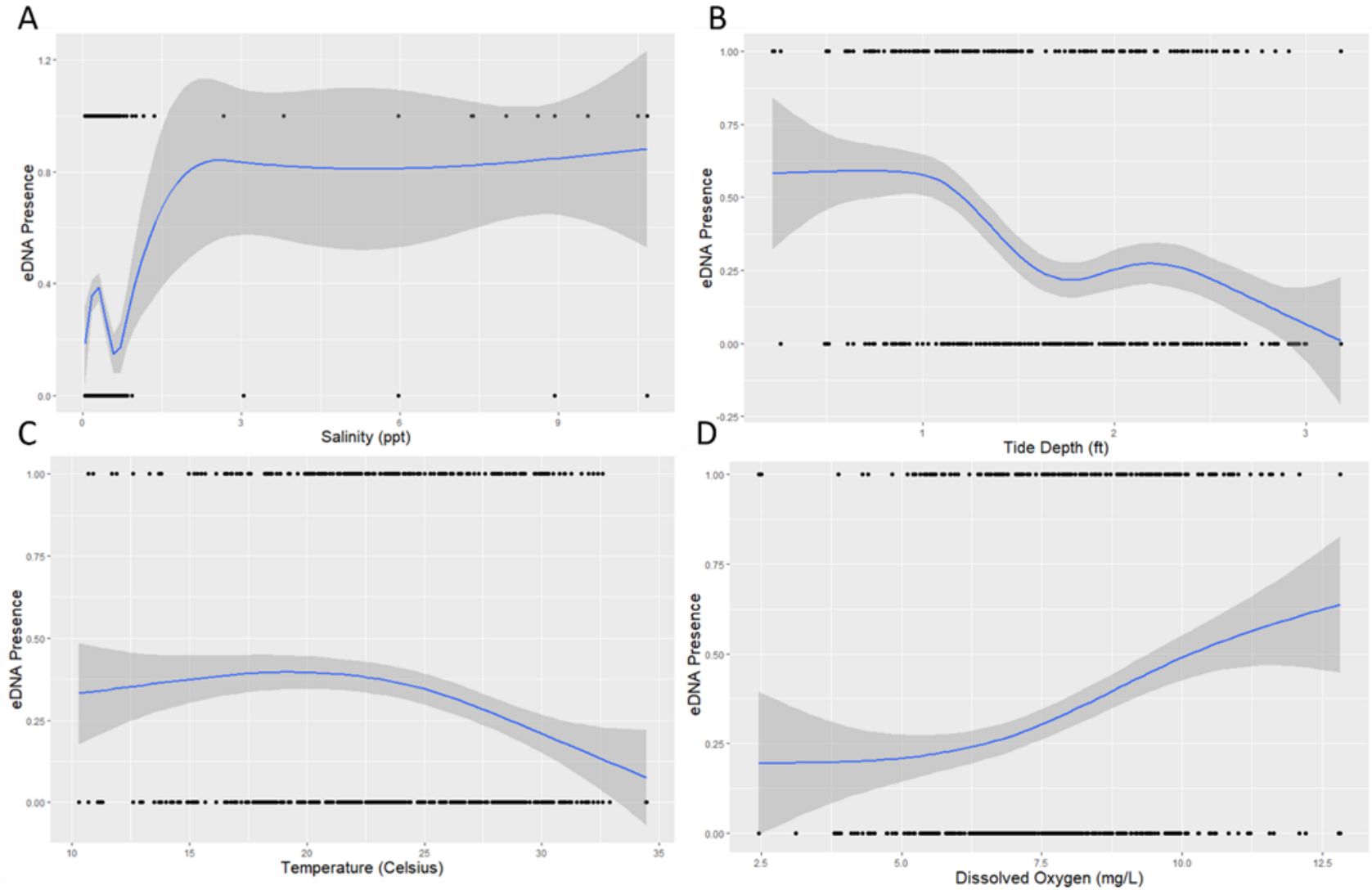

Figure 5. Generalized additive model smoothed regression outputs of eel eDNA presence affected by the predictor variables, salinity (PSU, panel A), tide depth (ft, panel B), water temperature (°C, panel C), and dissolved oxygen (mg/L, panel D).

Discussion

The plankton trawl provided no eel eDNA and no eel catch of any species. The plankton surveys were conducted in March, however the first glass eels caught in an eel ramp were documented in early January, therefore future plankton trawls should consider an earlier sampling window as early as October through February. Coordination with agencies that have large research vessels with electric winch systems is highly recommended for future plankton surveys due to the challenges of conducting bottom plankton tows such as the amount of line out and focus on passes and river mouths which tend to have heavy ship traffic.

Sites 119 had the highest percent positive detections of eel eDNA in eDNA samples compared to other sites. This site is also where the only juvenile eel were successfully caught in the eel ramp. The site, located on Lynn Bayou in Port Lavaca, Texas, had significantly higher salinity and was the site closest to the nearest Gulf pass, Pass Cavallo in Matagorda Bay. In addition, salinity was also shown to be associated with high eel eDNA presence at all sites in GAM modeling. This indicates that future studies may find more success if sites closer to the coast are targeted to locate eels and analyze eel eDNA presence, rather than sites further upstream.

As shown by both the eDNA evidence as well as the concurrent project utilizing eel ramps, glass American Eel were documented in early January – earlier than prior documentation of the only other glass American eel documented in the Northern Gulf of Mexico, which was in March in the pan handle of Florida (Oakley et al, 2021). Environmental DNA levels fluctuated over the year but increased coastwide around January of 2023 when glass eels and elver eels were first captured and then began to decrease again after the last eels were captured in April. Site 119 had the highest number of captured juvenile eels, both glass eel and elver stage, and at least one positive eDNA sample was consistently collected whenever eels were caught at this site. This combination of juvenile eel catch and increasing eel eDNA positive samples during this time period of January through April indicates a potential recruitment season for the species on the Gulf Coast, all though additional annual monitoring will be needed to confirm this. Nevertheless, this potential recruitment window is similar to recruitment timing of eels in the Southeastern Atlantic Coast, as glass eels are caught in Chesapeake Bay tributaries from February through June (Able and Fahay 1998, Fabrizio and Tuckey 2017), and glass eels in North Carolina are caught from November through early May (Powles and Warlen 2002).

In addition, evidence from GAM modeling further specifies the validity of this recruitment period. The model indicated that low temperature, high dissolved oxygen, and low tidal depth coincide with higher eel eDNA presence at all sites across the coast, and these conditions are emblematic of winter conditions along the Texas Gulf Coast. This shows how eel abundance in this region coincides with winter water conditions, and further supports a potential recruitment period of winter and early spring along the Texas Gulf Coast. The similarities between recruitment periods in Southeastern Atlantic estuaries and the data collected in this study and the concurrent eel ramp study help validate this potential Gulf of Mexico eel recruitment time period.

This consistency between juvenile eel catch and increasing positive eel eDNA samples at site 119 shows that eel presence may be linked to heightened levels of eDNA. As noted in Itakura et al (2019) *Anguilla japonica* – a closely related eel species to *Anguilla rostrata* – sheds a large amount of eDNA in the water compared to other fish species. This heightened eDNA release may also prove useful in determining presence and absence of eels across the state of Texas in general, rather than just detecting migrating eels. However, population, and more specifically, juvenile eel recruitment cannot be estimated via eDNA alone.

While the current study was only able to determine presence/absence of eels utilizing eDNA, work on the east Coast has shown that eDNA can be used to produce relative quantitative estimates of abundance when compared to numerical abundances of American eel as sampled using Electrofishing (Chin et al. 2021). These methodologies may prove useful in future eDNA-based research for this species in the Gulf of Mexico. Calculation of quantification of individuals or catch per unit effort using eDNA concentration levels is still in development and relatively unreliable. In addition, eDNA sampling does not allow for differentiation between life stages, however, in this case, the concurrent catch of juvenile eels indicates a possible recruitment pulse displayed by the increase in positive eDNA samples. Environmental DNA serves as a useful tool in detecting the presence or absence of potentially difficult to capture species, which can help in future studies analyzing both juvenile recruitment and overall American Eel population status in Texas waters. Although the percentage of positives increased during winter and early spring coastwide, the total number of positive eel eDNA samples may be underestimated due to site 119 being selected later in the study as an eDNA site, resulting in a potential bias. As site 104 produced no positive eel eDNA samples during the first six months of the study, 119 was selected as an eDNA site instead after glass eels were first caught at this location in January. Due to this, data collected at site 104 may introduce a bias of a lower overall percentage of positive eDNA samples during the first half of the year.

Future work is underway to continue monitoring select sites using eel ramps. With additional years of recruitment timing data gathered and additional glass eel catch sites located, future studies using eDNA to monitor long-term recruitment pulses can be considered. The overall percent of positive eDNA samples across all sites showed a seasonal trend, peaking between the months of January and March. The completion of this study has resulted in critical baseline information for detecting the spatial and temporal recruitment of American Eel to the Texas Gulf Coast. This information can assist natural resource agencies in determining the conservation and management needs of American Eel populations in Texas.

Literature Cited

Able, K.W., M.P. Fahay. 1998. The First Year in the Life of Estuarine Fishes in the Middle Atlantic Bight. Rutgers University Press, New Jersey. 342 pp.

Able, K.W., Fahay, M.P. 2010. Ecology of estuarine fishes. Temperate Waters of the Western North Atlantic. John Hopkins University Press. Baltimore, MD. 566 pp.

Able, K.W., Allen, D.M., Bath-Martin, G., Hare, J.A., Hoss, D.E., Marancik, K.E., Powles, P.M., Richardson, D.E., Taylor, J.C., Walsh, H.J., Warlen, S.M., and Wenner, C., 2011. Life history and habitat use of the speckled worm eel, *Myrophis punctatus*, along the east coast of the United States. Environmental Biology of Fishes 92, 237.

Baillie, S.M., McGowan, C., May-McNally, S., Leggat, R., Sutherland, B.J.G., Robinson, S. 2019. Environmental DNA and its applications to fisheries and oceans Canada: national needs and priorities. Canadian Technical Report of Fisheries and Aquatic Sciences 3329.

Baker, C.S., Steel, D., Nieukirk, S., Klink, H. 2019. Environmental DNA (eDNA) in the wake of the whales for detection and species information. Frontiers in Marine Science. 5: 1-11.

Cardas, J.B., Deconick, D., Marquez, I., Torre, P.P., Garcia-Vazquez, E., Machado-Schiaffino, G. 2020. New eDNA based tool applied to the specific detection and monitoring of the endangered European eel. Biological Conservation. 250.

Chin, S.C., J. Waldman, M. Bednarski, M. Camhi, J. LaBelle, and S.E. Alter. 2021. Relating American Eel *Anguilla rostrata* abundance to environmental DNA concentrations in the Bronx River. The N American Journal of Fisheries Management. https://doi.org/10.1002/nafm.10625.

Dutil, J.D., Dumont, P., Cairns, D.K., Galbraith, P.S., Verreault, G., Castonguay, M., Proulx, S. 2009. *Anguilla rostrata* glass eel migration and recruitment in the estuary and Gulf of St. Lawrence. Journal of Fish Biology, 74: 1970—1984.

Fabrizio, M.C., T.D. Tuckey. 2017. Estimating relative abundance of young-of-the-year American eel, *Anguilla rostrata*, in the Virginia tributaries of the Chesapeake Bay, spring 2016. Virginia Institute of Marine Science, College of William and Mary. https://doi.org/10.21220/V58C8P

Federal Register. 2015. Volume 80, No. 195. Department of Interior Fish and Wildlife Service. 50 CFR Part 17. Endangered and threatened wildlife and plants: 12-month findings on petitions to list 19 species as endangered or threatened species.

Harrison, A.J., Walker, A.M., Pinder, A.C., Briand, C., Aprahamain, M.W. 2014. A review of glass eel migratory behavior, sampling techniques and abundance estimates in estuaries: implications for assessing recruitment, local production and exploitation. Rev. Fish. Biol. Fisheries. https://doi.org/10.1007/s11160-014-9356-8.

Itakura H, Wakiya R, Yamamoto S, Kaifu K, Sato T, Minamoto T. 2019. Environmental DNA analysis reveals the spatial distribution, abundance, and biomass of Japanese eels at the river-basin scale. Aquatic Conserv: Mar Freshw Ecosyst. 29: 361–373. https://doi.org/10.1002/aqc.3058

Lacoursiere-Roussell, A., Rozabal, M., Bernatchez, L. 2016. Estimating fish abundance and biomass from eDNA: variability among capture methods and environmental conditions. Molecular Ecology Resources. 16: 1401—1414.

Moyer, Gregory & Bartron, Meredith & Galbraith, Heather & Grassi, Josh & Rees, Christopher. 2022. Development and validation of two environmental DNA assays for American Eel (*Anguilla rostrata*). Environmental DNA. 10.1002/edn3.369.

Oakley, J.W., Hansen, J., Molina, T., Curtis, S., and Guillen, G.J. 2021. Extensive field effort using a novel gear type to detect recruitment of American eel (*Anguilla rostrata*) in Texas. Prepared for Texas Parks and Wildlife Department, Contract 505077. EIH Report 21-002, 42 pp.

Oakley, J.W., G. Guillen, and S. Curtis. 2023. Establishing a network of eel ramps to monitor recruitment of glass and elver American Eel. Interim Performance Report to the Texas Parks and Wildlife Department. Contract # CA-0002501. Pp19.

Port, J.A., O'Donell, J.L, Romero-Maraccini, O.C., Leary, P.R., Litvin, S.Y., Nickols, K.J., Yamahara, K.M., Kelly, R.P. 2016. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. Molecular Ecology. 25: 527—541.

Postaire, B.D., Bakker, J., Gardiner, J., Wiley, T.R., Chapman, D.D. 2020. Environmental DNA detection tracks established seasonal occurrence of blacktip sharks (*Carcharhinus limbatus*) in a semi-enclosed subtropical bay. Scientific Reports. https://doi.org/10.1038/s.41598-020-68843-0.

Powles, P.M., S.M. Warlen. 2002. Recruitment season, size, and age of young American eels (*Anguilla rostrata*) entering an estuary near Beaufort, North Carolina. Fisheries Bulletin. 100: 299—306.

Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M., Gough, K.C. 2014. The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. Journal of Applied Ecology, 51: 1450—1459.

SEAMAP. 2016. SEAMAP Operations Manual for Trawl and Plankton Surveys. Gulf States Marine Fisheries Commission. Ocean Springs, MS.

Shepard, S. 2015. American Eel Biological Species Report. USFWS. Hadley, MA.

Smith. P.E., Richardson, S.L. 1977. Standard techniques for pelagic fish egg and larva surveys. FAO Tech. Paper No. 175, 100 pp.

TPWD. 2012a. Texas Conservation Action Plan (TCAP): Species of Greatest Conservation Need. Texas Parks and Wildlife Department. Accessed 10/02/2015. http://tpwd.texas.gov/huntwild/wild/wildlife_diversity/nongame/tcap/sgcn.phtml

TPWD. 2012b. Texas Conservation Action Plan 2012 - 2016: Overview. Editor, Wendy Connally, Texas Conservation Action Plan Coordinator. Austin, Texas.

United States Fish and Wildlife Service (USFWS). 2007. Endangered and threatened wildlife and plants - 12 month finding on a petition to list the American eel as threatened or endangered. Notice of 12-month petition finding. Federal Register 72:22, 4967-4997.

Weldon, L., O'Leary, C., Steer, M., Newton, L., Macdonald, H., Sergeant, S.L. 2020. A comparison of European eel *Anguilla anguilla* eDNA concentrations to fyke net catches in five Irish lakes. Environmental DNA, 2: 587-600.