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ARTICLE

# Comparison of American Fisheries Society (AFS) Standard Fish Sampling Techniques and Environmental DNA for Characterizing Fish Communities in a Large Reservoir

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## Abstract

Recently, methods involving examination of environmental DNA (eDNA) have shown promise for characterizing fish species presence and distribution in waterbodies. We evaluated the use of eDNA for standard fish monitoring surveys in a large reservoir. Specifically, we compared the presence, relative abundance, biomass, and relative percent composition of Largemouth Bass *Micropterus salmoides* and Gizzard Shad *Dorosoma cepedianum* measured through eDNA methods and established American Fisheries Society standard sampling methods for Theodore Roosevelt Lake, Arizona. Catches at electrofishing and gillnetting sites were compared with eDNA water samples at sites, within spatial strata, and over the entire reservoir. Gizzard Shad were detected at a higher percentage of sites with eDNA methods than with boat electrofishing in both spring and fall. In contrast, spring and fall gillnetting detected Gizzard Shad at more sites than eDNA. Boat electrofishing and gillnetting detected Largemouth Bass at more sites than eDNA; the exception was fall gillnetting, for which the number of sites of Largemouth Bass detection was equal to that for eDNA. We observed no relationship between relative abundance and biomass of Largemouth Bass and Gizzard Shad measured by established methods and eDNA copies at individual sites or lake sections. Reservoirwide catch composition for Largemouth Bass and Gizzard Shad (numbers and total weight [g] of

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fish) as determined through a combination of gear types (boat electrofishing plus gillnetting) was similar to the proportion of total eDNA copies from each species in spring and fall field sampling. However, no similarity existed between proportions of fish caught via spring and fall boat electrofishing and the proportion of total eDNA copies from each species. Our study suggests that eDNA field sampling protocols, filtration, DNA extraction, primer design, and DNA sequencing methods need further refinement and testing before incorporation into standard fish sampling surveys.

Standard methods of sampling fish communities can be biased and expensive (Hickley and Starkie 1985; Bonar et al. 2009), necessitating further refinement of current standard methods and development of new methods. This is especially applicable to large aquatic systems, where sampling fish communities can be highly complex, time consuming, and laborious (Hickley and Starkie 1985; Sutherland 2006). Recently, methods involving the examination of DNA in water samples (i.e., environmental DNA [eDNA]) have shown promise for characterizing fish species presence and distribution in waterbodies (Ficetola et al. 2008; Jerde et al. 2011; Minamoto et al. 2012; Goldberg et al. 2013; Laramie et al. 2015).

Environmental DNA consists of an organism's nuclear or mitochondrial DNA (released cellular material) that is found in both aqueous and terrestrial environments. Currently, eDNA may be effective for estimating species biomass through measurement of the number of eDNA copies in a water sample; this hypothesis follows the assumption that aquatic vertebrates release eDNA into the water (from feces, secretions, or tissues) in proportion to their biomass (Takahara et al. 2012; Thomsen et al. 2012). Although eDNA has been used successfully to characterize relative abundance and biomass of species in controlled settings and large rivers, these tests are limited (e.g., Jerde et al. 2011; Mahon et al. 2013; Pilliod et al. 2013). In addition, spatial variation in eDNA across a large standing waterbody is not well understood. Further evaluation of the procedure is necessary to identify its utility in standard fish monitoring surveys of standing waters. Furthermore, the relationship between data collected from eDNA surveys and the data collected through established fish capture surveys—especially for characterizing fish relative abundance and biomass in large standing waters—remains unclear.

If successful, rapid evaluation of fish relative abundance, biomass, and species composition by eDNA would allow fisheries managers to identify potential problems in fish communities at early stages and thus to apply management actions that would have a better likelihood of success. Field-applied eDNA methods would allow managers to monitor many large and small lakes and ponds at lower cost and at a faster rate than established fish sampling techniques. Additionally, sport fisheries can be compromised due to the presence of undesirable species. For example, Gizzard Shad *Dorosoma cepedianum* can be a valuable forage fish in northern lakes where the species cannot overwinter and where populations can be controlled (Michaletz 1998); however, in southern waters, Gizzard Shad can overpopulate and compete with desirable sport fishes, such as Largemouth Bass

*Micropterus salmoides*, Black Crappies *Pomoxis nigromaculatus*, and White Crappies *Pomoxis annularis*. In Theodore Roosevelt Lake (hereafter, Lake Roosevelt), Arizona, Gizzard Shad are rapidly expanding in distribution and abundance and are therefore becoming a concern for fisheries managers. Lake Roosevelt supports a popular sport fishery for Largemouth Bass, and since the recent boom in Gizzard Shad population growth, the Largemouth Bass populations have declined drastically. Gizzard Shad are expanding in the reservoir and are competing with Largemouth Bass and other sport fish for space and resources. Early detection is the most cost-effective management strategy to reduce the success of an invasion; however, once the species has become established, management can be expensive and time consuming (Pimentel et al. 2000; Leung et al. 2002; Jerde et al. 2011; Goldberg et al. 2013). The use of eDNA sampling methods may provide early warning of an invasion and may serve to monitor relative abundance and biomass of undesirable fishes after they have invaded. In addition, eDNA could be used to help identify locations of high abundances within the waterbody to aid in targeting removals.

Our objective was to compare fish presence, relative abundance, biomass, and species composition measured through eDNA methods and established American Fisheries Society (AFS) standard sampling methods in a large (8,698 ha) warmwater reservoir. Specifically, for Gizzard Shad and Largemouth Bass, we compared species detections obtained by use of established methods (boat electrofishing and gillnetting) to species detections obtained via eDNA methods at the same sites. For the two species, we also examined the relationship between AFS boat electrofishing/gillnetting CPUE versus total eDNA copies per individual sampling site and per area of the reservoir. Many studies have shown that CPUE is related to fish density (Hall 1986; Coble 1992; McInerney and Degan 1993; Hill and Willis 1994); therefore, we used CPUE and biomass per unit effort (BPUE) as measures of fish abundance. In addition, we investigated the relationship between the proportion of fish catch composition (number of fish captured and total weight [g] of fish) for each gear type and the proportion of total eDNA copies from Gizzard Shad and Largemouth Bass over the entire reservoir.

## METHODS

*Study site.*—Our study was conducted at Lake Roosevelt, which was formed by the creation of a dam completed in 1911

near the confluence of the Salt River and Tonto Creek (Ham 1995; Salt River Project 2015). Lake Roosevelt is located east of Phoenix in Gila County, Arizona, and is the upstream-most reservoir among a series of four reservoirs on the Salt River. It is the largest body of water located entirely in the state of Arizona and has a surface area of 8,698 ha, a length of 36 km, a shoreline length of 206 km, and a maximum depth of 57 m at 100% storage capacity (SRP 2015). The warm, monomictic reservoir stratifies between February and April, completely turns over by December (Ham 1995), and never freezes. The spring-fed headwaters of the reservoir are the Black River and the White River, which converge at the Salt River (ADWR 2014a, 2014b). The sparse vegetation surrounding the lake is characteristic of the Sonoran Desert Arizona Upland Region (i.e., saguaro *Carnegiea gigantea* and blue palo verde *Parkinsonia florida* forest), and limited within-lake cover is present.

The reservoir was originally created to store water for irrigation purposes. It has since become a large water resource for the Phoenix metropolitan area and a popular warmwater sport fishery for the state of Arizona. Lake Roosevelt supports many warmwater sport fish species, including the Largemouth Bass, Smallmouth Bass *Micropterus dolomieu*, Yellow Bass *Morone mississippiensis*, Black Crappie, sunfishes *Lepomis* spp., Channel Catfish *Ictalurus punctatus*, and Flathead Catfish *Pylodictis olivaris* (AZGFD 2013).

*American Fisheries Society standard fish sampling field methods.*—Lake Roosevelt was separated into three spatial strata for sampling: (1) Tonto Creek area, (2) mid-lake area, and (3) Salt River area (Figure 1). We randomly selected starting points for 15 approximately 600-s electrofishing sites and 10 gill-net sites within each stratum for both spring (April–June 2014) and fall (October–November 2014), resulting in a total of 90 electrofishing sites and 60 gill-net sites (Figure 1). Sampling sites chosen in the spring were different than those chosen in the fall, and gill-net sampling sites were selected separately from electrofishing transects. All sampling occurred when weather conditions were ideal (no rainstorms; little to no wind). We used a Garmin handheld GPS unit (Model GPSMAP78S Land & Sea; Garmin, Olathe, Kansas) to locate field sites that were selected from a map of the lake on Google Earth (Google Earth 2014).

We employed AFS standard sampling methods to sample fish communities in Lake Roosevelt. A 5.49-m, aluminum, flat-bottomed boat equipped with a Smith-Root VVP 15B electrofisher (Smith-Root, Vancouver, Washington) was used for all electrofishing surveys; for gill-net surveys, we used AFS standard core gill nets (Bonar et al. 2009). All sampling equipment for electrofishing and gill-net surveys met all AFS standard specifications for sampling warmwater fish in large standing waters (Miranda and Boxrucker 2009). We conducted boat electrofishing surveys parallel to the shoreline in water depths of approximately 0.91–1.83 m. We set gill nets perpendicular to the shoreline, with the near-shoreline end set

approximately 1–2 m from shore at various depths. Electrofishing was conducted at night in accordance with AFS standard procedures (water temperatures = 15–23°C; at night because water clarity > 1 m). However, due to the high-conductivity water, the frequency (40–55 Hz) and duty cycle (40–60% on-time) of the electrofishing unit had to be adjusted to effectively electrofish. Boat electrofishing surveys are not typically conducted in the fall according to AFS standard procedures; however, we conducted boat electrofishing surveys in the fall for purposes of comparison with data for spring. We set gill nets at night, spanning both crepuscular periods, and typically conducted gillnetting when water temperatures were 20°C or less; however, surface water temperatures sometimes exceeded 20°C. Electrofishing effort (seconds and starting/stopping points) was recorded at each transect, and gill-net effort was measured in net-nights (Miranda and Boxrucker 2009).

All captured fish were identified, measured (mm TL), and weighed (g). A Hydrolab Quanta (Hydrolab Corp., Austin, Texas) was used to record water temperature (°C) and pH at every gill-net and electrofishing site and to measure conductivity (µS) at electrofishing sites only.

*Environmental DNA field sampling methods.*—Prior to each sampling event, the boat and all sampling equipment were sanitized with a 10% bleach solution and were allowed to completely dry (Jacks et al. 2009; USFWS 2013). We collected all water samples immediately prior to setting gill nets or electrofishing. A 10-min period was permitted to allow fish to redistribute themselves before electrofishing surveys.

We marked and recorded the beginning of each electrofishing and gill-net site on the Garmin GPSMAP78S unit. We collected surface water samples at all sampling sites by holding a sterile, 1-L Nalgene bottle with sterile latex gloves and dipping it into the water off the bow of the boat. For electrofishing transects, we moved parallel to shoreline, dunking the bottle at four equidistant locations along the 600-s transect, and filled the bottle about one-fourth each time. This provided a composite water sample along the entire transect. At gill-net sites, we collected surface water samples perpendicular to the shoreline starting about 27 m away from shore, heading into the shore, and skimming the top of the water across the length of the net site. The 1-L Nalgene bottle was then capped, labeled, and placed in a sanitized cooler on ice. We put a control sample (deionized water) in the cooler that remained in the cooler during the entire sampling period and was processed on the last day of sampling to warn of any contamination from the point of collection to eDNA analysis. Temperature and pH were recorded at sample sites after water collection.

*Environmental DNA preservation methods.*—After all field sampling was completed for the night, the water samples remained on ice for preservation until they were processed for shipment. On the morning after field sampling (<16 h after water sample collection), each of the 1-L water samples was

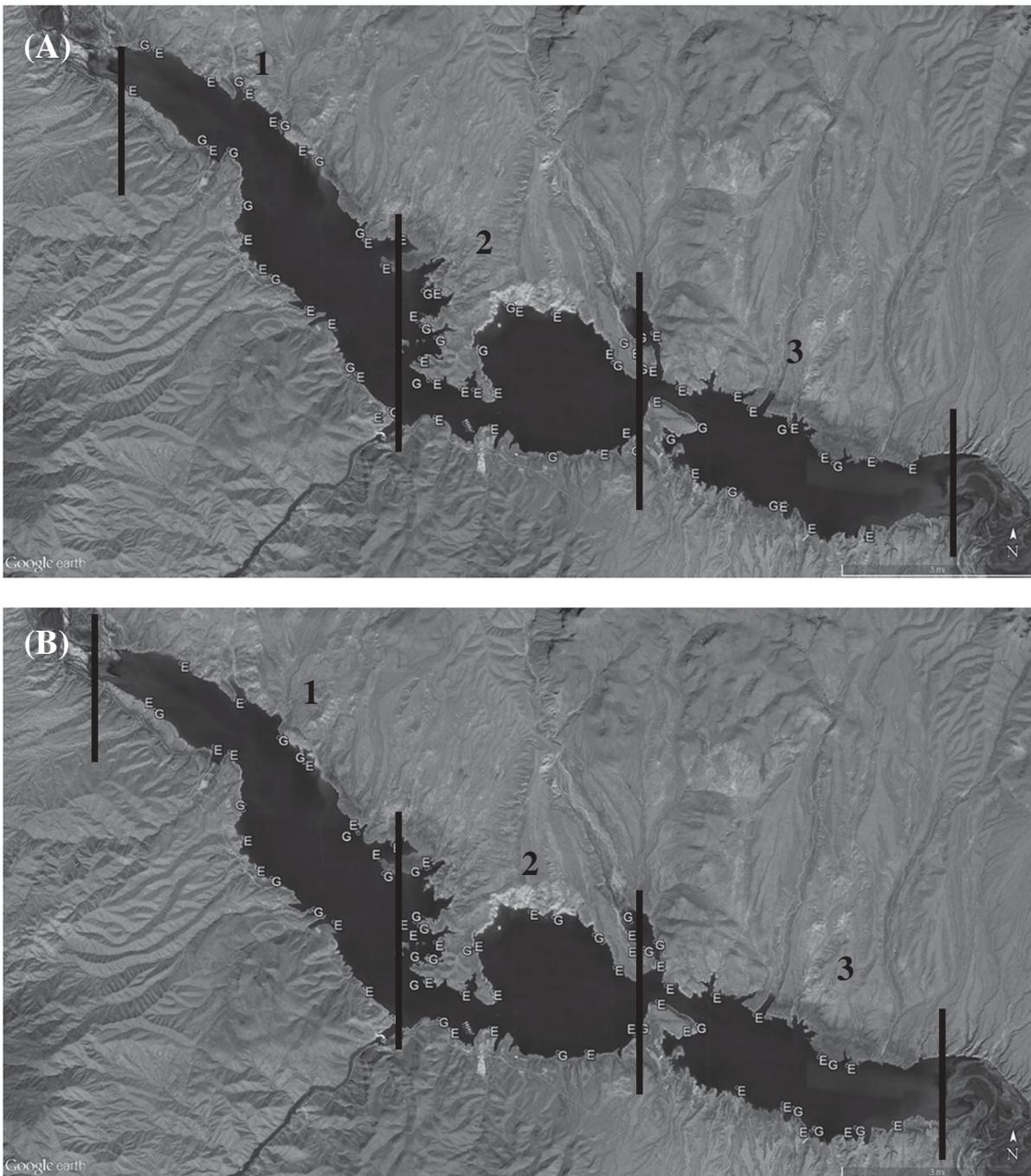


FIGURE 1. Boat electrofishing sites (E) and gillnetting sites (G) in Lake Roosevelt, Arizona, sampled via American Fisheries Society standard methods during (A) spring and (B) fall 2014. The separate strata used for field sampling and reservoir stratum analysis were the (1) Tonto Creek area; (2) mid-lake area; and (3) Salt River area.

processed separately according to sample site. Each water sample was divided into five 50-mL, conical centrifuge tubes. Into each tube, we poured 15 mL of the 1-L water sample, 1.5 mL of 3-M sodium acetate (molecular biology grade; Thermo Fisher Scientific), and 33.5 mL of 200-proof absolute ethanol (molecular biology grade; Fisher Scientific). Samples from one

sampling site were placed into a 0.95-L bag and were sealed to prevent cross-contamination. We then sealed three 0.95-L bags each into a 3.79-L bag and placed all the processed samples in the freezer ( $<0^{\circ}\text{C}$ ) until they were shipped ( $<6$  d later). For shipping, samples were placed in a Styrofoam cooler on ice packs and were shipped overnight to the U.S. Geological

Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC; La Crosse, Wisconsin) for DNA processing.

*Environmental DNA laboratory analysis methods.*—Samples from Lake Roosevelt were immediately stored at  $-20^{\circ}\text{C}$  upon arrival at USGS–UMESC until further processing. To concentrate individual samples into a pellet for DNA extraction, each sample was centrifuged at  $5,000 \times g$  for 30 min. The supernatant was poured off, and the 50-mL tubes were inverted for 10 min to decant excess liquid. The resulting pellets were re-suspended in 300  $\mu\text{L}$  of 100% molecular-grade ethanol. The four individual samples from a set (5–50-mL centrifuge tubes) were then consolidated into a 1.5-mL microcentrifuge tube. These tubes were centrifuged for 10 min at  $14,000 \times g$ , and the supernatant was aspirated and discarded. The resulting composite pellet was re-suspended in 250  $\mu\text{L}$  of gel solubilization buffer from the IBI gMAX Mini Genomic DNA Kit (IBI Scientific, Peosta, Iowa) and was transferred to a 2-mL microcentrifuge tube. All samples were stored at  $-80^{\circ}\text{C}$  until DNA extractions were conducted.

The DNA was extracted from samples by using the IBI gMAX Mini-Kit in accordance with the manufacturer's protocol for water samples. An extraction negative (100  $\mu\text{L}$  of molecular-grade deionized water) was co-extracted alongside each processed set of Lake Roosevelt samples. All samples were eluted to a final volume of 100  $\mu\text{L}$ . After extraction, samples were stored at  $-80^{\circ}\text{C}$  until quantitative PCR could be performed.

To confirm the specificity of the primers, they were tested against DNA from fishes typically found in Lake Roosevelt. Fin clips were obtained from the following nontarget species: Threadfin Shad *Dorosoma petenense*, Red Shiner *Cyprinella lutrensis*, Smallmouth Buffalo *Ictiobus bubalus*, and Bluegill *Lepomis macrochirus*. The DNA from each fin clip was extracted by using the IBI gMAX Mini-Kit via the tissue extraction protocol, with slight modification. We also included DNA from Largemouth Bass and Gizzard Shad fin clips to optimize our PCR conditions and serve as PCR positive controls.

Primers specific to Largemouth Bass and Gizzard Shad were designed using the National Center for Biotechnology Information's Primer-Basic Local Alignment Search Tool (Primer-BLAST; <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Two markers were designed for each species and consisted of forward and reverse primers and a species-specific probe that was fluorescently tagged with either fluorescein FAM or JOE. Two markers were created to ensure amplification of DNA from the species of interest. We tested our markers in vitro against the following nontarget species: Smallmouth Buffalo, Threadfin Shad, Golden Shiner *Notemigonus crysoleucas*, Bluegill, Red Shiner, Bigmouth Buffalo, Spotfin Shiner *Cyprinella spiloptera*, Sand Shiner *Notropis stramineus*, Longnose Dace *Rhinichthys cataractae*, Blacknose Dace *Rhinichthys atratulus*, Creek Chub *Semotilus atromaculatus*, White Sucker *Catostomus commersonii*, Brassy Minnow *Hybognathus hankinsoni*, and Emerald Shiner *Notropis atherinoides*. Primers were designed within the mitochondrial genome of each species, which is the typical target in eDNA studies (Wilcox et al. 2013; Farrington et al. 2015). Primers were chosen based on their dissimilarity to those of other closely related species, particularly to those of target species found within the same water system (Appendix Table A.1). The first marker for Largemouth Bass was located within the cytochrome-c oxidase I region (COI), and the second was located within the NADH dehydrogenase subunit 4 (ND4) region (Table 1). The first marker for Gizzard Shad was within the ND3 region, and the second was within the ND5 region (Table 1). The DNA extracts were quantified in four replicate quantitative PCRs containing 1  $\mu\text{L}$  of template, 800-nM primers, 250-nM double-quenched probe, and  $1 \times$  SensiFAST Probe No-ROX Master Mix (Bioline, Taunton, Massachusetts) in 25- $\mu\text{L}$  reaction volumes. Reactions were denatured at  $94^{\circ}\text{C}$  for 2 min, followed by 45 cycles of  $94^{\circ}\text{C}$  for 10 s,  $68^{\circ}\text{C}$  for 15 s, and  $64^{\circ}\text{C}$  for 20 s, along with a final extension at  $72^{\circ}\text{C}$  for 5 min. Additionally, we had three positive controls, one nontemplate control, and many field blanks for each eDNA sample. On each plate, we included a standard curve from 0 to 1,000,000 copies of our targeted DNA

TABLE 1. Oligonucleotide sequences and target DNA amplicon lengths used for quantitative PCR analysis of Largemouth Bass and Gizzard Shad environmental DNA from Lake Roosevelt, Arizona.

Species	Amplicon length (bp)	Oligonucleotide name	Oligonucleotide sequence
Largemouth Bass	107	Microps2_F	AGGCTACGGCATGATACG
		Microps2_R	TTGAGCCTGTTATGACTACTCC
		Microps2_Probe	6-FAM/GCCCCTTAC/ZEN/CAAGGAACTCA-Iowa Black FQ
Gizzard Shad	101	Doros1_F	ACTAGTCACTGTGTCGTGG
		Doros1_R	TCCTCTATTCGGCTCATTCC
		Doros1_Probe	6-FAM/TGCCATCCT/ZEN/TGTTCTTCTGAC-Iowa Black FQ

(G Block). Our three positive controls for each sample consisted of the 1  $\mu$ L of sample plus 100 copies of our template. Field negative controls and extraction negative controls were analyzed along with samples as well as two nontemplate controls on each plate by using a Mastercycler Realplex 2 Thermocycler (Eppendorf, Hauppauge, New York). All molecular methods and marker design and testing were carried out at the USGS–UMESC.

*Analysis.*—We created maps by using Google Earth (Google Earth 2014) to plot and visually examine the distribution of fish and eDNA in the entire reservoir. Gizzard Shad and Largemouth Bass fish counts and total eDNA copies at each site were plotted by using symbols of various sizes.

Correspondence in detection between eDNA and electrofishing or gillnetting methods was tested by using Pearson's chi-square analysis with a Yates correction for continuity ( $\alpha = 0.05$ ). In instances where frequencies in the contingency table were below 5, Fisher's exact test was used to examine correspondence. The detections of Gizzard Shad and Largemouth Bass from boat electrofishing or gillnetting and from eDNA methods were counted and cross-classified in  $2 \times 2$  contingency tables. The null hypothesis was that no relationship existed between species detections obtained by either electrofishing or gillnetting and the species detections from eDNA methods at individual sites.

We used within-season, paired-gear comparison methods (Peterson and Paukert 2009) to compare eDNA data with the data from established AFS gill-net and boat electrofishing methods. The assumption was that both the eDNA methods and the AFS standard methods sampled the same fish populations and assemblages. Regression analysis ( $\alpha = 0.05$ ) was used to examine the relationships between data of the following types at each site for each species (Gizzard Shad and Largemouth Bass): (1) boat electrofishing CPUE (fish/h) and total eDNA copies (composite number based on the total number of copies detected in all four PCR replicates); (2) boat electrofishing BPUE (g/h) and total eDNA copies; (3) gill-net CPUE (fish/net-night) and total eDNA copies; and (4) gill-net BPUE (g/net-night) and total eDNA copies.

For the per-stratum analysis, we calculated the average CPUEs and BPUEs of Largemouth Bass and Gizzard Shad captured in the boat electrofishing and gill-net surveys within each reservoir stratum during spring and fall 2014. The CPUE or BPUE was compared to the total eDNA copies collected in that area of the reservoir. The null hypothesis was that no relationship existed between Gizzard Shad and Largemouth Bass CPUE or BPUE from collections obtained with established gear types (electrofishing and gillnetting) and the total copies of eDNA from paired water samples either at individual sites or in overall lake sections (i.e., strata).

Finally, we compared the total catch composition (numbers and biomass) of Largemouth Bass and Gizzard Shad to the total eDNA copies sampled from Lake Roosevelt overall for

both spring and fall 2014. We used plot analysis to conduct comparisons for each gear type and for both gears combined. Program R (version 0.98.501) was used for all analyses (R Core Team 2012).

## RESULTS

We successfully collected eDNA of both target fish species in Lake Roosevelt. Our decontamination procedures were successful, as we did not detect Gizzard Shad or Largemouth Bass DNA in any of our field control samples. We also did not detect any target DNA in our extraction negative controls or no-template controls, with one exception. After re-analyzing that plate, all negative controls were clear; therefore, data from the second analysis of that plate were used in statistical comparisons.

### Temporal and Spatial Variation in Fish Catch and Environmental DNA

Fish catch and eDNA of Gizzard Shad were distributed across Lake Roosevelt and did not seem to be concentrated in any specific areas of the reservoir for spring and fall electrofishing surveys (Figure 2) or for spring and fall gill-net surveys (Figure 3). Largemouth Bass eDNA and fish distribution were highest in the eastern portion of the reservoir during spring electrofishing surveys; however, for fall electrofishing surveys, distribution was highly variable throughout the reservoir (Figure 4). Overall, Largemouth Bass catch and eDNA were highest near the two inflow sources (Tonto Creek and Salt River) in the eastern and western areas of the reservoir (Figures 4, 5).

Both the capture of fish in established gear and the number of eDNA copies varied seasonally and by gear type in Lake Roosevelt. Catch of Gizzard Shad and Largemouth Bass was higher in spring than in fall (Table 2). More Gizzard Shad eDNA copies were obtained in spring water samples than in fall water samples (Table 2); conversely, the numbers of eDNA copies for Largemouth Bass were highest in fall (Table 2).

### Species Detection using Established Gear versus Environmental DNA

Gizzard Shad were detected at all study sites in Lake Roosevelt by using boat electrofishing, gillnetting, eDNA, or a combination of methods. During both spring and fall, Gizzard Shad were detected at a higher percentage of sites based on eDNA methods than with boat electrofishing (Figure 6). In contrast, gill nets detected Gizzard Shad at more sites than eDNA for both spring and fall sampling (Figure 6). Although gillnetting detected Gizzard Shad at more gill-net sites, eDNA yielded an overall higher detection rate than traditional sampling methods at all sampled sites. We found that electrofishing and gillnetting detected Largemouth Bass at more sites than eDNA; the exception was fall gillnetting, which detected Largemouth Bass at an equal number of sites as eDNA (Figure 6).

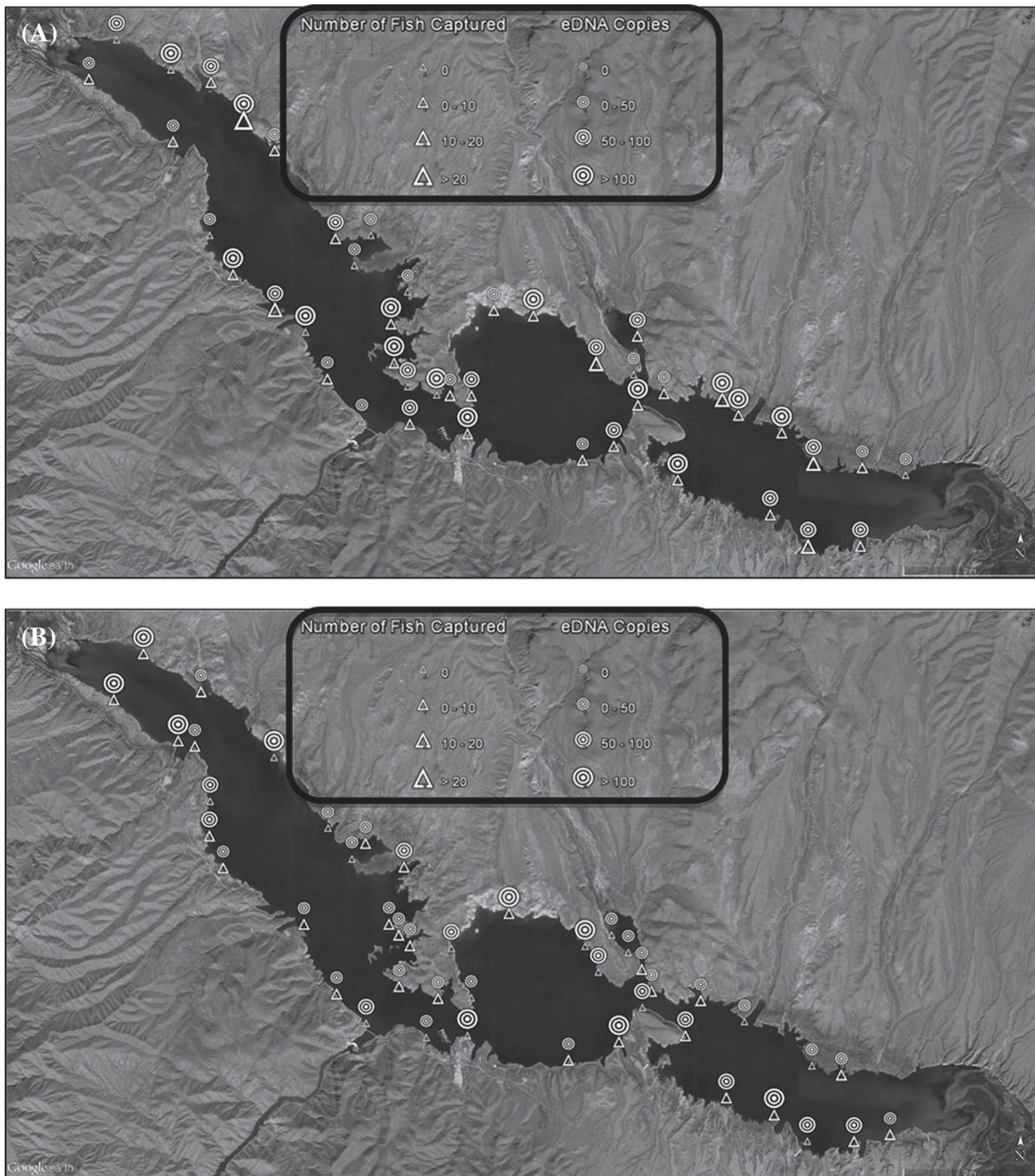


FIGURE 2. Aerial view of Lake Roosevelt, illustrating the lakewide distribution and abundance of Gizzard Shad as determined by boat electrofishing in comparison with the number of environmental DNA (eDNA) copies in water samples collected during (A) spring and (B) fall 2014.

We found no relationship between detections at specific sites with established gear and eDNA (Pearson's chi-square analysis and Fisher's exact test;  $\alpha = 0.05$ ). Detections of Gizzard Shad and Largemouth Bass via established gears were independent of detections based on the number of eDNA copies (Table 3).

#### Relationship between Relative Abundance Measured by Traditional Gear and Environmental DNA

We found no relationship (Table 4) between Gizzard Shad and Largemouth Bass CPUEs or BPUEs from spring and fall boat electrofishing and gillnetting at individual sampling sites and the number of eDNA copies from

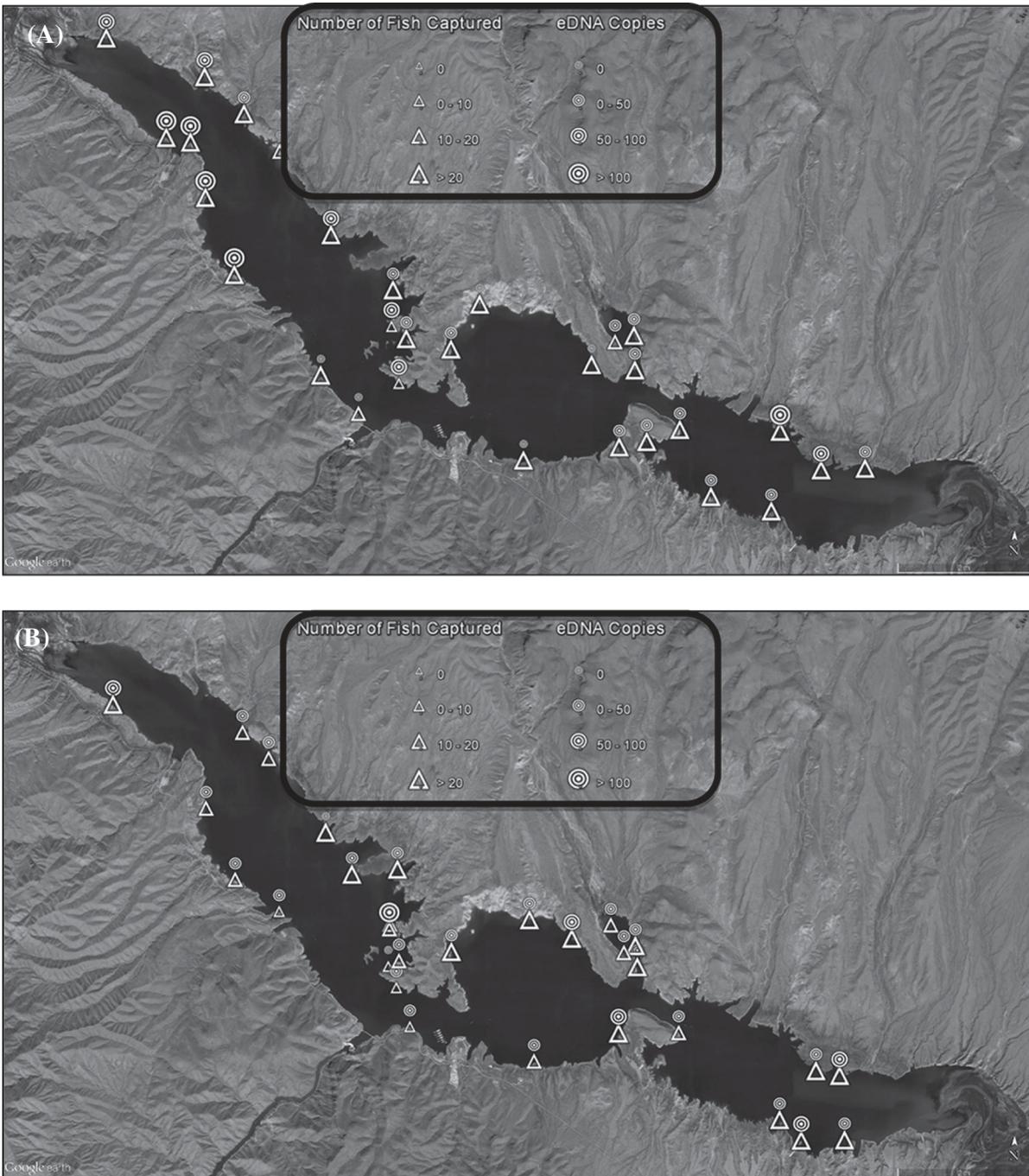


FIGURE 3. Aerial view of Lake Roosevelt, illustrating the lakewide distribution and abundance of Gizzard Shad as determined by gill-net sampling in comparison with the number of environmental DNA (eDNA) copies in water samples collected during (A) spring and (B) fall 2014.

adjacent water samples. Similarly, no relationship existed (Table 4) between the CPUEs or BPUEs of Gizzard Shad and Largemouth Bass sampled by spring boat electrofishing, fall boat electrofishing, and spring gillnetting in a given reservoir stratum and the number of eDNA copies in the same stratum. Additionally, no relationship was detected (Table 4) between Largemouth Bass CPUE or BPUE from

fall gillnetting in a stratum and the number of Largemouth Bass eDNA copies in the same stratum. Positive relationships were observed for spring gill-net CPUEs of Gizzard Shad ( $N = 3$ ,  $R^2 = 0.988$ ,  $P = 0.069$ ) and Largemouth Bass ( $N = 3$ ,  $R^2 = 0.988$ ,  $P = 0.071$ ) and their respective number of eDNA copies per stratum; however, the  $P$ -values were slightly above our significance level of 0.05 (Table 4;

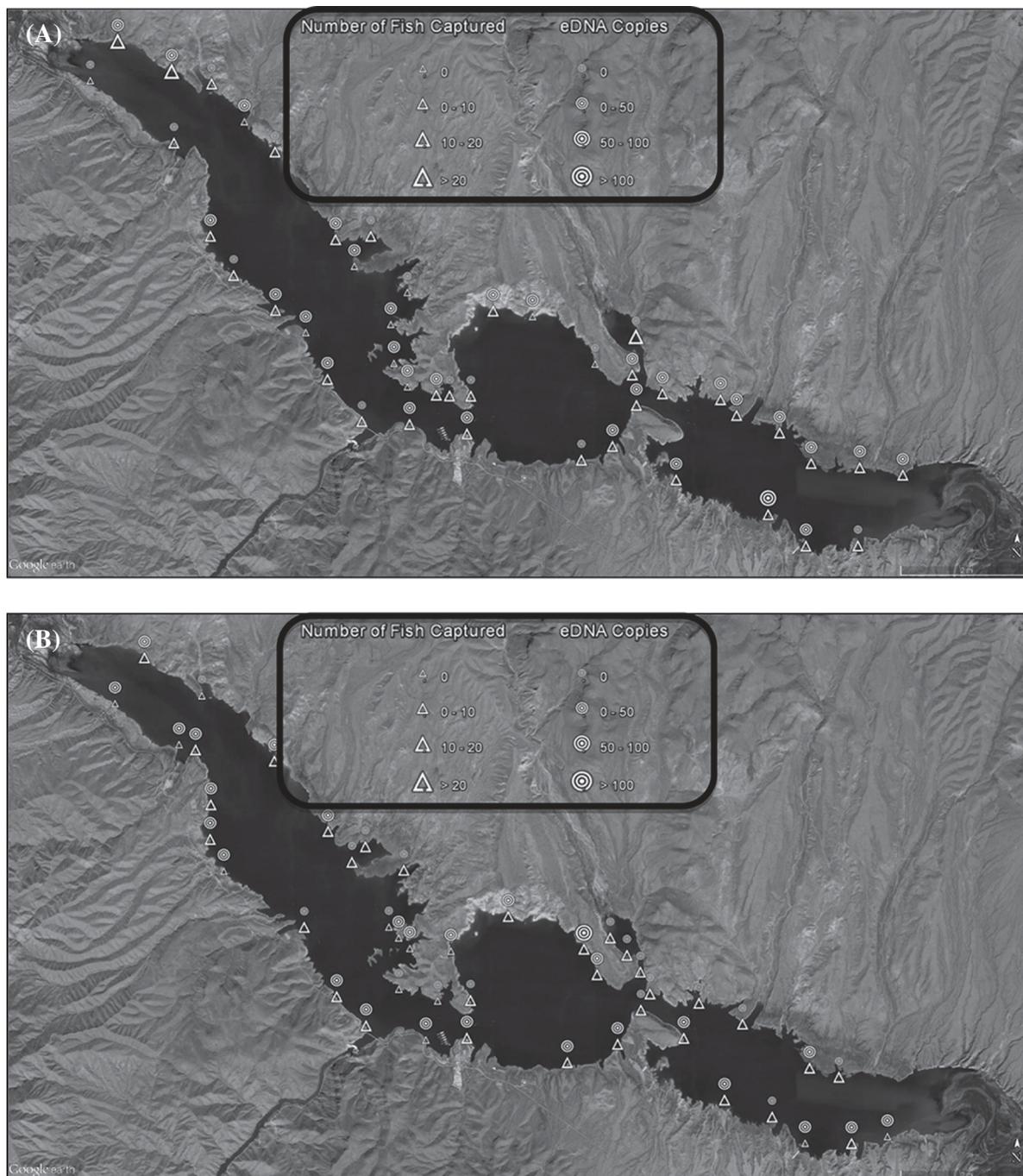


FIGURE 4. Aerial view of Lake Roosevelt, illustrating the lakewide distribution and abundance of Largemouth Bass as determined by boat electrofishing in comparison with the number of environmental DNA (eDNA) copies in water samples collected during (A) spring and (B) fall 2014.

shown in bold italics). No relationship existed between fall gill-net CPUE of Gizzard Shad and the number of DNA copies per stratum. Overall, we found no relationships between the number of eDNA copies and the CPUEs or BPUEs from either type of traditional gear at individual sites or strata during spring or fall.

#### Species Composition of Gizzard Shad and Largemouth Bass in the Entire Reservoir

Plot analysis suggested that reservoirwide catch composition of Largemouth Bass and Gizzard Shad (numbers and total weight [g] of fish) achieved through a combination of standard gear types (boat electrofishing plus gillnetting) was similar to

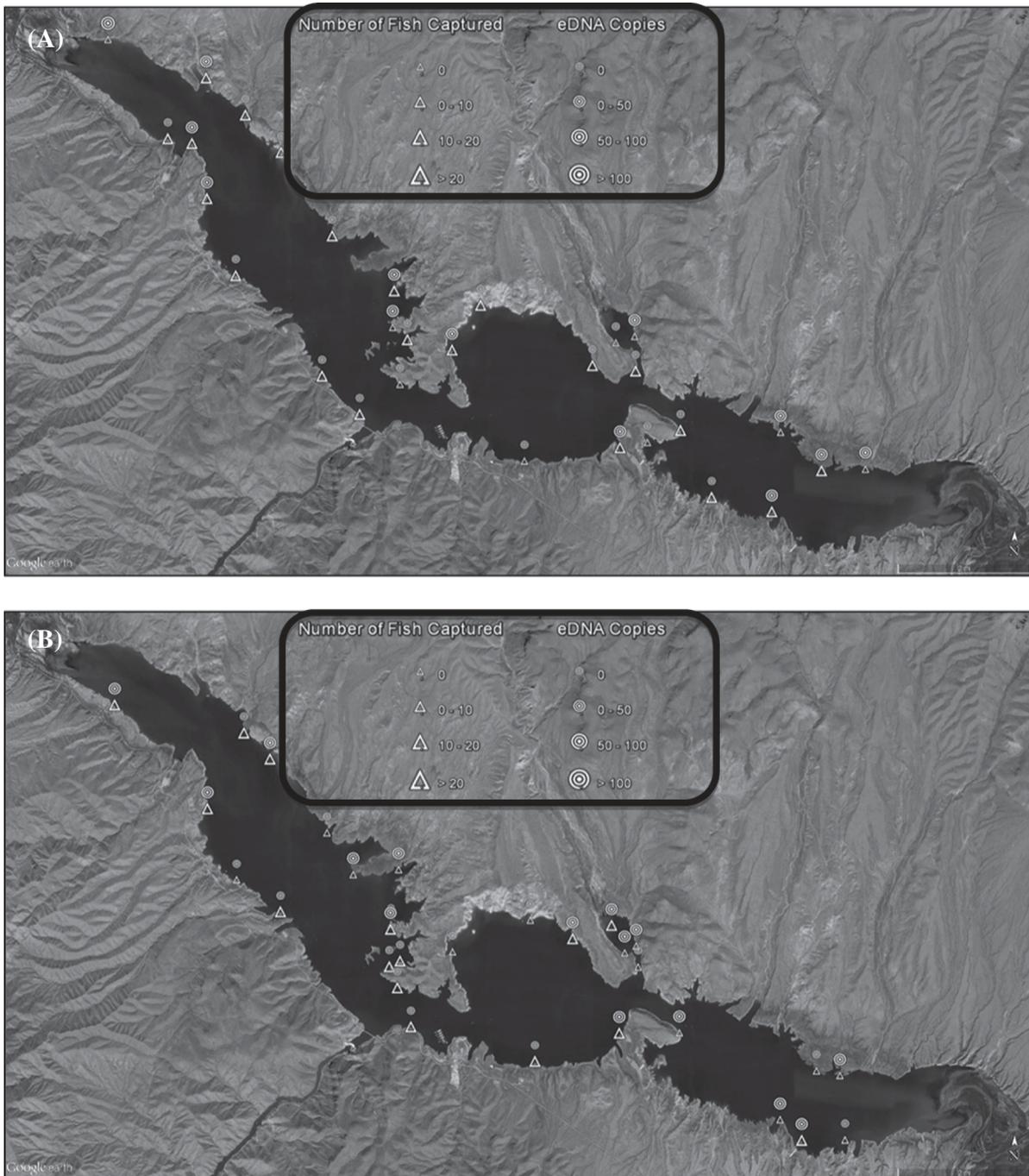


FIGURE 5. Aerial view of Lake Roosevelt, illustrating the lakewide distribution and abundance of Largemouth Bass as determined by gill-net sampling in comparison with the number of environmental DNA (eDNA) copies in water samples collected during (A) spring and (B) fall 2014.

the proportion of total eDNA copies from each species for both spring and fall field sampling (Figure 7). Likewise, spring and fall gill-net surveys portrayed a total catch composition (numbers and total weight) that was similar to the proportion of total eDNA copies for Largemouth Bass and Gizzard Shad (Figure 7). In contrast, a total lack of similarity was illustrated between the proportions of fish caught via spring and fall boat

electrofishing and the proportion of total eDNA copies from each species (Figure 7).

## DISCUSSION

This is the first known study to investigate the utility of eDNA for quantifying fish numbers, biomasses, and proportions

TABLE 2. Summary of total catch and total biomass of Gizzard Shad and Largemouth Bass obtained by using established gear and the total number of environmental DNA (eDNA) copies from paired water samples collected in Lake Roosevelt, Arizona.

Season	Gear type	Species	Total fish captured	Total fish biomass (g)	Total eDNA copies
Spring	Boat electrofishing	Gizzard Shad	202	88,849	5,875.39
		Largemouth Bass	153	65,197	253.91
Spring	Gillnetting	Gizzard Shad	1,096	409,697	2,430.15
		Largemouth Bass	46	22,673	40.92
Fall	Boat electrofishing	Gizzard Shad	99	34,712	3,071.16
		Largemouth Bass	92	37,434	350
Fall	Gillnetting	Gizzard Shad	703	196,796	896.91
		Largemouth Bass	28	15,974	108.91

in a large reservoir. We were able to successfully investigate the relationship between the number of eDNA copies from specific fish species and the abundance estimates (CPUE and BPUE) obtained for those species by using established gear types. Other studies have examined how established field methods compare with eDNA methods for estimating species abundance and presence (Dejean et al. 2012; Pilliod et al. 2013); however, our study was unique in examining the use of eDNA during regular fish monitoring in a large reservoir.

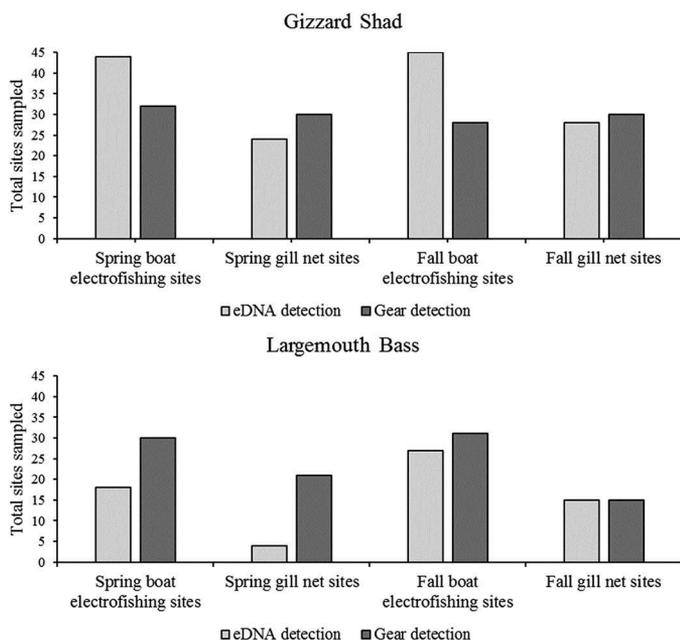


FIGURE 6. Species detections at Lake Roosevelt sites based on environmental DNA (eDNA) sampling versus species detections via established field sampling methods (boat electrofishing and gillnetting). During spring surveys, 44 paired sites (i.e., eDNA samples and standard gear) were sampled by boat electrofishing for a total of 8.20 survey hours, and 30 paired sites were sampled by gillnetting for a total of 30 net-nights. During fall surveys, 45 paired sites were sampled by boat electrofishing for a total of 8.08 survey hours, and 30 paired sites were sampled by gillnetting for a total of 30 net-nights.

### Temporal and Spatial Variation in Fish Catch and Environmental DNA

The number of DNA copies throughout the reservoir varied seasonally. These results suggest that eDNA field sampling methods should incorporate temporal components similar to those of established fish sampling methods, such as electrofishing and netting (Pope and Willis 1996; Strickler et al. 2015). In our study, spring field sampling revealed an overall higher yield of both fish catch and eDNA copies, suggesting that eDNA field sampling in Lake Roosevelt should be conducted during the spring to increase the chances of Gizzard Shad detection. However, the optimal sampling time frame for eDNA may vary among species and among aquatic systems.

The seasonal variation in the amount of eDNA in water samples may be attributed to changes in fish behavior and physiology and is influenced by many abiotic factors (e.g., changes in temperature, dissolved oxygen, turbidity, food supplies, and photoperiod; Pope and Willis 1996). Fish behavior, such as spawning, can increase the amount of eDNA present in the water, providing an overestimate of abundance. Littoral fishes (e.g., Largemouth Bass) are not as mobile in an aquatic system as pelagic fishes (e.g., Gizzard Shad) and, depending on the sampling technique, littoral fish may be underrepresented with eDNA sampling. In Lake Roosevelt, we collected surface water samples for eDNA, which may be a more ideal field sampling approach for pelagic species like the Gizzard Shad.

### Species Detection using Traditional Gear versus Environmental DNA

The distribution and abundance of Gizzard Shad and Largemouth Bass were variable throughout the aquatic system, and the effectiveness of established sampling methods or eDNA methods for detecting the presence of these fish in Lake Roosevelt varied depending on the gear and species. Although gill nets detected Gizzard Shad at more gillnetting sites, eDNA yielded an overall higher detection rate in all sampled sites within Lake Roosevelt than did traditional sampling methods. In addition, traditional sampling was more

TABLE 3. Results of Pearson's chi-square analysis and Fisher's exact test ( $\alpha = 0.05$ ) examining the relationship between detection of species (Gizzard Shad and Largemouth Bass) by using established fish sampling gears and detection via the number of eDNA copies.  $P < 0.05$  indicates a significant relationship.

Species	Survey type	Pearson's chi-square analysis			Fisher's exact test: $P$
		$\chi^2$	df	$P$	
Gizzard Shad	Boat electrofishing	–	1	–	1.000
Largemouth Bass	Boat electrofishing	0.415	1	0.52	0.618
Gizzard Shad	Gillnetting	–	1	–	1.000
Largemouth Bass	Gillnetting	0.025	1	0.874	0.793

effective at detecting Largemouth Bass at sites within Lake Roosevelt. Our results suggest that an understanding of the limits and biases of eDNA as a fish sampling technique is as important as such an understanding for traditional fish sampling gears. It is well known that gill nets and boat electrofishing possess gear bias and can be species and size selective (Rudstam et al. 1984; Jackson et al. 1995; Ruetz et al. 2007). Furthermore, susceptibility to any gear type depends on fish species, life stage, size in relation to the gear, habitat preference, schooling and swimming behavior, and feeding and activity level (Hayes 1983; Hubert 1983). The susceptibility of Gizzard Shad and Largemouth Bass to eDNA detection, as with detection by traditional sampling gear, may be attributed to the differences in species behavior. In the large standing waterbody that we studied, gillnetting was a more effective capture method for sampling Gizzard Shad, whereas boat electrofishing was more effective for capturing Largemouth Bass. This could be due to the difference in biology of these species; the Gizzard Shad is a pelagic species, whereas Largemouth Bass spend most of their time in the littoral zone (Miller 1960; Savino and Stein 1989). As a result, Gizzard Shad eDNA covers a wider surface area than Largemouth Bass eDNA.

#### Relationship between Relative Abundance Measured by Traditional Gear and Environmental DNA

The amount of eDNA collected during our spring and fall sampling in Lake Roosevelt did not reflect gill-net or electrofishing catches at individual sites or in each lake stratum. This could be due to biotic and abiotic factors that contribute to a patchy distribution of eDNA in Lake Roosevelt, such as fish behavior, microbial decomposition, recreational activities, wind (currents), UV radiation, water quality (dissolved oxygen, pH, and water temperature), inflow sources, lake mixing, and water fluctuations (Dejean et al. 2011; Barnes et al. 2014). Additionally, the amount of fish eDNA on the surface in a reservoir can vary during a given sampling period due to recreational activity, wind, inflow sources, lake mixing, and fluctuating water levels, which can unevenly distribute eDNA in an aquatic system. These human-caused and environmental factors all play a role in the amount and distribution of eDNA in a system.

Lastly, eDNA may not persist as long in Lake Roosevelt as in lentic systems that are colder, more protected from solar radiation, and more alkaline. Detectable amounts of eDNA decay faster in waters that are warmer, less protected from solar radiation, and neutral or acidic (Strickler et al. 2015). Dejean et al. (2011) found that eDNA in controlled environments were detectable for up to 30 d. Persistence of eDNA in aqueous environments is highly influenced by environmental variables and the abundance of the target species.

#### Species Composition of Gizzard Shad and Largemouth Bass in the Entire Reservoir

In previous studies, eDNA has been a useful tool for characterizing overall species composition in a waterbody (see Thomsen et al. 2012). Our plot analysis suggested that eDNA field methods can characterize species composition (Gizzard Shad and Largemouth Bass) in a manner similar to established methods; however, differences exist depending on the gear used in comparisons. As discussed above, the susceptibility of a species to a particular gear depends on many variables, such as fish species, life stage, size in relation to the gear, habitat preferences, schooling and swimming behavior, and feeding and activity levels (Hayes 1983; Hubert 1983). Employing a combination of sampling gears (e.g., electrofishing and gillnetting) has been proven as the best approach to characterize fish communities in aquatic systems (Fago 1998; Ruetz et al. 2007; Bonar et al. 2009). Use of a single sampling gear will not yield a complete species composition or capture all year-classes of a fish species in a waterbody. Data from a carefully selected combination of multiple gear types can preserve information and enhance the detection of differences among sites (Weaver et al. 1993). Further tests on multiple lakes will be needed to evaluate whether eDNA is consistent in detecting a variety of other fish species in other standing waterbodies.

#### Limitations of Environmental DNA Sampling in a Large Reservoir and Future Directions

Currently, eDNA sampling to characterize fish species abundance and biomass in large waterbodies is subject to many limitations. A number of important factors must be further addressed before eDNA can be used in standard fish sampling protocols. Contamination is a critically important consideration

TABLE 4. Summary of regression analyses per site and per reservoir stratum comparison of total environmental DNA (eDNA) copies and (1) nighttime boat electrofishing CPUE; (2) gillnetting CPUE; (3) nighttime boat electrofishing biomass per unit effort (BPUE); and (4) gillnetting BPUE ( $\alpha = 0.05$ ). The sample size ( $N$ ) represents the number of paired American Fisheries Society (AFS) boat electrofishing and eDNA sites and paired AFS gillnetting and eDNA sites for spring and fall 2014 field sampling in Lake Roosevelt, Arizona. All field data were collected in accordance with AFS standard protocols for boat electrofishing and gillnetting surveys on large standing waters (Miranda and Boxrucker 2009). Bold italics highlight relationships for which the  $P$ -values were slightly above our significance level of 0.05.

Standard field sampling method and season	Species	Regression equation	$N$	$R^2$	$P$
<b>Analysis per site</b>					
Spring boat electrofishing	Gizzard Shad	CPUE (fish/h) = 0.0102(total eDNA copies) + 24.573	44	0.004	0.675
Fall boat electrofishing	Gizzard Shad	CPUE (fish/h) = 0.0066(total eDNA copies) + 11.607	45	0.001	0.825
Spring boat electrofishing	Largemouth Bass	CPUE (fish/h) = -0.0247(total eDNA copies) + 19.492	44	0.001	0.963
Fall boat electrofishing	Largemouth Bass	CPUE (fish/h) = -0.2461(total eDNA copies) + 13.425	45	0.044	0.165
Spring gillnetting	Gizzard Shad	CPUE (fish/net-night) = 0.014(total eDNA copies) + 35.403	30	0.014	0.534
Fall gillnetting	Gizzard Shad	CPUE (fish/net-night) = 0.1079(total eDNA copies) + 20.208	30	0.065	0.175
Spring gillnetting	Largemouth Bass	CPUE (fish/net-night) = -0.1023(total eDNA copies) + 1.6729	30	0.039	0.297
Fall gillnetting	Largemouth Bass	CPUE (fish/net-night) = -0.0058(total eDNA copies) + 0.9545	30	0.001	0.896
Spring boat electrofishing	Gizzard Shad	BPUE (g/h) = 11.044(total eDNA copies) + 9,954	44	0.025	0.303
Fall boat electrofishing	Gizzard Shad	BPUE (g/h) = -6.782(total eDNA copies) + 4,669.3	45	0.008	0.554
Spring boat electrofishing	Largemouth Bass	BPUE (g/h) = -0.1481(total eDNA copies) + 63.717	44	0.001	0.840
Fall boat electrofishing	Largemouth Bass	BPUE (g/h) = -69.362(total eDNA copies) + 5,364	45	0.027	0.278
Spring gillnetting	Gizzard Shad	BPUE (g/net-night) = 3.1692(total eDNA copies) + 13,400	30	0.006	0.683
Fall gillnetting	Gizzard Shad	BPUE (g/net-night) = 24.83(total eDNA copies) + 5,817.5	30	0.079	0.133
Spring gillnetting	Largemouth Bass	BPUE (g/net-night) = -13.587(total eDNA copies) + 774.3	30	0.002	0.807
Fall gillnetting	Largemouth Bass	BPUE (g/net-night) = -23.233(total eDNA copies) + 616.81	30	0.019	0.472
<b>Analysis per reservoir stratum</b>					
Spring boat electrofishing	Gizzard Shad	CPUE (fish/h) = $6 \times 10^{-5}$ (total eDNA copies) + 26.064	3	0.001	0.997
Fall boat electrofishing	Gizzard Shad	CPUE (fish/h) = -0.0127(total eDNA copies) + 25.043	3	0.548	0.470
Spring boat electrofishing	Largemouth Bass	CPUE (fish/h) = -0.0277(total eDNA copies) + 17.351	3	0.067	0.832
Fall boat electrofishing	Largemouth Bass	CPUE (fish/h) = -0.0777(total eDNA copies) + 20.58	3	0.792	0.301
<b>Spring gillnetting</b>	<b>Gizzard Shad</b>	<b>CPUE (fish/net-night) = 0.011(total eDNA copies) + 27.654</b>	<b>3</b>	<b>0.988</b>	<b>0.069</b>
Fall gillnetting	Gizzard Shad	CPUE (fish/net-night) = 0.017(total eDNA copies) + 18.343	3	0.041	0.870
<b>Spring gillnetting</b>	<b>Largemouth Bass</b>	<b>CPUE (fish/net-night) = -0.1432(total eDNA copies) + 3.4871</b>	<b>3</b>	<b>0.988</b>	<b>0.071</b>
Fall gillnetting	Largemouth Bass	CPUE (fish/net-night) = -0.0254(total eDNA copies) + 1.8553	3	0.113	0.781
Spring boat electrofishing	Gizzard Shad	BPUE (g/h) = 0.8425(total eDNA copies) + 9,874.4	3	0.020	0.909
Fall boat electrofishing	Gizzard Shad	BPUE (g/h) = -3.2626(total eDNA copies) + 7,863.7	3	0.565	0.458
Spring boat electrofishing	Largemouth Bass	BPUE (g/h) = 0.1115(total eDNA copies) + 53.61	3	0.881	0.224

TABLE 4. Continued.

Standard field sampling method and season	Species	Regression equation	<i>N</i>	<i>R</i> <sup>2</sup>	<i>P</i>
Fall boat electrofishing	Largemouth Bass	BPUE (g/h) = -26.706(total eDNA copies) + 7,941	3	0.783	0.308
Spring gillnetting	Gizzard Shad	BPUE (g/net-night) = 31.2(total eDNA copies) + 111,292	3	0.938	0.160
Fall gillnetting	Gizzard Shad	BPUE (g/net-night) = -17.848(total eDNA copies) + 70,935	3	0.014	0.924
Spring gillnetting	Largemouth Bass	BPUE (g/net-night) = -800.49(total eDNA copies) + 18,476	3	0.824	0.276
Fall gillnetting	Largemouth Bass	BPUE (g/net-night) = -48.545(total eDNA copies) + 7,087	3	0.016	0.919

when using eDNA methods. To identify any potential contamination, we utilized both field controls and laboratory controls; no contamination was detected. The time frame in which the water samples are processed is another consideration. Our water samples were shipped for analysis, and the time from field collection to eDNA analysis was frequently up to 1 week. We took necessary precautions to reduce degradation of DNA by precipitating and preserving the eDNA immediately after field sampling; however, the effects of reducing the time between collection and eDNA processing are unknown.

We found no relationships between eDNA copies and the CPUE or BPUE from either traditional gear type during spring or fall at individual sites or in reservoir strata. Likewise, we observed that the proportions of Gizzard Shad or Largemouth Bass as determined by established sampling methods were only roughly similar to the proportions obtained with eDNA sampling. Future studies could investigate the utility of eDNA as a tool to quantify the proportions of a larger number and variety of fish species present in a large standing waterbody. Furthermore, the number of water samples required to characterize species composition or other metrics remains unclear. The number of water samples to collect will depend on waterbody size and project goals. In addition, eDNA sampling techniques may parallel established field sampling in that the more areas surveyed, the lower the variability in fish CPUE, BPUE, and species compositions.

It would be beneficial for future studies to further examine how the amount of eDNA shed by different species and life stages varies under a range of environmental conditions. For example, increased temperature affects fish metabolism, physiology, growth, and immune function (Engelsma et al. 2003; Person-Le Ruyet et al. 2004; Takahara et al. 2011), potentially increasing the amount of sloughed cells and mucus entering the water. Use of closed aquarium studies to examine the relative amounts of eDNA shed and retained by the species of interest (e.g., Largemouth Bass and Gizzard Shad) under various environmental conditions might be one way to examine the effects of these factors on eDNA persistence in a

waterbody and to reduce the variability in results. Furthermore, as discussed earlier, eDNA persistence varies under different environmental conditions. Modeling of eDNA persistence under a variety of temperatures and other environmental conditions and applying these results to field studies could be used to reduce variation in monitoring programs (Lacoursière-Roussel et al. 2016).

The collection of water samples for eDNA detection requires further assessment. In the present study, we sampled surface water, and we collected Gizzard Shad eDNA more successfully than Largemouth Bass eDNA. Sediment samples have proven to contain a higher proportion of eDNA than surface water samples (Turner et al. 2015); however, the DNA of species not currently occupying a waterbody could be present in the sediment. Depth profile sampling while avoiding the sediment is one method that should be considered, as it may lead to more accurate sampling of species that are currently present in the waterbody. Moreover, our methods for eDNA collection applied a random sampling approach, which worked well for a very abundant species (Gizzard Shad); however, to capture species at lower abundances, eDNA methods may require a more refined approach targeting the ideal habitat for the species of interest. Lastly, the relationship between species abundance, biomass, and composition measured by eDNA and those measured by established gears should be tested in other systems, especially those of different sizes, to evaluate whether waterbody size, fish species composition, and in-lake habitat factors affect the usefulness and accuracy of the technique over a wide range of systems. Field and laboratory methods for eDNA sampling to characterize fish communities must be further developed and improved before they can be incorporated into standard fish sampling protocols.

## Conclusions

The ease of eDNA sampling over established fish sampling methods makes it appealing to natural resource managers. Compared to the current established methods, eDNA sampling

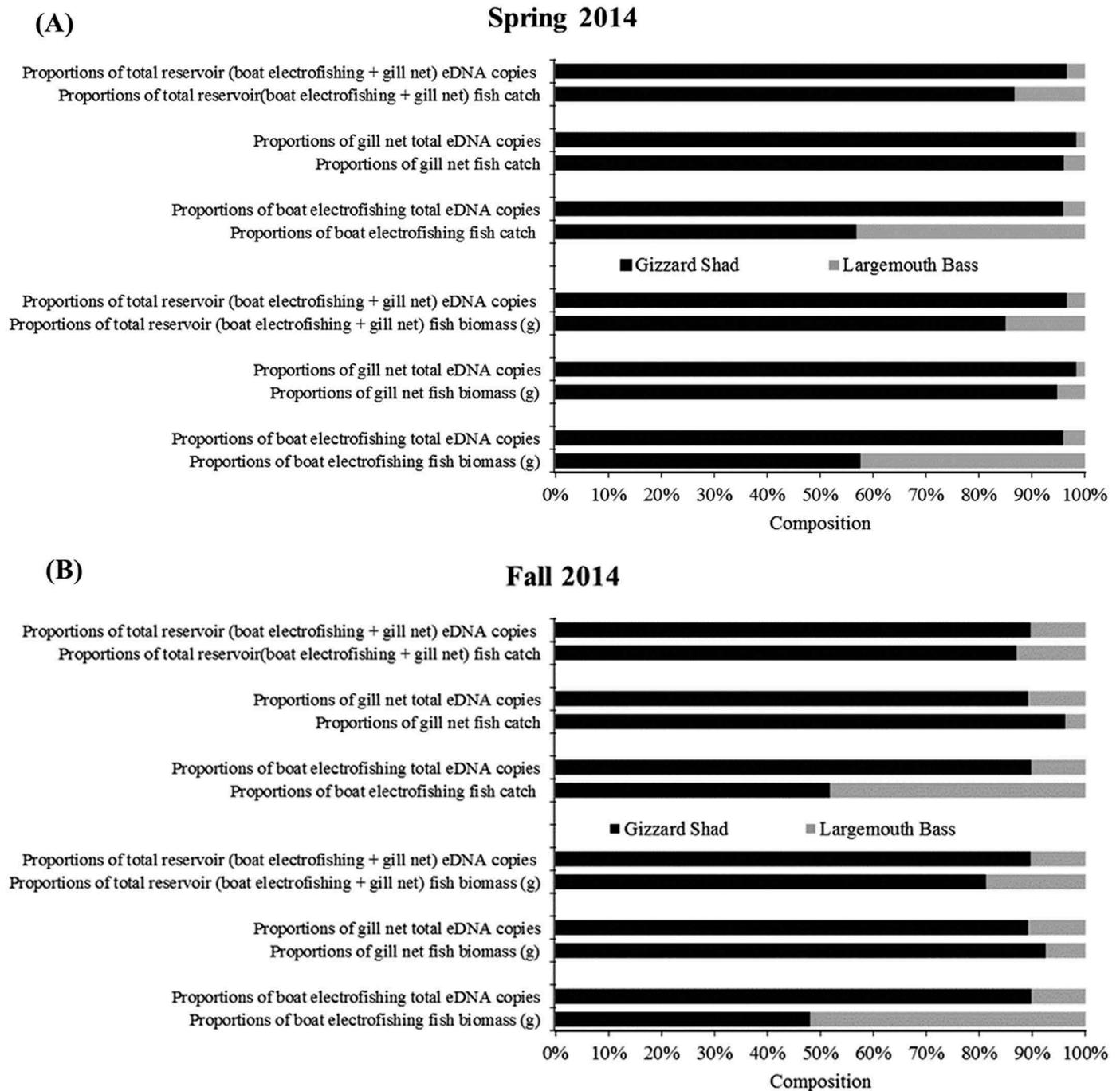


FIGURE 7. Comparison of Gizzard Shad and Largemouth Bass catch (number of fish captured) and biomass (weight [g] of fish captured) composition to environmental DNA (eDNA; total number of copies) composition in samples collected from Lake Roosevelt during spring and fall 2014.

can be less laborious, less time consuming, and more cost effective. Our study suggests that eDNA collections are not useful for comparisons within a large, mixed reservoir such as Lake Roosevelt; however, they may be useful for loosely characterizing relative abundance and biomass in a lake overall. Additionally, eDNA sampling may be useful at sites with

difficult access, such as remote sites, and in smaller waterbodies. This sampling technique has already been proven to detect species presence at very low abundances and to accomplish the detection of species while imposing little or no disturbance (Jerde et al. 2011; Goldberg et al. 2013; Takahara et al. 2013). However, quantification of fish

abundance using eDNA methods is currently limited and requires further investigation to identify limitations and benefits in fish monitoring programs. Furthermore, field sampling protocols, filtration, DNA extraction, primer design, and DNA analysis methods need further refinement and testing before incorporation into standard fish sampling surveys.

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### Appendix: Comparison Sequences Used to Create Environmental DNA Markers for Gizzard Shad and Largemouth Bass

TABLE A.1. GenBank accession numbers used for sequence comparison to create the Gizzard Shad and Largemouth Bass markers (COI = cytochrome-c oxidase I; ND4, ND5, ND6 = NADH dehydrogenase subunits 4, 5, and 6). Primer sequences match those of loci in GenBank sequences with accession numbers DQ536426.1 and DQ536425.1.

Accession number	Species and gene region targeted
GU225596.1	Longfin Gizzard Shad <i>Dorosoma anale</i> COI gene
NC_009580.1	Threadfin Shad <i>Dorosoma petenense</i> mitochondrion
NC_014689.1	Florida Largemouth Bass <i>Micropterus salmoides floridanus</i> mitochondrion
NC_020359.1	Green Sunfish <i>Lepomis cyanellus</i> mitochondrion
KJ554083.1	Pumpkinseed <i>Lepomis gibbosus</i> COI gene
KF930036.1	Orangespotted Sunfish <i>Lepomis humilis</i> COI gene
KF571550.1	Longear Sunfish <i>Lepomis megalotis</i> COI gene
HQ557406.1	Redspotted Sunfish <i>Lepomis miniatus</i> COI gene
HQ557139.1	Dollar Sunfish <i>Lepomis marginatus</i> COI gene
EU524700.1	Redbreast Sunfish <i>Lepomis auritus</i> COI gene
HQ579042.1	Redeye Bass <i>Micropterus coosae</i> COI gene
HQ579041.1	Spotted Bass <i>Micropterus punctulatus</i> COI gene
HQ024948.1	Bigmouth Buffalo <i>Ictiobus cyprinellus</i> COI gene
KJ552641.1	Black Bullhead <i>Ameiurus melas</i> COI gene
EU525102.1	Black Crappie <i>Pomoxis nigromaculatus</i> COI gene
HQ557150.1	Cutthroat Trout <i>Oncorhynchus clarkii</i> COI gene
HQ556940.1	Desert Sucker <i>Catostomus clarkii</i> COI gene
KF930344.1	Flathead Catfish <i>Pylodictis olivaris</i> COI gene
JX960911.1	Gila Trout <i>Oncorhynchus gilae</i> COI gene
HQ579040.1	Redear Sunfish <i>Lepomis microlophus</i> COI gene
KC146839.1	Banded Tilapia <i>Tilapia sparrmanii</i> COI gene
KF930366.1	Walleye <i>Sander vitreus</i> COI gene
KF930142.1	White Bass <i>Morone chrysops</i> COI gene
KF930303.1	White Crappie <i>Pomoxis annularis</i> COI gene
KF930144.1	Yellow Bass <i>Morone mississippiensis</i> COI gene
EU524425.1	Yellow Bullhead <i>Ameiurus natalis</i> COI gene
NC_012929.1	Arctic Grayling <i>Thymallus arcticus</i> mitochondrion
NC_000860.1	Brook Trout <i>Salvelinus fontinalis</i> mitochondrion
NC_024032.1	Brown Trout <i>Salmo trutta</i> mitochondrion
NC_003489.1	Channel Catfish <i>Ictalurus punctatus</i> mitochondrion
NC_004593.1	Northern Pike <i>Esox lucius</i> mitochondrion
NC_001717.1	Rainbow Trout <i>Oncorhynchus mykiss</i> mitochondrion
NC_008105.1	Roundtail Chub <i>Gila robusta</i> mitochondrion
NC_014353.1	Striped Bass <i>Morone saxatilis</i> mitochondrion
NC_019572.1	Yellow Perch <i>Perca flavescens</i> mitochondrion
FJ751827.1	Bigmouth Buffalo ND4 and ND5 genes
AY032633.1	Cutthroat Trout ND5 and ND6 genes
FJ751813.1	Desert Sucker ND4 and ND5 genes
HM991667.1	Flathead Catfish ND5 gene
FJ813509.1	Gila Trout ND4 gene
HM991666.1	Yellow Bullhead ND5 gene
NC_008643.1	Red Shiner <i>Cyprinella lutrensis</i> mitochondrion
NC_013071.1	Smallmouth Buffalo <i>Ictiobus bubalus</i> mitochondrion
NC_008107.1	Gizzard Shad <i>Dorosoma cepedianum</i> mitochondrion
DQ536426.1	Gizzard Shad mitochondrion
DQ536425.1	Largemouth Bass <i>Micropterus salmoides</i> mitochondrion