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Temperature May Play a More Important Role in Environmental DNA Decay than Ultraviolet Radiation

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Abstract: Environmental DNA (eDNA) preservation is crucial for biological monitoring using eDNA technology. The decay of eDNA over time in natural water bodies and the effects of temperature and ultraviolet (UV) radiation on the decay rate are largely unknown. In this study, the linear and exponential decay models were used to explore the relationship between residual eDNA content and decay time, respectively. It was found that the residual eDNA content treated with a higher temperature decreased by an average of 89.65% at the end of experiment, while those in the 4 °C treatment group remained stable. The higher decision coefficient (R^2) of the exponential decay models indicated that they could better reflect the decay of eDNA over time than linear. The difference in the decay rates of the exponential modes was slight between the 20 °C (25.47%) and 20 °C + UV treatment groups (31.64%), but both were much higher than that of the 4 °C group (2.94%). The results suggest that water temperature significantly affected the decay rate of eDNA, while UV radiation had little effect.

Keywords: eDNA; decay; qPCR; temperature; UV

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1. Introduction

Environmental DNA (eDNA) is released by organisms (mucus, saliva, secretion, feces, shed tissues and organs, etc.) into the environment such as water, soil and sediment [1–3]. eDNA technology can be applied to analyze biological information qualitatively or quantitatively by extracting and amplifying DNA fragments by high-throughput sequencing [4–6]. Compared with traditional survey methods of aquatic biodiversity, such as trawl, eDNA technology has a higher detection rate, especially for populations with low-density or unique life history by collecting only a small of water samples [7–9]. eDNA sampling is less destructive to avoid disturbance to rare and endangered species or their habitats during sampling [10,11]. This method has been applied in aquatic biology since 2008 [2] and has been widely used in species diversity monitoring, biomass assessment, and species invasion analysis in the water environment in recent years.

eDNA is easily degraded in the natural environment. Therefore, eDNA preservation is significant for eDNA analysis. Previous studies on the dynamics of eDNA decay typically were conducted by culturing target species in lab-scale microcosms and detecting the amount of residual eDNA in the environment after removing the target species [6,12,13]. In these studies, eDNA concentrations in the simulated experiments may not reflect the actual species abundance in natural environments and only the eDNA decay rates for the target species were examined specifically. It was observed that the eDNA decay rates varied greatly with the target species and the presetting experimental conditions. In particular, few studies have examined the dynamics of the bulk eDNA decay in natural environments.

Understanding of the bulk decay rates is of great importance for the determination of eDNA sample preservation time and method from sample collection in the field to DNA extraction in the lab. It is also crucial for making an eDNA sampling protocol.

Therefore, without introducing and removing target species, we assessed the bulk eDNA decay rate over time under different temperature and UV radiation conditions. The 16S rRNA gene of prokaryotic eDNA and 18S rRNA gene of eukaryotic eDNA were analyzed based on the real-time fluorescence quantitative PCR (qPCR) analysis to present the eDNA in a natural environment.

2. Materials and Methods

2.1. Sample Collection

Fifty liter water samples from the surface of Mati Lake (Tianjin, China) were collected with a water collector and immediately brought back to the laboratory on 4 October 2020, and 8 December 2020, with water temperatures of about 20 °C and 4 °C, respectively. The UV radiation in October was about 10 $\mu W/cm^2$ and was below 1.0 $\mu W/cm^2$ in December. Thus, three groups treated with 4 °C, 20 °C and 20 °C + ultraviolet radiation (10 $\mu W/cm^2$) were set up, respectively. Five liter water samples were added to each group and continuously cultured in sterile glassware. Water samples from glassware were filtered with 0.45 μm membranes on days 0, 1, 2, 3, 4, 5, 7, 9, 11 and 14 to extract eDNA. Each membrane was placed in a 50 mL sterile centrifuge tube and stored at −80 °C until the eDNA extraction.

2.2. Real-Time Fluorescence Quantitative PCR Analysis

The eDNA extraction was performed using FastDNA Spin Kit. PCR primers 338F/806R and 573F/951R were used to amplify the 16S rRNA gene of prokaryotic DNA and 18S rRNA gene of eukaryotic DNA in PCR reactions, respectively. After amplification and purification, the PCR products were attached to the pMD18-T plasmid vector (2692bp). Standard plasmids were then extracted after the blue-white screening. After sequencing the standard plasmids, the length of the ligated products and the plasmid copy number were obtained. After determining the initial concentration of the standard plasmid, a 10-fold gradient dilution process was performed. Then, plasmids with different concentration gradients were used as real-time fluorescence quantitative PCR templates. A quantitative standard curve was established by taking the cycle threshold (Ct) from 1 to 35 and the logarithm of the plasmid copy number. The standard curves must meet certain criteria, including $R > 0.98$ and a slope of −3 to −4. The experimental procedure was performed using ChamQ SYBR Color qPCR Master Mix (2X) with reaction conditions of 95 °C for 3 min, 95 °C for 5 s, 58 °C for 30 s and 72 °C for 1 min. At the end of the PCR reaction, the PCR products were heated from 60 °C to 95 °C. Double-stranded PCR products were opened, resulting in a decrease in fluorescence intensity. It obtained melt curves, which were used to assess whether their intercalating dye qPCR assays had produced single, specific products.

3. Results

3.1. qPCR Standard Curve Analysis and Melt Curves Analysis

As shown in Figure 1, the slope of the 18S rRNA gene standard curve was $K = -3.335$, decision coefficient $R^2 = 0.998$ and the regression equation was $y = -3.335x + 39.012$. Slope K of the 16S rRNA gene standard curve was $K = -3.3744$, decision coefficient $R^2 = 0.985$ and the regression equation was $y = -3.3744x + 40.991$. These results showed good linear relationships in the concentration range of the diluted plasmid standard DNA, indicating that the standard curves established in the study can accurately reflect the amplification of prokaryotic and eukaryotic eDNA in the environment.

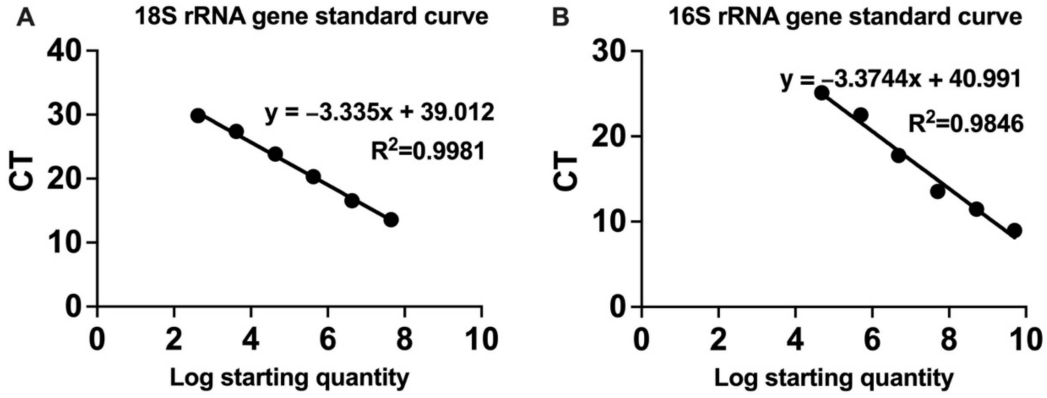

Figure 1. 18S rRNA gene qPCR standard curve (**A**) and 16S rRNA gene qPCR standard curve (**B**).

As shown in Figure 2, a single peak was observed in the melt curves of both the 18S rRNA gene of eukaryotic DNA and the 16S rRNA gene of prokaryotic DNA, which was interpreted as representing a pure, single amplicon.

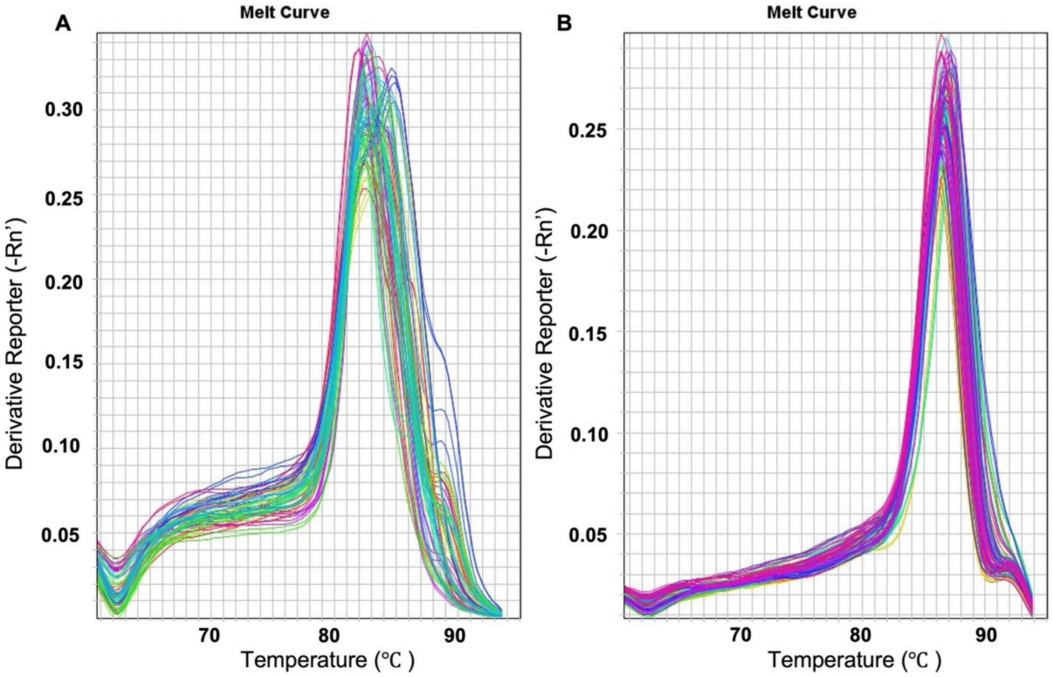

Figure 2. Melt curves from qPCR of 18S rRNA gene (**A**) and 16S rRNA gene (**B**).

3.2. eDNA Decay with Time

As shown in Figure 3A,B, for the 18S rRNA gene of eukaryotic DNA, the concentration of eDNA was 1.37×10^7 copies/mL for the October sample and 2.93×10^6 copies/mL for the December sample at the start of the experiment (0 days), respectively. In general, the residual eDNA content treated at a higher temperature showed clear decay trends, while those in the 4 °C treatment group remained stable. At the end of the experiment, the residual eDNA content in 20 °C and 20 °C + UV radiation treatment groups decreased by 97.11% and 98.54% for the October sample, 96.01% and 98.84% for the December sample, respectively. It can be observed that at the earliest decay stages (<3 days), the eDNA content had a slight increase for the three treatment groups, and those in the 20 °C treatment showed a higher increase speed than the 4 °C treatment. The main reason for this is that after sampling from the natural environment, some feces and tissues may remain in the sampling water and continuously release eDNA into the water in the first few days of

the experiment [12]. Besides, 20 °C is more conducive to the growth and reproduction of algae [13].

As shown in Figure 3C,D, for 16S rRNA gene of prokaryotic DNA, the initial eDNA content was 4.24×10^7 copies/mL in October and 8.50×10^6 copies/mL in December at the start of the experiment, respectively. Like eukaryotes, the residual eDNA content treated at a higher temperature showed obvious decreasing trends and those in the 4 °C treatment group remained stable. At the end of the experiment, the residual eDNA content remained 10^6 copies/mL in all the treatment groups. Specifically, the residual eDNA content in 20 °C and 20 °C + UV radiation treatment groups decreased by 89.24% and 87.49% for the October sample, 68.53% and 81.45% for the December sample, respectively.

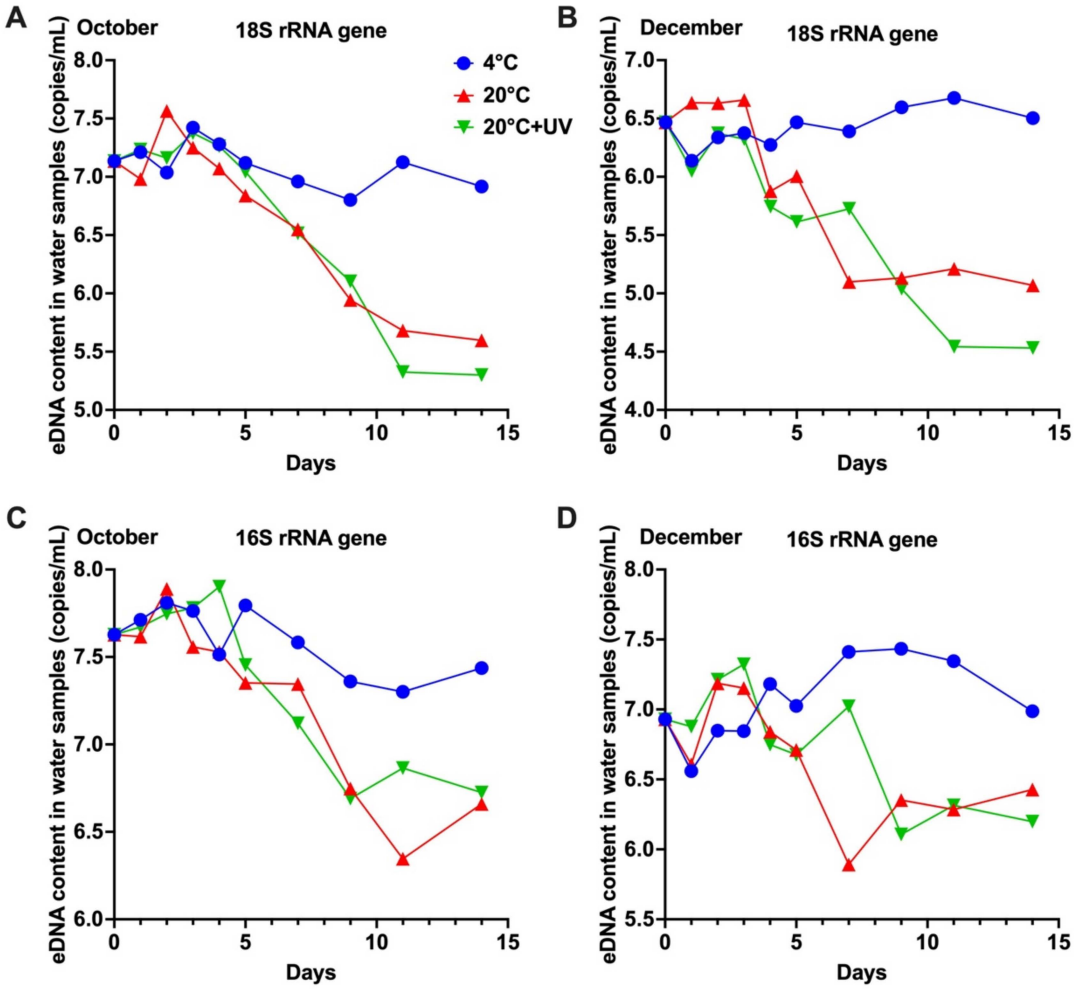

Figure 3. Residual eDNA content in the environment during the experiment period. (**A**,**B**) showed the concentration of 18S rRNA gene of eukaryotic DNA in the October and December samples, respectively. (**C**,**D**) showed the concentration of 16S rRNA gene of prokaryotic DNA in the October and December samples, respectively.

From 3 days to 14 days, the linear and exponential decay models were used to explore the relationship between residual eDNA content and decay time, respectively (Figures 4 and 5). It can be observed that the decision coefficient (R^2) value of the exponential decay model was higher, indicating that the exponential decay model could better reflect the relationship between the eDNA decay and time. In the exponential decay model, a fast-decay phase can be observed followed by a slow-decay phase (except for those in 4 °C treatment groups in December samples). The exponentially decreasing trend was consistent with the previous studies [12,14].

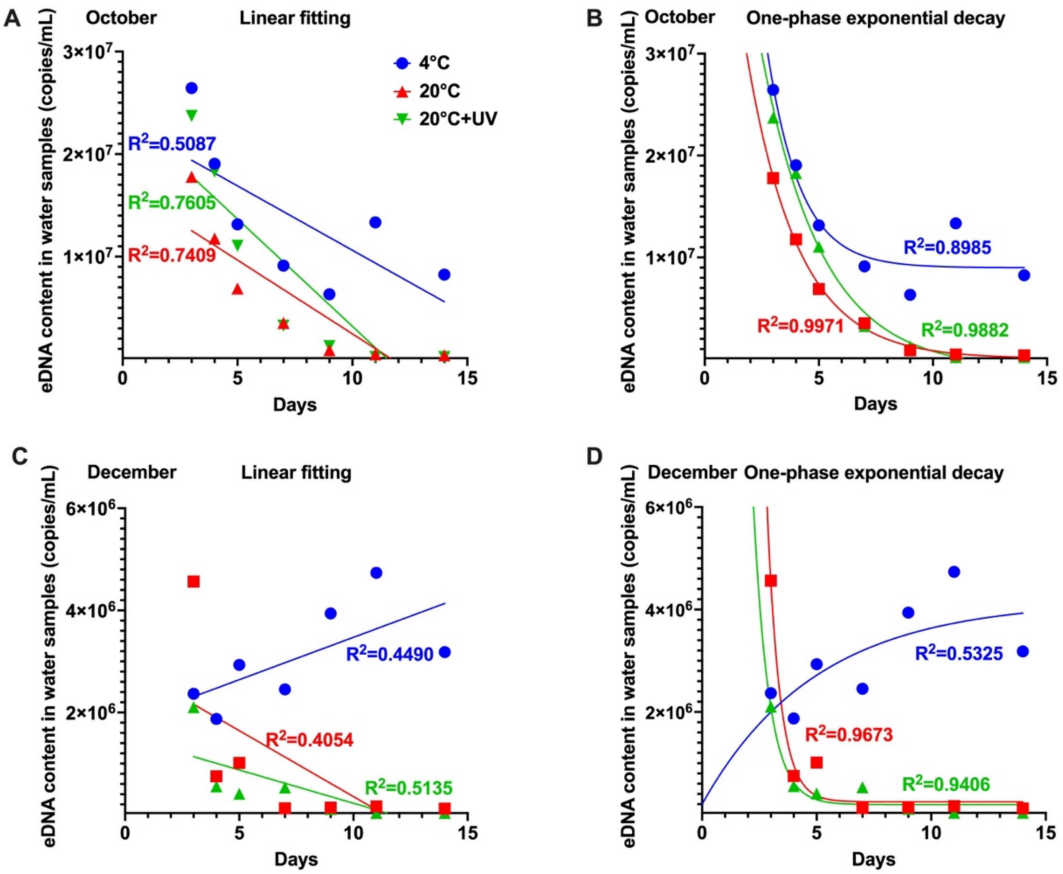

Figure 4. Relationship between residual eDNA content in the environment and decay time (from 3 days to 14 days) for 18S rRNA gene of eukaryotic DNA. (**A**,**B**) showed the linear fitting and one-phase exponential decay models in October samples, respectively. (**C**,**D**) showed the linear fitting and one-phase exponential decay models in December samples, respectively.

3.3. Effects of Temperature and Ultraviolet Radiation on eDNA Decay

By comparing the decay coefficients of the exponential model, the decay rates of the 20 °C and 20 °C + UV treatment groups were much higher than that of the 4 °C treatment group, while the differences between the 20 °C and 20 °C + UV treatment groups were very small (Table 1). Thus, temperature largely affects eDNA decay rate. Elevating water temperature may accelerate the eDNA decay. Several previous studies on eDNA decay rates addressed the same conclusion. For example, a tank experiment conducted by Jo et al. showed that the eDNA decay rate increased with temperature [15]. In another laboratory experiment, Kasai et al. quantified the temperature-dependent decay of the emitted eDNA and found that the concentration of eDNA showed that temperature had a positive effect on the decay rate from 10 to 30 °C [16]. Takahara et al. observed that eDNA concentrations decreased fastest at 25 °C, followed by 4 °C and −30 °C [17]. Similarly, Strickler et al. found that the eDNA decay rates were the lowest for the 5 °C treatment group compared with the 20 °C and 35 °C groups [18]. eDNA was primarily degraded by extracellular nucleases and microorganisms. Thus, the acceleration of the eDNA decay rate at a higher temperature may be driven by the increase in enzyme activity.

Table 1. The decay rate in the exponential decay models in the different treatment groups.

Treatment Groups	4 °C	20 °C	20 °C + UV
18S rRNA gene in October	10.59%	34.61%	43.45%
18S rRNA gene in December	−2.69%	33.31%	37.51%
16S rRNA gene in October	6.83%	18.79%	22.07%
16S rRNA gene in December	−2.99%	15.18%	23.54%
Average	2.94%	25.47%	31.64%

UV radiation may not affect the decay rate of eDNA at the same temperature (Figures 3 and 4). Mächler et al. (2018) observed that UV radiation did not affect the eDNA-based detection rates of macroinvertebrates in a near-natural mesocosm experiment with manipulating UV radiation [19]. Andruszkiewicz et al. (2017) found that there was no difference in marine fish communities as measured by eDNA metabarcoding between groups treated by high doses of sunlight suspended at the surface and by lower sunlight at depth beneath the water surface [20]. Opposite results have also been found in other studies. In a study by Pilliod et al. (2014), eDNA content in shade samples (without UV) was 11.5 times that of sun samples [21]. However, in their experiment, they were not separating the effects of light and temperature, that is, the UV levels and water temperature co-vary. Strickler et al. (2015) proposed that, independently, UV had positive effects on eDNA decay rate, but when combined with other environmental conditions such as pH and temperature, UV radiation did not affect decay rate, and eDNA decay rate in aquatic environments with higher elevations or lower latitude were more likely to be affected by UV radiation [18].

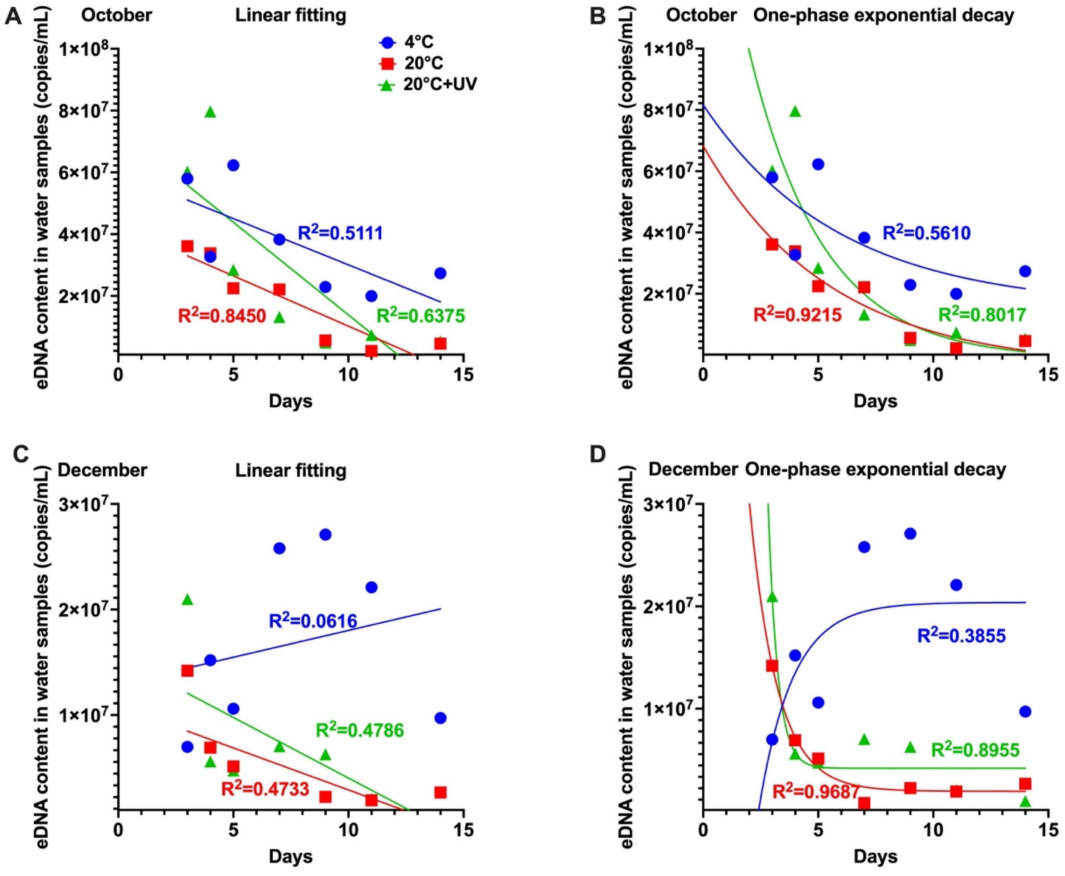

Figure 5. Relationship between residual eDNA content in the environment and decay time (from 3 days to 14 days) for 16S rRNA gene of prokaryotic DNA. (**A**,**B**) showed the linear fitting and one-phase exponential decay models in the October samples, respectively. (**C**,**D**) showed the linear fitting and one-phase exponential decay models in the December samples, respectively.

4. Conclusions

Sample preservation conditions are a prerequisite for the application of eDNA technology, and water temperature and UV radiation are two common influencing factors for sample preservation. In this study, the residual concentrations of prokaryotic and eukaryotic eDNA over time under different temperature and UV conditions were measured based on the qPCR analysis to identify the effects of water temperature and UV radiation on the eDNA decay. The results showed that the high temperature may accelerate the eDNA decay and the change can be regressed by exponential decay models. However, the UV radiation had little effect on the eDNA decay. Although other sample preservation factors (e.g., pH) and the ecological processes influencing on the factors may be further studied, the present study may provide helpful information on making protocol of eDNA sampling.

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