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The effect of artificial light at night on wild fish community: manipulative field experiment and species composition analysis using environmental DNA

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ABSTRACT

The use of artificial light at night (ALAN) has been increasing globally and has been reported to affect a wide range of organisms. However, the effects of ALAN on wild fish communities remain unknown. In this study, we investigated the effects of ALAN on the species distribution and composition of a fish community in a canal. We hypothesized that the fish species composition in areas subjected to ALAN would differ from that in the control area. To test this hypothesis, we conducted a three-week manipulative field experiment in which real-world ALAN conditions were simulated by illuminating the water surface at night using LED lights for two weeks. During the experiment, water samples were collected from ALAN and control conditions four times a week, from which environmental DNA (eDNA) were extracted. Additionally, the number of arthropods in the ALAN and control environments was recorded daily to investigate whether ALAN impacts distribution patterns of fish prey, which may have indirect effect on fish through changed prey-predator relationships. Collected water samples were analyzed using eDNA metabarcoding with MiFish primers and real-time PCR targeting six fish species to obtain qualitative and quantitative data on fish species composition. We compared the fish species composition data between the ALAN and control environments. Our results suggest that ALAN did not significantly influence the overall fish species composition and that the sampling location had a more significant impact. Our findings also point to the possibility that the effect of ALAN on habitat selection may vary depending on the diet of the individual fish. Overall, the effect of ALAN on fish was less significant than expected. By combining eDNA methods with manipulative field experiment, this study shows the applicability of eDNA methods in investigating the effect of pollutants and offers a promising area for future investigation.

1. Introduction

It is widely accepted that human activities affect ecosystems (Vitousek et al., 1997). One type of human activity that is rapidly increasing is the use of artificial light at night (ALAN) (Hölker et al., 2010). The surface area of the Earth illuminated by ALAN has increased rapidly over the past two decades. It is estimated that approximately 23 % of the land surface between 75 °N and 60 °S and 22 % of the coastal regions worldwide are exposed to ALAN (Davies et al., 2014; Falchi et al., 2016). While ALAN is necessary for human activities such as transportation and ensuring safety at night, it is also the cause of light pollution (Cabrera-Cruz et al., 2018). There are wide-ranging reports that light pollution is negatively influencing wildlife, including insect population, moth diversity, turtle distribution, and bird migration (Altermatt and Ebert, 2016; Cabrera-Cruz et al., 2018; Hu et al., 2018; Longcore and Rich, 2004; Owens et al., 2020). Concerning aquatic organisms, prior research indicated that ALAN could alter the behavior, activity patterns, and reproductive success of specific fish species such as *Amphiprion ocellaris, Girella laevifrons*, and *Gambusia affinis* (Fobert et al., 2019; Pulgar et al., 2019; Miner et al., 2021). Although these results

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indicate that ALAN can affect fish ecology, many are based on laboratory experiments conducted under controlled conditions (but see Vowles and Kemp, 2021). Moreover, these studies focused on a limited number of fish species as the subject of investigation. There are still few studies which have comprehensively targeted wild fish communities as the subject to investigate the effects of ALAN. Becker et al. (2013), which analyzed sonar videos to investigate whether ALAN altered the behavior and habitat selection of wild fish communities is a rare example of such research. However, the effects of ALAN were not discussed at the species level. Therefore, the potential effects of ALAN on wild fish communities in which multiple species coexist remain unknown (Becker et al., 2013; Vowles and Kemp, 2021; Davies et al., 2014).

Environmental DNA (eDNA) technique is a novel method for obtaining information on species distributions, biomass, and population sizes (Thomsen and Willerslev, 2015; Doi et al., 2017). eDNA techniques rely on extracting and analyzing DNA fragments contained in environmental samples, such as water, sediment, soil, or air, to detect species (Taberlet et al., 2018; Tsuji et al., 2019; Ruppert et al., 2019; Pawlowski et al., 2020). In this context, eDNA includes both intracellular and extracellular DNA released by organisms in the form of feces, saliva, urine, skin, hair, mucus, carcasses, eggs, and sperm (Taberlet et al., 2018; Ruppert et al., 2019; Pawlowski et al., 2020). This technique has developed rapidly in the past decade owing to recent advances in molecular science, such as high-throughput sequencing (HTS) technologies (Tsuji et al., 2019; Garlapati et al., 2019). eDNA techniques can be roughly divided into single-species detection and eDNA metabarcoding (Minamoto, 2022). While the former uses species-specific PCR primers and enables highly sensitive detection of presence–absence information and DNA quantification of a specific species, the latter uses universal PCR primers and enables the simultaneous analysis of DNA from multiple species (Minamoto, 2022). eDNA methods are starting to be used as tools for biodiversity monitoring and assessment because they are time and economically cost-effective compared to traditional survey methods, are non-invasive, and do not require taxonomic expertise to identify species (Biggs et al., 2015; Thomsen and Willerslev, 2015). There are past studies that have shown that eDNA techniques can have similar or sometimes even higher sensitivity in detecting organisms compared to conventional methods (Allen et al., 2021; Gehri et al., 2021; McElroy et al., 2020) and can be complementary to conventional surveys (Schenekar, 2023).

This study aimed to investigate whether and how ALAN affects the distribution of fish in a real-world environment. We conducted a manipulative field experiment to create an ALAN environment and compared the fish species composition between the ALAN and control environments to determine whether ALAN changed fish distribution. We used both eDNA metabarcoding and quantitative real-time PCR (qPCR) to analyze fish species composition. This is because eDNA metabarcoding allows analysis of the influence on overall species composition, whereas qPCR allows analysis of whether the influence on the individual species differed. In addition to the direct effect of ALAN on fish distribution, we also aimed to investigate whether ALAN impacted fish indirectly by changing the distribution pattern of prey. Terrestrial arthropods, which are known to be attracted to ALAN (Owens et al., 2020), can function as fish prey (Silveira et al., 2023; Francis and Schindler 2009; Milardi et al., 2016). We compared the abundance of terrestrial arthropods between ALAN and control environments to measure the significance of an indirect effect ALAN causes.

We hypothesized that the fish species composition in the ALAN and control environments would differ. Past research has shown that while some fish are drawn to light, others tend to avoid it (Inoue, 1972). Therefore, we expected our study to reflect this characteristic and hypothesized that fish species detected in ALAN environment samples would generally differ from those detected in control samples. We also hypothesized that terrestrial arthropod would be more abundant in ALAN environment and that carnivorous fish which consume arthropods would be attracted to ALAN conditions, thus changing the species composition and distribution of fish.

2. Materials and methods

2.1. Manipulative field experiment

The manipulative field experiment was conducted using a 600 m stretch of the central canal of Kahokugata, Ishikawa Prefecture, Japan (36.67408°N, 136.68610°E) (Fig. 1, Figure S1). The canal is in an agricultural landscape where little environmental change was observed along and near the 600 m stretch chosen as the experimental site. There are no streetlights or other lighting infrastructure near the study site that could have potentially disturbed the ALAN treatment experiment. The lightness level at the study site at night was between 0 ~ 0.3 lx depending on the weather condition. Illuminance light meter (AP-881D, AOPUTTRIVER) was used to take all measurements of light intensity. The canal width at the study site is 17 m, and the depth is 4.0 m (Kawata and Fukutomi, 2019). Canal flow was almost static.

The field experiment was conducted over three weeks in September 2021, in which ALAN treatment took place from September 9th to 16th (first treatment week) and from September 23rd to 30th (second treatment week). The 600 m stretch was divided into upstream (300 m) and downstream (300 m) sections. In the first treatment week, we created a 56 m-long ALAN treatment zone in the middle of the upstream section (Figs 2 and S1). Ten LED lights (YC-45U, GOODGOODS, Japan), five on each bank of the canal, were placed within the ALAN treatment zone at equal distances from each other (Fig. 2). The LED lights illuminated the water surface every night during the week of the manipulative field experiment to replicate the real-world ALAN environment. We used them at the "Mid" setting (50 % of full intensity). This created an ALAN condition of 70 lx where the water surface was hit directly and 0.5 lx at a 12.5 m distance from the gadget (Fig. 2). Fully charged, the LED lights provide light for more than 10 h. Thus, we charged them daily to ensure that the battery would not run out during the night, ensuring that the water surface was illuminated during the entire dark period from sunset to sunrise (Table S1). The downstream section was used as the control environment, and no treatment was added. The first treatment week was followed by a week without illumination in the study site, after which

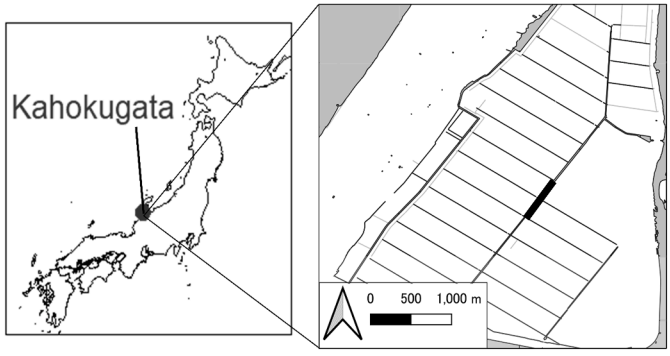

Fig. 1. Map of the study site. Location of Kahokugata, Japan, where the study site was located, in the left box, and the Kahokugata region showing the 600 m stretch used as the study (thick line), in the right box. Grey areas in the right map show the water and white areas show land. The maps were made using the "map" function of the "maps" package in R and data downloaded from the Geospatial Information Authority of Japan and edited using QGIS Desktop 3.28.2.

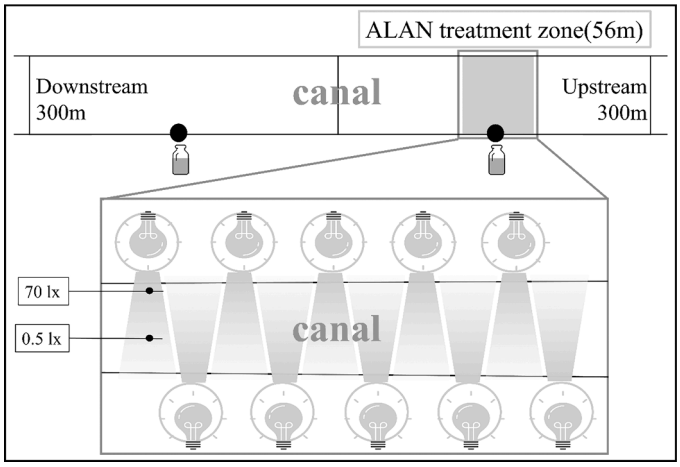

Fig. 2. Design of the ALAN treatment and the water sampling location. The location of the ALAN treatment zone was at the central part of downstream 300 m during the first week of treatment, and it was switched to the upstream 300 m for the second ALAN treatment week. The two water sampling locations are shown with black dots above the water bottle icons. The canal was 17 m in width, the light intensity during the ALAN treatment was 70 lx where the light was strongest and 0.5 lx at 12.5 m distance from the gadget.

the second treatment week was conducted. During the second treatment week, the location of the ALAN treatment and control zones were swapped to eliminate any potential effects of the location.

2.2. Sampling

2.2.1. Water sampling

In each of the two weeks of the field experiment, water samples for eDNA analysis were collected four times at the central point of each of the two 300 m sections (Table S2). Among the four times water samples were collected, the first was conducted as a control, thus, did not reflect fish species composition during the ALAN treatment. The remaining three samples were collected after the 3rd, 5th, and 7th night of ALAN treatment. All water samples were collected at sunrise immediately after ALAN treatment ended within ten minutes. This was to maximize the reflection of fish distribution during the ALAN treatment at night and to minimize the reflection of fish distribution during the daytime.

Two 1 L samples were collected from water surface within 50 cm of depth from each sampling location (Fig. 2). Water samples were collected using buckets with gloved hands and transferred to plastic bottles. All buckets and bottles were sterilized by bleaching and pre-washed with canal water to remove any potential bleach residue. Immediately after collection, 1 mL of 10 % benzalkonium chloride (Nippon Pharmaceutical, Tokyo, Japan) was added to all samples to suppress DNA degradation (Yamanaka et al., 2017). Sterile water (1 L) was used as a negative control to detect any contamination during the sampling process and was handled in the same manner as the other samples.

2.2.2. Arthropod number survey

A survey to investigate whether ALAN had affected the distribution of potential food sources of fish took place in the form of counting terrestrial arthropods. We placed six trays every 100 m on the canal bank along the 600 m stretch of the experimental site. The trays were placed 50 cm away from the water surface as measured on the first day of the experiment to avoid them being washed away, even when the canal width increased due to weather conditions. The trays were colorless and transparent, were 11 cm in height, 21 cm in width, and 31 cm in length. They were filled with water so that any arthropods that dropped into the tray could not escape. The trays were deployed every evening just before ALAN treatment started and the number of arthropods that had dropped into the trays were counted every morning at sunrise immediately after the ALAN treatment ended. Among the six trays, one was located at the center of the ALAN treatment zone, two were within 100 m of the ALAN treatment zone, and three were in the control zone.

2.3. Water filtration and extraction

The water samples were transported under cool-refrigerated conditions and processed in the laboratory within 30 h of collection. They were filtered through 0.7 μm GF/F filters with a 47 mm diameter (Whatman, Buckinghamshire, UK). Two filtrations took place for each sample. The total volume of filtered water depended on the degree of clogging caused by each sample and ranged from 600 to 700 mL for the collected water samples and was 1 L for all the negative control samples. The filters were stored at −25 °C until DNA extraction.

DNA was extracted from the filters following the protocol recommended by the eDNA Society (Minamoto et al., 2021) using a Salivette tube (Sarstedt, Numbrecht, Germany) and DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Briefly, two filters were placed in the basket of the Salivette tube. Subsequently, 400 μL of Buffer AL (Qiagen) and 40 μL of Proteinase K (Qiagen) were added to filters. The filters were then incubated for 30 min at 56 °C after which they were centrifuged at 3000 × *g* for 3 min. After adding 220 μL of TE buffer, the filters were recentrifuged at 3000 × *g* for 1 min. The DNA was purified using DNeasy Blood & Tissue Kit (Qiagen). The final recovered volume of the extracted DNA was 100 μL, and the extracted eDNA was stored at −25 °C until further analysis.

2.4. eDNA Metabarcoding

2.4.1. Initial PCR

Two PCR analyses were performed before library construction and sequencing, to first amplify the 12S rRNA gene region, and then to index sequence to allow identification of samples. The initial PCR was performed using the MiFish-U primers (forward: 5′-GTCGGT AAAACTCGTGCCAGC-3′; and reverse: 5′- CATAGTGGGG TATCTAATCCCAGTTTG-3′) (Miya et al., 2015). This primer set targets 163–185 bp of the 12S rRNA gene region and can be used to identify taxonomically diverse fish according to family, genus, and species (Miya et al., 2015). Four independent PCRs were carried out per sample, and the PCR reactions consisted of 1.25 μL of 10 × KOD Buffer (Toyobo, Osaka, Japan), 1.25 μL of dNTPs (2 mM each), 0.75 μL of $MgSO_4$ (25 mM), 312.5 nM each of forward and reverse primers, 0.25 μL of KOD Plus Neo (Toyobo), 1 μL of extracted DNA template, and 6.75 μL of sterile water, resulting in a total reaction volume of 12 μL. The samples collected on different days were processed as separate sets and handled on different PCR occasions to prevent cross-contamination. Thus, eight separate initial PCRs were conducted. Four negative control reactions that consisted of 1 μL of sterile water instead of extracted DNA templates were included on all occasions. The cycling conditions were as follows: 94 °C for 2 min; followed by 40 cycles of 98 °C for 10 sec, 65 °C for 30 sec, and 68 °C for 30 sec; and a final extension of 68 °C for 5 min.

2.4.2. Purification of initial PCR products

PCR products from four independent reactions of the same sample were pooled into one tube, and all PCR products were purified using SPRIselect magnetic beads (Beckman Coulter, Brea, CA, USA), according to the manufacturer's protocol, using an equal volume of SPRIselect solution. The concentration of purified PCR products was measured using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). All samples aside from the field negative controls and PCR negative controls were diluted to a final concentration of 0.1 ng/μL. Negative controls were diluted to the mean dilution percentage of the

collected samples on the same initial PCR occasion.

2.4.3. Second PCR

A second round of PCR was performed using primers with unique tags (8 bp) for each reaction. The total PCR reaction was 12 μL, comprising 6 μL of 2 × KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Inc., Wilmington, MA, USA), 0.3 μM each of indexed forward and reverse primers (forward: 5′-AATGATACGGCGACCACCGAGATCTACAXXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′ and reverse: 5′-CAAGCAGAAGACGGCATACGAGATXXXXXXXXXGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′, using unique indexes i5 and i7 represented by the octo-X segments) (Miya et al., 2015), 1 μL of diluted initial PCR product, and 1 μL of sterile water. The cycling conditions were as follows: 95 °C for 3 min; followed by 12 cycles of 98 °C for 20 sec and 72 °C for 20 sec; and lastly a final extension period of 5 min at 72 °C. PCR products were then pooled in equal quantities into a single 1.5 mL tube.

Using the E-gel SizeSelect 2 % gel (Thermo Fisher Scientific), DNA amplicons of target lengths from the pooled DNA products were size-selected. Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) was used to check the size distribution of amplicons. The size-selected PCR products were diluted to 4 nM following the measurement of their concentration using a Qubit 3.0 Fluorometer.

2.4.4. Sequencing and Bioinformatics

Preparation for the HTS was performed according to the manufacturer's instructions (Illumina, San Diego, CA, USA). The 4 nM library was further diluted to 50 pM using the resuspension buffer (RSB). PhiX Control v3 (Illumina) was diluted and added at a final concentration of 20 %. Finally, the library was sequenced on an iSeq 100 Sequencing System (Illumina) using iSeq i1 Reagent v2 (Illumina).

After sequencing, the data were processed and analyzed. First, the raw reads were analyzed using the USEARCH v10.0.240 (Edgar, 2010) in the following steps: 1) Merging of the pair-end reads using the command "fastq_mergepairs." All reads <100 bp were discarded at this point. Additionally, paired reads with >5 differences in the aligned region of about 65 bp were also discarded during this first step. 2) Removal of primer sequences from the merged reads using the command "fastq_truncate". 3) Quality filtration of reads using the command "fastq_filter." All reads with either >1 expected error or a length of <100 bp after removing primer sequences were deemed of too low quality for further analysis, thus, they were discarded at this point. 4) Dereplication of processed reads using the command "fastx_uniques." All singleton, doubleton, and tripleton sequences were removed from further analyses. 5) Denoising of dereplicated reads to generate amplicon sequence variants (ASVs) using the "unoise3" command. The ASVs that met at least one of the following criteria were discarded during this step: putative chimeric sequences, erroneous sequences, and ASVs with fewer than four reads. Finally, 6) remaining ASVs were assigned taxonomy to species level using the "usearch_global" command. The database is a compilation of 12S rRNA gene region sequences downloaded from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) on August 5, 2022. The taxonomical assignment was only accepted under the condition of >98.5 % identity (a two-nucleotide difference was acceptable) between reference and query sequences and >90 % query cover (Sakata et al., 2020).

Following the taxonomical assignment of ASVs, we performed the following species processing: 1) Reads detected from negative control samples were regarded as possible contamination. Therefore, following the common practice of eDNA metabarcoding data analysis, we subtracted the total number of species reads detected in the field and PCR-negative samples from the collected samples of the same set (Miya et al., 2020; Sato et al., 2021). 2) Reads of organisms other than fish were discarded because they were not the targets of this study.

2.5. qPCR

The DNA concentrations of selected species, i.e., *Channa argus, Cyprinus carpio, Planiliza haematocheilus, Oryzias sakaizumii, Pseudorasbora parva*, and *Rhodeus ocellatus ocellatus,* were quantified using qPCR. These six species were selected because of the following results of the metabarcoding: 1) They were detected from > 50 % of collected samples; 2) There are differences of sample numbers and relative read abundance (RRA) between ALAN treatment and control samples (see Figure S2 and S3); and 3) There were existing species-specific assays, or the development of species-specific assays were straightforward. Species-specific primers and probes developed in this study were used for qPCR targeting *Channa argus, Planiliza haematocheilus, Rhodeus ocellatus ocellatus*, and *Pseudorasbora parva* (see Supplementary text for the development of these assays), whereas previously developed primers and probes, for which the specificity was confirmed by Takahara et al. (2012) and Tsuji et al. (2018), were used for *Cyprinus carpio* and *Oryzias sakaizumii,* respectively.

All real-time qPCRs were then carried out in triplicate. The reaction mix comprised 900 nM each of forward and reverse primers, 125 nM of probe (Table 1), 10 μL of 2 × Taqman Environmental Master Mix 2.0 (ThermoFisher Scientific), 0.1 μL of AmpErase Uracil N-Glycosylase (ThermoFisher Scientific), and 2 μL of extracted DNA templates, resulting in a total volume of 20 μL. Three reactions of negative controls, containing 2 μL of distilled water (DW) instead of extracted DNA templates, were included in all qPCRs. The cycling conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 55 cycles of 95 °C for 15 sec and 60 °C for 1 min. A 4-point serial dilution (10:1) of linearized plasmids containing synthesized artificial DNA fragments of the target gene sequence were used as standard samples to generate the calibration curve. The concentrations of the four standard samples ranged from 3×10^1 to 3×10^4 DNA copies per reaction.

2.6. Statistical Analysis

All statistical analyses were performed using R version 4.1.1 (R Core Team, 2021).

2.6.1. Analysis for the results of eDNA metabarcoding

Three statistical analyses were conducted using the data obtained from eDNA metabarcoding. First, PERMANOVA was used to examine whether there was any difference between fish species composition detected from the ALAN treatment and control samples, and upstream and downstream samples. This is to see whether the variables (ALAN treatment and location of sampling) affect fish species composition. We performed two PERMANOVA analysis using Jaccard dissimilarity matrices with presence–absence data and Bray–Curtis dissimilarity matrices with RRA data. Both PERMANOVAs were conducted using 999 simulations. Multiple models were constructed with ALAN treatment of samples and location of sampling as explanatory variables with and without interactions (Table S5). Additionally, we performed PERMADISP to check whether the results obtained from the PERMANOVA analyses were due to variance or not. PERMANOVA and PERMADISP were performed using the "adonis2" function and "betadisper" function in the "vegan" package (Oksanen et al., 2020) in R.

Second, the RRA data of collected samples were used to calculate the Shannon diversity index using the "diversity" function in the "vegan" package (Oksanen et al., 2020). We used linear mixed model (LMM) to test whether Shannon diversity index varied between ALAN treatments and between sampling locations (Table S5) using the "lmer" function of "lme4" package (Bates et al., 2015) in R. ALAN treatment and sampling location were included as fixed predictors, and the sampling day was included as a random effect.

Third, the fish species detected using eDNA metabarcoding were divided into three groups according to their diet using the categorization of Taguchi (2021) and Kishi (2001): carnivorous (six species),

Table 1
Species-specific primers and probes used in this study.

primers/ probe	target species	primer sequence (5′~3′)	reference information
Lzh-CytB-F	*Planiliza haematocheilus*	TCCCTTACATCGGTGACGC	desigend for this study
Lzh-CytB-R		CCGAGGGGGTTGTTCG	
Lzh_cytb_p02		[FAM]-CATTCCACTTCCTCCTCCCCTTCGTTATT-[TAMRA]	
Roo-CyB-F	*Rhodeus ocellatus ocellatus*	CTACGCCATCTTACGATCCATTCCTA	desigend for this study
Roo-CyB-R		GGATGTTCTACAGGTATGCCGC	
Roo-CyB-Pmgb		[FAM]-CTTATTCTGAACCCTAGTGGCA-[NFQ]-[MGB]	
Channa_argus_CyB_F	*Channa argus*	CGTCGGCGTTATCTTGCTAC	desigend for this study
Channa_argus_CyB_F		CTGAAAACCCGCCTCAAATCC	
Channa_argus_CyB_P		[FAM]-CGTTGGCTATGTCCTCCCCTGGG-[TAMRA]	
Ppa-CytB-F	*Pseudorasbora parva*	CCCTACATAGGAGATACCCTGG	desigend for this study
Ppa-CytB-R		GTGTAGGAATAGGAGATGGATAACCG	
Ppa-CytB-P		[FAM]-TTCGCATTCCACTTCCTTCTCCCATTTATT-[TAMRA]	
Osa16S-F	*Oryzias sakaizumii*	ATCTTCAAGTAGAGGTGACAGACCA	Tsuji et al. (2018)
Osa16S-R		AACTCTCTTGATTTCTAGTCATTTGTGTC	
Osa16S-Pr		[FAM]-TGGATAGAAGTTCAGCCTC-[NFQ]-[MGB]	
CpCyB_496F	*Cyprinus carpio*	GGTGGGTTCTCAGTAGACAATGC	Takahara et al. (2012)
CpCyB_573R		GGCGGCAATAACAAATGGTAGT	
CpCyB_550p		[FAM]-CACTAACACGATTCTTCGCATTCCACTTCC-[TAMRA]	

herbivorous (three species), and omnivorous species (10 species) (Table S6). Then, the detection frequency and RRA were compared between ALAN treatment and control samples within each diet group. If a particular diet group showed consistency in both RRA and detection frequency, the magnitude of the trend was tested using Wilcoxon signed rank test. To perform the Wilcoxon signed rank test, RRA values and detection frequency of two samples collected on the same day same location were averaged to obtain single values for ALAN treatment and control samples. The obtained values for ALAN treatment and control samples were paired for each day and Wilcoxon signed rank test was performed for the sets of pairs to determine the significance of the difference between ALAN treatment and control samples. Additionally, principal component analysis (PCA) was performed to investigate whether the diet type of the fish was a factor in characterizing the samples. The result of the PCA was visualized as a biplot using the "ggbiplot" function in the "ggbiplot" package (Vincent and Vu 2011) in R.

2.6.2. Analysis of the qPCR results of selected species

For the six selected species, the DNA concentration in each sample was calculated as the mean of three qPCR reactions. The DNA concentrations of negative reactions were considered zero (Ellison et al., 2006). DNA concentration was log-transformed after adding one (Wu et al., 2023). Following this, LMM was performed separately for each species to investigate whether DNA concentration varied depending on the ALAN treatment of the samples (Table S5).

2.6.3. Analysis of the arthropod number survey results

We investigated whether sampled arthropod number varied between the ALAN treatments. The six trays were sorted into three groups depending on the ALAN treatment zone. The tray located in the center of the ALAN treatment zone was categorized as Group 1, two trays within 100 m of the ALAN treatment zone were categorized as Group 2, and the three trays placed in the control zone were categorized as Group 3. Thus, the smaller the group number, the closer it was to the center of the ALAN treatment zone. As the data were not normally distributed, Kruskal-Wallis test was performed to test the influence of tray group on arthropod abundance. Following this, Wilcoxon rank-sum test were performed for all possible pairs sets to identify the tray group with significant difference in abundance.

3. Results

3.1. eDNA metabarcoding

1,790,601 raw iSeq reads (37,304 ± 29,040 [mean ± SD]) were obtained from 48 samples, including 32 collected samples, 8 negative control field samples, and 8 negative control PCR samples. After processing, 1,528,490 reads were assigned taxonomy (31,667 ± 24,770 [mean ± SD]), of which 1,496,089 were assigned to fish taxa (31,168 ± 24,548 [mean ± SD]) (Tables S7 and S8). After subtracting read numbers detected from field negative control and PCR negative control samples, the total reads assigned to fish taxa in field samples were 1,477,788 (46,180 ± 13,606 [mean ± SD]). Approximately 0.43 % of the raw reads and 0.27 % of the reads assigned to fish taxa belonged to the field and PCR negative control samples, respectively.

19 fish species were identified (Table S9). They were *Cyprinus carpio, Carassius cuvieri, Carassius spp., Rhodeus ocellatus ocellatus/Rhodeus ocellatus kurumeus, Zacco platypus, Pseudorasbora parva, Gnathopogon elongatus elongatus, Misgurnus anguillicaudatus, Silurus asotus, Micropterus salmoides, Lepomis macrochirus, Gymnogobius castaneus, Rhinogobius spp., Tridentiger obscurus, Channa argus, Mugil cephalus, Planiliza haematocheilus, Hyporhamphus intermedius*, and *Oryzias sakaizumii* (Fricke et al., 2023). The dominant species were *Ca. cuvieri* and *Carassius spp.* which were detected in all 32 field samples. The other frequently detected species were *Cy. carpio, G. e. elongatus, O. sakaizumii, Pl. haematocheila, Ch. argus, Rhinogobius spp., Ps. parva, S. asotus*, and *Rho. ocellatus ocellatus/Rho. ocellatus kurumeus*, all of which were detected in more than 75 % of the field samples. Of the 15 out of 19 species with mean RRA >0.05 %, *Cy. carpio, O. sakaizumii, S. asotus*, and *Gy. castaneus* showed greater detection rate and mean RRA values in the ALAN samples than in the control samples (Figures S2 and S3). In contrast, *Pl. haematocheila, Ps. parva*, and *T. obscurus* showed greater detection rate and mean RRA values in the control samples that in the ALAN samples. *Mic. salmoides* was detected only in the ALAN samples (Figures S2 and S3).

The results of PERMANOVA based on Bray–Curtis dissimilarity matrices (with or without interaction) showed that fish species composition did not vary between ALAN treatments but varied between upstream and downstream sampling locations ($p < 0.01$, Table S10). PERMANOVA based on the Jaccard index showed that fish species composition did not differ between the ALAN treatment or sampling location.

The LMM showed that the Shannon diversity index calculated from different ALAN treatment samples was not significantly different ($p =$

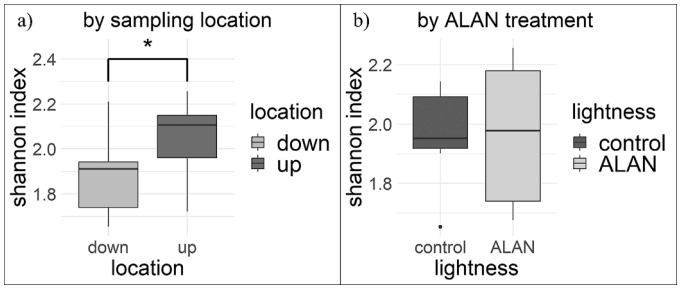

Fig. 3. Shannon index of fish species detected using metabarcoding by (a) the ALAN treatment of samples (left) and (b) by the location of the sampling point (right). The colored boxes indicate the interquartile range (N = 12 each). The median is indicated by the thick line within the colored boxes. The statistical difference between samples is indicated ($p < 0.05$, (*)) on the plot based on the LMM performed.

0.912, Table S10). In contrast, Shannon diversity index was significantly different between sampling locations ($p < 0.05$, Table S10). The boxplots depict this showing larger differences between sampling locations than between the ALAN treatments (Fig. 3). The coefficient of variation of the two samples collected on the same day same location ranged from 0.0018 to 0.107.

Carnivorous species had a consistent trend of having greater values in the ALAN treatment samples than in the control samples, while no other diet group showed a trend (Fig. 4). Their RRA and detection frequency both had larger values in ALAN treatment samples (Figs 4 and 5). However, the Wilcoxon signed rank test performed for carnivorous species showed that the difference of RRA and detection frequency between ALAN treatment and control samples were not statistically significant ($p = 0.173$ and 0.312, respectively). The ordination of PCA shows that the arrow of carnivorous species is pointing towards the direction where ALAN treatment samples are clustered as opposed to the direction of the cluster of control samples (Fig. 6).

3.2. qPCR

The R^2 values of the calibration curves were >0.983 for all the runs. The PCR efficiencies, values of the slopes, and intercepts ranged from 81.389 % to 124.887 %, −2.841 to −3.867, and 38.156 to 42.638, respectively (Table S11). *Cy. carpio* and *O. sakaizumii* were detected in all collected samples, and *Ps. parva, Pl. haematocheila, Rho. ocellatus ocellatus,* and *Ch. argus* were detected in most collected samples (Table S12).

The LMMs showed that none of the six fish species showed differences in DNA concentrations between the ALAN and control samples. However, the DNA concentrations of *Cy. carpio, O. sakaizumii*, and *Pl. haematocheila* were significantly different between sampling locations, with all three species showing greater abundances in downstream than in upstream samples ($p < 0.05$, 0.001, and 0.001, respectively, Table S10).

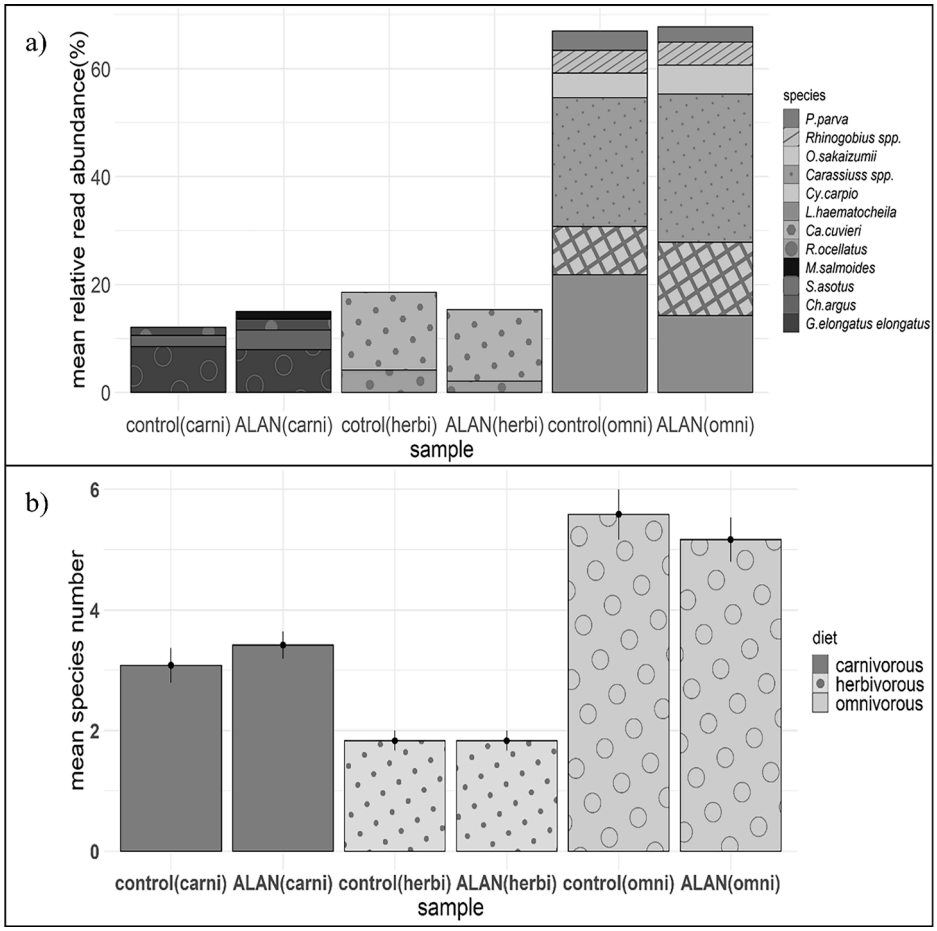

Fig. 4. a) Mean relative read abundance of 15 species with mean relative read abundances of >1 % between ALAN treatment and control samples by diet group. Carnivorous species are indicated as (carni), herbivorous species are indicated as (herbi), and omnivorous species are indicated as (omni), and b) Mean species number detected using eDNA metabarcoding from ALAN treatment and control samples by diet group.

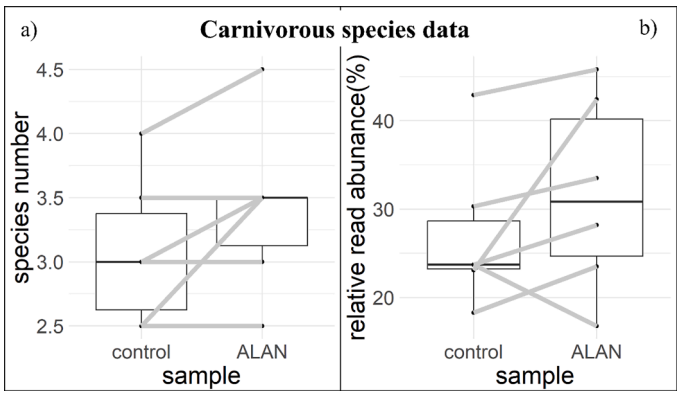

Fig. 5. The difference of a) detection frequency and b) RRA values of carnivorous species between ALAN treatment and control samples. The grey lines connect the data of the samples collected on the same day. The median is indicated by the line within the boxes.

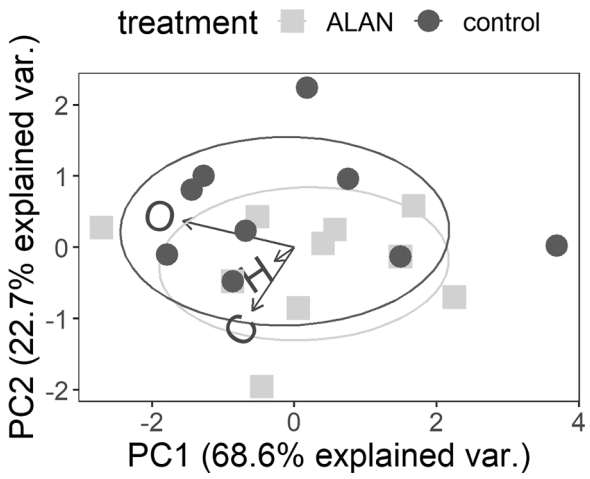

Fig. 6. Visualization of principal component analysis (PCA) on characterizing samples with diet type of fish. C, H, and O represent carnivorous, herbivorous, and omnivorous species, respectively. The dark and light are the 68 % ellipse of ALAN treatment samples and control samples, respectively. The length of the arrows indicates the contribution of the three diet types to the principal components.

3.3. Arthropod number survey

A total of 34 arthropods were collected during field experiments (Table S13). They represented different taxonomical groups, such as Scutigeromorpha, Orthoptera, Hemiptera, Hymenoptera, Coleoptera, and Diptera. Orthoptera, Hemiptera, Hymenoptera, Coleoptera, and Diptera have been shown to function as fish prey by Silvera et al. (2023). Following Kruskal-Wallis test ($p < 0.001$, df=2, Chi-squared=14.794), Wilcoxon rank sum tests showed that arthropod abundance varied significantly between ALAN treatment and control samples, with higher numbers sampled from group 1 than group 3 ($p < 0.001$, W = 290) and group 2 ($p < 0.01$, W = 471) (Fig. 7).

4. Discussion

This study investigated whether ALAN affects wild fish communities in a canal in Japan. In particular, we focused on the composition and distribution of the fish species. Contrary to our hypothesis, we found that the ALAN treatment levels used in this study did not alter the overall species composition of wild fish or distribution of six selected fish species at the study site. Sampling location was a significant factor in the

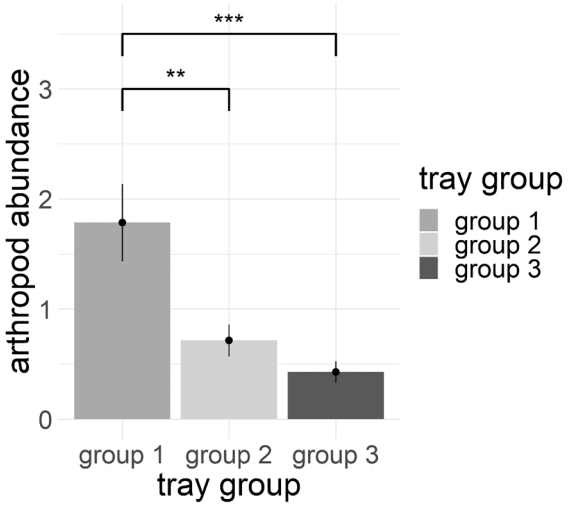

Fig. 7. Arthropod number sampled from trays belonging to group 1 (Located in the center of the ALAN treatment zone), group 2 (within 100 m of the ALAN treatment zone), and group 3 (placed at the opposite 300 m of the ALAN treatment zone). The statistical difference between groups is indicated ($p < 0.001$, (***) and $p < 0.01$(**)) on the plot based on the performed Wilcoxon rank sum tests.

composition and distribution of the wild fish community in our experiments. Although the effect of ALAN treatment on wild fish communities was not as significant as expected, some groups of fish, namely carnivorous species, may have been affected more by ALAN treatment than other groups.

The species composition of the fish community at the study site, the Shannon diversity of fish, and the DNA concentrations of the six selected fish species were not significantly different between the ALAN treatment and control samples. In contrast, larger differences in fish species composition and distribution were observed between samples collected from the upstream and downstream sampling points. It is generally accepted that eDNA concentration is positively correlated with fish abundance/biomass (Doi et al., 2017; Takahara et al., 2012). The fact that DNA concentrations were significantly higher in downstream samples can be interpreted as indicating that more fish are distributed downstream. Thus, it can be concluded that location had a greater effect on fish composition and distribution than the ALAN treatment. It is true that eDNA methods is still not robust enough to differentiate whether the difference in eDNA concentration was due to difference in abundance or biomass. However, stochastically it would be unnatural to consider that certain sized fish remained constantly in certain location throughout the three weeks.

Possible differences in some environmental conditions between the upstream and downstream sampling points may explain why location affected fish species composition and distribution. The apparent homogeneity of the site was one of the reasons for selecting this site. However, we cannot eliminate possible differences in some environmental factors that could affect fish diversity and distribution between the two sampling points.

This study showed that ALAN did not affect the overall fish community. However, the study points to a possibility that ALAN may have a greater effect on certain wild fish, namely carnivorous fish, than on others in selecting their habitat. Although statistically not significant, the metabarcoding results showed that the mean RRA and detection rate were both higher in ALAN samples than in control samples for carnivorous species (Figs, 4 and 5). Additionally, *Mic. salmoides*, a common carnivorous invasive species in Japan, were detected only in the ALAN samples. The PCA visualization depicted this trend, showing that the ALAN samples could be characterized by higher detection of carnivorous species (Fig. 6). These results may point to the possibility that

carnivorous species are more likely to be distributed under ALAN than under control conditions. Possible explanations for this are that the illuminated water conditions benefited carnivorous species that use visual senses to search for food (Rowe and Dean, 1998; Liang et al., 1998) and that the increase in arthropod abundance under ALAN conditions made it advantageous for carnivorous species that feed on insects (Huskey and Turingan, 2001; Katano et al., 2003) to have a higher success rate in finding food. Our results align with previous observations that the number of fish estimated to be carnivorous, based on their size, increases under illuminated water conditions (Becker et al., 2013). By using eDNA metabarcoding, which does not rely on the measurement of body size to identify species, our results provided more reliability that carnivorous fish prefer ALAN conditions.

Here, the adequacy of the temporal and physical design of the manipulative experiment will be discussed. Firstly, the physical design of the experiment was appropriate. This is because the distance of two sampling locations was enough to distinguish species composition of the two locations. Previous study suggests that 300 m of distance may not be enough to eliminate the possibility of collecting eDNA transported from upstream (Shogren et al., 2019). However, considering a recent meta-analysis which reviewed downstream transport of eDNA fragments (Jo and Yamanaka 2022) and the near static water flow at our study site, we deemed water collected downstream would not reflect the species composition of fish at the upstream sampling point.

Secondly, the temporal design of the experiment was appropriate for the following three reasons. Firstly, the duration of the experiment was adequate. Becker et al. (2013) collected data for nine nights of ALAN exposure with less than half of illuminated surface area compared to our study. Referring to this study, we assumed that 7 nights of ALAN exposure, 12 h per night, along a 56 m stretch would be acceptable as the design of the purpose of this study. Secondly, the sampling schedule of this study was adequate because the half-lives of eDNA is shown to be between 1 hour to 19 h in previous studies which was performed with similar water conditions with ours (over 20.0 °C) (Andruszkiewicz et al., 2020, Tsuji et al., 2017). Thus, by sampling at dawn directly after ALAN treatment, it can be considered that species composition at nighttime was strongly reflected in the samples in contrast to species composition during the daytime. Thirdly, the seasonal setting of the experiment was also appropriate. The possibility of the results of this study being under the influence of spawning period can be eliminated, as the spawning period of only one species, *Rho. ocellatus ocellatus*, among the 19 detected fish species coincide with the study period.

Lastly, the choice of the illumination gadget was appropriate for the aim of the study. Ten LED gadgets were used to illuminate the water surface. LEDs are the type of lighting that recorded the highest increase in demand during the past few years (Zissis et al., 2021). LEDs are predicted to have over 90 % share of lighting market share by 2030 (Zissis et al., 2021). Thus, to simulate a realistic ALAN condition LED would be the best choice of lighting. Moreover, the intensity of light used in this study was similar to previous studies investigating the effect of ALAN on fish (Vowles and Kemp 2021; Miner et al., 2021; Fobert et al., 2019). Thus, the lightness used in this study can be considered appropriate.

There are two limitations to this study. Firstly, the eDNA methods used in this study were limited to obtaining presence/absence and quantitative data on the DNA concentration of species. Therefore, this study was limited to investigating whether ALAN causes any disturbances in fish species composition and distribution. Other studies with a molecular scale of investigation have reported that ALAN influences the concentrations of glucose and melatonin in fish (Miner et al., 2021; Brüning et al., 2015). Recent advancements in eDNA techniques have enabled the detection of RNAs in water (Tsuri et al., 2021). mRNAs are thought to reflect the health and physiological condition of individuals more than DNAs because the expression patterns of mRNAs fluctuate depending on the physiological state (Tsuri et al., 2021; Veilleux et al., 2021). Thus, eRNA methods are regarded more advantageous than eDNA methods for assessing the physiological reactions of individuals to environmental stressors.

Secondly, the spatial and temporal scale of the study was small. We focused on short-term impact of ALAN on fish community, but longer duration experiment may have yielded different results. It is reported that in Europe, over 50 % of the coastline is under influence of light pollution (Davies et al., 2014). Compared to such spatial scale, the length of our manipulative experiment site was short. Larger scale experiment may have yielded different results.

The effect of ALAN on aquatic ecosystems can vary depending on water quality (Levine and MacNichol, 1982). The color of the water at our study site indicated that it contained large amounts of organic matter (Levine and MacNichol, 1982). Light penetration in such water differs from that in clear water (Levine and MacNichol, 1982). Although our results suggest that the effect of ALAN on fish species composition and distribution is not as strong as hypothesized, further research under other water depth and conditions is needed to accurately understand the effect of ALAN on wild fish communities.

Further understanding of the ecological effect of ALAN on aquatic organisms is essential to enable more informed decision-making regarding wildlife-friendly light infrastructure design. Human-wildlife encounters and conflicts are on an upward trend due to factors such as urbanization, habitat alterations, an increase in outdoor recreation, and the development of transport infrastructure (Messmer, 2009; Tablado and Jenni, 2017; Forman and Alexander, 1998). Policies to reduce the conflict between human activity and wildlife should be sought in such situations. Understanding whether and how ALAN affects wild fish communities will allow policymakers to consider nighttime lighting designs that maximize human benefit but minimize disturbance to wildlife. We believe that the accumulation of knowledge in this field will significantly contribute to a more harmonious coexistence between humans and wildlife.

5. Conclusions

To date, research on the effect of ALAN on fish in terms of changes in activity levels, rate of reproductive success, and behavioral patterns were mostly conducted in laboratory environments. In rare cases where the research was conducted outdoors, the investigation was limited to one species or targeted the whole fish community but did not identify the species at the study site. The effects of ALAN on wild fish communities remain poorly known.

To the best of our knowledge, this is the first study to investigate the effects of ALAN on wild fish communities by identifying fish at the species level using eDNA methods. The results revealed that the ALAN treatment used in this study did not alter the species composition of wild fish, but the location did. Additionally, there are some indications that ALAN does not affect all fish equally, but that its effect varies depending on the diet type. Despite these findings, there are still several areas of investigation. Further research is needed to gain a deeper and more comprehensive understanding of ALAN in wild fish ecology.

CRediT authorship contribution statement

Aisha Oyabu: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Writing – review & editing. **Luhan Wu:** Formal analysis, Investigation, Writing – review & editing. **Takehiro Matsumoto:** Formal analysis, Investigation, Writing – review & editing. **Natsumi Kihara:** Investigation, Writing – review & editing. **Hiroki Yamanaka:** Investigation, Writing – review & editing. **Toshifumi Minamoto:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: TMi and HY are the inventors of patents for the use of benzalkonium chloride in eDNA preservation.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2023.100457.

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