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ORIGINAL ARTICLE

Effect of biotic and abiotic factors on the production and degradation of fish environmental DNA: An experimental evaluation

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Abstract

Environmental DNA (eDNA) is a very promising approach to facilitate and improve the aquatic species monitoring, which is crucial for their management and conservation. In comparison with the plethora of monitoring studies in the fields, relatively few studies have focused on experimentally investigating the "ecology" of eDNA, in particular pertaining to processes influencing the detection of eDNA. The paucity of knowledge about its ecology hampers the use of eDNA analysis to its full potential. In this study, we experimentally evaluated the impact of several biotic and abiotic factors on the rate of production and degradation of eDNA. Individuals of three freshwater fish species (brown bullhead, tench, and yellow perch) with distinct ecology were placed in two types of water from the St. Lawrence River (Québec, Canada) with very distinct physicochemical characteristics and at three different temperatures. Water samples were then filtered at predetermined time intervals, and quantitative PCR was used to quantify the eDNA in each sample. We found that temperature, species, water types, and some interactions between these factors had a strong effect on the production and degradation of eDNA. The results of this study enhance our knowledge about the ecology of eDNA, thus improving eDNA data interpretation.

KEYWORDS
biomonitoring, conservation, ecology, eDNA, fish, temperature

1 | INTRODUCTION

Species monitoring is essential for effective management of aquatic resources as it provides crucial information about the distribution and abundance of species (Gibbs, 2000). While efficient and obviously useful, traditional methods used for fish population surveys (e.g., gillnets, seine, scuba diving, etc.) can be invasive, costly, time consuming, and selective. This may result in a biased estimation of species occurrence (Boivin-Delisle et al., 2021; Lodge et al., 2012; Sigsgaard et al., 2015). A more time-efficient tool that has been recently developed is environmental DNA (eDNA), which can also greatly reduce the cost of species monitoring by lowering both the cost of labor and material (Biggs et al., 2015; Deiner et al., 2021; Evans et al., 2017; Pochardt et al., 2020; Sengupta et al., 2019; Sigsgaard et al., 2015). Over the last decade, an ever-increasing number of studies have shown that eDNA is often as efficient or even

better than traditional methods at detecting the presence of species (Berger et al., 2020; Boivin-Delisle et al., 2021; Thomsen et al., 2012; Valentini et al., 2016; Wilcox et al., 2016; Wineland et al., 2019). This is particularly true for species with low population densities (Dougherty et al., 2016; Jerde et al., 2011; Lim et al., 2016; Lopes et al., 2020; Sigsgaard et al., 2015; Yonezawa et al., 2020).

However, the future of biomonitoring using eDNA needs to overcome present limitations of the method to reach its full potential (Lacoursière-Roussel & Deiner, 2021). For example, a better understanding of the causes behind false positive and negative detections is needed to avoid wrong and costly decisions based on erroneous results. Also, it is still challenging to make an accurate estimation of biomass or abundance. Several studies have reported that biomass is linked to the quantity of eDNA detected, but the relationship is less apparent in natural environments than in experimental studies (Lacoursière-Roussel et al., 2016; Ushio et al., 2018; Yates et al., 2019). Furthermore, the comparison of results with other studies can be tedious, which may complicate decision making for the management of aquatic resources. These limitations could be reduced with a better understanding of eDNA ecology, that is, the interactions between the environment and eDNA, which includes its origin, state, fate, and transport (Barnes & Turner, 2016; Harrison et al., 2019). In comparison with the ever-increasing number of empirical studies showing the efficiency of eDNA analysis for biomonitoring, relatively few have focused on studying the basis of eDNA ecology. Yet this is important to further investigate processes influencing eDNA detection for the improvement of this tool (Adams et al., 2019).

Production (associated with origin) and degradation (associated with fate) of eDNA are two of the major processes influencing the amount of eDNA of a given species present in the environment (Barnes & Turner, 2016; Goldberg et al., 2015). Recent studies have shown the effects of abiotic (e.g., pH, temperature, ultraviolet, and salinity; Afzali et al., 2021; Boivin-Delisle et al., 2021; Collins et al., 2018; Kasai et al., 2020; Mächler et al., 2018; Seymour et al., 2018; Strickler et al., 2015; Tsuji et al., 2017; Zulkefli et al., 2019) or biotic parameters (e.g., microbial activity and bivalve filtering; Friebertshauser et al., 2019; Lance et al., 2017; Mächler et al., 2018; Tsuji et al., 2017; Zulkefli et al., 2019) on eDNA fate, including degradation, but these studies have not taken eDNA production into consideration. On the other hand, production of eDNA has been studied alone as a function of different parameters such as biomass, temperature, diet, or life stage (Doi et al., 2017; Evans et al., 2016; Klymus et al., 2015; Lacoursière-Roussel et al., 2016; Maruyama et al., 2014; Takahara et al., 2012; Thalinger et al., 2021). Only a few studies have simultaneously considered both the production and degradation of eDNA to understand how their interaction influences the detection of species. For example, Sassoubre et al. (2016) quantified the production and degradation rate of three marine fishes and found that production rate could differ between species, but degradation rate was the same. Jo et al. (2020), and Jo et al. (2019) found that both fish biomass and temperature influenced the production and degradation rate of eDNA. Moreover, Allan et al. (2020) reported that the production and degradation of eDNA differed between different temperatures and animals with various forms, namely fish, shrimp, and jellyfish. Finally, experimental studies have compared the effect of a few parameters on eDNA detection in tap water (Bylemans et al., 2018; Pilliod et al., 2014; Strickler et al., 2015; Takahara et al., 2012) but rarely compared ecologically relevant types of water found in nature (e.g., Eichmiller et al., 2016).

Here, we aimed to experimentally assess how temperature, species, and two types of water with very distinct physicochemical characteristics can influence the production and degradation of eDNA. We collected and brought to the laboratory large volumes of two naturally occurring water masses from a large river and individuals of three fish species to compare their effects and interactions with three temperature conditions in a controlled environment experiment. As observed in past studies, we predicted that both production and degradation would increase at elevated temperatures (Jo et al., 2019, 2020; Lacoursière-Roussel et al., 2016) and that water mass and fish species would influence eDNA production and degradation but without any *a priori* regarding the directionality of the effects.

2 | MATERIAL AND METHODS

2.1 | Water and fish collection

We tested the effect of three temperatures, two water masses, and three freshwater fish species on the production and degradation of eDNA. To do so, we collected and brought to the laboratory water from the St. Lawrence River near Repentigny (Québec, Canada, 45°43'32.8"N, 73°27'26.1"W), where two very different water masses, namely "green" and "brown" waters, flow adjacent to one another without mixing (Laporte et al., 2020). Due to their different origins, these two water masses have very distinct physicochemical characteristics. The green water comes from the Great Lakes. It flows mainly on limestone substrate and is characterized by relatively low turbidity (1.3 NTU) and high conductivity (>250 μS/cm). The brown water originates from the Ottawa River, which runs on the Canadian Shield. Consequently, the brown water has a higher turbidity (4.2 NTU), lower conductivity (<160 μS/cm), and lower pH than the green water coming from the Great Lakes (Hudon, 2000; QFL Canada, 2005). Measurements of these parameters as well as that of temperature and chlorophyll *a* were taken at the time of water collection (Table S1). The water was transported to Université Laval (Québec City, QC) on May 30, 2019, and then stored in the closed tanks in a refrigerated room with light cycle following daylight (12 h light and 12 h dark) at 4°C for 3 weeks until fish were brought to the laboratory. The three species of freshwater fish used in this experiment were the brown bullhead (*Ameiurus nebulosus*), the tench (*Tinca tinca*), and the yellow perch (*Perca flavescens*). The tench originates from Eurasia. It is an invasive species in North America, and thus, considerable efforts are made to prevent this species from expanding its range. The yellow perch and the brown bullhead are two very important species for commercial and

TABLE 1 Sequences of primers and probes for TaqMan assays used to amplify DNA of each species

Species	Gene	Primer	Sequence 5′
Brown bullhead	Cyt *b*	Forward	CCCTCGTACAATGAATCTGAGGG
		Reverse	GTTTCATGTAAAAAGAGGGCATGTAAA
		Probe	ACCCGATTCTTCGCATTT
Yellow perch	COI	Forward	CAGGGGTTTCCTCAATTCTAGGT
		Reverse	CCAGCGGCAAGAACAGGTAGT
		Probe	CCAATATCAAACTCCCTTGTT
Tench	Cyt *b*	Forward	CAACCGCATTCTCGTCAGTAAA
		Reverse	CAAAAGGATATTTGTCCTCATGGC
		Probe	TCGCCCGAGGATTAT

Note: See Hernandez et al. (2020) for details.

recreational fishing in Eastern Canada (Bernatchez & Giroux, 2012, Fisheries and Ocean Canada, 2018). For each species, one-year-old juveniles between 5 and 10 cm (total length) were captured in the Baie-du-Fèbvre (part of the St. Lawrence R.) with a seine net. Sampling and experimental protocols were approved by Université Laval's Comité de protection des animaux, and sampling and transport were conducted with a scientific sampling license from the Ministère des Forêts, de la Faune et des Parcs du Québec (Permit number: 2019-04-04-019-17-SF).

2.2 | Experimental design

Experiments were performed at the LARSEM (Laboratoire Aquatique de Recherche en Sciences Environnementales et Médicales, Université Laval, Québec). The fish were left at room temperature for 24 h which allowed them to recover from the transportation. They were then acclimated for 18 h to the appropriate water mass and temperature before starting the experiment. Before transferring the fish into the water tanks, we sampled and filtered the water to ensure that there was no DNA from these species already present in the water that was taken from the St. Lawrence River. Five individuals of each species were placed together in the tanks filled with 20 L of either green or brown water, and the tanks were oxygenated with an air pump for the entire duration of the experiment. These tanks were placed in rooms set at three different temperatures (8°C, 16°C, and 24°C). Water temperature was measured daily to ensure that there was no variation in temperature for the duration of the experiment. Light followed a cycle of 12 h of day and 12 h of night in the experimental rooms. Four tank replicates of the same water mass and one negative control with water, but no fish were included for each temperature, which corresponds to a total of 30 tanks [(4 replicates + 1 control) x 2 water masses x 3 temperatures). The tanks were positioned randomly in the rooms at each temperature (Figure S1). After 4, 10, and 24 h following the transfer of fish into the tanks (i.e., the production phase), one water sample was collected in 300 ml sterilized plastic bottles that were stored on ice until filtration. Filtration was done within the hour following water sampling with a syringe and 0.7 µm GF/F glass microfiber filters. We filtered 250 ml of water, and these samples were immediately stored at −20°C until extraction. The bottles and the syringe filter holders used were all sterilized for 30 min in a 10% bleach (sodium hypochlorite) solution followed by 30 min under UV lights. The working surface was also bleached between each sample. To detect if contamination occurred during filtration, a negative control with only pure water was filtered at each sample time. After 48 h from the onset of the experiment, the fish were removed, and water samples were filtered immediately following fish removal (day 0). Water samples were filtered again 2, 4, 8, and 16 days later. These samples were part of the degradation phase, and the same method of filtration and addition of negative controls as described above was applied. The total number of samples used for the analysis was 192. This included 72 for the production phase (3 temperatures x 2 water masses x 3 time periods x 4 replicates) and 120 for the degradation phase (3 temperatures x 2 water masses x 5 time periods x 4 replicates). Samples taken before the start of the experiment and from the control tanks were excluded.

2.3 | DNA extraction and amplification

All pre-PCR manipulations were done in a separated area of the laboratory exclusively dedicated to this end to avoid any cross-contamination. Again, all nonsterile material used in extractions was sterilized for 30 min in a 10% bleach (sodium hypochlorite) solution followed by 30 min under UV lights. Bench surfaces were bleached between each extraction batch and each qPCR plate preparation. DNA extraction was performed using the QIAshredder and DNeasy Blood&Tissue kit (Qiagen) as detailed in Laporte et al. (2020). A negative extraction control tube with only reagents was added for each extraction batch. The eDNA was then quantified using the Taqman (Applied Biosystem) real-time qPCR method. The Taqman qPCR assays for each of the three species were designed in our laboratory (Hernandez et al., 2020), and the primers and probes are described in Table 1. Along with the assay of the three species, a SPUD assay (Sigma) was also used as

a positive control to evaluate if inhibitors were present in the samples. PCR amplifications were performed in a room separate from the pre-PCR room. The reaction for the amplification contained a final volume of 20 µl, which included 1.8 µl of each primer (10 µM), 0.5 µl of probe (10 µM), 10 µl of Environmental Master Mix 2.0 (Life Technologies), 3.9 µl of SPUD, and 2 µl of DNA. The cycling conditions were 2 min at 50°C, 10 min at 95°C, 50 cycles of 15 s at 95°C, and 60 s at 60°C. For each sample, six PCR replicates were made and six negative control wells containing pure water instead of DNA were added for each plate. A positive control with synthetic DNA (gBlocks) corresponding to the appropriate species was also added. This synthetic DNA consists of 500 base pairs that was designed from the COI sequence. A standard curve of five dilutions with a known concentration of synthetic DNA was used to precisely estimate the quantity of eDNA (number of copies) to each plate. The limit of detection (LOD) was estimated following the method described by Klymus et al. (2020) where the threshold is set at the lowest concentration where there is at least 95% of positive replicates.

2.4 | Statistical analyses

Before analysis, the quantity of eDNA was standardized by the biomass of each species in each tank (see Table S2). A log transformation with the natural logarithm (*e*) was then used to normalize the data. Also, only the average of technical replicates for each sample was used in the analysis. For the degradation samples, the data were also normalized, so that each sample would have the same quantity of eDNA at the beginning of the degradation phase (Day 0). To do so, we divided the quantity of eDNA of each sample by the highest value of eDNA quantity among all samples at Day 0, which gave us a ratio between 0 and 1. We then multiplied the quantity of eDNA for each sample by that ratio for every following sample time. This normalization allowed a direct and unbiased comparison of the quantity of eDNA detected in each of the different conditions. Following this normalization step, the value at Day 0 was the same for each sample and therefore not included in the subsequent statistical analyses. Statistical analyses were performed separately for the production and degradation phases. For each of these, a linear mixed-effects model was performed with R 3.6.3 (R Core Team, 2020) using the function *lme()* from the nlme library (Pinheiro et al., 2020). Time, species, temperature, and water type were included as fixed variables, and the tank replicates were included as a random effect. To control for the normalization done in the degradation phase, we also added the log-transformed absolute quantity of the eDNA detected as a covariate. The Tukey test for post-hoc analysis with a significant threshold of $p < 0.05$ was then used to assess the difference between each treatment. Following the linear mixed-effect analysis, we performed a model selection to see which significant variables had the most impact on the production and degradation of eDNA. Dataset is available in Appendix S2.

3 | RESULTS

3.1 | Quality control

Water temperature was successfully maintained constant (±0.5°C) for the duration of the experiment in each of the three temperature conditions. Prior to the start of the experiment, no eDNA from the three species was detected in the water in each of the six qPCR replicates. The eDNA concentration in negative control tanks ranged from 0% to 1% of that in treatment tanks taken at the same sampling time, and thus, cross-contamination was minimal and did not significantly influence the results. Also, no contamination was detected in filtration, extraction, and amplification negative controls. There was no inhibition detected during PCR amplification. The average assay efficiencies based on the standard curves were 94.6% for the brown bullhead, 96.5% for the yellow perch, and 95.2% for the tench. The limit of detection (LOD) of 100% for each of the three species was of 8 copies/reaction. However, the percentage of detection remained the same for the three species at 4 copies/reaction with a detection rate of 90%. Under 4 copies/reaction, detection probability differed between species (Table S3). For yellow perch and brown bullhead, the concentration of eDNA went below the LOD after 8 days during the degradation phase at 24°C while it took 16 days at 8°C and 16°C. For tench, it only took 2 days at 24°C, 4 days at 16°C, and 8 days at 8°C to reach LOD threshold.

3.2 | Production

Temperature, water mass, species, and time all had significant effects on quantity of eDNA detected during the production phase ($p < 0.001$). First, after adding fish to the tanks, the quantity of eDNA increased significantly from 4 h [average: 10.85 ln(copies)/L] to 10 h [average: 11.03 ln(copies)/L] after which it dropped to an average of 10.14 ln(copies)/L after 24 h (Figure 1a, Table S4). With respect to differences between species, the quantity of eDNA detected was highest for the yellow perch (average: 11.08 ln(copies)/L) and lowest for the tench (average: 10.08 ln(copies)/L). The difference between both brown bullhead and yellow perch vs. tench was significant but not between brown bullhead and yellow perch (Figure 1b, Table S4). There was a significant difference in the quantity of eDNA detected between the two types of water, with less eDNA detected in the green water (average: 10.46 ln(copies)/L) than in the brown water (average: 10.89 ln(copies/L; Figure 1c, Table S4). Finally, the quantity of eDNA detected between temperatures also differed with the highest quantity at 16°C (average: 10.92 ln(copies)/L) followed by a drop at 24°C. At 8°C and 24°C, the quantity of eDNA detected was not significantly different [10.47 and 10.64 ln(copies)/L, respectively] (Figure 1d, Table S4).

During this production phase, there were also several significant interactions between parameters (Table 2). First, the interaction between water mass and time showed that the quantity

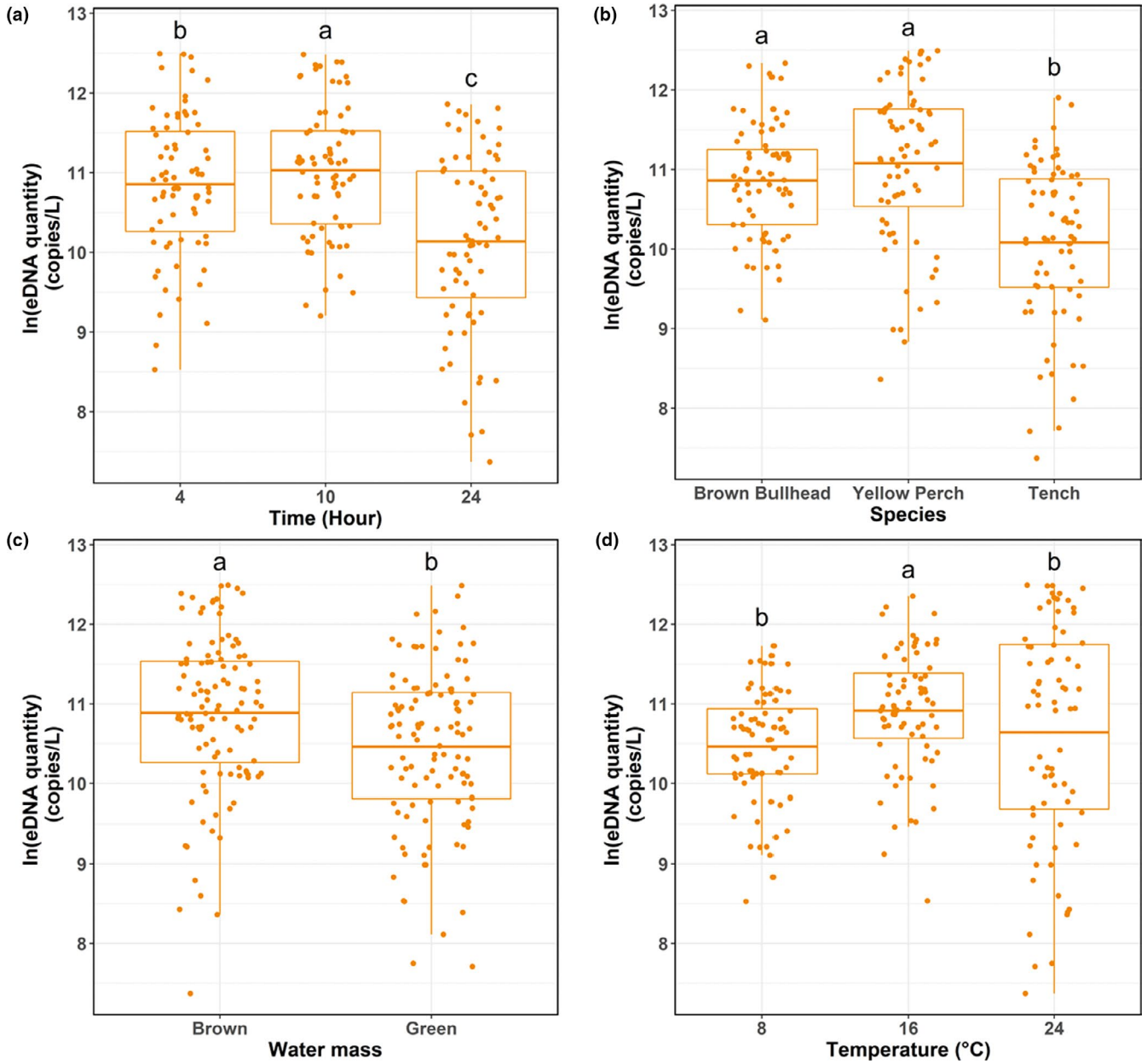

FIGURE 1 Quantity of DNA as a function of (a) time, (b) species, (c) type of water, and (d) temperature during the production phase. Only the average of PCR replicates was used, and each sample is represented by a dot. The middle line of the boxplot represents the mean, the box represents the first and third quartile (Q1 and Q3), and the whiskers represent the minimum (Q1 − 1.5 × IQR) and maximum (Q3 + 1.5 × IQR). Letters indicate significant difference between conditions. $p < 0.001$ for each parameter. Exact values for each condition can be found in Table S4

of eDNA was the same in both types of water after 4 h [green: 10.91 ln(copies)/L; brown: 10.80 ln(copies)/L], but diverged after 10 and 24 h, with a lower quantity of eDNA in the green water mass compared to the brown water mass (Figure 2, Table S5; $p < 0.001$). The interaction between time and temperature was also significant ($p < 0.001$). After 4 h, the quantity of eDNA detected at different temperatures was highest at 24°C [11.63 ln(copies)/L)] and lowest at 8°C [10.14 ln(copies)/L]. However, after 10 h, the quantity of eDNA increased at 8°C and 16°C [10.59 and 11.31 ln(copies)/L, respectively] but decreased at 24°C [11.19 ln(copies)/L]. After 24 h, the eDNA quantity at 8°C remained the same [10.66 ln(copies)/L] but decreased moderately at 16°C [10.64 ln(copies)/L] and abruptly at 24°C [9.12 ln(copies)/L] (Figure 3, Table S6). Finally, the interaction between species and temperature was only significant with a threshold of $p < 0.1$ instead of 0.05 used in the analysis ($p = 0.068$), but the observed trend was nevertheless informative. Thus, while the difference in quantity of eDNA detected between 8°C and 16°C was the same for all three species, the decrease in eDNA quantity detected between 16°C and 24°C for the brown bullhead was less pronounced compared to the other two species (Figure 4, Table S7). In the model selection, the first model included the interaction between temperature and

TABLE 2 Results of the lme model for the production phase

Parameter	DF	denDF	*F*-value	*p*-value
(Intercept)	1	108	71381.93	**<0.001**
Temperature	2	54	12.25	**<0.001**
Time	2	108	106.73	**<0.001**
Species	2	54	65.34	**<0.001**
Water	1	54	32.08	**<0.001**
Temperature:time	4	108	106.29	**<0.001**
Temperature:species	4	54	2.33	0.068
Time:species	4	108	5.66	**0.004**
Temperature:water	2	54	1.78	0.178
Time:water	2	108	24.73	**<0.001**
Species:water	2	54	2.67	0.078
Temperature:time:species	8	108	2.72	**0.009**
Temperature:time:water	4	108	21.71	**<0.001**
Temperature:species:water	4	54	0.20	0.939
Time:species:water	4	108	0.80	0.526
Temperature:time:species:water	8	108	0.87	0.548

Note: Time, temperature, species, and water were added as fixed effects while the replicates (tanks) were added as random effects. DF is the degree of freedom and denDF is the denominator degree of freedom. Significant *p*-values are in bold.

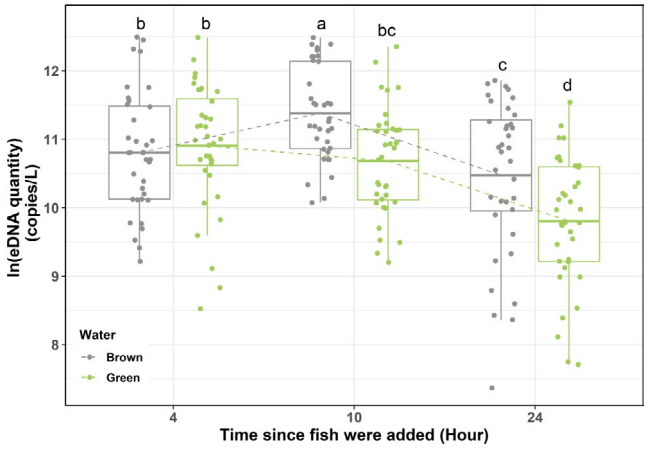

FIGURE 2 Quantity of DNA detected for each type of water at different sampling times during the production phase. The middle line of the boxplot represents the mean, the box represents the first and third quartile (Q1 and Q3), and the whiskers represent the minimum (Q1 − 1.5 × IQR) and maximum (Q3 + 1.5 × IQR). Only the average of PCR replicates was used, and each sample is represented by a dot. Letters indicate significant difference between conditions. $p < 0.001$. Exact values for each condition can be found in Table S5

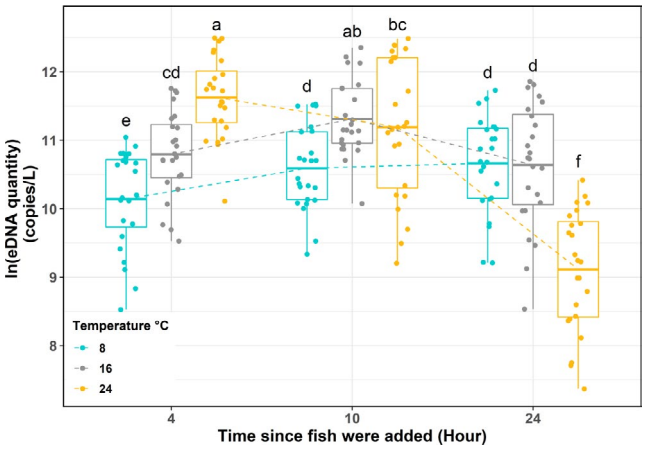

FIGURE 3 Quantity of DNA detected for each temperature (°C) at different sampling times during the production phase. The middle line of the boxplot represents the mean, the box represents the first and third quartile (Q1 and Q3), and the whiskers represent the minimum (Q1 − 1.5 × IQR) and maximum (Q3 + 1.5 × IQR). Only the average of PCR replicates was used, and each sample is represented by a dot. Letters indicate significant difference between conditions. $p < 0.001$. Exact values for each condition can be found in Table S6

time (Table 3). It has a weight of 1, which indicates that this interaction explains very well the variation of eDNA we observed in the production phase.

3.3 | Degradation

The quantity of eDNA detected just after the removal of the fish increased importantly in comparison to the quantity detected at the end of the production phase of the experiment. This could potentially be explained by the stress caused by handling the fish when removing them from the tanks (Eichmiller et al., 2016; Jo et al., 2019, 2020; Klymus et al., 2015). The quantity of eDNA at the beginning of the degradation phase (Day 0) was set at 12.16 ln(copies)/L for every sample after normalization. First, the parameter ln(eDNA), which is the nonnormalized but still

log-transformed quantity of eDNA, is significant (Table 4). This is expected because the nonnormalized quantity of eDNA still follows the values of the normalized quantity. The results showed that time, species, temperature, and type of water again all had a significant effect on the detection of eDNA ($p < 0.001$ for time, species, temperature, and $p = 0.002$ for type of water). After each sample time, the quantity decreased steadily until it reached an average of 0.81 ln(copies)/L on day 16 (Figure 5a, Table S8). The quantity of eDNA detected was significantly different between species, with yellow perch having the highest quantity at every sample time (average: 5.18 ln(copies)/L), followed by brown bullhead (average: 4.34 ln(copies)/L), and then the tench [average: 3.48 ln(copies)/L] (Figure 5b, Table S8). This indicates that the eDNA degradation rate was different for each species (fastest for the tench, intermediate for brown bullhead, and slowest for yellow perch). For the types of water, the quantity of eDNA detected was lower in green water [average: 4.11 ln(copies)/L] than in brown water [average: 4.56 ln(copies)/L], indicating that degradation was faster in green water (Figure 5c, Table S8). Finally, the quantity of eDNA detected was significantly different for each temperature (Figure 5d, Table S8). It was higher at 8°C [average: 5.86 ln(copies)/L] than at 16°C [average: 5.17 ln(copies)/L] and 24°C [average: 1.97 ln(copies)/L], which indicates that degradation rate increased with temperature (Figure 5d, Table S8). However, the difference in the quantity of eDNA detected between 16°C and 24°C was more pronounced than between 8°C and 16°C, despite the temperature interval being the same.

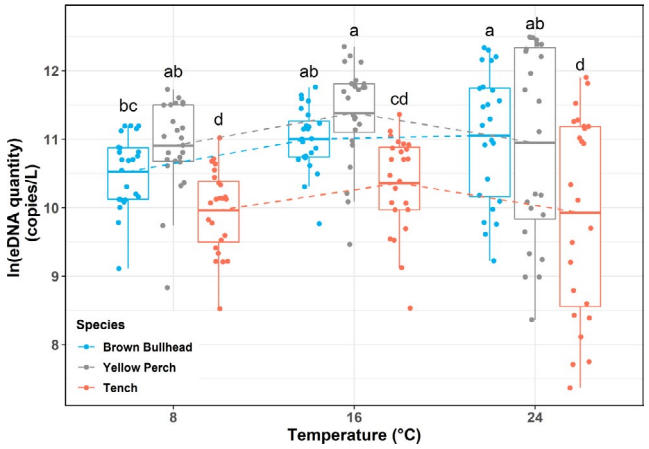

FIGURE 4 Quantity of DNA detected for each species at different temperatures during the production phase. The middle line of the boxplot represents the mean, the box represents the first and third quartile (Q1 and Q3), and the whiskers represent the minimum (Q1 − 1.5 × IQR) and maximum (Q3 + 1.5 × IQR). Only the average of PCR replicates was used, and each sample is represented by a dot. Letters indicate significant difference between conditions. $p = 0.068$. Exact values for each condition can be found in Table S7

TABLE 3 Results of the model selection for the production phase

Model	*K*	BIC	ΔBIC	ModelLik	BICWt
Temperature × time	10	522.12	0	1.00E+00	1.00E+00
Time + species + water	7	575.46	53.35	2.61E−12	2.61E−12
Temperature + time + species + water	9	579.26	57.14	3.90E−13	3.90E−13
Time + species	6	581.83	59.71	1.08E−13	1.08E−13
Temperature + time + species	8	586.23	64.12	1.19E−14	1.19E−14
Time × species	10	600.55	78.43	9.29E−18	9.29E−18
Species + water	5	604.47	82.35	1.31E−18	1.31E−18
Species	4	608.51	86.40	1.73E−19	1.73E−19
Temperature + species + water	7	610.01	87.90	8.19E−20	8.19E−20
Time + water	5	611.84	89.73	3.28E−20	3.28E−20
Time	4	613.67	91.56	1.31E−20	1.31E−20
Time × water	7	614.09	91.98	1.06E−20	1.06E−20
Temperature + species	6	614.44	92.33	8.93E−21	8.93E−21
Species × water	7	614.54	92.43	8.51E−21	8.51E−21
Temperature + time + water	7	618.54	96.43	1.15E−21	1.15E−21
Temperature + time	6	621.06	98.95	3.27E−22	3.27E−22
Temperature × species	10	633.98	111.87	5.11E−25	5.11E−25
Water	3	634.05	111.93	4.94E−25	4.94E−25
Temperature + water	5	640.94	118.83	1.57E−26	1.57E−26
Temperature	4	643.29	121.17	4.87E−27	4.87E−27
Temperature × water	7	651.55	129.44	7.82E−29	7.82E−29

Note: K is the number of parameters, ModelLik is the likelihood, and BICWt is the weight of each model.

TABLE 4 Results of the lme model for the degradation phase

Parameter	DF	denDF	*F*-value	*p*-value
(Intercept)	1	161	4025.87	**<0.001**
Temperature	2	54	306.74	**<0.001**
Time	3	161	7600.98	**<0.001**
Species	2	54	51.41	**<0.001**
Water	1	54	10.86	**0.002**
ln(eDNA)	1	161	6783.18	**<0.001**
Temperature:time	6	161	12.02	**<0.001**
Temperature:species	4	54	0.53	0.717
Time:species	6	161	7.30	**<0.001**
Temperature:water	2	54	7.19	**0.002**
Time:water	3	161	4.13	**0.007**
Species:water	2	54	0.75	0.478
Temperature:time:species	12	161	5.34	**<0.001**
Temperature:time:water	6	161	0.64	0.697
Temperature:species:water	4	54	0.17	0.954
Time:species:water	6	161	2.23	**0.043**
Temperature:time:species:water	12	161	0.86	0.590

Note: Time, Temperature, Species, and Water were added as fixed effects while the replicates (tanks) were added as random effects. DF refers to the degree of freedom and denDF is the denominator degree of freedom. ln(eDNA) is the non-normalized, but log-transformed quantity of eDNA to control for the normalization that was made in the degradation phase. Significant *p*-values are in bold.

Some of the interactions between parameters were also significant (Table 4). A first interaction was between temperature and time ($p < 0.001$). For instance, 2 days after the removal of fish from the tanks, the quantity of eDNA detected dropped more sharply at 24°C than at 8°C or 16°C, as seen during the production phase [average: 4.41, 8.89, and 10.43 ln(copies)/L, respectively]. After 2 days also, the quantity of eDNA was unexpectedly higher at 16°C than at 8°C. However, on the fourth day, the eDNA quantity remaining in the tanks became lower at 16°C than 8°C and remained lower until day 16, thus translating into an overall higher rate of degradation at 16°C than at 8°C, as predicted. The quantity of eDNA at 24°C remained the lowest at each sample time (Figure 6, Table S9). However, the rate of reduction in the quantity of eDNA (i.e degradation rate) at 24°C decreased with time such that the quantity of eDNA after day 16 became more similar with that at the other two temperatures and in fact not statistically different than that at 16°C (Figure 6). The interaction between the temperature and the type of water was also significant ($p = 0.005$). At 8°C and 16°C, there was no statistical difference between the quantity of eDNA detected in the brown and green waters. However, at 24°C, there was a significant difference between both water masses with less eDNA detected in the green water than in the brown water [average: 1.52 and 2.42 ln(copies)/L, respectively] (Figure 7, Table S10). In the model selection (Table 5), the first model included the temperature and the species. It has a weight >0.93, which indicates that these two variables explain very well the variation of eDNA quantity detected in the degradation phase.

4 | DISCUSSION

The goal of this study was to assess how temperature, species, and different types of water influence the production and degradation of eDNA. Overall, our results showed that temperature, fish species, and biophysicochemical characteristics of different types of water affect the production and/or degradation of eDNA, with some interactions between these factors. However, the temperature had the strongest impact on the quantity of eDNA detected in both the production and degradation while the effect of the species also influenced the detection during the degradation phase. This is one of the few studies in which both the production and degradation phases have been documented simultaneously within the experimental design. In the following sections, we discuss the degradation results first, putting into context how each parameter can influence the production phase. We then discuss how our results could help improve future eDNA experimental designs in natural environments.

4.1 | Effect of temperature

Temperature had a strong positive effect on eDNA degradation rate, which is coherent with previous studies. For instance, the literature reports an eDNA half-life (i.e., the time it takes for half of the quantity to be degraded) ranging from 20 h to 8.4 days for temperatures between 8°C and 12°C, from 9 h to 39.1 h between 15°C and 18°C, and from 5 h to 3.3 days between 20°C and 25°C

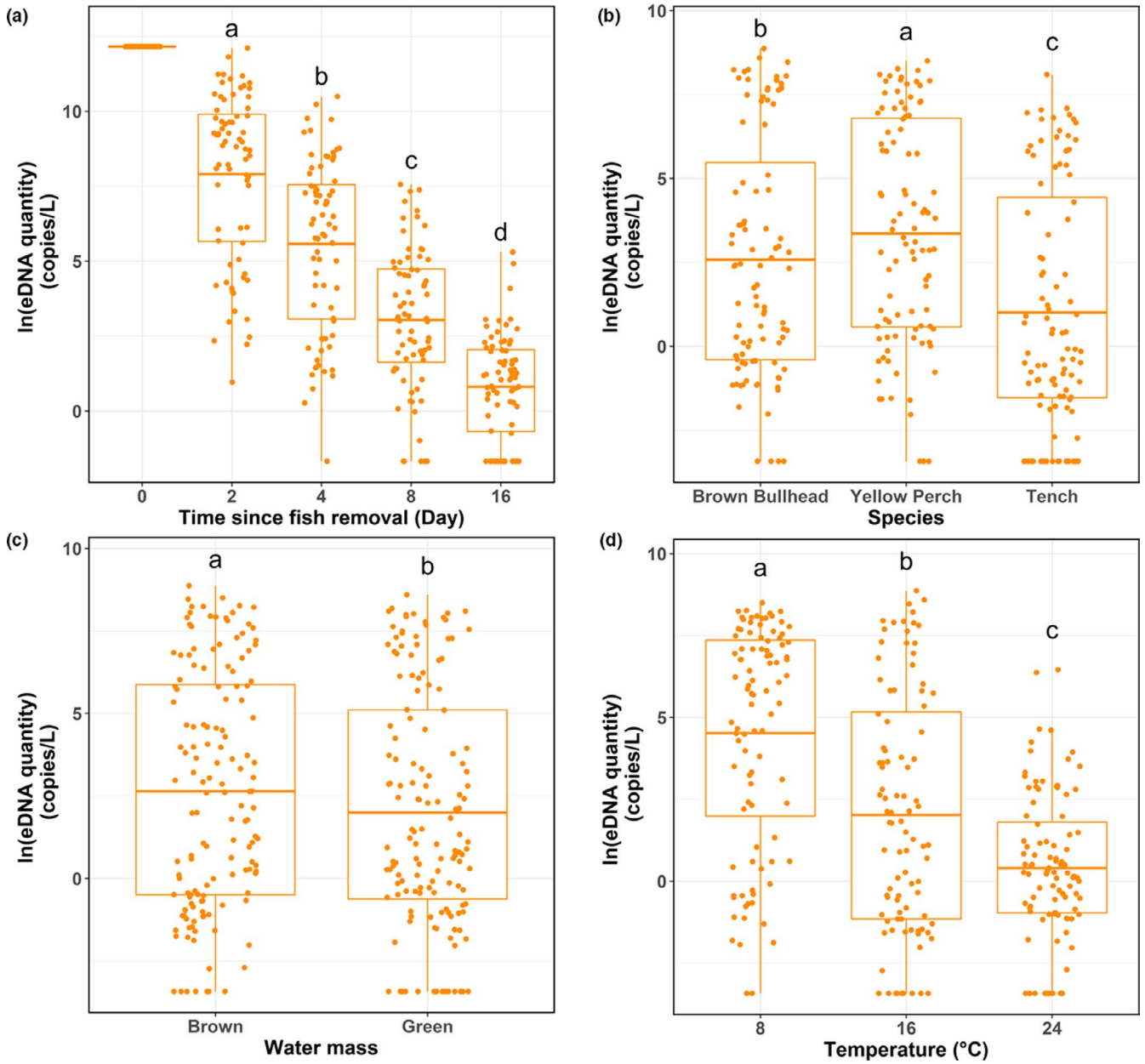

FIGURE 5 Quantity of DNA as a function of (a) time, (b) species, (c) type of water, and (d) temperature during the degradation phase. The middle line of the boxplot represents the mean, the box represents the first and third quartile (Q1 and Q3), and the whiskers represent the minimum (Q1 − 1.5 × IQR) and maximum (Q3 + 1.5 × IQR). Only the average of PCR replicates was used, and each sample is represented by a dot. Letters indicate significant difference between conditions. $p < 0.001$ for time, species, and temperature. $p = 0.002$ for water. Exact values for each condition can be found in Table S8

(Eichmiller et al., 2016; Jo et al., 2019, 2020; Kasai et al., 2020; Lance et al., 2017; Maruyama et al., 2014; Strickler et al., 2015; Tsuji et al., 2017). In our study, the half-life time was four days at 8°C and 16°C, and <2 days at 24°C. The half-life times for 8°C and 24°C are within the range of other studies, while it is higher than other studies for 16°C. This discrepancy is likely due to other parameters used in other published studies such as pH, biomass, salinity, or UV light. Indeed, the upper range between 15°C and 18°C is lower than the one of 20°C and 25°C in the literature, which is unexpected, and may be explained by environmental differences across studies for each temperature tested. Moreover, as in previous studies, we observed a nonlinear relationship between the degradation rate and the temperature with a much higher rate between 16°C and 24°C than between 8°C and 16°C (e.g., Lance et al., 2017; Tsuji et al., 2017). This could be explained by the association between higher temperature and microbial and enzymatic activity that could increase the degradation rate at higher temperatures instead of DNA denaturation alone (Barnes et al., 2014; Eichmiller et al., 2016; Jo et al., 2019; Tsuji et al., 2017). Altogether, our results support the idea that species occurrence could be detected on a longer time scale in colder environments (Thomsen & Willerslev, 2015).

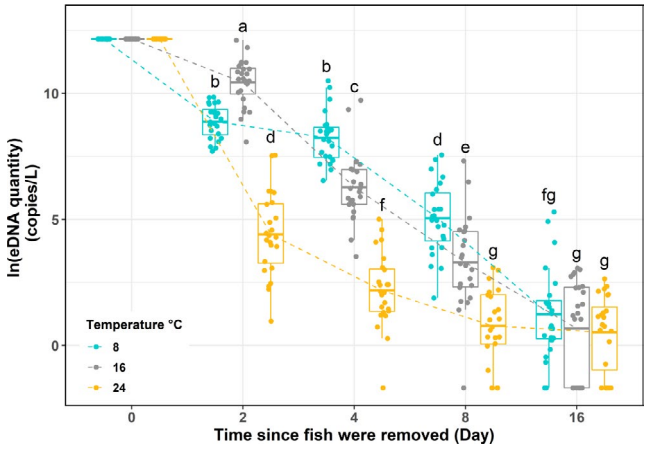

FIGURE 6 Quantity of DNA detected for each temperature at different sample times during the degradation phase. The middle line of the boxplot represents the mean, the box represents the first and third quartile (Q1 and Q3), and the whiskers represent the minimum (Q1 − 1.5 × IQR) and maximum (Q3 + 1.5 × IQR). Only the average of PCR replicates was used, and each sample is represented by a dot. Letters indicate significant difference between conditions. $p < 0.001$. Exact values for each condition can be found in Table S9

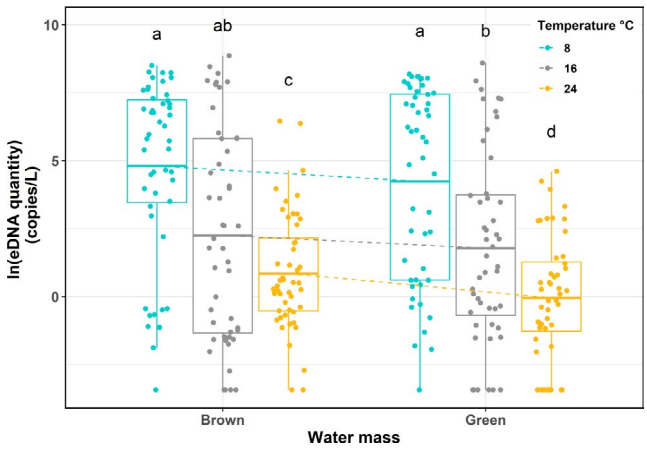

FIGURE 7 Quantity of DNA detected for each temperature in the two different water masses during the degradation phase. The middle line of the boxplot represents the mean, the box represents the first and third quartile (Q1 and Q3), and the whiskers represent the minimum (Q1 − 1.5 × IQR) and maximum (Q3 + 1.5 × IQR). Only the average of PCR replicates was used, and each sample is represented by a dot. Letters indicate significant difference between conditions. $p = 0.005$. Exact values for each condition can be found in Table S10

During the production phase, we found no overall difference between the quantity of eDNA detected at 8°C and 24°C, while the quantity at 16°C was significantly higher than either of these temperatures. This is likely caused by the interaction between temperature and time, which can be explained by the degradation rates observed during the degradation phase for each of the three temperatures. After 4 h, we detected more eDNA with increasing temperature, which corresponds to our expectation and what has been reported in other studies (Jo et al., 2019, 2020; Lacoursière-Roussel et al., 2016). However, even though the production rate is higher at 24°C, the degradation rate is also higher at this temperature. As such, our results suggest that after 24 h, the balance between a lower production and lower degradation rate at 8°C appears to be equivalent to the higher production and higher degradation rate at 24°C. As other studies reported a steady state after 3–25 h, and this balance between production and degradation was observed after 24 h, the quantity of eDNA detected appears to be stable (Bylemans et al., 2018; Nevers et al., 2018; Sassoubre et al., 2016). For instance, Takahara et al. (2012) and Klymus et al. (2015) also found no difference in eDNA quantity detected between different temperatures during an eDNA degradation experiment. As in our study, Takahara et al. (2012) sampled water 6 days after placing common carp (*Cyprinus carpio*) in tanks at 7°C, 15°C, and 25°C and found no difference for the two extreme temperatures. Moreover, Klymus et al. (2015) sampled water 14 days after placing bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) at 19°C, 25°C, and 31°C and found no difference among the three temperatures. Our results suggest that the lack of differences in eDNA concentration observed at different temperatures in those studies could be caused by the balance between production and degradation rates (production rate was not measured in those studies).

4.2 | Effect of species

During the degradation phase, the quantity of eDNA detected at each sample time differed for each of the three species, indicating that the degradation rate differed between them. This is in contrast with Sassoubre et al. (2016) and Kirtane et al. (2021), who found no difference of degradation rate among three marine fishes (northern anchovy (*Engraulis mordax*), Pacific sardine (*Sardinops sagax*), and Pacific chub mackerel (*Scomber japonicas*) for the first study and black sea bass (*Centropristis striata*), winter flounder (*Pseudopleuronectes americanus*), and summer flounder (*Paralichthys dentatus*) for the latter). In contrast, Allan et al. (2020) found that eDNA degradation rate differed importantly among species from very divergent taxonomic groups (fish, shrimp, and jellyfish). They hypothesized that these differences could be caused by different eDNA states being detected for the different taxa. For example, recent studies reported that longer eDNA fragments have a faster degradation rate than shorter ones (Jo & Minamoto, 2021; Jo et al., 2017; Moushomi et al., 2019). Furthermore, physiological differences could influence the state of eDNA being produced and thus its degradation rate. For instance, Kasai et al. (2020) proposed that the body mucus layer protection of eels may reduce its eDNA degradation rate compared to other fish species. In our study, all three species had distinct external characteristics that could hypothetically influence the origin or state of eDNA produced. For example, the brown bullhead is a species without scales, while the tench and yellow perch both have different types of scales, that is, ctenoid for the former and cycloid

TABLE 5 Results for the model selection for the degradation phase

Model	*K*	BIC	ΔBIC	ModelLik	BICWt
Temperature + species	8	510.85	0	1.00E+00	9.03E−01
Temperature + species + water	9	516.30	5.46	6.54E−02	5.90E−02
Temperature	5	517.34	6.50	3.88E−02	3.51E−02
Temperature + water	6	523.10	12.25	2.18E−03	1.97E−03
Temperature × water	8	524.07	13.23	1.34E−03	1.21E−03
Temperature × species	11	531.82	20.97	2.80E−05	2.52E−05
Temperature + time + species	10	533.33	22.48	1.31E−05	1.18E−05
Temperature + time + species + water	11	538.79	27.94	8.57E−07	7.73E−07
Temperature + time	8	539.32	28.47	6.57E−07	5.93E−07
Temperature × time	14	540.46	29.61	3.72E−07	3.35E−07
Temperature + time + water	9	545.15	34.30	3.56E−08	3.22E−08
Species	5	571.00	60.16	8.65E−14	7.81E−14
Water	4	572.30	61.45	4.53E−14	4.09E−14
Species + water	6	576.86	66.01	4.63E−15	4.18E−15
Species × water	8	586.29	75.45	4.14E−17	3.74E−17
Time	6	588.80	77.95	1.18E−17	1.07E−17
Time + species	8	593.51	82.66	1.12E−18	1.02E−18
Time + water	7	594.66	83.81	6.32E−19	5.71E−19
Time + species + water	8	599.36	88.51	6.03E−20	5.45E−20
Time × water	10	611.96	101.11	1.11E−22	9.98E−23
Time × species	14	614.85	104.00	2.61E−23	2.36E−23

Note: The log(eDNA) was also added in each model to control for the effect of the standardization. *K* is the number of parameters, ModelLik is the likelihood, and BICWt is the weight of each model.

for the latter (Bernatchez & Giroux, 2012; Brown et al., 2009; Scott & Crossman, 1973). Scales could rapidly sink at the bottom of the water column while epithelial cells or mucus produced could remain in suspension for a longer time, which could influence the detection (Sassoubre et al., 2016). More detailed studies are needed to determine if such external characteristics could influence the type, origin, and state of eDNA being produced and, in turn, the degradation rate among different fish species.

During the production phase, there was no difference between the quantity of eDNA detected for the yellow perch and the brown bullhead, but there was significantly less eDNA detected for the tench. Several studies also documented variation in the production rate among different species (Klymus et al., 2015; Sassoubre et al., 2016; Thalinger et al., 2021). Here, individuals of each of the three species were all aged 1+ year, so differences in age class or reproductive status was not likely the cause of this difference.

It is also possible that the production rate of eDNA is not the same among the three species of fish due to differences in activity. For instance, Thalinger et al. (2021) found that fish with a higher swimming activity released more eDNA than fish that were less active when comparing seven species (*Cottus gobio*, *Oncorhynchus mykiss*, *Phoxinus*, *Salvelinus fontinalis*, *Salmo trutta*, *Squalius cephalus*, and *Thymallus thymallus*). However, as we did not monitor the activity of the fish during the experiment, we cannot assess whether the tench was indeed less active than the two other species. Allometry could also play a role in explaining the difference in production rate across species because of metabolic rate or surface area that is not proportional to the size of the individuals (Stoeckle et al., 2020; Yates et al., 2020). Finally, there is also the possibility that the fast degradation rate of eDNA, as we have seen with the effect of temperature, contributed to the differences observed in the production phase. In particular, the tench had significantly less eDNA detected than either of the other two species both during the production and degradation phases.

Interestingly, the interaction between temperature and species was only marginally significant during the production phase, but the observed trend is nevertheless relevant as this might indicate that such interactions between temperature and taxa could be significant when comparing species with more distinct thermal preferendum and tolerance. Indeed, we used three species that are all very tolerant to high temperatures (Bernatchez & Giroux, 2012). However, for species with lower thermal tolerance, physiological stress could lead to different production rates between cold and warm temperatures, such as observed by Allan et al. (2020) who found a significant effect of the interaction between species and temperature on the production of eDNA.

4.3 | Effect of water characteristics

Overall, we found a significant difference in the quantity of eDNA detected over time between green and brown waters found in

the St. Lawrence River, and the significant interaction with temperature shows that the difference is mainly occurring at 24°C. Although not significant, we also observed a difference at 16°C, while no difference was detected at 8°C. This could be due to the slower degradation rate at a lower temperature. The difference in degradation rate between both types of water can be explained by the very distinct biophysicochemical characteristics of both types of water. It is known that several parameters such as pH, turbidity, nutrient availability, chlorophyll *a*, and dissolved organic carbon influence the degradation and detection of eDNA (Boivin-Delisle et al., 2021; Eichmiller et al., 2016; Seymour et al., 2018; Strickler et al., 2015; Tsuji et al., 2017; Williams et al., 2017; Wineland et al., 2019). Moreover, since water characteristics and origin are very different between the two types of water, microbial community is expected to be very distinct, although we could not document this in our study (Wang et al., 2018). This difference in microbial community could have a strong impact on eDNA detection, with some species that could be more efficient than others in degrading eDNA at different temperatures. Despite the differential effect of water characteristics observed in this study, it is important to keep in mind that very dynamic aquatic ecosystems such as the St. Lawrence River can show seasonal changes in water characteristics and associated microbial communities (Salter, 2018; Thalinger et al., 2021). Consequently, the differential effect of brown and green waters in this system on eDNA detection is also expected to vary seasonally.

During the production phase, the same difference in eDNA quantity was observed between both types of water. For the interaction with time, the quantity of eDNA detected was the same after 4 h, which suggests that production of eDNA could be the same in both types of water. The difference we observed after 4 h may then be caused by the strong effect of degradation, and in the same way, we observed for the temperature effect. However, there may still be an actual effect of water characteristics *per se* on eDNA production. As mentioned previously, several studies have documented how pH, turbidity, chlorophyll *a*, or dissolved oxygen can influence the detection of eDNA, but these studies did not always make the distinction between whether the difference is due to the production or degradation of eDNA. Other studies have shown that some of these parameters, such as pH or turbidity, can increase the physiological stress of individuals, increase their metabolism, and even change their behavior (Gray et al., 2014; Pistole et al., 2008; Vuorinen et al., 2004). Thus, it is plausible that water characteristics can also influence the production of eDNA.

4.4 | Improving eDNA experimental design in natural environments

By simultaneously documenting the dynamic of eDNA production and degradation in the same experiment, our study adds to the knowledge acquired by previous efforts in showing the quantity of eDNA detected in a water sample is influenced by both biotic and abiotic parameters. Our study also showed how degradation rate can impact the net production rate being detected, particularly at higher temperatures. For the species studied here at least, our results suggest that in temperate freshwater ecosystems, low temperatures (<8°C) can reduce the detection probability of rare species due to a slow production rate. Similarly, warm temperatures (>24°C) can also reduce detection probability due to the rapid degradation of eDNA. The detection probability appears to be higher at intermediate temperatures (e.g., around 16°C) where the balance between production and degradation is optimized. Thus, when the goal is to estimate species richness or to ensure a presence/absence of a species in a given location, we suggest a sampling campaign during which temperature is intermediate because of the higher potential of detection. However, depending on the information needed, sampling in cold or warm periods can also have their utility. For example, cold periods could allow to detect species on a longer timescale while warm periods provide information on a narrower timescale. The optimal temperature to conduct a sampling campaign may vary depending on other factors in the natural habitats, but this balance between warmer and colder temperatures can still occur despite changes in environmental conditions. Also, to avoid biases caused by seasonal changes in temperature and/or biophysicochemical conditions, it would be important to standardize as much as possible the environmental conditions when sampling is being carried out. For example, this could be done by sampling at the same time period every year (Lacoursière-Roussel et al., 2016). As reported in several other studies (e.g., Jo & Minamoto, 2021; Lacoursière-Roussel et al., 2016; Strickler et al., 2015), we observed complex interactions between factors influencing either eDNA production or degradation rate. For instance, during the production phase, a significant interaction between water mass and time indicated that while the net eDNA production increased with time in both types of water, it was less so in the green water than in the brown water mass. Also, during the production phase, the interaction between species and temperature showed that the difference in quantity of eDNA detected between species was not constant but instead varied with temperature. During the degradation phase, the significant interaction between temperature and the type of water indicated that while temperature influences the rate of eDNA degradation, this happened faster in the green water than the brown water mass. From an eDNA ecology point of view, this means that an accurate inference that a species was present in a given place and time represents a major challenge as that is an almost impossible endeavor to make general rules pertaining to the combined effect of various biotic and abiotic factors on the fate and persistence of eDNA in natural environments. Yet, this should not be perceived as a constraint limiting the usefulness of eDNA for monitoring purposes. Instead, as proposed by Lacoursière-Roussel and Deiner (2021), we should move beyond highlighting our lack of understanding and suggest what further research is needed before eDNA inferences can be used for rigorously answering specific ecological or management questions. Ultimately, predicting the fate and/or

persistence of eDNA in a given environment will require elaborating models that will include the various parameters that may influence the amount of eDNA and obtaining empirical estimates of values of such parameters that would be specific to a given combination of species and environmental conditions (e.g., Fukaya et al., 2021). For instance, while temperature is probably one of the most important factors influencing the persistence of eDNA and thus its detection probability, it will be very interesting to consider the potential interactions other factors could have with the temperature in future experimental studies. However, other aspects of the ecology of eDNA, such as the transport, can also greatly influence the detection of species. Incorporating these results of production and degradation rate to hydrodynamic models could greatly improve estimates of eDNA dispersion. For example, adding the degradation rate or persistence time of eDNA to these models could indicate the distance at which a species could be detected and thus allow to predict with more precision the localization of species of interest such as invasive or endangered species (Laporte et al., 2020). To conclude, our study provides useful information toward further improving our understanding of the ecology of eDNA in general. In turn, we hope that this will allow a better precision of models evaluating species detection as well as a better estimation of relative abundance toward improved management and conservation of freshwater fishes.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

ICA have made major contributions to the design of the study; the acquisition, analysis, and interpretation of the data; and writing of the manuscript. ML have made major contributions in the analysis and interpretation of the data; and writing of the manuscript. GC and JA have made major contributions to the conception and design of the study; the acquisition and interpretation of the data; and writing of the manuscript. LB have made major contributions to the conception and design of the study; the interpretation of the data; and writing of the manuscript.

DATA AVAILABILITY STATEMENT

Dataset is available on Dryad: https://doi.org/10.5061/dryad.7h44j0zvd

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