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Received: 27 January 2023 | Accepted: 4 June 2023

DOI: 10.1111/jfb.15476

JOURNAL OF FISH BIOLOGY fsbi

REGULAR PAPER

Comparative evaluation of loop-mediated isothermal amplification and PCR for detection of *Esox lucius* housekeeping genes for use in on-site environmental monitoring

Nivedhitha Jothinarayanan | Frank Karlsen | Lars Eric Roseng

Department of Microsystems, University of South-Eastern Norway, Horten, Vestfold, Norway

Correspondence
Lars Eric Roseng, Department of Microsystems, University of South-Eastern Norway, Raveien 215, Horten, Vestfold, 3184, Norway.
Email: lars.roseng@usn.no

Funding information
the Norwegian Micro- and Nano-Fabrication Facility, Grant/Award Number: 295864
NORFAB III

Abstract

Esox lucius (northern pike) is an invasive species in fresh water and causes extreme impacts in the local habitat. Northern pike easily replaces the local native species and disrupts the regional ecosystem. Traditionally, in connection with environmental monitoring, invasive species are identified using PCR through species-specific DNA. PCR involves many cycles of heating to amplify the target DNA and requires complex equipment; on the contrary, loop-mediated isothermal amplification (LAMP) entails isothermal amplification, which means the target needs to be heated to only one temperature between 60 and 65°C. In this study, the authors conducted a LAMP assay and a conventional PCR assay to determine which technique is less time consuming, more sensitive and reliable for use in real-time and on-site environmental monitoring. Mitochondrial gene cytochrome b, an essential factor in electron transport; histone (*H2B*), a nuclear DNA responsible for the chromatin structure; and glyceraldehyde 3-phosphate dehydrogenase involved in energy metabolism are taken as the reference genes for this article. The results show that LAMP is more sensitive and less time consuming than the conventional PCR, and thus it can be used for the detection of northern pike in aquatic ecosystems related to environmental monitoring.

KEYWORDS
amplification rate, environmental monitoring, housekeeping gene detection, invasive species, loop-mediated isothermal amplification, PCR, sensitivity

1 | INTRODUCTION

Esox lucius, a freshwater fish often seen in the Northern Hemisphere, is regarded as an invasive species in the south-eastern parts of Norway (regionally alien). It can easily create dense local populations by preying upon the small native species. The northern pike is a very effective predator that can disturb the local trout population and strongly affect the river pearl mussel (Bardal, 2019). Most invasive species threaten biodiversity and affect the agricultural economy. Therefore, it is important to identify such species up front to avoid damage (Haught & von Hippel, 2011), and monitoring their presence and distribution is essential to reduce the potential negative consequences. Such aquatic environments are monitored using nucleic acids (DNA/RNA) released into the environment by the species;

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such nucleic acids, collects directly in the form of water, reveal important information about past and present biodiversity (Bohmann *et al*., 2014; Rees *et al*., 2014). The high density of the northern pike population significantly affects the habitats of other species; therefore, the development of sensitive, rapid and reliable methods to examine environmental DNA (eDNA) is essential for fisheries management. Early detection of aquatic invasive species is important to assist fisheries managers. The current methods for eDNA analysis are time consuming and mostly incompatible with an early-phase evaluation.

PCR is currently the most used and adaptable analysis for DNA identification for monitoring the aquatic environment (Dejean *et al*., 2011; Piaggio *et al*., 2013). Nonetheless, the main disadvantage with the PCR is that it requires sophisticated laboratory equipment and also technical expertise, which are often unavailable in laboratories with poor resources. LAMP is a relatively new and unique technique that can overcome the disadvantages of PCR-based methods (Foo *et al*., 2020; Mori & Kanda, 2013; Niessen, 2015; Notomi *et al*., 2000). Inhibitory substances in complex biological samples can affect the amplification process, and this problem can be overcome using LAMP methods, which are simple and fast. In previous studies, the advantages and disadvantages of PCR and LAMP have been discussed in detail (Lau *et al*., 2017).

One of the significant factors is gene selection with less cross-homology of species, belonging to the same taxa. The genes selected here are based on the metabolic activity of species, with the least cross-homology. For example, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is one of the glycolytic proteins/enzymes that act in the reduction of NAD+ to $NADH_2$, which is thought to be expressed essentially as in the metabolic pathway. Furthermore, this gene plays an important role as an internal controller in gene expression patterns of fish development, especially in energy-intensive processes, correlation of egg quality and apoptosis (Sarropoulou *et al*., 2011).

The aim of the study is to compare the efficiency of the PCR and LAMP methods to determine which method is best suited for rapid and sensitive detection of *E. lucius* housekeeping genes. If the DNA amplification process is to be carried out automatically on a lab-on-a-chip platform, then the LAMP process will be easier to use than a PCR process that will require two temperatures for each amplification cycle. This study provides a useful approach for future eDNA technology that can be carried out by researchers involved in automatic aquatic environment monitoring.

2 | MATERIALS AND METHODS

2.1 | Nucleic acid extraction

The nucleic acid was extracted from *E. lucius* using the Boom's extraction method (NUCLISENS MINIMAG, BioMerieux, Lyon,

TABLE 1 The primer sequence for Cyt B

Sequence (5′-3′)	Primer type	Method
CTCCACAGCCTTCTCATCAGTCT	Forward	PCR (Fossøy *et al*., 2017)
TTCGGATAAGTCAGCCGTAGTTAA	Reverse	PCR (Fossøy *et al*., 2017)
TACACCACAGGGCTTGATA	F3	LAMP
GCATGGGCTGTAACGATAA	B3	LAMP
AGGGTGCCAATATCTTTGTGGTTCTCAGCCATCCTACCTG	FIP	LAMP
AGTCGGCACAGCCTTAAGCCTGGTCGTCACCTAAGAGA	BIP	LAMP
ATCAGCGTGTGATTGCCA	Loop F	LAMP
CCGAACTAAGCCAGCCAG	Loop B	LAMP

Abbreviation: LAMP, loop-mediated isothermal amplification.

TABLE 2 The primer sequence for H2B

Sequence (5′-3′)	Primer type	Method
TCTATAGTAGCCGCACGCTG	Forward	PCR
TCACTAAAGCCGCACTCTCG	Reverse	PCR
GCCGAGTGCATACATTGTA	F3	LAMP
TTTCAACGTTCACTAAAGCC	B3	LAMP
CATACTGGTCCTGCAACGCTCGAGAGGGATACAATGAATCC	FIP	LAMP
ACTGATTGTACCACGCCTTTGAGCACTCTCGTGACTTGATT	BIP	LAMP
CGATTCAAACAGGAACTGGTG	Loop F	LAMP
AGCCATGACTCGCAATGT	Loop B	LAMP

Abbreviation: LAMP, loop-mediated isothermal amplification.

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TABLE 3 The primer sequence for GAPDH

Sequence (5′-3′)	Primer type	Method
CTGGTGCTGGAATTGCACTG	Forward	PCR
TCAATGACGCGGTTGCTGTA	Reverse	PCR
CAGAGGACCAAGTTGTGTC	F3	LAMP
CAAGTCGAGGGCTAGAGT	B3	LAMP
GCTTGACAAAGTGGTCGTTCAGACA TTCGCTCCTCCATCT	FIP	LAMP
TACAGCAACCGCGTCATTGAGGTCG ATTGGCTTTACTCC	BIP	LAMP
GCAATTCCAGCACCAGCATC	Loop F	LAMP
GATGGCTCACATGACCACCAA	Loop B	LAMP

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LAMP, loop-mediated isothermal amplification.

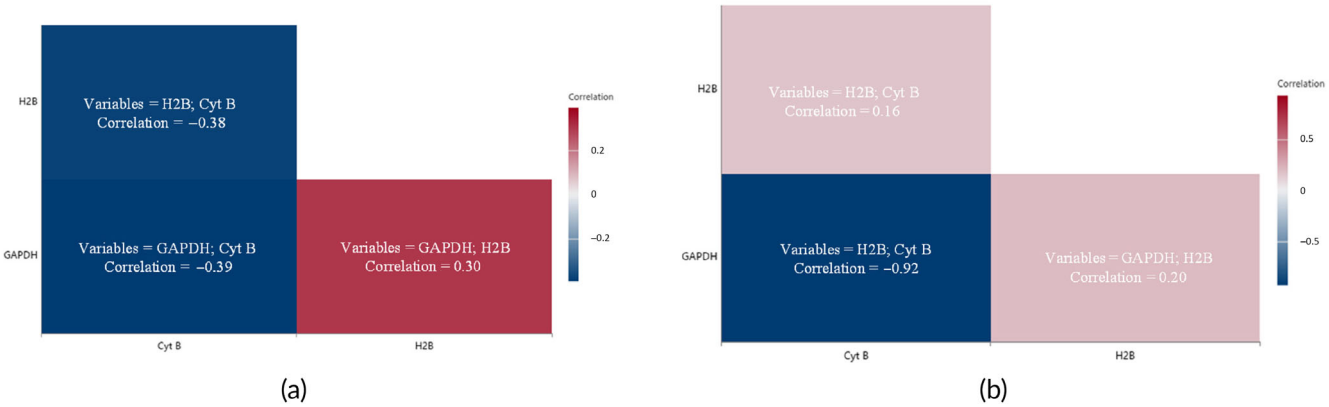

FIGURE 1 Correlation between the three genes of amplification for *Esox lucius* DNA using (a) PCR and (b) LAMP (loop-mediated isothermal amplification)

France). Two pikes weighing *c*. 3 kg were captured from lakes in Vestfold and Telemark County. To simulate typical biological, cellular debris or eDNA material, the DNA was obtained from various sources of northern pike, such as tissue, bone marrow, skin and eggs. This material was used for nucleic acid extraction using the protocol before further dilution. The protocol was strictly followed, including standard molecular quality procedures to eliminate sample contamination.

2.2 | Primer design and reconstitution

The primers and probes of *E. lucius* housekeeping genes were designed in LAMP designer (PREMIER Biosoft, San Francisco, CA, USA) for the respective LAMP analysis. The primers for PCR were designed by National Centre of Biotechnology (NCBI) online tool. The designed primers were commercially purchased from Eurofins Genomics (Denmark), and the sequences are presented in Tables 1–3. All selected genes of *E. lucius* and sequences were retrieved from the NCBI with reference numbers Cyt B (NC_004593.1), H2B-like mRNA (NM_001310976.1) and GAPDH mRNA (XM_010884975.4). Procured primers were reconstituted based on the data sheet provided.

2.3 | PCR analysis

PCR was performed in StepOnePlus (Applied Biosystems, Thermo Fisher Scientific, USA) with rox dye fluorescence. The temperature cycle is as follows: holding stage at 50°C for 2 min for Uracil-N-Glycosylase (UNG) activation (to remove contaminants), initiation of polymerase at 95°C for 10 min, followed by the cycling stage of 15 s at 95°C and 1 min at 60°C for 50 cycles. The final volume of the PCR reaction mixture was 20 μl with reconstituted primers along with Takyon PCR MasterMix (Eurogentec), for 2.5 μl of the template.

2.4 | LAMP assay

The temperature cycle was programmed in StepOnePlus (Applied Biosystems) for the LAMP analysis. The reaction mixture comprised 25 μl of 10× primer mix, commercially available WarmStart Master Mix,

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fluorescent dye and template DNA (New England Biolabs, USA). The Master Mix mainly consists of a combination of deoxynucleotide triphosphate, *Bst* DNA polymerase, Tris–HCl, KCl, Tween, $(NH_4)_2SO_4$, betaine and $MgSO_4$ in appropriate concentrations. The primer concentrations were 0.2, 0.4 and 1.6 for F3/B3, Loop F/B and FIP/BIP, respectively. The reaction was carried out at 65°C for 1 h and set with 80 cycles.

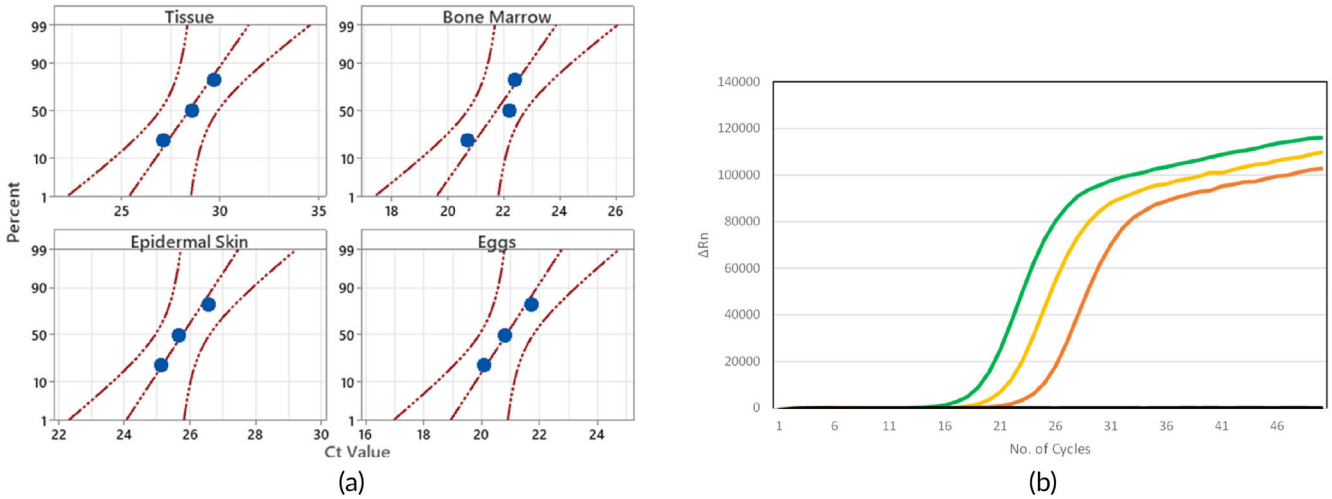

FIGURE 2 (a) Probability plot for different sources of samples: (i) tissue, (ii) bone marrow, (iii) epidermal skin and (iv) eggs tested in PCR analysis; (b) typical amplification graph for three sources of the samples tested with negative sample using PCR. —, Bone Marrow; —, Skin; —, Eggs; —, Negative control

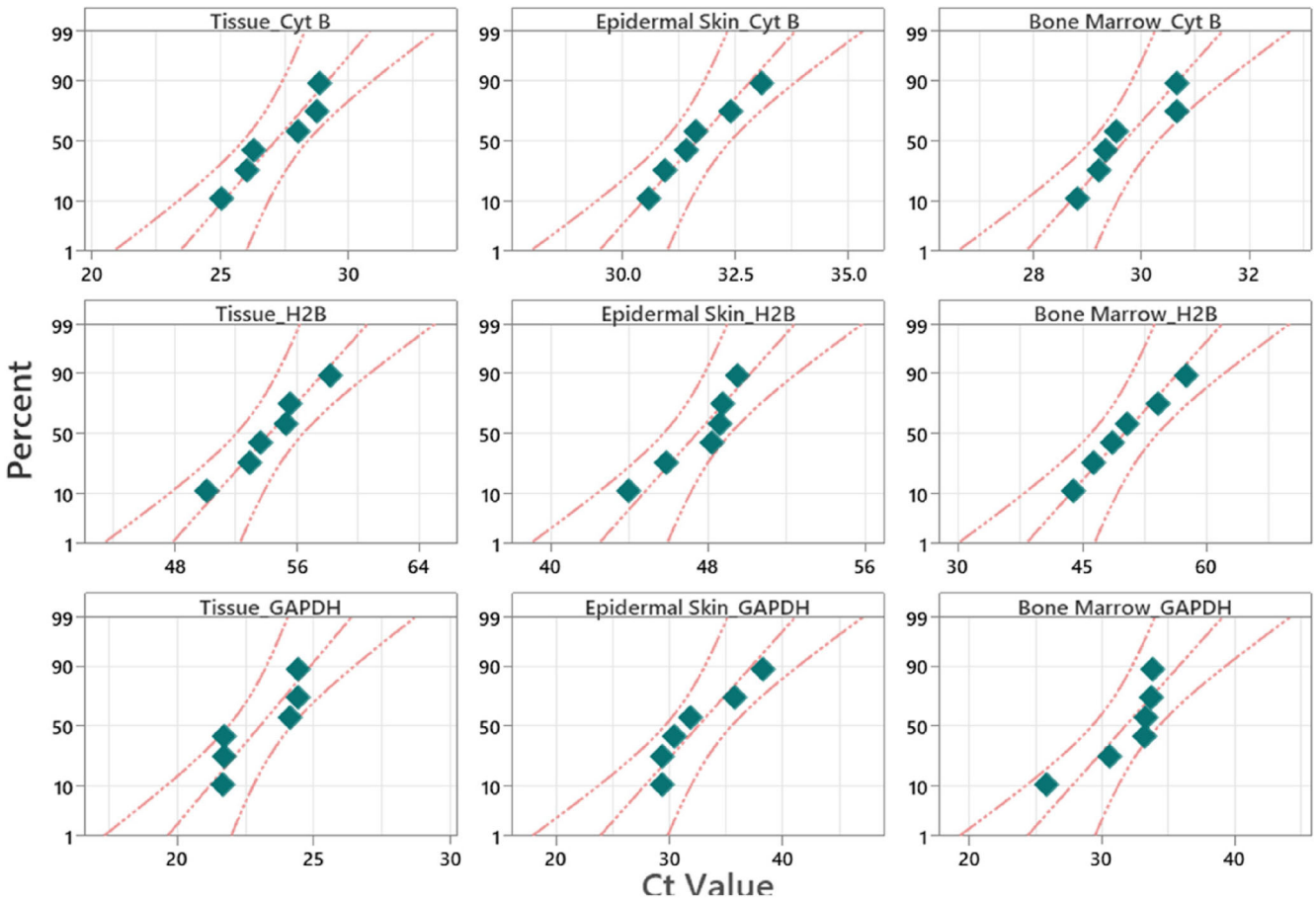

FIGURE 3 Probability plots for different sources of samples from *Esox lucius*, and three gene primers were tested using LAMP (loop-mediated isothermal amplification)

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2.5 | Analytical sensitivity analysis

The positive oligos of GAPDH gene were used for sensitivity analysis and serially diluted extracted DNA sample. The oligo sequence for GAPDH starts at 5′ TTTCAGTGGTTGACCTGAC and ends at ATAAAGGAAATCACAACTTGAA 3′. Serially diluted oligos and DNA template were used in LAMP and PCR to determine the lowest amount of the target that could be detected. Positive oligos were procured from Eurofins Genomics.

2.6 | Specificity test

The related potential DNA of eight other fish, including (a) *Scardinius erythrophthalmus*, (b) *Abramis brama*, (c) *Perca fluviatilis* and (d) *Rutilus rutilus*, along with *E. lucius* DNA were tested using LAMP analysis, and in addition, precision of primers was validated.

2.7 | Statistics

All the data were represented as mean ± S.E. from six experiments, except the probability test from PCR from three experiments. The statistical significance was determined using a sample *t*-test, correlation test and probability test (Minitab 21). The probability value (*P*-value) of 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Primer validation for DNA template

A key element in amplifying the target nucleic acid is the primers with enzymes and cofactor ions. Thus, the primers were designed with criteria for GC content, temperature and length of base pairs, their suitability for amplification and detection, except the Cyt B–PCR primers which were assessed and obtained from the Norwegian Institute for Natural Research (NINA) (Fossøy *et al.*, 2017). The target nucleic acid used in the study was extracted from *E. lucius* using the Boom's extraction method, as mentioned in Section 2.1. PCR and LAMP amplification results confirmed that the primers from three genes, Cyt B, H2B and GAPDH, were successfully validated in terms of the DNA template and negative control without amplification.

The Ct (cycle threshold) value of the three genes amplified using PCR was compared in correlogram using Pearson's method of correlation (Figure 1a). It revealed that the correlation coefficient was positive for GAPDH:H2B = 0.30, whereas that for GAPDH:Cyt B = −0.39 and

TABLE 4 Sample *t*-test for GAPDH positive oligos using PCR

Descriptive statistics					
Sample	***N***	**Mean**	**S.D.**	**S.E.**	**95% C.I. for *μ***
Positive oligo	6	19.04	3.89	1.59	(14.95; 23.12)
10^{-1} diluted	6	21.25	3.75	1.53	(17.32; 25.19)
10^{-2} diluted	6	20.19	10.22	4.17	(9.46; 30.91)
10^{-3} diluted	6	25.19	2.62	1.07	(22.44; 27.93)
10^{-4} diluted	6	22.01	11.04	4.51	(10.43; 33.60)
10^{-5} diluted	6	28.813	1191	0.486	(27.564; 30.063)
10^{-6} diluted	6	28.73	14.16	5.78	(13.88; 43.59)

Test		
Null hypothesis		**H_0: $\mu = 0.05$**
Alternative hypothesis		H_1: $\mu \neq 0.05$
Sample	***t*-Value**	***P*-value**
Positive oligo	11.95	0.000
10^{-1} diluted	13.86	0.000
10^{-2} diluted	4.83	0.005
10^{-3} diluted	23.52	0.000
10^{-4} diluted	4.87	0.005
10^{-5} diluted	59.17	0.000
10^{-6} diluted	4.96	0.004

Note: *μ*, population mean of positive oligo; 10^{-1}, diluted; 10^{-2}, diluted; 10^{-3}, diluted; 10^{-4}, diluted; 10^{-5}, diluted; 10^{-6}, diluted.
Abbreviation: GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

TABLE 5 Sample *t*-test for GAPDH positive oligos using LAMP

Descriptive statistics					
Sample	***N***	**Mean**	**S.D.**	**S.E.**	**95% C.I. for *μ***
Positive oligo	6	20.36	2.80	1.14	(17.42; 23.29)
10^{-1} diluted	6	23.67	3.66	1.49	(19.84; 27.51)
10^{-2} diluted	6	27.45	3.24	1.32	(24.05; 30.85)
10^{-3} diluted	6	31.40	3.97	1.62	(27.24; 35.57)
10^{-4} diluted	6	33.21	16.98	6.93	(15.39; 51.02)
10^{-5} diluted	6	44.65	4.97	2.03	(39.44; 49.87)
10^{-6} diluted	6	34.0	26.8	10.9	(5.9; 62.1)

Test		
Null hypothesis		**H_0: $\mu = 0.05$**
Alternative hypothesis		H_1: $\mu \neq 0.05$
Sample	***t*-Value**	***P*-value**
Positive oligo	17.77	0.000
10^{-1} diluted	15.83	0.000
10^{-2} diluted	20.73	0.000
10^{-3} diluted	19.35	0.000
10^{-4} diluted	4.78	0.005
10^{-5} diluted	21.99	0.000
10^{-6} diluted	3.11	0.027

Note: *μ*, population mean of positive oligo; 10^{-1}, diluted; 10^{-2}, diluted; 10^{-3}, diluted; 10^{-4}, diluted; 10^{-5}, diluted; 10^{-6}, diluted.
Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LAMP, loop-mediated isothermal amplification.

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H2B:Cyt B = −0.38 was negative. LAMP amplification Ct values yielded two positive correlation coefficients of GAPDH:H2B = 0.20 and H2B: Cyt B = 0.16 and a negative correlation coefficient of GAPDH:Cyt B = −0.92 (Figure 1b). The positive correlation relationship increases the chance of amplifying the two genes in multiplex with LAMP than PCR.

3.2 | PCR probability of amplification

The DNA samples extracted from different sources of northern pike were subjected to amplification using the obtained primers of Cyt B. A high percentage of amplification observed in the four forms of samples, (a) tissue, (b) bone marrow, (c) epidermal skin and (d) eggs, was scored using the probability plot test (Figure 2a) with normal 95% C.I. The fluorescence value (ΔRn) of the reporter dye from a standard exponential amplification phase for bone marrow, skin and eggs is presented (Figure 2b). Normal distribution reveals that in the tissue sample 90% of the amplification occurred at 28.48 Ct. In the bone marrow, epidermal skin and egg samples, 90% of the amplification occurred at cycle thresholds of 21.76, 25.79 and 20.87, respectively.

3.3 | LAMP probability of amplification

The authors performed a LAMP assay for all similar samples amplified by three gene primers in different periods and presented as a probability (95% C.I.) plot (Figure 3). In most cases, it fit the straight line, with standard statistical calculation derived using Pearson's method. The Cyt B gene in the tissue sample was amplified using LAMP at the rate of 90% with an average Ct value of 27.19. The higher amplification rate of the H2B gene decreased at the cycle threshold of 54.31 and GAPDH at 23.04 Ct. In the epidermal skin and bone marrow Ct values were very close to the S.D. of ±0.78. Only, the expression of H2B nuclear DNA occurred, nearly all the time, approximately after the

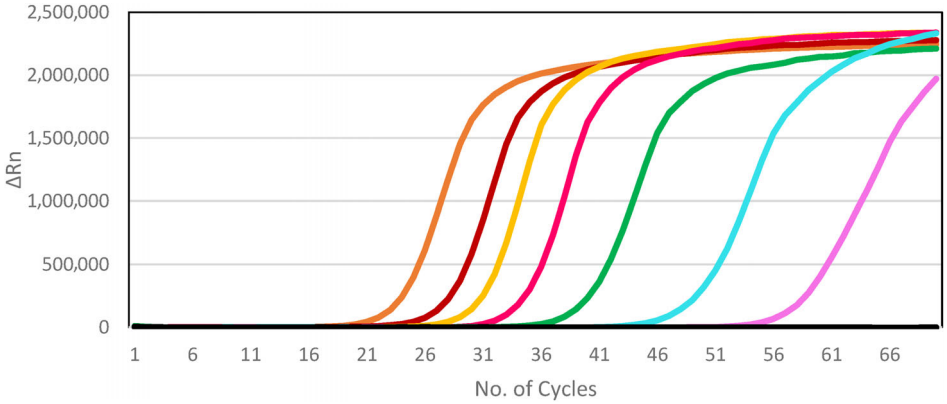

FIGURE 4 The amplification plot for GAPDH (glyceraldehyde 3-phosphate dehydrogenase) positive oligos using LAMP (loop-mediated isothermal amplification) analysis. —, Positive Oligo; —, 1:10000 dilution; —, 1:10 dilution; —, 1:100000 dilution; —, 1:100 dilution; —, 1:1000000 dilution; —, 1:1000 dilution; —, Negative control

TABLE 6 Sample *t*-test for DNA template tested for GAPDH primers using PCR

Descriptive statistics					
Sample	***N***	**Mean**	**S.D.**	**S.E.**	**95% C.I. for μ**
Pike DNA template	6	30.527	0.789	0.322	(29.699; 31.354)
10^{-1} diluted	6	33.972	0.869	0.355	(33.060; 34.884)
10^{-2} diluted	6	25.44	19.72	8.05	(4.74; 46.13)
10^{-3} diluted	6	6.54	16.02	6.54	(−10.27; 23.36)
10^{-4} diluted	6	0.000000	0.000000	0.000000	(0.000000; 0.000000)
Test					
Null hypothesis					**H_0: μ = 0.05**
Alternative hypothesis					H_1: μ ≠ 0.05
Sample			***t*-Value**		***P*-value**
Pike DNA template			94.65		0.000
10^{-1} diluted			95.60		0.000
10^{-2} diluted			3.15		0.025
10^{-3} diluted			0.99		0.367
10^{-4} diluted			–[a]		–[a]

Note: μ, population mean of pike DNA template; 10^{-1}, diluted; 10^{-2}, diluted; 10^{-3}, diluted; 10^{-4}, diluted.
Abbreviation: GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
[a]All values in columns are identical.

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42nd cycle, indicating that non-compatible ends are even amplified in the course of detection.

3.4 | Sensitivity test using PCR and LAMP

The sensitivity assay was performed using PCR and LAMP for serially diluted DNA template in comparison with positive oligos of diluted GAPDH. The sensitivity of GAPDH oligos for 10-fold dilution using PCR and LAMP occurred even at 10^{-6} dilution with a *P*-value of 0.004 and 0.027, respectively ($\mu = 0.05$), which is concluded from the sample *t*-test (Tables 4 and 5). Every dilution was amplified at increasing order of Ct values in the positive oligos (Figure 4). The amount of GAPDH oligo was 300 ng, and the whole lyophilized powder was suspended in 800 µl, with 0.199×10^{11} molecules. Therefore, it was expected that 1 µl reaction template should consist of 2.49×10^{7}

TABLE 7 Sample *t*-test for DNA template tested for GAPDH primers using LAMP

Descriptive statistics					
Sample	***N***	**Mean**	**S.D.**	**S.E.**	**95% C.I. for *µ***
Pike DNA template	6	24.302	0.941	0.384	(23.314; 25.289)
10^{-1} diluted	6	28.143	1170	0.478	(26.915; 29.372)
10^{-2} diluted	6	36.43	5.83	2.38	(30.31; 42.55)
10^{-3} diluted	6	36.0	28.3	11.6	(6.3; 65.7)
10^{-4} diluted	6	31.8	34.8	14.2	(−4.8; 68.3)
Test					
Null hypothesis					**H_0: $\mu = 0.05$**
Alternative hypothesis					H_1: $\mu \neq 0.05$
Sample			***t*-Value**		***P*-value**
Pike DNA template			63.14		0.000
10^{-1} diluted			58.80		0.000
10^{-2} diluted			15.28		0.000
10^{-3} diluted			3.11		0.026
10^{-4} diluted			2.23		0.076

Note: *µ*, population mean of pike DNA template; 10^{-1}, diluted; 10^{-2}, diluted; 10^{-3}, diluted; 10^{-4}, diluted.
Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LAMP, loop-mediated isothermal amplification.

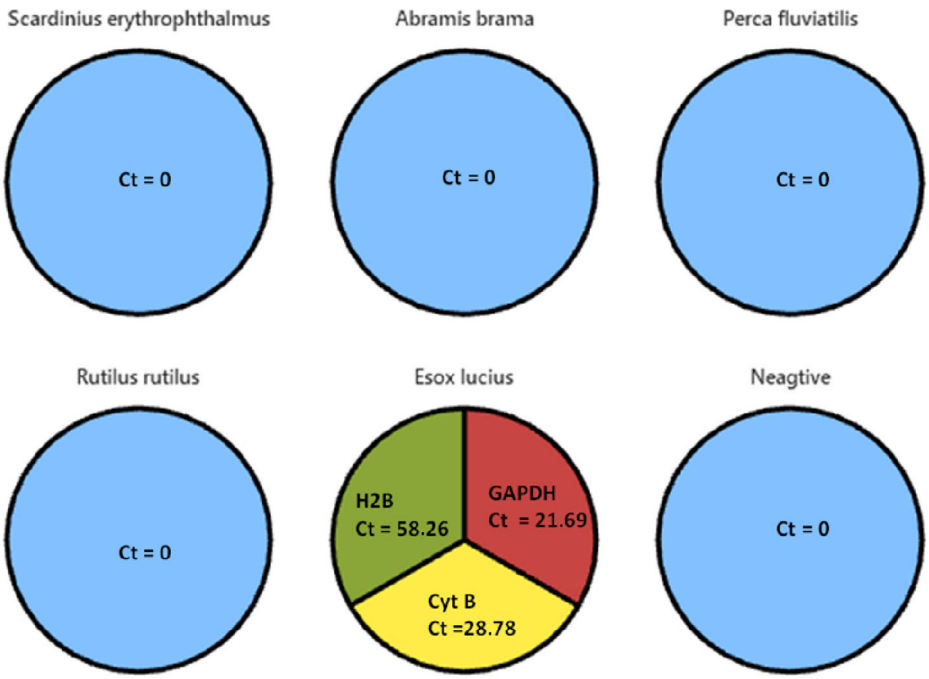

FIGURE 5 Specificity test for the designed primers for *Esox lucius* and compared with other different fish

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molecules. If it was diluted up to 10^{-6}, then it should contain 24 μl^{-1} molecules. The initial concentration of GAPDH oligos was found to be 6.2×10^{7} molecules per 2.5 µl; when it was serially diluted up to 10^{-6}, it consisted of 62 molecules per 2.5 µl for PCR analysis. Therefore, the least number of 62 molecules could amplify this appropriate reaction mixture. Similarly, LAMP analysis presented the exponential phase at all six dilutions, and 24 molecules per microlitre could be detected using the LAMP method with least dilution. This is a rather lower concentration of detection in LAMP compared to PCR. In the following DNA template using PCR analysis, the amplification stopped at 10^{-3} dilution, and *P*-value was 0.367, which is higher than the statistically significant value of 0.05 (Table 6). LAMP analysis for serially diluted DNA template was performed up to 10^{-4} and sometimes above that. The *P*-value for the sample was 0.026 for 10^{-3} dilution, and it rejected the null hypothesis at 10^{-4} dilution (Table 7). Therefore, the results of DNA template dilution indicate that sensitivity is significant up to 10^{-4} dilution for LAMP and less than 10^{-3} for PCR.

3.5 | Specificity using LAMP

For specificity, all three genes were used to detect *E. lucius* DNA along with eight other similar taxa. The LAMP assay was able to detect the target alone, when performed together with the other related potential targets. It further validates the primer precision in the detection of northern pike (Figure 5). Again, it confirms that the selected Cyt B, H2B and GAPDH gene expression is unique to *E. lucius*. The selected LAMP primers yielded specific amplification observed among other fish species, because the other fish species did not yield amplification and follow the straight line together with the negative control.

4 | DISCUSSION

The present study evaluated the amplification precision of two molecular methods, LAMP and PCR, in detecting northern pike housekeeping genes using the designed primers. To the best of the authors' knowledge, this is the first study to compare PCR and LAMP techniques for the detection of *E. lucius* (invasive species) for environmental monitoring applications. eDNA detection or identification is important for maintaining the regional ecosystem. From genetic marker selection to amplification technique, it is most important in eDNA detection to identify specific targets in diverse taxa of the ecosystem.

Compared to PCR, first, LAMP detection has the same specificity in identifying *E. lucius* and is considered inexpensive. Second, the results indicate, as seen in the sensitivity analysis, that LAMP has more sensitive accuracy and robustness when dealing with complex biological samples. The conventional PCR method has been extensively used in the investigation of aquatic organisms, in eDNA determination and in field studies when samples are collected for laboratory analyses (Barnes *et al*., 2014; Deiner & Altermatt, 2014; Pilliod *et al*., 2014). Currently, LAMP is a more suitable method for clinical diagnosis, in laboratories and in fields with limited resources (Khodaparast *et al*., 2022; Lim *et al*., 2021; Natoli *et al*., 2021). The idea of comparing the PCR and LAMP is to find the best technique for sensitivity, less time consumption and preferably a constant temperature for an automatic lab-on-a-chip analysis out in the field. The authors' research group is currently working on an automatic system for environmental monitoring using a lab-on-a-chip platform and an electronic instrument that controls the molecular biological processes on this chip. To keep the complexity as low as possible, it is therefore desirable to use an isothermal amplification process like LAMP.

Unlike PCR, LAMP does not require a heat denaturation step to convert double-stranded DNA into single-stranded DNA. The complementary template is synthesized by the activity of DNA polymerase with strong displacement (Parida *et al*., 2008). Previous reports state that, when DNA concentration decreases below 10^{-5} pg μl^{-1}, this is not possible to detect (Mauvisseau *et al*., 2019), where analytical sensitivity is most important at low sample quantities. The analytical sensitivity comparison between the conventional PCR, nPCR and qPCR methods and LAMP method for the detection of *Entamoeba histolytica* DNA showed excellent results for LAMP (Foo *et al*., 2020). The current LAMP results demonstrated reliable detection at DNA amounts (DNA extracted from tissue) of 1 µl of template from 10^{-1} to 10^{-4} dilution, which is higher than the PCR detection limit in the present study. The significance is lost after 10^{-3} dilution in PCR sensitivity test, but the positive oligo amplification in both PCR and LAMP found no noticeable difference in the setting of the detection limit, likewise, which was up to 10^{-6} dilution. LAMP analysis is a good substitute for conventional PCR-based methods, for its speed, sensitivity, uniform temperature requirements and visual assessment, making it more suitable than PCR for on-site detection in an automatic system. The limitation of LAMP, compared to PCR, is that LAMP is a relatively new method in eDNA determination. Because PCR has long been the established method, well-tested PCR primers for eDNA determination of many different species are available from recognized databases. PCR primers are established as primer bank (Wang *et al*., 2012); for example, Bold database has primers for different genes of specific species. Currently, such libraries with well-tested LAMP primers have not yet been built. Thus, the validated LAMP primers from this study will be definitely a benchmark for such databases in detecting *E. lucius*.

Another fact is that fish eDNA can be degraded in aquatic environments by several regional factors such as water temperature, pH, salinity and ultraviolet radiation and even by microbes and enzymes recognized as biotic factors. In addition, eDNA degradation is dependent on genomic properties such as target length, properties and origin of nucleic acid, *i.e*., mitochondrial or nuclear.

The idea developed here is to use the LAMP DNA amplification method for automatic gene-dependent identification of eDNA on-site in the field. LAMP is a modern isothermal DNA amplification technique that is well suited for use inside a lab-on-a-chip platform. Such an automatic analysis system arranged for execution on-site in the field can be operated by operators with lower requirements for

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molecular biology competence, compared to traditional DNA analyses in professional laboratories. Finally, the authors found that LAMP performance was also the best compared to PCR. The LAMP primers used in this study will be a support for building gene library databases for researchers, advisors and fisheries managers for development in LAMP detection of northern pike.

5 | CONCLUSIONS

In summary, in the comparison between LAMP and PCR assays, LAMP showed most significant sensitivity in detecting northern pike DNA template than PCR. The LAMP specificity test showed no amplification in closely related species, demonstrating the feasibility of LAMP as a potential on-site assay in the application of eDNA monitoring. Therefore, LAMP is a straightforward technique and cost effective for early detection of invasive species. Further, the authors recommend that the primers may be helpful for setting up the LAMP analysis in detecting the presence of *E. lucius* invasive species in fresh water.

ACKNOWLEDGEMENTS

We thank the Ministry of Education and Research (KD) and Norwegian Environment Agency for funding this work and the Norwegian Micro- and Nano-Fabrication Facility (project no.: 295864 NORFAB III) for instruments.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

ORCID

Lars Eric Roseng https://orcid.org/0000-0001-7644-5135

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How to cite this article: Jothinarayanan, N., Karlsen, F., & Roseng, L. E. (2023). Comparative evaluation of loop-mediated isothermal amplification and PCR for detection of *Esox lucius* housekeeping genes for use in on-site environmental monitoring. *Journal of Fish Biology*, 1–9. https://doi.org/10.1111/jfb.15476

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