EUROPEAN JOURNAL OF PHYCOLOGY, 2026
https://doi.org/10.1080/09670262.2026.2659221

A field-ready molecular workflow for sample-to-result detection of the harmful dinoflagellate species *Prorocentrum cordatum* (Prorocentraceae, Dinoflagellata) in coastal Brazilian waters

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ABSTRACT

Conventional monitoring of toxic phytoplankton is time consuming, requires large sample volumes and depends on expert taxonomic identification, highlighting the need for faster, more sensitive and widely accessible methods. The globally distributed dinoflagellate *Prorocentrum cordatum* contributes to harmful algal blooms, posing serious risks to marine ecosystems and coastal economies. This study presents a sample-to-result molecular assay for the detection of *P. cordatum* using the polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) targeting the ITS and *psbA* genes. Analyses were performed on small volumes of water (5–50 ml), from Santa Catarina Island, southern Brazil, which were concentrated with syringe filters and membranes. Sensitivity was evaluated through 10-fold serial dilutions of *P. cordatum* DNA, and specificity tested against three other potentially harmful dinoflagellates, *P. lima, P. hoffmannianum* and *Takayama acrotrocha*. Detection limits were 5.3×10^2 cells l^{-1} for ITS-PCR and 5.3×10^3 cells l^{-1} for *psbA*-PCR; and 5.3×10^5 and 5.3×10^6 cells l^{-1} for ITS-LAMP and *psb*A-LAMP, respectively. In field tests (n = 48, all $<10^6$ cells l^{-1}), *P. cordatum* was detected in 32 samples by ITS-PCR and 29 by *psb*A-PCR. In contrast, ITS-LAMP detected the species in only three samples, and *psb*A-LAMP in none. Overall, PCR proved highly sensitive and specific for detecting low cell densities, while LAMP, although less sensitive, offers a possible portable option for field monitoring during moderate-to-high bloom conditions.

HIGHLIGHTS

- *Prorocentrum cordatum* was isolated for molecular assay development.
- Biomass concentration was achieved using small volumes.
- PCR and LAMP assays targeting ITS and psbA were validated.

ARTICLE HISTORY Received 1 December 2025; Revised 25 March 2026; Accepted 7 April 2026

KEYWORDS Dinoflagellate; field samples; HAB; harmful algae; LAMP; PCR

Introduction

Prorocentrum cordatum (Ostenfeld) J.D.Dodge (Dinophyceae, synonyms *Prorocentrum minimum* (Pavillard) J.Schiller, *Exuviaella peisonins* J.Schiller and *Exuviaella pacifica* Kuz'mina) (Guiry *et al.*, 2023) is a bloom-forming marine microalga with a cosmopolitan distribution listed in the Taxonomic Reference List of Harmful Microalgae (Heil *et al.*, 2005; Lundholm *et al.*, 2009). In Brazil, *P. cordatum* has been reported mainly in southern states, occurring year-round in Paranaguá, Paraná, where it has been detected at high cell densities (Mafra *et al.*, 2006, 2024). In 2006 in Florianópolis (Alves *et al.*, 2010) and 2007 in Penha (Miotto & Tamanaha, 2012), Santa Catarina, *P. cordatum* was recorded at mollusc farms.

Although cases of human intoxication linked to *P. cordatum* have been reported, its toxicity and associated toxins remain uncertain, partly due to possible interactions with bacteria and biphasic blooms involving *Dinophysis acuminata* (Grzebyk *et al.*, 1997; Heil *et al.*, 2005). *Prorocentrum cordatum* has multiple nutrient uptake strategies and a mixotrophic metabolism, allowing it to thrive in nitrogen- and phosphorus-enriched environments, including hypoxic regions (Henrichs & Clegg, 2024), and to expand geographically in response to human activities (Stoecker *et al.*, 1997; Heil *et al.*, 2005; Glibert *et al.*, 2008; Khanaychenko *et al.*, 2019). Moreover, *P. cordatum* belongs to a group of organisms that benefit from coastal pollution, climate change and

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Published online 15 May 2026

ocean acidification (Findlay *et al.*, 2025), which increases its photochemical efficiency, energy flux and antioxidant capacity (Zhu *et al.*, 2024). Consequently, *P. cordatum* is considered a sentinel species of eutrophication in coastal ecosystems under the current climate emergency (Telesh *et al.*, 2021; Tenorio *et al.*, 2022).

In this context, several techniques are available for studying and identifying harmful algal species. Classical single-cell approaches based on light microscopy enable morphological identification and cell counting and provide high-resolution insights into cellular heterogeneity and strain variability, but they are time-consuming and difficult to scale. High-throughput single-cell analyses using next-generation flow cytometry, combined with genomic tools, enable detailed characterization of phytoplankton populations at unprecedented resolution (Hubert *et al.*, 2025). Omics-based approaches further expand detection capacity, with metabarcoding and metagenomics suited for ecosystem-scale monitoring, and transcriptomics for investigating bloom dynamics and toxin activity (Jacobs-Palmer *et al.*, 2021; Sawh *et al.*, 2025). However, the high cost and technical complexity of flow cytometry and omics methods limit their use in large-scale or long-term monitoring programmes.

Algal identification can also be achieved using molecular methods such as Polymerase Chain Reaction (PCR) and Loop-Mediated Isothermal Amplification (LAMP), which offer high sensitivity and specificity for dinoflagellates (Toldrà *et al.*, 2020). Quantitative PCR (qPCR), targeting the ITS rRNA as barcoding, have effectively detected *P. cordatum* in eastern Australian waters, advancing research on harmful microalgae (McLennan *et al.*, 2021). However, PCR, particularly when coupled with DNA sequencing, requires equipped laboratory and trained personnel, restricting its use in the field. By contrast, LAMP, which relies on isothermal amplification, requires minimal equipment and is well suited for on-site monitoring (Toldrà *et al.*, 2020). LAMP assays have already been developed for several harmful algal species. For example, Zhang *et al.* (2014) targeted the large subunit (LSU) of rDNA to detect *P. cordatum* using a rapid, low-cost, heat-based DNA extraction method from centrifuged biomass. Similarly, Lee *et al.* (2021) designed LAMP assays targeting the internal transcribed spacer (ITS) and LSU regions for *Ostreopsis* cf. *ovata* and *Amphidinium massartii*. Together, these studies highlight the potential of LAMP as a practical molecular tool for the field-based monitoring of harmful algal blooms.

Selecting an appropriate molecular marker is critical for nucleic acid amplification assays. The ITS region is widely used to identify harmful algal species due to its high variability (Granéli & Turner, 2006) and has been successfully applied in both PCR and LAMP assays for detecting various dinoflagellates, including *P. cordatum* (Zhang *et al.*, 2012; Huang *et al.*, 2017, 2020; Qin *et al.*, 2019; Wang *et al.*, 2019; Lee *et al.*, 2021; McLennan *et al.*, 2021; Yang *et al.*, 2024). Plastid genes are also potentially suitable for identifying phototrophic dinoflagellates but remain less explored. The D1 region of the chloroplast DNA (cpDNA) *psbA* gene has shown promise, revealing phylogenetic clustering of monophyletic species groups at the amino acid level (Takishita & Uchida, 1999; Granéli & Turner, 2006).

This study aimed to develop a sample-to-result molecular approach for monitoring *P. cordatum* and to evaluate its application in a confined coastal lagoon on the eastern side of Santa Catarina Island, within the municipality of Florianópolis, southern Brazil. Biomass was concentrated from small water volumes (5–50 ml) using a syringe filter and membrane-based protocol. Primers targeting the ITS and *psb*A genetic markers were designed for both PCR and LAMP assays, with sensitivity and specificity assessed. The assays were validated using natural and spiked field samples, and PCR amplicons were sequenced and analysed phylogenetically to confirm species identities.

Materials and methods

Sample collection and algal cultures

Four potentially toxic dinoflagellate strains were used in this study: *P. cordatum* (strain PCO-BVR-01), *Prorocentrum lima* (strain LM-049), *Prorocentrum hoffmannianum* (strain LM-059) and *Takayama acrotrocha* (strain TAC-BVR-01). *Prorocentrum cordatum* was collected with a phytoplankton net from Conceição lagoon, a coastal lagoon in Florianópolis, Brazil (Fig. 1). The lagoon ranges from mesotrophic to hypertrophic conditions (Silva *et al.*, 2017), favouring harmful algal blooms. *P. cordatum* (Supplementary fig. S1) was identified following Steidinger (1996) and isolated using the single-cell capillary method (Andersen, 2005). The other species were donated from the Laboratório de Ficologia (LAFIC-UFSC) and the Laboratório de Microalgas (CEM-UFPR).

P. cordatum and *T. acrotrocha* were cultured in 75 ml of modified f/2-Si medium, while *P. lima* and *P. hoffmannianum* were maintained in modified f/4-Si medium (Guillard, 1975). All cultures were grown at 20–22°C, salinity 30 psu, under ~100 µmol photons m^{-2} s^{-1} with a 12:12 h light-dark cycle and subcultured every 2 months. For cell counts, exponential-phase cultures were gently homogenized, fixed with 1% acid Lugol solution, and counted in triplicate using a Neubauer chamber (hemocytometer) under a light

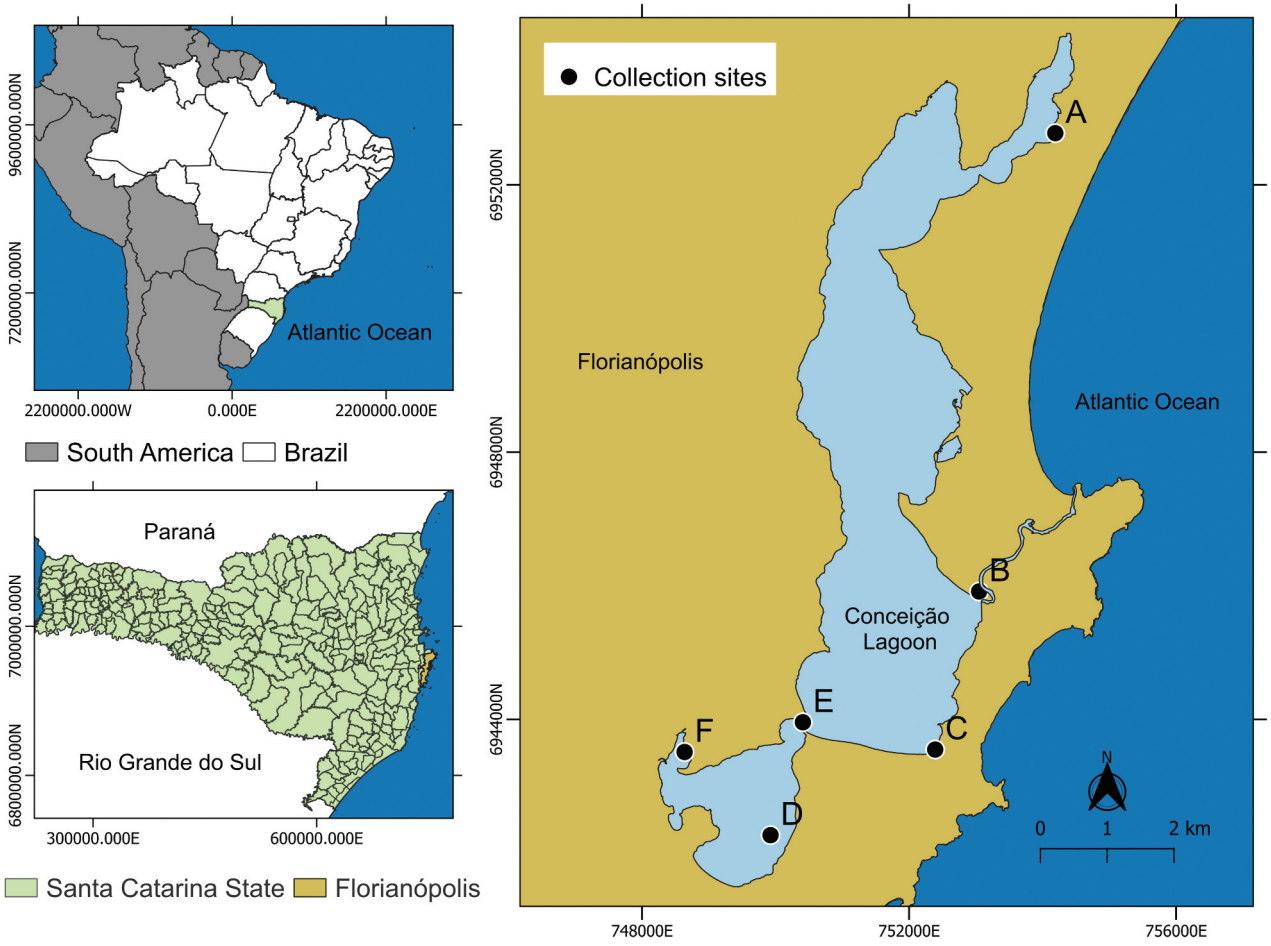

Fig. 1. Sampling sites for *Prorocentrum cordatum* monitoring in Conceição lagoon, Florianópolis – SC, Brazil. The top-left inset shows a map of Brazil with the state of Santa Catarina (SC) highlighted in green. Below, a magnified view of SC highlights the municipality of Florianópolis in brown. To the right, a detailed map of Florianópolis shows local water bodies in blue and sampling sites labelled alphabetically. The map's x- and y-axes represent UTM coordinates. Maps were generated using Quantum GIS (QGIS, version 3.34; QGIS Development Team, 2023).

microscope. Prior to counting, samples were examined by phase-contrast and dark-field microscopy to verify the absence of cell aggregates, confirming a random and homogeneous distribution of cells within the chamber fields.

Biomass concentration method and DNA extraction

Exponential-phase cultures and field samples were homogenized and concentrated using syringes (Descarpack), syringe filters (Millipore SX0002500 and SX0001300), and membranes (Millipore) (Supplementary fig. S2a). The biomass concentration protocol was adapted from Lee *et al.* (2021). For field samples, a 100 µm nylon pre-filter (Millipore NY1H2500) was used to remove macroalgae, zooplankton and other large particles (Supplementary fig. 2b). Subsequently, 10–60 ml of culture or field sample were drawn into syringes and filtered through a 0.22 µm polyether sulphone (Millipore GPWP02500) or mixed cellulose ester membrane (Millipore GSWP01300) to concentrate biomass (Supplementary fig. S2c). The biomass-containing filter was then rinsed with 2 ml of nuclease-free water to remove salts and dissolved substances. Membranes were dried on a paper towel for 2 min and stored at −80°C until further analysis (Supplementary fig. S2d).

Genomic DNA was extracted from retained biomass using either the DNeasy Blood & Tissue Kit (Qiagen) or a heat-based method. For the kit-based protocol, the entire membrane was eluted with AL buffer (Qiagen) and incubated following adaptations described by Eland *et al.* (2012). In the heat-based method, adapted from Zhang *et al.* (2014), the entire membrane was eluted in 100 µl of nuclease-free water and heated at 100°C for 10 min. DNA was quantified using the Qubit dsDNA BR Assay Kit (ThermoFisher Scientific, Q32851) on a Qubit 4 Fluorometer. All DNA extracts were stored at −20°C until use.

PCR primer design and assay

Two sets of PCR primers were developed to detect *P. cordatum*: one targeting the ITS1-5.8S-ITS2 region

of nuclear rDNA (PcorITS1 and PcorITS2, Table 1), and another targeting the *psb*A gene of chloroplast cpDNA (PcorpsbAF and PcorpsbAR, Table 1). Primers were designed using NCBI Primer-BLAST (Ye *et al.*, 2012) with sequences from GenBank (JX402086.1 and JX573313.1) and sequences generated in this study. Initially, a published primer set (McLennan *et al.*, 2021) targeting *P. cordatum* (ITSfwd and ITSrev, Table 1) was used to amplify a 664 bp fragment of the ITS1-5.8S-ITS2 rDNA region from DNA extracted from *P. cordatum* isolated in this study (see *Sample collection and algal culture*). The resulting amplicons (accession numbers: PV730001–PV730006) were sequenced and together with the reference sequence JX402086.1 served as templates for designing new primers (PcorITS1 and PcorITS2, Table 1) targeting a shorter 243 bp region. The *psb*A primers (PcorpsbAF and PcorpsbAR, Table 1) were designed based on the reference sequence JX573313.1, amplifying a 661 bp fragment of the D1 region.

PCR was performed in 25 µl reactions containing 1× buffer, 2 mM $MgCl_2$, 0.4 mM dNTPs, 0.4 µM of each primer, 1 U Hot Start Taq polymerase (Promega M7841), 10–20 ng DNA and nuclease-free water. Amplifications were run on a MiniAmp Plus Thermal Cycler (ThermoFisher Scientific) with the following programme: initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 45s, annealing at 60°C (ITS) or 65°C (*psb*A) for 45s, and 72°C for 45s; final extension at 72°C for 5 min. Products were visualized by electrophoresis on 1–1.5% agarose gels.

Sequencing and phylogenetic analysis

PCR amplicons generated with ITSfwd/ITSrev, PcorITS1/PcorITS2 and PcorpsbAF/PcorpsbAR were purified using the Wizard SV Clean-Up System (Promega A9281), following the manufacturer's instructions, and quantified with a NanoDrop Lite Plus spectrophotometer (ThermoFisher Scientific). Purified fragments were bidirectionally sequenced at the FIOCRUZ Sequencing Unit (RPT1A, Rio de Janeiro, Brazil) using Sanger sequencing on an ABI Prism 3730 DNA sequencer with the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems).

Chromatograms were visually inspected with CHROMAS version 2.4, and consensus sequences were assembled in SeqMan version 7.0. Species identity was confirmed using BLASTn, and sequences were aligned with ClustalW (Madeira *et al.*, 2024). Final sequences were deposited in GenBank (accession numbers: ITS: PV730001–PV730015; *psb*A: PV785871–PV785883).

Phylogenetic analysis of the *P. cordatum* *psb*A (PV785871–PV785883) and ITS (PV730001–PV730015) sequences from field and cultured samples were performed using the maximum-likelihood optimality criterion in IQ-TREE v2.4 (Nguyen *et al.*, 2015). The best-fit substitution models, TVM+F + I for *psb*A and K2P+G4 for ITS, were selected, and node support was assessed with 1000 bootstrap replicates. Resulting trees were visualized using iTOL (Letunic & Bork, 2021).

Design of LAMP primers and assay implementation

Consensus ITS1-5.8S-ITS2 (PV730001–PV730006) and *psb*A (PV785877–PV785880) sequences generated in this study were aligned with JX402086.1 and JX573313.1, respectively, using ClustalW (Madeira *et al.*, 2024). These alignments were used to design LAMP primers with the NEB LAMP Primer Design Tool v1.4.1 (https://lamp.neb.com/). Primer sets for both regions included a forward primer (F3), backward (reverse) primer (B3), forward inner primer (FIP) and backward (reverse) inner primer (BIP) (Notomi *et al.*, 2000) (Table 1).

Primers were diluted in nuclease-free water and combined into a 10× primer mix containing 16 µM each of FIP and BIP, and 2 µM each of F3 and B3. LAMP reactions were performed using the WarmStart® Colorimetric LAMP Master Mix (New England BioLabs, M1704) in a final volume of 10 µl, containing 5 µl of 2× Master Mix, 1 µl of 10× primer

Table 1. Sequences of primers used in this study.

Target (Assay)	Primer name	Sequence (5' → 3')
ITS (PCR)*	ITSfwd	TTCCGTAGGTGAACCTGCGG
	ITSrev	ATATGCTTAAATTCAGCGGGT
ITS (PCR)	PcorITS1	GTGGTCTCCACTCTCACATCT
	PcorITS2	CCCTGGCACACAGAAACA
*psb*A (PCR)	PcorpsbAF	AGCTGGAGTTGCATGTTGGA
	PcorpsbAR	TGGGAAGTTGTGTGCATTCCT
ITS (LAMP)	PcorITS-F3	TTCGCACGCATCCATTCG
	PcorITS-B3	ACATCCGTCGCTGAAAGTTG
	PcorITS-FIP	CTGCATGGGGGCATCGATGCTGAACAACAGTTGGTGAGGC
	PcorITS-BIP	TGTCCTTGCTGTCGGGTGTCAGAGTGGAGACCACTCAGG
*psb*A (LAMP)	PcorpsbA-F3	CATGGATCATTGGTAACTTCA
	PcorpsbA-B3	ACCATATACCTATTACTGGCC
	PcorpsbA-FIP	CATCCTCCTGTCCGAATATATAGCCTCTACTTGCAGAGACACTTG
	PcorpsbA-BIP	AGACATATAGCATATCAGCAGCACATGTAGGCTTCTTGAGTTGTT

*ITS Primer designed by McLennan *et al.* (2021).

mix and 4 µl of template DNA. Reactions were incubated at 65°C for 60 min in 0.2 ml microtubes in a dry bath (Kasvi).

For product detection, 1 µl of 1000× SYBR Green I (Invitrogen S7563) was added to the inner lid of each 0.2 ml tube before incubation and mixed into the reaction after the LAMP reaction was complete. Amplification was detected visually by SYBR Green I through both colour change observable to the naked eye and fluorescence under UV light and further confirmed by electrophoresis on a 2% agarose gel.

Specificity and sensitivity analyses of PCR and LAMP assays

For specificity testing, genomic DNA was extracted from four algal isolates, *P. cordatum, P. lima, P. hoffmannianum* and *T. acrotrocha* (see *Sample collection and algal culture* in Materials and methods), using the DNeasy Blood & Tissue Kit (Qiagen) (see *Biomass concentration method and DNA extraction* in Materials and methods). The DNA samples were tested using both PCR and LAMP assays, following the conditions described in the sections *PCR primer design and assay* and *Design of LAMP primers and assay implementation*, respectively.

To evaluate sensitivity, genomic DNA from *P. cordatum* (5.3×10^7 cells l^{-1} = 10 ng μl^{-1}) was serially diluted 10-fold (10 ng μl^{-1} to 10^{-6} ng μl^{-1}) and used as template for both PCR and LAMP assays.

A 4 ml aliquot of the environmental water, previously tested for the presence of *P. cordatum* by PCR and Utermöhl analysis, was spiked with 1 ml of *P. cordatum* culture in the exponential growth phase (1.2×10^7 cells l^{-1}) and 10-fold diluted down to 1.2×10^2 cells l^{-1} to further evaluate LAMP sensitivity in environmental conditions. Each dilution was tested in triplicate using the LAMP assay. Detection limits were estimated using probit regression analysis with MedCalc Statistical Software version 19.2.6 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org/).

Details of the visualization of PCR and LAMP products for both sensitivity and specificity analyses are provided in the sections *PCR primer design and assay* and *Design of LAMP primers and assay implementation*, respectively, both in Materials and methods.

Assay validation based on field-collected samples

A total of 48 environmental water samples was collected from six sites in Conceição Lagoon (Fig. 1; Table 2) during 2024 and 2025 to standardize field analyses. Both concentrated samples (phytoplankton net) and whole water samples (Van Dorn bottle) were included. The samples were grouped according to collection period: (i) January–February 2024, (ii) September 2024 and (iii) January 2025.

Group (i) comprised 20 concentrated samples collected from sites A–E. At each site, 5 ml and 50 ml of water were filtered through 13 mm and 25 mm syringe membranes, respectively. The 25 mm membranes were aseptically cut into small fragments using sterile scissors and transferred to 1.5–2.0 ml microcentrifuge tubes with sterile forceps for subsequent DNA extraction. DNA from the 5 ml samples was extracted using a heat-based protocol, whereas DNA from the 50 ml samples was extracted using the DNeasy Blood & Tissue Kit (Qiagen), following adaptations described in Materials and methods. Group (ii) included eight samples from sites D and F. Biomass was filtered onto 13 mm membranes until saturation. Concentrated samples saturated at 6–8 ml, while whole samples saturated at 6–10 ml (Table 2). Biomass was concentrated in duplicate for each collection method, and DNA was extracted using both the heat-based method and the DNeasy Blood & Tissue Kit. Group (iii) consisted of 20 concentrated and whole samples from sites A, B, C, D and F. As in Group (ii), biomass was filtered onto 13 mm membranes until saturation, with volumes ranging from 4–17 ml. DNA was extracted using only the heat-based method.

For cell quantification, each field sample was gently homogenized, and a subsample was fixed with 1% acid Lugol's solution. Fixed material was transferred to 10 ml Utermöhl chambers (limit of detection: 10^2 cells l^{-1}), settled for 24 hours, and analysed in triplicate using an inverted light microscope (Edler & Elbrächter, 2010).

DNA samples were tested with both PCR and LAMP assays targeting the ITS and *psb*A regions, following the conditions described in Materials and methods, using product visualization methods as described.

Results

Sensitivity and specificity

The ITS-PCR assay detected concentrations as low as 10^{-4} ng μl^{-1} (~5.3×10^2 cells l^{-1}), while the *psbA*-PCR detected down to 10^{-3} ng μl^{-1} (~5.3×10^3 cells l^{-1}) (Fig. 2). For LAMP, the ITS-based assay detected down to 10^{-1} ng μl^{-1} (~5.3×10^5 cells l^{-1}), whereas the *psbA*-LAMP assay detected only at 10° ng μl^{-1} (~5.3×10^6 cells l^{-1}) (Fig. 3). Overall, ITS assays were 10 times more sensitive than *psbA* assays.

Using spiked samples, the ITS-LAMP assay yielded positive results in all triplicates down to 1.2×10^5 cells l^{-1} (Fig. 4), with a detection limit of 2.4×10^4 cells l^{-1} ($p < 0.0001$) estimated by probit regression. For *psbA*-LAMP, all triplicates tested positive at 1.2×10^6 cells l^{-1}, with an estimated

Table 2. Validation of field samples collected in Conceição Lagoon. Environmental water samples collected from sites A–F in Conceição Lagoon (Fig. 1) during 2024 and 2025 and grouped by collection date: Group (i) January–February 2024, Group (ii) September 2024 and Group (iii) January 2025.

Collection date	Sample ID	Collection method	Collection site	Volume (ml)	Extraction method	PCR Analysis		LAMP Analysis		Utermöhl Quantification (cells l^{-1})
						ITS	*psb*A	ITS	*psb*A	
Group (i)	1	Concentrated	A	5	Heat	+	+	–	–	2.09×10^4
January 2024	2	Concentrated	B	5	Heat	+	+	–	–	2.38×10^4
	3	Concentrated	C	5	Heat	+	+	–	–	1.81×10^4
	4	Concentrated	D	5	Heat	+	+	+	–	1.87×10^5
	5	Concentrated	E	5	Heat	+	+	–	–	1.11×10^5
February 2024	6	Concentrated	A	5	Heat	–	–	–	–	1.43×10^3
	7	Concentrated	B	5	Heat	–	–	–	–	3.33×10^3
	8	Concentrated	C	5	Heat	–	–	–	–	8.55×10^3
	9	Concentrated	D	5	Heat	–	–	–	–	4.32×10^4
	10	Concentrated	E	5	Heat	+	–	–	–	9.50×10^3
January 2024	11	Concentrated	A	50	Kit	+	+	–	–	1.05×10^5
	12	Concentrated	B	50	Kit	+	+	–	–	1.19×10^5
	13	Concentrated	C	50	Kit	+	+	–	–	9.03×10^4
	14	Concentrated	D	50	Kit	+	+	+	–	9.33×10^5
	15	Concentrated	E	50	Kit	+	+	+	–	5.53×10^5
February 2024	16	Concentrated	A	50	Kit	–	–	–	–	7.13×10^3
	17	Concentrated	B	50	Kit	+	+	–	–	1.66×10^4
	18	Concentrated	C	50	Kit	+	+	–	–	4.28×10^4
	19	Concentrated	D	50	Kit	+	+	–	–	2.16×10^5
	20	Concentrated	E	50	Kit	+	+	–	–	4.75×10^4
Group (ii)	21	Concentrated	F	6	Kit	+	+	–	–	4.28×10^3
September 2024	22	Concentrated	F	6	Heat	+	+	–	–	4.28×10^3
	23	Concentrated	D	8	Kit	–	–	–	–	6.65×10^3
	24	Concentrated	D	8	Heat	+	+	–	–	6.65×10^3
	25	Whole	F	6	Kit	+	+	–	–	2.85×10^3
	26	Whole	F	6	Heat	+	+	–	–	2.85×10^3
	27	Whole	D	10	Kit	+	+	–	–	2.38×10^3
	28	Whole	D	10	Heat	+	+	–	–	2.38×10^3
Group (iii)	29	Concentrated	A	9	Heat	–	–	–	–	$<10^2$
January 2025	30	Concentrated	A	4	Heat	–	–	–	–	$<10^2$
	31	Whole	A	5	Heat	–	–	–	–	$<10^2$
	32	Whole	A	8	Heat	–	–	–	–	$<10^2$
	33	Concentrated	B	15	Heat	+	+	–	–	$<10^2$
	34	Concentrated	B	6	Heat	–	–	–	–	$<10^2$
	35	Whole	B	15	Heat	–	–	–	–	$<10^2$
	36	Whole	B	17	Heat	–	–	–	–	$<10^2$
	37	Concentrated	C	7	Heat	–	–	–	–	$<10^2$
	38	Concentrated	C	11	Heat	–	–	–	–	$<10^2$
	39	Whole	C	12	Heat	–	–	–	–	$<10^2$
	40	Whole	C	15	Heat	–	–	–	–	$<10^2$
	41	Concentrated	D	7	Heat	+	–	–	–	$<10^2$
	42	Concentrated	D	6	Heat	+	–	–	–	$<10^2$
	43	Whole	D	11	Heat	+	+	–	–	$<10^2$
	44	Whole	D	7	Heat	+	+	–	–	$<10^2$
	45	Concentrated	F	7	Heat	+	+	–	–	9.50×10^2
	46	Concentrated	F	5	Heat	+	+	–	–	3.80×10^3
	47	Whole	F	7	Heat	+	+	–	–	9.50×10^2
	48	Whole	F	8	Heat	+	+	–	–	1.90×10^3

Collection method: concentrated samples collected using a phytoplankton net, or whole water samples collected using a Van Dorn bottle; *Extraction method*: DNA extracted using the heat-based method, or the DNeasy Blood & Tissue Kit (Qiagen). + indicates that amplicons were detected in the assays; – indicates that no amplicons were detected.

detection limit of 329,092 cells l^{-1} ($p < 0.0001$) (Fig. 4).

Both PCR (Fig. 5) and LAMP (Fig. 6) assays targeting the ITS and *psb*A regions yielded specific amplifications for *P. cordatum*, with no detectable amplification in the other species.

Field sample validation in Conceição lagoon

Prorocentrum cordatum was identified in 32 of the 48 field-collected samples by Utermöhl quantification, with occurrences recorded across all six sampling sites (A–F, Fig. 1) in Conceição lagoon (Table 2). Among all sampling sites, D and F exhibited the highest cell densities in whole (quantitative) samples (samples 25–28 and 47–48), ranging from $>10^2$ to 2.85×10^3 cells l^{-1} (Table 2).

Group (i) concentrated samples (1–20, Table 2) were analysed using both heat- and kit-based DNA extraction methods to compare the two extraction approaches. PCR analysis detected *P. cordatum* in nine of the 10 samples extracted using the commercial kit (for both ITS and *psb*A markers), and in six and five samples extracted by heat treatment using ITS and *psb*A markers, respectively. LAMP assays detected *P. cordatum* in only three samples using the ITS marker: sample 4 (heat-based extraction) and samples 14 and 15 (kit-based extraction). All

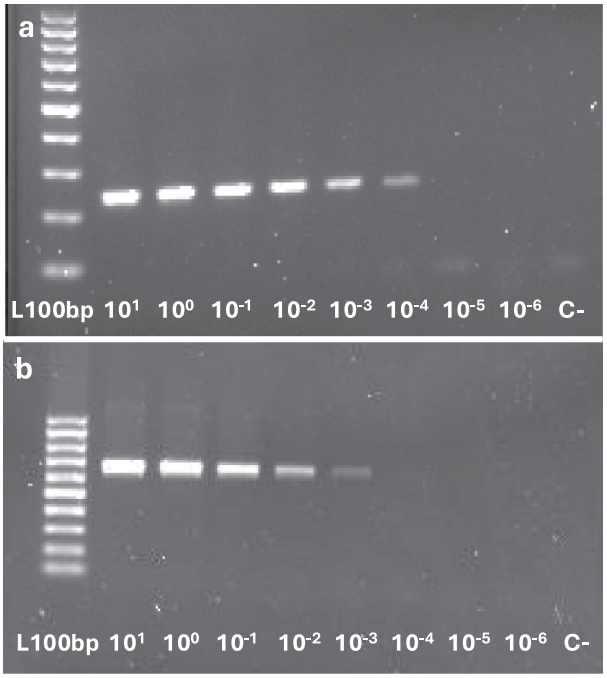

Fig. 2. Sensitivity of PCR assays. (a) ITS-PCR (243 bp) and (b) *psb*A-PCR (661 bp) assays tested with *Prorocentrum cordatum* DNA at concentrations ranging from 10^{1} to 10^{-6} ng µl^{-1}. Line L100 bp: 100 bp molecular marker (100 bp); lines 10 to 10^{-6}: serial dilutions of DNA; line C-: negative control (water only). The ITS-PCR assay demonstrated greater sensitivity than the *psb*A-PCR, detecting DNA concentrations as low as 10^{-4} ng µl^{-1} (approximately 5.3×10^{2} cells l^{-1}), while *psb*A-PCR detected down to 10^{-3} ng µl^{-1} (~5.3×10^{3} cells l^{-1}).

LAMP-positive samples had cell densities above 10^{5} cells l^{-1}. No amplification was observed with the *psb*A marker (Table 2, Supplementary fig. S3).

Group (ii) samples (21–28, Table 2) were analysed in duplicate using heat- and kit-based extraction methods, applied to concentrated and whole samples, in order to compare extraction and collection methods. PCR detected *P. cordatum* in seven of the eight samples with both ITS and *psbA* markers. In contrast, all LAMP assays were negative, consistent with Utermöhl counts of around 10^{3} cells l^{-1}, which fall below the LAMP detection limit (>10^{5} cells l^{-1}) (Table 2).

Group (iii) samples (29–48, Table 2) were analysed exclusively with heat-based DNA extraction to compare phytoplankton and Van Dorn-bottle collection methods. ITS-PCR detected *P. cordatum* in nine samples, while *psbA*-PCR detected it in seven (Table 2, Supplementary figs 3–4). Notably, concentrated sample 33 showed <10^{2} cells l^{-1} in the Utermöhl analysis, yet both PCR assays (ITS and *psbA*) successfully amplified *P. cordatum* DNA, despite this value being below the established PCR detection limit (>10^{2} cells l^{-1}). A similar result was observed in concentrated samples 41 and 42, where ITS-PCR detected *P. cordatum* despite Utermöhl counts of <10^{2} cells l^{-1}. The LAMP assays did not detect *P. cordatum* in any of the samples from this group.

Across all three sample groups (Table 2), PCR assays successfully detected *P. cordatum* in both

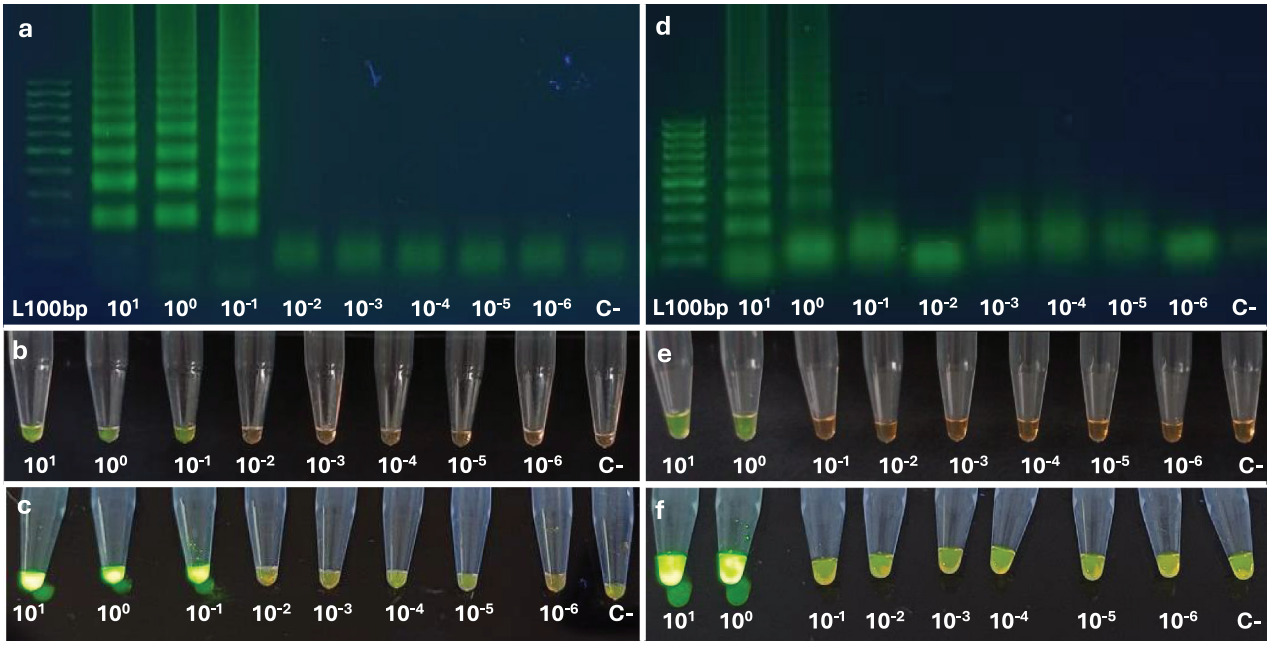

Fig. 3. Sensitivity of LAMP assays. Amplification of *Prorocentrum cordatum* DNA using ITS-LAMP and *psb*A-LAMP assays with DNA concentrations ranging from 10^{1} to 10^{-6} ng µl^{-1}, evaluated by: (a) and (d) electrophoresis on a 2% agarose gel; (b) and (e) visible colour change after staining with 1000 × SYBR Green I (Invitrogen S7563); and (c) and (f) fluorescence under UV light. Panels (a) to (c) show results for the ITS-LAMP assay, and (d) to (f) for the *psb*A-LAMP assay under the same conditions. Line L100bp: 100 bp molecular marker; lanes 10^{1} to 10^{-6}: serial DNA dilutions; lane C-: negative control (water only). The ITS-LAMP assay showed higher sensitivity, detecting DNA concentrations as low as 10^{-1} ng µl^{-1} (approximately 5.3×10^{5} cells l^{-1}), while the *psbA*-LAMP assay detected only at 10° ng µl^{-1} (5.3×10^{6} cells l^{-1}).

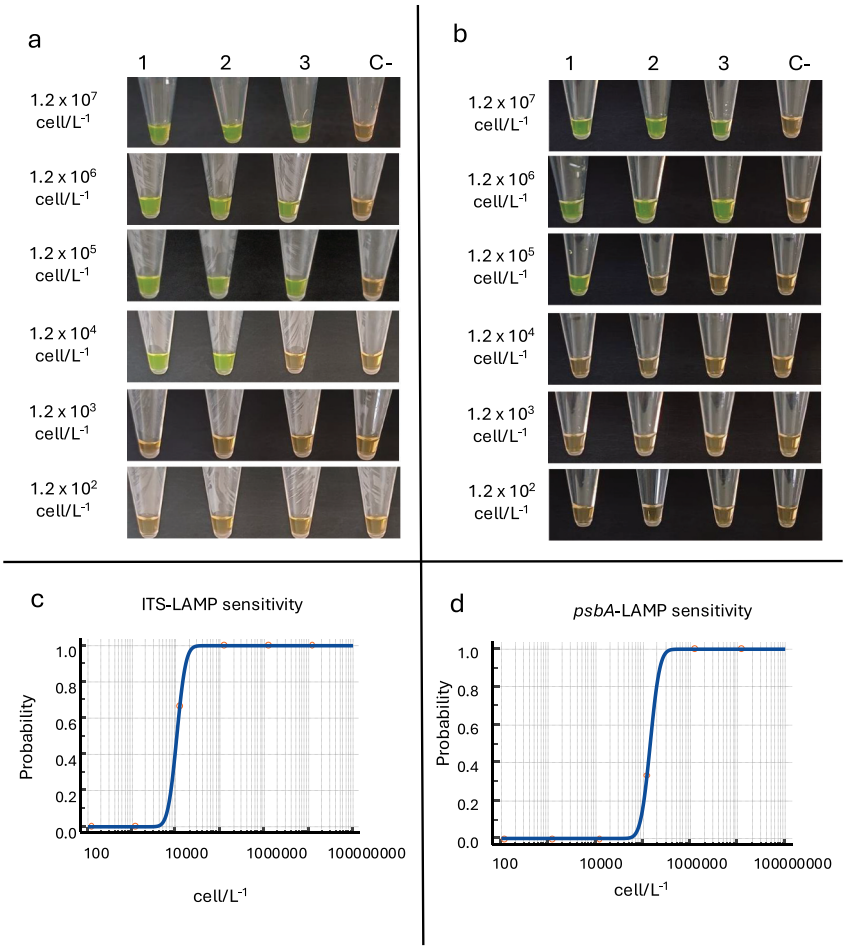

Fig. 4. Analytical sensitivity of LAMP assays. Colorimetric detection of *Prorocentrum cordatum* DNA using ITS-LAMP **(a)** and *psb*A-LAMP **(b)** assays, evaluated across concentrations from 1.2×10^7 to 1.2×10^2 cells l^{-1}, based on visible colour change after staining with 1000 × SYBR Green I (Invitrogen S7563). Each assay was run in triplicate (lines 1 to 3), with a negative control (C-, water only). Sensitivity for ITS **(c)** and *psbA* **(d)** assays were determined via probit regression. ITS-LAMP showed higher sensitivity, with all triplicates testing positive down to 1.2×10^5 cells l^{-1} and a detection limit of 24 012 cells l^{-1} ($p < 0.0001$), while the *psbA*-LAMP detected down to 1.2×10^6 cells l^{-1}, with a detection limit of 329 092 cells l^{-1} ($p < 0.0001$).

concentrated and whole samples, with ITS-PCR identifying the species in 32 samples and *psbA*-PCR in 29 (Table 2, Supplementary fig. S4–S5). PCR also proved more robust and sensitive than microscopy, detecting *P. cordatum* in samples 33 and 41–44 from Group (iii), despite Utermöhl counts indicating $<10^2$ cells l^{-1}. In contrast, LAMP assays returned positive results only in concentrated samples: ITS-LAMP detected *P. cordatum* in three cases (samples 4, 14 and 15), whereas *psb*A-LAMP detected none (Table 2, Supplementary fig. S3). These outcomes are consistent with spiked-sample trials, reflecting the lower sensitivity of *psb*A-LAMP (limit of detection ~10^6 cells l^{-1}) compared with ITS-LAMP (~10^5 cells l^{-1}) (Table 2, Fig. 4).

Positive amplicons from the *psb*A and ITS PCR assays in nine samples from Group (i) (11–15, 17–20, Table 2) were sequenced. NCBI database analysis confirmed >99% similarity to *P. cordatum*. In the phylogenetic trees, *psb*A and ITS sequences from these samples clustered with those from a *P. cordatum* culture isolated from Conceição lagoon, forming a well-supported monophyletic group (Fig. 7) and confirming species identity.

Discussion

Harmful algal bloom (HAB) events pose serious ecological, economic and public health risks, and their irregular occurrence, patchy distribution and rapid development make monitoring challenging (Anderson *et al.*, 2012; Wells *et al.*, 2015). Among detection methods, molecular techniques are particularly suitable for the monitoring and early detection of HAB-forming species due to their simplicity, efficiency and relatively low cost, making them ideal for field-based and point-of-care monitoring where rapid decision-making is essential (Zhang *et al.*, 2014; Toldrà *et al.*, 2020; Lee *et al.*, 2021).

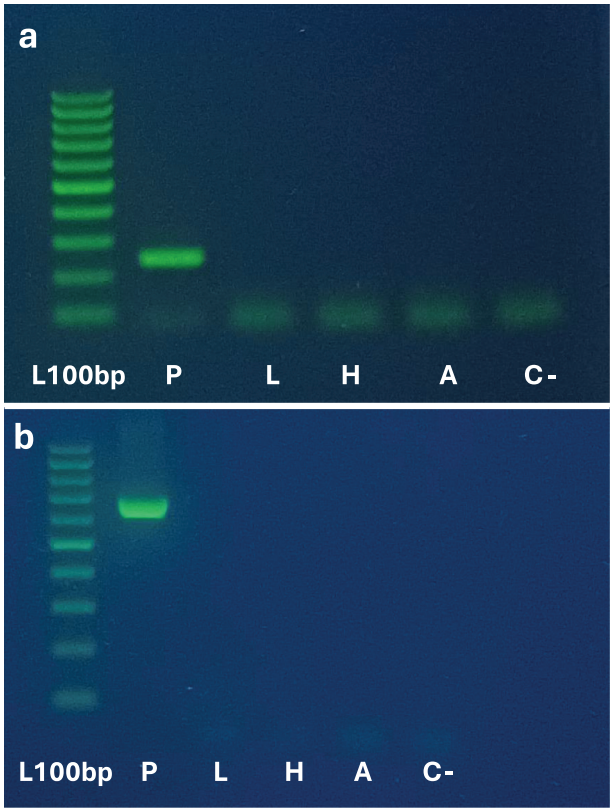

Fig. 5. Specificity of PCR assays. **(a)** ITS-PCR (243 bp) and **(b)** *psb*A-PCR (661 bp) assays were tested using DNA from *Prorocentrum cordatum* (P), *Prorocentrum lima* (L), *Prorocentrum hoffmannianum* (H) and *Takayama acrotrocha* (A) DNA at concentrations of 10 ng μl^{-1}. Line L100 bp: 100 bp molecular marker; line C-: negative control (water only). Both assays showed 100% specificity for *P. cordatum*, with no amplification observed for the other species.

In this study, molecular assays included both PCR and LAMP methods, targeting two molecular markers: ITS and *psb*A. For both assay types, ITS analyses were 10 times more sensitive than *psb*A, likely due to differences in gene copy number. The ribosomal cistron, which includes the ITS, is estimated at ~398 ± 184 copies per *P. cordatum* cell (Liu *et al.*, 2021), whereas *psb*A copy numbers in dinoflagellates are more variable and generally lower (Barbrook *et al.*, 2006; Iida *et al.*, 2009). Nevertheless, despite its lower sensitivity, the *psb*A-PCR assay still detected relatively low cell concentrations (5.3×10^3 cells l^{-1}), highlighting its potential as a low-copy genetic marker. These results align with previous molecular applications for harmful dinoflagellate detection, including LAMP and qPCR assays for *Prorocentrum* spp. (Chen *et al.*, 2013; Zhang *et al.*, 2014; McLennan *et al.*, 2021; Yang *et al.*, 2024), *Alexandrium* sp. (Wang *et al.*, 2008; Zhang *et al.*, 2012), *Amphidinium* spp. (Wang *et al.*, 2019; Lee *et al.*, 2021), *Chattonella* spp. (Qin *et al.*, 2019) and *Karenia* spp. (Zhang *et al.*, 2009; Huang *et al.*, 2020; Wang *et al.*, 2020). Accordingly, the ITS1 and ITS2 regions have previously been used for the specific detection of *P. cordatum* by qPCR (McLennan *et al.*, 2021), and, in line with these findings, our ITS-PCR assay also specifically amplified *P. cordatum*. In contrast, this study is the first to demonstrate specific amplification using the *psb*A-PCR assay, as this molecular marker has been comparatively less explored.

In addition to differences in molecular marker performance, this study also revealed variation between amplification methods, with PCR being up to 1000 times more sensitive than LAMP under the tested conditions. Differences in sensitivity between LAMP and PCR are known to be assay-dependent and influenced by factors such as primer design, enzyme performance, reaction configuration and the presence of inhibitors in environmental samples (Khan *et al.*, 2018; Meagher *et al.*, 2018). However, the lower sensitivity of LAMP may actually be advantageous for point-of-care surveillance, since dinoflagellates such as *P. cordatum* typically reach hazardous levels only at higher cell densities, being classified as potentially harmful at concentrations exceeding 10 000 cells l^{-1} (intermediate effects) and 100 000 cells l^{-1} (severe effects) (Mafra Jr. *et al.*, 2024). Consistent with these thresholds, our results obtained from spiked samples demonstrated that LAMP effectively detected moderate to high *P. cordatum* cell densities. Accordingly, our assays were optimized to target cell densities relevant to bloom events, which commonly exceed 10^6 cells l^{-1}, supporting their use for anticipating and monitoring HAB caused by *P. cordatum*, a species considered harmful due to its bloom-forming capacity and potential toxicity (Heil *et al.*, 2005; Lundholm *et al.*, 2009).

In South America, a bloom of *P. cordatum* was reported in Peru in 2017 (Tenorio *et al.*, 2022), while in Brazil cell densities reached up to 1.7×10^5 cells l^{-1} in Paranaguá Bay in 2003 (Mafra *et al.*, 2006). In Santa Catarina, the species has been recorded in Penha and Florianópolis, with densities reaching 7.2×10^3 cells l^{-1} (Alves *et al.*, 2010; Miotto & Tamanaha, 2012). These findings demonstrate that *P. cordatum* is a recurrent and widespread component of the coastal plankton community in southern Brazil, and its elevated abundance at sites D and F in Conceição Lagoon is probably linked to its mixotrophic behaviour and ability to thrive under eutrophic conditions characteristic of the lagoon's southern region (Stoecker *et al.*, 1997; Heil *et al.*, 2005; Glibert *et al.*, 2008; Silva *et al.*, 2017; Khanaychenko *et al.*, 2019). Although the observed

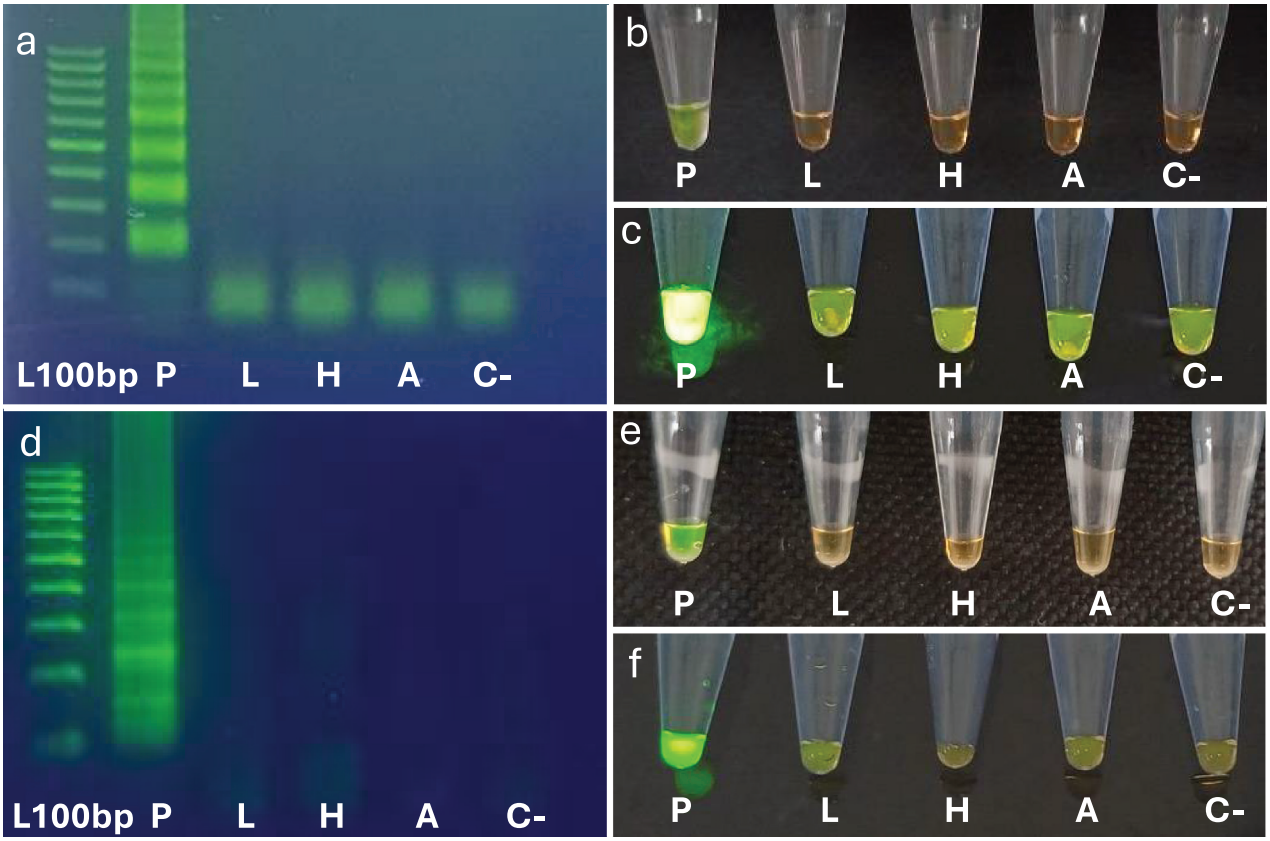

Fig. 6. Specificity of LAMP assays. Amplification of *Prorocentrum cordatum* (P), *Prorocentrum lima* (L), *Prorocentrum hoffmannianum* (H) and *Takayama acrotrocha* (A) DNA at concentrations of 10 ng μl^{-1}, assessed by: **(a)** and **(d)** electrophoresis on a 2% agarose gel; **(b)** and **(e)** visible colour change after staining with 1000 × SYBR Green I (Invitrogen S7563); and **(c)** and **(f)** fluorescence under UV light. Panels (a) to (c) show results for the ITS-LAMP assay, and panels (d) to (f) for the *psbA*-LAMP assay under the same conditions. Line L100 bp: 100 bp molecular marker; lane C-: negative control (water only). Both assays showed 100% specificity for *P. cordatum*, with no amplification observed for the other species.

densities may not pose an immediate ecological risk, they highlight the need for continued monitoring and a better understanding of local phytoplankton dynamics, reinforcing the role of *P. cordatum* as a potential sentinel species for eutrophication in the lagoon.

Addressing this need for effective monitoring, the present study established a syringe-based biomass concentration method to simplify and improve phytoplankton molecular analysis, enabling the detection of *P. cordatum* by both PCR and LAMP assays in concentrated and whole field samples using volumes as small as 5 ml (Table 2, Supplementary fig. S2). This approach aligns with current best practices, as sample filtration is regarded as the most appropriate strategy for molecular diagnostic and metagenomic applications, as it effectively concentrates target cells while preserving nucleic acid integrity. Conventional filtration protocols commonly rely on vacuum or peristaltic pumps operated until filter clogging, as reported by Hubert *et al.* (2025), resulting in moderate cell enrichment and requiring additional infrastructure. In contrast, the manual low-volume syringe filtration employed in this study enabled high concentrations of cells per membrane without the need for powered equipment. This approach is particularly well suited for field-based applications and monitoring programmes conducted under limited-resource conditions, while still yielding sufficient biomass for sensitive molecular detection.

This study presents a validated set of molecular methods for detecting *P. cordatum*, providing a rapid and broadly applicable tool for phytoplankton monitoring in coastal waters (Fig. 8). PCR and LAMP assays were complementary: PCR offered a highly sensitive and specific laboratory-based method capable of detecting *P. cordatum* at low cell densities, whereas LAMP demonstrated potential as a practical, field-friendly alternative suitable for detecting higher concentrations typical of bloom conditions. Across different sites and cell densities, both molecular markers were effective, with the ITS

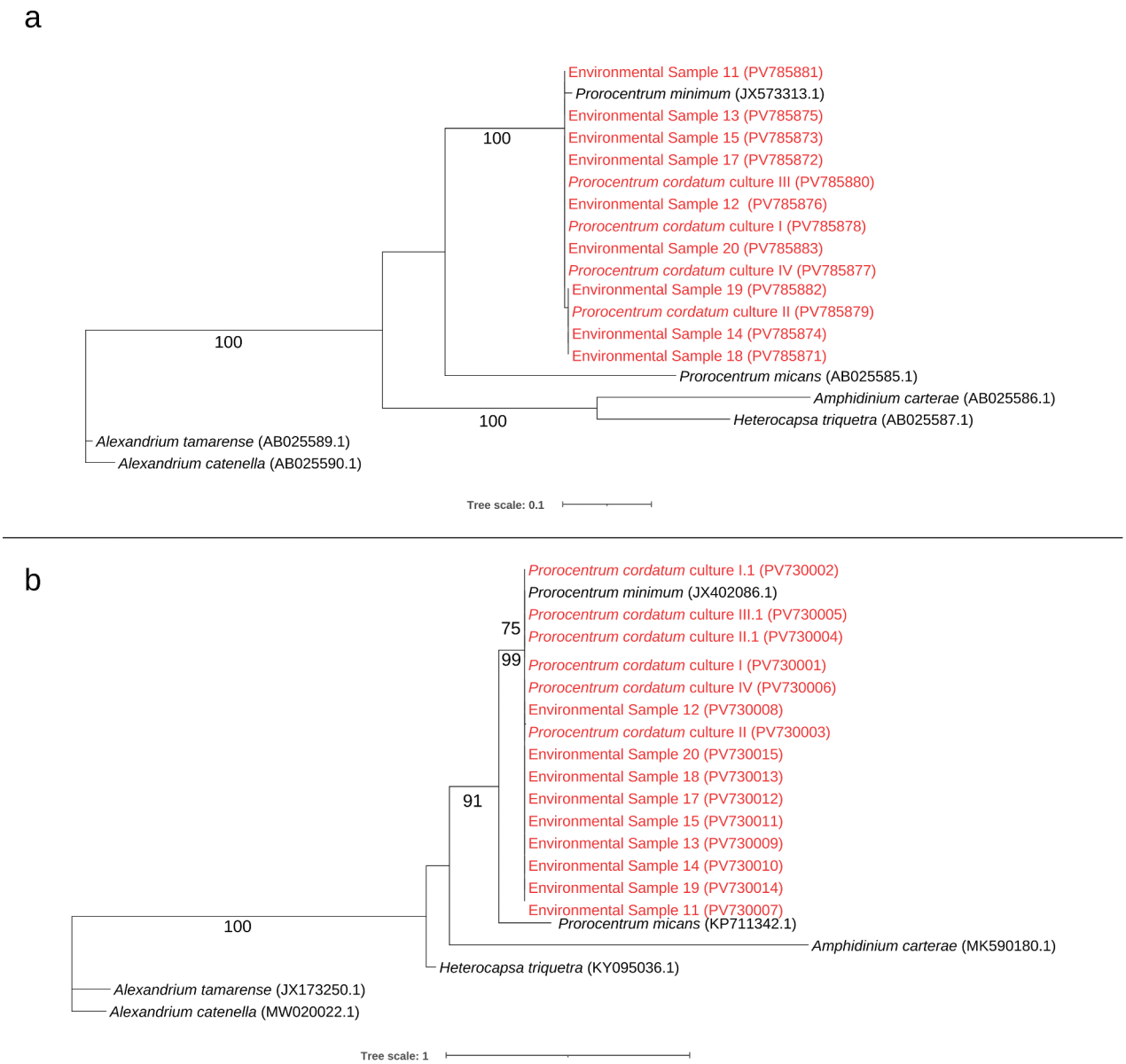

Fig. 7. Maximum likelihood analysis of *Prorocentrum cordatum* based on *psb*A **(a)** and ITS **(b)** sequences, constructed using the TVM+F + I and K2P+G4 models, respectively. The analysis included nine sequences from environmental samples (11–15 and 17–20, Table 2) obtained in this study. Additionally, six ITS and four *psb*A sequences from *P. cordatum* isolates in this study (–IV) were analysed, together with additional dinoflagellate sequences used as outgroups (accession numbers in parentheses). The results strongly indicate that all nine environmental samples are assignable to *P. cordatum*, as they share monomorphic *psb*A and ITS profiles and form a monophyletic clade with *P. cordatum* isolates from Florianópolis and South Korea (contigs JX573313.1 and JX402086.1). Bootstrap values (based on 1000 replicates) are shown at the nodes; only values above 75% are displayed.

region showing greater sensitivity than the *psb*A gene. Sequence comparisons (>99% similarity in NCBI) and phylogenetic analyses confirmed species identity for both markers. In addition, a simple and efficient sampling strategy was established for both concentrated and whole samples. Biomass could be concentrated using syringe filters with volumes as small as 5 ml, while a rapid, low-cost DNA extraction method requiring only nuclease-free water and a heat block proved effective in both field and laboratory settings. Together, these methods provide an adaptable workflow for detecting harmful dinoflagellates and offer practical support for environmental risk management and the investigation of *P. cordatum* dynamics in coastal lagoon systems.

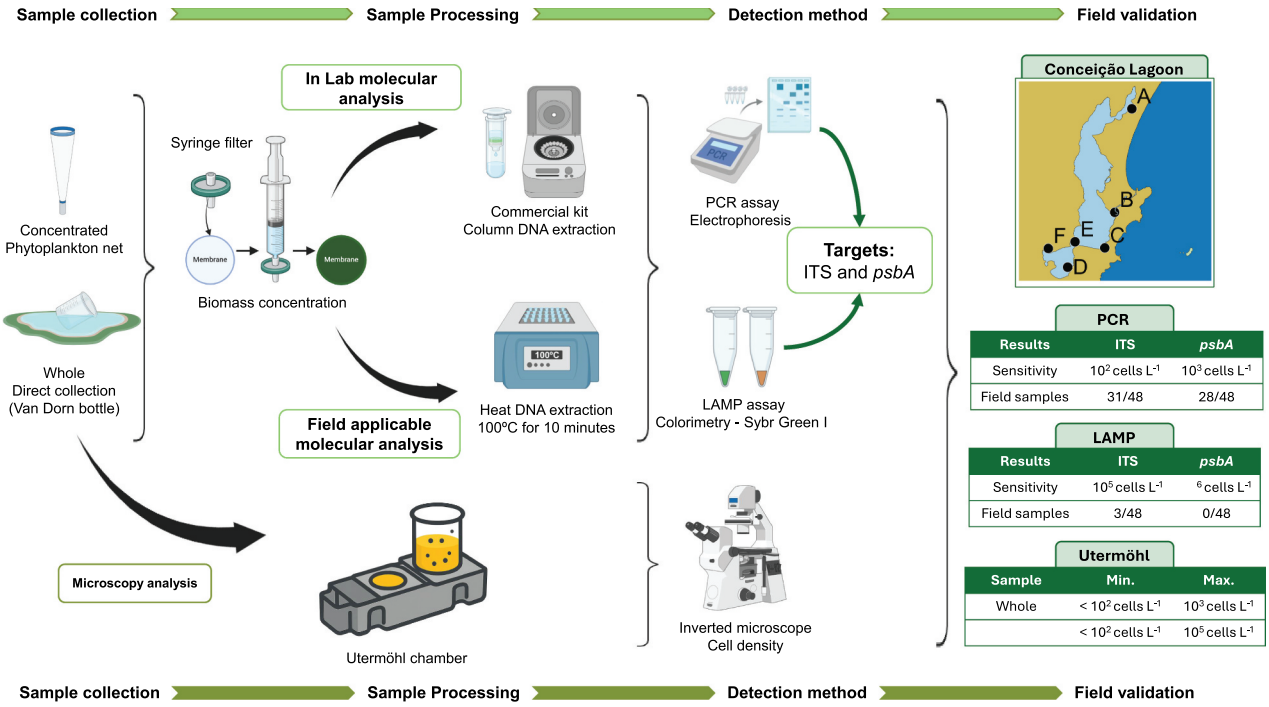

Fig. 8. *Prorocentrum cordatum* detection workflow. Concentrated water samples (collected using a phytoplankton net) and whole water samples (collected using Van Dorn bottles) from Conceição Lagoon, Florianópolis, Brazil, were analysed using two molecular methods (PCR and LAMP) and compared with gold-standard quantification by the Utermöhl chamber method. Biomass was concentrated using syringe filters, followed by either column-based or heat-based DNA extraction. PCR and LAMP assays targeting ITS (rDNA) and *psb*A (cpDNA) were then performed. The PCR assay enabled sensitive detection at low cell densities, whereas the LAMP assay detected higher cell concentrations.

Acknowledgements

The authors are grateful to PDTIS-FIOCRUZ for access to the DNA sequencing facility, LAMEB-UFSC for providing infrastructure and equipment and Dr Luiz L. Mafra Jr. from the Universidade Federal do Paraná for donating the algal cultures. We also acknowledge support from FAPESC, CNPq, CAPES and Rede Brasileira de Pesquisas sobre Mudanças Climáticas Globais (Rede Clima). This paper forms part of the PhD thesis of Andre Akira Gonzaga Yoshikawa, developed within the Programa de Pós-Graduação em Biologia Celular e do Desenvolvimento (PPGBCD) at the Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina [FAPESC/2022TR001374], IOC-FIOCRUZ and the Wellcome Trust [grant number: 207486/Z/17/Z].

Supplementary material

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at https://doi.org/10.1080/09670262.2026.2659221

Supplementary fig. S1. *Prorocentrum cordatum* from strain number PCO-BVR-01 observed under light microscopy using an Olympus BX-41 microscope at 100× magnification, with measurements performed in ImageJ. (a) Dorsal view of a cell, length = 15.229 µm; (b) dorsal view of an elongated cell, width = 9.251 µm; (c) ventral view of a cell, width = 13.742 µm.

Supplementary fig. S2. Biomass concentration by filtration. (a) Syringe (Descarpack) fitted with a syringe filter (Millipore SX0002500 and SX0001300); (b) Field samples pre-filtered using a 100 µm nylon membrane (Millipore, NY1H2500); (c) and (d) Mixed cellulose esters (MCE) 0.22 µm membrane (Millipore GSWP01300) before and after biomass concentration, respectively.

Supplementary fig. S3. ITS-LAMP field sample analysis. DNA at concentrations of 10 g μl^{-1} assessed by visible colour change after staining with 1000× SYBR Green I (Invitrogen S7563). Line L100bp: 100 bp molecular marker; lane C-: negative control (water only); C+: positive control of 10 ng $\mu l^{-1}$of *P. cordatum* gDNA.

Supplementary fig. S4. ITS-PCR (243bp) field sample analysis. DNA at concentrations of 10 ng μl^{-1}. Line L100 bp: 100 bp molecular marker; line C-: negative control (water, no DNA); C+: positive control of 10 ng μl^{-1} with *P. cordatum* gDNA.

Supplementary fig. S5. *psbA*-PCR (661bp) field sample analysis. DNA at concentrations of 10 ng μl^{-1}. Line L100 bp: 100 bp molecular marker; line C-: negative control (water, no DNA); C+: positive control of 10 ng μl^{-1} with *P. cordatum* gDNA.

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References

Alves, T.P., Schramm, M.A., Tamanaha, M.S. & Proença, L. A.O. (2010). Implementação e avaliação do monitoramento de algas nocivas e de ficotoxinas em um cultivo de moluscos em Florianópolis – SC. *Atlântica*, **32**: 71–78.

Andersen, R.A., Ed. (2005). *Algal Culturing Techniques*. Academic Press, Burlington.

Anderson, D.M., Cembella, A.D. & Hallegraeff, G.M. (2012). Progress in understanding harmful algal blooms (HABs): paradigm shifts and new technologies for research, monitoring, and management. *Annual Review of Marine Science*, **4**: 143–176.

Barbrook, A.C., Visram, S., Douglas, A.E. & Howe, C.J. (2006). Molecular diversity of dinoflagellate symbionts of Cnidaria: the *psbA* minicircle of *Symbiodinium*. *Protist*, **157**: 159–171.

Chen, G., Ma, C., Zhang, C., Zhou, J., Wang, Y., Wang, G., Zhang, B., Zu, Z. & Lu, D.D. (2013). A rapid and sensitive method for field detection of *Prorocentrum donghaiense* using reverse transcription-coupled loop-mediated isothermal amplification. *Harmful Algae*, **29**: 31–39.

Edler, L. & Elbrächter, M. (2010). The Utermöhl Method For Quantitative Phytoplankton Analysis. In *Microscopic and Molecular Methods For Quantitative Phytoplankton Analysis* (Karlson, B., Cusack, C. & Bresnan, E., eds.), 13–20. UNESCO, Paris.

Eland, L.E., Davenport, R. & Mota, C.R. (2012). Evaluation of DNA extraction methods for freshwater eukaryotic microalgae. *Water Research*, **46**: 5355–5364.

Findlay, H.S., Feely, R.A., Jiang, L.-Q., Pelletier, G. & Bednaršek, N. (2025). Ocean Acidification: another Planetary Boundary Crossed. *Global Change Biology*, **31**: e70238.

Glibert, P.M., Mayorga, E. & Seitzinger, S. (2008). *Prorocentrum minimum* tracks anthropogenic nitrogen and phosphorus inputs on a global basis: application of spatially explicit nutrient export models. *Harmful Algae*, **8**: 33–38.

Granéli, E. & Turner, J.T., Eds. (2006). *Ecology of Harmful Algae*. Ecological Studies. Springer, Berlin, Heidelberg.

Grzebyk, D., Denardou, A., Berland, B. & Pouchus, Y.F. (1997). Evidence of a new toxin in the red-tide dinoflagellate *Prorocentrum minimum*. *Journal of Plankton Research*, **19**: 1111–1124.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In *Culture of marine invertebrate animals: proceedings — 1st conference on culture of marine invertebrate animals, Greenport* (Smith, W.L. & Chanley, M.H., eds.), 29–60. Springer US, Boston.

Guiry, G.M., Guiry, M.D. & Guiry, G.M. (2023). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org/search/species/detail/?species_id=71381; searched on 16 January 2026.

Heil, C.A., Glibert, P.M. & Fan, C. (2005). *Prorocentrum minimum* (Pavillard) Schiller: a review of a harmful algal bloom species of growing worldwide importance. *Harmful Algae*, **4**: 449–470.

Henrichs, D. & Clegg, R.T. (2024). *Dead Zone Preference: Changes In Dinoflagellate Vertical Migration In the Presence of Hypoxia*. American Geophysical Union, Ocean Sciences Meeting, New Orleans. 18-23 February 2024 Poster No. CC14A-1186

Huang, H.-L., Gao, W.-F., Zhu, P., Zhou, C.-X., Qiao, -L.-L., Dang, C.-Y., Pang, J.-H. & Yan, X.-J. (2020). Molecular method for rapid detection of the red tide dinoflagellate *Karenia mikimotoi* in the coastal region of Xiangshan Bay, China. *Journal of Microbiological Methods*, **168**: 105801.

Huang, H.-L., Zhu, P., Zhou, C.-X., He, S. & Yan, X.-J. (2017). The development of loop-mediated isothermal amplification combined with lateral flow dipstick for detection of *Karlodinium veneficum*. *Harmful Algae*, **62**: 20–29.

Hubert, Z., Artigas, L.F., Li, -L.-L., Dédécker, C. & Monchy, S. (2025). Exploring the Regional Diversity of Eukaryotic Phytoplankton in the English Channel by Combining High-Throughput Approaches. *Microbiologyopen*, **14**: e70097.

Iida, S., Kobiyama, A., Ogata, T. & Murakami, A. (2009). Identification of transcribed and persistent variants of the *psbA* gene carried by plastid minicircles in a dinoflagellate. *Current Genetics*, **55**: 583–591.

Jacobs-Palmer, E., Gallego, R., Cribari, K., Keller, A.G. & Kelly, R.P. (2021). Environmental DNA metabarcoding for simultaneous monitoring and ecological assessment of many harmful algae. *Frontiers In Ecology and Evolution*, **9**: 612107.

Khan, M., Wang, R., Li, B., Liu, P., Weng, Q. & Chen, Q. (2018). Comparative Evaluation of the LAMP Assay and

PCR-Based Assays for the Rapid Detection of *Alternaria solani*. *Frontiers In Microbiology*, **9**: 2089.

Khanaychenko, A.N., Telesh, I.V. & Skarlato, S.O. (2019). Bloom-forming potentially toxic dinoflagellates *Prorocentrum cordatum* in marine plankton food webs. *Protistology*, **13**: 95–125.

Lee, E.S., Hwang, J., Hyung, J.-H. & Park, J. (2021). Detection of the benthic dinoflagellates, *Ostreopsis* cf. *ovata* and *Amphidinium massartii* (Dinophyceae), using loop-mediated isothermal amplification. *Journal of Marine Science and Engineering*, **9**: 885.

Letunic, I. & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, **49**: W293–W296.

Liu, Y., Hu, Z., Deng, Y., Shang, L., Gobler, C.J. & Tang, Y. Z. (2021). Dependence of genome size and copy number of rRNA gene on cell volume in dinoflagellates. *Harmful Algae*, **109**: 102108.

Lundholm, N., Bernard, C., Churro, C., Escalera, L., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø., Murray, S., Salas, R., Tillmann, U. & Zingone, A. (Eds.). (2009). *IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae*. https://www.marinespecies.org/hab.https://doi.org/10.14284/362; searched on 13 March 2025.

Madeira, F., Madhusoodanan, N., Lee, J., Eusebi, A., Niewielska, A., Tivey, A.R.N., Lopez, R. & Butcher, S. (2024). The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. *Nucleic Acids Research*, **52**: W521–W525. 10.1093/nar/gkae241

Mafra, L.L., Jr., Escobar, B.P. & Sobrinho, B.F. (2024). Harmful algae in the Paranaguá Estuarine Complex, Brazil: a spatio-temporal assessment. *Ocean and Coastal Research*, **72**: e24008.

Mafra, L.L., Jr., Fernandes, L.F. & Proença, L.A.O. (2006). Harmful algae and toxis in paranaguá bay, Brazil: bases for monitoring. *Brazilian Journal of Oceanography*, **54**: 1–16.

McLennan, K., Ruvindy, R., Ostrowski, M. & Murray, S. (2021). Assessing the use of molecular barcoding and qPCR for investigating the ecology of *Prorocentrum minimum* (Dinophyceae), a harmful algal species. *Microorganisms*, **9**: 510.

Meagher, R.J., Priye, A., Light, Y.K., Huang, C. & Wang, E. (2018). Impact of primer dimers and self-amplifying hairpins on reverse transcription loop-mediated isothermal amplification detection of viral RNA. *Analyst*, **143**: 1924–1933.

Miotto, M.C. & Tamanaha, M.S. (2012). Occurrence of potentially toxic and harmful thecate dinoflagellates in mussel farms at Penha municipality, SC. *Brazilian Journal of Aquatic Science and Technology*, **16**: 53–67.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**: 268–274.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, **28**: E63.

QGIS Development Team (2023). *QGIS Geographic Information System*. Open Source Geospatial Foundation. URL: https://qgis.org; searched on 02 February 2025.

Qin, Y., Chen, G., Zhang, C., Wang, Y. & Zhou, J. (2019). Development of loop-mediated isothermal amplification combined with a chromatographic lateral-flow dipstick for rapid detection of *Chattonella marina*. *Harmful Algae*, **89**: 101666.

Sawh, S.K., Merabet, S., Higazy, N., Béji, M., Sørensen, J. M., Range, P., Alqudah, A.M. & Daly Yahia, M.N. (2025). Environmental DNA metabarcoding in marine ecosystems: global advances, methodological challenges, and applications in the MENA region. *Biology*, **14**: 1467.

Silva, V.E.C., Franco, D., Fonseca, A.L., Fontes, M.L. & Donnangelo, A.R. (2017). Space-time evolution of the trophic state of a subtropical lagoon: lagoa da Conceição, Florianópolis Island of Santa Catarina, Brazil. *Revista Brasileira De Recursos Hídricos*, **22**: e10.

Steidinger, K.A. & Tangen, K. (1996). Dinoflagellates. In *Identifying Marine Diatoms and Dinoflagellates* (Tomas, C.R., ed.), 387–598. Academic Press, New York.

Stoecker, D.K., Li, A., Coats, D.W., Gustafson, D.E. & Nannen, M.K. (1997). Mixotrophy in the dinoflagellate *Prorocentrum minimum*. *Marine Ecology Progress Series*, **152**: 1–12.

Takishita, K. & Uchida, A. (1999). Molecular cloning and nucleotide sequence analysis of *psbA* from the dinoflagellates: origin of the dinoflagellate plastid. *Phycological Research*, **47**: 207–216.

Telesh, I., Schubert, H. & Skarlato, S. (2021). Abiotic stability promotes dinoflagellate blooms in marine coastal ecosystems. *Estuarine, Coastal and Shelf Science*, **251**: 107239.

Tenorio, C., Álvarez, G., Perez-Alania, M., Blanco, J.L., Paulino, C., Blanco, J. & Uribe, E. (2022). Bloom of *Prorocentrum cordatum* in Paracas Bay, Peru. *Diversity*, **14**: 844.

Toldrà, A., O'Sullivan, C.K., Diogène, J. & Campàs, M. (2020). Detecting harmful algal blooms with nucleic acid amplification-based biotechnological tools. *Science of the Total Environment*, **749**: 141605.

Wang, L., Chen, G.F., Zhang, C.Y., Wang, Y.Y. & Sun, R. (2019). Rapid and sensitive detection of *Amphidinium carterae* by loop-mediated isothermal amplification combined with a chromatographic lateral-flow dipstick. *Molecular and Cellular Probes*, **43**: 72–79.

Wang, L., Li, L., Alam, M.J., Geng, Y., Li, Z., Yamasaki, S. & Shi, L. (2008). Loop-mediated isothermal amplification method for rapid detection of the toxic dinoflagellate *Alexandrium*, which causes algal blooms and poisoning of shellfish. *FEMS Microbiology Letters*, **282**: 15–21.

Wang, L., Zhang, C., Chen, G., Wang, Y. & Fu, M. (2020). Development of a rapid screening test for *Karenia mikimotoi* by using loop-mediated isothermal amplification and lateral flow dipstick. *Journal of Applied Phycology*, **32**: 3143–3155.

Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M. & Cochlan, W.P. (2015). Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae*, **49**: 68–93.

Yang, C., Zhen, Y., Hou, J. & Mi, T. (2024). Development of a rapid detection method to *Prorocentrum Lima* by loop-mediated isothermal

amplification with hydroxy naphthol blue. *Marine Biotechnology*, **26**: 475–487.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S. & Madden, T.L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, **13**: 134.

Zhang, F., Ma, L., Xu, Z., Zheng, J., Shi, Y., Lu, Y. & Miao, Y. (2009). Sensitive and rapid detection of *Karenia mikimotoi* (Dinophyceae) by loop-mediated isothermal amplification. *Harmful Algae*, **8**: 839–842.

Zhang, F., Shi, Y., Jiang, K., Song, W., Ma, C., Xu, Z. & Ma, L. (2014). Rapid detection and quantification of *Prorocentrum minimum* by loop-mediated isothermal amplification and real-time fluorescence quantitative PCR. *Journal of Applied Phycology*, **26**: 1379–1388.

Zhang, F., Shi, Y., Jiang, K., Xu, Z. & Ma, L. (2012). Sensitive and rapid detection of two toxic microalgae *Alexandrium* by loop-mediated isothermal amplification. *Acta Oceanologica Sinica*, **31**: 139–146.

Zhu, Y., Lin, Q., Yang, Y., Xia, Y., Cai, H., Feng, X., Gonçalves, R.J. & Guan, W. (2024). Ocean acidification enhances the tolerance of dinoflagellate *Prorocentrum donghaiense* to nanoplastic-induced oxidative stress by modulating photosynthetic performance. *Frontiers In Marine Science*, **11**: 1494930.