CHEMICAL ENGINEERING TRANSACTIONS
VOL. 106, 2023
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Peck Loo Kiew, Hon Huin Chin

ISBN 979-12-81206-05-2; **ISSN** 2283-9216
DOI: 10.3303/CET23106094

Detection of Specific Harmful Marine Organisms in Seawater and Sediment Samples using eDNA

Hwa-Seong Son, Kun-Woo Yun, Min-Jun Seong, Mu-Chan Kim*

Department of Marine Environmental Engineering, Gyeongsang National University, 2 Tongyeonghaean-ro, Tongyeong, Gyeongnam, Republic of Korea.
Kmc81@gnu.ac.kr

Harmful organisms in the marine environment can have devastating impacts on fisheries, and marine ecosystems, and even cause harm to humans. Therefore, early detection and effective management are crucial. In recent years, eDNA monitoring has become an important tool for detecting aquatic organisms. Species-specific eDNA monitoring offers advantages over NGS methods, such as metabarcoding, as it is more cost-effective. This economic advantage has led to the widespread use of species-specific eDNA monitoring, particularly for detecting harmful organisms. In this study, eDNA monitoring was conducted for HABs, Cnidaria, and Echinodermata, which are designated as representative harmful organisms by the Republic of Korea. The monitoring was conducted in Masan Bay, South Korea, which is known to be heavily polluted in the past. The selective detection of harmful organisms in seawater using eDNA demonstrated the superiority of this technology over conventional morphological methods in terms of speed, accuracy, non-invasiveness, and non-destructiveness. Comparative analysis with microscopic examination of HABs further supported these advantages. The specific primers and monitoring methods presented in this study enable specific-species and selective detection, and they offer greater reproducibility compared to conventional morphological monitoring methods. Consequently, for the first time in South Korea, all 12 species of environmental factors and harmful organisms in Masan Bay were detected using eDNA, a method that relies less on specialized knowledge and is more cost-effective compared to existing methods. These findings hold great promise for environmental conservation, fisheries development, and the effective detection and management of harmful organisms in marine environments.

1. Introduction

Harmful species in the marine environment include Harmful Algal Blooms (HABs), Cnidaria, and Echinodermata, which have serious impacts on marine ecosystems. Especially, humans can be adversely affected through bioaccumulation when bivalves and fish ingest HABs and their toxins. Increased HABs from eutrophication and climate change have significant economic impacts, affecting fisheries productivity, stability, and marine tourism. Cnidaria and Echinodermata also have similar effects (Anderson et al., 2021).

Environmental DNA (eDNA) refers to DNA extracted from various environmental samples, including soil, sediment, and water. In contrast to traditional morphological identification methods that require specialized expertise, eDNA analysis involves sequencing DNA fragments for biomonitoring purposes. Researchers, industry, and governments are increasingly embracing the use of bioinformatics in eDNA investigations because of its convenience and safety. This approach offers several benefits, including high accuracy, cost-effectiveness, and ethical considerations (Berry et al., 2021). The detection of species diversity, community structure, and species changes through eDNA analysis has proven to be highly predictive for HABs (Liu et al., 2020).

There are 3 primary methods for eDNA monitoring. Firstly, NGS allows for the simultaneous analysis of large amounts of DNA. However, this method is costly, data processing can be challenging, and it requires expertise in biology (Pearson et al., 2021). Secondly, qPCR (Quantitative PCR) has gained popularity in research and monitoring due to its ability to quantify the amount of target DNA. It requires sophisticated equipment and specialized operation (Chin Chwan Chuong et al., 2022). Lastly, specific-species eDNA monitoring focuses on the qualitative analysis of specific species, particularly harmful or rare organisms. This method offers a more

Paper Received: 30 May 2023; Revised: 13 July 2023; Accepted: 27 July 2023
Please cite this article as: Son H.-S., Yun K.-W., Seong M.-J., Kim M.-C., 2023, Detection of Specific Harmful Marine Organisms in Seawater and Sediment Samples using eDNA, Chemical Engineering Transactions, 106, 559-564 DOI:10.3303/CET23106094

cost-effective alternative to NGS, and compared to qPCR, the established protocol makes it easier for non-experts to use, allowing for rapid and accurate detection.
In the South Korea, species have been designated as HABs, including *Dinophysis* spp., *Pseudo-Nitzschia* spp., *Alexandrium* spp., *Chattonella* spp., *Cochlodinium polykrikoides*. Cnidarians such as *Nemopilema nomurai, Aurelia aurita, Physalia physalis, Cubozoa*, and *Dactylometra quinquecirrha*, and Echinodermatas such as *Asterina pectinifera* and *Asteria amurensis* are also designated as harmful organisms. Masan Bay, located in South Korea, is known as one of the most polluted harbors in the region. The area has suffered from severe hypoxia and eutrophication for many years, resulting in recurring HABs (Lee et al., 2021). Several previous studies have focused on monitoring environmental factors or eDNA of single species associated with HABs. The study conducted eDNA monitoring of 12 species to determine the widespread distribution of harmful species in Masan Bay, South Korea, a semi-enclosed inland bay known as a polluted harbor. For the first time in Masan Bay, we also aimed to detect 12 harmful organisms simultaneously using a cost-effective eDNA method, which can provide valuable information that can help formulate effective management strategies for the 12 species.

2. Methods and Materials

2.1 Field Sampling Method

The fieldwork, involving sample collection, was conducted in Masan Bay in April 2023. Seawater samples were collected directly at a depth of 1 m using Van Dorn Water Sampler. The collected seawater was stored in a sterilized 4 L water bottle. Sediment samples were collected using a Van Veen Grab with a surface area of 0.1 m^2, allowing collection down to a depth of 1 cm (Table 1). During the field sampling, water temperature and pH were measured using a YSI 556MPS instrument (USA).

Table 1. Sampling site in the waters of Masan Bay, Korea

Location	Longitude	Latitude
St 1	35°10'11.3"N	128°35'21.8"E
St 2	35°08'14.3"N	128°36'00.8"E
St 3	35°05'38.9"N	128°39'07.9"E

2.2 Analyzing Environment Factors

The collected samples were promptly transported to the laboratory. A portion of the seawater sample (1 L) was fixed with Lugol's solution for species identification under a microscope. Chlorophyll-a (Chl-a) analysis involved filtering 1 L of the sample through a GF/F filter (Whatman) into a test tube with filter paper. Afterward, a solution of 15 mL 90 % acetone was added to the test tube, and the contents were vigorously vortexed on a shaker. The tube was then placed in a cold, dark chamber below 4 °C overnight. Afterward, the test tube was centrifuged at 3,000 rpm for 20 min, and the absorbance was measured at wavelengths 750, 665, 645, and 630 nm using a HUMAS HS-3300 instrument. The water filtered for Chl-a analysis was collected in 15 mL tubes for nutrient analysis and frozen until analysis. Quantification of nutrients such as dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP) was performed through automated analysis using a QuAAtro39 instrument. To determine suspended solids (SS), 1 L of the sample was filtered through a GF/F filter, and the filter was dried in a dry oven for a minimum of 2 hours. The filter weight was measured before and after filtration. Total nitrogen (T-N) and total phosphorus (T-P) were analyzed by preparing calibration curves according to the TN(SW) Method 10000 and TP(SW) Method 11000 kit protocols provided by the HUMAS manufacturer.

2.3 DNA Extraction, PCR Methods, and Visualization

In the laboratory, the collected seawater was filtered to 500 mL for each sampling point using a 0.8 μm cellulose ester filter (Aduatec MFC, USA). Subsequently, 60 μL of 0.2 M EDTA was added to 2940 μL of PBS buffer, and the mixture was spun at 150 rpm for 2 h at 20 °C. The eDNA collected from the filter was then transferred to a 1.5 mL microtube and centrifuged. The supernatant was discarded, and the remaining pellet was used for DNA extraction following the protocol of AccuPrep® Genomic DNA Kit (Bioneer, Korea). The collected sediments were stored at -20 °C until isolation. DNA extraction was performed as follows: 10 g of each sample was transferred to a conical tube, and 10 mL of 1x PBS was added. The sample was then sonicated for 5 min at 8,000 rpm using a HG-15A (DAIHAN, Korea). After centrifugation at 2,000 rpm for 15 min, the supernatant containing extracellular DNA (exDNA) was removed, and 10 mL of ethanol was added to the remaining precipitate and air-dried at room temperature for 24 h. After drying, dried samples were subjected to DNA extraction using the protocol provided by the ExgeneTM Soil DNA Mini (GeneAll, Korea) 3410A kit. The extracted eDNA from seawater and sediment samples was amplified by PCR using specific primers targeting

the 12 harmful organisms for species identification. The PCR amplification was performed using the following cycles: initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 sec, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at the specific primer annealing temperature, extension at 72 °C for 45 sec, and a final extension at 72 °C for 5 min. The amplified PCR products were visualized by running them on a 1 % agarose gel using 1X TAE buffer. DNA bands were identified by electrophoresis at 20 min intervals using a Gel Doc system (FluoroBox, Cellgentek, Korea).

2.4 Specific-species Primer Design

To detect the 12 harmful organisms, primers were designed by referring to the reference and the NCBI database (www.ncbi.nlm.nih.gov). Species-specific primers were designed by targeting the COI region (GQ120033.1) of *P. physalis*. Primer design was performed using Primer3 software (www.primer3.ut.ee). The selection of the top 10 similar species was done from the NCBI Blast tool, and their alignment was performed using the Bioedit program and ClustalW (www.genome.jp/tools-bin/clustalw). To ensure the specificity of the primers in a wide range of organisms, they were further validated by Blast analysis. The selected primers were synthesized by Bioneer (Korea). In the case of *Cubozoa*, the 16s rRNA region (JN184782.1) was targeted, while for *Dactylometra quinquecirrha*, the CYTB region of the mitochondrion (NC_020459.1) was selected. For the design of species-specific primers, the mitochondrial COX1 region of *Asterina pectinifera* (NC_001627.1) and *Asterias amurensis* (MZ435266.1) was utilized, following the same methodology described above.

Table 2: Masan Bay monitoring includes forward and reverse primers to detect HABs.

Scientific name	*Name*	*SEQUENCE (5' --> 3')*	Reference	Size(bp)
Cochlodinium polykrikoides	C.poly ITS-F	GACGCAGCGAAGTGTGATAA	(Lee et al., 2017)	643
	C.poly ITS-R	CAACGCCTTGACAAACAAGA		
Alexandrium spp.	sxtA4F	CTGAGCAAGGCGTTCAATTC	(Murray et al., 2011)	125
	sxtA4R	TACAGATMGGCCCTGTGARC		
Pseudo-Nitzschia spp.	5.8S rDNA F	CAGCGGTGGATGTCTAGGTTC	(Andree et al., 2011)	230
	5.8S rDNA R	GAACCTAGACATCCACCGCTG		
Dinophysis spp.	DINOCOX1F	AAAAATTGTAATCATAAACGCTTAGG	(Lin et al., 2002)	703
	DINOCOX1R	TGTTGAGCCACCTATAGTAAACATTA		
Chattonella spp.	HYKD-F	CTTGGTTGTTGTAGCGTCTT	(Sun et al., 2019)	343
	HYKD-R	GAGAGAGTGAGGTCAGCAGA		
Nemopilema nomurai	nomura F28s	ACTGTGAAACTGCGAATG	(Armani et al., 2014)	1,598
	nomura R28s	CCATTCAATCGGTAGTAGC		
Aurelia aurita	LCO1490	GGTCAACAAATCATAAAGATATTGG	(Kim et al., 2007)	643
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		
Physalia physalis	Physalia F	CCGGATATGGCCTTCCCTAG	this study	1,017
	Physalia R	TGGGCTCAGACGATAAAACCT		
Cubozoa	Cubozoa F	TGAGGCCTGCTCACTGATTT	this study	566
	Cubozoa R	TGCTGCTATTCAACATCGAGG		
Dactylometra quinquecirrha	Dactylometra F	AATGTCATTCTGAGGGGCCA	this study	1,152
	Dactylometra R	GGGTCTTCTACAGGTTGTGC		
Asterina pectinifera	Patiria F	AGAACTAGCGCAACCTGGAT	this study	1,554
	Patiria R	GGAGGATGACGTCGATGGAT		
Asterias amurensis	amurensis F	CAGATATGGCATTTCCCCGC	this study	1,104
	amurensis R	GTAGCGGCGGTAAAGTAAGC		

3. Results and discussion

Chl-a serves as a significant indicator of eutrophication and is employed as a reliable measure to forecast red tide occurrences in coastal waters (Hu Zuwu and Liu Ling, 2018). Microscopic observations showed that the most frequently observed HABs were *Alexandrium* spp. with the highest concentrations at St 2, St 1, and St 3. The species with the highest Chl-a content was *Pseudo-nitzschia* spp. with the highest concentration at St 3. Consequently, Chl-a content was in the order of St 2, St 3, and St 1 (Table 3). These results indicate that there may be spatial differences in HABs distribution and chlorophyll-a content among the survey sites. For nutrient concentrations, DIN was highest at St 1, St 3, and St 2, while DIP, T-N, and T-P were highest at St 1, St 2, and St 3, respectively.

Table 3: Analysis of Environmental Factors at the Masan Bay Site in April 2023

	Tem (℃)	pH	Chl-a (μg/L)	SS (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	NH_4-N (mg/L)	PO_4-P (mg/L)	T-N (mg/L)	T-P (mg/L)
St 1	14.40	7.78	0.351	0.002	0.042	0.004	0.139	0.031	0.022	0.027
St 2	14.53	7.63	0.974	0.004	0.032	0.004	0.005	0.026	0.015	0.018
St 3	14.49	7.75	0.590	0.004	0.022	0.102	0.052	0.024	0.013	0.000

Microscopic observations were conducted according to the guidelines in the Handbook of Marine Phytoplankton published by the National Academy of Fisheries Sciences, which served as an important reference for this study.(Dermastia et al., 2020)*Pseudo-nitzschia* referenced the paper. The dominant species on St. 1 were *Scrippsiella* spp. (27 %), *Alexandrium* spp. (26 %), *Gymnodinium* spp. (16 %), *Heterosigma acaciae* (16 %), and *Pseudonichia* spp. (15 %), with two HABs dominating. At St 2, *Alexandrium* spp. (31 %), Scrippsiella spp. (23 %), *Protoperidinium* spp. (16 %), *Gymnodinium* spp. (15 %), and *Chaetoceros* spp. were the dominant species, of which *Alexandrium*, HABs, was the dominant species. At St 3, the dominant species were *Alexandrium* spp. (25 %), *Scrippsiella* spp. (24 %), *Pseudo-nitzschia* spp. (22 %), *Gymnodinium* spp. (17 %), and *Protoperidinium* spp. (12 %), with two HABs as the dominant species.

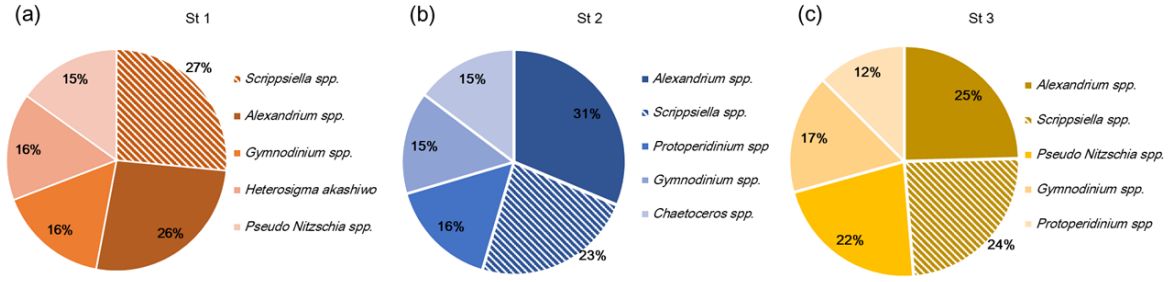

Figure 1: The Top 5 Dominant Species Classified by Morphological Identification under the Microscope in Masan Bay: (a) St 1, (b) St 2, and (c) St 3

A total of 12 species of harmful organisms were detected in Masan Bay. Nevertheless, the harmful organism *C. polykrikoides* was not observed under the microscope and no eDNA was detected in St 1(Table 4.). It was observed under the microscope in St 2 and St 3, and its eDNA was detected earlier than expected in both seawater and sediment samples. *Alexandrium* spp. accounted for the most abundant HABs species as counted by the morphological method, microscopy (TS100F, Nikon, Japan), followed by *Pseudo-nitzschia* spp., *Dinophysis* spp., *Chattonella* spp., and *C. polykrikoides*. The HABs detected in the sediments were present as cysts, but it is possible that mixtures accumulated over a long period of time.

Table 4: A Comparison of Traditional and eDNA Monitoring Methods for HABs in Seawater and Sediments.

		HABs				
		Cochlodinium polykrikoides	*Alexandrium* spp.	*Pseudo-Nitzschia* spp.	*Dinophysis* spp.	*Chattonella* spp.
St 1	Morphology	-	+	+	+	+
	Seawater	-	+	+	+	+
	Sediment	-	+	+	+	+
St 2	Morphology	+	+	+	+	-
	Seawater	+	+	+	+	+
	Sediment	+	+	+	-	+
St 3	Morphology	+	+	+	+	+
	Seawater	+	+	+	+	+
	Sediment	-	+	+	+	+

Morphologically, the Cnidaria were easy to recognize in the surface layer, but difficult to identify in the middle and lower layers. For Cnidaria, only the full *A. aurita* was morphologically observed in St 1, and no Cnidaria was morphologically observed in St 2 and St 3. *N. nomura*i was only found in seawater, *A. aurita* was found in both seawater and sediment, and *P. physalis, Cubozoa*, and *D. quinquecirrha* were not observed in seawater but eDNA was detected in sediment (Table 5).

Table 5: A Comparison of Traditional and eDNA Monitoring Methods for Cnidaria in Seawater and Sediments.

		Cnidaria				
		Nemopilema nomurai	*Aurelia aurita*	*Physalia physalis*	*Cubozoa*	*Dactylometra quinquecirrha*
St 1	Morphology	NA	+	NA	NA	NA
	Seawater	+	+	-	-	-
	Sediment	-	+	+	+	+
St 2	Morphology	NA	NA	NA	NA	NA
	Seawater	+	+	-	-	-
	Sediment	-	+	+	+	+
St 3	Morphology	NA	NA	NA	NA	NA
	Seawater	+	+	-	-	-
	Sediment	-	+	+	+	+

Morphologic identification of starfish is difficult unless the diver is able to identify them directly. Echinodermata is primarily observed in the warmer months, and *A. amurensis* has not been found in seawater or sediment samples. However, eDNA analysis revealed the presence of *A. pectinifera* DNA only in the sediment at St 1. It is possible that echinoderm eDNA could be detected even though there are no shellfish farms near the sampling sites. Therefore, while morphological identification of Echinodermata species is difficult, the detection of Echinodermata eDNA in the sediment samples suggests the possible presence of Echinodermata (Table 6).

Table 6: A Comparison of Traditional and eDNA Monitoring Methods for Echinodermata in Seawater and Sediments.

		Echinodermata	
		Asterina pectinifera	*Asterias amurensis*
St 1	Morphology	NA	NA
	Seawater	-	-
	Sediment	+	-
St 2	Morphology	NA	NA
	Seawater	-	-
	Sediment	-	-
St 3	Morphology	NA	NA
	Seawater	-	-
	Sediment	-	-

The simultaneous detection of 12 species of harmful organisms confirmed the presence of harmful organisms at the sampling point, and the detection of harmful organisms' eDNA in the sediment samples allowed us to predict the likely presence of cysts. In this study, specific primers were developed and used to detect specific species of Cnidaria and Echinodermata rather than using universal primers. Validation of these specific primers was performed by the NCBI. Using the developed primers, eDNA could be detected in both seawater and sediment samples, confirming the usefulness of eDNA as a tool for monitoring harmful organisms. Morphological monitoring of harmful organisms is difficult and inefficient, especially for non-experts in species identification. In contrast, the eDNA approach provides an efficient and non-invasive way to monitor these species (Sahu et al., 2022). Seawater plays an important role in marine ecosystems and biodiversity, and changes in nutrient levels can have a major impact on biodiversity. Nevertheless, a variety of factors actively influence eDNA detection in seawater environments. In the surface layer, eDNA degradation occurs at varying rates due to factors such as currents, pH, temperature, and UV light. In comparison, the sediment layer is less affected by environmental variables than the surface layer, allowing DNA to be preserved for longer periods of time. In addition, sediments are often composed of mixtures of different materials that have accumulated over a long period of time. In this study, sediment samples were centrifuged to remove light extracellular DNA so that the precipitated intracellular DNA (iDNA) could be extracted. This approach is not only predictive of the precipitated intracellular DNA in the sediment but also indicative of the potential presence of cysts. Thus, eDNA detection is sensitive to environmental changes and serves as a useful tool for monitoring harmful species.

4. Conclusion

Traditionally, previous studies have mainly focused on the detection or monitoring of single species of HABs or the analysis of specific environmental factors. In this study, we designed specific primers for each of the 12 identified harmful organisms and performed environmental factor analysis. This study represents the first attempt to monitor the temperature, pH, nutrient levels, and eDNA of 12 harmful organism species in Masan

Bay. Continued monitoring is necessary to assess species diversity in response to nutrient changes and to identify seasonal variations in eDNA. The detection of 12 harmful species and the assessment of environmental factors are the first results of this study, so we will be conducting additional experiments over the summer to investigate changes in eDNA, and we expect that these follow-up experiments will yield more comprehensive and robust data that can contribute to advancing knowledge in this field. Thus, Continuous analysis of eDNA and environmental factors of harmful organisms in the marine environment is expected to help fisheries and harmful organism control by identifying distribution patterns. They underscore the need for further research and observation in the future.

Acknowledgments

This work supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2021R1F1A1051818).

References

Anderson, D. M., Fensin, E., Gobler, C. J., Hoeglund, A. E., Hubbard, K. A., Kulis, D. M., Landsberg, J. H., Lefebvre, K. A., Provoost, P., Richlen, M. L., Smith, J. L., Solow, A. R., Trainer, V. L., 2021, Marine harmful algal blooms (HABs) in the United States: History, current status and future trends, Harmful Algae, 102, 101975.

Andree, K. B., Fernández-Tejedor, M., Elandaloussi, L. M., Quijano-Scheggia, S., Sampedro, N., Garcés, E., Camp, J., Diogène, J, 2011, Quantitative PCR coupled with melt curve analysis for detection of selected *Pseudo-nitzschia* spp.(Bacillariophyceae) from the northwestern Mediterranean Sea, Applied and environmental microbiology, 77(5), 1651-1659.

Armani, A., Giusti, A., Castigliego, L., Rossi, A., Tinacci, L., Gianfaldoni, D., Guidi, A., 2014, Pentaplex PCR as screening assay for jellyfish species identification in food products, Journal of agricultural and food chemistry, 62(50), 12134-12143.

Berry, O., Jarman, S., Bissett, A., Hope, M., Paeper, C., Bessey, C., Schwartz, M. K., Hale, J., Bunce, M., 2021, Making environmental DNA (eDNA) biodiversity records globally accessible, Environmental DNA, 3(4), 699-705.

Chin Chwan Chuong, J. J., Rahman, M., Ibrahim, N., Heng, L. Y., Tan, L. L., Ahmad, A., 2022, Harmful microalgae detection: Biosensors versus some conventional methods, Sensors, 22(9), 3144.

Dermastia, T. T., Cerino, F., Stanković, D., Francé, J., Ramšak, A., Tušek, M. Ž., Beran, A., Natali, V., Cabrini, M., Mozetič, P., 2020, Ecological time series and integrative taxonomy unveil seasonality and diversity of the toxic diatom Pseudo-nitzschia H. Peragallo in the northern Adriatic Sea, Harmful Algae, 93, 101773.

Hu Z., Liu L., 2018, Prediction of Water Pollution by Nutrients Based on Eutrophication Evaluation, Chemical Engineering Transactions, 71, 667-672.

Kim, Sook-Yang, and Eun-Seob Cho., 2007, Molecular phylogeny of moon jellyfish *Aurelia aurita* Linnaeus collected from Yeosu waters in Korea based on nuclear and mitochondrial DNA sequences, Journal of Life Science, 17(3), 318-327.

Lee, H. G., Kim, H. M., Min, J., Kim, K., Park, M. G., Jeong, H. J., Kim, K. Y., 2017, An advanced tool, droplet digital PCR (ddPCR), for absolute quantification of the red-tide dinoflagellate, *Cochlodinium polykrikoides* Margalef (Dinophyceae), Algae, 32(3), 189-197.

Lee, Y. W., Park, M. O., Kim, S. G., Kim, S. S., Khang, B., Choi, J., Lee, D., Lee, S. H., 2021, Major controlling factors affecting spatiotemporal variation in the dissolved oxygen concentration in the eutrophic Masan Bay of Korea.", Regional Studies in Marine Science, 46, 101908.

Lin, S., Zhang, H., Spencer, D. F., Norman, J. E., Gray, M. W., 2002, Widespread and extensive editing of mitochondrial mRNAs in dinoflagellates, Journal of molecular biology, 320(4), 727-739.

Liu, Q., Zhang, Y., Wu, H., Liu, F., Peng, W., Zhang, X., Chang, F., Xie, P., & Zhang, H., 2020, A review and perspective of eDNA application to eutrophication and HAB control in freshwater and marine ecosystems, Microorganisms, 8(3), 417.

Murray, S. A., Wiese, M., Stüken, A., Brett, S., Kellmann, R., Hallegraeff, G., Neilan, B. A., 2011, sxtA-based quantitative molecular assay to identify saxitoxin-producing harmful algal blooms in marine waters, Applied and environmental microbiology, 77(19), 7050-7057.

Pearson, L. A., D'Agostino, P. M., Neilan, B. A., 2021, Recent developments in quantitative PCR for monitoring harmful marine microalgae, Harmful algae, 108, 102096.

Sahu, A., Kumar, N., Singh, C. P., Singh, M., 2022, Environmental DNA (eDNA): Powerful Technique for Biodiversity Conservation, Journal for Nature Conservation, 126325.

Sun, Y. J., Chen, G. F., Zhang, C. Y., Guo, C. L., Wang, Y. Y., & Sun, R., 2019, Development of a multiplex polymerase chain reaction assay for the parallel detection of harmful algal bloom-forming species distributed along the Chinese coast, Harmful Algae, 84, 36-45.