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Application of Radiolabeled Technique in Indonesia: A Receptor Binding Assay (RBA) Approach for Analysing Paralytic Shellfish Poisoning (PSP) Toxins in Green Mussels from Lampung Bay

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ABSTRACT

The Receptor Binding Assay (RBA), using radiolabeled [^{3}H]-saxitoxin as a tracer, was applied to detect Paralytic Shellfish Poisoning (PSP) toxins in green mussels (*Perna viridis*) from Lampung Bay, Indonesia. Sampling was conducted during Harmful Algal Bloom (HAB) events in 2012, 2014, and 2015, which coincided with mass fish mortality linked to red tide phenomena. The RBA achieved a markedly lower detection limit (0.2 µg STX eq. /100 g) than the conventional Mouse Bio-Assay (MBA) of 40 µg STX eq. /100 g and offered greater sensitivity, specificity, and faster analysis without ethical constraints. Environmental parameters such as nutrients, water quality, and plankton community composition were also monitored to elucidate ecological drivers of toxin production. The findings represented that RBA was a reliable and efficient tool for PSP toxin monitoring, providing early-warning capability for HAB-related risks in tropical marine ecosystems.

INTRODUCTION

Harmful Algal Blooms (HABs) are a global concern due to their impacts on marine ecosystems and human health. [1]. Among the most dangerous HAB-related toxins are those responsible for Paralytic Shellfish Poisoning (PSP), a syndrome caused by the ingestion of shellfish contaminated with Saxitoxin (STX). and its analogs [2]. STX is a potent neurotoxin produced by certain dinoflagellate species, including *Alexandrium spp., Gymnodinium catenatum*, and *Pyrodinium bahamense*. These toxins can accumulate in filter-feeding shellfish, posing a significant risk to human consumers [3].

The occurrence of HABs in the waters of Hurun Bay, part of Lampung Bay, was first reported on December 13, 2012 [4]. A mass fish mortality event was observed in parts of Lampung Bay, particularly along the South Lampung coast and extending to the southern coast of Pesawaran District [1]. Thousands of fish, including both farmed and wild species, were found dead. Investigations by the Center for Development of

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Marine Aquaculture in Lampung suggested that the mass mortality was caused by an algal bloom, predominantly of the *dinoflagellate species Cochlodinium polykrikoides*. According to reports, the bloom began gradually in October 2012 and peaked in December 2012, severely impacting fish farming operations in floating cages [4]. The phenomenon was accompanied by a red discolouration of the water, commonly referred to as "red tide." In addition to *C. polykrikoides*, algal blooms are a recurring phenomenon in the waters off the western coast of Lampung Bay. Sidabutar documented the presence of *P. bahamense*, a dinoflagellate species abundant in Hurun Bay, a part of the west of Lampung Bay [1,5].

These planktonic organisms can produce STX and its derivatives, potent neurotoxins that remain stable across a wide range of temperatures. Shellfish, being filter feeders, can bioaccumulate these toxins without being adversely affected. However, humans consuming contaminated shellfish may experience PSP, which can be fatal. Reports indicate that the frequency of HAB events in Indonesian waters has been increasing [6].

The detection of PSP toxins in shellfish has traditionally relied on the Mouse Bioassay (MBA), which, while effective, has several limitations, including low sensitivity and ethical concerns. In recent years, alternative methods such as the Receptor Binding Assay (RBA) have been developed [7]. The RBA method, which uses radiolabeled STX to compete with unlabeled toxins for receptor binding, offers a more sensitive and rapid approach to toxin detection [8].

The objective of this study was to quantify STX levels in shellfish from Hurun Bay, part of Lampung Bay, using the RBA method. By comparing the results with regulatory limits, we aim to provide valuable data for the management of PSP risks in the region.

THEORY/CALCULATION

Mechanism of STX and RBA

STX is a potent neurotoxin produced by certain marine dinoflagellate species, such as *Alexandrium spp., G. catenatum*, and *P. bahamense var. compressum*. These microscopic algae are responsible for the production of PSP, which can accumulate in filter-feeding shellfish. When humans consume contaminated shellfish, they may experience PSP, a syndrome characterized by neurological symptoms such as muscle paralysis, numbness, and, in severe cases, respiratory failure [9,10].

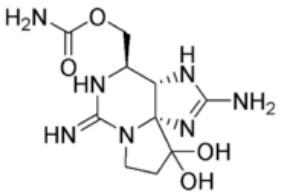

Fig. 1. The structure of saxitoxin [10].

The molecular structure of STX (Fig. 1) consists of a complex heterocyclic ring system with the chemical formula $C_{10}H_{17}N_7O_4$ and a molecular weight of 299.29 g/mol.

STX is an incredibly potent toxin, being approximately 100 times more poisonous than strychnine, 1,000 times more lethal than the synthetic nerve agent sarin, and 2,000 times more toxic than sodium cyanide [8]. Its toxicity arises from its ability to bind to voltage-gated sodium channels in nerve cells, blocking the influx of sodium ions and preventing the propagation of nerve impulses.

RBA principle

The Receptor Binding Assay (RBA) is a highly sensitive and specific method for detecting STX and its analogs in shellfish. The principle of RBA is based on the competitive interaction between radiolabeled STX (T*) and unlabeled STX (T) for binding to specific pharmacological receptors, such as voltage-gated sodium channels. This competitive binding enables the quantification of toxin concentrations in unknown samples [11,12].

When an unlabeled toxin (T) is added to the incubation mixture either as a standard or an unknown sample it competes with the radiolabeled toxin (T*) for binding sites on the receptor. This competitive interaction leads to the generation of two distinct complexes: the radiolabeled toxin-receptor complex (T*R) and the unlabeled toxin-receptor complex (TR). As more unlabeled toxin is introduced, the amount of T*R diminishes, a trend illustrated in Fig. 2.

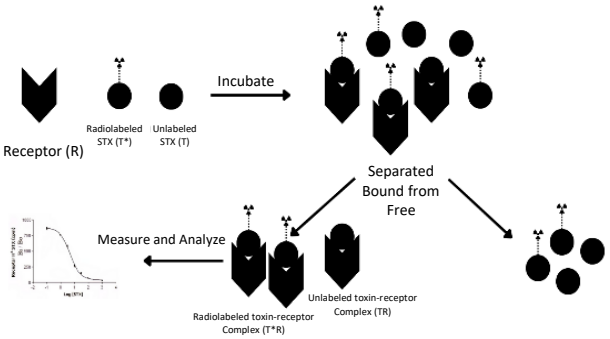

Fig. 2. A schematic representation of interactions in competitive binding assays. [11].

The amount of radiolabeled complex formed is quantified using a liquid scintillation counter. By comparing the reduction in radiolabeled complex formation to a control reaction (which lacks unlabeled toxin), a competition curve is generated. This curve enables the quantification of toxin concentrations in unknown samples [11,12].

Quantification of STX

The competitive binding process can be described by the following relationship in Eq. (1):

$$\frac{B_i}{B_0} = \frac{1}{1 + \frac{[T]}{K_d}} \quad (1)$$

In this assay, B_i corresponds to the quantity of radiolabeled toxin-receptor complex (T*R) formed when unlabeled toxin (T) is present, while B_0 represents the amount of T*R generated in the absence of T. K_d (the equilibrium dissociation constant) characterizes the binding affinity between the toxin and receptor. As the concentration of unlabeled toxin increases, the formation of T*R decreases due to competitive binding, enabling the quantification of saxitoxin levels in unknown samples.

The competition curve (Fig. 3) is used to determine the concentration of saxitoxin in unknown samples. The linear range of the curve, corresponding to the B_i/B_0 value between 0.2 and 0.7, is used for accurate quantification. The results are expressed as micrograms of saxitoxin equivalents per 100 grams of shellfish tissue (µg STX eq./100 g) [2,11].

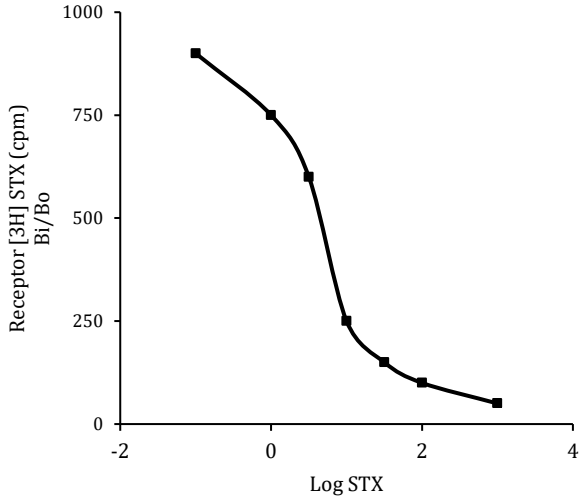

Fig. 3. Radiolabeled and unlabeled toxin competition curve.

Toxicity and exposure pathways

Saxitoxin poisoning primarily occurs through ingestion and, less commonly, through respiratory exposure. Consumption of contaminated shellfish is the primary route of exposure, leading to the rapid onset of poisoning PSP symptoms, including tingling sensations, numbness, and progressive muscle paralysis. Experimental studies have demonstrated the extreme toxicity of STX, with lethality observed at very low doses; pigs succumb following intramuscular injections of 5 µg/kg, while mice exhibit fatal outcomes after intraperitoneal injections at the same dose [13]. Emergency treatment for PSP involves providing artificial respiration, as there is no specific antidote for saxitoxin poisoning [10]

METHODOLOGY

Study site

Sampling was carried out at the locations depicted in Fig. 4, with samples collected in 2015. The sampling sites were located within the geographical coordinates of 5°27'45.57"S to 5°33'10.49"S latitude and 105°15'29.15"E to 105°16'14.74"E longitude.

Fig. 4. Sampling site in Hurun Bay, Lampung.

EXPERIMENTAL SECTION

The laboratory technique used to evaluate STX through the RBA was adapted from previous research [11,14-16] with improvements made to certain aspects.

Materials

The shellfish samples analyzed in this study were green mussels (*Perna viridis, Mytilidae*), Radiolabeled [^{3}H] STX and STX di-HCl were obtained from American Radiolabeled Chemicals

(ARC), while rat brain tissue, used as the receptor membrane source, was sourced from the National Animal Center, Mahidol University. MOPS (3-(N-morpholino) propanesulfonic acid) buffer was purchased from Advanced Bioreagent. Additional chemicals included trimethylamine hydrochloride ($[CH_3]_3N(Cl)CH_2CH_2OH$, >98%, Sigma-Aldrich), 0.1 N HCl (37%, Sigma-Aldrich), double-distilled water (ddH_2O), and deionised water. The experimental setup included a range of essential laboratory equipment and consumables. Pipetting operations were performed using adjustable-volume micropipettes (1-1000 µL range) with disposable tips, along with multi-channel pipettors (5-200 µL capacity) for high-throughput processing. Liquid scintillation counting was conducted using a Scintiverse cocktail with either conventional or microplate-compatible scintillation counters.

Filtration steps employed 96-well microfilter plates featuring 0.65 µm Durapore membranes and Type B glass fiber filters (Millipore MSFB N6B 50), processed using a MultiScreen vacuum manifold (Millipore MAVM 096 01) connected to vacuum pumps. Sample handling utilized 15 mL and 50 mL conical centrifuge tubes, supported by reagent reservoirs and 96-well mini-dilution tube arrays for efficient sample preparation.

Additional equipment included temperature control devices (−80°C freezers and refrigerators), mixing apparatus (vortex mixers), centrifugation systems for 15 mL tubes, and basic laboratory tools such as 1 L volumetric flasks, 10 mL graduated cylinders, and sealing tape (Millipore MATA HCL00). pH measurements were obtained using either electronic meters or pH paper, with hot plates were available for heating requirements. The comprehensive setup ensured that all experimental procedures could be performed with appropriate precision and reproducibility.

Water quality parameters measured at the sampling sites included pH, nitrite, nitrate, phosphate, and ammonia.

PROCEDURE

Mussel samples treatment

Mussel samples were carefully collected and transported to the laboratory under controlled conditions to minimize contamination. Upon arrival, the shells were cleaned thoroughly to remove any external debris or contaminants. The mussels were shucked, drained, and dissected to extract the soft tissue. The mussel tissue was homogenized in a blender for three minutes to ensure sample uniformity. The samples were stored at -20°C until further treatment.

5 g of homogenized sample was added to 5.0 mL of 0.1N HCl in a 15 mL conical tube and vortexed to ensure complete dispersion. The pH of the mixture was maintained in the range 3.0 – 4.0 to prevent localized alkalinization and subsequent toxin degradation. The mixture in the tube with a loosened cap was immersed in the boiling water for five minutes, then removed and left to cool. The entire contents of the tube were moved to a graduated cylinder and volumetrically diluted to a final volume of 10 mL. After gentle swirling to ensure homogeneity, the mixture was allowed to settle until the supernatant became clear. Subsequently, 5 - 7 mL of the cleared supernatant was transferred to a centrifuge tube and spun for 10 minutes at 3000 rpm. The resulting supernatant was collected and transferred to a sterile centrifuge tube. The resulting extracts were stored at -20°C until the RBA was used for additional analysis.

Getting the stock and standards solution ready

Assay buffer solution (pH 7.4) containing 100 mM MOPS and 100 mM choline chloride**.** To prepare the MOPS-choline chloride buffer, 20.9 g of MOPS and 13.96 g of choline chloride were dissolved in 900 mL of deionized water under constant stirring. The pH of the solution was carefully adjusted to 7.4 by titration with either 0.1 N HCl or 0.1 N NaOH. After pH adjustment, the solution was brought to a final volume of 1 L by adding deionized water. The completed buffer solution was stored at 4°C until required for experimental use.

Rat brain membrane preparation. The pH of a membrane kit was adjusted to 7.4 by dilution with 100 mM MOPS and 100 mM choline chloride buffer at 40°C. The working stock prepared using this method had a protein concentration of 1.0 mg/mL. To achieve an in-well concentration of 0.5 mg/mL, the working stock was further diluted in the assay plate before use. The dilution was achieved by mixing 2 mL of the membrane stock with 18 mL of MOPS/choline chloride buffer. Before use, the mixture was vigorously vortexed and then placed on ice.

STX’s typical operating solution without a label. The concentration of the STX diHCl standard is 268.8 µM (100 µg/mL). A bulk standard curve could be prepared in advance and stored at 4°C for

up to one month. The dilution series listed in Table 1 needs to be made.

Check for the Standard (STD) Curve. A reference standard for the STX assay was made in 0.0003M HCl at a concentration of 1.8×10^{-8} M STX (corresponding to 3.0×10^{-9} M STX in the assay). For long-term storage, this standard was divided into 1 mL parts and kept at -80°C. Aliquots can be kept stable for up to a month at 4°C after being thawed for regular use. By verifying daily performance, this reference standard acts as a quality control (QC) check to guarantee the assay's correctness and consistency.

[^{3}H]-STX radioligand solution**.** The [^{3}H]STX stock solution was supplied at multiple concentration specifications: 50 µCi/ampoule, 21 Ci/mmol specific activity, 0.1 mCi/mL, or 4.17 µM. Daily working solutions were prepared fresh by diluting the stock to 15×10^{-9} M in MOPS-choline chloride buffer (100 mM each). This working solution was subsequently diluted in assay wells to achieve a final test concentration of 2.5×10^{-9} M.

Assay Procedure. The assay was performed in a 96-well microfilter plate, beginning with a 35 µL MOPS buffer addition to pre-wet each filter membrane. Triplicate wells received 35 µL aliquots of either STX standards, quality control samples, or test samples, followed by sequential addition of 35 µL [^{3}H]STX working solution (dispensed via multichannel pipette) and 105 µL diluted membrane suspension, as shown in Table 2. The plate was incubated at 4°C for 1 hour to facilitate binding, after which the reaction mixture was vacuum-filtered using a MultiScreen manifold. To remove unbound components, each well was washed with 200 µL MOPS buffer (pH 7.4) prior to further analysis. This standardized protocol ensured consistent binding conditions across all samples while maintaining optimal assay sensitivity.

After applying a vacuum to completely evacuate all liquid from the wells, each well was washed twice with 210 µL of ice-cold assay buffer using an 8-multichannel pipette. The plastic base of the plate was then removed, and the plate was blotted on absorbent paper to eliminate residual liquid. The counting cassette was prepared by securely sealing its bottom with adhesive tape. Using the 8-multichannel pipette, 50 µL of OptiPhase scintillation cocktail was precisely dispensed into each well. The plate was sealed with adhesive tape and allowed to equilibrate at room temperature for 30 minutes. Finally, radioactivity was quantified using a Perkin Elmer MicroBeta2™ microplate scintillation counter, with a counting duration of 1 minute per well to ensure accurate measurement.

Table 1. The Standard Solution.

	Dilutions	Tube Concentration	Plate Concentration
A	$6x10^{-6}$ M (Prepared)	6×10^{-6} M	1×10^{-6} M
B	500 µL of A + 4.5 mL 0.003 M HCl	6×10^{-7} M	1×10^{-7}M
C	1.5 mL of B + 3.5 mL 0.003 M HCl	1.8×10^{-7} M	3×10^{-8} M
D	500 µL of B + 4.5 mL 0.003 M HCl	6×10^{-8} M	1×10^{-8} M
E	500 µL of C + 4.5 m 0.003 M HCl	1.8×10^{-8} M	3×10^{-9} M
F	500 µL of D + 4.5 mL 0.003 M HCl	6×10^{-9} M	1×10^{-9} M
G	500 µL of F + 4.5 mL 0.003 M HCl	6×10^{-10} M	1×10^{-10} M
H	5 mL 0.003 M HCl	0 M	Reference

Table 2. Plate Layout.

	1 2	3	4	5	6
A	STX Std 1000 × 10^{-9} M in-assay		QC (3.0×10^{-9} M STX)		
B	STX Std 100 × 10^{-9} M in-assay		Reference (0.003 N HCl)		
C	STX Std 30 × 10^{-9} M in-assay		S-1	S-1	S-1
D	STX Std 10 × 10^{-9} M in-assay		S-2	S-2	S-2
E	STX Std 3 × 10^{-9} M in-assay		S-3	S-3	S-3
F	STX Std 1 × 10^{-9} M in-assay		S-4	S-4	S-4
G	STX Std 0.1 × 10^{-9} M in-assay		S-5	S-5	S-5
H	Reference (0.003 N HCl)				

Data analysis

The competitive binding data were analyzed using GraphPad Prism® software with a one-site receptor competition model. Toxin concentrations, expressed as 10^{-9} M STX eq., were determined using a standardized curve-fitting approach: (1) logit transformation of normalized binding data (B_i/B_0), (2) plotting against logarithmic STX concentrations, and (3) nonlinear regression analysis to generate the best-fit curve. Unknown sample concentrations were calculated by solving the regression equation for x using each sample's logit-transformed y-value (B_i/B_0). Final results were converted to µg STX eq./100 g shellfish tissue using Eq. (2), calculating STX concentrations in shellfish samples [17].:

$$Concentration = \frac{[STX]cal \times Dilution\ factor \times Volume}{Sample\ Weight} \times 100 \qquad (2)$$

where [STX]cal represents the STX concentration determined from the regression equation, and the dilution factor, volume, and sample weight are adjusted accordingly.

Assay quality control

To ensure assay validity and accuracy, three critical quality control parameters were implemented. First, the Hill slope of the competition curve was required to fall within the range of −.8 to −.2, reflecting specific binding at a single receptor site. Deviations outside this range indicated assay nonlinearity, necessitating repetition. Second, the QC standard (nominal concentration: 3.0×10^{-9} M) had to yield measured values between 2.1 and 3.9×10^{-9} M (±30%). Failure to meet this criterion invalidated the entire assay. Third, sample measurements were subject to strict acceptance criteria: (1) only dilutions with Bi/B_0 values between 0.2 – 0.7 (linear range) were quantified; (2) samples with $Bi/B_0 < 0.2$ required further dilution and reanalysis, whereas those with $Bi/B_0 > 0.7$ were reported as below the detection limit; and (3) replicate measurements had to exhibit ≤30% relative standard deviation (RSD) to ensure precision. Adherence to these controls guaranteed the reliability and traceability of all reported toxin concentrations.

RESULTS AND DISCUSSION

Validity of the method

The assays were conducted in triplicate. The resulting calibration curve is depicted in Fig. 5. The competitive binding assay was performed under standardized conditions: radioligand: [^{3}H]STX (final concentration 1.5×10^{-9} M), reference Standard FDA-certified saxitoxin dihydrochloride (STX·2HCl), biological matrix rude rat brain membrane preparation, assay format: 96-well microplate system, detection Platform PerkinElmer MicroBeta²™ microplate scintillation counter. All measured parameters adhered to the established quality control criteria for the method: the EC_{50} value was determined to be $1.64 \pm 0.27 \times 10^{-9}$ M (relative standard deviation, RSD = 12.11%); the Hill Slope of the curve was −1.027.

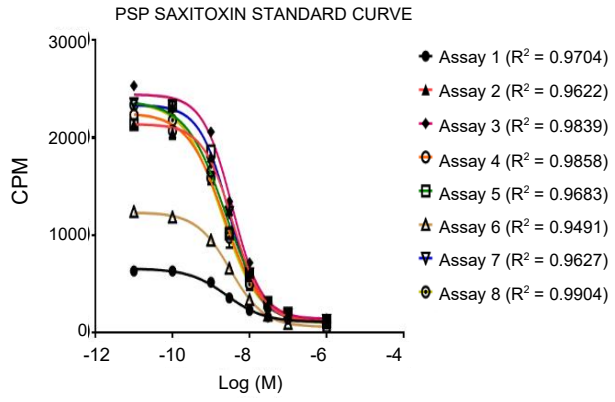

Fig. 5. Calibration curve from assays.

The linearity of the method was evaluated by plotting the measured values against the expected values of STX concentrations, as illustrated in Fig. 6.

Plotting the measured results against the anticipated STX concentrations allowed for the determination of the method's linearity. The linearity of the method is illustrated in Fig. 6 with an R^2 value of 0.9962, and the resultant curve showed a high degree of correlation.

Table 3 presents the results of routine enumeration conducted across varying STX concentrations (0-1000 × 10^{-9} M). The RSD between assays remained approximately 30%, indicating high precision across all measurements. Elevated variability (RSD > 30%) in measurements involving the standard toxin would compromise the precision and reliability of sample quantification. Therefore, maintaining RSD values below this threshold is essential to ensure the accuracy and reproducibility of the assay.

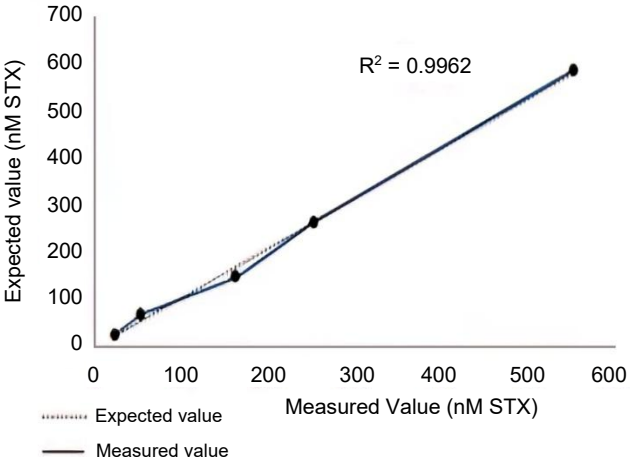

Fig. 6. Plotting the measured and predicted levels of STX.

Table 3. Data count per minute (cpm) in various concentration STX standard.

STX std conc'n (10^{-9} M)	CPM 1	CPM 2	CPM 3	CPM AVE	SD	RSD (%)
1000	17	25	19	20	4.16	20.48
100	34	44	35	38	5.51	14.62
30	61	52	75	63	11.59	18.50
10	90	97	89	92	4.36	4.74
3	205	197	168	190	19.47	10.25
1	332	335	330	332	2.52	0.76
0.1	502	476	464	481	19.43	4.04
REF	501	447	457	468	28.73	6.13
QC	47	58	55	53	5.69	10.66

Application of the RBA technique for the determination of PSP toxins in samples.

Shellfish specimens collected from Hurun Bay, Lampung, were evaluated for PSP toxins using the validated RBA protocol. Toxin concentrations were quantified in µg STX eq. Per 100 g tissue through competitive binding curves analyzed with GraphPad Prism® software (GraphPad Software Inc.). As detailed in Table 4, the method demonstrated robust analytical performance: intra-assay precision (RSD < 30%) and inter-assay reproducibility (RSD range: 1.94–19.04%) met established validation criteria. These results confirm the RBA's reliability for monitoring PSP toxins in marine samples, supporting its application in food safety surveillance.

Table 4. STX equivalent in shellfish (green mussel) samples.

Sample location	µg STX eq./kg sm	AVE Sample Toxicity	µg STX eq./100 g sm	SD	RSD
Pasaran Island 1	22.91 30.31 27.01	26.74	2.674	3.71	13.88
Pasaran Island 2	17.61 19.89 18.51	18.67	1.867	1.15	6.17
Ujung Bom	16.77 17.10 16.45	16.77	1.677	0.33	1.94
Hurun Bay	579.55 422.85 422.85	475.08	47.508	90.47	19.04
Kubur Island	19.48 18.14 15.98	17.87	1.787	1.77	9.89

Water quality

Water quality parameters have been regularly monitored by the Center for Marine Aquaculture in Pesawaran, Lampung. The average measurements obtained are presented in Table. 5.

Table 5. Data parameters of water quality.

Year	Location	Parameter				
		pH	NO_2 mg/L	NO_3 mg/L	NH_3 mg/L	PO_4 mg/L
2012	Hurun Bay 1	7.98	0.002	0.058	0.014	0.011
2014	Ujung Bom	7.99	0.175	0.055	0.516	1.398
	Kubur Island	8.01	0.037	0.069	0.156	0.021
	Hurun Bay	8.02	0.085	0.063	0.259	0.044
2015	Ujung Bom	7.99	0.118	0.408	0.062	0.158
	Pasaran Island 1	8.03	0.187	0.385	0.265	0.114
	Pasaran Island 2	8.05	0.138	0.459	0.070	0.087
	Hurun Bay 1	8.01	0.110	0.642	0.082	0.094
	Hurun Bay 2	8.10	0.097	0.623	0.076	0.061

The nutrient dynamics observed in Hurun Bay, Ujung Bom, Kubur Island, and Pasaran Island from 2012–2015 emphasize conditions that can influence the occurrence of HABs and the accumulation of PSP toxins in local bivalves.

The stable pH range (7.98–8.10) suggests that carbonate chemistry was within normal limits for supporting phytoplankton growth. However, nutrient enrichment particularly nitrate (NO_3^-), ammonia (NH_3), and phosphate (PO_4^{3-}) showed substantial increases in 2014–2015, especially at Bom tip and Hurun bay. Elevated nitrate (>0.6 mg/L) and phosphate (>0.1 mg/L) are critical drivers of eutrophication, stimulating rapid phytoplankton proliferation, including toxin-producing *dinoflagellates* such as *Alexandrium spp* [18,19]. Similarly, the high ammonia level recorded at Bom tip in 2014 (0.516 mg/L) reflects the correlation between nutrient enrichment and certain phytoplankton proliferation caused the risk of future harmful algal blooms under unchecked anthropogenic inputs [20].

These nutrient conditions align with ecological risk factors for PSP events. Although the measured STX levels in shellfish from Hurun Bay remained below the regulatory thresholds of the European Union (EU) and Food and Drug Administration (FDA), the increasing nutrient loads indicate a higher future probability of HAB outbreaks and subsequent PSP toxin accumulation. The observed link between toxin production and STX gene responses in *Alexandrium pacificum* under different NO_3^- conditions indicates that nitrogen plays a direct role in the STX biosynthesis pathway [21,22].

The current PSP toxin concentrations remain within safe limits; however, the nutrient dynamics in Lampung's coastal waters reflect increasing eutrophication pressure.

Phytoplankton abundances

The obtained results indicated that there are several types of plankton that influenced the occurrence of red tides. They included *Alexandrium cattenella, Gymnodinium sp, Dinophysys, Proro-centrum sp, P. bahamense,* and *Pseudo-nitzchia*. The types of plankton identified in the study are shown in Fig. 7.

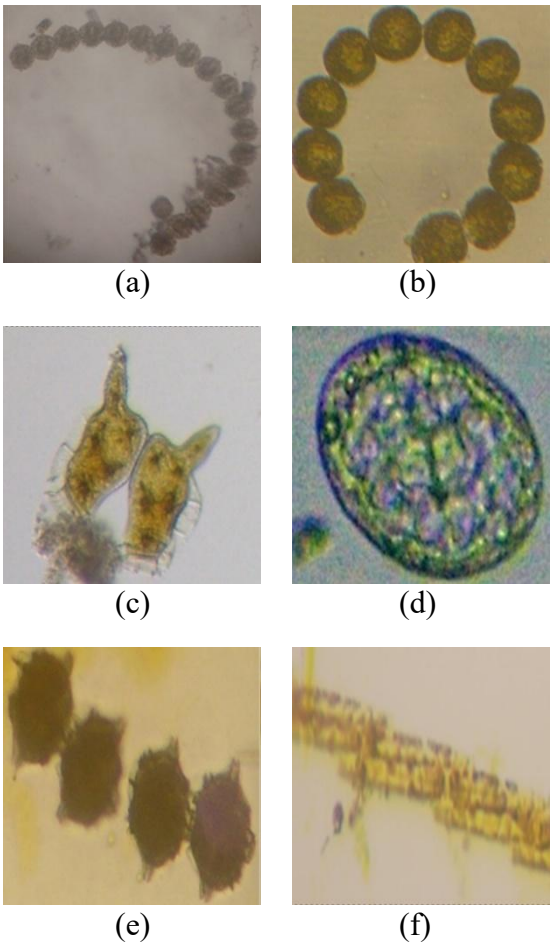

Fig. 7. Light micrographs of phytoplankton observed at 200 × magnification, a = *Alexandrium cattenella*; b = *Gymnodinium sp*; c = *Dinophysis sp.*; d = *Pyrodinium bahamense*; e = *Prorocentrum sp* ; f = *Pseudo-nitzchia.*

In 2014, *A.catenella, Dinophysis sp., Gymnodinium sp., Prorocentrum sp., Pyrodinium sp.,* and *Pseudo-nitzschia* were identified at various locations, including Ujung Bom, Kubur Island, Lahu Island, and Maitem. The highest population of *Pseudo-nitzschia* (1,635 cells/L) and *Prorocentrum sp*. (3,270 cells/L) was recorded at Kubur Island and Lahu Island, respectively. Meanwhile, *A. catenella* exhibited its highest population density (9,810 cells/L) at Kubur Island, as shown in Table. SVI.

During the 2015 monitoring, *Pseudo-nitzschia, Dinophysis sp., Gymnodinium sp., Prorocentrum sp.,* and *Pyrodinium sp*. were observed at Ujung Bom, Pasaran Island 1, Pasaran Island 2, Hurun Bay 1, and Hurun Bay 2. The highest populations of *Pseudo-nitzschia* (1,234 cells/L) and *Pyrodinium sp.* (1,480 cells/L) were recorded at Ujung Bom and Pasaran Island 1, respectively. As shown in Table S VII, the abundance of algal cells ranged as follows: *Prorocentrum sp*. (110–3,270 cells/L), *Gymnodinium sp.* (0–1,635 cells/L), and *Pyrodinium sp.* (0–1,840 cells/L). These algae possess the potential to produce various harmful toxins, including PSP, neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), as well as hemolytic and hemagglutinating effects [23]. These toxins effectively accumulate in the flesh of filter-feeding organisms, such as oysters, in bloom-affected areas. While the marine organisms themselves remain unaffected by the toxins, they serve as vectors, transferring these harmful substances to humans through the consumption of contaminated seafood [9].

As filter feeders, shellfish serve as bioindicators for detecting contamination of marine biota by PSP toxins. Therefore, periodic monitoring is crucial. Due to the high toxicity of saxitoxin, there is a need for sensitive and rapid analytical methods to detect its presence in shellfish accurately [13]. Ecologically, shellfish play a crucial role in marine food webs, feeding on plankton and other suspended food particles, while also acting as prey for fish, birds, marine mammals, and various invertebrates [6].

In Indonesia, radiolabeled techniques have been extensively developed for receptor binding studies, radiopharmaceutical development, and molecular interaction analyses. Previous research demonstrated the successful synthesis of radioiodinated bioligands and their application in receptor binding assays using Scintillation Proximity Assay (SPA) methodology to quantify receptor affinity with high sensitivity and selectivity [24]. By extending these proven radiolabeling and receptor binding approaches to marine toxins including phycotoxins and ichthyotoxins that act through specific protein ligand interactions it is now feasible to develop rapid, quantitative assays for marine toxin monitoring in coastal environmental and food safety contexts.

CONCLUSION

This study validated the RBA as a sensitive, precise, and reproducible method for detecting STX in shellfish from Lampung Bay. The assay consistently achieved high linearity (R^2 = 0.9962), acceptable precision (RSD < 30%), and a detection limit of 0.2 µg STX eq./100 g, far superior to the mouse bioassay (40 µg STX eq./100 g). Analysis of local shellfish samples revealed toxin levels below international safety thresholds, although one site exhibited elevated concentrations, underscoring the need for continued monitoring. Integration of toxin analysis with environmental and phytoplankton data represented that this work emphasizes RBA as a robust tool for food safety surveillance and early detection of harmful algal bloom events in Indonesia and comparable marine regions.

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AUTHOR CONTRIBUTION

Untung Sugiharto: Conceptualization, Validation, Resources, Project administration, Visualization, Supervision, Writing - original draft, Writing - review & editing. Eti Rohaeti: Conceptualization, Methodology, Data curation, Validation, Formal analysis, Investigation, Visualization, Supervision, Writing - original draft, Writing - review & editing. Henny Purwaningsih: Conceptualization, Methodology, Data curation, Validation, Formal analysis, Investigation, Visualization, Supervision, Writing - original draft, Writing - review & editing. Ali Arman Lubis: Conceptualization, Methodology, Data curation, Validation, Formal analysis, Investigation, Visualization, Supervision, Writing - original draft, Writing - review & editing. Tri Retno Dyah Larasati: Data curation, Formal analysis, Validation, Investigation. Dienda Shintianata: Data curation, Formal analysis, Validation, Investigation. Faza Putri Andini: Data curation, Formal analysis, Validation, Investigation. Sonia Saraswati Meiliastri: Data curation, Formal analysis, Validation, Investigation. Muawanah: Data curation, Formal analysis, Validation, Investigation.

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