Marine Pollution Bulletin 185 (2022) 114340

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Co-occurrence of pectenotoxins and *Dinophysis miles* in an Indonesian semi-enclosed bay

Sem Likumahua [a,b,c,*], M. Karin de Boer [a,d], Bernd Krock [e], Willem M. Tatipatta [b], Malik S. Abdul [b], Anita G.J. Buma [a]

[a] Center for Isotope Research-CIO Oceans, Energy and Sustainability Research Institute Groningen, Faculty of Science and Engineering, University of Groningen, Nijenborgh 7, 9747AG Groningen, the Netherlands
[b] Centre for Deep Sea Research, The National Research and Innovation Agency (BRIN), Jl. Y. Syaranamual Guru-guru, Poka, 97233 Ambon, Indonesia
[c] Collaborative Research Center for Aquatic Ecosystem of Eastern Indonesia, Pattimura University, Jl. Ir. M. Putuhena, Poka, 97233 Ambon, Indonesia
[d] Beta Science Shop, Faculty of Science and Engineering, University of Groningen, Nijenborgh 6, 9747AG Groningen, the Netherlands
[e] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

ARTICLE INFO

Keywords:
Shellfish toxins
Algal bloom
Lipophilic
Dinophysis miles
Pectenotoxins
Ambon Bay

ABSTRACT

The study aims to unravel the variability of *Dinophysis* spp. and their alleged toxins in conjunction with environmental drivers in Ambon Bay. Phytoplankton samples, lipophilic toxins and physiochemical water properties were analysed during a 1.5-year period. Three *Dinophysis* species (*D. miles*, *D. caudata*, and *D. acuminata*) were found in plankton samples, of which *D. miles* was the most abundant and persistently occurring species. Pectenotoxin-2 (PTX2) and its secoacid (PTX2sa) were detected throughout, and PTX2sa levels strongly correlated with *D. miles* cell abundance. The toxin showed a positive correlation with temperature, which may suggest that *D. miles* cells contain rather constant PTX2sa during warmer months. Dissolved nitrate concentrations were found to play a major role in regulating cell abundances and toxin levels. This study adds adequate information regarding marine biotoxins and potentially toxic species for future Harmful Algal Bloom management in Ambon and Indonesia at large.

1. Introduction

The dinoflagellate genus *Dinophysis* Ehrenberg is known to have a wide distribution range in marine environments worldwide, but it is found in high densities mostly in coastal waters (Hallegraeff et al., 2003). Some species may inhabit narrow environmental ranges whereas others have a broad distribution range due to their ability to acclimate to different temperature and salinity ranges (reviewed by Reguera et al., 2012). The genus performs photosynthesis using plastids obtained from their preys, and a recent study showed that *Dinophysis miles* can have plastids from different algae (Qiu et al., 2011). Some *Dinophysis* species are found to co-occur during their proliferations in natural waters. In tropical waters, blooms of *D. caudata* often co-occur with other *Dinophysis* species such as *D. acuminata* and *D. miles* (Marasigan et al., 2001). In natural marine phytoplankton communities, *Dinophysis* species mostly occur at very low cell numbers (<100 cells L^{-1}), yet, they can reach high densities of approximately 10^5 cells L^{-1} in coastal waters (Hallegraeff and Lucas, 1988; Reguera et al., 2012). *Dinophysis* distribution in water column and bloom formation are mostly regulated by physical drivers such as temperature, tides, wind forced water movements, upwelling and downwelling (Campbell et al., 2010; Escalera et al., 2010; Díaz et al., 2013; Hattenrath-Lehmann et al., 2013; Whyte et al., 2014; Moita et al., 2016; Ruiz-Villarreal et al., 2016).

Given their tendency to thrive in coastal waters, *Dinophysis* spp. may cause major problems to shellfish industries and public health around the world (Lindahl et al., 2007; Pizarro et al., 2009). Yasumoto et al. (1985) found that even at low abundances such as a few hundred cells L^{-1} the genus was able to produce detectable toxin levels. Ten species of the genus are associated with the production of diarrhetic shellfish toxins (DSTs) including okadaic acid (OA) and its derivatives dinophysistoxins (DTXs), as well as pectenotoxins (PTXs) (Reguera et al., 2014). DSTs accumulate in filter feeders, leading to human poisoning with symptoms of nausea, vomiting, diarrhea, chills and abdominal pain after consuming the contaminated biota (Pizarro et al., 2008; Hess, 2010; Li

* Corresponding author at: Centre for Deep Sea Research, The National Research and Innovation Agency (BRIN), Jl. Y. Syaranamual Guru-guru, Poka, 97233 Ambon, Indonesia.
E-mail address: sem.likumahua@brin.go.id (S. Likumahua).

https://doi.org/10.1016/j.marpolbul.2022.114340
Received 4 September 2022; Received in revised form 29 October 2022; Accepted 2 November 2022

et al., 2014; Reguera et al., 2014; Hu et al., 2017). Environmental drivers such as salinity, temperature, light, and nutrient concentration combined with genetic factors have shown effects on toxin production of marine dinoflagellates including *Dinophysis*, which may also lead to variations in toxin profiles and cell quotas (Cembella and John, 2006; Mafra et al., 2014 and references therein). Yet, information regarding *Dinophysis* spp. and their toxin variability in the field is rare (Reguera et al., 2014). Culture experiments revealed variations of toxin production, content and level, occurring at different growth phases while also varying among strains (reviewed by Reguera et al., 2014). A culture study of *D. accuminata* demonstrated that toxins are produced at maximum levels during the exponential phase and remain stable or only slightly increase in the stationary phase. The differences among growth phases may be caused by the imbalance between toxin production and cell division (Tong et al., 2011).

Previous field and laboratory studies have shown that *Dinophysis* species are the only dinoflagellate genus that is able to produce pectenotoxins (PTXs) (reviewed by Reguera et al., 2014; Fabro et al., 2016). PTXs are cyclic polyether lactones that differ from each other structurally due to the difference of oxidation degree, spiroketal ring arrangements, and the lactone opening ring, which was found to be associated with toxicity reductions (Quilliam, 2003; Reguera et al., 2014; Fabro et al., 2016). PTXs and especially pectenotoxin-2 (PTX2) are generally found in combination with OA and DTXs in shellfish associated with DSP events (Dominguez et al., 2010). Yet, only OA and DTXs are proven as the main DSP causatives (Vale and Sampayo, 2002), since no diarrheagenic symptoms are observed after PTX2 oral administration to laboratory rodents (Fabro et al., 2016). To date, direct effects of PTX2 on human illness are debatable, even though other laboratory studies have shown that both oral and injection of PTX2 equivalents can damage livers of mice (Aune et al., 2002; Miles et al., 2004; Espiña and Rubiolo, 2008; Li et al., 2014). In addition, PTX2 showed negative effects on reproduction and recruitment of the Pacific oyster as well as reduction of forage fish reproduction and fitness (Rountos et al., 2019; Gaillard et al., 2020). Ferron et al. (2016) showed that PTX2 acts as cytotoxic in human metabolisms, inducing DNA damage and some key xenobiotic-metabolizing enzymes (XME) (Alarcan et al., 2017). PTX2 is metabolized into other PTX derivatives in shellfish, of which pectenotoxin-2 seco acid (PTX2sa) is one of the alleged converted products, generated when hydrolysis of the lactone moiety occurs (Suzuki et al., 2001; Miles et al., 2004). These toxins have been reported elsewhere around the world, yet, no information was available for Indonesian waters (Vale and Sampayo, 2002; MacKenzie et al., 2005; Fernández et al., 2006; Wilkins et al., 2006; Amzil et al., 2007; Farrell et al., 2018; Sibat et al., 2018).

Occurrences of toxic dinoflagellates in Indonesian waters have been recorded since 1970s. Since then, their proliferations have shown severe impacts on marine biota, fishery industries and human health (Wouthuyzen et al., 2004; Thoha et al., 2007; Aditya et al., 2013). In Ambon Bay, Eastern Indonesia, human illnesses and casualties were generally due to paralytic shellfish poisoning (PSP) events associated with blooms of *Pyrodinium bahamense* var. *compressum*. As a result, a dinoflagellate-monitoring program has been installed in Ambon Bay to monitor the abundance and distribution of potentially toxic species. Other than *Pyrodinium bahamense*, monitoring results (data unpublished) have revealed the presence of other potentially toxic species such as *Gymnodinium catenatum*, *Alexandrium* spp., *Dinophysis acuminata*, *D. caudata*, *D. tripos*, *D. miles* and the diatom species *Pseudo-nitzschia* spp. (Likumahua et al., 2019, 2020). However, associated toxin information of these species is rare both for the area as well as for the country at large. Thus, this present study focused on *Dinophysis* species observations as well as their alleged associated toxins in Ambon Bay (Fig. 1), eastern Indonesia.

In an earlier study, we for the first time detected PTXs (PTX2 and PTX2sa) in phytoplankton samples collected from Ambon Bay. These results, based on a 7-month field campaign in 2018, showed a weak correlation between PTX2 levels and *Dinophysis* spp. cell abundances (Likumahua et al., 2020). In addition, PTX2sa levels were scarcely

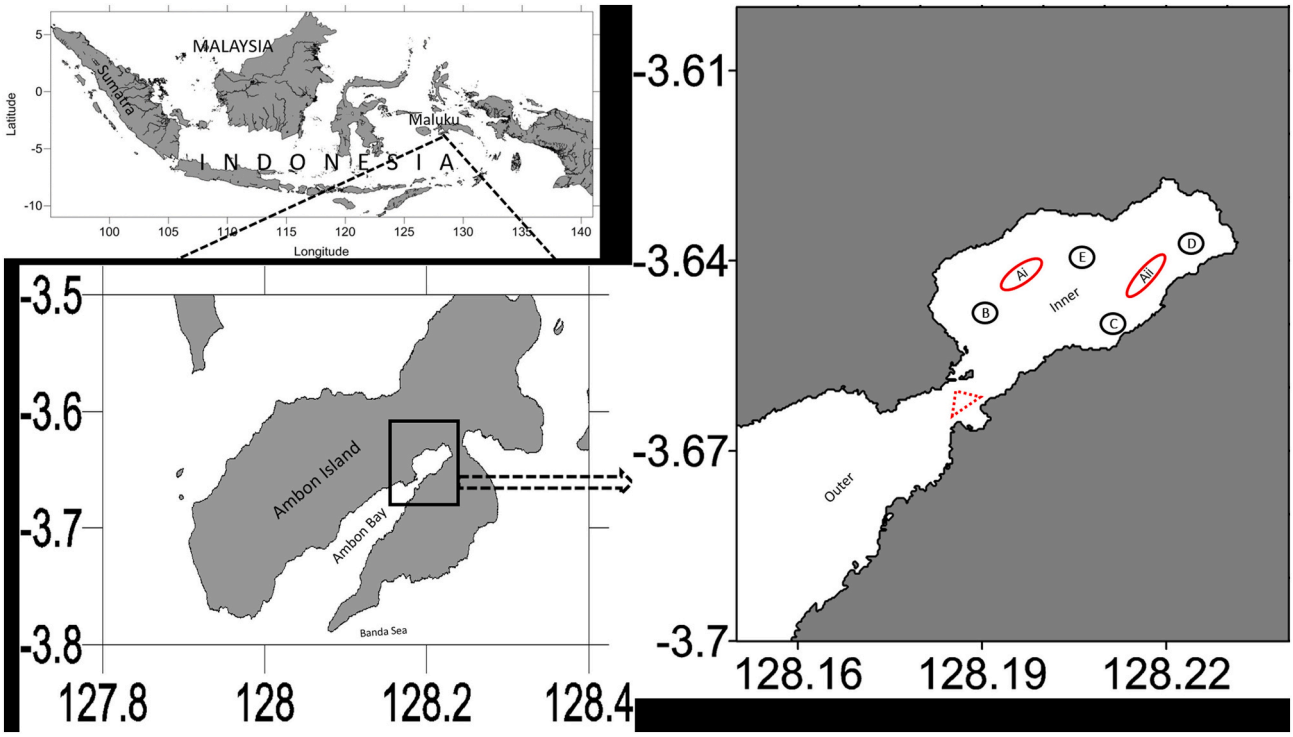

Fig. 1. Map of Ambon Bay with the location of the six sampling stations (B, C, D and E were sampled in 2018; and two red stations (Ai and Aii) were sampled in 2019). The red triangle shows the location of the sill. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

detected and only found during the dry season. Following up on this first campaign, biological, toxicological and physiochemical sampling was continued, in order to further understand the dynamics of *Dinophysis* spp. and its relationships with PTXs under varying environmental conditions. Given the lack of information on *Dinophysis* species in Indonesian waters, a 1.5-year data set was assembled based on sampling campaigns between 2018 and 2019 in Ambon Bay. The objective of this study was thus to investigate dynamics of *Dinophysis* species and their associated toxins in relation with environmental drivers. Therefore, the present research was expected to establish valuable baseline data to aid adequate formulation of a management system to deal with harmful algal blooms and associated toxic events nationwide.

2. Materials and methods

2.1. Study area

Ambon Island (3°38′17″S and 128°07′02″E) (Fig. 1) is located in Maluku Province, eastern Indonesia, and has roughly 450,000 inhabitants. The area is influenced by a tropical climate, showing both dry and wet seasons. The dry season is associated with the northwest monsoon occurring between October and March, and is characterized by low precipitation and relatively high air temperatures. The wet season is characterized by high precipitation and relatively low air temperatures, occurring during the southeast monsoon between March and September. The period of transformation from the dry to wet season is known as Transition I (between March and May), and from the wet to dry season is Transition II (between September and November). The island has a semi-enclosed estuary, called Ambon Bay, that has a narrow and shallow sill (Fig. 1) dividing the bay into two parts (outer and inner bay) while limiting water circulation between both sections.

2.2. Sampling

Samples were collected in the inner bay between January 2018 and June 2019. Four stations (B, C, D and E (Fig. 1)) were selected from the standard LIPI (Lembaga Ilmu Pengetahuan Indonesia - Indonesian Institute of Sciences) monitoring program and sampled on a weekly-based scheme between January and July during 2018 (1st campaign), which encompassed the first dry season, transition I and the first wet season. During expected phytoplankton peaks of each season, samples were collected twice a week. After the first intensive sampling period, a second less frequent sampling scheme (2nd campaign) was followed, to cover the transition II in 2018 between September and November, the second dry season (December 2018 to February 2019), the second transition I (March and May 2019) and the beginning of the second wet season (June 2019). Samples were collected fortnightly during this second scheme, at two stations (Ai, representing stations B and E; Aii, representing stations C and D) (Fig. 1). These stations were determined based on highly similar characteristics shared by the two nearby stations as sampled during the earlier campaign (Likumahua et al., 2019). 168 samples were collected during the first campaign based on 42 field-sampling days and 38 samples during the second phase based on 19 sampling days. Thus, the total amount of samples collected during the 1.5-year sampling period was 206 ($n = 206$). The total time spent for boating and sampling was approximately <2 h, prior to sample processing in the Centre for Deep Sea Research (CDSR) - LIPI laboratories.

2.3. Phytoplankton sampling and analysis

Phytoplankton samples were collected using a Hydro-Bios plankton net (Ø 40 cm, length 100 cm, 20 μm mesh size), which was deployed twice through the upper 20 m of the water column. Samples were transferred to 500 mL bottles and adjusted to a final volume of 400 mL with filtered seawater. Forty milliliters of concentrated net sample was fixed with acidic lugol iodine solution and one drop of formalin (4 % final concentration) to inactivate bacteria. The cell abundance of *Dinophysis* spp. was determined by counting 10 mL fixed sample using Uthermöhl sedimentation chambers (Hydro-Bios, Kiel-Germany). Cells were let to settle at the bottom of chambers for at least 12 h prior to analysis at 200× magnification using an inverted microscope (LH 50A, Olympus, Japan). The whole surface of the chamber was scanned for the analysis and the final cell density was expressed in cells per net tow (cells NT^{-1}). Chlorophyll-a (chl-a) levels were obtained from the data recorded by a fluorescence sensor mounted on a CTD (see below), which had been calibrated by spectrophotometry. A prior study had shown a strong positive correlation between the chl-a fluorescence from the CTD and net haul levels (Likumahua et al., 2019). The average 0–2 m chl-a concentration was calculated as the surface level, and the average 0–20 m was calculated to express the average water column chl-a level.

2.4. Physiochemical water properties

A compact Conductivity, Temperature, Depth (CTD) (Alec ASTD 687, Kobe-Japan) was deployed to measure depth, temperature, salinity, turbidity, density and chlorophyll-a (chl-a) fluorescence. Temperature and density profiles were used to calculate mixed layer depth (MLD) and stratification indices using the threshold method as described by González-Pola et al. (2007) and Somavilla et al. (2017).

Water samples for nutrient analysis were collected with a 3.5 L Niskin bottle at two depths, representing water layers above (2 m) and below (20 m) the thermocline, if present. Water was pre-filtered on board using a 20 μm filter mesh to eliminate large plankton, organic material and sediment particles. Filtered water samples were transferred to pre-cleaned (deionized water) 600 mL bottles and stored in a dark insulated box, filled with seawater at ambient temperature. In the lab, samples were filtered immediately through 0.2 μm pore size, 47 mm Ø nylon membrane filters (Fisherbrand, EU) using a vacuum pump with 0.3 bar maximum pressure, and stored at −20 °C until analysis. Dissolved nutrients, nitrate, phosphate and silicate were measured using a spectrophotometer (UV–Vis Shimadzu 1700, Kyoto-Japan) following Strickland and Parsons (1972), and ammonium following APHA (1998).

2.5. Toxin analysis

Cell pellets for the toxin analysis were obtained from 300 mL (1st campaign) and 360 mL (2nd campaign) of the concentrated net-tow samples by centrifugation at 12,100 ×*g* for 20 min and transferred to 2 mL centrifugation tubes (Eppendorf, Hamburg-Germany). Pellets were then stored at −20 °C prior to analysis. Approximately 0.9 g lysing matrix D and 500 μL methanol was added to the pellets followed by sample homogenization and toxin extraction at maximum speed (6.5 m s^{-1}) for 45 s in a homogenizer (FastPrep Bio101, Thermo Savant, Illkirch, France). Homogenized samples were centrifuged at 16,100 ×*g* at 4 °C for 15 min, after which the supernatants were transferred to 0.45 μm pore size spin filters (Milipore Ultrafree, Eschborn, Germany) and centrifuged for 30 s at 5700 ×*g*. Filtrates were transferred to HPLC vials for toxin analysis by liquid chromatography – tandem mass spectrometry (LC-MS/MS) for DSTs (okadaic acid (OA), DTX1, and DTX2) and PTXs. Samples of the first campaign were analysed on LC-MS/MS system consisting of a liquid chromatograph (LC1100, Agilent, Waldbronn, Germany) coupled to a hybrid triple quadrupole-linear ion trap tandem mass spectrometer (API 4000 QTrap, Sciex, Darmstadt, Germany) as detailed in Krock et al. (2008). In brief, 5 μL of sample were injected into the chromatographic system equipped with a C8 analytical column (Hyperclone 3 μm BDS C8 130 Å 50 × 2 mm, Phenomenex, Aschaffenburg, Germany), maintained at 20 °C. The flow rate was 0.2 mL min^{-1} and gradient elution was performed by eluents A (water, formic acid, and ammonium formate) and B (acetonitrile, formic acid, and ammonium formate). Detection of toxin was performed in the selected reaction monitoring (SRM) scan carried out in positive-ion mode with selected mass transitions (OA, DTX-2: m/z 822 > 223, DTX-1: m/z 836 > 237,

PTX-2: m/z 876 > 213, and PTX-2sa: m/z 894 > 213). Samples of the second campaign were analysed on a UHPLC system (Acquity/Xevo TQ-XS, Waters, Eschborn, Germany). Separation of the methanol extract was performed after injection of 0.5 μL sample on RP C18 column (Purospher® STAR RP-18 endcapped (2 μm) Hibar®, Waters), which was maintained at 40 °C. The flow rate was 0.6 mL min^{-1} and gradient elution with two eluents (eluent A: water; eluent B: acetonitrile/water (9:1 v/v), both containing 6.7 mM ammonia). The linear gradient was initialized after 1.5 min isocratic elution with 70 % A to 10 % A until 3.5 min. This condition was maintained until 4.0 min and the gradient was then returned to initial conditions until 4.1 min. These settings were retained up to 5.0 min (=total running time). The SRM same transitions as mentioned above were used.

The detection limit of each toxin was determined separately for each campaign according to the different mass specs methods used (Table 1), and the limits were expressed in nanogram per net tow (ng NT^{-1}).

2.6. Statistical analysis

Data used to display physiochemical and biological time series was obtained by calculating average values of all stations for each parameter. This was done since spatial variabilities were very low and no significant differences were found among stations (data not shown). However, data from all stations were used for all statistical analyses, which were done using the Minitab 18 package. The multivariate analysis of Principal Component Analysis (PCA) and Spearman's rank were employed as statistical tools in this study. Data was standardized to eliminate physical dimensions before the ordination in the PCA according to Legendre and Birks (2012). A PCA score plot was performed to investigate the discrimination among seasons, based on all data (physicochemical and biological data). Meanwhile, PCA loading plots coupled with non-parametric Spearman's rank correlation analyses were employed to analyse relationship among parameters for the whole sampling period and individual seasons separately based on the score plot results. In this way, visible regulations of environmental drivers on both cell abundances and toxin levels could be obtained. The Spearman rank coefficient correlations (r) are categorized as follows: $r > 0.6$ is strong, r between 0.6 and 0.4 is medium, and $r < 0.4$ is weak. Regression analysis was done after omitting all zeros in the data (where toxins were not detected) to display a clear linear correlation between cell abundances and toxins once a strong correlation was found and the data was distributed normally.

3. Results

3.1. Season grouping

The time resolved PCA score plot of all parameters revealed clear clustering of two main seasons (dry and wet) and transition period I (Trn I) from dry to wet as well as period II (Trn II) from wet to dry (Fig. 2). The results showed that the dry season and the first transition (Trn I) in 2018 were grouped together whereas the second transition period of 2018 (Trn II) distinctly separated from the other seasons. Thus, these three distinct periods (dry, wet and Trn II) were distinguished in each time series graph and statistical analyses. In addition, since the majority of data of the dry season of 2019 had a different distribution from the dry season of 2018, both periods were statistically analysed separately. Three data points of Trn I of 2018 fell in the wet season area and were subsequently grouped with the wet season since these data belonged to dates where the season started.

Table 1
Limit of detection (LoD) for DSTs, PTX2 and PTX2sa.

Toxin types	LoD (ng NT^{-1})	
	1st campaign	2nd campaign
Okadaic acid (OA)	5.91	0.65
Dinophysis toxin – 1 (DTX1)	2.94	0.82
Dinophysis toxin – 2 (DTX2)	3.23	1.48
Pectenotoxin – 2 (PTX2)	0.37	0.40
Pectenotoxin – 2 seco acid (PTX2sa)	0.41	0.50

3.2. Physiochemical properties

The average (0–20 m) salinity ranged between 32.53 and 33.84, where highest values were found between the wet season and the second transition period (Trn II) in 2018 (Fig. 3A). During 2018, the average salinity showed an upward trend and subsequently decreased gradually during the dry season 2019 (Fig. 3A). Highest average (0–20 m) water temperatures were recorded during the dry season, and a considerable decrease occurred during the wet season for both years (Fig. 3A). Water temperature increased during the second transition period of 2018 (Trn II), between September and November. The average (0–20 m) water temperature ranged between 26.37 and 30.06 °C.

A high variability in average mix layer depth (MLD) was observed in the inner bay during the sampling period (Fig. 3B). The similarly high variation was observed spatially at all stations (data not shown). Shallow MLDs frequently occurred during the second transition period (Trn II) in 2018 and the first half of the dry season of 2019. Generally, mixed layer depth (MLD) ranged between 0.5 and 20 m. Meanwhile, the average stratification index ranged between 0.08 and 5.18 kg m^{-3} (Fig. 3B). A slight upward trend in stratification index was observed during the dry and wet season of 2018, and unclear trends were shown during the subsequent period at the end of the year as well as the following year. At the beginning of the dry season of 2019, water columns were stratified. However, high stratification indices were frequently found during the wet season in both years.

The average phosphate concentration varied during the sampling period, which ranged from 0.002 to 1.65 μM at the surface (2 m) and from 0.15 to 2.21 μM near the bottom (20 m) (Fig. 4A). An unclear pattern was shown over time, yet, a small decreasing trend was found during the end of the wet season for both years. In general, phosphate concentrations were higher at the bottom 20 m than at the water surface. Similarly, high average nitrate concentrations were observed near the bottom, which showed upward trends between the second half of the dry season and the wet period in both years (Fig. 4B). Yet, the concentration at the surface showed an unclear trend. The average concentration ranged between 0.003 and 1.35 μM at the surface, and between 0.19 and 5.73 μM near the bottom (Fig. 4B). Unlike phosphate and nitrate, the average silicate concentration at the surface was higher than near the bottom (Fig. 4C). During the dry and wet season of 2018, the concentration at both depths showed a slight upward trend, reaching the highest level at the peak of the wet season. During the second transition period (Trn II) of 2018, the concentration dropped considerably and reached the lowest levels for both depths (Fig. 4C). The average silicate concentration ranged from 0.31 to 49.62 μM and from 0.10 to 47.95 μM at the surface and the bottom 20 m, respectively. Average ammonium concentrations were mostly comparable for both layers during the sampling period, except for January 2019 where the bottom 20 m levels were much higher than those at the surface (Fig. 4D). The average ammonium concentration ranged from 1.36 to 12.67 μM at the surface and from 1.21 to 16.48 μM near the bottom. An increasing trend was found in 2018 during the dry and wet season, which subsequently dropped considerably during the second transition period (Trn II). Meanwhile, no clear trend was observed during the following year (Fig. 4D).

3.3. Biological parameters

A slightly upward trend of the average (0–20 m) chl-a (representing total phytoplankton biomass) was observed in 2018, during which highest levels were observed in the wet season followed by decreases

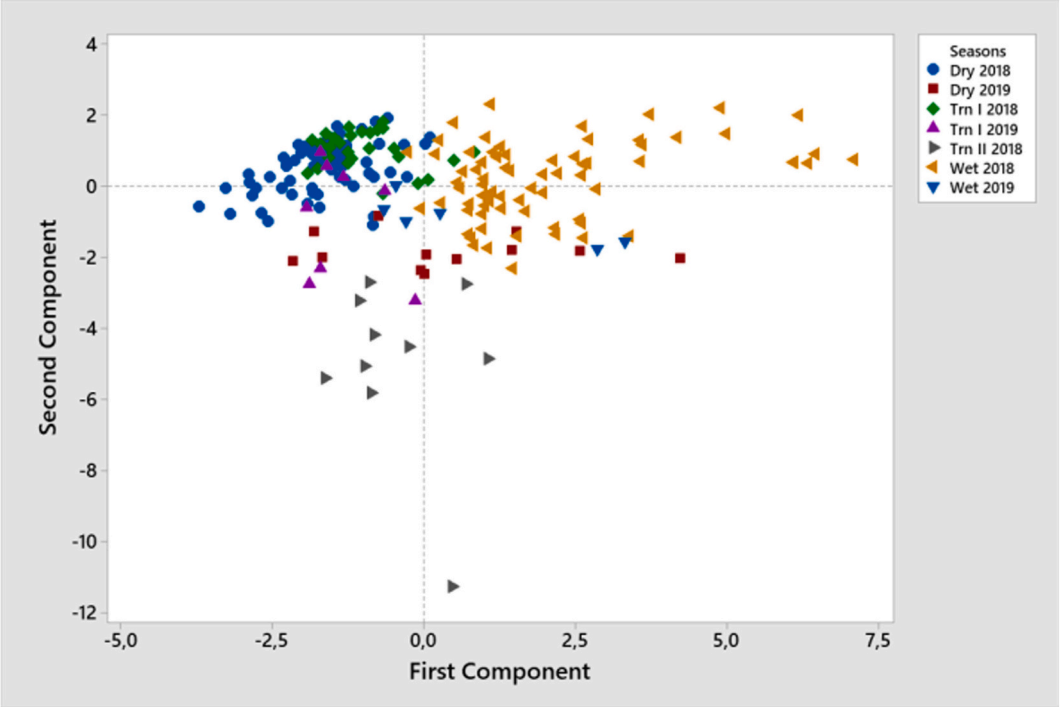

Fig. 2. Score plot PCA analysis for seasonal grouping based on all water properties and biological data collected in Ambon Bay between January 2018 and July 2019 (n = 206).

during the second transition period (Trn II) (Fig. 5A). The average (0–20 m) level in general was higher than the surface concentration, with exceptions between January and February 2019 where surface concentrations were high. The average chl-a level ranged from 0.16 to 5.11 mg m^{-3} and from 0.35 to 3.02 mg m^{-3} at the surface and the average (0–20 m), respectively (Fig. 5A).

Three *Dinophysis* species (*D. miles*, *D. caudata* and *D. acuminata*) were observed during the sampling period (Fig. S1), of which *D. miles* was the most dominant species in terms of net cell numbers (Fig. 5B). The highest *D. miles* abundance was found in the second transition period where it co-occurred with high *D. caudata* cell densities. Meanwhile, *D. acuminata* was found in very low cell densities, roughly comprising between 7 and 10 % of the total *Dinophysis* population. Average cell abundances of *D. miles* ranged between 50 cells NT^{-1} and 12.82 × 10^3 cells NT^{-1}, *D. caudata* ranged from not observed to 7.83 × 10^3 cells NT^{-1}, and *D. acuminata* ranged from not observed to 60 cells NT^{-1}. The abundance of *D. miles* showed a clear upward trend during 2018 and a slight increase during the dry season of 2019. During the dry season, cell abundances of *D. miles* were different for both years, since cell densities in 2019 were higher than in 2018 (Fig. 5B). The two other species showed no clear trends either during the whole sampling period or during a certain season.

Lipophilic toxin analysis revealed the absence of okadaic acid (OA) and *Dinophysis* toxins (DTX1 and DTX2). Yet, PTX2 and its seco acid (PTX2sa) were frequently detected above the LoD in plankton pellets. The average PTX2 level was detected between the LoD and 43.48 ng NT^{-1}, and PTX2sa was detected between the LoD and 15.11 ng NT^{-1} (Fig. 5C). PTX2 levels were found to be very low during the dry season of 2018 and continued to increase slightly during the wet season. Meanwhile, inversely, PTX2sa levels were higher during the dry season and lower during the wet season of 2018 (Fig. 5C). Both toxins reached maximum levels during transition period II in 2018. Higher PTX2sa levels were also detected during the subsequent year, especially during the dry season of 2019.

ICES (2006) recommended that toxin cell quotas can be calculated for dominant species only if the cell numbers are higher than 10^4 cells NT^{-1}. Therefore, the toxin cell quota calculation of the dominant *D. miles* in this study was generated from 2 data points (10.34 × 10^3 cells NT^{-1} and 20.5 × 10^3 cells NT^{-1}). PTX2 cell quotas were 0.01 pg $cell^{-1}$ and 0.34 pg $cell^{-1}$, and PTX2sa cell quotas were 0.44 pg $cell^{-1}$ and 1.14 pg $cell^{-1}$.

3.4. Relationship between biological factors and environmental drivers

When considering the whole sampling period, the PCA loading plot and the Spearman rank analysis showed a positive correlation between *D. miles* and PTX2 ($r = 0.54$; $p = 0$), while a weak relationship was found with PTX2sa ($r = 0.23$; p = 0) (Fig. 6 and Table 2). Cell abundances of *D. caudata* showed a weak correlation with PTX2sa ($r = 0.27$; $p = 0$), and no correlation was found with PTX2. Temperature negatively influenced both *D. miles* abundances and PTX2 levels, yet positively correlated with PTX2sa (Table 2). Meanwhile, nitrate was the only nutrient that showed correlation with *D. miles* and both toxins (Fig. 6 and Table 2). Stratification represented a physical factor that had a weak impact on *D. miles* and PTX2.

Judging from the PCA score plot analysis for the dry season of 2018, *D. miles* positively correlated with both toxins, yet PTX2sa was stronger than PTX2 (Fig. S2A). The Spearman rank showed similar results as the PCA, of which the PTX2sa correlation index was stronger than PTX2 ($r = 0.42$ and $r = 0.30$; $p = 0$, respectively) (Table 2). Silicate concentrations at both depths correlated with *D. miles* and PTX2 while no correlation was found with PTX2sa. Medium correlations were found between PTX2 and ammonium at both depths. During the dry season of 2019, *D. miles* was only correlated with PTX2sa level and no relation was found with PTX2 (Fig. S2B and Table 2). Phosphate concentrations positively influenced *D. miles* cell abundances ($r = 0.63$; $p < 0.05$) and PTX2sa levels ($r = 0.73$ and 0.80; p < 0.05). In contrast, silicate and ammonium showed negative correlations with *D. miles* and PTX2sa (Table 2). Linear regression analyses of *D. miles* cell abundances versus PTX2sa levels showed strong relationships during the dry season of 2019 and for the total dry season of both years (Fig. 7).

Weak correlations were found between *Dinophysis* cell abundances

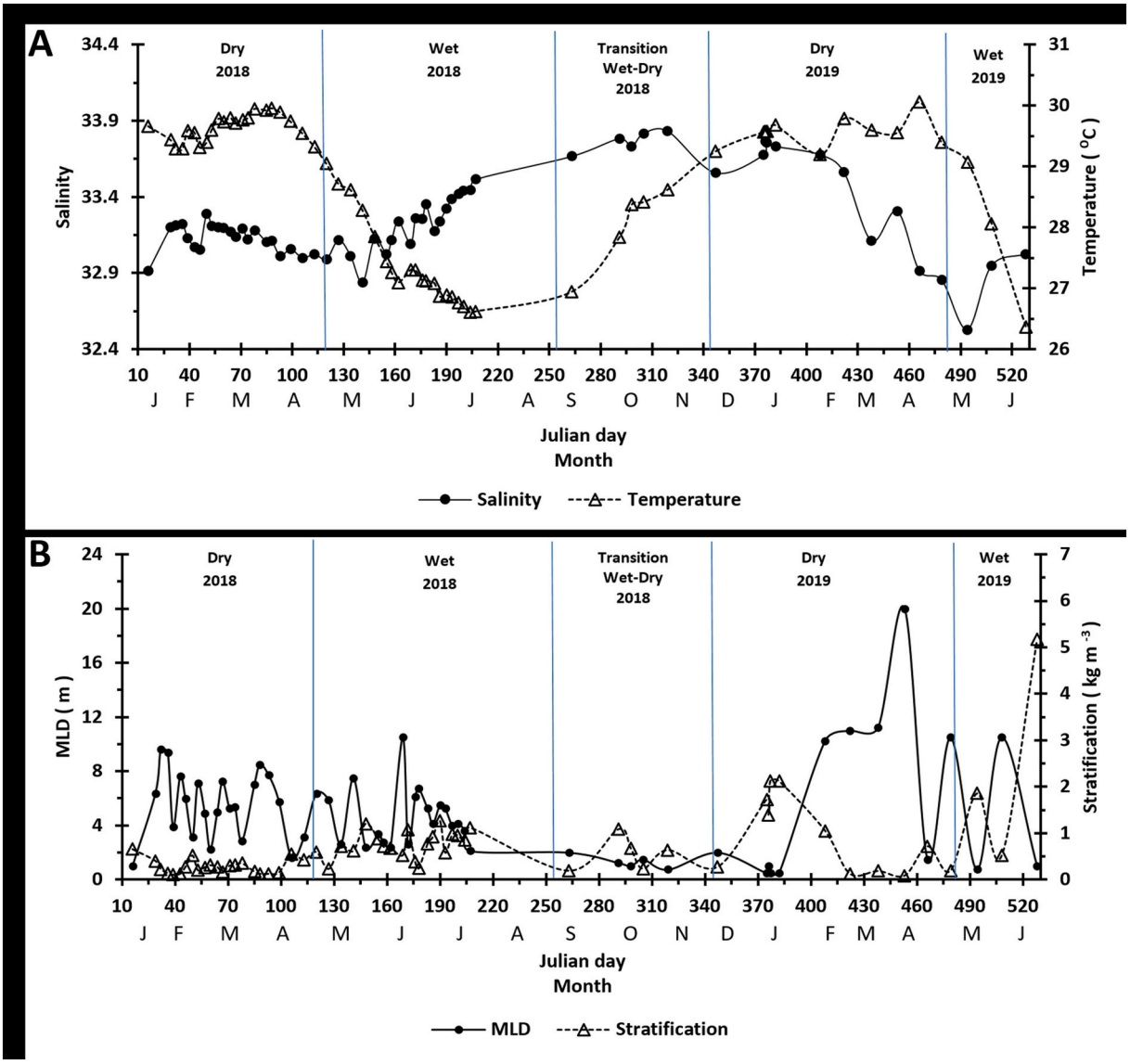

Fig. 3. Temporal variability (average of all stations) of water physical properties in Ambon Bay (2018–2019). A: average (0–20 m) temperature and salinity, B: mixed layer depth (MLD) and stratification. Vertical blue lines indicate boundaries of each season for both years, as determined by PCA score plot of all measured parameters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(*D. miles* and *D. caudata*) and PTX2 levels during the whole wet season (Fig. S2C and Table 2). Phosphate and nitrate concentrations positively correlated with *D. miles* cell abundances, and in reverse silicate showed a negative relationship (Table 2). When cell abundances and toxin levels were high in the second transition period, *D. miles* and *D. caudata* were strongly correlated ($r = 0.75$; $p = 0$), yet no clear influence of nutrients on biological factors (cells and toxins) were found (Fig. S2D and Table 2). Both *Dinophysis* species (*D. miles* and *D. caudata*) showed a strong negative correlation with PTX2 levels ($r = -0.75$ and -0.83; $p = 0$, respectively). Conversely, *D. miles* abundances positively correlated with PTX2sa levels ($r = 0.61$; $p = 0$).

4. Discussion

PTX2 and PTX2sa were successfully detected and correlated with *Dinophysis* cell abundances, which implies that the population present in Ambon Bay only produces pectenotoxins. The assembled 1.5-year data collected in Ambon Bay during 2018–2019 confirmed the persistent occurrence with high abundances of *D. miles* compared to two other *Dinophysis* species and the species cell density strongly correlated with pectenotoxin levels. Interestingly, this extended data set (compared with the earlier 7 months campaign, Likumahua et al., 2020) revealed high PTX2sa abundances in the dry season and the second transition period, during which strong correlations were found between the toxin and *D. miles* cell abundance. Thus, this finding may indicate that *D. miles* cells not only contain PTX2, but also that they are likely to contain a stable PTX2sa level under a combination of environmental conditions, typical for those periods.

4.1. Physicochemical water properties

The tropical Ambon Island is highly influenced by seasonal monsoons, which influence water properties in Ambon Bay during upwelling and downwelling. Cold nutrient-rich water was previously detected in the inner bay during the wet season as the result of upwelling processes in the Banda Sea (Likumahua et al., 2019, 2020). During this period, water temperature decreased considerably and coincided with high salinity levels, which were also found in the present study. High saline water coupled with low temperatures were detected during the wet seasons of both years, indicating the seasonal variability of water

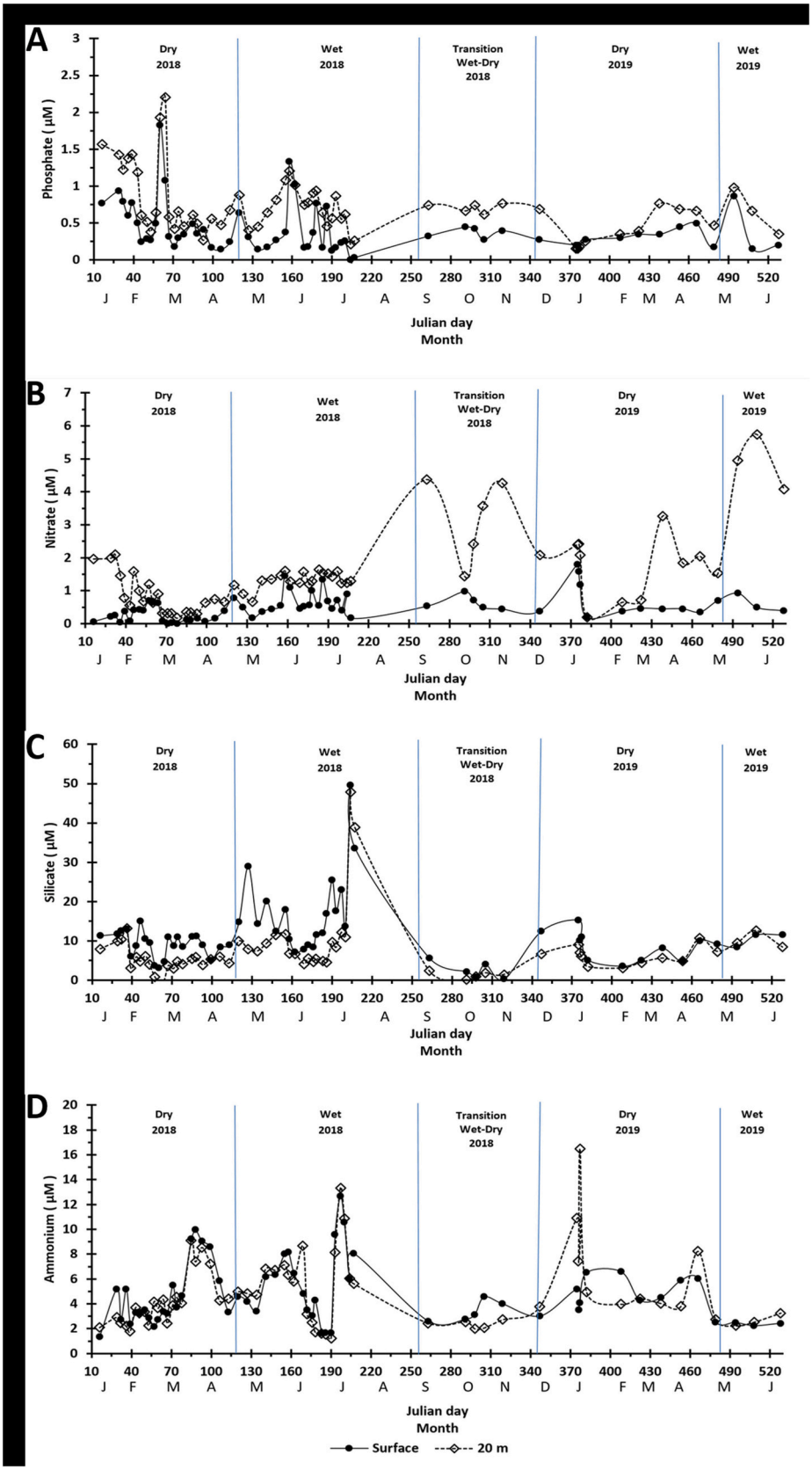

Fig. 4. Temporal variability (average of all stations) of major nutrients at two water depths in Ambon Bay (2018–2019). Vertical blue lines indicate boundaries of each season for both years, as determined by PCA score plot of all measured parameters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

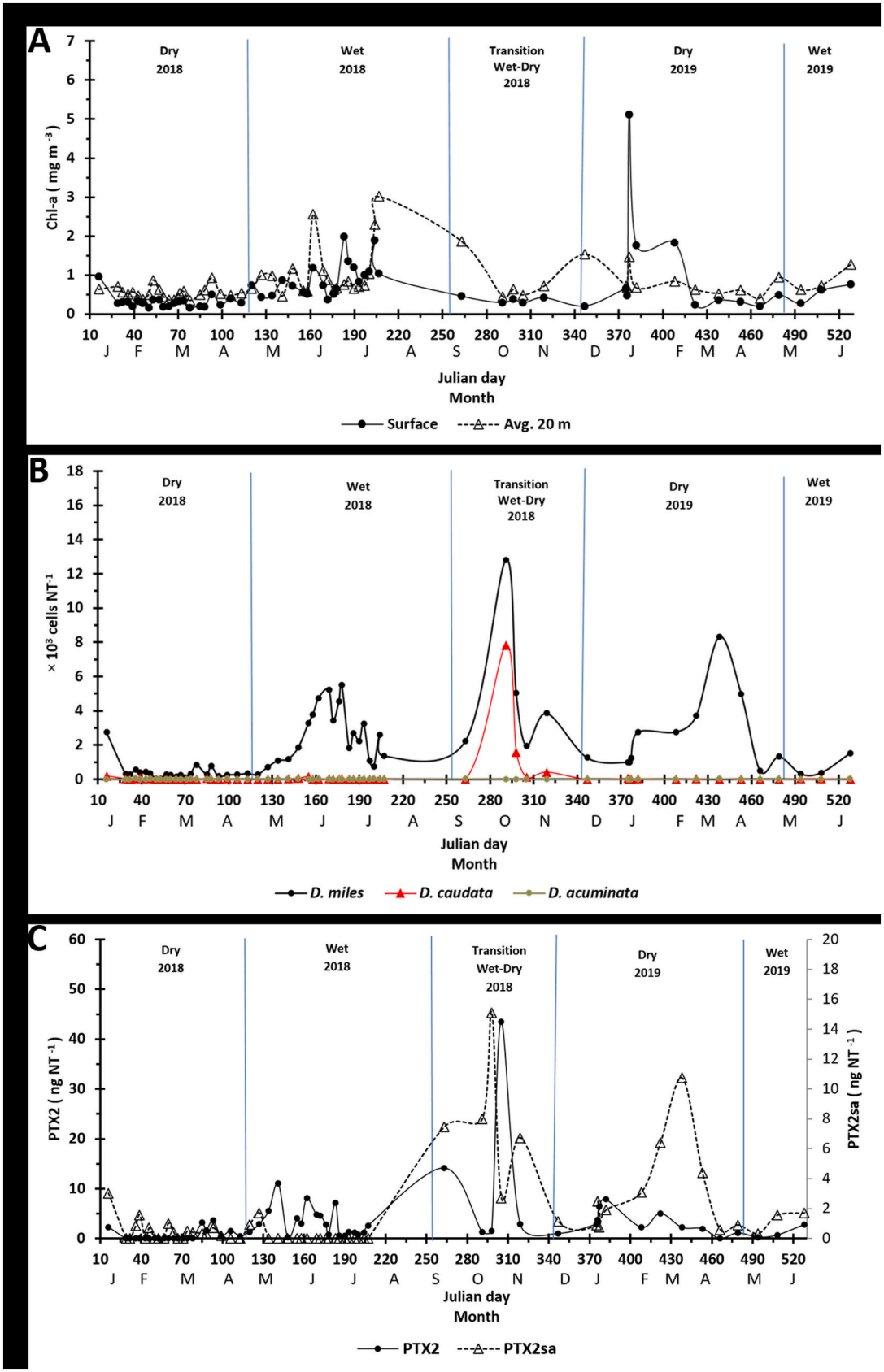

Fig. 5. Temporal variability (average of all stations) of biological parameters in Ambon Bay (2018–2019). Vertical blue lines indicate boundaries of each season for both years, as determined by PCA score plot of all measured parameters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

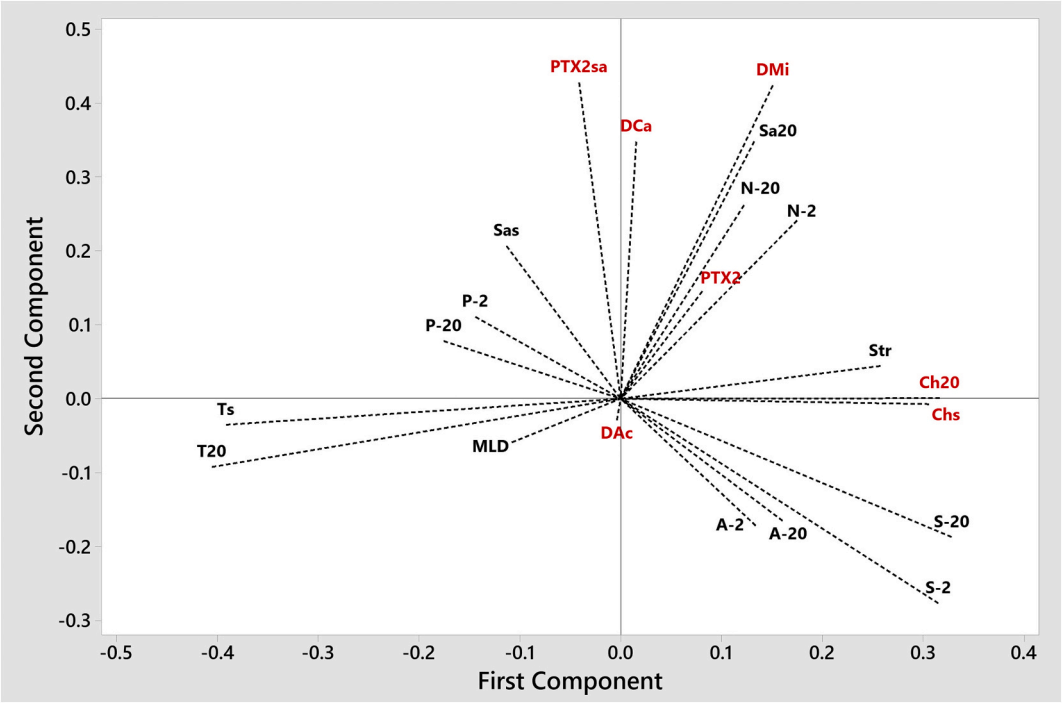

Fig. 6. The PCA loading plot of all data (2018 and 2019) determining correlation between biological parameters (red letters) and environmental drivers ($n = 206$). Abbreviations: DMi (*Dinophysis miles*), DCa (*Dinophysis caudata*), DAc (*Dinophysis acuminata*), PTX2 (pectenotoxin-2), PTX2sa (pectenotoxin-2 secoacid), T20 (temperature average 0–20 m), Ts (temperature at surface), S20 (salinity average 0–20 m), Sas (salinity at surface), Chl20 (chlorophyll-a average 0–20 m), Chls (chlorophyll-*a* at surface), P-2 (phosphate at surface 2 m), P-20 (phosphate at 20 m), N-2 (nitrate at surface 2 m), N-20 (nitrate at 20 m), S-2 (silicate at surface 2 m), S-20 (silicate at 20 m), A-2 (ammonium at surface 2 m), A-20 (ammonium at 20 m), MLD (mixed layer depth) and Str (stratification). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

physical properties in Ambon Bay. High nutrient concentrations during the dry season, especially between January and February of 2018 had been discussed previously as the impact of water mixing triggered by strong winds (Likumahua et al., 2019, 2020). Similar high nutrient conditions were found during the dry season of 2019, except for phosphate in January during which its concentration was low. This might be related to nutrient uptake by phytoplankton, as high diatom densities and *Gonyaulax* sp. proliferations were frequently recorded during January of 2019 (data not shown). High nutrient (phosphate, nitrate and ammonium) concentrations during the end of the dry season of 2019 (Fig. 4A, B & D) were likely linked to high runoffs triggered by high precipitation between March and May (Meteorological and Climate Bureau measurement, data not shown). Thus, an interannual variability of nutrient concentrations was observed in this study. The assembled 1.5-year nutrient data in this study imply that eutrophication in the bay might be regulated by runoffs during high precipitation months coupled with wind-triggering mixing and upwelling during the southeast monsoon. Regardless of overall differences between monsoonal seasons and interannual variability, as discussed above, strong influences of local human activities such as aquaculture, persistent wastewater discharges from land and ships are likely to affect nutrient concentrations. These multi nutrient sources combined with seasonal physical variability strongly hampers the explanation and prediction of biological processes including *Dinophysis* cell abundances and toxin levels in the bay.

4.2. Possible distribution of Dinophysis miles in Southeast Asia

Throughout the sampling period, *D. miles* was found to be persistent in the bay, yet, no blooms were formed by the species. Its cell densities during our study were relatively low compared to cell abundances found in Sapian Bay, the Philippines (Marasigan et al., 2001). In their study, *D. miles* reached bloom conditions (±5000 cells L^{-1}) and was found to be the dominant *Dinophysis* species, since its densities were two times higher than the co-occurring *D. caudata*. This finding was similar to our results, since *D. miles* dominated *Dinophysis* communities followed by *D. caudata*. In addition, earlier observations obtained from the long-term monitoring program by LIPI (since 2008) showed frequent occurrences of *D. miles* dominating *Dinophysis* species in Ambon Bay (data not shown). Very little is known about the global distribution of *D. miles*, yet, the species seems restricted to tropical waters and the Indo-West Pacific region (Gul and Saifullah, 2010; reviewed by Reguera et al., 2012; Reguera et al., 2014). Thus, our study specifically provides valuable information on the occurrence of this potentially toxic *Dinophysis* species in tropical Southeast Asian bays.

4.3. Dinophysis miles and pectenotoxins

DSTs such as OA, DTXs and PTXs are common toxin components produced by *Dinophysis* species (Yasumoto et al., 1980; Li et al., 2014; Reguera et al., 2014; Hu et al., 2017; Reguera and Blanco, 2019). OA and DTXs were not detected in our phytoplankton samples, yet PTXs (PTX2 and PTX2sa) were successfully detected and were only found to correlate with *D. miles* cell abundance. In contrast, the Philippines *D. miles* strain was found to produce OA and DTX1 (Marasigan et al., 2001). The presence of *D. acuminata* in our samples was not followed by the detection of OA and DTXs. Yet, many studies both in the field and in the lab have shown that *D. acuminata* produces these DSP toxin components (Bazzoni et al., 2018; Gao et al., 2017; Jiang et al., 2018; Mackenzie, 2019; Wolny et al., 2020). A review study on *D. acuminata* along the Chilean coast showed a consistent detection of PTX2 while other toxins were absent, implying that the species was the pectenotoxin producer (Díaz et al., 2022). High PTX2 levels in their study coincided with high abundance of *D. acuminata* cells, while other species were minor in abundance. In our study, *D. miles* was likely the potential producer of PTX2 since it was the dominant species that had much higher

Table 2
Spearman rho correlation analyses among *Dinophysis* species, PTXs and environmental drivers at 95 % ($p < 0.05$) confidence level. Abbreviations: PTX2 (pectenotoxin-2), PTX2sa (pectenotoxin-2 seco acid), T20 (temperature average 0–20 m), Ts (Temperature surface), S20 (salinity average 0–20 m), Sas (salinity surface), Chl20 (chlorophyll-*a* average 0–20 m), Chls (chlorophyll-*a* surface), P-2 (phosphate at surface 2 m), P-20 (phosphate 20 m), N-2 (nitrate at surface 2 m), N-20 (nitrate 2 m), S-2 (silicate at surface 2 m), S-20 (silicate 20 m), A-2 (ammonium at surface 2 m), A-20 (ammonium 20 m), MLD (mixed layer depth).

	All data/all years ($n = 206$)			Dry season 2018 ($n = 98$)			Dry season 2019 (n = 20)			Wet season ($n = 78$)			Transition II ($n = 10$)		
	D. miles	PTX2	PTX2sa	*D. miles*	PTX2	PTX2sa	*D. miles*	PTX2	PTX2sa	*D. miles*	PTX2	PTX2sa	*D. miles*	PTX2	PTX2sa
D. acuminata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D. caudata	0.53*	–	0.27*	0.30*	–	–	–	–	–	–	0.22	–	0.75*	−0.83*	–
PTX2	0.54*	–	–	0.30*	–	0.40	–	–	–	0.30	–	–	−0.75*	–	–
PTX2sa	0.23*	–	–	0.42*	–	–	0.63	–	–	−0.25	–	–	0.61*	–	–
T20	−0.51*	−0.34*	0.30*	–	–	–	–	–	–	–	0.22	–	–	–	–
Ts	−0.44*	−0.27*	0.32*	–	–	–	–	–	–	–	0.30	–	–	–	–
S20	0.31*	–	0.16	–	−0.45*	−0.23	–	–	–	–	−0.23	−0.21	–	–	–
Sas	–	–	0.26*	–	–	–	–	–	–	0.30*	–	–	–	–	–
Chl20	0.40*	0.51*	–	–	0.46*	–	–	–	–	–	–	–	–	–	–
Chls	0.46*	0.50*	–	–	0.25	–	–	–	−0.57	–	–	–	–	–	–
P-2	–	−0.22*	–	–	–	–	–	–	0.80	0.30	–	–	–	–	–
P-20	–	−0.24	–	–	−0.40*	–	0.63	–	0.73	0.41*	–	−0.21	–	–	–
N-2	0.40*	0.30*	–	–	–	–	–	–	–	0.40*	–	–	–	–	–
N-20	0.41*	0.23*	0.20*	0.20*	–	–	–	–	–	0.20*	–	0.34*	–	–	–
S-2	–	–	−0.30*	0.30*	0.31*	–	–	–	−0.62	−0.50*	–	–	–	–	–
S-20	–	–	−0.21*	0.33*	0.23	–	−0.63	–	−0.59	−0.20	–	–	–	–	–
A-2	–	0.20*	–	–	0.43*	–	–	–	–	–	–	–	–	–	–
A-20	–	0.24*	–	–	0.53*	–	−0.61	–	−0.64	–	–	–	–	–	–
MLD	–	–	−0.24*	–	–	–	–	–	0.72	–	–	−0.30*	–	–	–
Stratification	0.30*	0.24*	–	–	–	–	–	–	–	−0.40	0.25*	0.30	0.80*	−0.76*	–

Confidence level: * ≤ 0.01.

abundances compared to *D. acuminata* and *D. caudata*.

Earlier studies of *D. caudata* revealed that the species produced only PTX2 in considerable amounts while OA and DTXs were below threshold levels or not detected (Fernández et al., 2006; Mafra et al., 2014; Li et al., 2015). In addition, *Dinophysis* spp. has been found to have intra-specific differences in toxin profiles (Reguera and Pizarro, 2008). Hence, based on our 1.5-year results, it was likely that *Dinophysis* species in Ambon Bay might be genetically different from strains found in the Philippines or other regions. Variations in toxin content may also be influenced by cell responses to varying environmental conditions as found in different locations (Reguera et al., 2012 and references therein). In support of this, Uchida et al. (2018) demonstrated variations in toxin profiles among strains of the same *Dinophysis* species and also between different species, collected from different Japanese locations.

Previously, the 7-month data set (Likumahua et al., 2020) showed a weak correlation between *D. miles* cell abundance and PTX2 level due to the observed non-synchronized peaks of both variables (Likumahua et al., 2020). Increasing cell abundance was not followed by elevated PTX2 levels (e.g. between June and July of 2018), yet, conversely, high toxin levels were found during the period when cell numbers decreased (e.g. between day 85 and 100 (March and April of 2018); and between day 127 and 145 in May of 2018) (Fig. 5B & C). The present extended data set collected during a period of 1.5-year showed similar non-synchronized peaks between *D. miles* cells and PTX2 levels (e.g. between day 280 and 310; and between day 385 and 460, Fig. 5B & C) leading to weak correlations between both (Table 2). A similar non-synchronized pattern between cell abundance and toxicity levels was observed in a field study in Southern Brazil, during which *Dinophysis* spp. was more toxic when cell densities were low (Mafra et al., 2014). In addition, a culture study of *D. acuminata* showed that high toxicity levels occurred at low cell densities (Basti et al., 2018). In our study, the lack of synchronization between cell densities and toxin levels might furthermore be explained by the co-occurrences of the three species which might contain different toxin cell quotas, or exhibit different life stages or responses to environmental conditions (Tong et al., 2011; Nielsen et al., 2012; Nielsen et al., 2013; Mafra et al., 2014; Reguera et al., 2014; Basti et al., 2015; Ajani et al., 2016).

Likewise, PTX2sa was found to have a weak positive relationship with *D. miles* cell abundances when considering the whole research period. Yet, strong relationships were found during the dry season and the second transition period. Generally, during these two particular periods, PTX2sa was detected at higher levels than those observed during the wet season or than those of PTX2. PTX2sa is thought to be mostly present in filter feeder flesh as the hydrolyzed product of PTX2, generated enzymatically (Vale and Sampayo, 2002; Miles et al., 2004; MacKenzie et al., 2012; Nielsen et al., 2016; Blanco et al., 2018). Enzymatic conversion of PTX2 into PTX2sa was also detected in disrupted net-haul samples damaging *Dinophysis* cells (Suzuki et al., 2001; Fernández-Puente et al., 2004). Yet, PTX2sa was also detected in live picked *D. acuta* cells (Puente et al., 2004) as well as in other *Dinophysis* species in natural seawater (Fux et al., 2010; Li et al., 2010; Trefault et al., 2011; McCarthy et al., 2014; He et al., 2020). Interestingly, strong correlations were found between PTX2sa and *D. tripos* cell abundances from net-haul plankton samples collected in Argentinean shelf waters (Fabro et al., 2015). They argued that PTX2sa in their samples was not an artefact due to disrupting sample processing since the toxin was only detected in samples containing *D. tripos* while absent in samples with *D. acuminata*. This led to the conclusion that *D. tripos* from this area was a direct PTX2sa producer.

Considering the same net-haul method for collecting plankton samples as used in the previous study (Fabro et al., 2015), PTX2sa in our study is likely to be associated with *D. miles* as the only possible producer. Given the fact that *D. miles* was the most abundant and persistently occurring species in Ambon Bay, it is not likely that other *Dinophysis* species were contributing to elevated PTX2sa levels. However, the association between PTX2sa and *D. miles* was only found under

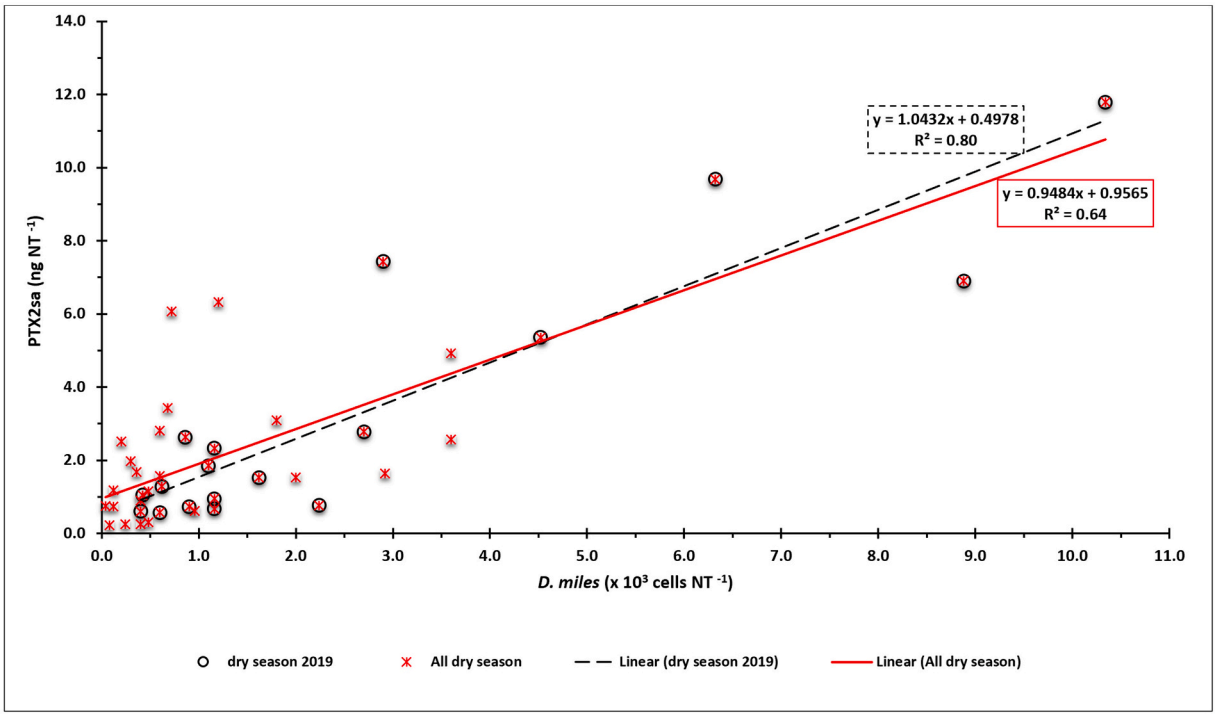

Fig. 7. Linear regression models of *Dinophysis miles* cell abundances versus PTX2sa levels during the dry season 2019 and the total of dry season samples (2018 and 2019).

certain environmental conditions since the strong correlations were only observed during the dry season and the second transition period. PTX2sa was positively correlated with temperature during the whole sampling period. Hence, a relatively high water temperature might be one of the environmental factors that triggered these strong relationships. High temperatures persisted during the dry season and elevated temperatures were recorded during the transition of the wet to dry season of 2018, during which PTX2sa was frequently detected and its level was relatively high. Conversely, when PTX2 was abundant during the wet season (lower temperature) of 2018, PTX2sa was not detected. This might imply that there were no positive relationships hinting to enzymatic conversion. Takahashi et al. (2007) reported high PTX2 and PTX2sa levels associated with *D. caudata* cell abundance in North Stradbroke Island, Australia. Yet, they argued that PTX2sa was the PTX2 breakdown product due to disrupting sampling methods. In our study, PTX2sa was only observed during warmer months and absent during the wet season, indicating that sampling artefacts were unlikely to contribute to PTX2 conversion. However, to confirm our finding regarding PTX2sa production, culture experiments on the *D. miles* strain from Ambon Bay are needed. In addition, other possibilities such as predator presence, grazing impacts and cell rupture should be explored to unravel toxin variability and the PTX2sa production by this species.

4.4. Factors controlling cell and toxin variations

In our previous study, temperature was negatively associated with *D. miles* cell abundances and PTX2 levels while nutrient concentrations and physical drivers such as MLD, stratification, light availability and tide showed positive correlations (Likumahua et al., 2020). In the present extended data set, similar associations were observed during the whole sampling period. However, physiochemical driver impacts on biological parameters, in particular cell abundances and toxins, varied among seasons. These indicate that Ambon Bay has specific seasonal and hydrological influences on *Dinophysis* species as well as cellular toxin content and levels, which had been shown by previous field studies (Reguera et al., 2014 and references therein; Ajani et al., 2016; Díaz et al., 2016; Ruiz-Villarreal et al., 2016). Despite the variability in environmental impacts, nitrate concentrations, especially the level at 20 m showed a strong correlation with *D. miles* abundance and both toxin levels. A culture study on *D. accuminata* showed that nitrate did not regulate its cellular growth judging from transcriptome experiments (Hattenrath-Lehmann et al., 2021). Since the present data statistically showed correlations between this nutrient and *D. miles*, we might not able to precisely display the direct effects. Possibly, other environmental drivers coinciding with elevated nitrate levels, not analysed by us, influenced *D. miles* cells and toxins in Ambon Bay. Yet, further studies of *D. miles* in batch culture are needed to unravel this. No other studies are available explaining *D. miles* behavior in stratified water column in terms of vertical migration due to depth differences in nutrient availability. Due to the lack of knowledge on *D. miles* biology and ecophysiology, species specific information on growth response as a function of environmental conditions as well as (variability in) toxin quota is urgently needed.

5. Conclusions

Three *Dinophysis* species were successfully identified, of which *D. miles* was the most abundant in Ambon Bay. The observed dynamics do not allow for accurate prediction of *Dinophysis* outbreaks in Ambon, Bay, likely due to the high variability and human influences in this rather enclosed and heavily used bay. Therefore, year-round monitoring is of extreme importance. The 1.5-years data set showed positive relationships between *D. miles* and both PTX2s whereas other *Dinophysis* toxins were absent. Specifically, cell abundances were found to be linearly related to PTX2sa productions during the dry season. Therefore, our data suggest that *D. miles* is likely a PTX2sa producer under certain environmental conditions in Ambon Bay, in particular at elevated temperature. Generally, the presence of pectenotoxin in Ambon Bay reveals *D. miles* as the potential producer and nitrate was the main factor regulated both cell density and toxins during the overall sampling period. Given the fact that two other *Dinophysis* species (*D. caudata* and *D. acuminata*) were OA and DTXs producers found elsewhere, their

dynamics in Ambon Bay need to be monitored since those toxins might likely be detected once cell densities exceed a certain threshold. Finally, species and strain specific information regarding *D. miles* and its toxins is urgently needed given its presence in Indonesian and other East-Asian waters, to confirm the present findings. Thus, it is important to perform mono algal ecophysiological culture experiments of this species.

CRediT authorship contribution statement

Sem Likumahua: Conceptualization, sampling design, sample collection, lab work and analysis, data curation, statistical analysis, writing the original draft and revising the manuscript.

M. Karin de Boer: Sampling design, review (commentary) and editing, supervision.

Bernd Krock: Toxin analysis and interpretation, review and editing.

Willem M. Tatipatta: Sample collection for water physical properties.

Malik S. Abdul: Sample collection and analysis for nutrients.

Anita G.J. Buma: Supervision, conceptualization, sampling design, review (commentary) and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgements

We thank Eduard Moniharapon for operating the CTD, Christophe Brochard for performing qualitative microscopic analysis, and Ronald Visser for preparing and setting up equipment in Ambon. Especially, we would like to thank Dr. Augy Syahailatua for allowing us to work in the LIPI's plankton laboratory during our campaign in Ambon. This work was supported and funded by LPDP program (Nomor: PRJ-1007/LPDP.3/2016), the Indonesian Ministry of Finance and partially funded by the Helmholtz-Gemeinschaft Deutscher Forschungszentrum through the research program "Changing Earth - Sustaining our Future" of the Alfred Wegener Institut-Helmholtz Zentrum für Polar- und Meeresforschung.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114340.

References

Aditya, V., Koswara, A., Fitriya, N., Rachman, A., Sidabutar, T., Thoha, H., 2013. Public awareness on harmful algal bloom (HAB) in Lampung Bay. Mar. Res. Indones. 38 (2), 71–75.

Ajani, P., Larsson, M.E., Rubio, A., Bush, S., Brett, S., Farrell, H., 2016. Modelling bloom formation of the toxic dinoflagellates Dinophysis acuminata and Dinophysis caudata in a highly modified estuary, southeastern Australia. Estuar. Coast. Shelf Sci. 183, 95–106.

Alarcan, J., Dubreil, E., Huguet, A., Hurtaud-Pessel, D., Hessel-Pras, S., Lampen, A., Fessard, V., Le Hegarat, L., 2017. Metabolism of the marine phycotoxin PTX-2 and its effects on hepatic xenobiotic metabolism: activation of nuclear receptors and modulation of the phase I cytochrome P450. Toxins 9 (7), 212.

Amzil, Z., Sibat, M., Royer, F., Masson, N., Abadie, E., 2007. Report on the first detection of pectenotoxin-2, spirolide-A and their derivatives in french shellfish. Mar. Drugs 5 (4), 168–179.

Aune, T., Sorby, R., Yasumoto, T., Ramstad, H., Landsverk, T., 2002. Comparison of oral and intraperitoneal toxicity of yessotoxin towards mice. Toxicon 40, 77–82.

Basti, L., Suzuki, T., Uchida, H., Kamiyama, T., Nagai, S., 2018. Thermal acclimation affects growth and lipophilic toxin production in a strain of cosmopolitan harmful alga Dinophysis acuminata. Harmful Algae 73, 119–128.

Bazzoni, A.M., Mudadu, A.G., Lorenzoni, G., Soro, B., Bardino, N., Arras, I., Sanna, G., Vodret, B., Bazzardi, R., Marongiu, E., Virgilio, S., 2018. Detection of Dinophysis species and associated okadaic acid in farmed shellfish: a two-year study from the western Mediterranean area. J. Vet. Res. 62 (2), 137–144.

Blanco, J., Álvarez, G., Rengel, J., Díaz, R., Mariño, C., Martín, H., Uribe, E., 2018. Accumulation and biotransformation of Dinophysis toxins by the surf clam Mesodesma donacium. Toxins 10 (8), 314.

Campbell, L., Olson, R.J., Sosik, H.M., Abraham, A., Henrichs, D.W., Hyatt, C.J., Buskey, E.J., 2010. First harmful dinophysis (dinophyceae, dinophysiales) bloom in the US is revealed by automated imaging flow cytometry 1. J. Phycol. 46 (1), 66–75.

Cembella, A., John, U., 2006. Molecular physiology of toxin production and growth regulation in harmful algae. In: Ecology of Harmful Algae. Springer, Berlin, Heidelberg, pp. 215–227.

Díaz, P.A., Reguera, B., Ruiz-Villarreal, M., Pazos, Y., Velo-Suárez, L., Berger, H., Sourisseau, M., 2013. Climate variability and oceanographic settings associated with interannual variability in the initiation of Dinophysis acuminata blooms. Mar. Drugs 11 (8), 2964–2981.

Díaz, P.A., Ruiz-Villarreal, M., Pazos, Y., Moita, T., Reguera, B., 2016. Climate variability and Dinophysis acuta blooms in an upwelling system. Harmful Algae 53, 145–159.

Díaz, P.A., Álvarez, G., Pizarro, G., Blanco, J., Reguera, B., 2022. Lipophilic toxins in Chile: history,producers and impacts. Mar. Drugs 20 (2), 122.

Dominguez, H.J., Paz, B., Daranas, A.H., Norte, M., Franco, J.M., Fernández, J.J., 2010. Dinoflagellate polyether within the yessotoxin, pectenotoxin and okadaic acid toxin groups: characterization, analysis and human health implications. Toxicon 56 (2), 191–217.

Escalera, L., Reguera, B., Moita, T., Pazos, Y., Cerejo, M., Cabanas, J.M., Ruiz-Villarreal, M., 2010. Bloom dynamics of Dinophysis acuta in an upwelling system: in situ growth versus transport. Harmful Algae 9 (3), 312–322.

Espiña, B., Rubiolo, J.A., 2008. Marine toxins and the cytoskeleton: pectenotoxins, unusual macrolides that disrupt actin. FEBS J. 275 (24), 6082–6088.

Fabro, E., Almandoz, G.O., Ferrario, M.E., Hoffmeyer, M.S., Pettigrosso, R.E., Uibrig, R., Krock, B., 2015. Co-occurrence of Dinophysis tripos and pectenotoxins in Argentinean shelf waters. Harmful Algae 42, 25–33.

Fabro, E., Almandoz, G.O., Ferrario, M., Tillmann, U., Cembella, A., Krock, B., 2016. Distribution of Dinophysis species and their association with lipophilic phycotoxins in plankton from the Argentine Sea. Harmful Algae 59, 31–41.

Farrell, H., Ajani, P., Murray, S., Baker, P., Webster, G., Brett, S., Zammit, A., 2018. Diarrhetic shellfish toxin monitoring in commercial wild harvest bivalve shellfish in New South Wales,Australia. Toxins 10 (11), 446.

Fernández, M.L., Reguera, B., González-Gil, S., Míguez, A., 2006. Pectenotoxin-2 in single-cell isolates of Dinophysis caudata and Dinophysis acuta from the Galician Rías (NW Spain). Toxicon 48 (5), 477–490.

Fernández-Puente, P., Sáez, M.J.F., Hamilton, B., Lehane, M., Ramstad, H., Furey, A., James, K.J., 2004. Rapid determination of polyether marine toxins using liquid chromatography-multiple tandem mass spectrometry. J. Chromatogr. A 1056, 77–82.

Ferron, P.J., Hogeveen, K., De Sousa, G., Rahmani, R., Dubreil, E., Fessard, V., Le Hegarat, L., 2016. Modulation of CYP3A4 activity alters the cytotoxicity of lipophilic phycotoxins in human hepatic HepaRG cells. Toxicol. in Vitro 33, 136–146.

Fux, E., González-Gil, S., Lunven, M., Gentien, P., Hess, P., 2010. Production of diarrhetic shellfish poisoning toxins and pectenotoxins at depths within and below the euphotic zone. Toxicon 56, 1487–1496.

Gaillard, S., Le Goïc, N., Malo, F., Boulais, M., Fabioux, C., Zaccagnini, L., Carpentier, L., Sibat, M., Réveillon, D., Séchet, V., Hess, P., Hegaret, H., 2020. Cultures of Dinophysis sacculus, D. acuminata and pectenotoxin 2 affect gametes and fertilization success of the Pacific oyster, Crassostrea gigas. Environ. Pollut. 265, 114840.

Gao, H., An, X., Liu, L., Zhang, K., Zheng, D., Tong, M., 2017. Characterization of Dinophysis acuminata from the Yellow Sea, China, and its response to different temperatures and Mesodinium prey. Oceanol. Hydrobiol. Stud. 46 (4), 439–450.

González-Pola, C., Fernández-Díaz, J.M., Lavín, A., 2007. Vertical structure of the upper ocean from profiles fitted to physically consistent functional forms. Deep-Sea Res. Pt. I 54 (11), 1985–2004.

Gul, S.A.D.A.F., Saifullah, S.M., 2010. Taxonomic and ecological studies on three marine genera of Dinophysiales from Arabian Sea shelf of Pakistan. Pak. J. Bot. 42 (4), 2647–2660.

Hallegraeff, G.M., Lucas, I.A.N., 1988. The marine dinoflagellate genus Dinophysis (Dinophyceae): photosynthetic, neritic and non-photosynthetic, oceanic species. Phycologia 27 (1), 25–42.

Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), 2003. Manual on Harmful Marine Microalgae. Monographs on Oceanographic Methodology. UNESCO Publishing.

Hattenrath-Lehmann, T.K., Marcoval, M.A., Berry, D.L., Fire, S., Wang, Z., Morton, S.L., Gobler, C.J., 2013. The emergence of Dinophysis acuminata blooms and DSP toxins in shellfish in New York waters. Harmful Algae 26, 33–44.

Hattenrath-Lehmann, T.K., Nanjappa, D., Zhang, H., Yu, L., Goleski, J.A., Lin, S., Gobler, C.J., 2021. Transcriptomic and isotopic data reveal central role of ammonium in facilitating the growth of the mixotrophic dinoflagellate,Dinophysis acuminata. Harmful Algae 104, 102031.

He, X., Chen, J., Wu, D., Wang, J., Xin, M., Liu, L., Sun, P., Wang, B., 2020. Occurrence, distribution, source, and influencing factors of lipophilic marine algal toxins in Laizhou Bay, Bohai Sea,China. Mar. Pollut. Bull. 150, 110789.

Hess, P., 2010. Requirements for screening and confirmatory methods for the detection and quantification of marine biotoxins in end-product and official control. Anal. Bioanal. Chem. 397, 1683–1694.
Hu, T., LeBlanc, P., Burton, I.W., Walter, J.A., McCarron, P., Melanson, J.E., Strangman, W.K., Wright, J.L., 2017. Sulfated diesters of okadaic acid and DTX-1: self-protective precursors of diarrhetic shellfish poisoning (DSP) toxins. Harmful Algae 63, 85–93.
ICES, 2006. Report on the ICES/IOC Workshop on New and Classic Techniques for the Determination of Numerical Abundance and Biovolume of HAB Species – Evaluation of the Cost, Time-efficiency and Intercalibration Methods (WKNCT), 22–27 August 2005, Kristineberg, Sweden. ICES CM 2005/C:10.
Jiang, H., Kulis, D.M., Brosnahan, M.L., Anderson, D.M., 2018. Behavioral and mechanistic characteristics of the predator-prey interaction between the dinoflagellate Dinophysis acuminata and the ciliate Mesodinium rubrum. Harmful Algae 77, 43–54.
Krock, B., Tillmann, U., John, U., Cembella, A.D., 2008. LC-MS/MS on board ship – tandem mass spectrometry in the search for phycotoxins and novel toxigenic plankton from the North Sea. Anal. Bioanal. Chem. 392, 797–803.
Legendre, P., Birks, H.J.B., 2012. From classical to canonical ordination. In: Tracking Environmental Change Using Lake Sediments. Springer, Dordrecht, pp. 201–248.
Li, Z., Mengmeng, G., Shouguo, Y., Qingyin, W., Zhijun, T., 2010. Investigation of pectenotoxin profiles in the Yellow Sea (China) using a passive sampling technique. Mar. Drugs 8 (4), 1263–1272.
Li, X., Li, Z., Chen, J., Shi, Q., Zhang, R., Wang, S., Wang, X., 2014. Detection, occurrence and monthly variations of typical lipophilic marine toxins associated with diarrhetic shellfish poisoning in the coastal seawater of Qingdao City,China. Chemosphere 111, 560–567.
Li, A., Sun, G., Qiu, J., Fan, L., 2015. Lipophilic shellfish toxins in Dinophysis caudata picked cells and in shellfish from the East China Sea. Environ. Sci. Pollut. Res. 22 (4), 3116–3126.
Likumahua, S., de Boer, M.K., Krock, B., Nieuwenhuizen, T., Tatipatta, W.M., Hehakaya, S., Imu, L., Abdul, M.S., Moniharapon, E., Buma, A.G., 2019. First record of the dynamics of domoic acid producing Pseudo-nitzschia spp. in Indonesian waters as a function of environmental variability. Harmful Algae 90, 101708.
Likumahua, S., de Boer, M.K., Krock, B., Hehakaya, S., Imu, L., Müller, A., Max, T., Buma, A.G., 2020. Variability of dinoflagellates and their associated toxins in relation with environmental drivers in Ambon Bay, eastern Indonesia. Mar. Pollut. Bull. 150, 110778.
Lindahl, O., Lundve, B., Johansen, M., 2007. Toxicity of Dinophysis spp. in relation to population density and environmental conditions on the Swedish west coast. Harmful Algae 6 (2), 218–231.
Mackenzie, L.A., 2019. A long-term time series of Dinophysis acuminata blooms and associated shellfish toxin contamination in Port Underwood, Marlborough Sounds, New Zealand. Toxins 11 (2), 74.
MacKenzie, L., Beuzenberg, V., Holland, P., McNabb, P., Suzuki, T., Selwood, A., 2005. Pectenotoxin and okadaic acid-based toxin profiles in Dinophysis acuta and Dinophysis acuminata from New Zealand. Harmful Algae 4 (1), 75–85.
MacKenzie, L., Selwood, A., Marshall, C., 2012. Isolation and characterization of an enzyme from the Greenshell ™ mussel Perna canaliculus that hydrolyses pectenotoxins and esters of okadaic acid. Toxicon 60, 406–419.
Mafra, L.L., dos Santos Tavares, C.P., Schramm, M.A., 2014. Diarrheic toxins in field-sampled and cultivated Dinophysis spp. cells from southern Brazil. J. Appl. Phycol. 26 (4), 1727–1739.
Marasigan, A.N., Sato, S., Fukuyo, Y., Kodama, M., 2001. Accumulation of a high level of diarrhetic shellfish toxins in the green mussel Perna viridis during a bloom of Dinophysis caudata and Dinophysis miles in Sapian Bay, Panay Island, the Philippines. Fish. Sci. 67 (5), 994–996.
McCarthy, M., van Pelt, F.N., Bane, V., O'Halloran, J., Furey, A., 2014. Application of passive (SPATT) and active sampling methods in the profiling and monitoring of marine biotoxins. Toxicon 89, 77–86.
Miles, C.O., Wilkins, A.L., Munday, R., Dines, M.H., Hawkes, A.D., Briggs, L.R., Sandvik, M., Jensen, D.J., Cooney, J.M., Holland, P.T., Quilliam, M.A., MacKenzie, A.L., Beuzenberg, V., Towers, N.R., 2004. Isolation of pectenotoxin-2 from Dinophysis acuta and its conversion to pectenotoxin-2 seco acid, and preliminary assessment of their acute toxicities. Toxicon 43, 1–9.
Moita, M.T., Pazos, Y., Rocha, C., Nolasco, R., Oliveira, P.B., 2016. Toward predicting Dinophysis blooms off NW Iberia: a decade of events. Harmful Algae 53, 17–32.
Nielsen, L.T., Krock, B., Hansen, P.J., 2012. Effects of light and food availability on toxin production, growth and photosynthesis in Dinophysis acuminata. Mar. Ecol. Prog. Ser. 471, 37–50.
Nielsen, L.T., Krock, B., Hansen, P.J., 2013. Production and excretion of okadaic acid, pectenotoxin-2 and a novel dinophysistoxin from the DSP-causing marine dinoflagellate Dinophysis acuta–effects of light, food availability and growth phase. Harmful Algae 23, 34–45.
Nielsen, L.T., Hansen, P.J., Krock, B., Vismann, B., 2016. Accumulation, transformation and breakdown of DSP toxins from the toxic dinoflagellate Dinophysis acuta in blue mussels, Mytilus edulis. Toxicon 117, 84–93.
Pizarro, G., Escalera, L., González-Gil, S., Franco, J.M., Reguera, B., 2008. Growth, behaviour and cell toxin quota of Dinophysis acuta during a daily cycle. Mar. Ecol. Prog. Ser. 353, 89–105.
Pizarro, G., Paz, B., González-Gil, S., Franco, J.M., Reguera, B., 2009. Seasonal variability of lipophilic toxins during a Dinophysis acuta bloom in Western Iberia: differences between picked cells and plankton concentrates. Harmful Algae 8 (6), 926–937.
Puente, P.F., Sáez, M.J.F., Hamilton, B., Furey, A., James, K.J., 2004. Studies of polyether toxins in the marine phytoplankton, Dinophysis acuta, in Ireland using multiple tandem mass spectrometry. Toxicon 44 (8), 919–926.
Qiu, D., Huang, L., Liu, S., Lin, S., 2011. Nuclear, mitochondrial and plastid gene phylogenies of Dinophysis miles (Dinophyceae): evidence of variable types of chloroplasts. Plos one 6 (12), e29398.
Quilliam, M.A., 2003. Chemical method for lipophilic shellfish toxins. In: Hallegraeff, G. M., Anderson, D.M., Cembella, A.D. (Eds.), Manual on Harmful Marine Microalgae. UNESCO Publishing, Paris, France, pp. 211–245.
Reguera, B., Blanco, J., 2019. Dinophysis toxins: distribution, fate in shellfish and impacts. Toxins. https://doi.org/10.3390/toxins11070413.
Reguera, B., Pizarro, G., 2008. Planktonic flagellates which produce polyether toxins of the old "DSP Complex". In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection, 2nd ed. CRC Press, London, pp. 257–284. 798 pp.
Reguera, B., Velo-Suárez, L., Raine, R., Park, M.G., 2012. Harmful Dinophysis species: a review. Harmful Algae 14, 87–106.
Reguera, B., Riobó, P., Rodríguez, F., Díaz, P., Pizarro, G., Paz, B., Franco, J.M., Blanco, J., 2014. Dinophysis toxins: causative organisms, distribution and fate in shellfish. Mar. Drugs 12 (1), 394–461.
Rountos, K.J., Kim, J.J., Hattenrath-Lehmann, T.K., Gobler, C.J., 2019. Effects of the harmful algae, Alexandrium catenella and Dinophysis acuminata, on the survival, growth, and swimming activity of early life stages of forage fish. Mar. Environ. Res. 148, 46–56.
Ruiz-Villarreal, M., García-García, L.M., Cobas, M., Díaz, P.A., Reguera, B., 2016. Modelling the hydrodynamic conditions associated with Dinophysis blooms in Galicia (NW Spain). Harmful Algae 53, 40–52.
Sibat, M., García-Portela, M., Hess, P., 2018. First identification of a C9-diol-ester of okadaic acid in Dinophysis acuta from Galician Rías Baixas (NW Spain). Toxicon 153, 19–22.
Somavilla, R., González-Pola, C., Fernández-Díaz, J., 2017. The warmer the ocean surface, the shallower the mixed layer. How much of this is true? J. Geophys. Res.C 122 (9), 7698–7716.
Suzuki, T., MacKenzie, L., Stirling, D., Adamson, J., 2001. Pectenotoxin-2 seco acid: a toxin converted from pectenotoxin-2 by the New Zealand Greenshell mussel,Perna canaliculus. Toxicon 39 (4), 507–514.
Takahashi, E., Yu, Q., Eaglesham, G., Connel, D.W., McBroom, J., Costanzo, S., Shaw, G. R., 2007. Occurrence and seasonal variations of algal toxins in water, phytoplankton and shellfish from north Stradbroke Island, Queensland,Australia. Mar. Environ. 64, 429–442.
Thoha, H., Adnan, Q., Sidabutar, T., Sugestiningsih, 2007. Note on the occurrence of phytoplankton and its relation with mass mortality in the Jakarta Bay, May and November 2004. Makara Sains 11 (2), 63–67.
Tong, M., Kulis, D.M., Fux, E., Smith, J.L., Hess, P., Zhou, Q., Anderson, D.M., 2011. The effects of growth phase and light intensity on toxin production by Dinophysis acuminata from the northeastern United States. Harmful Algae 10, 254–264.
Trefault, N., Krock, B., Delherbe, N., Cembella, A., Vásquez, M., 2011. Latitudinal transects in the southeastern Pacific Ocean reveal a diverse but patchy distribution of phycotoxins. Toxicon 58 (5), 389–397.
Uchida, H., Watanabe, R., Matsushima, R., Oikawa, H., Nagai, S., Kamiyama, T., Baba, K., Miyazono, A., Kosaka, Y., Kaga, S., Matsuyama, Y., Suzuki, T., 2018. Toxin profiles of okadaic acid analogues and other lipophilic toxins in Dinophysis from Japanese coastal waters. Toxins 10 (11), 457.
Vale, P., Sampayo, M.A.D.M., 2002. First confirmation of human diarrhoeic poisonings by okadaic acid esters after ingestion of razor clams (Solen marginatus) and green crabs (Carcinus maenas) in Aveiro lagoon, Portugal and detection of okadaic acid esters in phytoplankton. Toxicon 40, 989–996.
Whyte, C., Swan, S., Davidson, K., 2014. Changing wind patterns linked to unusually high dinophysis blooms around the Shetland Islands,Scotland. Harmful Algae 39, 365–373.
Wilkins, A.L., Rehmann, N., Torgersen, T., Rundberget, T., Keogh, M., Petersen, D., Hess, P., Rise, F., Miles, C.O., 2006. Identification of fatty acid esters of pectenotoxin-2 seco acid in blue mussels (Mytilus edulis) from Ireland. J. Agric. Food Chem. 54 (15), 5672–5678.
Wolny, J.L., Egerton, T.A., Handy, S.M., Stutts, W.L., Smith, J.L., Whereat, E.B., Bachvaroff, T.R., Henrichs, D.W., Campbell, L., Deeds, J.R., 2020. Characterization of Dinophysis spp. (Dinophyceae, Dinophysiales) from the mid-Atlantic region of the United States. J. Phycol. 56 (2), 404–424.
Wouthuyzen, S., Tan, C.K., Ishizaka, J., Son, T.P.H., Ransi, V., Tarigan, S., Sediadi, A., 2004. Monitoring of algal blooms and massive fish kill in the Jakarta Bay, Indonesia using satellite imageries. Month. 2005, 2006–2007.
Yasumoto, T., Oshima, Y., Sugawara, W., Fukuyo, Y., Oguri, H., Igarashi, T., Fujita, N., 1980. Identification of Dinophysis fortii as the causative organism of diarrhetic shellfish poisoning. Bull. Jpn. Soc. Sci. Fish. 46, 1405–1411.
Yasumoto, T., Murata, M., Oshima, Y., Sano, M., Matsumoto, G., Clardy, J., 1985. Diarrhetic shellfish toxins. Tetrahedron 41, 1019–1025.