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Harmful algae in the Paranaguá Estuarine Complex, Brazil: a spatio-temporal assessment

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ABSTRACT

Harmful algal blooms (HABs) can negatively affect coastal water quality, aquatic fauna, and human health from the consumption of toxin-contaminated seafood. Estuaries are especially prone to the incidence of these noxious events and sensitive to the associated damage. This study represents a comprehensive overview of previous investigations reporting the occurrence of harmful algae and/or phycotoxins in the Paranaguá Estuarine Complex (CEP), in Southern Brazil. Secondary data from 2002 to 2021 were combined with new results obtained from widely spaced sampling campaigns performed from 2018 to 2019 to assess the periods and estuarine sectors at greater risk for the incidence of HABs. In total, about 600 water/sediment samples containing harmful microalgae and 675 tissue samples of phycotoxin-contaminated fauna were analyzed. The most frequent and abundant species of potentially toxic microalgae were dinoflagellates belonging to the species complexes *Dinophysis acuminata* and *Prorocentrum lima*, producers of diarrhetic shellfish toxins (DSTs), and diatoms of the genus *Pseudo-nitzschia*, producers of amnesic shellfish toxins (ASTs). Okadaic acid, a DST, was by far the most common toxin in the fauna, reaching higher levels in primary consumers such as bivalve mollusks (mussels, oysters, and clams), zooplankton, and suspension-feeding crustaceans (ghost shrimps), and moderate to low levels in cephalopods, fishes, gastropods, echinoderms, sea turtles, seabirds, and cetaceans. Less rainy periods (winter-early spring) were associated with the highest incidence of DSTs and blooms of their producing algae *P. lima* and *Dinophysis* spp., mainly in the middle region of Paranaguá Bay and on the shallow continental platform adjacent to the estuarine mouth. However, certain HAB-forming species may be more successful in inner estuarine areas (*Prorocentrum cordatum*) and during different periods, such as late summer—early fall (*Pseudo-nitzschia* spp.). Continuous monitoring of harmful algae and toxins is therefore highly recommended to mitigate the effects of HABs in the entire estuary.

Keywords: Algal blooms, Marine phytoplankton, Toxin accumulation, Brazilian coast, Subtropical estuary

INTRODUCTION

Regardless of the great ecological importance of phytoplankton in the direct or indirect maintenance of life in aquatic environments, some species can cause deleterious effects on the environment and public health. These are usually associated with events of extraordinary population growth under favorable environmental and ecological conditions, known as harmful algal blooms (HABs). However, some species can be harmful even at low densities, such as some species of the genus *Dinophysis*, which can become harmful at densities below 200

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cells L^{-1} due to the amount of intracellular toxins and the associated toxicity (Yasumoto et al., 1980). Other studies have indicated that climate change has caused changes in the composition of benthic and planktonic communities (Glibert, 2019; Gobler, 2020). As a consequence, an increase in the frequency and distribution of HABs has been observed in some regions, both in continental and marine waters (reviewed in Glibert, 2020). Cases of human intoxication, resulting from contaminated food or direct contact with algal toxins, have also increased over the past decades (Hallegraeff et al., 2021).

HABs can be categorized according to their harmful effects, namely: (i) species that are not toxic, but form a large biomass and can kill marine organisms by depleting oxygen from the water; (ii) species that are not harmful to human health, but can negatively affect other organisms via mechanical effects (damage or clogging of gills); (iii) species that produce toxins causing seafood poisoning, such as amnesic, diarrheic, neurotoxic or paralytic shellfish poisoning (ASP, DSP, NSP, and PSP syndromes, respectively), azaspiracid poisoning (AZP), and ciguatera poisoning (CP); (iv) toxin-producing species that affect human health upon direct contact with the water during blooms or with aerosols containing toxins transported to the beach; and (v) species whose harmfulness to human health is not known, but which show toxicity in bioassays and are likely to represent possible damage (Lassus et al., 2016; Moreira-González et al., 2021a). The main route of human intoxication is the consumption of suspension-feeding organisms that accumulate large amounts of toxins in their tissues, but other organisms, such as gastropods, cephalopods, crustaceans, and fish, can also act as vectors. Algal toxins (i.e., phycotoxins) are thermostable compounds and thus cooking contaminated seafood does not reduce their toxicity (van Dolah, 2000).

The subtropical coastal environments of Southern Brazil present a vast range of suitable conditions for the establishment of HABs, such as a high influx of nutrients, abundant light availability, and wide temperature and salinity fluctuations throughout the year. Therefore, not rarely, the right combination of conditions allows some harmful species to outcompete others and bloom in large estuaries, including the Paranaguá Estuarine Complex (CEP) (Mafra et al., 2006, 2014, 2019) and Guaratuba Bay (Mafra et al., 2014; Tibiriçá et al., 2015) in Paraná State, and Babitonga Bay (Mafra et al., 2014; Alves et al., 2018) and several other smaller embayments and bights in Santa Catarina State (Proença et al., 2007; Tavares et al., 2009; Vianna et al., 2023). In these areas, blooms of the DSP-causing dinoflagellate *Dinophysis acuminata* complex, which have been recorded almost annually in the region, and of the ASP-causing diatoms *Pseudo-nitzschia* spp. may occasionally contaminate seafood and cause episodes of human intoxication, both on a local (Fernandes et al., 2013; Alves et al., 2018; Vianna et al., 2023) and regional (Proença et al., 2017; Mafra et al., 2019) scale. *D. acuminata* blooms may occur throughout the year, even though larger and more intense events typically occur in winter and early spring, when cloud cover and precipitation are lower, and salinity and water column stability are higher in coastal areas (Tavares et al., 2009; Tibiriçá et al., 2015; Proença et al., 2017; Alves et al., 2018; Mafra et al., 2019; Vianna et al., 2023). In contrast, higher cell abundances of toxigenic *Pseudo-nitzschia* spp. (*P. calliantha*, *P. multiseries*, and *P. pesudodelicatissima*) are usually recorded in summer and early fall, when nutrient concentrations and turbulence are at maximum levels in this region (Mafra et al., 2006; Fernandes et al., 2013; Tibiriçá et al., 2015; Vianna et al., 2023). Moreover, blooms of the fish-killing raphidophyte *Heterosigma akashiwo* and the diatom *Coscinodiscus wailesii* (Proença and Fernandes, 2004), as well as the occurrence of several other potentially harmful planktonic algae, including PSP-causing *Alexandrium* spp. and *Gymnodinium catenatum* (Mafra et al., 2006; Tavares et al., 2009; Menezes et al., 2018), the presence of benthic toxigenic species like *Amphidinium* spp., *Coolia* spp., *Prorocentrum lima* complex, and *Ostreopsis* cf. *ovata* (Moreira-González et al., 2018, 2019, 2020; Tibiriçá et al., 2019, 2020), and the detection of multiple emerging phycotoxins compounds in bivalve mollusks (Schramm et al., 2010; Futigami et al., 2021) pose a continuous risk to marine life and human health in the southern Brazilian coast.

Among the southern Brazilian coastal areas subject to HAB events, the CEP stands out for being, at the same time, particularly favorable to the formation of HABs and vulnerable to their harmful effects. Several harmful algal species have been documented in the CEP (Table 1), occasionally forming blooms that negatively affect economic activities, marine life, and human health (e.g. Proença and Fernandes, 2004; Mafra et al., 2019). Therefore, it is necessary to fully characterize the occurrence of HABs and their effects in order to better understand the triggering mechanics and predict future blooms in this area. The present work reviews the literature on the occurrence of HAB-forming species and toxin contamination in the CEP, and systematically assesses, using primary and secondary data, the distribution of such occurrences in space and time, ultimately contributing to risk assessment, possible mitigation, and/or preparation for future events.

Table 1. List of HAB-forming species reported in the CEP, grouped by Type 1-3 risk categories (see Methods for details), along with their respective potential harmful mechanisms and the cell densities (cells L^{-1}) associated with each attributed risk level. Within each harmful category ("risk type"), lower cell densities linked to mild effects are highlighted in green, medium cell densities/intermediate effects in yellow, and higher cell densities/severe effects in red. ASTs, DSTs, PSTs: amnesic, diarrhetic, and paralytic shellfish toxins, respectively; ATXs: anatoxins; MCs: microcystins; YTXs: yessotoxins.

Risk	Species*	Potential Effect	Level 1	Level 2	Level 3	Level 4	Level 5
Type 1	*Dinophysis acuminata*	DSTs	-	-	-	100-1000	>1000
	Dinophysis caudata	DSTs	-	-	-	100-1000	>1000
	Dinophysis fortii	DSTs	-	-	-	100-1000	>1000
	Dinophysis rotundata	DSTs	-	-	-	100-1000	>1000
	Dinophysis tripos	DSTs	-	-	-	100-1000	>1000
	Gymnodinium catenatum	PSTs	-	-	-	presence	>1000
	Phalacroma rotundatum	DSTs	-	-	-	100-1000	>1000
	Prorocentrum lima	DSTs	-	-	-	100-1000	>1000
	Pseudo-nitzschia spp.	ASTs	-	-	-	<100.000	>100.000
Type 2	*Amphora* spp.	ASTs	-	50.000-80.000	80.000-100.000	>100.000	-
	Anabaena spp.	ATXs, MCs		<8000	8000-10.000	>10.000	
	Cochlodinium spp.	Fish mortality	-	<8000	8000-10.000	>10.000	-
	Dinophysis spp.	DSTs	-	<100	100-1000	>1000	-
	Gonyaulax spp.	PSTs	-	<8000	8000-10.000	>10.000	-
	Gyrodinium spp.	Fish mortality	-	<10.000	10.000-100.000	>100.000	-
	Lingulodinium spp.	YTXs	-	<10.000	10.000-100.000	>100.000	-
	Nitzschia spp.	ASP	-	50.000-80.000	80.000-100.000	>100.000	-
	Phalacroma spp.	DSP		<100	100-1000	>1000	-
	Polykrikos spp.	Fish mortality		<8000	8000-10.000	>10.000	-
	Prorocentrum cordatum	Unknown toxins		<10.000	10.000-100.000	>100.000	-
	Prorocentrum spp.	Multiple toxins	-	<10.000	10.000-100.000	>100.000	-
Type 3	*Coscinodiscus* spp.	High biomass	<10.000	10.000-100.000	>100.000	-	-
	Coscinodiscus wailesii	High biomass	<8000	8000-10.000	>10.000	-	-
	Trichodesmium spp.	Unknown toxins	<8000	8000-10.000	>10.000	-	-

*Raphidophytes were excluded because they are seldom found/counted in preserved samples.

METHODS

Study area

The CEP is a tidally dominated estuarine system (Noernberg et al., 2006) located in Paraná State, Southern Brazil (48°25'W, 25°30'S; Figure 1). This large water body (612 km²) represents a unique environment, surrounded by the Atlantic Forest and composed of large mangrove swamps, mudflats, and sandy beaches. It can be divided into six regions: Laranjeiras Bay, Antonina Bay, Pinheiros Bay, Paranaguá Bay, the central section (i.e., the mixing sector), and the adjacent continental shelf (Figure 1).

Artisanal fishing and small-scale aquaculture are the main activity of more than 50 fishing communities in the area, with fishing periods throughout the year, but mostly during summer (Lana et al., 2001). Such practices and the livelihood of these coastal communities are especially vulnerable to environmental alterations (Angulo et al., 2004), with climate change and anthropogenic activities such as deforestation, sewage disposal, and maritime ports representing great risks (Lana et al., 2001; Pellizzari, 2008; Negrello-Filho et al., 2018).

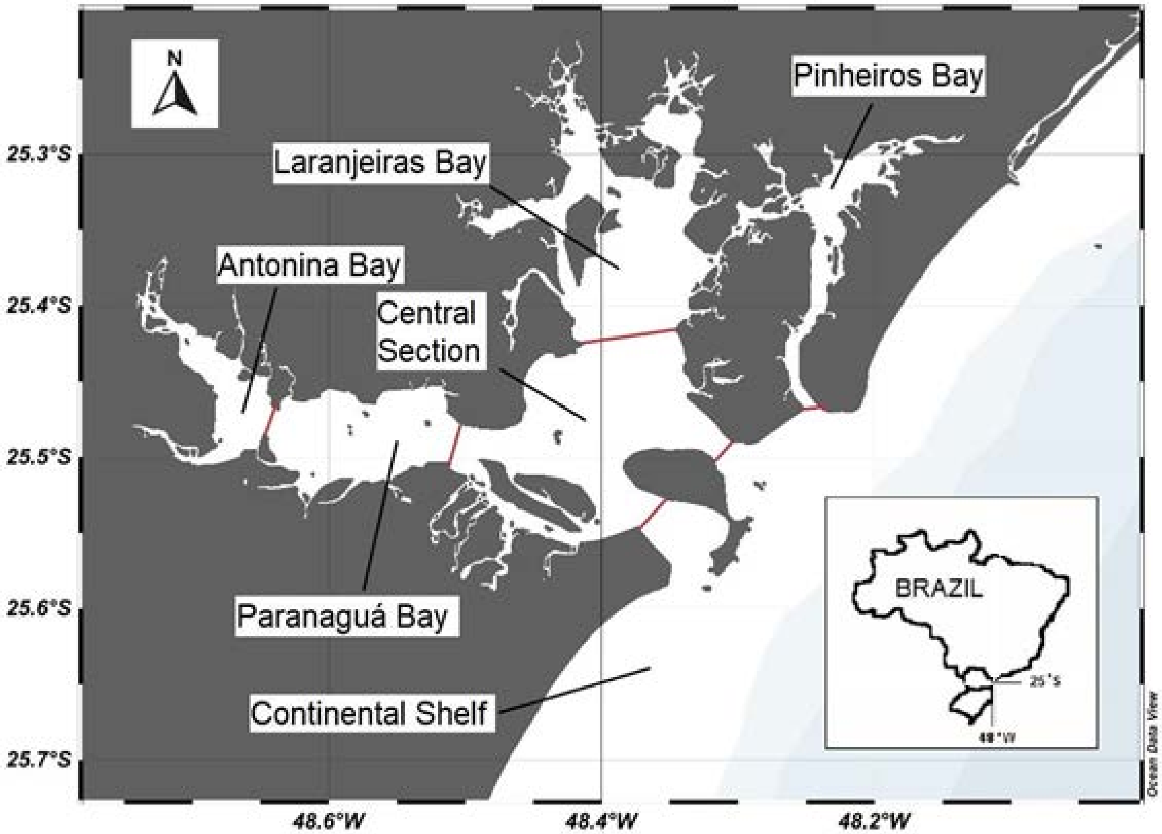

Figure 1. Paranaguá Estuarine Complex, in Southern Brazil.

The CEP is known to harbor more than 200 species of fish fauna, a richness higher than other systems in Brazil and around the world (Passos et al., 2012). The zooplankton assemblages with the highest densities (up to 82,000 organisms m^{-3}) occur in poly- and mesohaline sectors of the bay, in areas of maximum chlorophyll-*a* concentrations, and their distribution follows a salinity gradient (Lopes et al., 1998). Local hydrodynamics and phytoplanktonic primary productivity are mainly influenced by freshwater runoff, tidal currents, and dominant winds (Lana et al., 2001; Angulo et al., 2004; Marone et al., 2005). The region has a humid subtropical climate (Cfa; Köppen's classification), with a mean annual rainfall of 2500 mm (maximum 5300 mm) and around 85% average air humidity, and these regimes vary seasonally, with the driest period usually beginning in late austral fall and lasting until late winter (Figure 2), when the average salinity of the CEP ranges from 20 to 34 and the water temperature from 18 to 25°C (Lana et al., 2001).

LITERATURE REVIEW AND SECONDARY DATA ANALYSIS

This study represents a comprehensive overview of previous investigations reporting the occurrence of harmful algae and/or phycotoxins in the CEP area, including those specifically focusing on HABs—both planktonic and benthic species—and more general investigations on the ecology and taxonomy of microalgae. Values of cell densities for potentially harmful microalgal species (cells L^{-1} for phytoplankton or cells g^{-1} for microphytobenthos) and toxin concentrations in marine organisms (ng g^{-1}) were retrieved from published studies, technical reports, and theses, which were analyzed together with the primary data obtained as described in Section 2.3. The geographical location, sampling date, and salinity values associated with each occurrence were recorded to investigate spatio-temporal variability and determine the estuarine regions and periods with the highest incidence of HABs and their related negative effects, such as toxin accumulation along the trophic chain. The analysis was divided into two periods: "early studies," which use secondary data from investigations performed before 2010; and "recent studies," which include secondary data from 2010 to 2021 coupled with primary data obtained from 2018 to 2019.

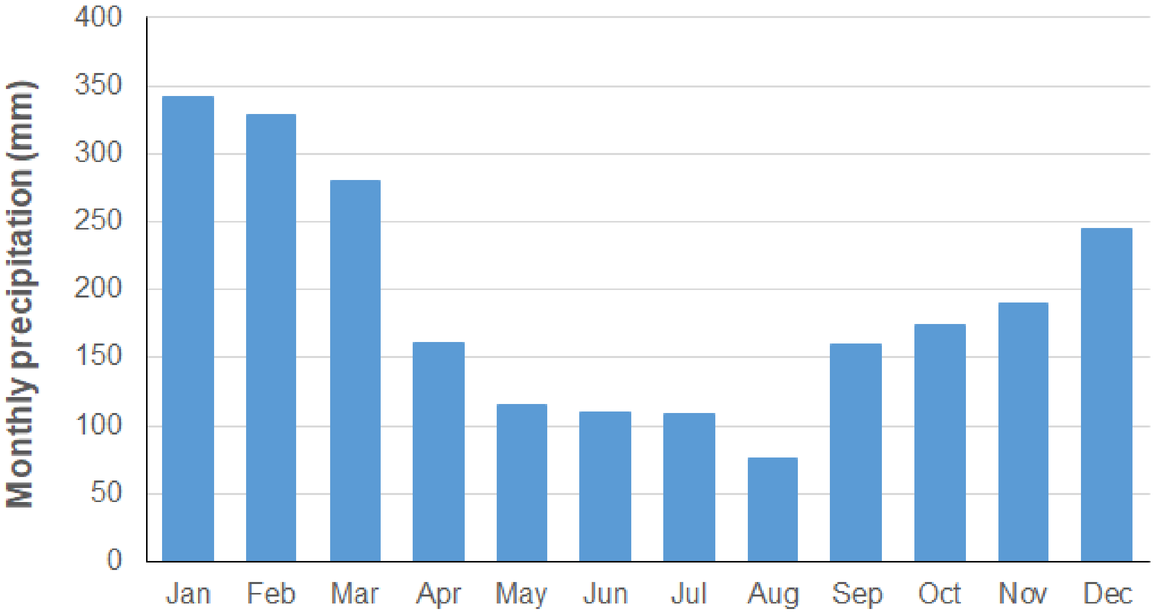

Figure 2. Mean monthly rainfall (mm) in Paranaguá city, from 1991 to 2020. Data source: INMET (https://portal.inmet.gov.br/normais).

Before 2010, studies on HABs and toxins were scarce in the CEP. The data analyzed here (referred to as "early studies") came from three early phytoplankton ecological studies from the 1980s to the early 2000's (Brandini, 1988; Brandini and Fernandes, 1996; Anderle, 2004) and three investigations focusing more specifically on HAB species (Proença and Fernandes, 2004; Mafra et al., 2006; Procopiak et al., 2006). By integrating the data from those studies, the maximum cell densities of selected planktonic HAB species and the respective periods of higher cell abundance in the CEP area could be analyzed.

Recently, studies on HABs and toxins have become more frequent and geographically expanded in the CEP. In the present study, the planktonic HAB species existing in the CEP from 2010 to 2021 were categorized according to their potential harmful effects (risks) into three categories: (i) Type 1: bloom-forming species capable of producing toxins that affect both marine organisms and human health; (ii) Type 2: toxigenic genera and species that may affect marine biota and/or human health, depending on the environmental conditions and the strain/species involved; and (iii) Type 3: species that may affect marine organisms and the environment at high cell density (Leem and Dornfeld, 1996; Hallegraeff et al., 2003; Lassus et al., 2016). Risk levels were assigned to each species within each category, depending on the cell densities usually linked to mild, intermediate, or severe negative effects (Table 1), as reported in various HAB studies. Then, the risk levels were applied to the cell density values reported in recent investigations in the CEP area (Ávila, 2011; APPA, 2013; Carvalho, 2013; Brustolin et al., 2014; Mafra et al., 2014, 2015a, 2015b, 2019; Tranker, 2014; Nolli, 2018), and to the primary data generated during the present study. Maps were prepared using the Ocean Data View program (Schlitzer, 2021). The map construction began with the preparation of a datasheet containing the geographical positions and sampling dates of each occurrence. Based on the cell density values obtained at each point and date, a risk level was attributed using the classification detailed in Table 1. Subsequently, a surface map model was generated for the CEP region using the pre-existing cartographic data layer. Finally, the sampling points representing the risk levels were added and the map was finalized by spreading the data over a gridded field, using an average weight for each value, automatic scale lengths, shading, and color grading.

Using an expanded dataset with secondary cell density data from 2002 to 2017 (Ávila, 2011; APPA, 2013; Brustolin et al., 2014; Carvalho, 2013; Mafra et al., 2006, 2014, 2015a, 2015b, 2019; Tranker, 2014; Moreira-González et al., 2020; Nolli, 2018) and primary data from 2018 to 2019 (detailed in Section 2.3), boxplot charts were created to better visualize the temporal (monthly) variability in the abundance of the most frequent HAB species in the CEP: *C. wailesii* (n = 64 occurrences), *Pseudo-nitzschia* spp. (n = 95), *P. lima* complex (n = 127), *P. cordatum* (n = 202), and *D. acuminata* complex (n = 243). In addition, cell abundances of the same species, along with those of *Dinophysis caudata* (n = 20), *Trichodesmium* spp. (n = 24), and *Gymnodinium* spp. (n = 68), were correlated with the corresponding salinity values obtained from the same expanded dataset.

Finally, a toxin dataset was assembled from secondary data composed of toxin levels (ng g^{-1}) accumulated in marine organisms sampled from October 2012 to October 2017 in the central section and the adjacent continental shelf of the CEP (Mafra et al., 2014, 2015a, 2015b, 2019; Nolli, 2018; Moreira-González et al., 2021b, 2023). Toxin levels were compared between marine animals representing different trophic levels (zooplankton, bivalve mollusks, gastropods, cephalopods, crustaceans, echinoderms, fishes, sea turtles, seabirds, and cetaceans), and temporal (monthly) variability was assessed for different bivalve species using boxplot charts. Graphs were generated in R Studio (2022), version 4.2.1. No statistical analyses were performed because data were unbalanced and obtained from different studies, using multiple sampling and analytical protocols.

PHYTOPLANKTON IDENTIFICATION AND QUANTIFICATION: 2018-2019 PERIOD

Subsurface water samples were collected along the CEP from 2018 to 2019. Sampling was performed in different periods: (1) September and October 2018; (2) April to June 2019; and (3) October and November 2019. Periods 1 and 3 represented the early rainy season in this region, while Period 2 coincided with the late rainy season (Figure 2). Moreover, field surveys were conducted in different sectors of the CEP, namely: northern sector, encompassing Laranjeiras Bay and Pinheiros Bay (n = 9 sampling points); western sector, encompassing Antonina Bay and Paranaguá Bay (n = 7), the central sector (n = 3), and the adjacent continental shelf (n = 3) (Figure 1).

The identification and counting of microphytoplankton cells (> 20 µm) were performed by the Utermohl sedimentation technique (1958), using a Zeiss Axio Vert.A1 inverted microscope. Due to the large amount of sediments present in the samples, a volume of 7 to 10 mL was settled for approximately 12 hours. Then, the total abundance of phytoplankton was determined using transects throughout the counting chamber under 100× and 200× magnification, to reduce the statistical error associated with the procedure (Edler and Elbrächter, 2010). Thereafter, the number of cells counted per chamber was converted into cells per liter, considering the microscope magnification (100× or 200×), the total area of the chamber, the estimation area counted (chamber or transect), and the volume of sample settled.

In this study, only potentially harmful algal taxa were identified at the species level; all other microalgae were identified only at the genus level. Afterwards, the harmful species were categorized into risk levels (Levels 1 to 5) according to their harmful potential (Types 1 to 3) (Table 1). The sum of all species from a given sampling site and date was used to compose maps representing the distribution and relative abundance of potentially harmful microalgae classified as Type I, II, and III in the CEP. Individual maps were generated for each sampling period.

SPATIO-TEMPORAL DISTRIBUTION OF HAB SPECIES AND TOXINS

HAB species: Early studies

Several potentially harmful species have been recorded in the CEP, however, few studies have reported their presence and abundance at this site before 2010. The authors of early studies frequently reported high cell densities of diatoms that can form high biomass blooms, including *Cylindrotheca closterium*, *Leptocylindrus minimus*, and *Skeletonema costatum*. These species were all classified as potentially harmful by Procopiak et al. (2006), due to the possibility of causing ecological damage during massive bloom events. Mafra et al. (2006), based on samples collected bimonthly over 14 months from 2002 to 2003, was the first to specifically investigate the distribution, abundance, and toxicity of harmful microalgae in the CEP. Several species classified as potentially harmful to the environment or human health (Hallegraeff et al., 2003; Lassus et al., 2016) were detected during the study. These included the dinoflagellates *D. acuminata* and *G. catenatum*, which produce diarrhetic and paralytic shellfish toxins (DSTs and PSTs), respectively, and *Prorocentrum cordatum* (previously classified as *P. minimum*), which may be toxic to invertebrate larvae. Other potentially harmful species included the ichthyotoxic raphidophytes *Heterosigma akashiwo* and *Chattonella* spp., the primnesiophyte *Phaeocystis* spp., which is potentially toxic to larvae and invertebrates, and three species of diatoms that produce amnesic shellfish toxins (ASTs)—*Pseudo-nitzschia calliantha*, *P. multiseries*, and *P. pungens*—besides other locally important harmful microalgae, such as the potentially toxic cyanophyte *Trichodesmium erythraeum* and the invasive diatom *C. wailesii*, whose blooms can modify the trophic structure in the water column and cause hypoxia near the bottom.

In early studies, the greatest diversity and abundance of HAB species were found in the central sector of the estuary, close to its mouth, where cell density peaks of 3.2×10^5 cells L^{-1} of *Pseudo-nitzschia* spp. and 11.4×10^5 cells L^{-1} of *Phaeocystis* spp. were recorded (Mafra et al., 2006). Harmful dinoflagellates, such as *G. catenatum* and those belonging to the *D. acuminata* complex, and raphidophytes of the genus *Chattonella*, were also recorded with lower cell densities in the same sector (Table 2). Since the 1980s and 1990s, high cell densities of potentially harmful microalgae have been recorded in the outermost sectors of the CEP and adjacent coastal areas (Brandini, 1988; Brandini and Fernandes, 1996), some of which have been related to ecological and economic damage associated with events of mass mortality of aquatic organisms, such as the blooms of *C. wailesii* in 1991 and *H. akashiwo* in 2001 (Proença and Fernandes, 2004). In the outermost portion of the CEP, the typically higher salinity and transparency values seem to benefit the growth of several potentially harmful species of microalgae (Mafra et al., 2006). In addition, microalgal populations growing on the continental shelf near the mouth of the CEP can be brought to the outermost areas of the estuary during certain periods of the year. In general, the highest cell densities of the most harmful species were detected in spring and/or late summer to early fall, at the beginning and end of the rainy season (Figure 2), respectively (Table 2).

HAB species: Recent data (2010-2021)

In 2010, the establishment of the Microalgae Laboratory (LaMic) at the Center for Marine Studies of the Federal University of Paraná (CEM-UFPR) in Pontal do Paraná, PR — at the estuarine mouth — fostered research on HABs and phycotoxins in the CEP. Studies became more frequent, extensive, and diverse, embracing both planktonic and benthic species, as well as toxin production and accumulation in the fauna. The most frequent and abundant potentially toxic microalgal species in the CEP from 2010 to 2021 were the dinoflagellates *Dinophysis* spp., especially those belonging to the *Dinophysis acuminata* species complex (*D. acuminata*, *D. ovum*), and the *Prorocentrum lima* species complex (*P. caipirignum*, *P. lima*), producers of the DSTs okadaic acid (OA) and dinophysistoxins (DTXs), and the diatoms of the genus *Pseudo-nitzschia*, producers of the AST domoic acid.

Table 2. Potentially harmful planktonic algae reported in the Paranaguá Estuarine Complex before 2010, maximum cell density measured and periods of occurrence.

Species	Maximum density	Periods		Reference
Coscinodiscus wailesii	5.6×10^{4} cells L^{-1}	April	Late rainy season	Fernandes and Brandini (2004)
	1.4×10^{4} cells L^{-1}	February to April		Mafra et al. (2006)
Chatonella spp.	3.6×10^{4} cells L^{-1}	June October	Late rainy season Early rainy season	Mafra et al. (2006)
Dinophysis acuminata complex	4.6×10^{3} cells L^{-1}	September to December	Early rainy season	Mafra et al. (2006)
Gymnodinium catenatum	6.4×10^{3} cells L^{-1}	August and October	Late rainy season	Mafra et al. (2006)
Prorocentrum cordatum	1.3×10^{5} cells L^{-1}	June to September	Dry season	Anderle (2004)
	1.7×10^{4} cells L^{-1}	October to April	Rainy season	Mafra et al. (2006)
Pseudo-nitzschia spp.	1.5×10^{4} cells L^{-1} 3.2×10^{4} cells L^{-1}	February to April January to March	Rainy season	Anderle (2004) Mafra et al. (2006)
	14.4×10^{4} cells L^{-1}	March to August	Rainy to dry season	Fernandes and Brandini (2004)
Phaeocystis spp.	1.1×10^{6} cells L^{-1}	June to September September to December	Dry season Early rainy season	Mafra et al. (2006)

During the regular phytoplankton monitoring as part of the environmental assessment related to the operational activities of the seaports in the CEP, quarterly sampling campaigns from 2014 to 2021 revealed the presence of 21 species of potentially harmful microalgae in Antonina and Paranaguá Bays, although never in cell densities characterizing blooms (APPA, 2021). Among these, the most frequent species were *Skeletonema costatum* (present in 100% of the samples; also the most abundant species), *Cylindrotheca closterium* (100%), *Tripos fusus* (94.3%), *Leptocylindrus danicus* (85.7%), *Tripos furca* (65.7%), *Dinophysis caudata* (57.1%), *Trichodesmium erythraeum*

(57.1%), *Asterionellopsis glacialis* (54.3%), *Chaetoceros convolutus* (51.4%), *Prorocentrum cordatum* (48.6%), and *Leptocylindrus minimus* (45.7%). Other potentially harmful species were detected less frequently, such as *Gonyaulax spinifera* (28.6%), *Gymnodinium catenatum* (28.6%), *Alexandrium minutum* (8.6%), *Cerataulina pelagica* (8.6%), *Dinophysis tripos* (5.7%), *Trichodesmium thiebautii* (5.7%), and *Dactyliosolen fragilissimus* (2.8%). Most species represent a low environmental risk because they cause damage to the biota only during events with extremely intense biomass accumulation. However, the relatively frequent presence of potentially invasive species that produce PSTs (*G. catenatum* and *A. minutum*), in addition to those that produce DSTs (*D. caudata* and *D. tripos*) and other toxic compounds (*G. spinifera*, *P. cordatum*, *T. erythraeum*, and *T. thiebautii*) pose a constant risk to the estuarine fauna and their consumers, including local human populations.

From 2010 to 2017, less rainy periods (Figure 2) were associated with a higher incidence of microalgal blooms with greater harmful potential in the CEP, mainly in the intermediate region of Paranaguá Bay and on the shallow continental platform adjacent to the mouth of the CEP (Figure 3). However, when cell counts from the exceptional 2016 bloom of the *D. acuminata* complex are excluded, thus representing a more typical situation in this region, the high-incidence zone is more restricted to the Paranaguá Bay area during drier periods (Figure 4). During the rainy season, the inner portions of the estuarine central sector also present a relatively greater risk of HAB development (Figure 3 and Figure 4). Typically, the central sector provides the most favorable nutrient, light, and salinity conditions for phytoplankton growth in the CEP. Phytoplankton growth is limited by low light penetration in the inner estuarine areas and low nutrient concentrations — mainly nitrogen compounds — in the outer portion, which is affected by the oligotrophic Tropical Water that dominates the adjacent continental shelf during most of the year (Brandini et al., 1988; Lana et al., 2001; Machado et al., 1997).

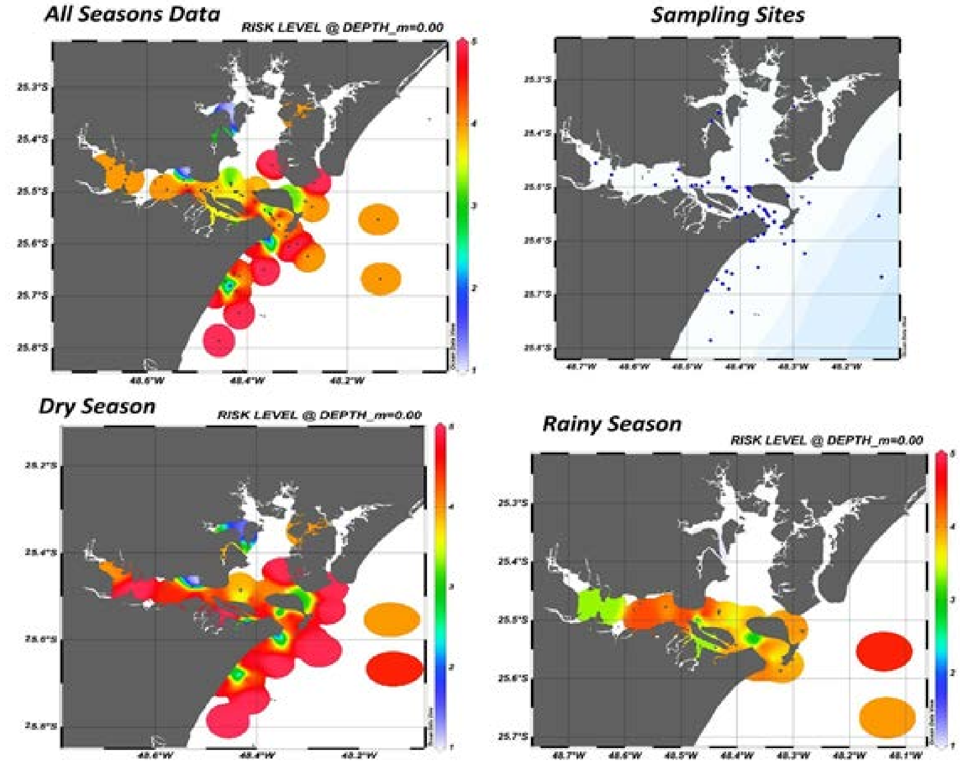

Figure 3. Risk assessment for the incidence of harmful algal blooms in the Paranaguá Estuarine Complex. The risk index increases from 1 (purple) to 5 (red) according to the cell density and harmful effects of algal species occurring in the area, based on historical data from 2010 to 2017 (see Methods for details).

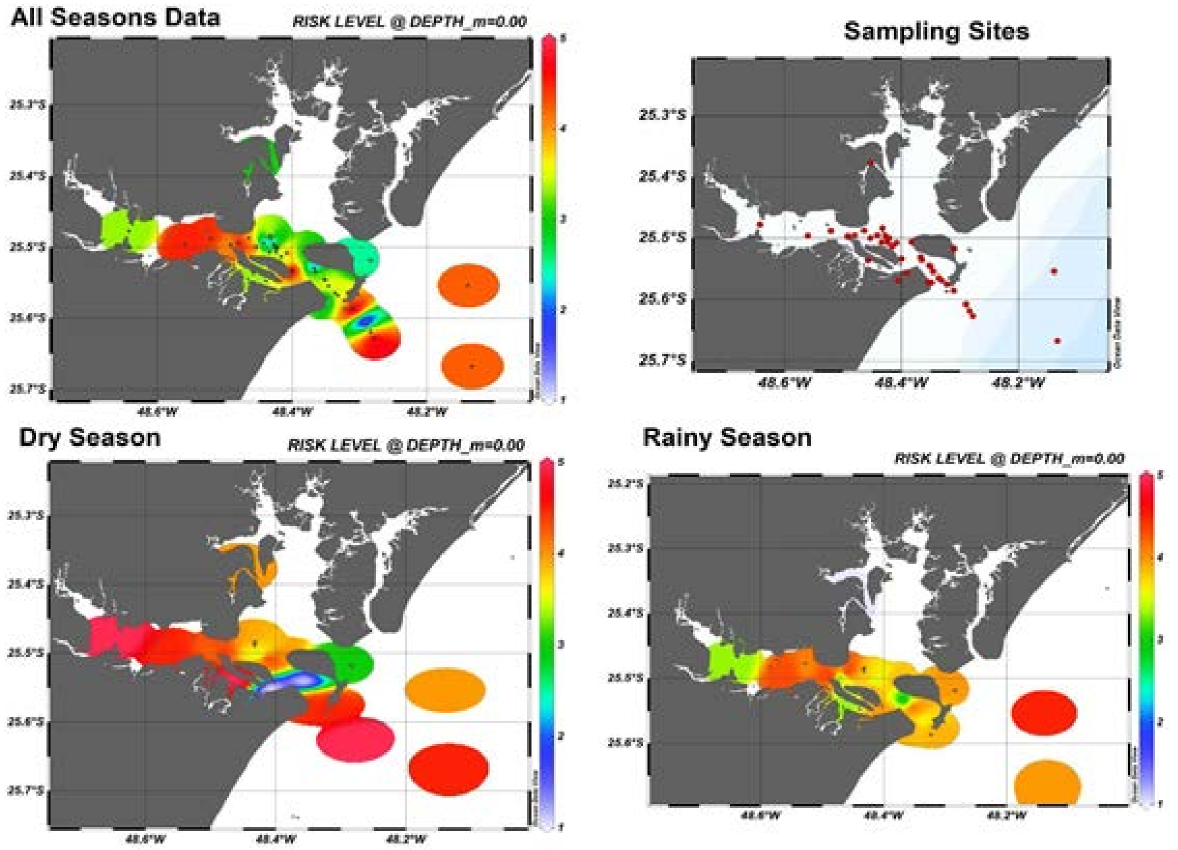

Figure 4. Risk assessment for the incidence of harmful algal blooms in the Paranaguá Estuarine Complex. The risk index increases from 1 (purple) to 5 (red) according to the cell density and harmful effects of algal species occurring in the area, based on historical data from 2010 to 2017, excluding the massive 2016 *Dinophysis acuminata* bloom (see Methods for details).

Based on the primary data collected here over a wider spatial coverage in 2018-2019, a higher incidence of potentially harmful microalgae was diagnosed during the end of the rainy season (austral fall; Figure 5B) compared with the beginning of the same period (austral spring; Figure 5 A, C). During the springs of 2018 and 2019, only potentially harmful algal species classified as Type 3—i.e., those that can only affect aquatic organisms at extremely high cell abundance—were recorded in the estuary (Figure 6). Again, the shallow continental shelf adjacent to the outer portion of the CEP, as well as the intermediate sectors of the estuary, were characterized as the areas under the highest risks associated with the negative effects caused by the proliferation of potentially harmful microalgae (Figure 5 and Figure 6).

Higher water temperature and greater nutrient input linked to heavier summer rainfall are expected to stimulate phytoplankton growth in the CEP, even though light penetration is lower due to higher turbidity within the estuary, and some degree of photoinhibition may exist at the surface in the adjacent coastal waters during this period (Brandini, 1985; Brandini et al., 1988; Machado et al., 1997; Lana et al., 2001; Mafra et al., 2006). In addition, grazing might play a major role in controlling phytoplankton growth in the outer estuarine sectors, at least during part of the summer and winter (Lopes et al., 1998).

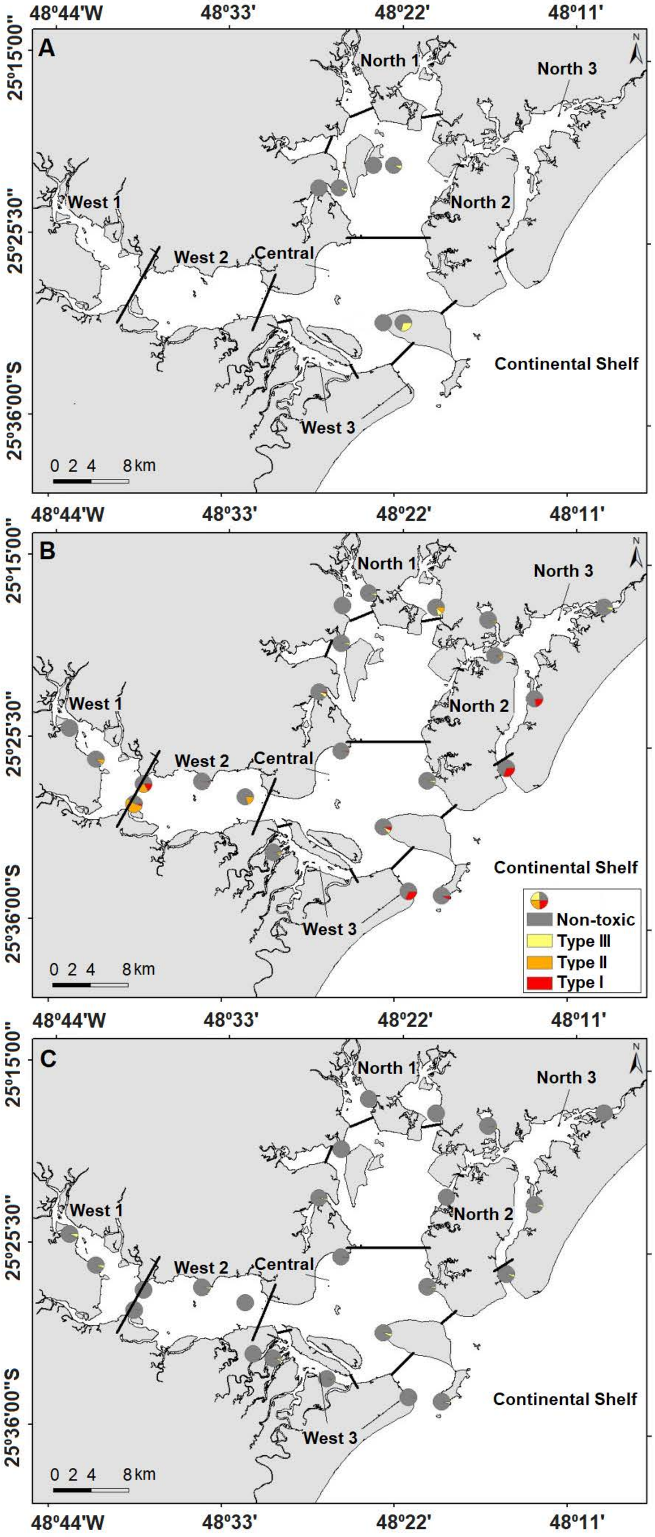

Figure 5. Dominance of non-harmful (gray area in the pie charts) and potentially harmful microalgal species (red: Type 1; orange: Type 2; yellow: Type 3; see Methods for details) along the Paranaguá Estuarine Complex during: (A) early 2018-2019 rainy season; (B) late 2018-2019 rainy season; and (C) early 2019-2020 rainy season.

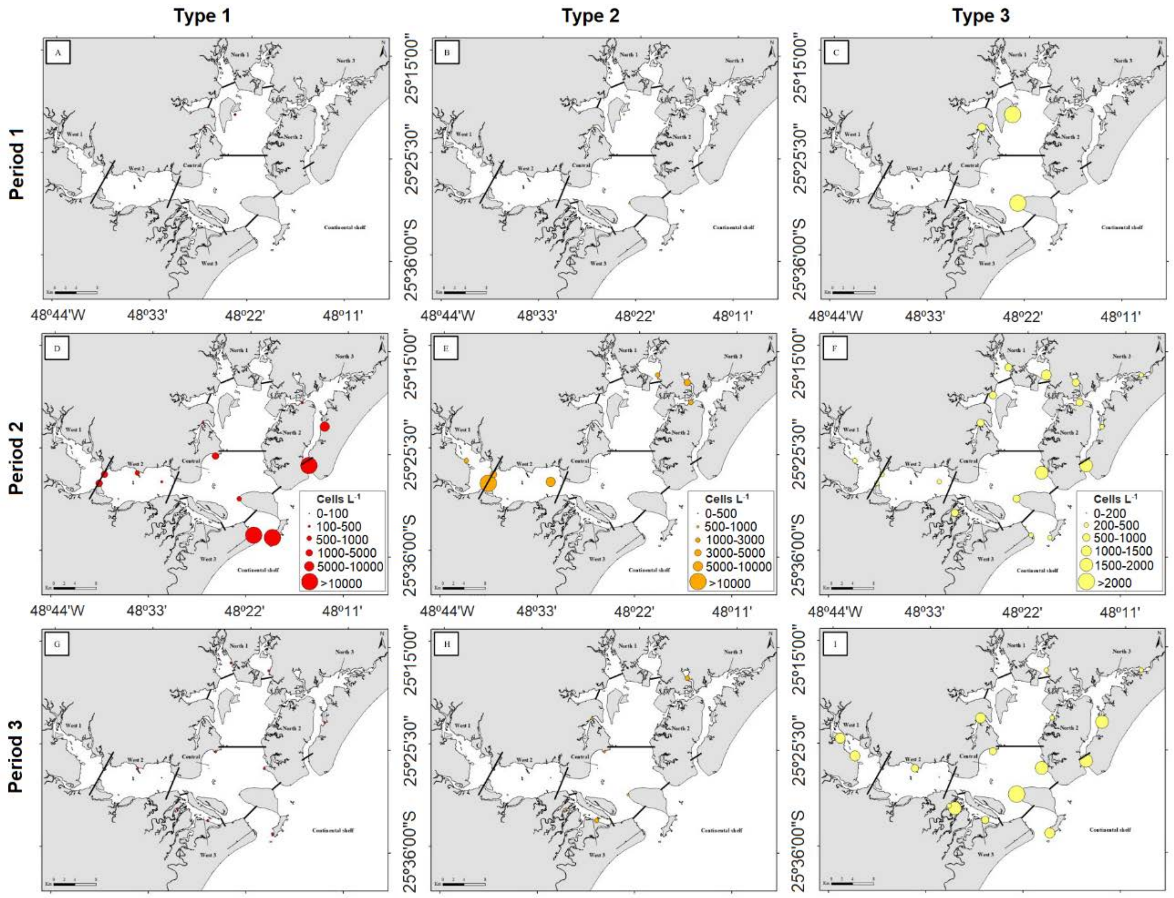

Figure 6. Abundance (cells L^{-1}) of potentially harmful microalgae (red: Type 1; orange: Type 2; yellow: Type 3 - see Methods for details) found in the Paranaguá Estuarine Complex during: (A-C) Period 1: early 2018-2019 rainy season; (D-F) Period 2: late 2018-2019 rainy season; and (G-I) Period 3: early 2019-2020 rainy season.

For some dinoflagellates—the most diverse and dominant HAB-forming group in this area—excess turbulence may constitute an additional limiting factor during most of the winter, as recorded in the CEP (Mafra et al., 2006) and similar subtropical estuaries (Tibiriçá et al., 2015; Alves et al., 2018). In general, this leaves the transition seasons (spring and fall) with the best opportunities for the development of most HAB species in the CEP (Mafra et al., 2006; present study). The exact periods and estuarine zones that favor the growth of each HAB-forming taxon, however, may depend on more specific requirements, including intrinsic nutrient and light adaptations, as well as trophic relationships with competitors, grazers, and prey, in the case of heterotrophic and mixotrophic species (Mafra et al., 2016, 2019).

Among the most frequent harmful species, *D. acuminata* occurred more abundantly in winter and spring (Figure 7), with the first period mainly related to an anomalous bloom event recorded in 2016. In that year, a very intense, large-scale bloom of the *D. acuminata* complex was recorded in June, lasting, with relatively low cell abundances, until August. The bloom was associated with a less saline and colder body of water, under the exceptional influence of the Rio da Prata plume (Proença et al., 2017; Alves and Mafra 2018; Mafra et al., 2019; Sobrinho, 2020), reaching the highest cell densities ever recorded for any *Dinophysis* species worldwide (Mafra et al., 2019). The massive bloom, which moved north from Argentina/Uruguay (Proença et al., 2017), reached its maximum cell abundance (10^5-10^6 cells L^{-1}) over the coast of Paraná State, decaying thereafter as *D. acuminata* cells were actively grazed by mixotrophic and heterotrophic dinoflagellates and ciliates (Sobrinho, 2020). On that occasion, the highest *D. acuminata* cell densities were recorded on the continental shelf and in the outer sector of the CEP. In a typical year, in contrast, local populations of *D. acuminata* reach their maximum cell abundances (10^3-10^4 cells L^{-1}) within the estuary (intermediate portions) during spring, as reported in the CEP (Mafra et al., 2006) and in the neighboring estuaries of Guaratuba Bay (Tibiriçá et al., 2015) and Babitonga Bay (Parizzi et al., 2016). Usually, *D. acuminata* develop more abundantly within the estuary during drier and less windy periods, when the water column becomes stratified and their prey—the mixotrophic ciliate *Mesodinium rubrum*—is available (Tibiriçá et al., 2015; Parizzi et al., 2016). Like other toxigenic *Dinophysis* species occurring in the region, such as *D. caudata* and *D. tripos* (Mafra et al., 2014), *D. acuminata* is more abundant at medium salinity values (20-30) in Brazilian subtropical estuaries (Mafra et al., 2006; Tibiriçá et al., 2015; Parizzi et al., 2016). In the CEP, *D. acuminata* cell abundances are historically higher at salinities ranging from 23 to 27, while *D. caudata* has typically occurred at slightly greater salinity values (Figure 8).

Other potentially harmful dinoflagellates occurring in the CEP are also associated with medium or low salinity values, such as the *Prorocentrum lima* complex and *Prorocentrum cordatum*, respectively (Figure 8). While *P. lima* is a benthic dinoflagellate typically associated with tidal mudflats in the central section of the estuary (Brustolin et al., 2014; Moreira-González et al., 2020), *P. cordatum* is a planktonic species that blooms in the low salinity waters of the CEP (Mafra et al., 2006) and elsewhere (Fan et al., 2003; Khanaychenko et al., 2019). It is noteworthy that *Prorocentrum cordatum* can be found at high cell abundances throughout the year in the CEP, except in winter (Figure 7), when salinities are typically higher within the estuary (Lana et al., 2001). Cells belonging to the *P. lima* species complex, which have been less frequently recorded, are more abundant in spring-summer. However, these benthic cells are only occasionally found in the phytoplankton assemblage, after resuspension from their substrates—sediments or macrophytes. Likewise, low cell densities of the benthic dinoflagellate *Ostreopsis* cf. *ovata* can eventually be found in suspension in the outer estuarine sectors and along the adjacent continental shelf. This species, which produces the potent neurotoxin palytoxin and analogues, reaches significant cell densities in the late summer, growing on macroalgae and corals around the Currais Archipelago, only ~10 km from the estuarine mouth (Tibiriçá et al., 2019).

The dinoflagellates *Gymnodinium* spp., the cyanobacteria *Trichodesmium* spp., and the diatoms *Coscinodiscus wailesii* and *Pseudo-nitzschia* spp. can be found at relatively high cell densities in a wide salinity range (from ~20 to 35) in the CEP (Figure 8). Of these, *C. wailesii* reaches higher cell abundances in winter and spring, when it may form high biomass blooms associated with copious mucus production and negatively affect the environment (Proença and Fernandes, 2004) and artisanal fishing activities (L. L. Mafra, pers. obs.). In contrast, *Pseudo-nitzschia* spp., the other potentially harmful diatoms recorded in this region, are usually more abundant in summer and fall (Figure 7). In fact, bloom cell densities (10^5-10^6 cells L^{-1}) of *Pseudo-nitzschia* spp. have been recorded during late summer/early fall in the CEP (Mafra et al., 2006; Nolli, 2018) and in the neighboring state of Santa Catarina, where bivalves became contaminated at unsafe levels with ASTs (Fernandes et al., 2013).

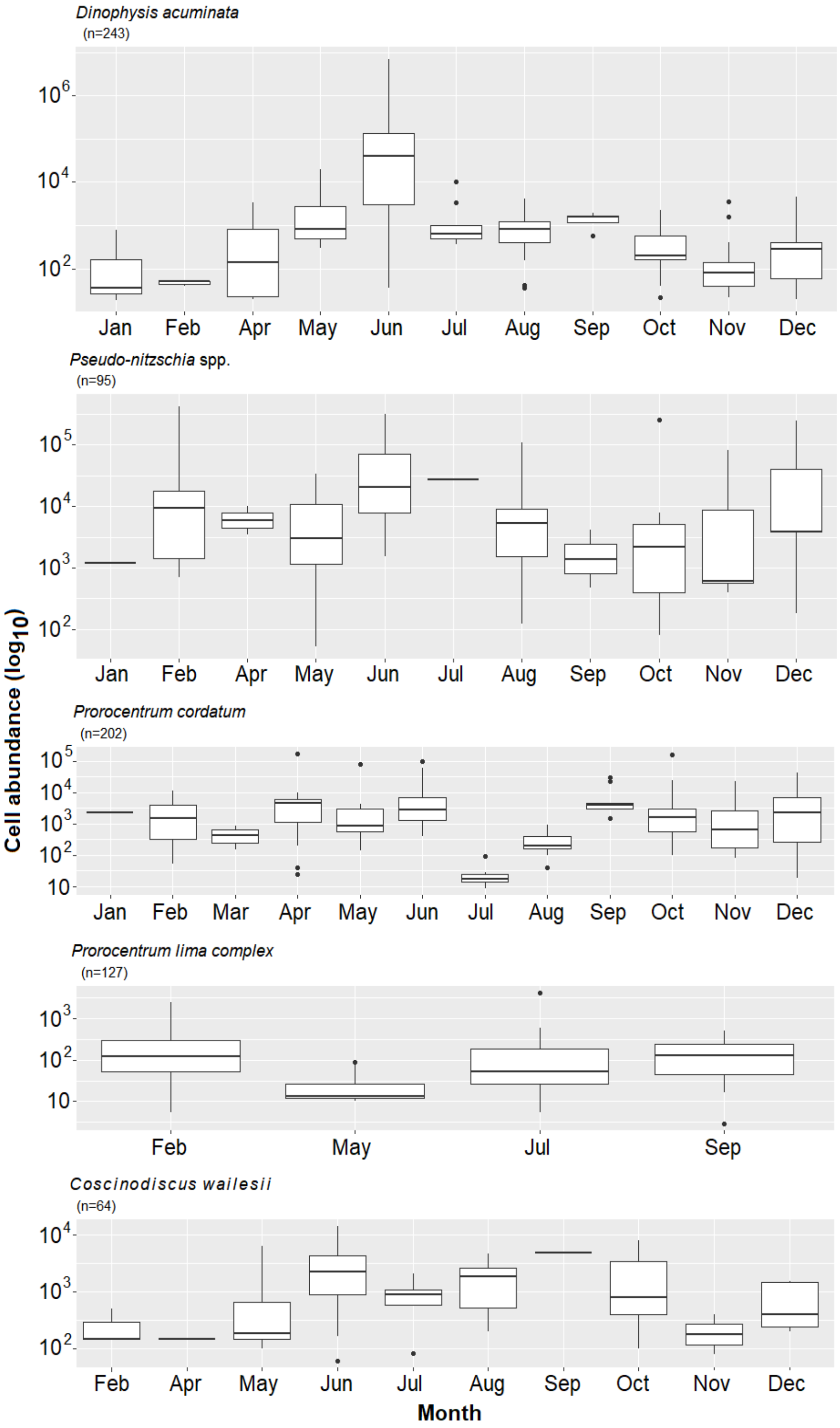

Figure 7. Temporal variability in the abundance of the most frequent HAB species in the Paranaguá Estuarine Complex (cells g^{-1} for the *Prorocentrum lima* complex; cells L^{-1} for the others).

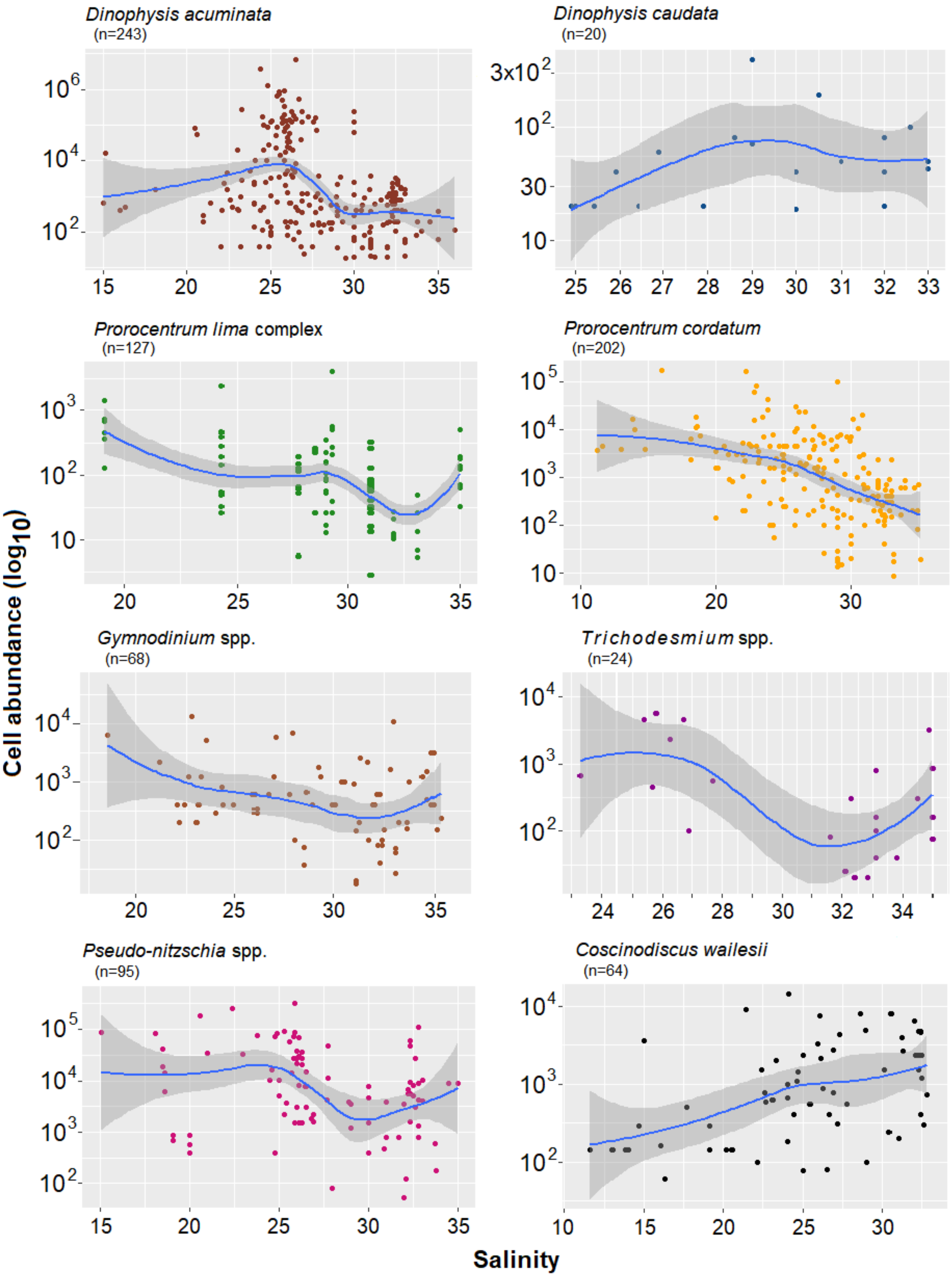

Figure 8. Relationship between salinity and cell abundance of the most frequent HAB species in the Paranaguá Estuarine Complex (cells g^{-1} for the *Prorocentrum lima* complex; cells L^{-1} for the others).

TOXIN ACCUMULATION IN THE FAUNA

Algal lipophilic toxins, mostly the DST okadaic acid (OA), have been frequently detected in marine animals in the CEP area (Mafra et al., 2014, 2015a, 2015b, 2019; Nolli, 2018; Moreira-González et al., 2021, 2023), sometimes at levels unsafe for human consumption (i.e., 160 ng OA g^{-1}). Other lipophilic toxins have been found at lower levels (8.8-87 ng g^{-1}) in plankton or accumulated in bivalve and cephalopod mollusks sampled in the CEP, including pectenotoxin-2 (PTX-2), PTX-2 seco acid, 7-*O*-palmytoyl OA, 7-*O*-palmytoyl dinophysistoxin-1 (DTX-1) (Mafra, et al., 2015a), and yessotoxin (YTX) (L. L. Mafra, unpublished results).

In this study, data from 675 samples of marine animals contaminated with OA in the CEP area, obtained from October 2012 to October 2017, were reassessed. There was no indication of biomagnification of this compound in local food webs. The highest levels of OA were measured in whole tissues of primary consumers, such as bivalves, zooplankton, and the suspension-feeding ghost shrimp *Callichirus major* (Mafra et al., 2019) (Figure 9). Lower OA concentrations can accumulate in specific organs/tissues of secondary and tertiary consumers, including the digestive glands (DGs) of gastropods (Mafra et al., 2019), the DGs, gills, kidneys, digestive tracts (DTs: intestine + stomach), and arms of octopuses *Octopus vulgaris* (Mafra et al., 2015a), the gizzards, DTs, gonads, fat tissues, and flesh of the fish *Mugil liza* (Mafra et al., 2019), as well as the DTs and livers of the fish *Cetengraulis edentulus* (Mafra et al., 2014) and the DTs of the sea turtle *Chelonia mydas* (Moreira-González et al., 2023) (Figure 9). Trace levels of the toxin could also be detected in the livers of top-level predators, such as the penguin *Spheniscus magellanicus* and the dolphin *Sotalia guianensis*, and in detritivorous echinoderms, *Mellita quinquiesperforata* (Mafra et al., 2019) (Figure 9).

The concentrations of okadaic acid in bivalves vary in a similar pattern to the *D. acuminata* cell abundance in the CEP over the studied period. Higher OA levels (10^{2}-10^{3} ng g^{-1}) were measured in June-July, when the massive bloom of the *D. acuminata* complex was recorded in the region, with moderate levels (10^{1}-10^{2} ng g^{-1}) recorded during spring (Figure 10). This dinoflagellate species thus represents the main risk for the incidence of DSP in the CEP area. In addition, the resuspension of *P. lima* cells from the bottom, and the co-ocurrence of *D. caudata* and *D. tripos* in the phytoplankton assemblage may constitute secondary sources of DST accumulation in seafood. Besides DSTs, *D. caudata* may produce PTX, creating a possible scenario for multi-toxin accumulation in bivalves, especially in those that accumulate higher toxin levels. In the present study, *Perna perna* mussels accumulated much higher OA levels than other bivalve species, including mangrove oysters (*Crassostrea* spp.) and clams (*Anomalocardia brasiliana*), over comparable sampling periods (Figure 10). The greater capacity for DST accumulation in *P. perna* has been demonstrated under controlled laboratory conditions and in other places where it coexists with oysters (Mafra et al., 2015b). Even so, oysters may still reach unsafe OA levels when the abundance of toxigenic cells is sufficiently high in the water column (Mafra et al., 2019).

CONCLUSION

Subtropical estuaries are very dynamic environments, where physical processes and chemical conditions are highly variable at different spatial and temporal scales. Consequently, phytoplankton species with different growth requirements and competitive abilities respond differently to these variations. Ecological succession events are therefore highly expected and variable over time. In this context, many harmful microalgal species may become dominant and negatively affect the environment and related human activities at specific periods and estuarine regions. In the CEP, less rainy periods have been associated with a higher incidence of microalgal blooms with greater harmful potential, especially in the central portion of the estuary and on the adjacent shallow continental platform.

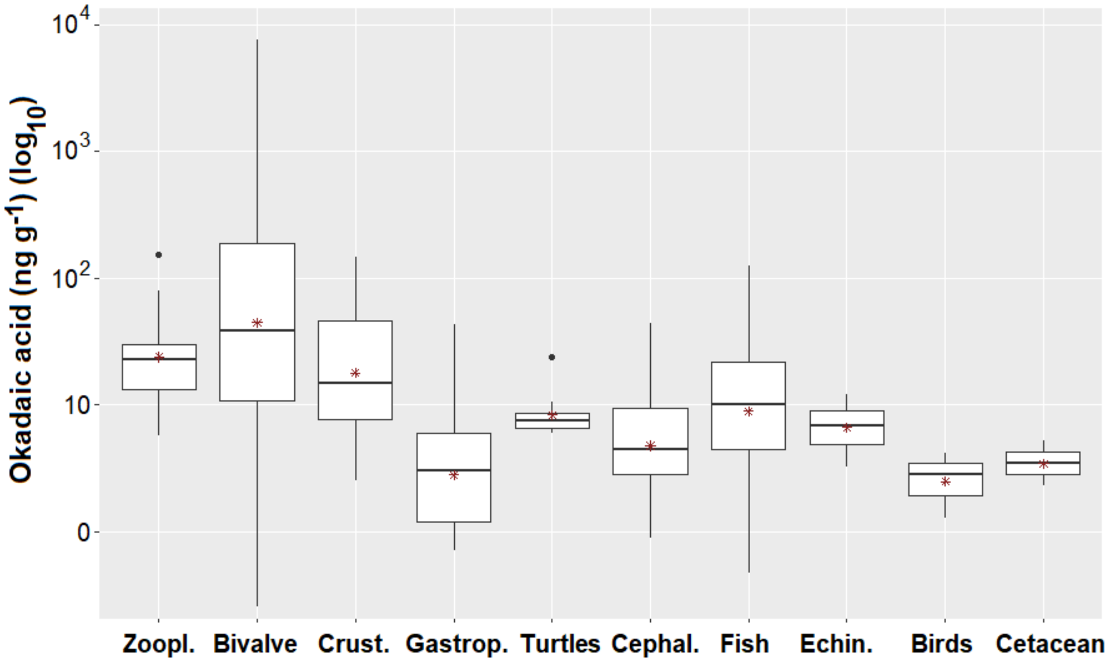

Figure 9. Levels of the diarrhetic toxin okadaic acid accumulated in consumers of different trophic levels occurring in the Paranaguá Estuarine Complex: zooplankton ("Zoopl."), bivalve mollusks ("Bivalve": clams, mussels, oysters), crustaceans ("Crust.": crabs, shrimps, ghost shrimps), sea turtles ("Turtles"), cephalopods ("Cephal.": squids, octopuses), fishes ("Fish"), echinoderms ("Echin.": sand dollars), seabirds ("Birds": penguins) and cetaceans ("Cetacean": dolphins). The horizontal lines inside the boxes represent the median values; the red asterisks indicate the average values.

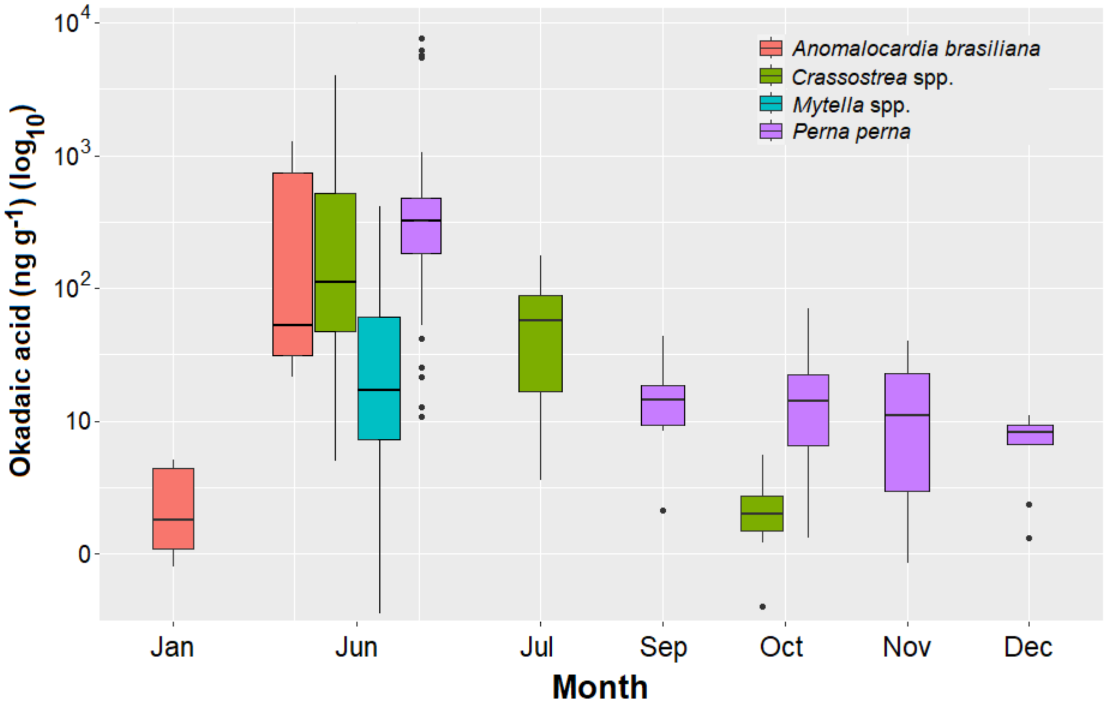

Figure 10. Temporal variability in the okadaic acid levels accumulated in different species of bivalve mollusks occurring in the Estuarine Complex of Paranaguá. Horizontal lines inside the boxes represent the median values.

However, certain HAB-forming species may be more successful in other estuarine areas and under different environmental conditions, which in turn may show large interannual variability, mediated by large-scale climatic events such as El Niño-Southern Oscillation (ENSO). Furthermore, climate change and anthropogenic interference in natural cycles may intensify the frequency and magnitude of HAB events, making them more difficult to predict. Continuous monitoring of harmful algae and toxins is thus highly recommended and should include areas that have been less frequently (or never) sampled in the estuary.

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AUTHOR CONTRIBUTIONS

L.L.M.Jr.: Conceptualization, Methodology, Data Curation, Formal Analysis, Visualization, Writing - Original Draft, Review & Editing, Funding Acquisition, Investigation, Resources, Supervision.

B.P.E.; B.F.S.: Data Curation; Formal Analysis; Visualization; Writing - Review & Editing.

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