Assessment of dinoflagellate diversity using DNA metabarcoding reveals toxic dinoflagellate species in Australian coastal waters

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ABSTRACT

Harmful algal blooms (HABs) present severe risks to marine ecosystems, wildlife, human health and economies globally. This study investigates the diversity and abundance of the primary HAB group—dinoflagellates—in Hervey Bay, Queensland, Australia, a region notable for its ecological uniqueness and high susceptibility to HABs. By employing DNA metabarcoding targeting the 18S rRNA V8-V9 regions, we aimed to provide a comprehensive overview of dinoflagellate communities across various substrates and locations, identifying both toxic and non-toxic species. Our findings revealed 66 distinct genera, including known toxin producers such as *Alexandrium*, *Gambierdiscus*, *Karenia* and *Prorocentrum*, with some toxic species detected in Australian waters for the first time. Additionally, we assessed the abundance of these toxic species and examined the influence of environmental factors on their occurrence. This study emphasises the importance of ongoing monitoring and ecological assessments to manage HAB impacts in vulnerable coastal areas such as Hervey Bay.

1. Introduction

Harmful algal blooms (HABs) are a threat to global marine environments, affecting wildlife and human health, along with substantial economic loss (Gobler, 2020; Yan et al., 2024; Zahir et al., 2024). HAB is a broad and general term that includes any harmful micro or macro algae, including the focus of this study, toxic dinoflagellates (TDs) (United Nations, 2020; HAIS-UNESCO, 2022). Dinoflagellates are a major group of aquatic eukaryotic phytoplankton that are highly diverse globally (Taylor et al., 2008; Bravo and Figueroa, 2013). They are crucial primary producers within the marine food web, providing food and energy for many marine organisms (Bravo and Figueroa, 2013). There are over 2500 known species of dinoflagellates and many of these are known to produce toxins (Taylor et al., 2008; Zonneveld and Pospelova, 2015). Some are the causative organisms for seafood poisonings in humans such as, ciguatera poisoning (CP), paralytic shellfish poisoning (PSP), azaspiracid shellfish poisoning (AZP) and diarrhetic shellfish poisoning (DSP), just to name a few (Kadiri and Isagba, 2018;

Bravo et al., 2019). Although toxin production isn't unique to dinoflagellates, it is estimated they account for 75–80 % of toxin producing eukaryotic phytoplankton species (Janouškovec et al., 2017). Their large genomes, complexity of their toxin production, unresolved taxonomic relationships and challenges in detection, all contribute to the unresolved questions regarding dinoflagellate biology and ecology (Bravo and Figueroa, 2013; Janouškovec et al., 2017; Gornik et al., 2019).

Dinoflagellate lifecycles are complex and are poorly understood in many taxa, so it is vital to understand these life history traits in order to understand their ecology. More than 10 % of known marine dinoflagellate species produce cysts as part of their life cycle. Cysts can remain dormant in the sediment until conditions favour vegetative growth in the water column (Bravo and Figueroa, 2013; Zonneveld and Pospelova, 2015). Three well-known genera of TD are *Alexandrium* (producer of saxitoxins; paralytic shellfish poisoning), *Gambierdiscus* (producer of ciguatoxins; ciguatera poisoning) and *Karenia* (producer of brevetoxins; neurotoxic shellfish poisoning and red tides). Some *Alexandrium* species

have a cyst stage that germinates into zygote cells, which then divide into gametes. It is unknown if *Gambierdiscus* species have a cyst stage, whereas some species of *Karenia*, such as *K. brevis* found in the Gulf of Mexico, is known to lack a cyst stage (Steidinger, 2009). *K. brevis* reproduces through binary fission (single parent cell dividing into identical daughter cells) and continues to grow (bloom) and produce toxins until conditions are unfavourable (Steidinger, 2009; Persson et al., 2013). While *Alexandrium* blooms in high numbers within the water column, *Gambierdiscus* attaches to macro-algal hosts (referred to as benthic toxic dinoflagellates, or BTDs) and can produce harmful toxins even at low abundance. The complexity, unknowns and diversity of dinoflagellate life cycles, coupled with the fact that some toxic species can be harmful without blooming or reaching high abundance, highlights the importance of considering various habitat substrates within an ecosystem when studying these organisms.

Like most HABs, TD growth is influenced by numerous natural factors such as upwelling, circulation patterns and river dynamics, as well as anthropogenic pressures such as heightened eutrophication and intensified destructive weather events. Reef degradation, often a consequence of such disturbances, can reduce the populations of herbivorous fish that help control algal growth. This fosters algal proliferation and in turn increases the abundance of epiphytic dinoflagellates (Sellner et al., 2003; Gobler, 2020). Notably, within the context of climate change, visible trends indicate a rise in TD occurrences; however, this surge is paralleled by an increased interest in such research and the development of innovative detection techniques (Sellner et al., 2003). Therefore, although there is evidence to show increased and proliferated TDs because of anthropogenic pressures, the overall assumption of this increase in the light of climate change remains uncertain and requires further research.

One location that has been affected by HABs, including TDs, is Hervey Bay (Holmes et al., 2021; Milham-Scott, 2021), which is situated on the east coast of Queensland, Australia (Fig. 1). Hervey Bay is an ecologically unique and diverse ecosystem (Bengtson Nash et al., 2005), providing a sheltered stop-over point for migrating humpback whales that are nurturing calves and has extensive seagrass meadows that provide the ideal feeding grounds for large dugong and turtle populations (Campbell and Mckenzie, 2001; Holmes et al., 2021). In addition, Hervey Bay is an important Ballot's saucer scallop (*Ylistrum balloti*) harvesting area. Although, in recent years there has been a decline in scallop numbers forcing a closure of the fishery in 2021 (State of Queensland, 2020). The economy and ecology of Hervey Bay are intertwined, as a healthy marine ecosystem is crucial for local recreational and commercial fisheries, along with its tourism industry (Milham-Scott, 2021). Hervey Bay lies on the southern border of the Great Barrier Reef Marine Park (GBRMPA) (Butler et al., 2013) and the reefs are subject to terrestrial runoff and sedimentation from the Mary and Burrum rivers. Such anthropogenic pressures, coupled with increased flooding and cyclone events have decreased water quality significantly within the bay (Milham-Scott, 2021). These factors have led to the destruction of 1000 km^2 of seagrass and triggered HABs, posing risks for both wildlife and human health (Butler et al., 2013; Milham-Scott, 2021). For example, Hervey Bay is the only known location on the east coast of Australia where ciguatera poisoning, caused by species from the BTD genus *Gambierdiscus*, has been repeatedly reported, prompting strict fishing regulations within the bay for certain fish species (Holmes et al., 2021). Additionally, large mixed algal blooms have resulted in beach closures due to their potential to cause skin and respiratory symptoms in humans (Wolanski, 2014; Milham-Scott, 2021). Despite these impacts, little research has been conducted on dinoflagellate communities in Hervey Bay. Therefore, revealing their diversity is crucial to fully understand their potential threat within the bay.

Conventional detection of TDs has relied on labour-intensive techniques and the expertise of highly skilled taxonomists for accurate identification. However, even with expertise of taxonomically skilled individuals, the morphological similarities among species present considerable challenges. Moreover, research efforts have predominantly been confined to narrow geographical scopes and small-scale sampling, further constraining our understanding of these organisms (Anderson, 2009; Medlin and Orozco, 2017). In recent years DNA metabarcoding has become a widely accepted method, enabling the detection of numerous species simultaneously, over a large spatial area and providing insights into community composition on targeted taxa (Harper et al., 2018; Pawlowski et al., 2022; Perkins et al., 2024). Numerous studies have employed metabarcoding to investigate the diversity and spatial distribution of TDs (Fu et al., 2021; Huang et al., 2021; Kobari et al., 2021). However, many studies primarily analyse water samples, which can lead to a misinterpretation of the true diversity of toxic dinoflagellates (TDs) in a given location. This is problematic because many TDs are benthic or epiphytic, residing on substrates such as macro-algae or sediment, or exist in cyst stages that are not easily detectable in water samples. As a result, focusing solely on water samples can result in the non-detection of some species, leading to an incomplete understanding of their diversity and distribution.

Therefore, to fill these knowledge gaps, this study aims to investigate the diversity and abundance of dinoflagellates in Hervey Bay by utilising multiple sample substrates (water, macro-algae and sand) collected from three distinct locations on the western coastline of Hervey Bay. These locations include Dayman Point, Piabla Beach and Burrum Heads, which represent diverse habitats such as sand bars, rocky shores, seagrass meadows, river mouth and intertidal corals. Additionally, this study aims to identify toxic dinoflagellate species present in these locations and examine if changes in environmental conditions influence their distribution and abundance across these substrates and locations.

2. Methods

2.1. Sample collection and processing

Samples were collected from water, macro-algae, and sand at three different locations on the western coastline of Hervey Bay, Queensland, Australia (Fig. 1). The choice of these sampling stations was based on their distinct benthic habitat types and ecological significance, as classified by the Queensland Government's habitat maps and zoning plans (The State of Queensland, 2023). Dayman Point (25°17′08.9″S, 152°54′27.4″E) is located at the entrance to the Great Sandy Straight and is characterised by sand bars and subtidal seagrass meadows, Piabla Beach (25°16′39.1″S, 152°50′27.8″E) has rocky shores and intertidal corals, and Burrum Heads (25°11′00.9″S, 152°36′57.2″E) is located the mouth of Burrum River which has intertidal and subtidal seagrass meadows. All samples were collected in coastal waters at approximately 2 m depth during low tide, ensuring that the sites were always submerged, rather than exposed during tidal fluctuations, and were representative of shallow coastal environments rather than deeper offshore waters.

Different methods were used to collect samples for each substrate from Dayman Point, Piabla Beach and Burrum Heads. To target planktonic dinoflagellates, water samples were collected first so that the macro-algae and sand were not disturbed. Five replicates (approximately 50 m apart) of 300 mL of seawater were collected and mixed with 100 mL Longmire preservation buffer (Williams et al., 2016; Edmunds and Burrows, 2020). Next, to target benthic-epiphytic dinoflagellates, five replicate samples of 500 g of mixed, submerged, macro-algae were detached from benthic substrates (e.g. rocks and corals) and collected in zip lock bags (36 × 48 cm) from 1 to 2 m depth. Individual macro-algal species were not specifically targeted; instead, mixed samples were collected from various locations to avoid introducing bias into the study. The bags were then closed and the contents shaken vigorously to dislodge any dinoflagellates from their algal hosts. The water contained within the bag was filtered through 150 μm mesh to remove larger macroalgae (Smith et al., 2017). Each of the five replicates contained
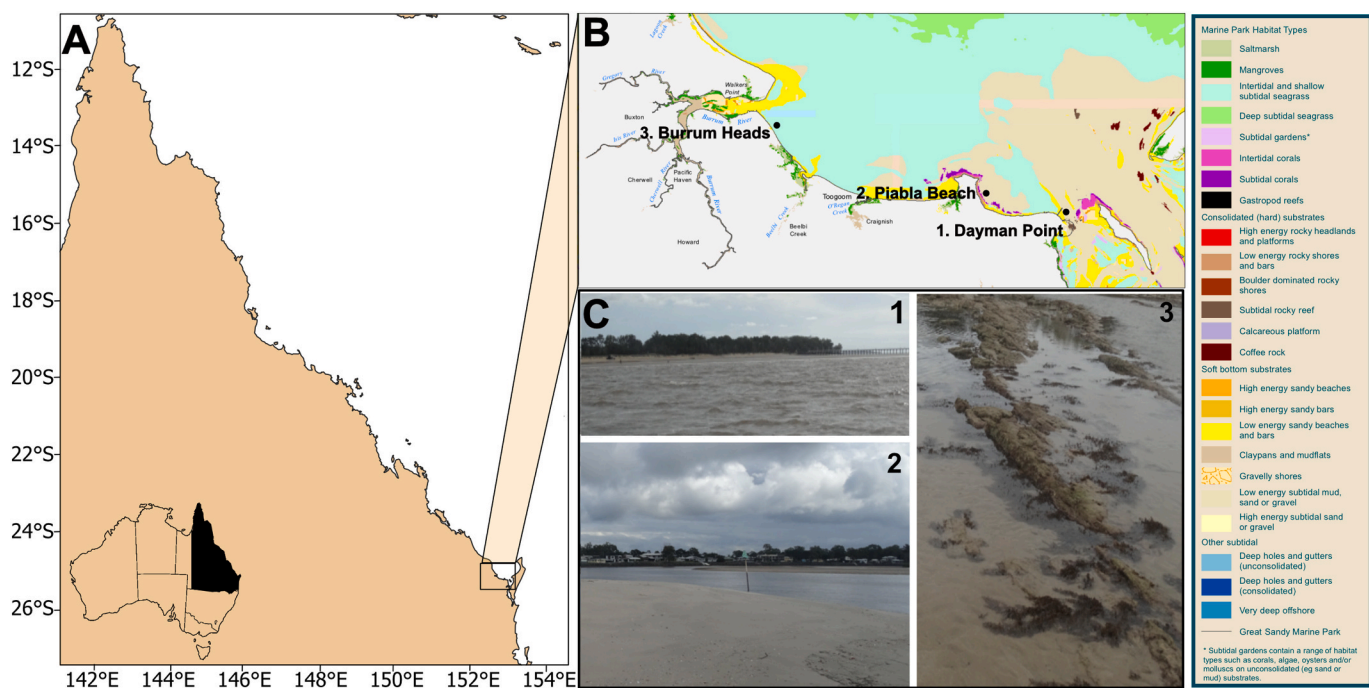
Fig. 1. A) Sampling sites in Hervey Bay, Queensland, Australia. B) The black dots represent the three sampling locations, 1) Dayman Point, 2) Piabla Beach and 3. Burrum Heads, with the Marine Park habitat types indicated in the panel on the right. C) Images from the three sampling locations.

300 mL of filtered micro-algal samples and added to 100 mL of Longmire buffer. In addition, one field blank per site and substrate was collected, consisting of 300 mL MilliQ water and 100 mL Longmire's preservation buffer. All samples were stored at room temperature prior to DNA extraction. This preservation method has been demonstrated to keep DNA stable for up to three months at tropical ambient temperature of 26.3 °C (Cooper et al., 2022). Finally, to target dinoflagellates that are benthic or in cyst form, 1 to 2 g (approximately 2 mL) of sand samples were collected from the top 2 cm of substrate, directly beneath the water and algae samples, using a sterilised spoon. Five replicates were taken per site and stored in a 50 mL tube containing 10 mL of DESS preservation which is recommended for preserving sediment DNA (Pawlowski et al., 2022). A negative control for each site was taken using artificial sand that had been bleached, washed, dried and UV sterilised. The samples were immediately chilled and transported back to the laboratory for extraction. Water temperature, salinity, pH, dissolved oxygen and conductivity levels were recorded at each site using a multiparameter water quality probe (Supp. Table 1). Five measurements were taken for each parameter at each site to represent values for each field replicate, and the average of these values was used for analysis.

2.2. DNA extraction, PCR amplification, and high-throughput sequencing

All DNA extractions were conducted in the Environmental DNA (eDNA) laboratory at James Cook University, Townsville, Australia. The preserve, precipitate, lyse, precipitate, purify (PPLPP) method was used to extract DNA from 100 mL of water and filtered macro-algae samples (Edmunds and Burrows, 2020). The MuDNA extraction protocol by Sellers et al. (2018) was used to extract DNA from sand samples subsequent to the removal of the DESS buffer. Two DNA extraction controls were also processed for each batch in addition to the samples and field controls. All DNA extracts were purified after extraction to remove environmental inhibitors using the DNeasy PowerClean Pro Cleanup Kit (Qiagen®).

The V8-V9 region of the 18S rRNA gene was amplified by Polymerase Chain Reaction (PCR) using Alveolata-specific primers (394 bp):

18SV8FAlveolata 5′-GCCCTTAGATGTTCTGGGCT-3′ and 18SV9RAlveolata3 5′-TGTTACGACTTCTCCTTCCTCTAAG-3′ (Funaki et al., 2022). The primers were modified to include Illumina Nextera overhang adapters (Illumina, San Diego, CA, USA) for downstream sequencing. PCR reactions were performed using a 25 μL reaction consisting of 11 μL SYBR Green Master Mix (ThermoFisher, AU), 7.7 μL of UltraPure H20, 1.65 μL of each primer (10 mM). Cycling conditions comprised an initial denaturation step at 94 °C for 2 min, followed by 40 cycles: 98 °C for 10 s, 58 °C for 30 s, 68 °C for 25 s, followed by a final extension step at 68 °C for 5 min. A custom gBlock was used as a positive control, containing the sequence of the dinoflagellate *Gambierdiscus holmesii* (found in Australia; Kretzschmar et al., 2019) with a 5 bp reverse compliment within the sequence to differentiate true detections from laboratory contamination (Sup. Fig. 1). Also, a negative control was used to test the specificity of the primer using a gBlock from *Chattonella marina*, which is a globally common HAB species from the superphylum Stramenopiles and should not be detected with the Alveolata-specific primer set (Supp. Fig. 2). In total, 61 samples including field samples, field blanks, extractions blanks, positive and negative controls and PCR blanks were amplified in triplicate giving a total of 183 samples. The library preparation and MiSeq 500 cycle sequencing was performed on an Illlumina MiSeq Platform (Illumina Inc. USA) at the Australian Genome Research Facility (AGRF).

2.3. Data processing and statistical analysis

The sequenced results were analysed using the DADA2 software package in R-studio (Callahan et al., 2017; R Core Team, 2018). Sequence processing was conducted with specific parameter settings, including a maximum allowed number of expected errors (maxEE) set to (2, 2), maximum number of ambiguous bases (maxN) set to 0, quality score truncation threshold (truncQ) set to 2, minimum sequence length (minLen) set to 150, removal of PhiX contaminant sequences (rm.phix) enabled, compression of output files (compress) enabled and single-threaded processing (multithread) set to FALSE. Sequences were then merged with an overlap of at least 12 bases, identical in the overlap

region and chimeric sequences were removed. The resulting unique sequences were then aligned with SILVA Release 132 SSU, targeting the region 18S rDNA V8-V9 region (Glöckner et al., 2017). Amplicon Sequence Variants (ASVs) with a total read count above 10 and a percent identity higher than 97 % were then kept for further analysis.

In order to visualise phylogenetic relationships and relative abundance (percentage of reads) of families within the orders belonging to the class Dinophyceae, a Newick tree was constructed in iTol (Letunic and Bork, 2007) following published Dinophyceae phylogenetic relationships (Takishita et al., 2003; Skovgaard et al., 2007; Jensen and Daugbjerg, 2009; Orr et al., 2012; Gong et al., 2017; Hoppenrath, 2017; Janouškovec et al., 2017; Gornik et al., 2019; Tillmann et al., 2021). The relative abundance of families present in each order was calculated using the package 'Tidyverse' in R-Studio. All subsequent analyses were performed in R-Studio (R Core Team, 2018; H. Wickham et al., 2019).

The 'VennDiagram' package was used to visualise the number of dinoflagellate genera present within each substrate and location (Chen and Boutros, 2011). In order to evaluate diversity measures we first standardised the data by aggregating the information across sites, biological replicates, and PCR replicates using the 'dplyr' package (Sola et al., 2022). We then performed a standardised function to calculate the minimum reads within the group, which were then standardised by dividing each value by the minimum reads. Using the 'Vegan' package (Dixon, 2003), Shannon diversity was calculated for each combination of site, substrate, biological replicate and PCR replicate. Additionally, the data was organised based on location and substrate, and mean diversity and abundance were summarised. Again, using the 'vegan' package (Dixon, 2003), a Principal Component Analysis (PCA) was performed using the standardised reads, to investigate patterns and relationships in dinoflagellate diversity across geographical locations and substrates. Subsequently, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) to assess the influence of geographical location and substrate type on the observed variations in the PCA results.

Next, using information from IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (HAIS-UNESCO, 2022) and the U.S. National Office for Harmful Algal Blooms (U.S. National Office for Harmful Algal Blooms, 2024) we identified any toxic dinoflagellates and developed a table with relevant information on each. A bubble plot was then used to visualise harmful dinoflagellates relative abundance across locations and substrates using 'ggplot2'package (Wickham, 2014). Finally, a correlation analyses between species and environmental variables, as well as among pairs of environmental variables, were performed using base R functions for correlation calculations. Pearson's correlation coefficient was performed to assess the strength and direction of these relationships, focusing on *p*-values less than 0.05 to determine statistical significance. Subsequently, the scale function was applied to standardise correlation values within the range (−1, 1). Visualisation of the correlation results was achieved through the creation of a heatmap-like plot using the 'ggplot2' package (Wickham, 2014), offering an overview of relationships between taxa and environmental parameters within the dataset.

3. Results

3.1. Dinoflagellate composition and relative abundance in Hervey Bay

A total of 8,135,831 raw sequence reads were obtained from samples collected from the three sampling locations and three different substrates in Hervey Bay, Queensland, Australia. After data processing using the DADA2 pipeline (Callahan et al., 2017), 2,440,980 reads passed quality control thresholds (Supp. Table 2). Among these were 1,328,387 reads across 1341 ASVs belonging to the superphylum Alveolata. All ASVs belonging to the phylum Dinoflagellata (the focus of this study) were retained, leaving 864,363 reads across 806 ASVs. The class Dinophyceae dominated the read count with 97 % (839,227) of the

total dinoflagellate reads across 790 ASVs, with the rest of the ASVs belonging to the class Bacillariophyceae (Supp. Fig. 3). Within the class Dinophyceae there are 29 families across 8 orders (Fig. 2), with the most abundant orders being Gymnodiniales (55 %), Peridiniales (13.4 %) and Gonyaulacales (9.31 %). It is important to note that the relative abundance presented here refers to percentage of the read count rather than actual read count or absolute cell abundance.

3.2. Diversity and distribution of dinoflagellates in sample substrates and locations

A total of 66 distinct genera of dinoflagellates were detected. Of these, 31 genera were detected across all substrates and locations (Fig. 3). The macro-algae substrate contained 14 unique genera (e.g. *Gambierdiscus*, *Nusuttodinium* and *Sinophysis*) whereas three genera (*Abedinium*, *Lessardia* and *Oodinium*) were only found in water samples and only one genus (*Galeidinium*), was identified in the sand substrate (Supp. Table 1). There were 12 genera shared between water and macro-algae and only five genera were identified in both algae and sand (Fig. 3). Four genera were unique to Dayman Point: *Amphidinium*, *Galeidinium*, *Gyrodiniellum*, and *Azadinium* (Supp. Table 2). Piabla Beach also had four unique genera: *Sinophysis*, *Nusuttodinium*, *Diplosalis* and *Thecadinium* (Supp. Table 2). Whereas Burrum Heads had six unique genera: *Gambierdiscus*, *Oodinium*, *Abedinium*, *Lessardia*, *Roscoffia*, and *Podolampas* (Supp. Table 2). There were nine genera shared between Dayman Point and Piabla Beach, 11 genera shared between Dayman Point and Burrum Heads and one genus (*Protodinium*) common only to Piabla Beach and Burrum Heads (Fig. 3; Supp. Table 2).

Burrum heads exhibited similar diversity levels in water and algae substrates among the five biological replicates. The facets representing these substrates were closely grouped, indicating minimal variance in diversity among replicates. In contrast, the sand samples from Burrum heads displayed a significant degree of diversity variance among biological replicates, with substantial facets highlighting greater variation within PCR replicates. This pattern was similar within the sampling location Piabla beach, except there was more variability in diversity within algae samples. These patterns are less obvious within the sampling location Dayman point, with greater variation in diversity within biological and PCR replicates across all three substrates (Fig. 4).

By exploring the variation in the data, most sampling points clustered near zero on the PCA, indicating similar but little variation between each site and substrate, but notable exceptions were observed. At Piabla Beach, variations were observed in the sand and algae substrates. Specifically, the Piabla Beach (algae) is positioned at the far end of PC1 (x-axis), indicating a significant distinction in that principal component, while Piabla Beach (sand) shows elevated values along PC2 (y-axis), reflecting unique characteristics in the second principal component. The Permutational Multivariate Analysis of Variance (PERMANOVA) results revealed significant differences attributed to both location (F = 61.42, *p* = 0.001) and substrate (F = 227.63, p = 0.001), indicating that these factors significantly contribute to the variation observed in the data. Although significant, only 1.57 % (R2 = 0.0157) of the total variability is explained by differences in location and 5.82 % (R2 = 0.058) of the variability is explained by the substrates. The residual variation was substantial (R2 = 0.93), suggesting that factors beyond location and substrate also play a role in the overall variability (Fig. 5).

3.3. Toxic dinoflagellates in Hervey Bay

Out of the 806 ASVs belonging to the phylum Dinoflagellata, only 36 ASVs were known TDs, representing 15 separate species. Seven of the 15 TDs had a single ASV representing each species, whereas the other eight species had multiple ASVs representing each species potentially representing higher genetic diversity or representing cryptic diversity among the species (Table 1). The 15 species were mainly distributed across two locations, Burrum Heads and Dayman Point, with only *Karenia bidigitata*
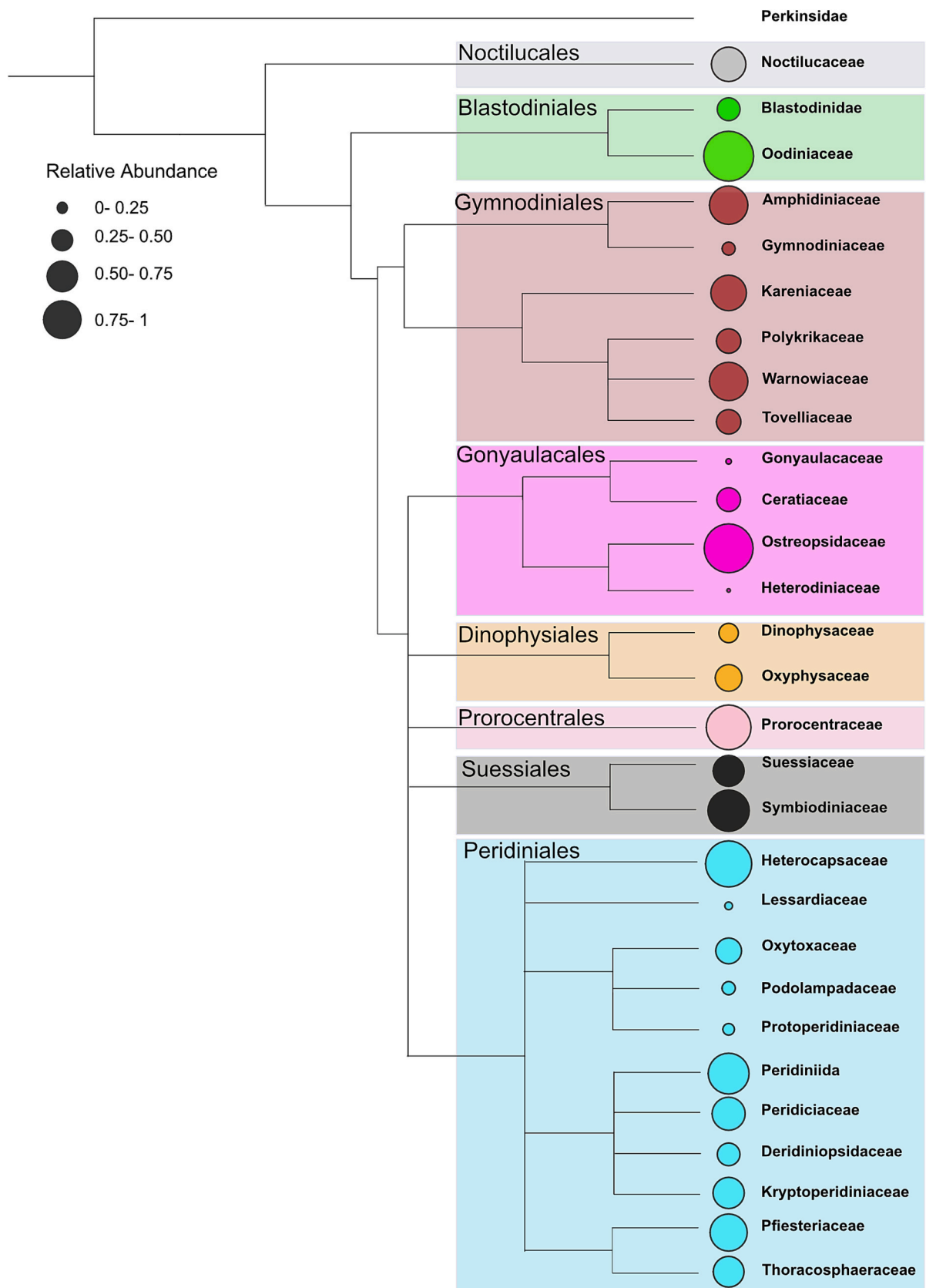
Fig. 2. A Newick tree and bubble plot representing broad phylogenetic relationships and relative abundance of the 8 orders and 29 families within the major dinoflagellate class Dinophyceae sequenced in this study. The orders are labelled at the top left corner of each coloured box and each leaf size represents the relative abundance (percentage of reads) of each family within the order it belongs. The order Perkinsidae was used as an outgroup.
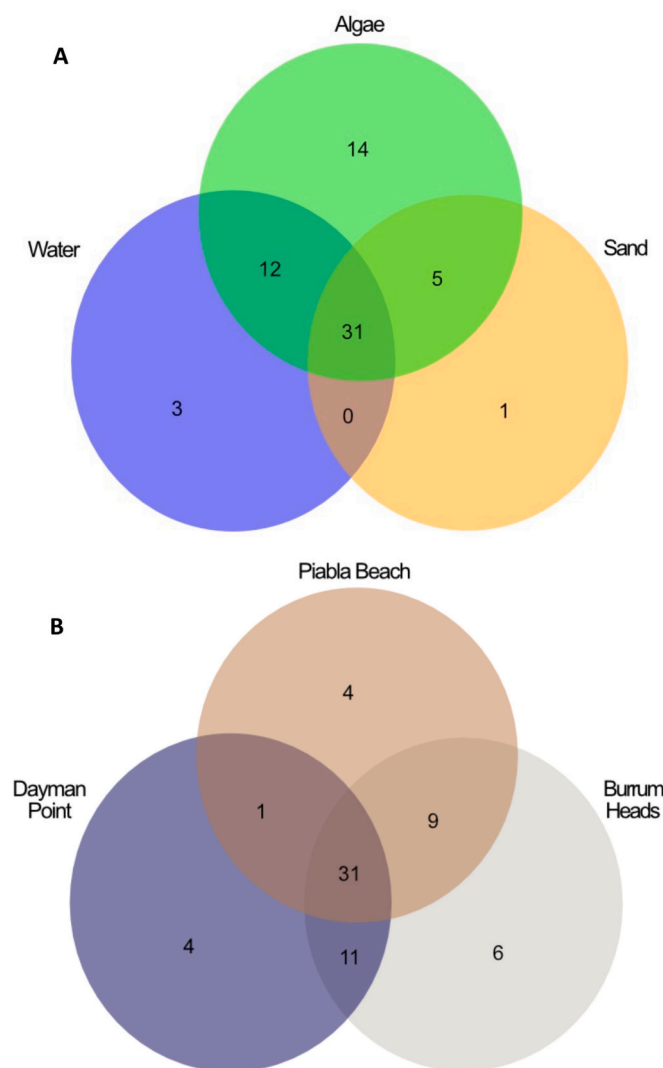
Fig. 3. Venn diagrams showing the 66 genera of dinoflagellates detected within different substrates (A) and locations (B) in Hervey Bay, Queensland, Australia.

and *Prorocentrum lima* detected in Piabla Beach. None of the species were found across all samples, however *Karenia bidigitata* was the most abundant species and was the only species to be found within each location and detected in each of the substrates. All the species except for *Alexandrium pseudogoniaulax* were detected in the macro-algal substrate and only four species were detected in the sand, *Karenia bidigitata*, *Gonyaulax spinfera*, *Amphidome languida* and *Akashiwo sanguinea* (Fig. 6).

3.4. Environmental correlations with toxic dinoflagellates in Hervey Bay

Among the toxic dinoflagellate species, *Akashiwo sanguinea* and *Prorocentrum lima* showed a particularly strong negative correlation with conductivity. *Akashiwo sanguinea*, *Alexandrium affine* and *Alexandrium lustitancium* showed the strongest positive correlation with dissolved oxygen. *Prorocentrum lima* showed the strongest positive correlation with water temperature. *Akashiwo sanguinea*, *Alexandrium affine* and *Alexandrium lusitanicum* demonstrated positive correlations with pH, suggesting their potential for proliferation in eutrophic conditions.

In terms of the environmental parameters, conductivity and temperature exhibited a strong negative correlation, which is consistent with the well-documented relationship in aquatic systems, where increasing temperature tends to lower water conductivity due to

changes in ion solubility (Nahhal et al., 2021). As expected, salinity displayed a strong positive correlation with conductivity, since salinity is derived from conductivity and both reflect ion concentration in the water (Vineis et al., 2011). Additionally, both pH and salinity demonstrated strong negative correlations with temperature, a trend often observed in aquatic environments where rising temperatures lead to decreased pH levels (Gobler, 2020; Garcia-Soto et al., 2021). Moreover, salinity, in conjunction with conductivity, exhibited a positive correlation with pH, reflecting the complex interplay between these parameters in aquatic marine ecosystems (Fig. 7). While these relationships are not the primary focus of the study, they highlight how certain environmental parameters correlate and their importance for dinoflagellate growth should be investigated.

4. Discussion

4.1. Dinoflagellate composition in Hervey Bay

Our study reveals a rich diversity of dinoflagellates within Hervey Bay, Queensland, Australia, shedding light on their distribution across various substrates and locations. Through comprehensive metabarcoding analysis, we identified a total of 66 distinct genera of dinoflagellates, across 12 families and eight orders, showcasing the complexity of these microorganisms within the bay's ecosystem.

Sampling across different substrates proved crucial in capturing the full spectrum of dinoflagellate diversity. Although approximately half off all genera were found across all substrates, the other half of the genera were detected in either a combination of two substrates or found in only one. Notably, our findings highlight the exclusive presence of *Galeidinium rugatum* in sand substrates. *G. rugatum* was first described in 2005 from Palau and their life cycle consists of a sand-dwelling, nonmotile phase, with a short (1 h) motile stage (Tamura et al., 2005). Additionally, the genus *Oodinium*, a parasitic dinoflagellate that infects fish and starts its life cycle in the water column until it finds a suitable host fish (Gómez and Skovgaard, 2015), was detected only in the water samples, consistent with the pre-parasitic stage. Furthermore, *Gambierdiscus* was detected only on the macro-algal samples which is consistent with its epiphytical life-cycle (Kohli et al., 2015; Bravo et al., 2019).

The spatial variation in dinoflagellate community structures across different locations in Hervey Bay underscores the significant influence of environmental and habitat differences in shaping these communities. For instance, the distinct assemblages observed at Burrum Heads and Piabla Beach suggest that site-specific conditions are critical determinants of dinoflagellate distribution. Burrum Heads site is directly next to the mouth of the Burrum river, with major changes and influences throughout the year due to variable rainfall affecting nutrient input (Bengtson Nash et al., 2005; Butler et al., 2013). Previous studies in estuarine environments show how changes in environmental conditions effect the diversity and abundance of dinoflagellates (da Silva Nunes et al., 2023; María Trigueros and Orive, 2001; Millette et al., 2021; Nwe et al., 2021).

Similarly, the unique genera detected at Piabla Beach likely reflect the distinctive environmental conditions of this location, which may support the growth of dinoflagellate species that are absent at other sites. Piabla Beach is characterised by rocky shores and intertidal corals which is distinct from the other two site habitats. Coral reefs have been shown to have distinct dinoflagellate communities, with many forming symbiotic relationships, parasitic or epiphytic, using corals and surrounding macroalgae as benthic structures (LaJeunesse, 2002; Apprill and Gates, 2007; Stat et al., 2008; Almazán-Becerril et al., 2015; Biessy et al., 2021). This is supported by our detection of genera such as *Sinophysis* that are known benthic inhabitants of coral reef ecosystems (Almazán-Becerril et al., 2015).
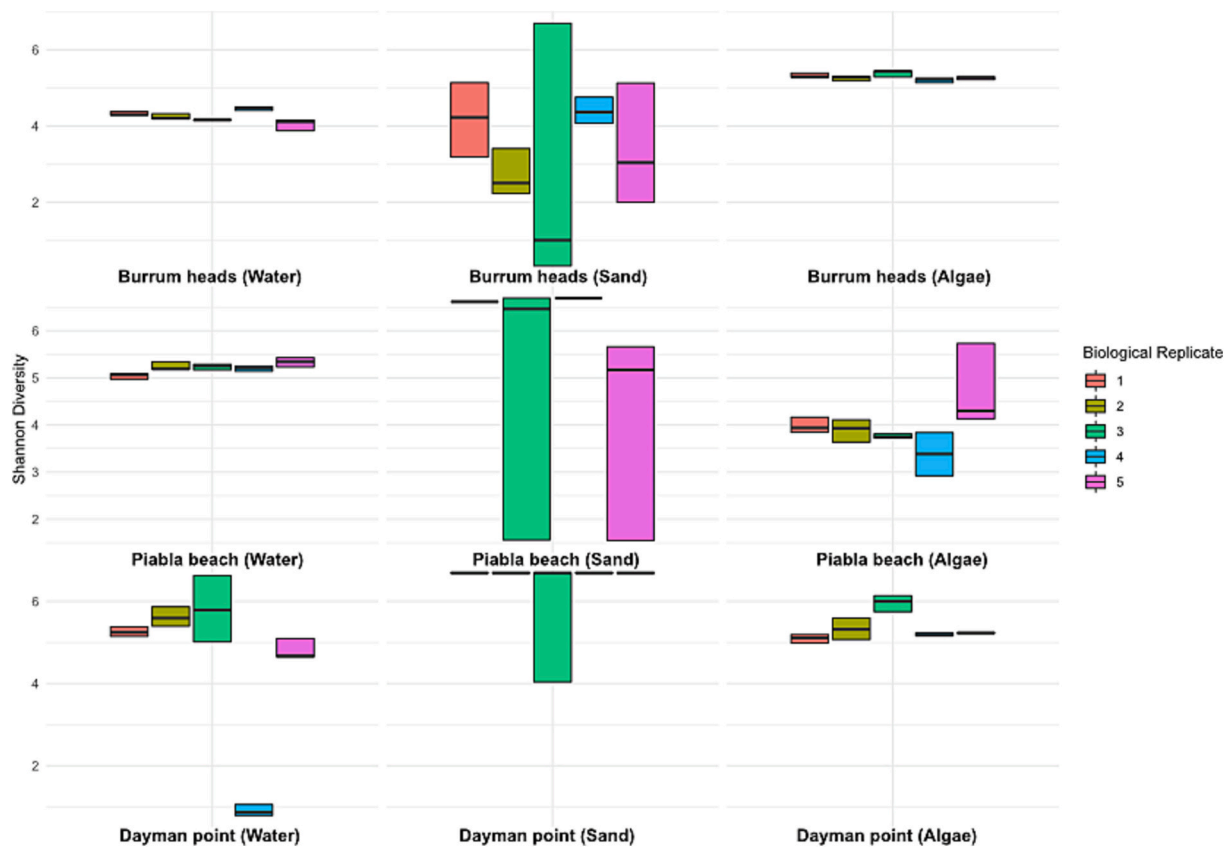
Fig. 4. Boxplots showing the Shannon diversity index for each combination of location and substrate, with separate facets for biological replicates and the variance in each facet represented by the differences in diversity measures between PCR replicates.
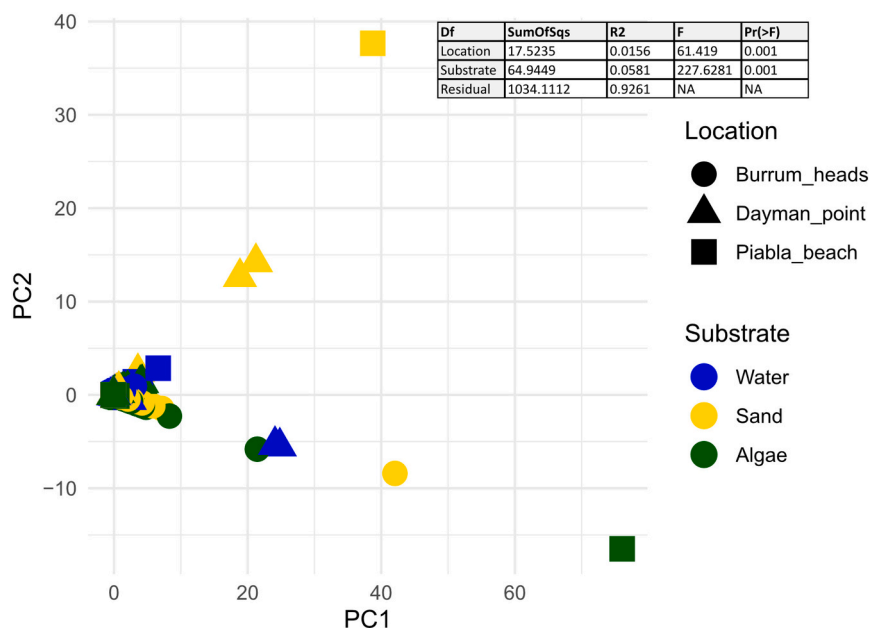

Df	SumOfSqs	R2	F	Pr(>F)
Location	17.5235	0.0156	61.419	0.001
Substrate	64.9449	0.0581	227.6281	0.001
Residual	1034.1112	0.9261	NA	NA

Fig. 5. The PCA plot displays dinoflagellate community diversity across substrates and locations. Points represent unique sampling sites, coloured by substrate and shaped by location. PC1 and PC2 capture variance. PERMANOVA highlight significant influences on community composition.

4.2. Diversity and distribution of dinoflagellates in Hervey Bay

The variability in alpha diversity across different substrates and locations, further indicates that not only the type of substrate, but also the local environmental conditions at each site contribute to the observed diversity patterns. For example, Burrum Heads exhibited consistent diversity levels in water and macro-algae substrates among biological replicates, suggesting a more stable and uniform dinoflagellate community. This stability contrasts sharply with the significant variance in diversity observed in sand samples from both Burrum Heads and Piabla

Table 1

ASVs of 15 toxic harmful algal bloom (HAB) dinoflagellates species identified from Hervey Bay, Queensland, Australia based on 18S V8-V9 rRNA sequencing. In the instance where species had multiple ASV IDs, the first representative (ASV with the highest number of reads) has been listed for the table. The 'harmful effect' details the toxins, including acids and other chemicals they produce, which negatively impact humans and wildlife. Information was taken from IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae and the U.S. National Office for Harmful Algal Blooms. Information on first detections in Australia is to the best of our knowledge from exploring the literature and based on the Census of Australian Marine Dinoflagellates (McCarthy, 2013).

ASV_ID	ASV no.	Species	Harmful effect	Human impacts	Wildlife impacts	GenBank no.	ID%	Coverage %	First time detected in Australian waters
92	2	*Akashiwo sanguinea*	Mycosporine-like amino acids (MAAs)-Red tides	Suspected respiratory irritant	Shellfish and seabird mortality	AY421770	100	100	No
1024	1	*Alexandrium affine*	Saxitoxins (STX)-Paralytic shellfish Poisoning (PSP)	Human illness and fatality when contaminated shellfish is consumed	Non-known but accumulates in shellfish	JF906996	100	100	No
1959	1	*Alexandrium lusitanicum*[a] (Junior synonym of *Alexandrium minutum*)	Saxitoxins (STX)-Paralytic shellfish Poisoning (PSP)	Human illness and fatality when contaminated shellfish is consumed	Non-known but accumulates in shellfish	JF906999	99.28	100	No
2048	1	*Alexandrium andersonii*	Saxitoxins (STX)-Paralytic shellfish Poisoning (PSP)	Human illness and fatality when contaminated shellfish is consumed	Fish mortality	JF521620	100	100	Yes
2588	1	*Alexandrium pseudogoniaulax*[a]	Goniodomin A (GDA)-red tides	Human illness and fatality when contaminated shellfish is consumed	Non-known but accumulates in shellfish	JF521638	100	100	No
549	3	*Amphidoma languida*	Azaspiracids-Azaspiracid Shellfish Poisoning (AZP)	Human illness when contaminated shellfish is consumed	Non-known but accumulates in shellfish	KR362880	97.16	100	Yes
2576	1	*Azadinium spinosum*	Azaspiracids-Azaspiracid Shellfish Poisoning (AZP)	Human illness when contaminated shellfish is consumed	Non-known but accumulates in shellfish	JX559885	100	100	Yes
728	2	*Cochlodinium polykrikoides*	Allelopathic chemicals-Red tides	Non-known	Fish mortality	EU418959	99.64	100	No
9	6	*Gambierdiscus carpenteri*[a]	Maitotoxins (MTX) and possibly ciguatoxins (CTX)-Ciguatera poisoning	Human illness when contaminated seafood is consumed	Non-known but CTXs accumulates in fish and shellfish	EF202911	100	100	No
86	4	*Gonyaulax spinifera*	Yessotoxin (YTX)[b] - red tides	Non-known	Shellfish mortality	FR865625	99.28	100	No
28	6	*Karenia bidigitata* (=*K. bicuneiformis*)	Phycotoxins-Red tides and possibly brevetoxins	Non-known	Fish and invertebrate mortality	HM067002	100	100	No
680	3	*Karenia mikimotoi*	Gymnocin-A, Gymnocin-B, Hemolysinf. PUFA	Non-known	Fish and invertebrate mortality	KU314866	100	100	No
1033	1	*Karenia papilionacea*	Phycotoxins-Red tides and possibly brevetoxins	Non-known	Fish and invertebrate mortality	HM067005	100	100	No
213	3	*Noctiluca scintillans*	Accumulation of ammonium and reduction of dissolved oxygen-Red tides	Non-known	Fish, invertebrate and coral mortality	GELK01045336	99.28	100	No
1722	1	*Prorocentrum lima*	Okadaic acids, dinophysistoxins (DTX-1 and DTX-2), prorocentrolide and a Fast-Acting Toxin (FAT)-Diarrhetic Shellfish Poisoning (DSP)	Human illness	Shellfish mortality	EU196419	100	100	No

[a] Not enough evidence this species is responsible for human or wildlife impacts but species from the same genus are known to produce harmful toxins responsible for harmful effects.

[b] Yessotoxins (YTXs) were thought to be associated with diarrhetic shellfish poisoning (DSP) until recently. However, recent evidence suggests YTXs should be excluded from DSP toxin groups and research regarding their threat to humans in ongoing.
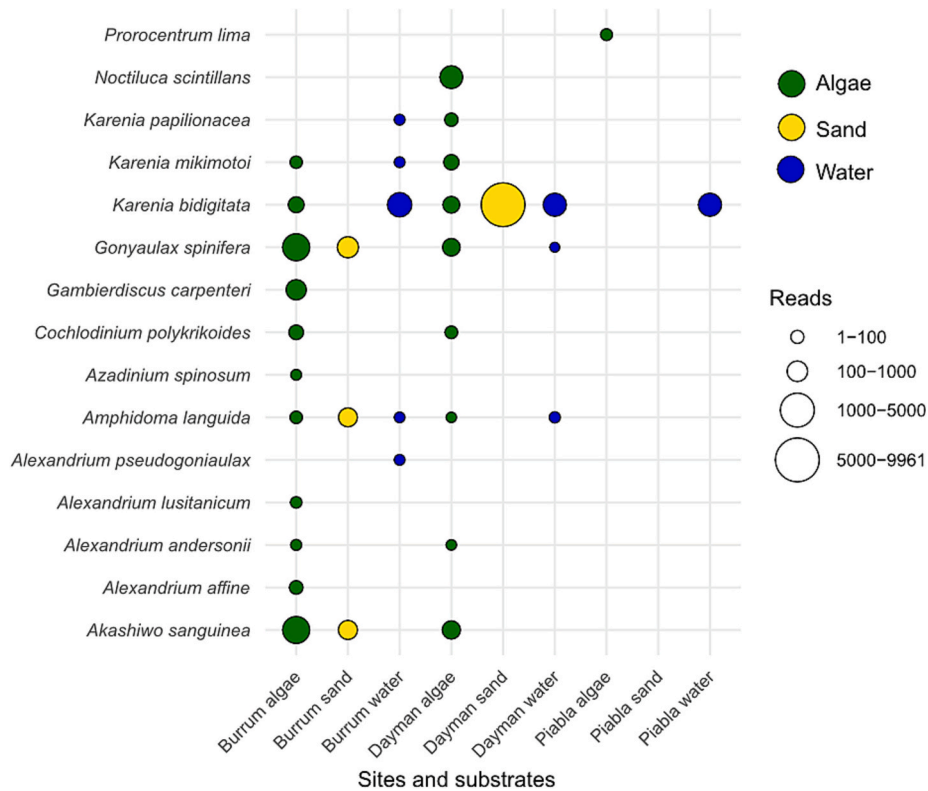
Fig. 6. A bubble plot showing toxic dinoflagellate abundance (total reads) across locations and substrates.
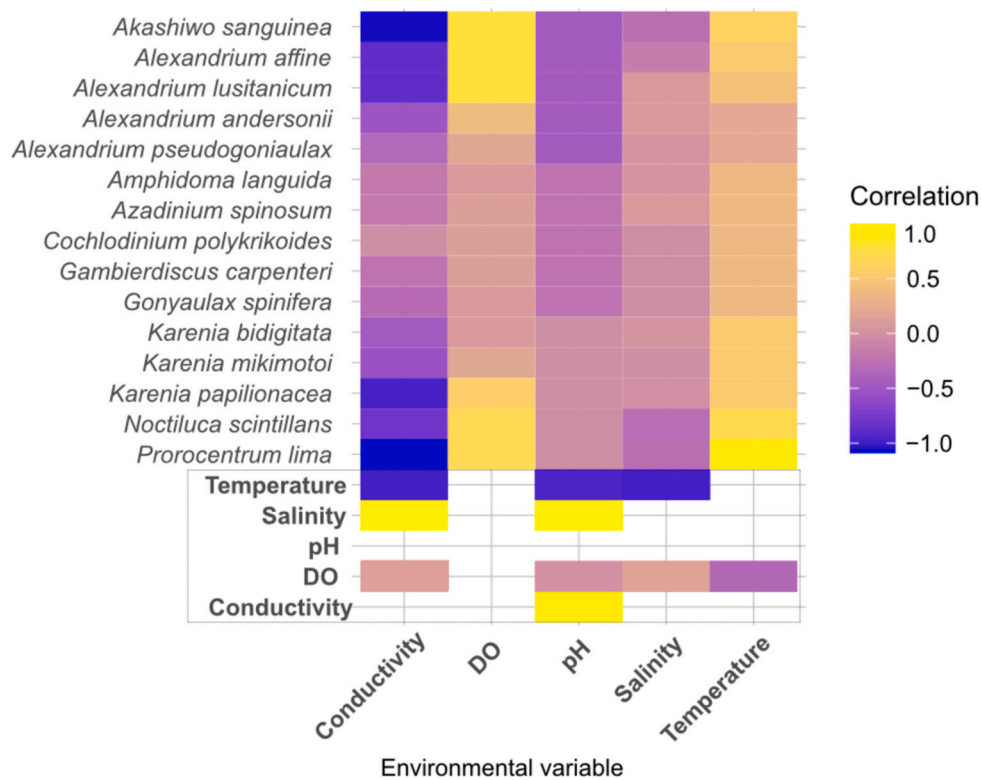

Fig. 7. Heat map showing correlations between the 15 toxic dinoflagellate species along with environmental parameters. Correlations are also indicated between the environmental parameters. Pearson's correlation coefficient was used to assess the strength and direction of these relationships, focusing on *p*-values less than 0.05. Yellow shows a positive correlation (1.0) and blue shows a negative correlation (−1.0). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Beach. It is possible that the diversity of dinoflagellates detected in the sand may be lower than expected for two reasons. First, only approximately 10 % of known dinoflagellates form cysts (Dale, 2001; Coyne and Craig Cary, 2005; Bravo and Figueroa, 2013), which means many species might not be present in the sediment samples in this dormant form. Second, the cysts that are present are more durable and can remain dormant for months or even years, potentially making them more resistant to breakdown during DNA extraction (Dale, 2001; Coyne and Craig Cary, 2005; Bravo and Figueroa, 2013). This resistance could result in lower DNA yields, thereby reducing the detection of dinoflagellate diversity in the samples.

The Principal Component Analysis (PCA) and Permutational Multivariate Analysis of Variance (PERMANOVA) further underscore the influence of both substrate and location on dinoflagellate community composition. Although both location and substrate significantly contributed to the variation in dinoflagellate communities, the large residual variation suggests that additional, unmeasured factors may also play a role in shaping these communities. This residual variation could be attributed to micro-scale environmental gradients, interactions with other microorganisms, or temporal variations in environmental conditions, such as seasonal changes in water temperature or nutrient availability (María Trigueros and Orive, 2001; Al-Azri et al., 2007; Yang et al., 2012; Nwe et al., 2021; Piontkovski et al., 2021; Ramilo et al., 2021). For instance, the notable variations observed at Piabla Beach in both algae and sand samples, with algae positioned at the far end of PC1 and sand along PC2, highlight the complexity of environmental interactions at this location and the need for further investigation into the specific factors driving these differences.

4.3. Toxic dinoflagellates in Hervey Bay

This study is the first to investigate and detect toxic dinoflagellates in Hervey Bay, uncovering 15 species, some of which were recorded in Australian waters for the first time. Notably, species from the genera *Alexandrium*, *Azadinium*, and *Amphidoma* were among those newly detected in Australia, underscoring the threat these organisms pose to local marine ecosystems, human health and fisheries. Their presence highlights the importance of ongoing monitoring and research to better understand and mitigate the risks associated with toxic dinoflagellates in Australia.

Alexandrium species are among the most significant TDs due to their severe health impacts. In our study, we detected four *Alexandrium* species: *A. affine*, *A. lusitanicum*, *A. andersonii*, and *A. pseudogonyaulax*. All four *Alexandrium* species were detected at the Burrum Heads site, which is situated at the river mouth. Previous studies have shown correlations between heavy rainfall and river runoff with the initiation and prolongation of toxic *Alexandrium* blooms (Weise et al., 2002; Anderson et al., 2005; Spatharis et al., 2007), indicating the vulnerability of this location to future *Alexandrium* blooms. The positive correlation between *A. affine* and *A. lusitanicum* with pH in our study aligns with the concept that eutrophic conditions, which often arise from nutrient inputs, can favour the growth of certain TDs (Bui et al., 2024; Lan et al., 2024). In addition, both *A. affine* and *A. lusitanicum* exhibited a negative correlation with dissolved oxygen (DO), which is often lower in river mouths due to increased organic matter from runoff (Garcia-Soto et al., 2021; Paerl, 2023). This further supports the idea that the conditions at Burrum Heads, characterised by nutrient influx and potentially reduced oxygen levels may create a favourable environment for these *Alexandrium* species to thrive.

Interestingly, three of these species (*A. affine*, *A. lusitanicum* and *A. andersonii*) were primarily found in macro-algae samples rather than in water or sand, despite *Alexandrium* not being typically considered epiphytic. This unexpected finding raises questions about their presence in macro-algae. A study by Biessy et al. (2021) explored diversity of eukaryotic organisms on benthic cyanobacterial mats (complex microbial assemblages comprising bacteria, micro and macro algae,

eukaryotic organisms and inorganic material) surrounding six islands of the South Pacific using DNA metabarcoding. They found two of the islands had high abundance of *Alexandrium* reads, showing their potential benthic/epiphytic nature. However, this data could not differentiate whether the *Alexandrium* detected were in the form of resting cysts or vegetative cells, highlighting the importance of sampling different substrates as the complexity and life cycles of many TDs are still poorly understood. By including macro-algae our sampling strategy, we were able to detect *Alexandrium* spp. where they might otherwise be missed.

In our study we detected three species from the genus *Karenia*: *K. bidigitata*, *K. mikimotoi* and *K. papilionacea*. *K. bidigitata* (=*K. bicuneiformis*) was most abundantly found across all locations and within all substrates out of the *Kerenia* spp. *K. bidigitata* (=*K. bicuneiformis*) has been to shown to produce brevetoxins, however there has been no harmful effects on marine mammals and humans reported to date (Ok et al., 2023, 2024). Nevertheless, the closely related *K. brevis* (known producer of harmful brevetoxins) regularly bloom in the gulf of Mexico, causing morality to manatees, sea turtles, dolphins and shellfish (Capper et al., 2013; Brammer-Robbins et al., 2022). Although there is no current evidence of such impacts in Hervey Bay, studies haven't specifically investigated this and there is a lack of data on potential brevetoxin-related mortalities. Given Hervey Bay's large population of dugongs, its role as a stopover for migrating humpback whales and its ecological importance for many other marine organisms (Milham-Scott, 2021; Marsh and Cleguer, 2024), this area warrants careful monitoring to detect any emerging threats from *Karenia* species.

Karenia papilionacea was detected in water samples from Burrum and macro-algae samples from Dayman. This species produces phycotoxins and is associated with red tides and possibly brevetoxins (not confirmed), leading to fish and invertebrate mortality. However, no human impacts have been reported. The phylogeny of *K. papilionacea*, like much of the *Karenia* genus has been subject to regular revision. In Japan, species described as *K. brevis* in previous studies are more similar to *K. papilionacea*, suggesting possible misidentification within the literature (Haywood et al., 2004; Brand et al., 2012; Kim et al., 2023).

Karenia mikimotoi was detected in both macro-algae and water samples from Burrum and Dayman sites. *K. mikimotoi* can adapt to various environmental conditions, enabling it to form lethal blooms (Li et al., 2019), potentially explaining the lack of environmental correlations within our results. There have been no fish kills directly attributed to *K. mikimotoi* in Hervey Bay, with only one report of fish kills in southeast Tasmania, Australia, in 1989 (Hallegraeff, 1992). However, the ability of these species to produce multiple toxins and form large, mixed species harmful blooms poses significant risks to marine ecosystems and human health. The complex and evolving taxonomy of *Karenia* spp. further complicates management efforts and highlights the necessity of accurately identifying and understanding these TDs.

Over the last few decades, blooms of *A. sanguinea* have increased in both geographical distribution and frequency (Tang and Gobler, 2015; Xu et al., 2017). Blooms have been reported in North and South America (Badylak et al., 2014; Jones et al., 2017; Sonia Sánchez et al., 2018), Ireland (O'Boyle and McDermott, 2014), Korea (Kang et al., 2021; Kim et al., 2024), Japan (Katano et al., 2011) and China (Yang et al., 2012; Luo et al., 2017; Chen et al., 2019). Recently, it has been established that *A. sanguinea* can produce resting cysts, which may facilitate its rapid global expansion (Tang and Gobler, 2015). Studies have shown that blooms of *A. sanguinea* have been associated with mortalities of shellfish, finfish and marine birds (Jessup et al., 2009; Xu et al., 2017). In our study, *A. sanguinea* was detected in both macro-algae and sand substrates at Burrum and Dayman Point, highlighting its adaptability to different environmental conditions. This finding aligns with other studies that have documented the ecological versatility and impact of this species in coastal regions (Badylak et al., 2014; Kang et al., 2021; Kim et al., 2024).

Two species associated with red tides which were detected in our

study are *Noctiluca scintillans* and *Cochlodinium polykrikoides*. *N. scintillans*, known for causing red tides in temperate, subtropical and tropical coastal waters globally, was detected only in macro-algae samples from Dayman Point. This species can attach to substrates using its tentacles during certain non-motile stages of its life cycle (Fukuda and Endoh, 2006), which might explain its presence on algal substrates rather than in adjacent water samples. Similarly, *C. polykrikoides*, recognised for its allelopathic effects and linked to numerous fish kills worldwide (Kim et al., 2000; Vargas-Montero et al., 2006; Imai and Kimura, 2008; Sakamoto et al., 2021), was found at a relatively low abundance in our study. No significant correlations with environmental parameters were observed for either species but their presence should be further monitored.

This study has identified two producers of azaspiracids: *Azadinium spinosum* and *Amphidoma languida*. *A. spinosum* was exclusively found in macroalgae samples collected from the Burrum River mouth, while *A. languida* was detected across various substrates and locations. Azaspiracids which cause Azaspiracid Shellfish Poisoning (AZP) have become widespread in regions including Europe, North Africa, New Zealand and eastern Canada, but there have been no previous reports from Australian waters (Twiner et al., 2008; Tillmann et al., 2012; Balci et al., 2023). Although Hervey Bay is not currently known for large-scale shellfish aquaculture, it is a scallop harvesting area. There has been evidence of multiple species of scallops accumulating azaspiracids (Braña Magdalena et al., 2003; López-Rivera et al., 2010; Ji et al., 2018; State of Queensland, 2020) and presents a danger to seafood consumers. Currently, there is a harvesting ban on scallops in Hervey Bay but if the fishery opens again, the presence of these species raises important considerations for further monitoring.

Only one species of *Gambierdiscus* was detected in our study; *G carpenteri*. *G. carpenteri* has not been definitively linked to ciguatoxin (CTX) production but is known to produce maitotoxins (MTXs), though their role in CP remains unclear (Kohli et al., 2014; Vacarizas et al., 2018). In our study, *G. carpenteri* was identified in algae at Burrum Heads, a site on the west coast of within Hervey Bay on the mouth of the Burrum river. The absence of *G. carpenteri* in other substrates, such as water or sand, is consistent with the typical epiphytic nature of *Gambierdiscus* species, which are often associated with benthic environments. Although *Gambierdiscus* spp. are not usually associated with non-coral sites at the mouth of the river, past studies have shown the adaptability of *G. carpenteri* into new environments along with their tolerance to changing environments (Kohli et al., 2014; Sparrow et al., 2017).

Hervey Bay, in particular Platypus Bay (East side of Hervey Bay), is the only known location on the east coast of Australia where ciguateric fishes have been repeatedly caught, particularly from Spanish mackerel (*Scomberomorus commerson*) and barracuda (*Sphyraena jello*) (Holmes et al., 2021). However, our sampling did not cover Platypus Bay, where fishing is restricted due to the prevalence of ciguatera (Sydney Fish Market, 2015). The detection of *G. carpenteri* at Burrum Heads, however, suggests the potential presence of *Gambierdiscus* species across various areas of Hervey Bay, including regions not typically associated with high ciguatera risk. Understanding the distribution and toxin profiles of *Gambierdiscus* species is essential for assessing the risks associated with CP. Further research is needed to elucidate the role of *G. carpenteri* in CP and to conduct additional monitoring across Hervey Bay, particularly in areas like Platypus Bay, to determine the presence and potential risks of other *Gambierdiscus* species.

Our detection of *P. lima*, known to be responsible for to Diarrhetic Shellfish Poisoning (DSP) in humans, was only detected in the macro-algae substrate which is consistent with the life cycle of the species, as it is considered a benthic toxic dinoflagellate that attaches to macro-algae (David et al., 2018). We only detected *P. lima* in Piabla Beach which is characterised by rocky benthos and intertidal corals. Again, this is consistent with their habitat distribution documented in previous studies (Lim et al., 2019; Moreira-González et al., 2019; Tarazona-Janampa et al., 2020). Additionally, *P. lima* demonstrates strong positive correlation with conductivity and a negative correlation with temperature in our study. This suggests that variations in environmental conditions may drive their proliferation. The negative correlation with temperature may indicate that rising temperatures could hinder *P. lima* growth, particularly since the water temperature in Hervey Bay was observed to range between 20 and 25 °C during our study. This aligns with findings from Aquino-Cruz et al. (2018), which showed that the growth rate of *P. lima* and its production of Diarrhetic Shellfish Poisoning (DSP) toxins (okadaic acid, OA; dinophysistoxin 1, DTX1) peak at similar temperatures. Their study indicated lower growth rates at extremes of temperature (5 and 30 °C). This has broader implications for regions that are not currently associated with toxic dinoflagellates such as *P. lima*; as ocean temperatures increase, these areas may become favourable to the proliferation of dinoflagellates and the production of toxins (Pan et al., 1999; D'ors et al., 2016; Aquino-Cruz et al., 2018; Paerl, 2023). While there are currently no studies directly assessing the effects of global warming on toxic dinoflagellates in Hervey Bay, this emphasises a critical knowledge gap that warrants further investigation. Given the significant impact of *P. lima* on human health and the shellfish industry, continuous monitoring of this species is crucial. Understanding its environmental triggers and distribution patterns will aid in predicting and managing future outbreaks, ultimately helping to protect both public health and the economy.

Gonyaulax spinifera (producer of yessotoxins; YTXs) was detected in two locations, Burrum and Dayman Point, across all substrate types examined: macro-algae, sand and water. The presence of *G. spinifera*, can pose significant risk to shellfish aquaculture. For example, in Autumn 2004, the production of several mussel farms along the Emilia-Romagna coasts (north-western Adriatic Sea) was suspended due to high YTX levels associated with the presence of *G. spinifera* (Riccardi et al., 2009). Additionally, similar occurrences have been reported in New Zealand, where *G. spinifera* has been tied to elevated YTX levels in shellfish (Rhodes et al., 2006). The detection of this species across multiple substrates within Hervey Bay highlights its widespread distribution and underscores the importance of ongoing monitoring efforts to mitigate the risks associated *G. spinifera*.

5. Implications for management, future research, and conclusions

5.1. Ecological and public health risks in Hervey Bay

The findings of this study underscore the critical need for enhanced management strategies and ongoing research to address the ecological and public health risks posed by TDs in Hervey Bay. As a historically important harvesting area for Ballot's saucer scallop, it is crucial to monitor TDs that impact shellfisheries, including *Azadinium spinosum* and *Amphidoma languida*, which are known to cause Azaspiracid Shellfish Poisoning (AZP), as well as *Alexandrium* spp., responsible for potentially lethal paralytic shellfish poisoning (PSP), and *Prorocentrum lima*, associated with diarrhetic shellfish poisoning (DSP). Although there has been a temporary closure of the scallop fishery due to declining numbers, the potential for future scallop harvesting or other shellfish aquaculture in the region necessitates continuous monitoring of these toxic taxa to protect public health and seafood safety.

Furthermore, dinoflagellates from the genus *Gambierdiscus* present additional risks in Hervey Bay. The presence of *Gambierdiscus* spp. in areas not typically associated with ciguatera poisoning (CP) raises concerns about potential hotspots for ciguatoxin accumulation in fish. Given that Hervey Bay is a popular destination for recreational fishing, an increase in CP cases could threaten seafood safety and negatively impact local economies dependent on fishing activities.

These dinoflagellates not only pose direct threats to human health through contaminated seafood but also have potential repercussions for local marine life. The detection of species from the genus *Karenia*, known to cause mortality in cetaceans, sirenians, and turtles in other

regions (Jessup et al., 2009; Steidinger, 2009; Capper et al., 2013; Fire et al., 2015), highlights a significant risk to local populations of whales, dugongs, dolphins and turtles. It is crucial to prioritise further research on the toxins produced by these harmful dinoflagellates and their effects on these ecologically and culturally significant species. By understanding these impacts, we can better inform management strategies aimed at preserving both marine biodiversity and public health in Hervey Bay.

5.2. Broader conclusions and future directions

DNA metabarcoding proved to be a highly effective method for detecting and mapping the distribution of various toxic dinoflagellates in Hervey Bay, enabling a comprehensive assessment of dinoflagellate diversity and abundance across different substrates and locations. This method provides significant advantages over traditional techniques, allowing for detailed insights into the presence and relative abundance of multiple species simultaneously.

However, it is important to note that our study provides only a snapshot of dinoflagellate diversity in Hervey Bay, as temporal sampling was not incorporated. Dinoflagellate populations can undergo significant changes due to seasonal variations and environmental shifts. Additionally, the abundance metrics presented in this study refer to sequencing reads rather than absolute cell counts, highlighting the need for future studies to investigate the correlation between read counts and actual cell abundance.

Future research should build on these findings by incorporating temporal sampling to capture the dynamics of dinoflagellate communities over time and providing a more comprehensive understanding of their ecological patterns and responses to changing environmental conditions. While metabarcoding offers valuable insights into community composition, targeted assays may be more effective for monitoring specific toxic dinoflagellates once their presence is detected, allowing for sensitive and precise assessments of immediate issues.

Furthermore, investigations into the ecological roles and life cycles of less understood dinoflagellate species, such as *Azadinium* spp. and *Noctiluca scintillans*, could be facilitated by the comprehensive data provided by metabarcoding. Expanding research to include areas like Platypus Bay, where CP risks are known, will be crucial in assessing the full extent of *Gambierdiscus* distribution. By integrating these approaches, we can enhance our ability to manage and mitigate the impacts of TDs, ultimately protecting both marine ecosystems and human health.

CRediT authorship contribution statement

Joseph C. Perkins: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kyall R. Zenger:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Shannon R. Kjeldsen:** Writing – review & editing, Resources, Methodology. **Yang Liu:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Jan M. Strugnell:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Investigation, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.117319.

Data availability

Data will be made available on request.

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