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Distribution and potential toxicity of benthic harmful dinoflagellates in waters of Florida Bay and the Florida Keys

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Abstract

Few studies have been carried out on benthic dinoflagellates along the Florida Keys, and little is known about their distribution or toxicity in Florida Bay. Here, the distribution and abundance of benthic dinoflagellates was explored in northern and eastern Florida Bay and along the bay and ocean sides of the Florida Keys. Isolates were brought into culture and their toxicity was tested with oyster larvae bioassays. Seven genera were detected, including *Prorocentrum*, *Coolia*, *Ostreopsis*, *Amphidinium*, *Gambierdiscus*, *Fukuyoa* (all included potentially toxic species) and *Sinophysis*. In general, distribution increased with water temperature and nutrient availability, especially that of phosphate. This study documented the first record of *Coolia santacroce* in the Florida Keys. Potential toxic effects of *Gambierdiscus caribaeus*, the abundance of which exceeded 1000 cells g^{-1} fw at some sites, were established using oyster larvae as a bioassay organism. These findings suggest a potential risk of ciguatera fish poisoning in this area.

Keywords

benthic HABs, Florida Bay, temperature, nutrients, *Gambierdiscus*, *Coolia santacroce*; bioassays

1. Introduction

Until recently, concerns about harmful algal blooms (HABs) in Florida were predominantly associated with the toxic dinoflagellate *Karenia brevis* that, while a naturally occurring species along Florida's Gulf Coast, may be increasing over time, and that can result in massive economic and environmental impacts (e.g., Brand and Compton, 2007). In 2017 to early 2019, the west coast of Florida experienced one of the largest and persistent such blooms in more than a decade, resulting in massive economic impacts and massive mortalities of sea turtles, manatees and fish (Glibert, 2019a). This planktonic dinoflagellate produces a group of potent natural neurotoxins, the brevetoxins, which can cause illness and mortalities in fish, seabirds and marine mammals, while humans are susceptible to those toxins by eating contaminated seafood or inhaling contaminated aerosols (Brovedani et al., 2016). In eastern Florida, from Lake Ocheechobee to the eastern lagoons and west through the Calooshachee River, blooms of the cyanobacterium *Microcystis aeruginosa* have also been increasing, with substantial blooms developing over the most recent summers (Rosen et al., 2017). The eastern lagoons have also recently experienced outbreaks of *Aureoumbra lagunensis*, the brown-tide pelagophyte (Gobler et al., 2013; Kang et al., 2015). Moving southward to Florida Bay, the main HAB problems have been related to picocyanobacteria blooms that have been associated with food web disruptions and declines in submerged aquatic vegetation (Berry et al., 2015; Glibert et al., 2004, 2009). Large picocyanobacterial blooms, dominated by *Synechococcus* sp. have periodically occurred in Florida Bay, including the summer of 2017. Other HABs in Florida have also been recognized, including *Pyrodinium* which has been recorded in the Indian lagoon of eastern Florida (Badylak et al., 2004; Landsberg et al., 2006; Phlips et al., 2006). However, in general, the prevalence of, or potential harm that can be caused by, benthic dinoflagellates in Florida has been less well appreciated.

Ciguatera Fish Poisoning (CFP) is one of the most relevant seafood-borne illnesses worldwide caused by the ingestion of fish species claimed to bioaccumulate lipophilic ciguatoxins in the food web (Friedman et al., 2017; Litaker et al., 2010; Núñez-Vázquez et al., 2018). Some benthic HABs

(BHABs) involved in ciguatera poisoning have been described from Florida (Friedman et al., 2017; Radke et al., 2015). Ciguatoxins, and other related biotoxins such as maitotoxins, are produced by benthic dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa,* generally recorded in association with other toxic benthic dinoflagellates such as *Prorocentrum*, *Ostreopsis*, *Coolia* and *Amphidinium* species (e.g. Berdalet et al., 2017; Berdalet and Tester, 2018; Parsons et al., 2012). Indeed, despite the number of studies on benthic dinoflagellate assemblages in tropical and subtropical areas, attention is often given almost exclusively, on species belonging to the genera *Gambierdiscus/Fukuyoa* and *Ostreopsis*, which are usually studied individually (e.g. Litaker et al., 2010; Nascimento et al., 2012; Nishimura et al., 2013; Rhodes et al., 2017; Rodríguez et al., 2017), while the whole benthic dinoflagellate community has been investigated only in some areas (e.g. (Berdalet and Tester, 2018; Boisnoir et al., 2019; Hoppenrath et al., 2013; Richlen and Lobel, 2011; Skinner et al., 2013; Yong et al., 2018).

Environmental factors, both abiotic and biotic, are known to influence several aspects of benthic HAB dinoflagellates, from their proliferation, toxin production and accumulation to their effects on environment and human health. Growth, distribution and abundance of CFP-associated dinoflagellates are largely temperature driven and expected to shift in response to climate induced changes as ocean temperatures rise (Kibler et al., 2015; Tester et al., 2010). Nevertheless, many studies have recently highlighted that other environmental factors besides temperature affect abundances of benthic dinoflagellates, including hydrodynamics, water depth, nutrient concentrations, substrate availability and allelochemical interactions (Accoroni et al., 2017a; Boisnoir et al., 2018; Larsson et al., 2019; Pichierri et al., 2017; Richlen and Lobel, 2011; Yong et al., 2018). In particular, nutrients and temperature commonly interact, impacting algal growth and community composition in different ways (Peñuelas et al., 2013), affecting the chemical and physical properties of aquatic systems (Sterner and Grover, 1998; Xia et al., 2016), in turn affecting physiological processes, including those of HABs (Berges et al., 2002; Glibert and Burford, 2017; Glibert, 2019b; Raven and Geider, 1988). Many studies have highlighted the important role of the availability of both inorganic and organic nutrient concentrations on the growth, abundances and distribution of benthic dinoflagellates (Accoroni et al., 2017b; Glibert et al., 2012; Parsons and Preskitt, 2007; Skinner et al., 2013).

Florida Bay is located between the southern end of the Florida mainland (i.e. the Florida Everglades) and the Florida Keys, USA. Florida Bay has witnessed many ecological changes in the past decades. Since the onset of industrialization in the 1880s, the health of the Florida Bay ecosystem has been negatively impacted on both decadal (e.g. increasing eutrophication) and centurial (e.g. changes in land use and water management practices within southern Florida) time-scales (Briceño and Boyer, 2010; Fourqurean and Robblee, 1999). Phytoplankton of Florida Bay are generally considered to be limited by availability of phosphorus (P) in the eastern region of the Bay and by nitrogen (N) in the western region, although different sources of N and P have been shown to stimulate different components of the algal and bacterial communities (Glibert et al. 2004). Agricultural and human development have been intensive in recent decades in southern Florida and the Florida Keys and have changed nutrient inputs to Florida Bay (Lapointe and Clark, 1992; Rudnick et al., 2005; Shangguan et al., 2017). As a warm, subtropical, shallow coastal lagoon with increasing nutrient enrichment, it is thought to be a prime area for the occurrence of, if not proliferation of, BHABs. Yet, in contrast to the number of studies conducted in Florida Bay with respect to the nutrient regime and planktonic microalgae, toxic benthic dinoflagellates have been comparatively understudied.

The aim of the present study was to assess the distribution, species abundance, and composition of potentially toxic benthic dinoflagellates along the Florida Keys and in Florida Bay related to different environmental factors, in an attempt to characterize conditions favoring their presence and abundances. Species were also isolated, brought into culture, identified based on molecular and morphological features, and their toxicity was tested with bioassay experiments with oyster larvae.

2. Materials and methods

2.1. Study area and sampling locations

Samples were collected in several distinct regions of Florida Bay: in eastern Florida Bay, in the enclosed sub-lagoons of the northern Bay at the southern edge of the Everglades, in the waters along the Bay side and enclosed canals of Florida Keys, and along the ocean side of Florida Keys (Fig. 1). Florida Bay is shallow (average depth 1.5 m), approximately 2200 km^2 in area, and connected to the Everglades wetland system at its northern boundary (Florida, USA). It has an open boundary with the Gulf of Mexico in the west, but the connection with the Atlantic Ocean on the eastern and southern boundaries is restricted to narrow tidal passes in the Florida Keys. The Bay has an average annual temperature of 25 °C with a mean monthly low temperature of 20 °C in January and a mean monthly high temperature of 28 °C in August (Fourqurean and Robblee, 1999), and two distinct meteorological seasons: a November–April dry season and a May–October rainy season (Duever et al., 1994). Riverine input in the northern boundary is considerable in the wet season, and the major freshwater input is through slow sheet flow from the Everglades watershed (Lee et al., 2007). Sample collection occurred in the eastern and northern edge of Florida Bay at 5 stations (Garfield Bight, Terrapin Bay, Duck Key, Big Key and Barnes Sound) (Fig. 1).

Along the northern border of Florida Bay, at the interface between the Everglades and the northern Florida Bay, there are a series of saline lakes. These lakes function as quasi-Intermittent Closed-Open Lakes and Lagoons (ICOLLs) (Frankovich et al., 2011; Maher et al., 2011; Shangguan et al., 2017; Suzuki et al., 1998) are connected to each other, and receive limited or even reversed flow during the dry season. They are considered to be particularly sensitive to changes in salinity and nutrients that are occurring, or may occur, due to ongoing Everglades restoration projects (Shangguan et al., 2017). There are two distinct interconnected sets, or chains, of these quasi-ICOLLs, each of which was previously characterized as poised at different stable states (*sensu* Scheffer and Carpenter, 2003). One ICOLL chain is eutrophic and in a planktonic-dominated state (West Lake, Long Lake), while the second ICOLL chain is more oligotrophic and in a benthic-dominated state (Seven Palm Lake, Middle Lake; based on the overall assessment of chlorophyll *a* (chl *a*) and submerged aquatic vegetation (SAV) (Bricker et al., 2008; Frankovich et al., 2011; Shangguan et al., 2017; USEPA, 2005). Both the eutrophic and oligotrophic ICOLLs were sampled (Fig. 1).

At the southern border of Florida Bay lies the Florida Keys. The Florida Keys supports a large seasonal population which grew nearly 200% from the 1970s, with many homes located on man-made canals. Along the southern border of the Florida Keys lies the Atlantic Ocean, but there are few inlets across the Keys and natural beaches are experiencing considerable erosion. Samples were collected at 11 sites along the bay-side edge of Florida Keys, residential canals, and at 4 ocean-side beaches (Fig. 1).

Hereafter these sites are designated as: FB (Florida Bay), ICOLLs (enclosed sub-lagoons), FBK (FB-side Florida Keys) and OK (ocean-side Florida Keys).

2.2. Sampling and sample treatment

Sampling occurred in April and in October 2014, i.e. at the end of the dry and wet seasons, respectively. The intent was to encompass both wet and dry seasons in one year. Surface temperature and salinity were measured at the time of sampling with a YSI Model 85 Handheld Dissolved Oxygen, Conductivity, Salinity and Temperature System (Yellow Spring, OH USA). Samples for BHAB dinoflagellates were collected from macroalgae and seagrass samples either by wading into shallow waters or by snorkeling. Where feasible, macrophytes were collected in replicate at approximately 0.5 m depth. Although the macroalgae varied from location to location, the most common genera collected included various rhodophytes, i.e. *Acanthophora spicifera* (M.Vahl) Børgesen, *Ceramium ciliatum* (J.Ellis) Ducluzeau, *Hypnea musciformis* (Wulfen) J.V.Lamouroux, *Palisada perforata* (Bory) K.W.Nam and *Polysiphonia* sp., and an ulvophyte, *Halimeda incrassata* (J.Ellis) J.V.Lamouroux. Seagrass samples were collected from beds of *Thalassia testudinum* K.D.Koenig, *Syringodium filiforme* Kützing and *Ruppia maritima* Linnaeus. Undisturbed samples were collected following the protocol recommended by Totti et al. (2010).

Briefly, in the field, the samples of macroalgae and seaweeds (*ca.* 10 g fresh weight) were vigorously shaken in ~100 mL of seawater, in wide-necked HDPE sample bottles or plastic bags to dislodge the epiphytic cells. These sample bottles and/or bags were immediately returned to the laboratory on Key largo where they were drained into flasks and the thalli were then repeatedly rinsed with filtered seawater, until all the cells of benthic dinoflagellates were removed from the substratum, as confirmed by microscopy. After homogenisation, two subsamples were then taken: one sample (250 mL), for cell counting, was fixed by adding neutralized formaldehyde (0.8%) and stored at 4 °C in darkness until counting was performed; the other sample (250 mL) was used for the strain isolation (see below). Finally, the macrophytes were weighed to determine fresh and dry weight, and their area calculated using a conversion factor obtained from the ratio of fresh-weight/surface as described in Accoroni et al. (2011).

Water samples were collected near the sampled macroalgae, making effort to avoid sediment resuspension, to analyse (i) the presence of benthic dinoflagellate species resuspended in the water column (250 mL water samples stored as described above), and (ii) nutrient concentrations (collected in triplicate in 500 mL polyethylene bottles). Upon return to the laboratory (< 1 hr), samples for the analysis of dissolved inorganic and organic nutrients were filtered using GF/F Whatman filters (pre-rinsed by filtering 60 mL of seawater and precombusted at 450 °C) and stored in polyethylene bottles at -22 °C until the analysis. Subsamples (4 mL) for the analysis of total nutrients were stored at the same conditions without filtration.

2.3. Microscope analysis

The identification and counting of dinoflagellate cells were carried out using an inverted microscope (Nikon Eclipse TE2000-U) equipped with phase contrast at 200× magnification. Sub-samples (5-25 mL) were settled in combined sedimentation chambers after homogenization, according to the Utermöhl sedimentation method (Edler and Elbrächter, 2010). Identification and counting were performed on 10 to 30 random fields, across 1 to 2 transects, or the whole sedimentation chamber, in order to count a representative cell number. Abundance values were expressed as both cells cm^{-2} and cells g^{-1} fw of macroalga.

2.4. Strain isolation, DNA extraction, amplification, and sequencing

Monoclonal strains of 5 dinoflagellates, including *Prorocentrum rhathymum*, *Coolia santacroce*, *Amphidinium carterae*, *Gambierdiscus caribaeus* and *Prorocentrum hoffmannianum*, were isolated from the epiphytic microphytobenthos, following the capillary pipette method (Hoshaw and Rosowski, 1973) and their identity confirmed molecularly (see below). After an initial growth in microplates, cells were cultured at 27 ± 0.1 °C under a 12:12 h L:D photoperiod and an irradiance of 90-100 µmol m^{-2} s^{-1}, in modified, silica-free, f/4 medium (Guillard, 1975) prepared with autoclaved natural seawater (salinity 35). Trace metals, iron, vitamins (H, B1 and B12) and HEPES pH 7.1 were added at levels corresponding to f/2 medium.

Genomic DNA was extracted from a 30 mL culture of each strain in logarithmic growth phase. The sample was centrifuged at 4,000 × g for 20 min and DNA was extracted using the CTAB (*N-cetyl-N,N,N*-trimethylammoniumbromide) method (Richards et al., 2001). Extracted DNA was subjected to polymerase chain reaction (PCR) for the amplification of the hypervariable (D1/D3) 28S ribosomal DNA region using the primers DIR (forward: 5′-ACC CGC TGA ATT TAA GCA TA 3′) and DIR-3Ca (reverse: 5′-ACG AAC GAT TTG CAG GTC AG-3′) (Lenaers et al., 1989). PCR was carried out in a MyCycler BioRad Thermal Cycler, following the cycle conditions described in Percopo et al. (2013) for the 28S rDNA amplification. Briefly, the reaction mix was subjected to an initial denaturation at a temperature of 94 °C for 5 min, followed by 34 cycles of denaturation at 94 °C for 1 min, at 55 °C for 1 min and 40 s and a final step at 72°C for 5 min. A final extension step was carried out for 5 min.

PCR products were visualized and quantified in 1.5% agarose gels stained with GelRed^{TM} (Biotium, Hayward, CA, USA) using Low DNA Mass Ladder (Invitrogen, Carlsbad, CA, USA) as reference, and visualized under UV light. PCR products with expected lengths and yields were purified and directly Sanger-sequenced by Macrogen Europe (Amsterdam, The Netherlands). Sequences were compared to the NCBI database by BLAST search with default settings (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This GenBank screening confirmed the strains isolated as *Prorocentrum rhathymum* (PRKL0414), *Coolia santacroce* (CSKL0414), *Amphidinium carterae* (ACKL0414), *Gambierdiscus caribaeus* (GCKL0414) and *Prorocentrum hoffmannianum* (PHKL0414).

The sequence of the strain CSKL0414 (*Coolia santracroce*) was aligned with 27 *Coolia* spp. sequences retrieved from Genbank and a strain of *Ostreopsis* cf. *ovata* (OOAN0601) was used as outgroup. Alignments were made by ClustalW implemented in Bioedit 7.0.

Two independent analyses were used to construct the phylogeny tree: maximum likelihood (ML) and Bayesian inference (BI). The best substitution and rate heterogeneity model was the Generalised time-reversible evolution model with gamma distribution (GTR+G) determined through Partitionfinder 2.0 (Lanfear et al., 2016) using Bayesian Information Criterion (Claeskens and Hjort, 2010). ML analysis was carried out with RaXML (Randomized Accelerated ML) performed through Cipress portal (Miller et al., 2011).

The robustness of the clades recovered was evaluated by 1,000 bootstrap pseudo-replicates.

Bayesian analyses were carried out using MrBayes 3.2 (Ronquist et al., 2012) with 3,000,000 Markov chain Monte Carlo generations with a sample frequency of 1,500. The 50% majority rule consensus tree was constructed using burn-in of 25% and posterior probabilities were calculated to measure tree strength. Pairwise distance estimations were conducted with MEGA package (Kumar et al., 2016).

2.5. Bioassay determination of putative toxicity

Oyster larvae (provided by the Oyster Hatchery at Horn Point Laboratory (UMCES, MD, USA) were used as bioassay organisms to assess potential toxicity of the obtained strains of benthic dinoflagellates.

The procedure used broadly followed that of Lin et al. (2017). Briefly, spawning oysters were collected in filtered natural seawater with a salinity of 10 and a temperature of 28 °C. The larvae were tested within 4 h of fertilization, in triplicate, in 3 mL 12-well culture plates with flat bottoms. The oyster larvae (60 larvae mL^{-1}) were exposed to a concentration of 900 cells mL^{-1} of log phase cultures of *Prorocentrum rhathymum*, *Coolia santacroce* and *Amphidinium carterae* and 90 cells mL^{-1} of *Gambierdiscus caribaeus* and *Prorocentrum hoffmannianum* (due to their wider size and biomass). After 48 hrs of exposure of the larvae to the algal cultures, each well was fixed with dilute acid Lugol's solution, and the samples were analyzed using an Utermöhl chamber (Edler and Elbrächter, 2010) through an inverted light microscope (Nikon Eclipse TE2000-U) at 100×. Survived larvae (i.e. those having the normal D-hinge shape) were counted and compared to those in the control with no dinoflagellate cells to estimate larval mortality.

2.6. Scanning electron microscope analysis

Some preserved subsamples (1 mL), obtained from both field and culture samples, were dehydrated by immersion in ethanol at increasing gradations (10%, 30%, 50%, 70%, 80%, 90%, 95%, and 100%). After 24 hrs in absolute ethanol, they were filtered on a Nucleopore polycarbonate filter and dried using a Critical Point Dryer (Polaron CPD 7501, Quorum Technologies, Newhaven, UK). The filters were then placed on stubs and coated with gold-palladium in a Sputter Coater (Polaron SC 7640, Quorum Technologies, Newhaven, UK) for observation under the scanning electron microscopy (FE-SEM; Zeiss Supra 40) (EHT =15.00 kV).

2.7. Nutrient analysis

Concentrations of NO_x^- (NO_3^- + NO_2^-), NH_4^+, and dissolved inorganic phosphorus (DIP) were measured using autoanalysis techniques. Concentrations of TDN and TDP were determined by persulfate oxidation (Solórzano and Sharp, 1980 for TDP; Valderrama, 1981). Concentrations of DON and DOP were determined by the difference between dissolved total and inorganic fractions.

2.8. Statistical analysis

All statistical analyses were conducted using Statistica (StatSoft Inc., Tulsa, OK, USA) software. The Shapiro-Wilks test was used to check data for normal distribution, while the Levene's test was used to assess homogeneity of variance. Non-parametric tests were used when tests did not reveal homogeneous variances and/or normal distributions. Correlations among environmental parameters and benthic dinoflagellate abundances were examined using Spearman-Rank order correlation, while Kruskal-Wallis ANOVA was used to assess differences in (i) the environmental parameters and (ii) the benthic dinoflagellate distribution between ICOLL, FB, FBK and OK stations and seasons (wet and dry). A Principal Component Analysis (PCA) was performed on a correlation matrix of ranked physical and chemical variables to characterize the different stations based on environmental features. Temperature, salinity, nutrient concentration (i.e. TDN, DON, DIN, TDP, DOP and DIP) and their ratios (TDN:TDP and DIN:DIP) were chosen as the best variables to characterize the environmental conditions of different stations and abundance values of benthic dinoflagellates were used as supplementary variables.

Comparisons between Spearman's and Pearson's correlations were made to determine both monotonic and linear relationships between abundances of benthic dinoflagellate recorded in water column and benthic substrata. Finally, differences in terms of larval mortality of oyster among benthic dinoflagellate cultures were assessed through an ANOVA test. When significant differences for the main effect were detected ($p < 0.05$), a post-hoc Tukey's pairwise test was also performed.

3. Results

3.1. Environmental parameters

In the study period, water temperature fluctuated from a minimum value of 25.8 °C in OK2 in the dry season to a maximum of 31.4 °C in FBK3 in the wet season (Fig. 2A), showing values significantly different between the two seasons (average values 28.1±1.5 and 29.5±1.1°C, respectively for dry and wet season) (Kruskal Wallis, $p < 0.01$). Significantly lower temperature values were observed in the ICOLL than in FBK sites (Kruskal Wallis, $p < 0.01$). Salinity ranged from 8.1 (Seven Palm Lake) to 44.8 (Garfield Bight) (average values 31.1±11.3 and 34.4 ± 6.9, respectively for dry and wet season) (Fig.2A), Significantly lower salinity values were observed in the ICOLL sites compared with sites in FB, FBK and OK (Kruskal Wallis, $p < 0.05$, 0.001 and 0.05, respectively).

Total Dissolved Nitrogen (TDN), and its component fractions varied widely between sites and seasons. Highest TDN concentrations were observed in the ICOLL and FB sites and much lower in the FBK and OK sites (Fig. 2B); significantly lower TDN values were observed in OK than in ICOLLs and in FB (Kruskal Wallis, both $p < 0.001$). Most of the TDN was composed of DON across all stations. Moreover, significantly lower DON values were observed in OK than in ICOLLs and in FB (Kruskal Wallis, both $p < 0.001$) and significant lower values were observed in FBK than in FB (Kruskal Wallis, $p < 0.05$). Dissolved Inorganic Nitrogen (DIN), obtained as the sum of NO_x^- and NH_4^+, ranged from 0.75 to 19.63 μmol l^{-1} (in OK2 in the dry season and in FB2 in the wet season, respectively, Fig. 2B) and showed values significantly different between the two seasons (Kruskal Wallis, $p < 0.01$).

Total dissolved P (TDP) also varied considerably between sites. Significantly higher TDP values were observed in OK than in ICOLLs and in FBK (Kruskal Wallis, both $p < 0.05$). Much of this variability was due to the variability in dissolved inorganic P (DIP), which ranged from 0.053 to 1.855 μmol l^{-1} (in Seven Palm Lake in dry season and in OK1 in wet season, respectively, Fig. 1C), and showed values significantly different between the two seasons (Kruskal Wallis, $p < 0.001$). Significantly higher DIP values were observed in OK than in ICOLLs and in FB (Kruskal Wallis, $p < 0.001$ and 0.05, respectively) and significantly higher values were observed in FBK than in ICOLLs (Kruskal Wallis, $p < 0.01$).

Molar ratios of DIN:DIP and TDN:TDP ranged from 3 to 108 and from 9 to 404, respectively (Fig. 2D). Significantly lower DIN:DIP and TDN:TDP values were observed in OK than in ICOLLs, FB and FBK (Kruskal Wallis $p < 0.05$, 0.01 and $p < 0.001$, respectively for DIN:DIP and $p < 0.001$, 0.001 and $p < 0.05$, respectively for TDN:TDP).

Water temperature was positively correlated with DIN ($r_s = 0.45$, $n=37$, $p<0.01$), and negatively correlated with DOP ($r_s = -0.38$, $n=37$, $p<0.01$). Salinity was negatively correlated with TDN:TDP ($r_s = -0.33$, $n=37$, $p<0.05$). and positively correlated with DIP ($r_s = 0.60$, $n=37$, $p<0.001$) and TDP ($r_s = 0.38$, $n=37$, $p<0.05$).

3.2. Composition, abundances and spatial distribution of benthic dinoflagellates

Species belonging to the genera *Ostreopsis*, *Prorocentrum*, *Gambierdiscus, Fukuyoa*, *Amphidinium*, *Sinophysis*, and *Coolia* were recorded during both seasonal surveys with different abundances in space and time. Significantly higher abundances of benthic dinoflagellates were observed in OK than in ICOLLs, FB and FBK (Kruskal Wallis, $p < 0.001$, 0.001 and 0.05, respectively). In general, *Prorocentrum* species represented the most abundant taxa, representing on average 37% of the entire dinoflagellate community (Fig 3), with the highest abundances recorded in OK3 in the dry season (683 cells cm^{-2}, corresponding to 35,352 cells g^{-1} fw). In particular, *Prorocentrum rhathymum* was detected at each site and sampling period, with the only exception in Big Key in the dry season. This species represented the 24% of the dinoflagellates (Fig. 3) with the highest abundances recorded in OK3 in dry season (250 cells cm^{-2}, corresponding to 12,934 cells g^{-1} fw). Significantly higher abundances of *P. rhathymum* were observed in OK than in ICOLLs and FB (Kruskal Wallis, both $p < 0.01$). *Prorocentrum lima* represented the 9% of the entire dinoflagellate community (Fig. 3) with the highest abundances recorded in OK3 in dry season (333 cells cm^{-2}, corresponding to 15,520 cells g^{-1} fw). Significant higher abundances were observed in OK than in ICOLLs (Kruskal Wallis, $p < 0.05$). *Prorocentrum hoffmannianum* was one of the less abundant species within the *Prorocentrum* genus (4%, Fig. 3), with the highest abundance recorded in OK3 in the dry season (100 cells cm^{-2}, corresponding to 5,173 cells g^{-1} fw). Significantly higher abundances were observed in OK than in ICOLLs and FB (Kruskal Wallis, both $p < 0.05$). Finally, among the

Prorocentrum species, *Prorocentrum* cf. *emarginatum* was the least represented in the dinoflagellate community (0.4% Fig. 3), with the highest abundances recorded in OK4 in the wet season (28 cells cm^{-2}, corresponding to 590 cells g^{-1} fw).

Next to *Prorocentrum rhathymum*, the most represented taxa were *Coolia* spp. and *Ostreopsis* spp. They represented on average 21 and 20% of the BHABs, respectively (Fig. 3). Their highest abundances were recorded in OK3 in the dry season (1,634 cells cm^{-2}, corresponding to 84,500 cells g^{-1} fw and 533 cells cm^{-2}, corresponding to 27,592 cells g^{-1} fw, respectively). *Coolia* showed significantly higher abundances in OK than in ICOLLs and FB (Kruskal Wallis, both $p < 0.01$). *Ostreopsis* abundances were higher in OK than in the other stations, significantly compared with FB (Kruskal Wallis, $p < 0.05$).

Amphidinium spp., represented on average 16% of the entire community (Fig. 3), and had abundances not exceeding 60 cells cm^{-2}, with the only exception in the wet season in OK1 (1081 cells cm^{-2}, corresponding to 55,900 cells g^{-1} fw).

Gambierdiscus spp. were sporadically recorded and represented on average 5% of the entire dinoflagellate community (Fig. 3), reaching the highest abundances in OK1 in the dry season (325 cells cm^{-2}, corresponding to 16,800 cells g^{-1} fw). *Fukuyoa* spp. were rarely recorded, with the highest abundances in FBK7 in the dry season (3 cells cm^{-2}, corresponding to 78 cells g^{-1} fw).

Sinophysis spp. were scarcely represented in the dinoflagellate community with the highest abundances recorded in FBK7 in the wet season (13 cells cm^{-2}, corresponding to 389 cells g^{-1} fw).

Among the benthic dinoflagellate community, some thin walled cysts were sporadically recorded, with the highest abundances in FBK7 in the wet season (8 cells cm^{-2}, corresponding to 229 cells g^{-1} fw).

Mean abundances of the benthic dinoflagellate taxa (as well as their total abundances) estimated over the sampling period were statistically different between the seasons only for *Sinophysis* (Kruskal Wallis, $p < 0.05$). Considering the two seasons, in the dry season the highest dinoflagellate abundance was observed in OK3 (2867 cells cm^{-2}, corresponding to 132,786 cells g^{-1} fw) in which

Coolia spp. represented the 57% of the entire benthic dinoflagellate community, followed by *Ostreopsis* spp. *P. lima*, *P. rhathymum*, *P. hoffmannianum* and *Amphidinium* spp. (19, 12, 9, 3 and 1% respectively; Fig. 4A,C). Although *Coolia* spp. were recorded with the highest abundances in this season (1634 cells cm^{-2}, corresponding to 84,500 cells g^{-1} fw), the community was dominated by *Ostreopsis* spp. and *Gambierdiscus* spp., both with values up to 62% of the entire dinoflagellate community in OK4 and OK1, respectively.

In the wet season, the highest abundances of BHABs were observed in OK1 (1256 cells cm^{-2}, corresponding to 64,949 cells g^{-1} fw) where *Amphidinium* spp. represented 86% of the entire benthic dinoflagellate community, followed by *P. rhathymum* and *Coolia* spp. (12, and 1% respectively; Fig. 4B,D). Although *Amphidinium* spp. was recorded with the highest abundances (1081 cells cm^{-2}, corresponding to 55,900 cells g^{-1} fw), in this season the community was dominated by *P. rhathymum* with values up to 100% of the entire dinoflagellate community in Barnes Sound, followed by *Amphidinium* spp. and *Ostreopsis* spp. with values up to 86% in OK1 and 82% in OK2 of the entire dinoflagellate community, respectively.

Ultrastructural (SEM) observations and rDNA characterizations in selected field and culture samples revealed the presence of benthic dinoflagellate taxa not revealed by light microscopy: *Ostreopsis heptagona, Coolia santacroce*, *Amphidinium carterae*, *Gambierdiscus caribaeus.*

Because of its recent description (Karafas et al., 2015) and because this study is the first to document its occurrence in the Florida Keys, the identification of *Coolia santacroce* (Fig. 5) was more extensively examined. Comparisons of its sequences were made to the NCBI database by BLAST, and a phylogeny tree with 27 *Coolia* spp. retrieved from Genbank was also constructed. Analyses of ML and BI yielded identical topologies; only the ML tree is shown with bootstrap and posterior probability values at nodes (Fig. 6). ML and BI analysis revealed the strain of this study (CSKL0414) fell into *C. santacroce* lineage with a well-supported bootstrap value (87). There was a small p-distance value (0.063) between CSKL0414 and holotype strain of *C. santacroce* (KP172271), while smallest p-distance value (0.019) was between the other three *C. santacroce* strains (KT288057, KT288058, KT288059). Indeed, the mean p-distance estimation calculated between CSKL0414 and the other *C. santacroce* strains was 0.072. Furthermore, *C. santacroce* group is closely related to *C. monotis* (mean p-distance value 0.136). Indeed, they clustered together with well supported bootstrap value of 71 and strongly supported posterior probability of 0.98.

3.3. Relationships between benthic dinoflagellate abundances and environmental parameters

The PCA analysis showed the percentages of explained variance of the first two components (PCA1 and PCA2) were 42.75 and 27.00%, respectively (Fig. 7). The first component (PCA1) shows the DIP and TDP gradient associated with salinity, which is opposite to that of DON, TDN and N:P ratio (expressed as both TDN:TDP and DIN:DIP), distinguishing the spatial gradient (ICOLLs-FB-FBK-OK). The second axis (PCA2) shows the water temperature gradient associated with DIN which is opposite to that of DOP and clearly distinguishes the seasons.

The total benthic dinoflagellate abundances were negatively correlated with N (i.e. TDN, DON and N:P ratio (both DIN:DIP and TDN:TDP), while a positive correlation was found with the water temperature and DIP (Table 1). All the most abundant dinoflagellate taxa showed a significant correlation with nutrient concentration (negative for N (TDN and DON) and positive for DIP), while only the species of the genera *Prorocentrum* and *Amphidinum* showed a clear positive correlation with water temperature and/or salinity (Table 1A). On the contrary, *Sinophysis* abundances showed a negative correlation with P (mainly DOP). These relationships were consistent during the dry season (Table 1B), while only the negative correlation with N (TDN), and N:P ratio (both DIN:DIP and TDN:TDP) persisted in the wet season (Table 1C).

3.4. Benthic versus planktonic cells

Comparisons were made between abundances of these BHABs and dinoflagellates of comparable genera in the plankton. Significant positive correlation was found between benthic and planktonic abundances of *Ostreopsis* spp., *Gambierdiscus* spp., *Coolia* spp., *Prorocentrum rhathymum*, *P.*

lima, and *P. hoffmannianum* (Table 2). Comparisons between Pearson's and Spearman's correlations of benthic and planktonic abundances showed a linear rather than monotonic relationship (except *P. rhathymum* and *Ostreopsis* spp. which did not show a linear relationship at all).

Interpreting the slope of the fitting line of these relationships suggests that certain benthic dinoflagellates were able to detach more easily than others from a colonized substrate. Taxa ranked as follows: *P. rhathymum*, *Gambierdiscus* spp., *P. hoffmannianum*, *P. lima* and *Coolia* spp. (7.41, 3.97, 0.78, 0.55 and 0.26, respectively). Moreover, some toxic planktonic dinoflagellates were detected in the water column that were not seen in the benthos (Table 3).

3.5. Toxic effects of cultured benthic dinoflagellates on larval growth

The species brought into culture were used in oyster larval bioassays to test for toxicity. Larval mortality in the whole experiment ranged between 13 and 39% (Fig. 8). The treatment with *Gambierdiscus caribaeus* induced a clear larval mortality with values (38±2%) significantly higher than those of the control and those of other treatments (Tukey HSD test, $p < 0.05$ for *Prorocentrum hoffmannianum* and $p < 0.01$ for *P. rhathymum*, *C. santacroce*, *A. carterae* and the control). While the treatments with *Coolia santacroce* and *Amphidinium carterae* showed larval mortality with lower values (17±3 and 18±4%, respectively) than those of the control (20±4%), those with *Prorocentrum hoffmannianum* and *P. rhathymum* had slightly, but not significant higher mortality (26±4 and 22±5%) than the control.

At the end of the 48-bioassay exposure, the abundances of *P. rhathymum*, *C. santacroce* and *A. carterae* had decreased by 17±1, 97±3 and 75±12%, respectively, while those of *G. caribaeus* and *P. hoffmannianum* cells remained stable.

4. Discussion

Benthic dinoflagellates are generally under-studied (Hoppenrath et al., 2014) but are now being investigated in new locations because of the rising awareness of their potential health, economic and ecological impacts at a global scale (Berdalet et al., 2017; Berdalet and Tester, 2018). Several BHAB species are known to produce toxins causing human poisonings through the consumption of contaminated marine organisms (Friedman et al., 2017; Lee et al., 2016; Randall, 2005) or via the formation of toxic bio-aerosols (Ciminiello et al., 2014; Vila et al., 2016). Although Florida Bay is a well-studied area, few studies have been carried out on benthic dinoflagellates along the Florida Keys (e.g. Norris et al., 1985; Parsons et al., 2017), especially in northern region to the saline lakes (the quasi-ICOLLs). Moreover, the role of environmental parameters on the toxic benthic dinoflagellate community of this region of Florida has not been previously investigated.

This study showed that the composition and abundances of the benthic dinoflagellate community were strongly affected by water temperature and nutrient availability. First, as expected, a significant positive correlation was found with the water temperature and total dinoflagellate abundances (especially for *Prorocentrum rhathymum* and *P. lima*, which represented the most abundant taxa). In particular, water temperature seems to have different impact on the benthic dinoflagellate abundances as a function of the seasons: increasing temperature values positively affected BHAB abundances in the dry season when temperatures are near optimum values for many benthic dinoflagellate species, while its influence came less in wet season when temperature may exceed the thermal optimum (e.g. Morton et al., 1992). Second, P strongly influenced the spatial distribution of the benthic dinoflagellates. Indeed, the abundances of benthic dinoflagellate clearly followed the west-east N:P ratio gradient as significantly higher abundances of benthic dinoflagellates were observed along the Florida Keys (especially on the oceanside) than in the rest of Florida Bay, i.e. where P was more available. All of the abundant benthic dinoflagellate taxa were positive correlated with DIP and negatively correlated with N (i.e. TDN, DON) and N:P ratio. The important role of P availability (associated to low N:P ratios) on dinoflagellate growth has previously been well studied highlighting that the onset of dinoflagellate blooms is often associated with N:P ratios even lower than Redfield, sometimes from an injection of P-rich water (Accoroni et al., 2015; Anderson et al., 2002; Fang, 2004; Glibert et al., 2012; Hodgkiss and Ho, 1997; Li et al., 2009; Riegman, 1995).

Florida Bay has undergone, and continues to undergo major changes in nutrient loads. Florida Bay experienced substantial ecological degradation in the late 1980s and although subsequently recovering, there have been environmental concerns since then. In September 2005, a phytoplankton bloom (dominated by *Synechococcus*) was initiated in the eastern bay region from a series of hurricanes (Katrina, Rita and Wilma) and by disturbance (mangrove removal, sediment resuspension, etc.) due to construction of the causeway connecting the mainland and Florida Keys (Glibert et al. 2009). This bloom resulted in the release of P which sustained the bloom for 3 years after which the system did not return to pre-bloom conditions, but instead formed a new baseline with significantly higher chlorophyll concentrations (Millette et al. 2018). One reason for the sustained increase in chlorophyll is a presumed loss of SAV (Glibert et al. 2009, Millette et al. 2018), driving the system toward a more planktonic condition. In 2012 the Comprehensive Everglades Restoration Plan was initiated, bringing increased flow into northern Florida Bay, influencing the productivity of the ICOLLs (Shangguan et al. 2017). Those lakes that has been oligotrophic prior to the project experienced an approximate doubling of phytoplankton biomass as nutrient ratios converged on Redfield proportions, and a shift to smaller sized cells and a loss of diatoms as NO_x^- availability declined. The other set of these lakes, previously highly eutrophic, sustained a significant, nearly 50%, decline in overall phytoplankton biomass, particularly larger sized cells, which, in turn, increased downstream. These findings have established that altered flow in Florida Bay affects not only salinity but also nutrients, in terms of total loads and proportions, and these changes can create conditions where phytoplankton assemblages change in composition. As a whole, Florida Bay has been a benthic dominated system with P as a controlling nutrient, but this is a system under multiple stresses and highly responsive to changes in flow and nutrients. Understanding how the benthic community responds to these changes is important.

Accoroni et al. (2015), building on the conceptual framework of Glibert et al. (2012), suggested that blooms of BHABs may form when stimulated by a ‘‘flush’’ of nutrients or organic materials that may allow a latent resident population to flourish. After that growth rate increase, bloom biomass is able to increase often accompanied by N:P values greater than Redfield. At this point, these blooms are able to sustain high biomass levels, not necessarily by high growth rates, but through other mechanisms, including (i) metabolic dissipatory strategies that allow the maintenance of cellular nutrient and energy balance in an environment where energy flow and nutrients are provided at unbalanced proportions and (ii) allelopathic and mixotrophic interactions with other species. This conceptual model was supported by data on *Ostreopsis* in the northern Adriatic Sea. Thus, adaptive physiology may enable the maintenance of BHAB taxa at less than maximal growth rates and at non-optimal N:P ratios.

In this study, seven genera of benthic dinoflagellates were detected, *Amphidinium*, *Coolia*, *Fukuyoa, Gambierdiscus*, *Ostreopsis*, *Prorocentrum* and *Sinophysis.* All these genera, except *Sinophysis*, include potentially toxic species. These genera have already been reported in other studies previously carried out in Florida Keys (Norris et al., 1985), and in other tropical Atlantic areas such as Bahamas Archipelago (Bomber et al., 1988), Belize (Faust, 2000, 1993), Gulf of Mexico (Tester et al., 2013), Cuba (Delgado et al., 2006), Porto Rico (Ballantine et al., 1988), and Virgin Islands (Carlson and Tindall, 1985).

Prorocentrum is typically regarded as one of the dominant genera among the benthic dinoflagellates (Delgado et al., 2006; Glibert et al., 2012; Okolodkov et al., 2014; Richlen and Lobel, 2011; Tester et al., 2014) as observed in this study, where it showed the highest percent abundances among the benthic dinoflagellate community. It was mainly represented by *P. rhathymum* (that is a well-known okadaic acid (OA) producer in Florida Bay, An et al., 2010), followed by *P. lima*, *P. hoffmannianum* and *Prorocentrum* cf. *emarginatum* (all known for producing Diarrhetic Shellfish

Poisoning (DSP) toxins including the lipid-soluble polyether compounds OA, methylokadaic acid, and/or dinophysistoxins, other OA derivatives, and prorocentrolides, a water-soluble ‘‘fast-acting toxin’’; Lee et al., 2016). *Prorocentrum* was detected in each site in this study reaching maximum abundances (10^4 cells g^{-1} fw) comparable to those reported in other studies performed in other tropical environments (Boisnoir et al., 2019; Delgado et al., 2006; Díaz-Asencio et al., 2019; Okolodkov et al., 2014) and higher than those reported in some others (10^3 cells g^{-1} fw, Tester et al., 2014; 10^2 cells g^{-1} fw, Yong et al., 2018).

Observed maximum abundances of *Coolia* spp. in this study (i.e. 10^4 cells g^{-1} fw) were higher than those observed in the Gulf of Mexico and in the Lesser Antilles (Caribbean Sea) (10^3 cells g^{-1} fw, (Boisnoir et al., 2019; Okolodkov et al., 2007). This genus is globally distributed in tropical and temperate coastal and estuarine waters but most species are found in tropical regions (Leaw et al., 2016). Reports of toxin production in *Coolia* have been often inconsistent and only *C. tropicalis* and *C. malayensis* are considered to be certainly toxic to date (Mohammad-Noor et al., 2013; Rhodes et al., 2014).

Recently, a new species, *C. santacroce* was described from the U.S. Virgin Islands and the Bahamas showing cytotoxic effects on human derived cells *in vitro* (Karafas et al., 2015; Karafas and Tomas, 2015). This study documented the first record of *C. santacroce* in the Florida Keys. Molecular analyses performed aligning the strain CSKL0414 of *Coolia* from this study with 27 *Coolia* spp. sequences retrieved from Genbank, showed that the strain CSKL0414 fell within *C. santacroce* group, closely related with the other strains of this group. *C. monotis* and *C. santacroce* were resolved as sister taxa and 5 independent lineages, *C. monotis*, *C. santacroce*, *C. canariensis*, *C. tropicalis* and *C. malayensis*, are recognizable in agreement with Karafas et al. (2015) and Karafas and Tomas (2015).

Species of the genus *Ostreopsis* include those able to produce toxins mostly belonging to the palytoxin group (Accoroni et al., 2016; Brissard et al., 2015; Ciminiello et al., 2006; Holmes et al., 1988; Lenoir et al., 2004; Meunier et al., 1997; Nakajima et al., 1981; Uchida et al., 2013;

Yasumoto et al., 2007). In this study, *Ostreopsis* spp. were mostly recorded along the Florida Keys, reaching maximum abundances (10^4 cells g^{-1} fw) comparable to those recorded in the island of Hawaiʻi (Parsons and Preskitt, 2007) and higher than those previously reported in the same area and in other tropical environments, i.e. 10^2 cells g^{-1} fw in the Florida Keys, the northwestern coast of Cuba and Malaysia (Bomber et al., 1988; Delgado et al., 2006; Tester et al., 2014), 10^3 cells g^{-1} fw in the Gulf of Mexico, Puerto-Rico, Guadeloupe and Belize (Ballantine et al., 1985; Boisnoir et al., 2018; Faust, 2009; Okolodkov et al., 2007), but lower than in the Lesser Antilles (Caribbean Sea) and south central Cuba (10^5 cells g^{-1} fw, Boisnoir et al., 2019; Díaz-Asencio et al., 2019). These benthic marine dinoflagellates have been commonly associated with tropical areas where they show the highest diversity (Parsons et al., 2012; Rhodes, 2011), while their appearance in temperate latitudes is considered a recent phenomenon (Corriero et al., 2016; Zenetos et al., 2005). However, it is noteworthy that the abundances reached in some temperate areas are considerably higher (10^6 cells g^{-1} fw e.g., Mangialajo et al., 2011; Vassalli et al., 2018) than those reported in tropical ones, causing serious problems on human health, mainly due to inhalation of sea water droplets containing *Ostreopsis* cells or fragments and/or aerosolized toxins, or cutaneous contact with cells, causing skin and eye irritations (Gallitelli et al., 2005; Tichadou et al., 2010; Vila et al., 2016). On the contrary, in tropical areas *Ostreopsis* is strongly suspected to be involved in several events of clupeotoxism, a human intoxication after consuming contaminated crustaceans and fish characterized by symptomology similar to that of ciguatera but with a much higher mortality rate (e.g. Onuma et al., 1999; Randall, 2005), but other types of intoxications common in temperate areas associated with high *Ostreopsis* abundances are uncommon. Among the *Ostreopsis* species present in the study area, *Ostreopsis heptagona* have been identified by ultrastructural (SEM) observations. This species was previously determined to be toxic as methanol extracts of a culture isolated from Knight Key (Florida) were weakly toxic to mice (Babinchak, according to Norris et al., 1985).

Amphidinium species are among the most abundant benthic dinoflagellates in intertidal or estuarine marine sandy sediments from tropical to temperate ecosystems (Hoppenrath et al., 2014; Jørgensen et al., 2004; Murray and Patterson, 2002) and they produce many different types of bioactive compounds (Murray et al., 2012), with a wide range of toxicological impacts. Observed maximum abundances of *Amphidinium* spp. in this study (i.e., 10^4 cells g^{-1} fw) were comparable to those recorded in the Gulf of Mexico (Okolodkov et al., 2007), while in the Lesser Antilles (Caribbean Sea) the highest abundance only reached 10^2 cells g^{-1} (Boisnoir et al., 2019).

In this study, *Gambierdiscus* abundances reached values up to 10^4 cells g^{-1} fw. In the Gulf of Mexico, the Caribbean Sea and French Polynesia, *Gambierdiscus* is reported with maximum abundances ranging from 10^3 to 10^5 cells g^{-1} fw (Ballantine et al., 1988, 1985; Bomber et al., 1989; Delgado et al., 2006; Díaz-Asencio et al., 2019; Okolodkov et al., 2014; Yasumoto et al., 1980). Lower abundances (10^2 cells g^{-1} fw or less) have been recorded in the Lesser Antilles (Boisnoir et al., 2019) and in Rawa Island, Malaysia (Yong et al., 2018). Although the majority of ciguatera cases in Florida are caused by fish caught in the Bahamas and the Florida Keys (Radke et al., 2015), in the present study, *Gambierdiscus* cells were sporadically recorded and represented only a small part of the entire dinoflagellate community. Nevertheless, in the dry season at two stations along the oceanside of the Florida Keys (i.e. OK1 and OK4), *Gambierdiscus* abundances exceeded 1,000 cells g^{-1} fw, the threshold cell density where a bloom begins to input significant amounts of toxin into the food web causing CFP (Litaker et al., 2010), posing a risk of CFP in this area. However, *Gambierdiscus* toxicity differs widely among species (Litaker et al., 2017; Pisapia et al., 2017), implying CFP risk is dependent on both increased cell abundance and the proportion of higher toxic species. Among the *Gambierdiscus* species occurring in the study area, the presence of *Gambierdiscus caribaeus* has been confirmed by molecular analysis. This species is already known for its toxicity due to the production of toxins involved in causing CFP (Holland et al., 2013; Pisapia et al., 2017).

The toxicity tests performed in this study revealed potential toxic effects for *Gambierdiscus caribaeus*, suggesting a release of putative toxin(s) into the medium. Although release of toxin may have been due to the lysis of *Gambierdiscus* cells caused to the low salinity (10) of the bioassay tests, larval mortality was observed without a decrease of dinoflagellate cells (i.e., oyster larvae did not feed on *G. caribaeus*). On the contrary, although *P. hoffmannianum* is known to produce high concentration of OA, this dinoflagellate did not induce a high mortality, most probably due to the fact that oyster larvae did not feed on *P. hoffmannianum* and OA is not released in the growth medium (Accoroni et al., 2018).

The benthic dinoflagellates recorded in this study have been often detected in water column as well. Results showed that certain benthic dinoflagellates were able to detach more easily than others from the benthic substrate and hence occupy the planktonic zone, i.e. *P. rhathymum*, *Gambierdiscus* spp., *P. hoffmannianum*, *P. lima* and *Coolia* spp. (from the most detachable to the less one). For these dinoflagellates, correlations of benthic and planktonic abundances showed a linear rather than monotonic relationship, suggesting that where many cells are on the benthic substrata, they also colonize the water column. On the contrary, results of *Ostreopsis* benthic and planktonic abundances did not reveal a linear relationship, suggesting that *Ostreopsis* cells have a predominantly benthic behavior but can be transferred in water column under certain environmental conditions. Indeed, several studies have highlighted that both benthic and planktonic abundances of *Ostreopsis* are highly affected by wave action, since they are only loosely attached to the substrata and can be easily removed and re-suspended in the water column (reviewed in Accoroni and Totti, 2016). The fact that many of those benthic toxigenic species can be easily recorded in water column may make them relatively more available as food resources for filter-feeding shellfish than when they grow on benthic substrata, increasing the risk of human intoxication through seafood consumption.

5. Summary and Conclusions

This study has documented the diversity of BHAB taxa in Florida Bay, the northern Florida Bay ICOLLs, and along both the bay-side and ocean-side of the Florida Keys. A diverse suite of taxa was enumerated across two seasons and potential toxicity was characterized in bioassay tests. One species (*Coolia santacroce*) was newly identified for the region. Although much attention has focused on the large scale *Karenia brevis* blooms in the eastern Gulf of Mexico and Florida's western coast, and more recently the cyanobacterial blooms that have proliferated from Lake Ocheechobee (*Microcystis* sp.) to Florida Bay (*Synechococcus* sp.), these finding suggest that BHABs may be another potential problem for Florida's waters. Both temperature and nutrients (especially P) were associated with abundances of these BHAB taxa, and thus with climate change and continued anthropogenic nutrient pressures, the abundance of these species will likely not be diminishing in the near future.

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Table 1. Spearman-Rank order correlation between water temperature (°C), salinity, nutrients (Total Dissolved Nitrogen (TDN), Dissolved Organic Nitrogen (DON), Dissolved Inorganic Nitrogen (DIN), Total Dissolved Phosphorus (TDP), Dissolved Organic Phosphorus (DOP) Dissolved Inorganic Phosphorus (DIP) and Nitrogen:Phosphorus ratios: organic ratio (DON:DOP) and inorganic ratio (DIN:DIP)) and abundances of benthic dinoflagellate taxa (*Prorocentrum* cf. *emarginatum*, *P. hoffmannianum*, *P. lima*, *P. rhathymum*, *Coolia*, *Ostreopsis*, *Amphidinium*, *Gambierdiscus, Fukuyoa*, thin-walled cysts, *Sinophysis*) throughout (A) the entire study period and in (B) dry and (C) wet seasons separately. Values indicated in italic are significant at $p < 0.05$, those in bold italic are significant at $p < 0.01$, those in bold italic and underlined are significant at $p < 0.001$.

	Ostreopsis	*Gambierdiscus*	*Fukuyoa*	*Coolia*	*Amphidinium*	*P. rhathymum*	*P. lima*	*P. hoffmannianum*	*P.* cf. *emarginatum*	thin walled cysts	*Sinophysis*	TOTAL
A) TOTAL												
water temperature (°C)	0.23	0.11	0.01	0.26	0.29	***0.43***	***0.42***	0.17	0.17	0.17	0.19	*0.36*
salinity	0.22	0.02	0.18	0.32	*0.40*	0.23	***0.62***	*0.38*	*0.42*	0.08	0.19	0.31
TDN	***-0.49***	***-0.52***	-0.04	***-0.73***	-0.24	***-0.53***	-0.31	***-0.62***	-0.15	-0.22	-0.26	***-0.63***
DON	*-0.41*	***-0.57***	-0.04	***-0.69***	-0.21	***-0.52***	-0.29	***-0.60***	-0.18	-0.22	-0.27	***-0.60***
DIN	*-0.37*	0.07	-0.06	-0.15	0.13	*0.15*	-0.09	*-0.36*	0.08	-0.07	0.21	0.02
TDP	0.27	0.23	0.10	0.24	0.13	0.10	0.11	0.23	-0.20	-0.05	*-0.41*	0.29
DOP	0.17	-0.06	0.15	-0.05	-0.22	-0.18	0.07	0.07	-0.27	0.00	***-0.54***	-0.11
DIP	0.32	0.30	-0.10	***0.43***	***0.45***	***0.49***	*0.40*	***0.51***	0.22	-0.11	0.27	***0.66***
TDN:TDP	***-0.53***	***-0.53***	-0.12	***-0.71***	-0.27	***-0.46***	*-0.34*	***-0.61***	-0.11	-0.19	-0.10	***-0.61***
DIN:DIP	*-0.42*	-0.31	-0.05	*-0.38*	-0.14	-0.28	-0.20	***-0.46***	-0.04	-0.07	0.09	***-0.45***
B) DRY												
water temperature (°C)	*0.47*	0.36	0.23	*0.48*	0.17	*0.53*	0.43	0.26	0.11	0.40	/	*0.48*
salinity	*0.51*	0.11	0.27	***0.58***	0.39	0.44	***0.72***	*0.57*	0.42	0.09	/	*0.50*
TDN	*-0.54*	*-0.48*	-0.05	***-0.67***	-0.36	***-0.81***	*-0.55*	***-0.68***	-0.04	-0.17	/	***-0.84***
DON	*-0.47*	*-0.50*	-0.08	***-0.61***	-0.30	***-0.78***	*-0.48*	***-0.65***	0.01	-0.19	/	***-0.80***
DIN	-0.18	0.30	0.22	-0.03	-0.05	0.14	-0.12	-0.32	-0.18	0.23	/	0.08
TDP	0.22	0.26	-0.02	0.18	0.13	*0.47*	0.36	*0.52*	0.27	-0.03	/	*0.48*
DOP	-0.06	0.02	-0.14	-0.13	-0.05	0.11	0.01	0.26	0.13	-0.16	/	0.11
DIP	*0.53*	0.40	0.04	*0.52*	0.31	***0.75***	***0.62***	***0.64***	0.22	0.06	/	***0.79***
TDN:TDP	*-0.53*	*-0.50*	-0.10	***-0.62***	-0.36	***-0.85***	***-0.62***	***-0.69***	-0.15	-0.20	/	***-0.87***
DIN:DIP	-0.41	-0.24	0.11	-0.29	-0.19	*-0.51*	-0.43	*-0.49*	-0.31	0.13	/	*-0.57*
C) WET												
water temperature (°C)	0.15	-0.01		0.04	0.27	0.10	0.31	-0.07	0.02	0.00	-0.05	0.15
salinity	-0.17	-0.21		-0.11	0.36	-0.10	0.43	-0.10	0.37	0.05	0.24	-0.12
TDN	*-0.49*	*-0.57*		***-0.77***	-0.17	-0.27	-0.07	***-0.62***	-0.23	-0.26	-0.45	*-0.54*
DON	-0.38	***-0.65***		***-0.72***	-0.18	-0.22	-0.03	*-0.59*	-0.28	-0.20	-0.37	-0.47
DIN	-0.45	-0.03		-0.30	0.06	-0.21	-0.39	***-0.66***	-0.05	-0.31	-0.19	-0.38
TDP	0.26	0.25		0.35	0.30	-0.07	0.07	0.18	-0.23	-0.31	-0.28	0.35
DOP	0.28	-0.31		0.08	-0.17	0.05	0.37	0.19	-0.13	-0.05	-0.16	0.10
DIP	0.19	0.30		0.35	*0.61*	-0.09	-0.03	0.24	0.10	-0.26	0.16	0.32
TDN:TDP	*-0.48*	*-0.56*		***-0.81***	-0.33	-0.24	-0.22	***-0.64***	-0.21	-0.15	-0.35	***-0.63***
DIN:DIP	-0.40	-0.33		*-0.53*	-0.26	-0.34	-0.24	*-0.57*	-0.05	-0.26	-0.20	***-0.64***

Table 2. Spearman-Rank order (r_s) and Pearson (r) correlation between abundances of benthic dinoflagellate taxa in the benthos and in the plankton (*Prorocentrum hoffmannianum*, *P. lima*, *P. rhathymum*, *Coolia*, *Ostreopsis*, *Gambierdiscus*). Only taxa with significant results are shown. Values indicated in bold italic are significant at $p < 0.01$, those in bold italic and underlined are significant at $p < 0.001$.

	Spearman's r_s	Pearson's r
Ostreopsis	***0.69***	0.17
Gambierdiscus	***0.81***	***0.99***
Coolia	***0.53***	***0.83***
P. rhathymum	***0.62***	***0.42***
P. lima	***0.53***	***0.59***
P. hoffmannianum	***0.54***	***0.57***

Table 3. Toxic planktonic dinoflagellates identified in the study area, and relative abundance.

' < 10 cells L^{-1}; + = 10 cells L^{-1}; ++ = 10^2 cells L^{-1}; +++ = 10^3 cells L^{-1}; = ++++ = 10^4 cells L^{-1}.

STATION	*Prorocentrum cordatum*	*Pyrodinium bahamense*	*Protoceratium reticulatum*	*Gonyaulax spinifera*	*Dinophysis* sp.	*Margalefidinium polykrikoides*
Seven Palm Lake	++++	+++	++	++		
Long Lake	++++					
Middle Lake	++++	+++	++		++	
West Lake	++++		++			
Barnes sound	++	+				
Big Key	++	++		+		
Duck Key	++	++				
Garfield Bight	+++	+	+	+		
Terrapin Bay	+++	+++	++			
FBK1	++	++				
FBK2	++					
FBK3	++					
FBK4	++	+				'
FBK5	++	++				'
FBK6			++			
FBK7	++		++			
OK1						
OK2	++	++	'			
OK3	++		+			
OK4	++	++	+			++

Figure captions

Fig.1. Map of the study area, showing the location of the sampling station along Florida bay and the Florida Keys (Florida, USA). (●) ICOLLs (1: Seven Palm Lake; 2: Middle Lake, 3: West Lake, 4: Long Lake). (■) FB stations (1: Barnes Sound; 2: Duck Key; 3: Terrapin Bay; 4: Garfield Bight; 5: Big Key). Florida Keys stations: (◆) FB-side (1: FBK1; 2: FBK2; 3: FBK3; 4: FBK4; 5: FBK5; 6: FBK6; 7: FBK7) and (▲) ocean-side (1: OK1; 2: OK2; 3: OK3; 4: OK4).

Fig. 2. Spatial variability of environmental parameters at sampling area in dry (full bars and circles) and wet (crossed bars and squares) seasons. (A) Salinity (bars, left y-axis) and water temperature (circles and squares, right y-axis). (B) Nitrogen fractions (μmol L^{-1}): Total Dissolved Nitrogen (TDN), with the detail of Dissolved Organic Nitrogen (DON, darker) and Dissolved Inorganic Nitrogen (DIN, lighter). (C) Phosphorus fractions (μmol L^{-1}): Total Dissolved Phosphorus (TDP), with the detail of Dissolved Organic Phosphorus (DOP, darker) and Dissolved Inorganic Phosphorus (DIP, lighter). (D) Nitrogen:Phosphorus ratios: organic ratio (DON:DOP, darker) and inorganic ratio (DIN:DIP, lighter). Environmental parameters' data for Middle Lake, West Lake and Long Lake in wet season are unavailable.

Fig. 3. Relative abundance (%) of each taxon within the benthic dinoflagellate community at the entire sampling area.

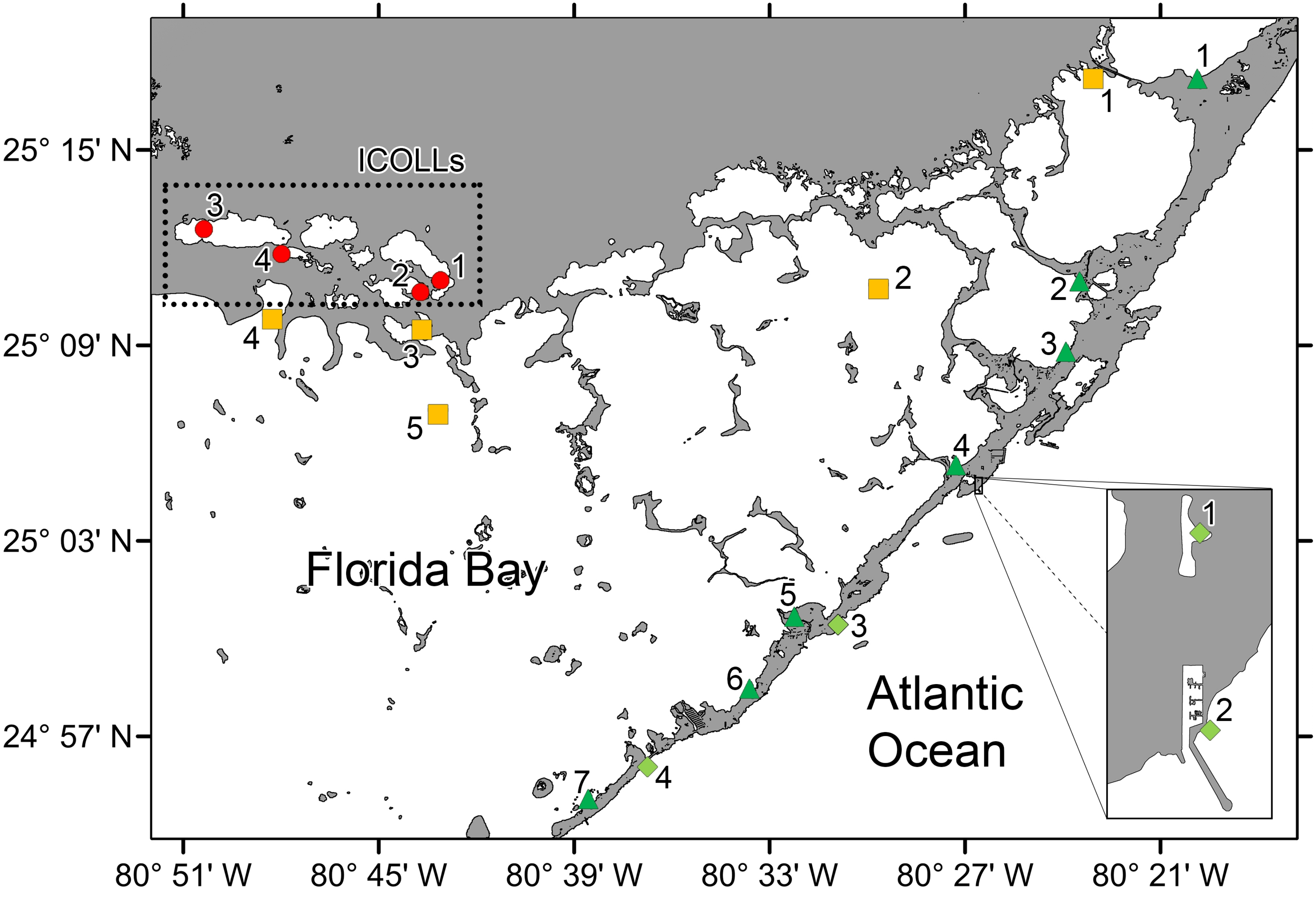
Fig. 4. Spatial trend in abundances benthic dinoflagellate community at the sampling area in dry (A, C) and (B, D) wet seasons. (A, B) Absolute (cells cm^{-2}) and (C, D) percent abundances (%) of toxic benthic dinoflagellate taxa (*Prorocentrum* cf. *emarginatum*, *P. hoffmannianum*, *P. lima*, *P. rhathymum*, *Coolia*, *Ostreopsis*, *Amphidinium*, *Gambierdiscus, Fukuyoa*, thin-walled cysts). Data from grey-colored stations are unavailable.

Fig. 5. Scanning electron microscopy images of *Coolia santacroce* (CSKL0414) collected from the Florida Keys in (A) epithecal and (B) ventral/lateral view (sulcus and cingulum are visible). Scale bars = 2µm.

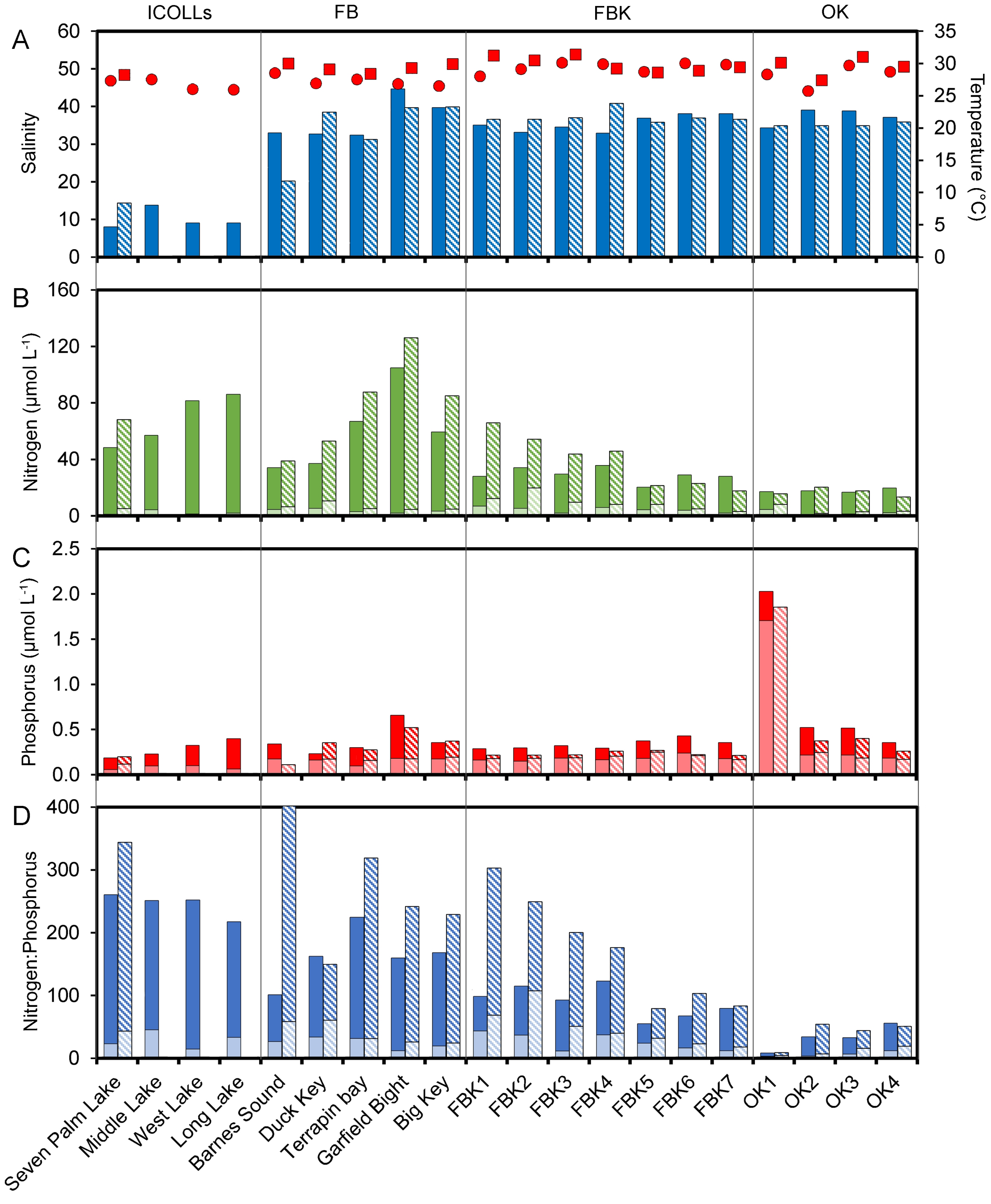
Fig. 6. Bayesian phylogenetic tree of the genus *Coolia* inferred from LSU ribosomal gene sequences. The tree is rooted with *Ostreopsis* cf. *ovata* OOAN0601 as outgroup. Nodes are annotated with ML bootstrap values followed by posterior probabilities. Strain sequenced in this study is in bold.

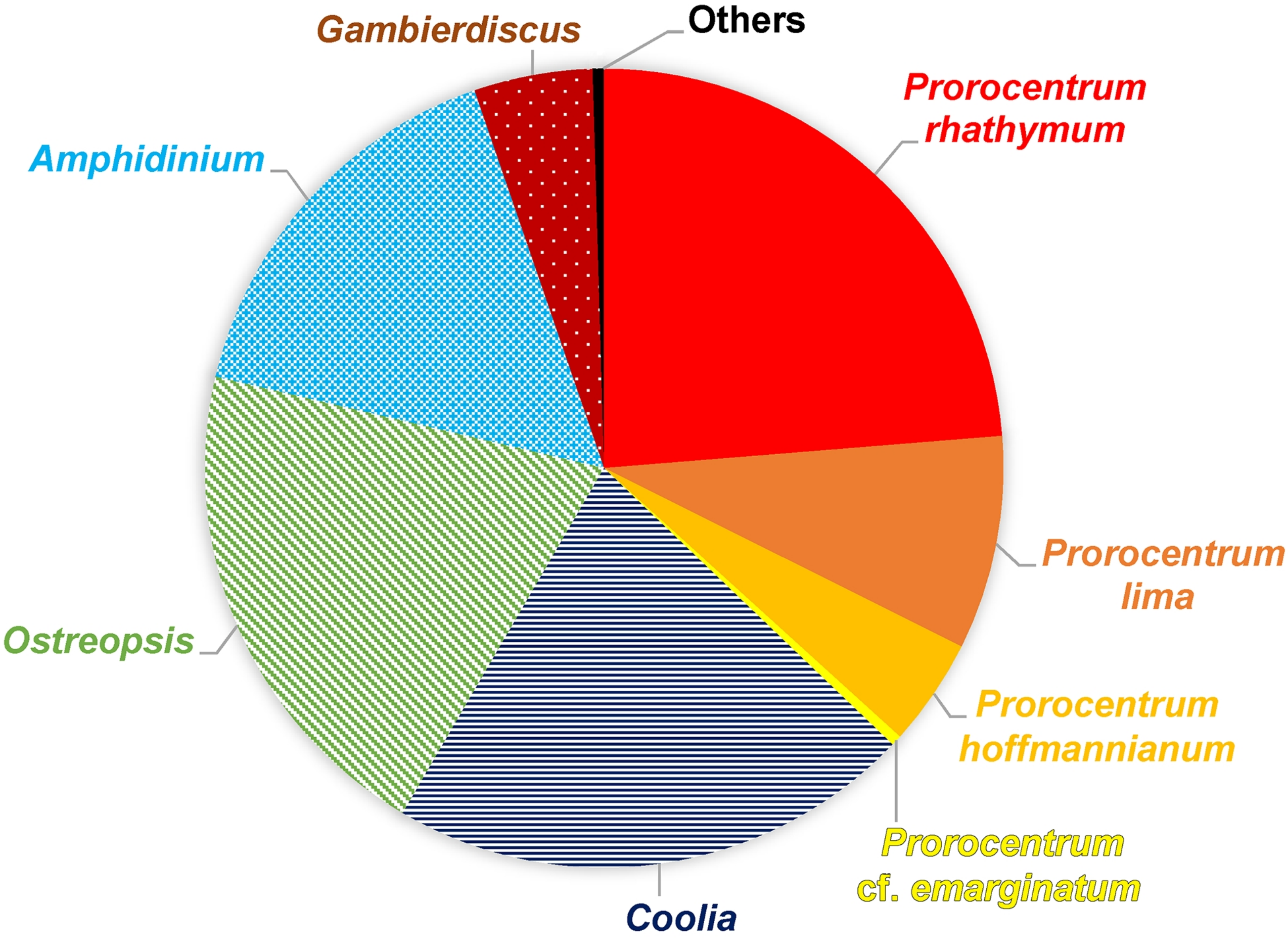
Fig. 7. Principal Component Analysis (PCA) based on correlation matrix of ranked environmental variables and abundance values of the most represented taxa (> 0% of the entire community) of the benthic dinoflagellate community used as supplementary variables. (A) Loading plot (T = temperature, S = salinity, TDN = Total Dissolved Nitrogen, DON = Dissolved Organic Nitrogen, DIN = Dissolved Inorganic Nitrogen, TDP = Total Dissolved Phosphorus, DOP = Dissolved Organic Phosphorus, DIP = Dissolved Inorganic Phosphorus, TDN:TDP = total dissolved N:P, DIN:DIP = dissolved inorganic N:P; Ostr = *Ostreopsis*, Gamb = *Gambierdiscus*, Cool = *Coolia*, Amph = *Amphidinium*, P.rhat = *Prorocentrum rhathymum*, P.lima = *Prorocentrum lima*, P.hoff = *Prorocentrum hoffmannianum*). (B) Score plot ((●) ICOLLs: 7 Palm = Seven Palm Lake, Middle = Middle Lake, West = West Lake, Long = Long Lake; (■) FB stations: Barnes = Barnes Sound, Duck = Duck Key, Terrapin = Terrapin Bay, Garfield = Garfield Bight, Big = Big Key; Florida Keys stations: (◆) FB-side: FBK1, FBK2, FBK3, FBK4, FBK5, FBK6, FBK7, (▲) ocean-side: OK1, OK2, OK3, OK4. D: dry season, W = wet season).

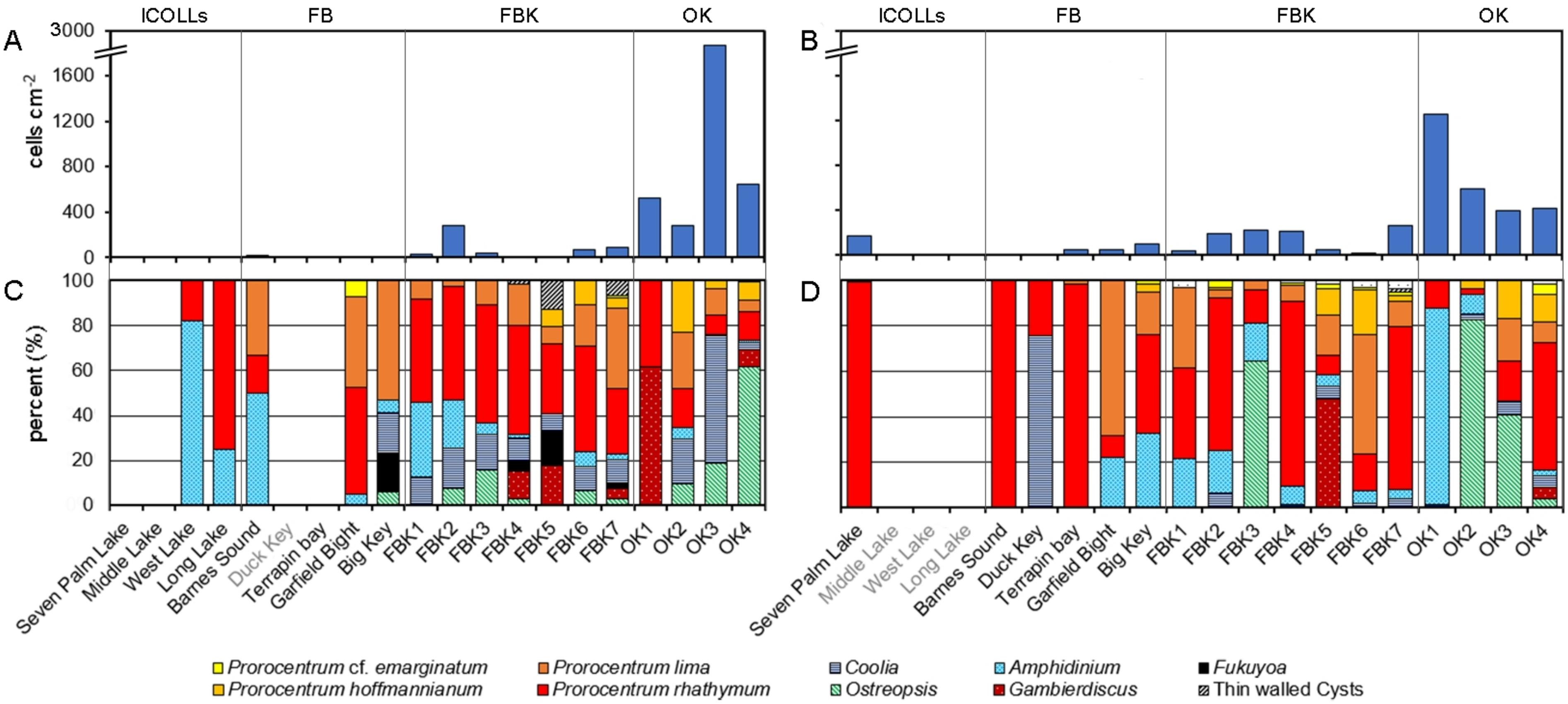
Fig. 8. Mortality of *Crassostrea virginica* larvae after 2 d of exposure to *Gambierdiscus caribaeus*, *Prorocentrum hoffmannianum*, *Prorocentrum rhathymum*, *Coolia santacroce* and *Amphidinium carterae*. Control treatments (CTRL) contained *Crassostrea virginica* larvae alone. Different letters show significant differences between treatments (Tukey HSD test: a<c, $p< 0.01$; b<c, $p< 0.05$)

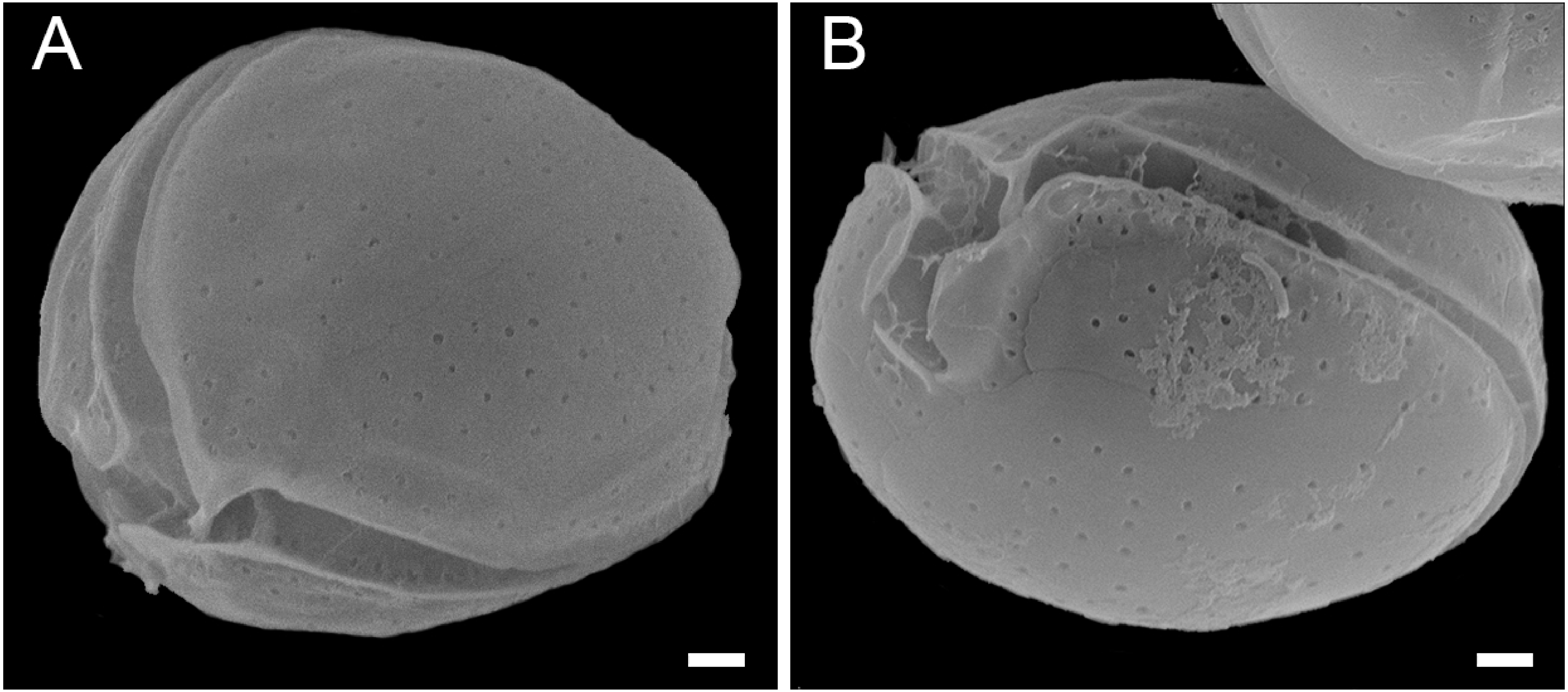


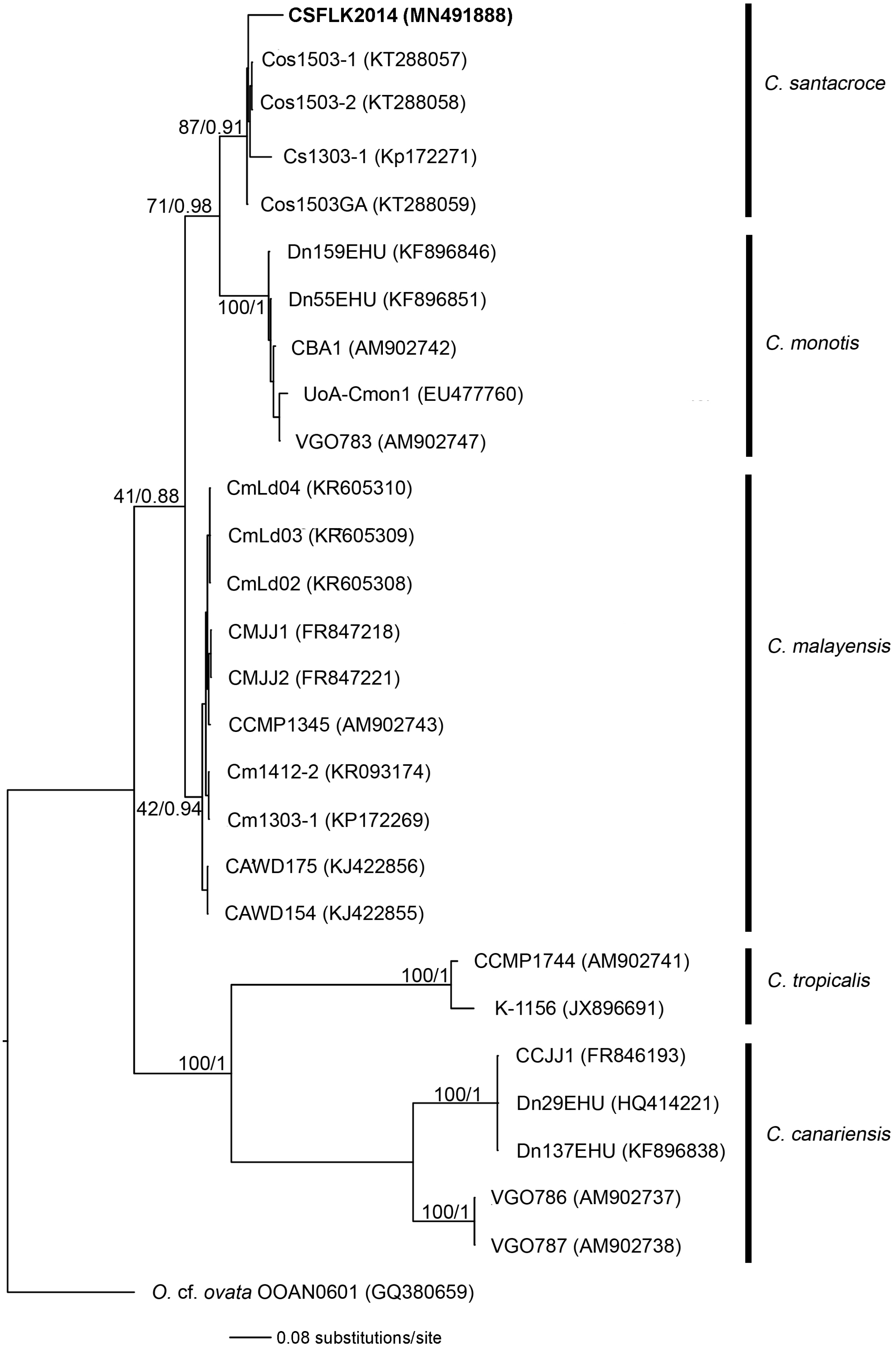
25° 15' N
25° 09' N
25° 03' N
24° 57' N
80° 51' W
80° 45' W
80° 39' W
80° 33' W
80° 27' W
80° 21' W
ICOLLs
Florida Bay
Atlantic Ocean
1
2
3
4
5
6
7

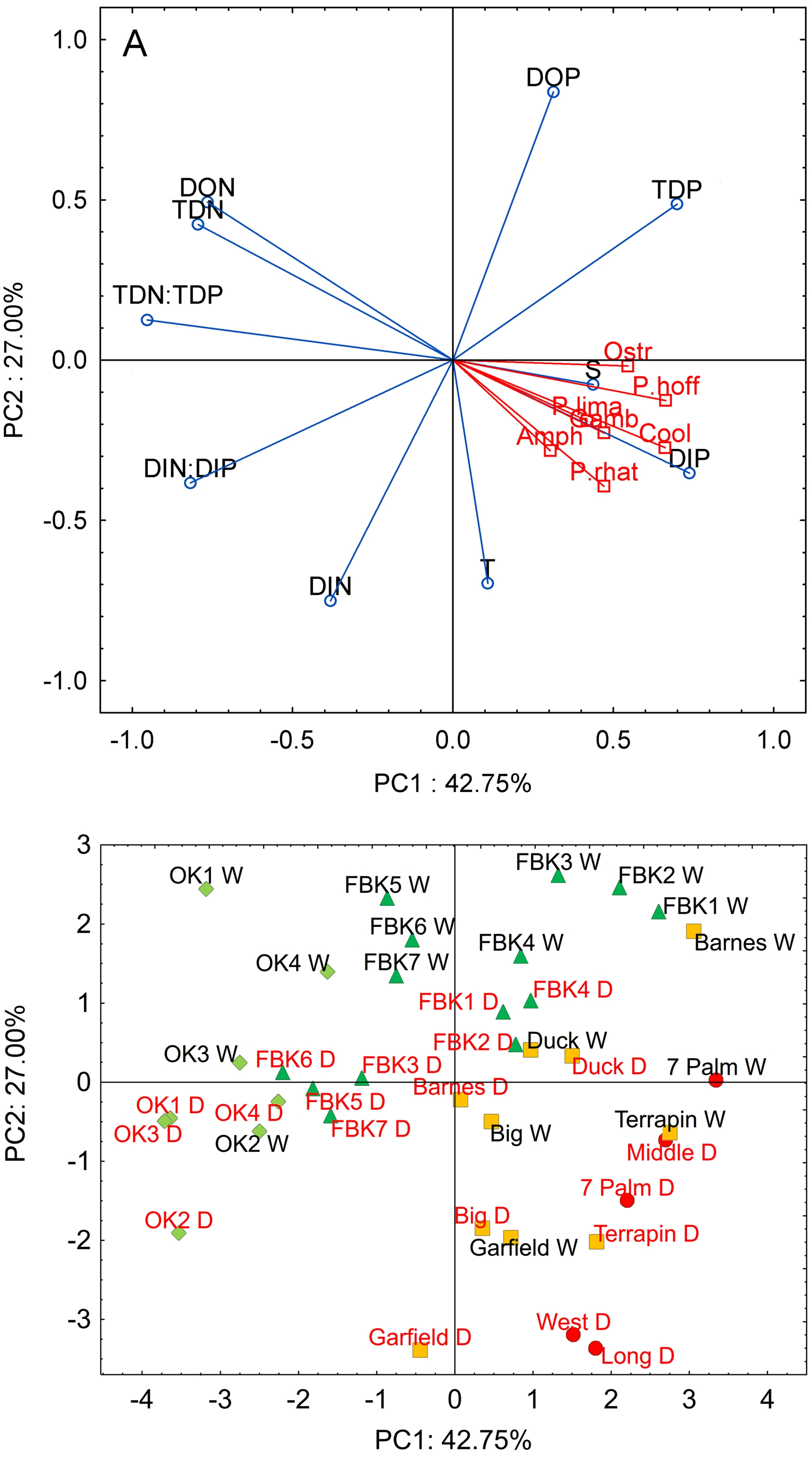

ICOLLs
FB
FBK
OK
A
Salinity
Temperature (°C)
0
10
20
30
40
50
60
0
5
10
15
20
25
30
35
B
Nitrogen (µmol L-1)
0
40
80
120
160
C
Phosphorus (µmol L-1)
0.0
0.5
1.0
1.5
2.0
2.5
D
Nitrogen:Phosphorus
0
100
200
300
400
Seven Palm Lake
Middle Lake
West Lake
Long Lake
Barnes Sound
Duck Key
Terrapin bay
Garfield Bight
Big Key
FBK1
FBK2
FBK3
FBK4
FBK5
FBK6
FBK7
OK1
OK2
OK3
OK4

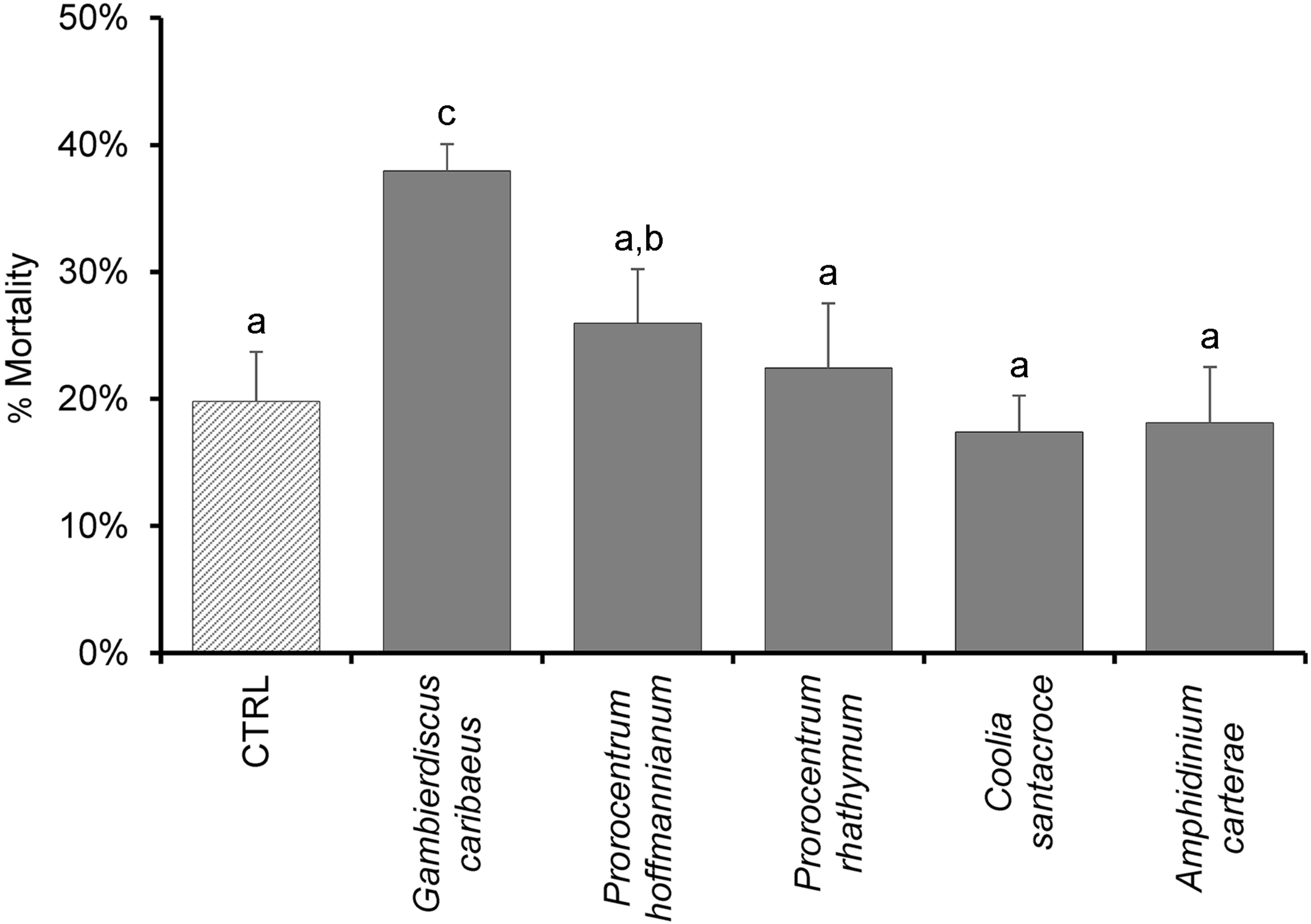

Gambierdiscus
Others
Prorocentrum rhathymum
Amphidinium
Prorocentrum lima
Ostreopsis
Prorocentrum hoffmannianum
Prorocentrum cf. emarginatum
Coolia


ICOLLs
FB
FBK
OK
A
B
C
D
cells cm^{-2}
3000
1600
1200
800
400
0
percent (%)
100
80
60
40
20
0
Seven Palm Lake
Middle Lake
West Lake
Long Lake
Barnes Sound
Duck Key
Terrapin bay
Garfield Bight
Big Key
FBK1
FBK2
FBK3
FBK4
FBK5
FBK6
FBK7
OK1
OK2
OK3
OK4
Prorocentrum cf. emarginatum
Prorocentrum hoffmannianum
Prorocentrum lima
Prorocentrum rhathymum
Coolia
Ostreopsis
Amphidinium
Gambierdiscus
Fukuyoa
Thin walled Cysts


A
B


CSFLK2014 (MN491888)
Cos1503-1 (KT288057)
Cos1503-2 (KT288058)
Cs1303-1 (Kp172271)
Cos1503GA (KT288059)
Dn159EHU (KF896846)
Dn55EHU (KF896851)
CBA1 (AM902742)
UoA-Cmon1 (EU477760)
VGO783 (AM902747)
CmLd04 (KR605310)
CmLd03 (KR605309)
CmLd02 (KR605308)
CMJJ1 (FR847218)
CMJJ2 (FR847221)
CCMP1345 (AM902743)
Cm1412-2 (KR093174)
Cm1303-1 (KP172269)
CAWD175 (KJ422856)
CAWD154 (KJ422855)
CCMP1744 (AM902741)
K-1156 (JX896691)
CCJJ1 (FR846193)
Dn29EHU (HQ414221)
Dn137EHU (KF896838)
VGO786 (AM902737)
VGO787 (AM902738)
O. cf. *ovata* OOAN0601 (GQ380659)
87/0.91
71/0.98
100/1
41/0.88
42/0.94
100/1
100/1
100/1
100/1
C. santacroce
C. monotis
C. malayensis
C. tropicalis
C. canariensis
0.08 substitutions/site


A
DOP
TDP
DON
TDN
TDN:TDP
Ostr
S
P.hoff
P.lima
Gamb
Cool
Amph
DIP
P.rhat
DIN:DIP
T
DIN
1.0
0.5
0.0
-0.5
-1.0
PC2 : 27.00%
PC1 : 42.75%
OK1 W
FBK5 W
FBK3 W
FBK2 W
FBK1 W
Barnes W
FBK6 W
FBK4 W
OK4 W
FBK7 W
FBK1 D
FBK4 D
FBK2 D
Duck W
OK3 W
FBK6 D
FBK3 D
Duck D
7 Palm W
Barnes D
OK1 D
OK4 D
FBK5 D
Terrapin W
OK3 D
OK2 W
FBK7 D
Big W
Middle D
7 Palm D
OK2 D
Big D
Terrapin D
Garfield W
West D
Garfield D
Long D
3
2
1
0
-1
-2
-3
-4
4
PC2: 27.00%
PC1: 42.75%


50%
40%
30%
20%
10%
0%
% Mortality
a
c
a,b
a
a
a
CTRL
Gambierdiscus caribaeus
Prorocentrum hoffmannianum
Prorocentrum rhathymum
Coolia santacroce
Amphidinium carterae