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*Original*

Distribution and potential toxicity of benthic harmful dinoflagellates in waters of Florida Bay and the Florida Keys / Accoroni, Stefano; Totti, Cecilia; Romagnoli, Tiziana; Giulietti, Sonia; Glibert, Patricia M.. - In: MARINE ENVIRONMENTAL RESEARCH. - ISSN 0141-1136. - 155:(2020).  
[10.1016/j.marenvres.2020.104891]

*Availability:*

This version is available at: 11566/285345 since: 2024-03-26T15:54:07Z

*Publisher:*

*Published*

DOI:10.1016/j.marenvres.2020.104891

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

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25

27   **Abstract**

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

39

40   **Keywords**

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

42

## 43    **1. Introduction**

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

66    Ciguatera Fish Poisoning (CFP) is one of the most relevant seafood-borne illnesses worldwide  
67    caused by the ingestion of fish species claimed to bioaccumulate lipophilic ciguatoxins in the food  
68    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

95 availability of both inorganic and organic nutrient concentrations on the growth, abundances and  
96 distribution of benthic dinoflagellates (Accoroni et al., 2017b; Glibert et al., 2012; Parsons and  
97 Preskitt, 2007; Skinner et al., 2013).

98 Florida Bay is located between the southern end of the Florida mainland (i.e. the Florida  
99 Everglades) and the Florida Keys, USA. Florida Bay has witnessed many ecological changes in the  
100 past decades. Since the onset of industrialization in the 1880s, the health of the Florida Bay  
101 ecosystem has been negatively impacted on both decadal (e.g. increasing eutrophication) and  
102 centurial (e.g. changes in land use and water management practices within southern Florida) time-  
103 scales (Briceño and Boyer, 2010; Fourqurean and Robblee, 1999). Phytoplankton of Florida Bay are  
104 generally considered to be limited by availability of phosphorus (P) in the eastern region of the Bay  
105 and by nitrogen (N) in the western region, although different sources of N and P have been shown  
106 to stimulate different components of the algal and bacterial communities (Glibert et al. 2004).

107 Agricultural and human development have been intensive in recent decades in southern Florida and  
108 the Florida Keys and have changed nutrient inputs to Florida Bay (Lapointe and Clark, 1992;  
109 Rudnick et al., 2005; Shangguan et al., 2017). As a warm, subtropical, shallow coastal lagoon with  
110 increasing nutrient enrichment, it is thought to be a prime area for the occurrence of, if not  
111 proliferation of, BHABs. Yet, in contrast to the number of studies conducted in Florida Bay with  
112 respect to the nutrient regime and planktonic microalgae, toxic benthic dinoflagellates have been  
113 comparatively understudied.

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

119

## 120 **2. Materials and methods**

## 121 2.1. Study area and sampling locations

122 Samples were collected in several distinct regions of Florida Bay: in eastern Florida Bay, in the  
123 enclosed sub-lagoons of the northern Bay at the southern edge of the Everglades, in the waters  
124 along the Bay side and enclosed canals of Florida Keys, and along the ocean side of Florida Keys  
125 (Fig. 1). Florida Bay is shallow (average depth 1.5 m), approximately 2200 km<sup>2</sup> in area, and  
126 connected to the Everglades wetland system at its northern boundary (Florida, USA). It has an open  
127 boundary with the Gulf of Mexico in the west, but the connection with the Atlantic Ocean on the  
128 eastern and southern boundaries is restricted to narrow tidal passes in the Florida Keys. The Bay has  
129 an average annual temperature of 25 °C with a mean monthly low temperature of 20 °C in January  
130 and a mean monthly high temperature of 28 °C in August (Fourqurean and Robblee, 1999), and two  
131 distinct meteorological seasons: a November–April dry season and a May–October rainy season  
132 (Duever et al., 1994). Riverine input in the northern boundary is considerable in the wet season, and  
133 the major freshwater input is through slow sheet flow from the Everglades watershed (Lee et al.,  
134 2007). Sample collection occurred in the eastern and northern edge of Florida Bay at 5 stations  
135 (Garfield Bight, Terrapin Bay, Duck Key, Big Key and Barnes Sound) (Fig. 1).  
136 Along the northern border of Florida Bay, at the interface between the Everglades and the northern  
137 Florida Bay, there are a series of saline lakes. These lakes function as quasi-Intermittent Closed-  
138 Open Lakes and Lagoons (ICOLs) (Frankovich et al., 2011; Maher et al., 2011; Shangguan et al.,  
139 2017; Suzuki et al., 1998) are connected to each other, and receive limited or even reversed flow  
140 during the dry season. They are considered to be particularly sensitive to changes in salinity and  
141 nutrients that are occurring, or may occur, due to ongoing Everglades restoration projects  
142 (Shangguan et al., 2017). There are two distinct interconnected sets, or chains, of these quasi-  
143 ICOLs, each of which was previously characterized as poised at different stable states (*sensu*  
144 Scheffer and Carpenter, 2003). One ICOLL chain is eutrophic and in a planktonic-dominated state  
145 (West Lake, Long Lake), while the second ICOLL chain is more oligotrophic and in a benthic-  
146 dominated state (Seven Palm Lake, Middle Lake; based on the overall assessment of chlorophyll *a*

147 (chl *a*) and submerged aquatic vegetation (SAV) (Bricker et al., 2008; Frankovich et al., 2011;  
148 Shangguan et al., 2017; USEPA, 2005). Both the eutrophic and oligotrophic ICOLLs were sampled  
149 (Fig. 1).

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

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

158

## 159 2.2. Sampling and sample treatment

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

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

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

192

### 193 *2.3. Microscope analysis*

194 The identification and counting of dinoflagellate cells were carried out using an inverted  
195 microscope (Nikon Eclipse TE2000-U) equipped with phase contrast at 200× magnification. Sub-  
196 samples (5-25 mL) were settled in combined sedimentation chambers after homogenization,  
197 according to the Utermöhl sedimentation method (Edler and Elbrächter, 2010). Identification and  
198 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<sup>-2</sup> and cells g<sup>-1</sup> 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<sup>-2</sup> s<sup>-1</sup>, 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<sup>TM</sup> (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

225 directly Sanger-sequenced by Macrogen Europe (Amsterdam, The Netherlands). Sequences were  
226 compared to the NCBI database by BLAST search with default settings  
227 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). This GenBank screening confirmed the strains isolated as  
228 *Prorocentrum rhathymum* (PRKL0414), *Coolia santacroce* (CSKL0414), *Amphidinium carterae*  
229 (ACKL0414), *Gambierdiscus caribaeus* (GCKL0414) and *Prorocentrum hoffmannianum*  
230 (PHKL0414).

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

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

240 The robustness of the clades recovered was evaluated by 1,000 bootstrap pseudo-replicates.  
241 Bayesian analyses were carried out using MrBayes 3.2 (Ronquist et al., 2012) with 3,000,000  
242 Markov chain Monte Carlo generations with a sample frequency of 1,500. The 50% majority rule  
243 consensus tree was constructed using burn-in of 25% and posterior probabilities were calculated to  
244 measure tree strength. Pairwise distance estimations were conducted with MEGA package (Kumar  
245 et al., 2016).

246

#### 247 2.5. Bioassay determination of putative toxicity

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

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

262

## 263 2.6. Scanning electron microscope analysis

264 Some preserved subsamples (1 mL), obtained from both field and culture samples, were dehydrated  
265 by immersion in ethanol at increasing gradations (10%, 30%, 50%, 70%, 80%, 90%, 95%, and  
266 100%). After 24 hrs in absolute ethanol, they were filtered on a Nucleopore polycarbonate filter and  
267 dried using a Critical Point Dryer (Polaron CPD 7501, Quorum Technologies, Newhaven, UK). The  
268 filters were then placed on stubs and coated with gold-palladium in a Sputter Coater (Polaron SC  
269 7640, Quorum Technologies, Newhaven, UK) for observation under the scanning electron  
270 microscopy (FE-SEM; Zeiss Supra 40) (EHT =15.00 kV).

271

## 272 2.7. Nutrient analysis

273 Concentrations of NO<sub>x</sub><sup>-</sup> (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>), NH<sub>4</sub><sup>+</sup>, and dissolved inorganic phosphorus (DIP) were  
274 measured using autoanalysis techniques. Concentrations of TDN and TDP were determined by  
275 persulfate oxidation (Solórzano and Sharp, 1980 for TDP; Valderrama, 1981). Concentrations of  
276 DON and DOP were determined by the difference between dissolved total and inorganic fractions.



277

## 278 2.8. Statistical analysis

279 All statistical analyses were conducted using Statistica (StatSoft Inc., Tulsa, OK, USA) software.  
280 The Shapiro-Wilks test was used to check data for normal distribution, while the Levene's test was  
281 used to assess homogeneity of variance. Non-parametric tests were used when tests did not reveal  
282 homogeneous variances and/or normal distributions. Correlations among environmental parameters  
283 and benthic dinoflagellate abundances were examined using Spearman-Rank order correlation,  
284 while Kruskal-Wallis ANOVA was used to assess differences in (i) the environmental parameters  
285 and (ii) the benthic dinoflagellate distribution between ICOLL, FB, FBK and OK stations and  
286 seasons (wet and dry). A Principal Component Analysis (PCA) was performed on a correlation  
287 matrix of ranked physical and chemical variables to characterize the different stations based on  
288 environmental features. Temperature, salinity, nutrient concentration (i.e. TDN, DON, DIN, TDP,  
289 DOP and DIP) and their ratios (TDN:TDP and DIN:DIP) were chosen as the best variables to  
290 characterize the environmental conditions of different stations and abundance values of benthic  
291 dinoflagellates were used as supplementary variables.  
292 Comparisons between Spearman's and Pearson's correlations were made to determine both  
293 monotonic and linear relationships between abundances of benthic dinoflagellate recorded in water  
294 column and benthic substrata. Finally, differences in terms of larval mortality of oyster among  
295 benthic dinoflagellate cultures were assessed through an ANOVA test. When significant differences  
296 for the main effect were detected ( $p < 0.05$ ), a post-hoc Tukey's pairwise test was also performed.

297

## 298 3. Results

### 299 3.1. Environmental parameters

300 In the study period, water temperature fluctuated from a minimum value of 25.8 °C in OK2 in the  
301 dry season to a maximum of 31.4 °C in FBK3 in the wet season (Fig. 2A), showing values  
302 significantly different between the two seasons (average values  $28.1 \pm 1.5$  and  $29.5 \pm 1.1$  °C,

303 respectively for dry and wet season) (Kruskal Wallis,  $p < 0.01$ ). Significantly lower temperature  
 304 values were observed in the ICOLL than in FBK sites (Kruskal Wallis,  $p < 0.01$ ). Salinity ranged  
 305 from 8.1 (Seven Palm Lake) to 44.8 (Garfield Bight) (average values  $31.1 \pm 11.3$  and  $34.4 \pm 6.9$ ,  
 306 respectively for dry and wet season) (Fig.2A), Significantly lower salinity values were observed in  
 307 the ICOLL sites compared with sites in FB, FBK and OK (Kruskal Wallis,  $p < 0.05$ , 0.001 and 0.05,  
 308 respectively).  
 309 Total Dissolved Nitrogen (TDN), and its component fractions varied widely between sites and  
 310 seasons. Highest TDN concentrations were observed in the ICOLL and FB sites and much lower in  
 311 the FBK and OK sites (Fig. 2B); significantly lower TDN values were observed in OK than in  
 312 ICOLLs and in FB (Kruskal Wallis, both  $p < 0.001$ ). Most of the TDN was composed of DON  
 313 across all stations. Moreover, significantly lower DON values were observed in OK than in ICOLLs  
 314 and in FB (Kruskal Wallis, both  $p < 0.001$ ) and significant lower values were observed in FBK than  
 315 in FB (Kruskal Wallis,  $p < 0.05$ ). Dissolved Inorganic Nitrogen (DIN), obtained as the sum of  $\text{NO}_x^-$   
 316 and  $\text{NH}_4^+$ , ranged from 0.75 to 19.63  $\mu\text{mol l}^{-1}$  (in OK2 in the dry season and in FB2 in the wet  
 317 season, respectively, Fig. 2B) and showed values significantly different between the two seasons  
 318 (Kruskal Wallis,  $p < 0.01$ ).  
 319 Total dissolved P (TDP) also varied considerably between sites. Significantly higher TDP values  
 320 were observed in OK than in ICOLLs and in FBK (Kruskal Wallis, both  $p < 0.05$ ). Much of this  
 321 variability was due to the variability in dissolved inorganic P (DIP), which ranged from 0.053 to  
 322 1.855  $\mu\text{mol l}^{-1}$  (in Seven Palm Lake in dry season and in OK1 in wet season, respectively, Fig. 1C),  
 323 and showed values significantly different between the two seasons (Kruskal Wallis,  $p < 0.001$ ).  
 324 Significantly higher DIP values were observed in OK than in ICOLLs and in FB (Kruskal Wallis,  $p$   
 325  $< 0.001$  and 0.05, respectively) and significantly higher values were observed in FBK than in  
 326 ICOLLs (Kruskal Wallis,  $p < 0.01$ ).  
 327 Molar ratios of DIN:DIP and TDN:TDP ranged from 3 to 108 and from 9 to 404, respectively (Fig.  
 328 2D). Significantly lower DIN:DIP and TDN:TDP values were observed in OK than in ICOLLs, FB

329 and FBK (Kruskal Wallis  $p < 0.05$ ,  $0.01$  and  $p < 0.001$ , respectively for DIN:DIP and  $p < 0.001$ ,  
330  $0.001$  and  $p < 0.05$ , respectively for TDN:TDP).

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

335

### 336 3.2. Composition, abundances and spatial distribution of benthic dinoflagellates

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

355 *Prorocentrum* species, *Prorocentrum* cf. *emarginatum* was the least represented in the  
 356 dinoflagellate community (0.4% Fig. 3), with the highest abundances recorded in OK4 in the wet  
 357 season (28 cells cm<sup>-2</sup>, corresponding to 590 cells g<sup>-1</sup> fw).  
 358 Next to *Prorocentrum rhathymum*, the most represented taxa were *Coolia* spp. and *Ostreopsis* spp.  
 359 They represented on average 21 and 20% of the BHABs, respectively (Fig. 3). Their highest  
 360 abundances were recorded in OK3 in the dry season (1,634 cells cm<sup>-2</sup>, corresponding to 84,500 cells  
 361 g<sup>-1</sup> fw and 533 cells cm<sup>-2</sup>, corresponding to 27,592 cells g<sup>-1</sup> fw, respectively). *Coolia* showed  
 362 significantly higher abundances in OK than in ICOLLs and FB (Kruskal Wallis, both  $p < 0.01$ ).  
 363 *Ostreopsis* abundances were higher in OK than in the other stations, significantly compared with  
 364 FB (Kruskal Wallis,  $p < 0.05$ ).  
 365 *Amphidinium* spp., represented on average 16% of the entire community (Fig. 3), and had  
 366 abundances not exceeding 60 cells cm<sup>-2</sup>, with the only exception in the wet season in OK1 (1081  
 367 cells cm<sup>-2</sup>, corresponding to 55,900 cells g<sup>-1</sup> fw).  
 368 *Gambierdiscus* spp. were sporadically recorded and represented on average 5% of the entire  
 369 dinoflagellate community (Fig. 3), reaching the highest abundances in OK1 in the dry season (325  
 370 cells cm<sup>-2</sup>, corresponding to 16,800 cells g<sup>-1</sup> fw). *Fukuyoa* spp. were rarely recorded, with the  
 371 highest abundances in FBK7 in the dry season (3 cells cm<sup>-2</sup>, corresponding to 78 cells g<sup>-1</sup> fw).  
 372 *Sinophysis* spp. were scarcely represented in the dinoflagellate community with the highest  
 373 abundances recorded in FBK7 in the wet season (13 cells cm<sup>-2</sup>, corresponding to 389 cells g<sup>-1</sup> fw).  
 374 Among the benthic dinoflagellate community, some thin walled cysts were sporadically recorded,  
 375 with the highest abundances in FBK7 in the wet season (8 cells cm<sup>-2</sup>, corresponding to 229 cells g<sup>-1</sup>  
 376 fw).  
 377 Mean abundances of the benthic dinoflagellate taxa (as well as their total abundances) estimated  
 378 over the sampling period were statistically different between the seasons only for *Sinophysis*  
 379 (Kruskal Wallis,  $p < 0.05$ ). Considering the two seasons, in the dry season the highest dinoflagellate  
 380 abundance was observed in OK3 (2867 cells cm<sup>-2</sup>, corresponding to 132,786 cells g<sup>-1</sup> fw) in which

381 *Coolia* spp. represented the 57% of the entire benthic dinoflagellate community, followed by  
 382 *Ostreopsis* spp. *P. lima*, *P. rhathymum*, *P. hoffmannianum* and *Amphidinium* spp. (19, 12, 9, 3 and  
 383 1% respectively; Fig. 4A,C). Although *Coolia* spp. were recorded with the highest abundances in  
 384 this season (1634 cells cm<sup>-2</sup>, corresponding to 84,500 cells g<sup>-1</sup> fw), the community was dominated  
 385 by *Ostreopsis* spp. and *Gambierdiscus* spp., both with values up to 62% of the entire dinoflagellate  
 386 community in OK4 and OK1, respectively.

387 In the wet season, the highest abundances of BHABs were observed in OK1 (1256 cells cm<sup>-2</sup>,  
 388 corresponding to 64,949 cells g<sup>-1</sup> fw) where *Amphidinium* spp. represented 86% of the entire  
 389 benthic dinoflagellate community, followed by *P. rhathymum* and *Coolia* spp. (12, and 1%  
 390 respectively; Fig. 4B,D). Although *Amphidinium* spp. was recorded with the highest abundances  
 391 (1081 cells cm<sup>-2</sup>, corresponding to 55,900 cells g<sup>-1</sup> fw), in this season the community was  
 392 dominated by *P. rhathymum* with values up to 100% of the entire dinoflagellate community in  
 393 Barnes Sound, followed by *Amphidinium* spp. and *Ostreopsis* spp. with values up to 86% in OK1  
 394 and 82% in OK2 of the entire dinoflagellate community, respectively.

395 Ultrastructural (SEM) observations and rDNA characterizations in selected field and culture  
 396 samples revealed the presence of benthic dinoflagellate taxa not revealed by light microscopy:  
 397 *Ostreopsis heptagona*, *Coolia santacroce*, *Amphidinium carterae*, *Gambierdiscus caribaeus*.  
 398 Because of its recent description (Karafas et al., 2015) and because this study is the first to  
 399 document its occurrence in the Florida Keys, the identification of *Coolia santacroce* (Fig. 5) was  
 400 more extensively examined. Comparisons of its sequences were made to the NCBI database by  
 401 BLAST, and a phylogeny tree with 27 *Coolia* spp. retrieved from Genbank was also constructed.  
 402 Analyses of ML and BI yielded identical topologies; only the ML tree is shown with bootstrap and  
 403 posterior probability values at nodes (Fig. 6). ML and BI analysis revealed the strain of this study  
 404 (CSKL0414) fell into *C. santacroce* lineage with a well-supported bootstrap value (87). There was a  
 405 small p-distance value (0.063) between CSKL0414 and holotype strain of *C. santacroce*  
 406 (KP172271), while smallest p-distance value (0.019) was between the other three *C. santacroce*

407 strains (KT288057, KT288058, KT288059). Indeed, the mean p-distance estimation calculated  
408 between CSKL0414 and the other *C. santacroce* strains was 0.072. Furthermore, *C. santacroce*  
409 group is closely related to *C. monotis* (mean p-distance value 0.136). Indeed, they clustered together  
410 with well supported bootstrap value of 71 and strongly supported posterior probability of 0.98.

411

### 412 3.3. Relationships between benthic dinoflagellate abundances and environmental parameters

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

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

428

### 429 3.4. Benthic versus planktonic cells

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

433 *lima*, and *P. hoffmannianum* (Table 2). Comparisons between Pearson's and Spearman's  
434 correlations of benthic and planktonic abundances showed a linear rather than monotonic  
435 relationship (except *P. rhathymum* and *Ostreopsis* spp. which did not show a linear relationship at  
436 all).  
437 Interpreting the slope of the fitting line of these relationships suggests that certain benthic  
438 dinoflagellates were able to detach more easily than others from a colonized substrate. Taxa ranked  
439 as follows: *P. rhathymum*, *Gambierdiscus* spp., *P. hoffmannianum*, *P. lima* and *Coolia* spp. (7.41,  
440 3.97, 0.78, 0.55 and 0.26, respectively). Moreover, some toxic planktonic dinoflagellates were  
441 detected in the water column that were not seen in the benthos (Table 3).

### 442 443 3.5. Toxic effects of cultured benthic dinoflagellates on larval growth

444 The species brought into culture were used in oyster larval bioassays to test for toxicity. Larval  
445 mortality in the whole experiment ranged between 13 and 39% (Fig. 8). The treatment with  
446 *Gambierdiscus caribaeus* induced a clear larval mortality with values ( $38 \pm 2\%$ ) significantly higher  
447 than those of the control and those of other treatments (Tukey HSD test,  $p < 0.05$  for *Prorocentrum*  
448 *hoffmannianum* and  $p < 0.01$  for *P. rhathymum*, *C. santacroce*, *A. carterae* and the control). While  
449 the treatments with *Coolia santacroce* and *Amphidinium carterae* showed larval mortality with  
450 lower values ( $17 \pm 3$  and  $18 \pm 4\%$ , respectively) than those of the control ( $20 \pm 4\%$ ), those with  
451 *Prorocentrum hoffmannianum* and *P. rhathymum* had slightly, but not significant higher mortality  
452 ( $26 \pm 4$  and  $22 \pm 5\%$ ) than the control.

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

456

#### 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



483 previously been well studied highlighting that the onset of dinoflagellate blooms is often associated  
484 with N:P ratios even lower than Redfield, sometimes from an injection of P-rich water (Accoroni et  
485 al., 2015; Anderson et al., 2002; Fang, 2004; Glibert et al., 2012; Hodgkiss and Ho, 1997; Li et al.,  
486 2009; Riegman, 1995).

487 Florida Bay has undergone, and continues to undergo major changes in nutrient loads. Florida Bay  
488 experienced substantial ecological degradation in the late 1980s and although subsequently  
489 recovering, there have been environmental concerns since then. In September 2005, a  
490 phytoplankton bloom (dominated by *Synechococcus*) was initiated in the eastern bay region from a  
491 series of hurricanes (Katrina, Rita and Wilma) and by disturbance (mangrove removal, sediment  
492 resuspension, etc.) due to construction of the causeway connecting the mainland and Florida Keys  
493 (Glibert et al. 2009). This bloom resulted in the release of P which sustained the bloom for 3 years  
494 after which the system did not return to pre-bloom conditions, but instead formed a new baseline  
495 with significantly higher chlorophyll concentrations (Millette et al. 2018). One reason for the  
496 sustained increase in chlorophyll is a presumed loss of SAV (Glibert et al. 2009, Millette et al.  
497 2018), driving the system toward a more planktonic condition. In 2012 the Comprehensive  
498 Everglades Restoration Plan was initiated, bringing increased flow into northern Florida Bay,  
499 influencing the productivity of the ICOLLs (Shangguan et al. 2017). Those lakes that has been  
500 oligotrophic prior to the project experienced an approximate doubling of phytoplankton biomass as  
501 nutrient ratios converged on Redfield proportions, and a shift to smaller sized cells and a loss of  
502 diatoms as  $\text{NO}_x^-$  availability declined. The other set of these lakes, previously highly eutrophic,  
503 sustained a significant, nearly 50%, decline in overall phytoplankton biomass, particularly larger  
504 sized cells, which, in turn, increased downstream. These findings have established that altered flow  
505 in Florida Bay affects not only salinity but also nutrients, in terms of total loads and proportions,  
506 and these changes can create conditions where phytoplankton assemblages change in composition.  
507 As a whole, Florida Bay has been a benthic dominated system with P as a controlling nutrient, but

508 this is a system under multiple stresses and highly responsive to changes in flow and nutrients.  
 509 Understanding how the benthic community responds to these changes is important.  
 510 Accoroni et al. (2015), building on the conceptual framework of Glibert et al. (2012), suggested that  
 511 blooms of BHABs may form when stimulated by a “flush” of nutrients or organic materials that  
 512 may allow a latent resident population to flourish. After that growth rate increase, bloom biomass is  
 513 able to increase often accompanied by N:P values greater than Redfield. At this point, these blooms  
 514 are able to sustain high biomass levels, not necessarily by high growth rates, but through other  
 515 mechanisms, including (i) metabolic dissipatory strategies that allow the maintenance of cellular  
 516 nutrient and energy balance in an environment where energy flow and nutrients are provided at  
 517 unbalanced proportions and (ii) allelopathic and mixotrophic interactions with other species. This  
 518 conceptual model was supported by data on *Ostreopsis* in the northern Adriatic Sea. Thus, adaptive  
 519 physiology may enable the maintenance of BHAB taxa at less than maximal growth rates and at  
 520 non-optimal N:P ratios.  
 521 In this study, seven genera of benthic dinoflagellates were detected, *Amphidinium*, *Coolia*,  
 522 *Fukuyoa*, *Gambierdiscus*, *Ostreopsis*, *Prorocentrum* and *Sinophysis*. All these genera, except  
 523 *Sinophysis*, include potentially toxic species. These genera have already been reported in other  
 524 studies previously carried out in Florida Keys (Norris et al., 1985), and in other tropical Atlantic  
 525 areas such as Bahamas Archipelago (Bomber et al., 1988), Belize (Faust, 2000, 1993), Gulf of  
 526 Mexico (Tester et al., 2013), Cuba (Delgado et al., 2006), Porto Rico (Ballantine et al., 1988), and  
 527 Virgin Islands (Carlson and Tindall, 1985).  
 528 *Prorocentrum* is typically regarded as one of the dominant genera among the benthic dinoflagellates  
 529 (Delgado et al., 2006; Glibert et al., 2012; Okolodkov et al., 2014; Richlen and Lobel, 2011; Tester  
 530 et al., 2014) as observed in this study, where it showed the highest percent abundances among the  
 531 benthic dinoflagellate community. It was mainly represented by *P. rhathymum* (that is a well-  
 532 known okadaic acid (OA) producer in Florida Bay, An et al., 2010), followed by *P. lima*, *P.*  
 533 *hoffmannianum* and *Prorocentrum* cf. *emarginatum* (all known for producing Diarrhetic Shellfish

534 Poisoning (DSP) toxins including the lipid-soluble polyether compounds OA, methylokadaic acid,  
 535 and/or dinophysistoxins, other OA derivatives, and prorocentrolides, a water-soluble “fast-acting  
 536 toxin”; Lee et al., 2016). *Prorocentrum* was detected in each site in this study reaching maximum  
 537 abundances ( $10^4$  cells  $g^{-1}$  fw) comparable to those reported in other studies performed in other  
 538 tropical environments (Boisnoir et al., 2019; Delgado et al., 2006; Díaz-Asencio et al., 2019;  
 539 Okolodkov et al., 2014) and higher than those reported in some others ( $10^3$  cells  $g^{-1}$  fw, Tester et al.,  
 540 2014;  $10^2$  cells  $g^{-1}$  fw, Yong et al., 2018).

541 Observed maximum abundances of *Coolia* spp. in this study (i.e.  $10^4$  cells  $g^{-1}$  fw) were higher than  
 542 those observed in the Gulf of Mexico and in the Lesser Antilles (Caribbean Sea) ( $10^3$  cells  $g^{-1}$  fw,  
 543 (Boisnoir et al., 2019; Okolodkov et al., 2007). This genus is globally distributed in tropical and  
 544 temperate coastal and estuarine waters but most species are found in tropical regions (Leaw et al.,  
 545 2016). Reports of toxin production in *Coolia* have been often inconsistent and only *C. tropicalis*  
 546 and *C. malayensis* are considered to be certainly toxic to date (Mohammad-Noor et al., 2013;  
 547 Rhodes et al., 2014).

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

557 Species of the genus *Ostreopsis* include those able to produce toxins mostly belonging to the  
 558 palytoxin group (Accoroni et al., 2016; Brissard et al., 2015; Ciminiello et al., 2006; Holmes et al.,  
 559 1988; Lenoir et al., 2004; Meunier et al., 1997; Nakajima et al., 1981; Uchida et al., 2013;

560 Yasumoto et al., 2007). In this study, *Ostreopsis* spp. were mostly recorded along the Florida Keys,  
561 reaching maximum abundances ( $10^4$  cells  $g^{-1}$  fw) comparable to those recorded in the island of  
562 Hawai'i (Parsons and Preskitt, 2007) and higher than those previously reported in the same area and  
563 in other tropical environments, i.e.  $10^2$  cells  $g^{-1}$  fw in the Florida Keys, the northwestern coast of  
564 Cuba and Malaysia (Bomber et al., 1988; Delgado et al., 2006; Tester et al., 2014),  $10^3$  cells  $g^{-1}$  fw  
565 in the Gulf of Mexico, Puerto-Rico, Guadeloupe and Belize (Ballantine et al., 1985; Boisnoir et al.,  
566 2018; Faust, 2009; Okolodkov et al., 2007), but lower than in the Lesser Antilles (Caribbean Sea)  
567 and south central Cuba ( $10^5$  cells  $g^{-1}$  fw, Boisnoir et al., 2019; Díaz-Asencio et al., 2019). These  
568 benthic marine dinoflagellates have been commonly associated with tropical areas where they show  
569 the highest diversity (Parsons et al., 2012; Rhodes, 2011), while their appearance in temperate  
570 latitudes is considered a recent phenomenon (Corriero et al., 2016; Zenetos et al., 2005). However,  
571 it is noteworthy that the abundances reached in some temperate areas are considerably higher ( $10^6$   
572 cells  $g^{-1}$  fw e.g., Mangialajo et al., 2011; Vassalli et al., 2018) than those reported in tropical ones,  
573 causing serious problems on human health, mainly due to inhalation of sea water droplets  
574 containing *Ostreopsis* cells or fragments and/or aerosolized toxins, or cutaneous contact with cells,  
575 causing skin and eye irritations (Gallitelli et al., 2005; Tichadou et al., 2010; Vila et al., 2016). On  
576 the contrary, in tropical areas *Ostreopsis* is strongly suspected to be involved in several events of  
577 clupeotoxism, a human intoxication after consuming contaminated crustaceans and fish  
578 characterized by symptomology similar to that of ciguatera but with a much higher mortality rate  
579 (e.g. Onuma et al., 1999; Randall, 2005), but other types of intoxications common in temperate  
580 areas associated with high *Ostreopsis* abundances are uncommon. Among the *Ostreopsis* species  
581 present in the study area, *Ostreopsis heptagona* have been identified by ultrastructural (SEM)  
582 observations. This species was previously determined to be toxic as methanol extracts of a culture  
583 isolated from Knight Key (Florida) were weakly toxic to mice (Babinchak, according to Norris et  
584 al., 1985).

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

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

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

618 The benthic dinoflagellates recorded in this study have been often detected in water column as well.  
619 Results showed that certain benthic dinoflagellates were able to detach more easily than others from  
620 the benthic substrate and hence occupy the planktonic zone, i.e. *P. rhathymum*, *Gambierdiscus* spp.,  
621 *P. hoffmannianum*, *P. lima* and *Coolia* spp. (from the most detachable to the less one). For these  
622 dinoflagellates, correlations of benthic and planktonic abundances showed a linear rather than  
623 monotonic relationship, suggesting that where many cells are on the benthic substrata, they also  
624 colonize the water column. On the contrary, results of *Ostreopsis* benthic and planktonic  
625 abundances did not reveal a linear relationship, suggesting that *Ostreopsis* cells have a  
626 predominantly benthic behavior but can be transferred in water column under certain environmental  
627 conditions. Indeed, several studies have highlighted that both benthic and planktonic abundances of  
628 *Ostreopsis* are highly affected by wave action, since they are only loosely attached to the substrata  
629 and can be easily removed and re-suspended in the water column (reviewed in Accoroni and Totti,  
630 2016). The fact that many of those benthic toxigenic species can be easily recorded in water column  
631 may make them relatively more available as food resources for filter-feeding shellfish than when  
632 they grow on benthic substrata, increasing the risk of human intoxication through seafood  
633 consumption.

634

## 635 **5. Summary and Conclusions**

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

647

#### 648 **Acknowledgements**

649 SA thanks the Università Politecnica delle Marche that funded one-year postdoc fellowship  
650 concerning the Ecology of harmful dinoflagellates at the University of Maryland Center for  
651 Environmental Science (MD, USA). Field work was conducted in conjunction with studies on  
652 nutrient changes in Florida Bay and in the ICOLLs supported by funds from the South Florida  
653 Water Management District to PMG. We thank J. Alexander, S. Kelly, M. Blaha, and J. Stachelek  
654 for field work assistance, and the Horn Point Laboratory Analytical Services for analytical  
655 assistance as well as 2 reviewers for helpful comments. This is contribution number XXXX from  
656 the University of Maryland Center for Environmental Science.

657

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 1098



1099 Table 1. Spearman-Rank order correlation between water temperature (°C), salinity, nutrients (Total  
 1100 Dissolved Nitrogen (TDN), Dissolved Organic Nitrogen (DON), Dissolved Inorganic Nitrogen (DIN),  
 1101 Total Dissolved Phosphorus (TDP), Dissolved Organic Phosphorus (DOP) Dissolved Inorganic  
 1102 Phosphorus (DIP) and Nitrogen:Phosphorus ratios: organic ratio (DON:DOP) and inorganic ratio  
 1103 (DIN:DIP)) and abundances of benthic dinoflagellate taxa (*Prorocentrum* cf. *emarginatum*, *P.*  
 1104 *hoffmannianum*, *P. lima*, *P. rhathymum*, *Coolia*, *Ostreopsis*, *Amphidinium*, *Gambierdiscus*, *Fukuyoa*,  
 1105 thin-walled cysts, *Sinophysis*) throughout (A) the entire study period and in (B) dry and (C) wet  
 1106 seasons separately. Values indicated in italic are significant at  $p < 0.05$ , those in bold italic are  
 1107 significant at  $p < 0.01$ , those in bold italic and underlined are significant at  $p < 0.001$ .

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

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1113 Table 2. Spearman-Rank order ( $r_s$ ) and Pearson ( $r$ ) correlation between abundances of benthic  
 1114 dinoflagellate taxa in the benthos and in the plankton (*Prorocentrum hoffmannianum*, *P. lima*, *P.*  
 1115 *rhathymum*, *Coolia*, *Ostreopsis*, *Gambierdiscus*). Only taxa with significant results are shown. Values  
 1116 indicated in bold italic are significant at  $p < 0.01$ , those in bold italic and underlined are significant  
 1117 at  $p < 0.001$ .

|                         | Spearman's $r_s$   | Pearson's $r$      |
|-------------------------|--------------------|--------------------|
| <i>Ostreopsis</i>       | <b><u>0.69</u></b> | 0.17               |
| <i>Gambierdiscus</i>    | <b><u>0.81</u></b> | <b><u>0.99</u></b> |
| <i>Coolia</i>           | <b><u>0.53</u></b> | <b><u>0.83</u></b> |
| <i>P. rhathymum</i>     | <b><u>0.62</u></b> | <b><u>0.42</u></b> |
| <i>P. lima</i>          | <b><u>0.53</u></b> | <b><u>0.59</u></b> |
| <i>P. hoffmannianum</i> | <b><u>0.54</u></b> | <b><u>0.57</u></b> |

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1120 Table 3. Toxic planktonic dinoflagellates identified in the study area, and relative abundance.  
 1121 ' < 10 cells L<sup>-1</sup>; + = 10 cells L<sup>-1</sup>; ++ = 10<sup>2</sup> cells L<sup>-1</sup>; +++ = 10<sup>3</sup> cells L<sup>-1</sup>; +++++ = 10<sup>4</sup> cells L<sup>-1</sup>.

| STATION         | <i>Prorocentrum<br/>cordatum</i> | <i>Pyrodinium<br/>bahamense</i> | <i>Protoceratium<br/>reticulatum</i> | <i>Gonyaulax<br/>spinifera</i> | <i>Dinophysis<br/>sp.</i> | <i>Margalefidinium<br/>polykrikoides</i> |
|-----------------|----------------------------------|---------------------------------|--------------------------------------|--------------------------------|---------------------------|--|
| 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             | ++                               | ++                              | +                                    |                                |                           | ++                                       |

1122

1123 **Figure captions**

1124 Fig.1. Map of the study area, showing the location of the sampling station along Florida bay and the  
1125 Florida Keys (Florida, USA). (●) ICOLLs (1: Seven Palm Lake; 2: Middle Lake, 3: West Lake, 4:  
1126 Long Lake). (■) FB stations (1: Barnes Sound; 2: Duck Key; 3: Terrapin Bay; 4: Garfield Bight; 5:  
1127 Big Key). Florida Keys stations: (◆) FB-side (1: FBK1; 2: FBK2; 3: FBK3; 4: FBK4; 5: FBK5; 6:  
1128 FBK6; 7: FBK7) and (▲) ocean-side (1: OK1; 2: OK2; 3: OK3; 4: OK4).

1129  
1130 Fig. 2. Spatial variability of environmental parameters at sampling area in dry (full bars and circles)  
1131 and wet (crossed bars and squares) seasons. (A) Salinity (bars, left y-axis) and water temperature  
1132 (circles and squares, right y-axis). (B) Nitrogen fractions ( $\mu\text{mol L}^{-1}$ ): Total Dissolved Nitrogen  
1133 (TDN), with the detail of Dissolved Organic Nitrogen (DON, darker) and Dissolved Inorganic  
1134 Nitrogen (DIN, lighter). (C) Phosphorus fractions ( $\mu\text{mol L}^{-1}$ ): Total Dissolved Phosphorus (TDP),  
1135 with the detail of Dissolved Organic Phosphorus (DOP, darker) and Dissolved Inorganic  
1136 Phosphorus (DIP, lighter). (D) Nitrogen:Phosphorus ratios: organic ratio (DON:DOP, darker) and  
1137 inorganic ratio (DIN:DIP, lighter). Environmental parameters' data for Middle Lake, West Lake  
1138 and Long Lake in wet season are unavailable.

1139

1140 Fig. 3. Relative abundance (%) of each taxon within the benthic dinoflagellate community at the  
1141 entire sampling area.

1142

1143 Fig. 4. Spatial trend in abundances benthic dinoflagellate community at the sampling area in dry (A,  
1144 C) and (B, D) wet seasons. (A, B) Absolute ( $\text{cells cm}^{-2}$ ) and (C, D) percent abundances (%) of toxic  
1145 benthic dinoflagellate taxa (*Prorocentrum* cf. *emarginatum*, *P. hoffmannianum*, *P. lima*, *P.*  
1146 *rhathymum*, *Coolia*, *Ostreopsis*, *Amphidinium*, *Gambierdiscus*, *Fukuyoa*, thin-walled cysts). Data  
1147 from grey-colored stations are unavailable.

1148

1149 Fig. 5. Scanning electron microscopy images of *Coolia santacroce* (CSKL0414) collected from the  
1150 Florida Keys in (A) epithecal and (B) ventral/lateral view (sulcus and cingulum are visible). Scale  
1151 bars = 2µm.

1152

1153 Fig. 6. Bayesian phylogenetic tree of the genus *Coolia* inferred from LSU ribosomal gene sequences.  
1154 The tree is rooted with *Ostreopsis* cf. *ovata* OOAN0601 as outgroup. Nodes are annotated with ML  
1155 bootstrap values followed by posterior probabilities. Strain sequenced in this study is in bold.

1156

1157 Fig. 7. Principal Component Analysis (PCA) based on correlation matrix of ranked environmental  
1158 variables and abundance values of the most represented taxa (> 0% of the entire community) of the  
1159 benthic dinoflagellate community used as supplementary variables. (A) Loading plot (T =  
1160 temperature, S = salinity, TDN = Total Dissolved Nitrogen, DON = Dissolved Organic Nitrogen,  
1161 DIN = Dissolved Inorganic Nitrogen, TDP = Total Dissolved Phosphorus, DOP = Dissolved Organic  
1162 Phosphorus, DIP = Dissolved Inorganic Phosphorus, TDN:TDP = total dissolved N:P, DIN:DIP =  
1163 dissolved inorganic N:P; Ostr = *Ostreopsis*, Gamb = *Gambierdiscus*, Cool = *Coolia*, Amph =  
1164 *Amphidinium*, P.rhat = *Prorocentrum rhathymum*, P.lima = *Prorocentrum lima*, P.hoff =  
1165 *Prorocentrum hoffmannianum*). (B) Score plot ((●) ICOLLs: 7 Palm = Seven Palm Lake, Middle =  
1166 Middle Lake, West = West Lake, Long = Long Lake; (■) FB stations: Barnes = Barnes Sound, Duck  
1167 = Duck Key, Terrapin = Terrapin Bay, Garfield = Garfield Bight, Big = Big Key; Florida Keys  
1168 stations: (◆) FB-side: FBK1, FBK2, FBK3, FBK4, FBK5, FBK6, FBK7, (▲) ocean-side: OK1, OK2,  
1169 OK3, OK4. D: dry season, W = wet season).

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1171 Fig. 8. Mortality of *Crassostrea virginica* larvae after 2 d of exposure to *Gambierdiscus caribaeus*,  
1172 *Prorocentrum hoffmannianum*, *Prorocentrum rhathymum*, *Coolia santacroce* and *Amphidinium*  
1173 *carterae*. Control treatments (CTRL) contained *Crassostrea virginica* larvae alone. Different letters  
1174 show significant differences between treatments (Tukey HSD test: a<c,  $p < 0.01$ ; b<c,  $p < 0.05$ )

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