

1 **Sentinels for future coral reef conditions: assessment of environmental**
2 **variability and water quality in semi-enclosed inland bays in the southern**
3 **Caribbean**

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13 **Abstract**

14 The mangrove-seagrass-coral reef continuum is of immense ecological and socio-economic
15 importance, supporting biodiversity, carbon storage, coastal protection, fisheries, and tourism. The
16 presence of extreme environmental conditions along this continuum could support adaptive refugia for
17 climate-sensitive taxa such as reef-building corals but physicochemical conditions are rarely assessed
18 at sufficient spatiotemporal resolution. Furthermore, coastal development and low water quality
19 increasingly threaten these interconnected coastal ecosystems. Yet, time-integrated pollution
20 monitoring is absent at most locations. Here, we used a multi-disciplinary approach to assess benthic
21 cover, coral diversity, and >20 abiotic parameters characterizing two mangrove- and seagrass-
22 dominated inland bays and two nearby coral reefs in Curaçao (southern Caribbean) during the cool,
23 dry season and warm, wet season. This was combined with time-integrated pollution monitoring using
24 bioindicators to assess nutrients and trace metal pollution (inland bays only), and passive samplers and
25 bioassays to assess organic chemical pollution (all four sites) during the wet season. This approach
26 revealed a previously undocumented extent of strong diel and seasonal environmental variability in the
27 two inland bays, with temperature, pH and dissolved oxygen frequently reaching values predicted
28 under moderate-to-severe future climate scenarios. In addition, the inland bays had greater nutrient
29 concentrations (especially ammonium) and ecotoxicological risks than the nearby reefs during the wet
30 season due to run-off, industrial- and wastewater effluents, ports and boating. Overall, our findings
31 show that Curaçao's inland bays have significant potential to serve as natural laboratories to study the
32 effects of future ocean conditions on resident taxa *in situ*. This however applies within the context of
33 strong diel fluctuations and with the caveat of co-occurring stressors. Our work confirms the important
34 role of mangrove and seagrass habitats as resilience hotspots for climate-sensitive taxa but also
35 highlights the urgent need to improve monitoring, water quality and protection of these valuable
36 habitats along the mangrove-seagrass-coral reef continuum.

37 **Keywords:** Tropical semi-enclosed bays, environmental variability, climate change, multiple
38 stressors, passive sampling, bioassay battery

39

40 **Introduction**

41 Tropical mangrove forests, seagrass beds and coral reefs are among the most diverse and productive
42 ecosystems on the planet and can form complex cross-ecosystem connections through biological,
43 chemical, and physical interactions (Nagelkerken et al., 2008; Ogden, 1980). Mangrove forests, for
44 example, trap sediments and regulate carbon and nutrient cycles, while seagrass meadows play crucial
45 roles as nurseries, thus stimulating the food web and promoting biodiversity (Alongi, 1990; McDevitt-
46 Irwin et al., 2016). Mangroves and seagrass beds further provide biogeochemical services for corals
47 threatened by climate change by mitigating heat-stress-induced bleaching via shading (Rogers, 2017;
48 Yates et al., 2014). At the same time, they also generate extreme temperature, pH and oxygen
49 environments. This may drive acclimatization or adaptation of resident corals to future ocean
50 condition (Camp et al., 2016; Unsworth et al., 2012). Coral reefs, on the other hand, create habitat for
51 numerous marine species (Fisher et al., 2015) and provide shoreline protection, which in turn ensures
52 low energy currents into seagrass beds and mangrove forests (Guannel et al., 2016). Consequently, the
53 mangrove-seagrass-coral reef continuum offers not only essential ecological services but also
54 important socio-economic benefits, such as tourism and fisheries (Costanza et al., 1997; Spalding et
55 al., 2017; Torre-Castro & Rönnbäck, 2004). However, despite their importance, these habitats have
56 experienced extensive deterioration over the past century due to the combined effects of global and
57 local stressors, including climate change, overfishing, habitat destruction, coastal development, and
58 low water quality (e.g. Collier & Waycott, 2014; Gilman et al., 2008; Hughes et al., 2017).

59 Climate change impacts significantly threaten mangrove forests, seagrass beds and coral reefs. For
60 example, sea-level rise, increased cyclonic activity and changes in precipitation are among the key
61 threats to mangrove forests (Friess et al., 2022), whereas warming and marine heatwaves pose the
62 greatest threat to seagrass beds (Marbà et al., 2022). Yet, coral reefs are arguably one of the
63 ecosystems that are most vulnerable to multiple climate change stressors because marine heatwaves
64 can lead to mass coral bleaching and mortality on regional to global scales (Eakin et al., 2019; Hughes
65 et al., 2017), while acidification lowers the calcification rates of many coral species (Kornder et al.,
66 2018). In addition, deoxygenation and acute low oxygen events (i.e., hypoxia, defined as $<2 \text{ mg L}^{-1}$)
67 have recently been recognized as emerging threats to coral reefs that will be exacerbated under future
68 ocean warming and intensifying local stressors (Altieri et al., 2017; Pezner et al., 2023). However, in
69 recent years, coral communities have been discovered that persist under extreme environmental
70 conditions that frequently reach or even exceed temperature, pH and oxygen levels predicted to occur
71 under future climate scenarios (Burt et al., 2020; Camp et al., 2018). Several of these naturally extreme
72 environments occur along the mangrove-seagrass-coral reef continuum, such as mangrove lagoons
73 (Camp et al., 2019; Stewart et al., 2022), seagrass beds (Camp et al., 2016), and semi-enclosed
74 lagoons/bays (Maggioni et al., 2021; Vermeij et al., 2007), and can act as natural laboratories to

75 understand mechanisms of climate change resistance and adaptation. Yet, many sites that could act as
76 potential natural laboratories and resilience hotspots remain undocumented due to a lack of high-
77 resolution abiotic data. Furthermore, while these extreme environments can provide critical insights
78 into how resident coral communities respond to future climate conditions *in situ*, the impacts of global
79 stressors in combination with local stressors have received less attention. It is, therefore, important to
80 identify and study natural laboratories where global and local stressors occur *in tandem* to study
81 mechanisms of climate change resistance under more realistic multi-stressor scenarios.

82 With human population growth near coral reefs exceeding the global average (Wong et al., 2022),
83 local anthropogenic stressors increasingly impact habitats along the mangrove-seagrass-coral reef
84 continuum but are often poorly monitored. Changes in land use and coastal development result in
85 contaminated terrestrial runoff into marine waters (Burt & Bartholomew, 2019; Fabricius, 2005;
86 Valiela et al., 2001), which exposes tropical marine organisms to excess nutrients, sediments and
87 chemical contaminants (Jones & Kerswell, 2003; Nalley et al., 2023; Peters et al., 1997). In addition,
88 declines in water quality can be caused by untreated wastewater discharges, industrial activities and
89 boating, introducing various pollutants such as pharmaceuticals, personal care products, trace metals,
90 anti-fouling agents and oil products (Schaffelke et al., 2005). Yet, the effects of these compounds on
91 corals, mangroves and seagrasses remain poorly understood (Lewis et al., 2011; Nalley et al., 2021,
92 2023). Furthermore, water quality monitoring programs are often absent or limited to spot sampling
93 which is inadequate to capture the complex spatiotemporal dynamics of many pollutants (e.g. Den
94 Haan et al., 2016).

95 These challenges can be overcome via the use of time-integrated sampling approaches. For example,
96 within biomonitoring, seagrass or macroalgae can be used to assess nutrient and trace metal content
97 (Fabricius et al., 2012; Govers et al., 2014a, 2014b). Similarly, passive sampling devices have been
98 developed to assess (low) concentrations of organic chemical pollution in aquatic environments which
99 sequester chemicals by diffusion of molecules over extended periods (Vrana et al., 2005).
100 Comprehensive water quality assessments therefore ideally combine time-integrated pollution
101 sampling via bioindicators or passive samplers with high-frequency measurements of physicochemical
102 parameters such as pH and dissolved oxygen from continuous logger measurements. Extracts from
103 passive samplers can be used for toxicity assessment, where responses of organisms (*in vivo*) or cell
104 lines (*in vitro*) are measured in bioassays, known as effect-based methods or bioanalytical tools, to
105 study the effects of exposure to mixtures of chemicals in the environment (De Baat et al., 2020; Neale
106 et al., 2021).

107 The goal of this study was two-fold. First, we aimed to conduct a comprehensive, high-frequency
108 assessment of key physicochemical parameters across the mangrove-seagrass-coral reef continuum to
109 determine the full range of environmental “extremeness” during both dry and wet seasons.

110 Temperature, light, salinity, dissolved oxygen, pH, carbonate chemistry and nutrients were measured
111 on two coral reefs and in two adjacent, semi-enclosed inland bays that feature extensive mangrove
112 stands and seagrass meadows. The second goal was to conduct time-integrated pollution monitoring at
113 all four sites using both bioindicators and passive sampling to gain insight into water quality and
114 potential ecotoxicological risks to marine life. Passive sampling was paired with a battery of two *in*
115 *vivo* and two *in vitro* bioassays to assess the mixture toxicity of organic chemicals. Subsequently,
116 effect-based trigger values were used to categorize the water quality profile across the mangrove-
117 seagrass-coral reef continuum. This state-of-the-art monitoring approach allowed us (1) to establish an
118 environmental baseline against which future monitoring can be benchmarked, and (2) to assess
119 whether these inland bays could serve as previously unrecognized natural laboratories and resilience
120 hotspots for corals under climate change. Importantly, our study provides a powerful water quality
121 framework that can be used by natural resource managers to monitor habitats along the mangrove-
122 seagrass-coral reef continuum and conserve their high ecological and socio-economic value (Barbier et
123 al., 2011).

124 **Material and Methods**

125 **Study sites**

126 Four sites on the island of Curaçao, southern Caribbean, were studied in March and
127 October/November 2020, which represent the cooler dry season and the warmer wet season,
128 respectively. The environmental variability and water quality were measured in two inland bays, Santa
129 Martha Bay (SMB) (12°16'27.34"N, 69°07'39.66"W) with an average depth of 3 m and Spaanse
130 Water Bay (SWB) with an average depth of 5-12 m (12°04'15.35"N, 68°51'30.61"W), and two nearby
131 fringing reef sites, Santa Martha Reef (SMR) (12°15'59.56"N, 69°07'39.66"W) and Director's Bay,
132 referred to hereafter as 'Spaanse Water Reef' (SWR) (12°03'53.48"N, 68°51'36.69"W) (Figure 1).
133 The two inland bays were selected based on their comparability in geomorphology, size, proximity to
134 and availability of a reference reef site, ease of access, and presence of (similar) coral communities,
135 seagrass and mangroves (Debrot et al., 1998; van Duyl, 1985; Vermeij et al., 2007). The bays differ in
136 the degree of local anthropogenic stressors. Spaanse Water Bay has experienced significant coastal
137 development over the last decades, resulting in residential use with insufficient sewage infrastructure,
138 causing pollution and eutrophication. In contrast, Santa Martha Bay is located in a sparsely populated
139 area with an abandoned resort undergoing reconstruction, and is thus much less impacted by local
140 stressors than Spaanse Water Bay.

141

142 **Benthic community assessment**

143 Benthic community surveys for all sites were conducted in March 2020 using continuous line transects
144 to characterize the immediate area surrounding the abiotic monitoring stations (see below). Given the
145 large area of the inland bays and their heterogenous nature, the measured benthic cover is therefore
146 only representative of the monitoring area, not the bays as a whole. In each site, six 9.9 m transects
147 were randomly placed up to 50 m away from the cement block with environmental data loggers.
148 Photos of the benthic community adjacent to the transect line were taken every 20 cm using a high-
149 resolution camera (Panasonic Lumix DMC-TZ70) and the benthic cover underneath every 1 cm was
150 analysed. A total of eleven categories were identified to describe the benthic community: hard coral,
151 soft coral, seagrass, macroalgae, soft sediment, rubble, hard substrate, upside-down jellyfish
152 (*Cassiopea* sp.), sea urchin (*Diadema* sp.), invertebrates and other. *Diadema* sp. and *Cassiopea* sp.
153 were analysed as separate categories and not included in the "invertebrates" category as these
154 organisms may have important ecological functions associated with coral development or influence
155 physicochemical conditions (Edmunds & Carpenter, 2001; Welsh et al., 2009). Scleractinian corals
156 were identified to species level when possible. The benthic cover was calculated according to the

157 following formula: % *benthic cover* $X = \frac{\text{Total distance cover } X}{\text{Total distance of line}} \times 100$

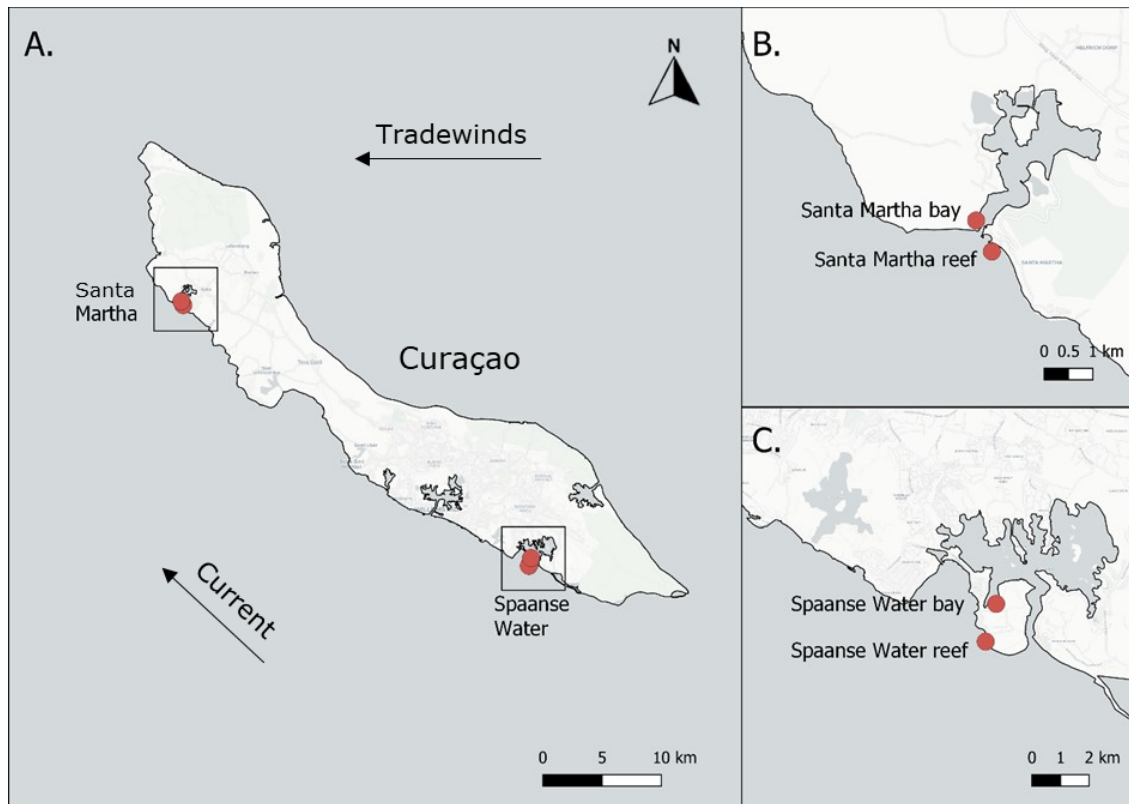


Figure 1. A. Map of Curaçao with sampling sites shown in red. Closer view of Santa Martha Bay and nearby fringing reef (B) and Spaanse Water Bay and nearby fringing reef (C).

158 **Environmental monitoring of temperature, salinity, pH_T, DO and PAR**

159 Temperature, conductivity and dissolved oxygen (DO) were continuously logged, whereas pH and
160 photosynthetically active radiation (PAR) were semi-continuous logged over two weeks during the dry
161 season (12-03-2020 to 21-03-2020) and five weeks during the wet season (28-10-2020 to 02-12-2020)
162 at all four sites. Logging frequency was 15 min for the conductivity and PAR loggers, and five min for
163 the pH and DO loggers.. The combined temperature and conductivity loggers (Odyssey® Conductivity
164 and Temperature) were calibrated with the factory-provided calibration equations using the Odyssey
165 data logging software. pH loggers (HOBO® pH and Temperature Data Logger (MX2501)) were
166 retrieved and calibrated every other day using TRISbuffer at two different temperatures to determine
167 seawater pH on the total scale (pH_T) as described in Dickson et al., (2007). TRIS buffers were
168 purchased from A.G. Dickson. Calibration of the DO loggers (HOBO® Dissolved Oxygen Logger
169 (U26-001)) was done by placing them in 100% saturated saltwater, whereafter they were placed in 0%
170 saturated saltwater (obtained by adding sodium sulphite). PAR loggers (Odyssey® Xtream
171 Photosynthetic Active Radiation Logger) were calibrated underwater via simultaneous deployment
172 with a Hydrolab DS5 Sonde (OTT Messtechnik GmbH & Co., Germany) equipped with a Li-Cor 4 pi
173 spherical sensor for 12 hours (7 am – 7 pm). Loggers were placed on a cement block, ensuring that the

174 PAR sensor and conductivity cell were vertically mounted (Figure S1a). The loggers were cleaned of
175 debris every other day with a toothbrush. Further details are given in the Supplemental Information.

176 **Seawater carbonate chemistry**

177 Discrete water samples were collected in both dry and wet seasons on multiple random days to
178 determine seawater carbonate chemistry (via total alkalinity and pH_T) and nutrient concentrations.
179 Total alkalinity was analysed using potentiometric titration on a Metrohm 716 DMS Titrino (Herisau,
180 Switzerland). Seawater pCO_2 and aragonite saturation state (Ω_{arag}) were calculated from in situ pH_T ,
181 temperature, salinity and total alkalinity using the Carb function (flag = 8) in the seacarb package
182 (version = 3.2.14) in R (2021.09.0).

183 **Nutrients and trace metals**

184 Nitrate, nitrite, ammonium and phosphate concentrations ($\mu\text{mol L}^{-1}$) were measured using a Skalar
185 SAN⁺⁺ system autoanalyzer. In addition, seagrass leaves were used as bioindicators for nutrient
186 pollution (Govers et al., 2014a) and trace metal pollution (Govers et al., 2014b) because they integrate
187 environmental conditions over time and therefore record ecologically relevant levels (Katharina E.
188 Fabricius et al., 2012). Seagrass leaves were only collected from the two inland bay sites and only in
189 the wet season (November 2020). Ten shoots (1 m distance between shoots) of *Thalassia testudinum*
190 and *Halophila stipulacea* with roots were collected in Spaanse Water Bay, whereas only 10 shoots of
191 *Halophila stipulacea* were collected in Santa Martha Bay due to the absence of *Thalassia testudinum*.
192 The seagrass shoots were split into roots, rhizomes and leaves, after which only the leaves were used.
193 The dried seagrass leaves were then analysed for trace metal concentrations (As, Cd, Co, Cr, Cu, Fe,
194 Ni, P, Pb, Se, Zn) and ratios and percentages of carbon (C), nitrogen (N) and phosphate (P). Further
195 details are given in the Supplemental Information.

196 **Sediment traps**

197 Replicate sediment traps were deployed in the wet season only (November 2020) to collect sediment
198 particles in the water column during 16 and 19 days at all four sites (see Table S1 for dates). Sediment
199 traps were constructed following the recommendations of Storlazzi et al., (2011) for coral reef
200 environments. To determine if trapped sediments were from local resuspension or transportation,
201 benthic sediment at the base of each trap was also sampled. After retrieval of the sediment traps, the
202 content was dried at 60 °C for 48 hours and analysed for weight and particle size characteristics: >1
203 mm, 500 to <1000 μm , 250 to <500 μm , 125 to <250 μm , 63 to <125 μm and <63 μm . The sediment
204 trap collection rate ($\text{mg cm}^2 \text{day}^{-1}$) was calculated as the weight of sediment trapped (mg) divided by
205 the number of days the trap was deployed and the surface area of the trap (cm^2). The amount of
206 organic matter was determined by burning the sediment samples in the muffle furnace at 450°C for 6
207 hours. The organic matter trap collection rate was calculated in the same way as the sediment trap
208 collection rate. Further details are provided in the Supplemental Information.

209 **Effect-based chemical water quality assessment**

210 Passive sampling of organic pollutants - Polar organic chemical integrative samplers (POCIS)
211 (Alvarez et al., 2004) containing 200 mg of hydrophilic-lipophilic balance (HLB) sorbent were
212 deployed for approximately four weeks at all four sites (see Table S1 for dates) during the wet season
213 for the passive sampling of organic chemicals. Forty POCIS were constructed according to De Baat et
214 al., (2020) and stored at 4°C in food-grade Mylar zip lock bags until deployment (except during
215 transport to Curaçao). At each site, seven POCIS were attached to a stainless steel frame and retained
216 in a stainless steel cage. The cage was placed on a cement block and deployed at the monitoring
217 stations next to the cement block with loggers (Figure S1b). After retrieval, each POCIS was
218 disassembled and the HLB sorbent of all 7 POCIS per site was pooled in a 50 mL Greiner tube and
219 stored at -20 °C until extraction. The HLB sorbent was freeze-dried for 3 days at -52 °C to remove any
220 remaining water and extracted according to De Baat et al., (2020) with slight modifications. Further
221 details on the extraction of HLB sorbent and calculations of sampled water volumes by POCIS are
222 provided in the Supplemental Information.

223 Bioassays - A battery of four bioassays was exposed to the POCIS extracts to determine levels of
224 chemical pollution at the four sites (Table 1). Before application in the bioassays, the POCIS extracts
225 were evaporated under a gentle stream of N₂ to dryness and reconstituted in dimethyl sulfoxide
226 (DMSO). A whole organism (*in vivo*) algal bioassay was performed with the marine alga *Dunaliella*
227 *tertiolecta* at the University of Amsterdam to assess photosynthetic inhibition (Sjollem et al., 2014)
228 using pulse amplitude modulation (PAM) fluorometry according to de Baat et al., (2018). An *in vivo*
229 bacterial bioluminescence inhibition assay using *Allivibrio fischeri* was performed at the Vrije
230 Universiteit Amsterdam according to Hamers et al., (2001) to assess bacterial luminescence inhibition
231 (commercially known as the Microtox® assay). Dose-response curves for the *in vivo* assays were
232 made in GraphPad Prism (GraphPad Software Inc., version 9.1.2.226, San Diego, CA, USA) and the
233 half maximal effective concentration (EC₅₀), expressed as the relative enrichment factor of the extract
234 in the bioassays, was determined by non-linear regression with a built-in log-logistic model. Toxicity
235 was expressed in toxic units (TU) and was calculated by 1/EC₅₀.

236 Two *in vitro* chemically activated luciferase gene expression (CALUX) reporter gene assays were
237 applied to assess the activation of the estrogen and aryl hydrocarbon receptors (ER α and AhR),
238 respectively. The two CALUX assays represent specific toxic endpoints and can serve as proxies for
239 specific sources of pollution (Burg et al., 2013; De Baat et al., 2020). Estrogen receptor agonism (ER α
240 CALUX), indicative of endocrine disruption, was used as a proxy for wastewater pollution (Välitalo et
241 al., 2016) and aryl hydrocarbon receptor agonism (PAH CALUX), indicative of the induction of
242 xenobiotic metabolism, as a proxy for industrial pollution (Neff et al., 2005). The ER α and PAH
243 CALUX assays were performed by the BioDetection Systems laboratory (Amsterdam, The
244 Netherlands) as described by De Baat et al., (2020). The effects of the extracts in the assays were

245 expressed as bioanalytical equivalent (...EQ/L) of the reference compounds of the assays using the
 246 estimated sampled water volume of the passive samplers (Table 1). Further details on the bioassays are
 247 provided in the Supplemental Information.

248 Effect-based trigger values (EBT) - Effect-based trigger values (EBTs) were used to identify potential
 249 ecotoxicological risks based on the bioassay responses (Neale et al., 2023). The EBTs available in the
 250 scientific literature are commonly derived for risk assessments in freshwater systems. The presently
 251 used EBTs were derived by Brion et al., (2019) (ER α), Escher et al., (2018) (PAH) and van der Oost et
 252 al., (2017) (algal and bacterial inhibition) (Table 1). To render these EBTs suitable for the present
 253 marine risk assessment, an additional safety factor of 10 was applied to account for the generally
 254 higher sensitivity of marine organisms to toxicants (RIVM, 2015), resulting in interim effect-based
 255 trigger values (iEBT) (Table 1). To calculate ecotoxicological risk quotients, the bioassay responses
 256 were divided by their respective EBTs or iEBTs, where an effect-based risk quotient ≥ 1 indicated a
 257 potential ecotoxicological risk (De Baat et al., 2020). Cumulative ecotoxicological risk for each site
 258 was subsequently obtained by summing the four effect-based risk quotients for each site. This sum
 259 allowed the ranking of sites based on their ecotoxicological risk, where the highest sum of a site is
 260 assumed to represent the highest chemical burden, resulting from organic chemical pollution.

Table 1. Overview of bioassay battery applied to assess toxicity at four sites in Curaçao. TU = toxic unit, ...EQ/L = equivalent concentration of the reference compound, n/a = not available.

^a = Van der Oost et al. (2017), ^b = Escher et al. 2018, ^c = Brion et al. (2019), ^d = this study.

	Bioassay	Endpoint	Reference compound	EBT	iEBT ^d	Unit
<i>in vivo</i>	Bacterial bioluminescence inhibition	Luminescence inhibition	n/a	0.05 ^a	0.005	TU
	Algal	Photosynthetic inhibition	n/a	0.05 ^a	0.005	TU
<i>in vitro</i>	PAH	Xenobiotic metabolism	benzo(a)pyrene	6.21 ^b	0.621	ng BEQ/L
	ER α	Estrogenic activity	17 β -estradiol	0.28 ^c	0.028	ng EEQ/L

261

262 **Statistical analysis**

263 Values in the raw dataset were examined for the presence of extreme outliers and excluded when they
 264 exceeded 3x the interquartile range (IQR). The effect of habitat (bay vs reef) and season (dry vs wet)
 265 on the daily average and the daily variability of all environmental parameters (i.e. temperature, pH,
 266 DO, salinity and PAR) was statistically analysed using the nonparametric Scheirer-Ray-Hare
 267 extension of the Kruskal-Wallis test with a post-hoc Dunn test because the assumptions of normality
 268 were violated (Scheirer et al., 1976). The assumptions of a normal distribution and equal variance
 269 were tested with a Shapiro-Wilks test and F-test, respectively. The Scheirer-Ray-Hare extension with a
 270 post-hoc Dunn test was also used to test for differences in average nutrient concentrations in the water
 271 samples between habitat and season since the assumptions of normality and homogeneity of variance

272 were not met. This also applied to the total alkalinity and carbonate chemistry data. All statistical
273 analyses were performed using Rstudio software (Version 2022.07.2+576 "Spotted Wakerobin"). P-
274 values ≤ 0.05 were considered significant.

275

276 Results

277 Benthic community assessment

278 Benthic cover surrounding the monitoring stations in Spaanse Water Bay was dominated by seagrass
279 and macroalgal meadows ($48\% \pm 7$ SE and $23\% \pm 3$ SE, respectively), while all other monitoring
280 locations were dominated by rubble and soft sediment (Figure 2). Spaanse Water Bay was
281 characterized by the presence of the benthic upside-down jellyfish *Cassiopea* sp. (0.08%), whereas
282 Santa Martha Bay had a high prevalence of the echinoid *Diadema* sp. (0.98%). Benthic community
283 surveys revealed a strong difference in coral cover between inland bays (< 1%) and reefs (~ 8%)
284 (Figure 2). Specifically, coral cover (all species included) in Spaanse Water Bay was 12 times lower
285 than in Spaanse Water Reef and 5.5 times lower in Santa Martha Bay than in Santa Martha Reef.
286 Similarly, coral diversity was substantially lower in the bays than on reef flats. In total, four coral
287 species were identified in the monitoring area at both bays, whereas 15 species (including one
288 unknown) were identified in the monitoring area on the reefs (Table S5).

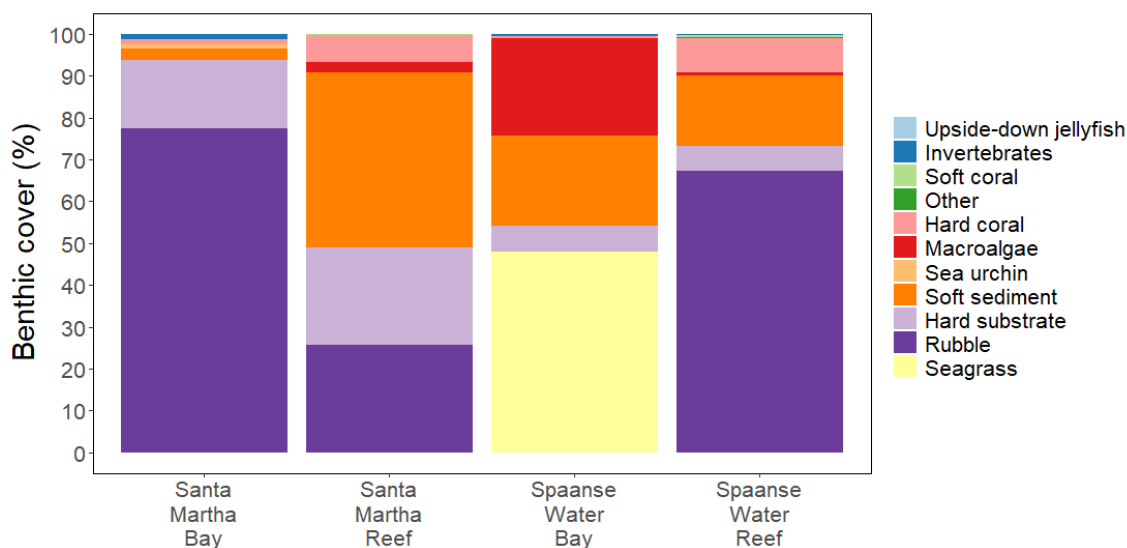


Figure 2. The benthic community at the four sites, expressed as cover percentage (%). The ‘invertebrates’ category comprises anemones, sponges, crustaceans (e.g. *Panulirus argus* and *Mithrax spinosissimus*) and feather duster worms. The ‘other’ category includes benthic cyanobacterial mats (BCM) and dead coral.

289 Temperature, salinity, pH_T, DO and PAR

290 The assessment of physicochemical conditions revealed that the bays not only had significantly
291 different average conditions but were also much more variable than the reefs (Figure 3, Tables S6-8).
292 Furthermore, strong differences between the dry and wet seasons were also observed for most
293 parameters, while some showed complex interactive effects.

294 The average temperature in the bays was significantly higher (up to 0.8°C) than on the reefs ($p =$
295 0.043) (Figure 3a, Tables S6, S7). The daily temperature variability was significantly higher in the
296 bays (up to 2.6 °C in the dry season and up to 2 °C in the wet season) compared to only up to 0.8 °C
297 in the dry and wet season on the reefs ($p = \leq 0.001$) (Figure 3a, Tables S6, S8). In addition,
298 temperature was more variable in the dry compared to the wet season ($p = 0.003$) (figure 3a, Tables
299 S6, S8). Notably, maximum temperatures exceeded the local coral bleaching threshold of 29.0°C
300 (MMM = 28 °C + 1 °C; Coral Reef Watch, virtual station Aruba, Curaçao, and Bonaire, NOAA 2020-
301 2021) by up to 1.4°C in the bays during the warmer dry season, while this was not the case for the
302 reefs (Table S6). Average temperatures were generally warmer during the wet than the dry season but
303 daily temperature variability did not differ significantly between the seasons ($p = 0.074$) (Figure 3,
304 Tables S6-S8).

305 The average salinity was not significantly different between bays and reefs ($p = 0.680$), however, there
306 was a significant difference between seasons ($p = 0.005$), with higher values in the dry (by 0.85 ppt)
307 compared to the wet season (Figure 3b, Tables S6, S7). Daily variability of salinity differed
308 significantly between both habitats ($p \leq 0.001$) and seasons ($p = 0.002$), but no significant interactive
309 effect was found ($p = 0.944$). Salinity was generally more variable in the bays than on reefs (up to 7.8
310 ppt versus up to 1.7 ppt daily), and also during the wet compared to the dry season (up to 3.4 ppt
311 versus up to 2.4 ppt daily) (Figure 3b, Tables S6, S8).

312 The reefs had a significantly higher average pH_T than the bays ($p = 0.033$), although most sites had a
313 relatively similar average pH_T within 8.03–8.08 during both seasons (Figure 3, Tables S6, S7). The
314 only exception was Spaanse Water Bay during the wet season which had the lowest average pH_T
315 found (7.95) (Figure 3c, Table S6). Average pH_T did not differ significantly between the dry and wet
316 seasons (Figure 3c, Tables S6, S8). In contrast, the daily pH_T variability tended to be higher in the
317 bays than the reefs, but only during the wet season ($p = 0.03$), and variability was generally higher
318 during the wet compared to the dry season (Figure 3c, Tables S6, S8).

319 The average dissolved oxygen (DO) concentration was significantly higher (2.2%) on the reefs than in
320 bays ($p = 0.002$), and also 3.0% higher during the dry than wet season ($p \leq 0.001$) (Figure 3d, Tables
321 S6, S7). The daily DO variability differed significantly between habitats ($p = \leq 0.001$) and between
322 seasons ($p = \leq 0.001$), with 56.6% higher daily DO variability in the bays than on the reefs and during
323 the wet season compared to the dry season. A significant interactive effect between habitat and season
324 was observed for the daily variability of DO ($p = 0.028$) but not for average DO concentration (Figure
325 3d, Tables S6-S8). During the dry season, Spaanse Water Bay reached dissolved oxygen levels as low
326 as 3.72 mg L⁻¹, while Santa Martha Bay reached DO levels as low as 2.58 mg L⁻¹ during the wet
327 season.

328 Both average photosynthetically active radiation (PAR) and daily variability in PAR differed
 329 significantly between habitat and season (all $p \leq 0.001$), however, no interactive effect was found
 330 between habitat and season ($p = 0.957$; $p = 0.661$) (Figure 3e, Tables S6-S8). The bays experienced
 331 31.5 % lower PAR than the reefs, and PAR was 33% lower during the wet than the dry season. The
 332 maximum PAR was up to $\sim 2100 \mu\text{mol m}^{-2} \text{s}^{-1}$ on the reefs but only up to $\sim 1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the
 333 bays (Figure 3e, Table S6).

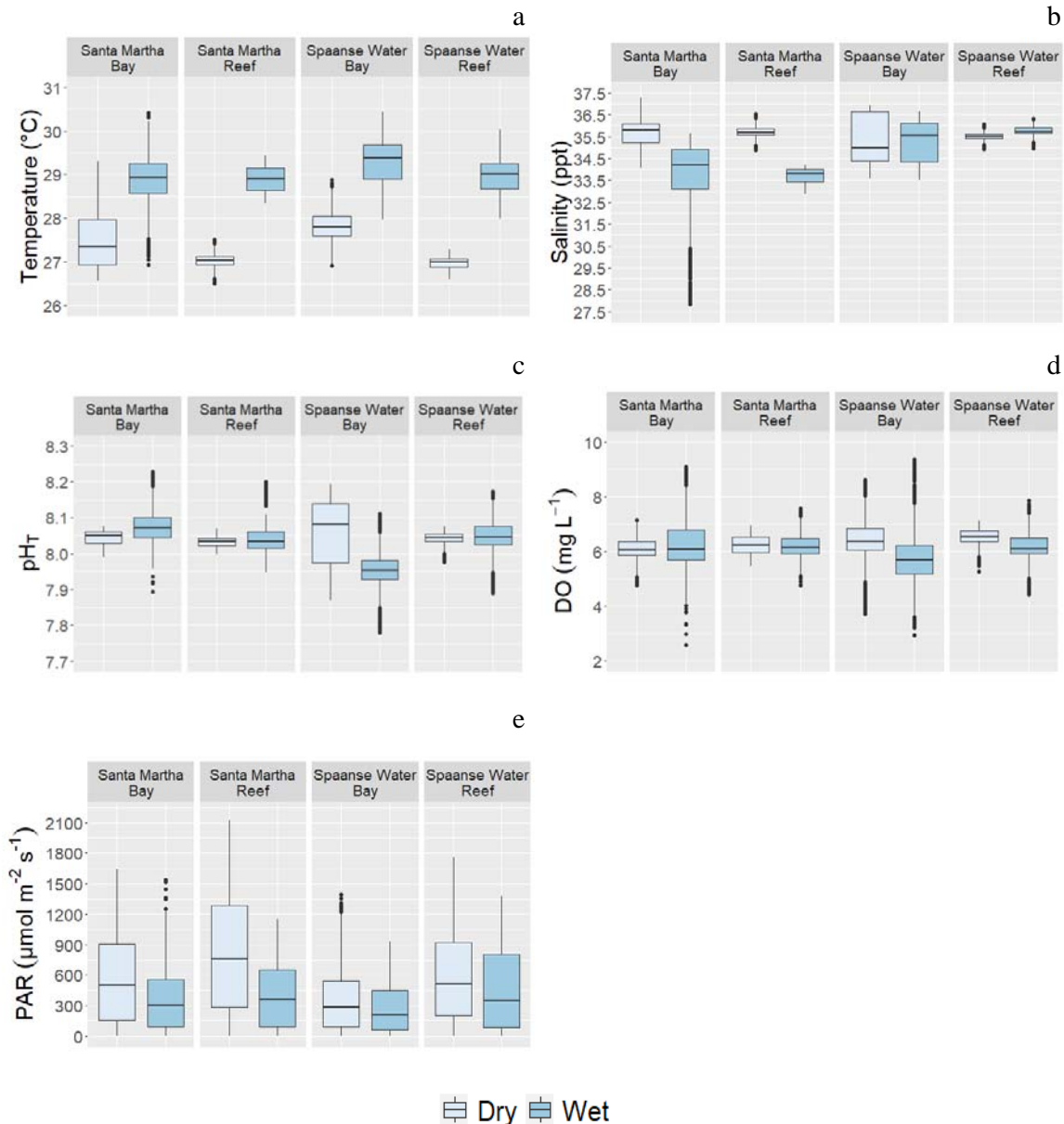


Figure 3. Physicochemical conditions measured in March 2020 (cool, dry season) and Oct/Nov 2020 (warm, wet season). a. temperature ($^{\circ}\text{C}$), b. salinity (ppt), c. pH_T d. dissolved oxygen (mg L^{-1}), e. photosynthetically active radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Boxes span the first to third quartiles; the horizontal line inside the boxes represents the median, and black dots represent the outliers beyond the range of 3 times the IQR as extreme outliers were excluded that exceeded 3 times the IQR. Whiskers represent the range of the data that falls within 1.5 times the IQR. See also Table S1.

334 Seawater carbonate chemistry

335 These results are described in more detail in the Supplementary Materials (Figure S5, Table S12).

336 Seawater nutrient concentrations

337 Overall, nutrient levels were significantly higher during the wet than the dry season and the bays
338 tended to have higher nutrient levels than the reefs, particularly during the wet season (Figure 4, Table
339 S9). Nevertheless, some of these effects differed for the different types of nutrients. Both nitrate and
340 phosphate concentrations were 81% and 24% higher during the wet than the dry season, respectively,
341 but did not differ significantly between habitats (Figure 4a, b; Table S9). Similarly, ammonium
342 concentrations were also 68% higher during the wet than the dry season but were the only nutrient
343 type that differed significantly between habitats, with the bays having 13% higher ammonium
344 concentrations than the reefs (Figure 4c; Table S9).

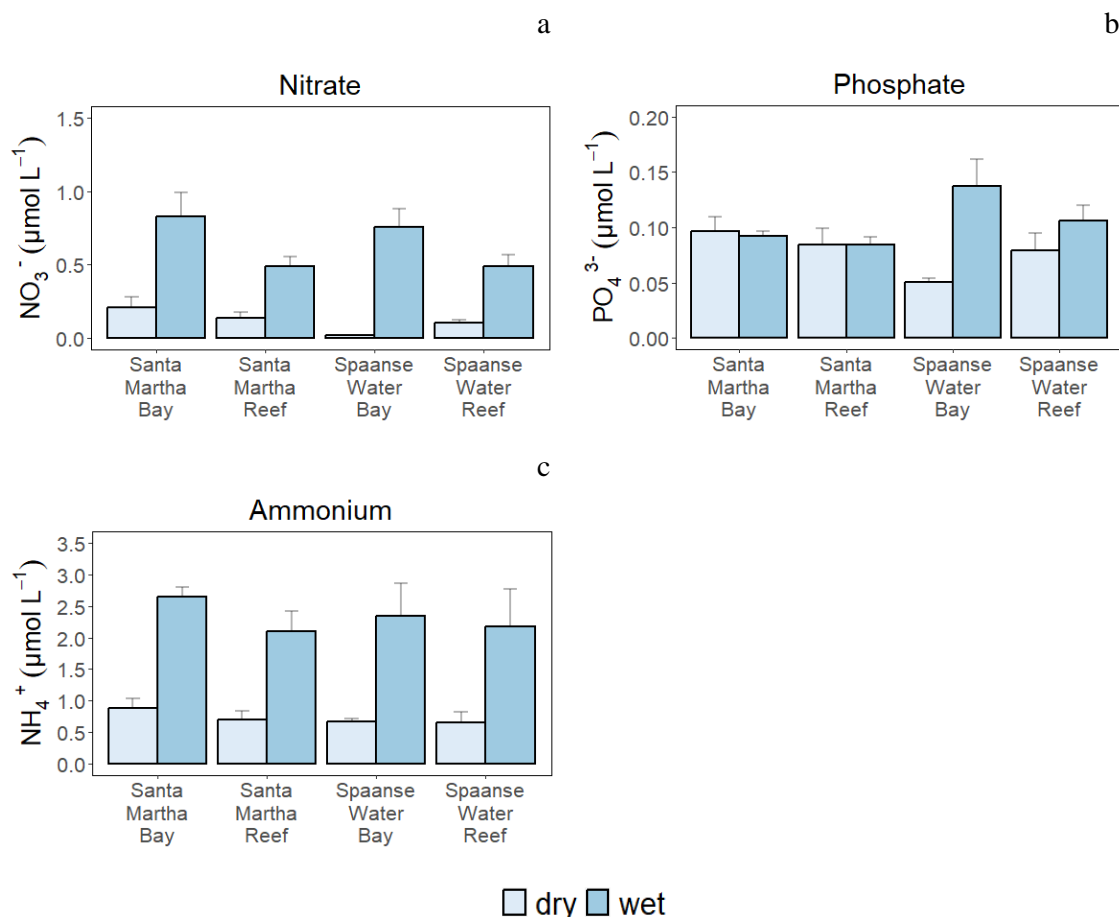


Figure 4. Nutrient concentrations measured in March 2020 (cool, dry season) and Oct/Nov 2020 (warm, wet season). a. Nitrate (NO_3^-), b. Phosphate (PO_4^{3-}) c. Ammonium (NH_4^+). Bars represent means \pm the standard error (SE).

345 **Seagrasses as indicators of nutrient and trace metal pollution**

346 In contrast to the water samples, the seagrass samples were only used to examine bay-specific nutrient
347 and trace metal pollution as seagrass was only found in the bays. These results are described in more
348 detail in the Supplementary Materials (Tables S10 and S11).

349 **Sediment trap collection rates**

350 Average trap collection rates during the wet season ranged from 0.94 to 3.23 mg cm⁻² day⁻¹, whereby
351 Santa Martha Reef had the highest rate (3.23 mg cm⁻² day⁻¹), and Spaanse Water Bay the lowest (0.94
352 mg cm⁻² day⁻¹) (Figure 5a). The reefs received overall higher sediment loads than the bays, however,
353 the sediment particles in the bays had a higher organic matter content (~11.5% versus ~4.5% on the
354 reefs) (Figure 5b). Santa Martha Bay sediment particles had the highest organic matter content (0.29
355 mg cm⁻² day⁻¹), which was ~ 50% higher compared to the other three sites. The traps collected a high
356 percentage of the smaller particles as a percentage of the total weight, whereas the opposite was true
357 for the sediment collected from the benthos (Figure S3). Furthermore, trapped sediment on the reefs
358 was dominated by smaller sizes compared to the bays. The benthic sediment showed the opposite,
359 with bays having a higher percentage of large particles than the reefs, particularly Santa Martha Bay.

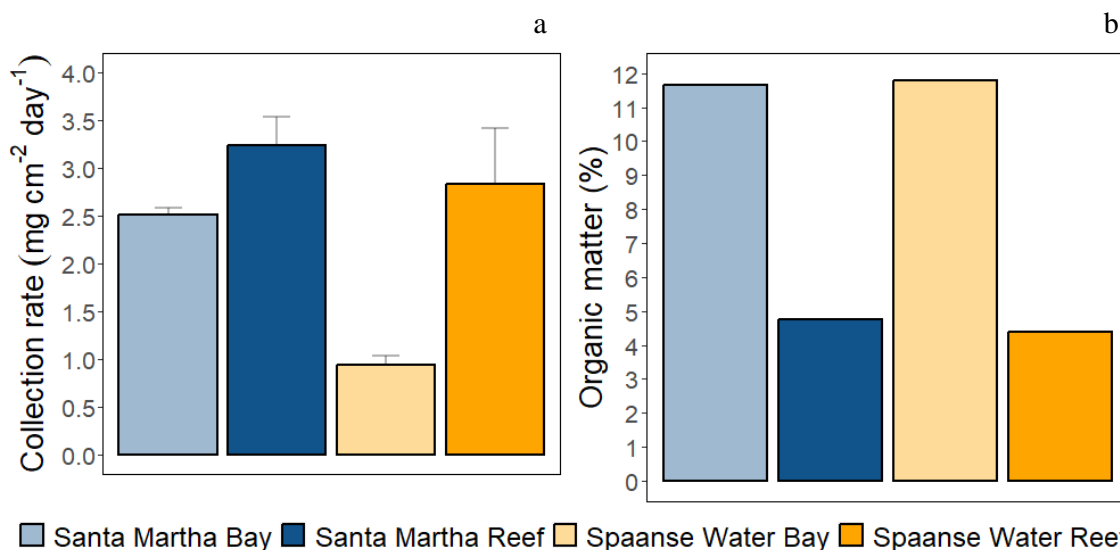


Figure 5. a. Average trap collection rates (mg cm⁻² day⁻¹) for all four sites during the warm wet season (Oct-Nov 2020), Shown is mean ± SD. b. Organic matter percentage of the total sediment weight accumulated in the sediment trap.

360 **Effect-based chemical water quality assessment**

361 All bioassays met their respective validity criteria and responses were observed in all bioassays. While
362 the EBT was exceeded at none of the sites (Figure S4), the more conservative iEBT was exceeded
363 (Figure 4). For the algal bioassay, responses above the iEBT threshold value were not detected but the
364 passive sampler extracts from the bays caused higher responses than those from the reefs, and Spaanse
365 Water Reef extracts caused no bioanalytical response at all. In the bacterial bioluminescence inhibition
366 assay, the iEBT threshold value was exceeded at Santa Martha Bay, Santa Martha Reef and Spaanse

367 Water Bay, while the response at Spaanse Water Reef was on par with the iEBT. Again, the highest
368 responses for this assay were detected at the bay sites. The ER α CALUX assay, a proxy for
369 wastewater, showed the highest response in Spaanse Water Bay but the iEBT was exceeded at none of
370 the sites. The PAH CALUX assay revealed that industrial pollution was present at each site, but only
371 Spaanse Water Bay exceeded the iEBT. The bays showed usually higher responses in comparison to
372 the reefs, which is also reflected in the exceedance of the iEBT in the bioassays (Figure 4).

373 A summary of the bioassays battery responses is shown in a heat map (Table 7). Spaanse Water Bay
374 extracts caused the highest responses in all bioassays, followed by Santa Martha Bay, Santa Martha
375 Reef and Spaanse Water Reef. Hence, the bays exhibited a higher cumulative mixture toxic pressure
376 than the reefs.

377

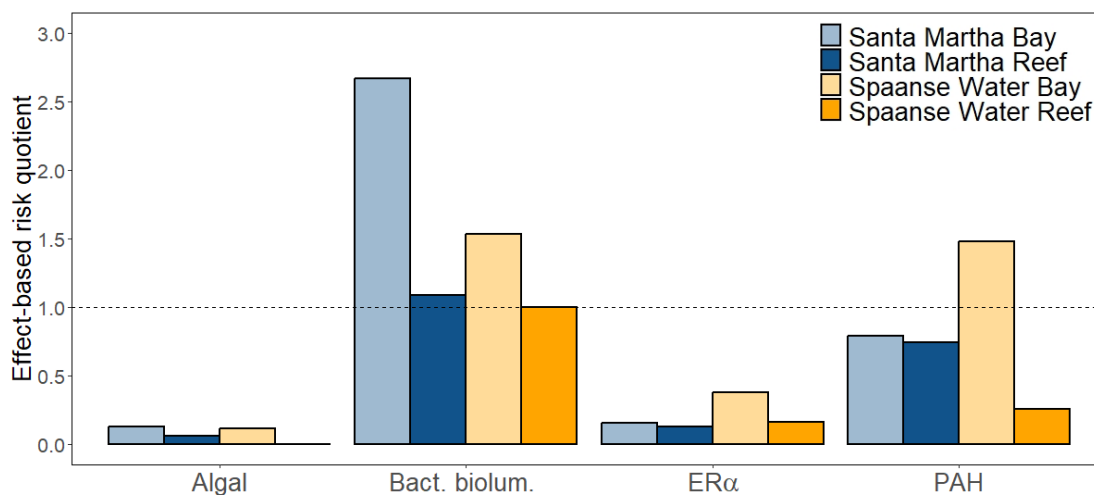


Figure 6. Responses of four bioassays at four sites during the wet season (Oct/Nov 2020). The dotted line represents an effect-based risk quotient ≥ 1 , derived by dividing the bioassay response by the intermediate effect-based trigger value for the marine environment (iEBT). Exceedance of this value indicates a potential ecotoxicological risk. Algal = Algal bioassay, Bact. biolum. = Bacterial bioluminescence inhibition assay, ER α = Estrogen receptor α CALUX (a proxy for wastewater), PAH = polycyclic aromatic hydrocarbon CALUX (a proxy for industrial pollution).

378

379

Table 2. Heat map depicting the responses of four bioassays and the sum of the effect-based risk quotients for four sites during the wet season (Oct/Nov 2020). The colour gradient reflects the bioassay response value, in which red indicates the highest value (i.e. higher pollution levels) and green the lowest value (i.e. lower pollution levels) for that specific bioassay.

Bioassay		Santa Martha Bay	Santa Martha Reef	Spaanse Water Bay	Spaanse Water Reef
<i>in vivo</i>	Algal				
	Bact. bioluminescence inhibition				
<i>in vitro</i>	ER α CALUX				
	PAH CALUX _o				
Sum of effect-based risk quotient		3.74	2.02	3.50	1.42

380

381 **Discussion**

382 **Are Curaçao's inland bays potential analogues for future ocean conditions?**

383 The presently studied inland bays represent thermally extreme environments because they were overall
384 up to 0.8°C warmer than the reefs and had high daily temperature variability year-round (up to 2.6°C)
385 (Fig. 3a, Table S6). This makes them somewhat comparable to, though less extreme than, other semi-
386 enclosed lagoons, such as the Bouraké lagoon in New Caledonia (up to 6.5°C, Maggioni et al., 2021)
387 or the mangrove lagoons in the Great Barrier Reef with a temperature range up to 7.7°C across three
388 months compared to 3.5°C in this study (Camp et al., 2019). However, when compared to a mangrove
389 site Belize, where the average daily temperature variance was 0.58°C (Lord et al., 2021), the inland
390 bays can be considered a more extreme site in the Caribbean Sea. Importantly, temperatures in the
391 bays already exceeded the full annual monthly temperature range for the reefs (25.7°C – 28.0°C; Coral
392 Reef Watch, virtual station Aruba, Curaçao, and Bonaire, NOAA, 2021) during the cool dry season.
393 Furthermore, maximum inland bay temperatures during the warm, wet season were in line with the
394 predicted mid-century sea surface temperature (global open ocean) under the intermediate emission
395 scenario SSP2-4.5 (IPCC, 2019, 2021).

396

397 While mangroves, seagrasses and the organisms associated with these habitats are typically adapted to
398 such dynamic temperature regimes, they represent extreme and potentially stressful temperatures for
399 corals that have much lower thermal tolerance. Specifically, the local coral bleaching threshold is only
400 ~29°C (NOAA, 2021) whereas upper thermal limits of tropical seagrass species range from 32-38°C
401 (Marbà et al., 2022). Thus, inland bay corals were exposed to temperatures exceeding their bleaching
402 thresholds already during the cool dry season and spent most of their time at or above the bleaching
403 threshold temperature during the warm wet season (Table S6). It should be noted here that
404 temperatures during the 2020 wet season were warmer than usual (NOAA, 2021), but while some
405 corals on the reefs were bleached at greater depth (10-15 m), this was not observed in the inland bays
406 (although some corals appeared pale; CDJ, personal observation). These findings suggest that inland
407 bay corals have greater heat tolerance than reef corals which is likely due to regular exposure to more
408 variable temperatures (Palumbi et al., 2014; Rivest et al., 2017; Safaie et al., 2018; Schoepf et al.,
409 2015).

410

411 The inland bays showed much larger daily fluctuations in DO compared to nearby reefs in both
412 seasons, whereas for pH_T this was the case only in the wet season (Figure 3c, d, Table S6). This strong
413 daily variability is driven largely by the high cover of seagrass, macroalgae, and mangroves in the
414 bays (Figure 2), combined with long seawater residence times due to restricted exchange with the open
415 ocean. These benthic communities influence the pH_T and DO by performing photosynthesis by day
416 and respiration by night (Delille et al., 2000; Hendriks et al., 2014). Although corals also

417 photosynthesize, the daily oscillations in pH_T and DO are typically much smaller in a coral-dominated
418 habitat as the increase of pH_T and DO by photosynthesis of endosymbionts is counteracted by
419 calcification and respiration of associated coral reef organisms (Anthony et al., 2013; Cryer et al.,
420 2023; Page et al., 2016). The observed daily pH variability of up to 0.25 units is lower than the 0.69
421 daily range in the semi-enclosed lagoon system in Bouraké, New Caledonia (Maggioni et al., 2021).
422 The pH range of 0.34 units found during the monitoring period in the inland bays was higher than the
423 pH range of 0.21 units found in a mangrove forest in Panama (0.21 units) (Stewart et al., 2022).
424 Similarly, the observed daily DO variability of up to 5.91 mg L^{-1} is comparable to 4.91 mg L^{-1} in the
425 semi-enclosed lagoon system in Bouraké, New Caledonia (Maggioni et al., 2021), but the average DO
426 found in the inland bays (6.1 mg L^{-1}) is higher than the average DO (3.74 mg L^{-1}) found in a
427 mangrove forest in Panama (Stewart et al., 2022). The persistence of corals in such extreme
428 environments is remarkable given their high sensitivity to low pH (Albright & Langdon, 2011;
429 Kornder et al., 2018) and to some degree low DO (Altieri et al., 2017; Johnson et al., 2021). The
430 inland bays are, therefore, ideal locations to investigate how such strong diel variability modulates
431 resistance to acidification and severe de-oxygenation or hypoxia (Altieri et al., 2021; Rivest et al.,
432 2017).

433 While average pH and DO levels in the inland bays were largely within the range of present-day
434 conditions, the strong diel fluctuations of DO and pH led to regular but temporary exposure to future
435 ocean conditions for resident organisms (Table S6). The most extreme site was Spaanse Water Bay
436 where even average pH_T (7.95) was substantially reduced during the warm wet season, reaching levels
437 predicted by ~2050 under the high-emission SSP3-7.0 scenario (IPCC, 2021). Minimum pH_T values
438 ranged from 7.78 to 7.87, which compares to predicted end-of-century pH levels of 7.9 and 7.75 under
439 the SSP3-7.0 and SSP2-4.5 scenarios, respectively (IPCC, 2021). Similarly, the low average DO
440 recorded in Spaanse Water Bay during the wet season (5.75 mg L^{-1}) represented a 10% decrease
441 compared to the dry season, which is more than the predicted global oxygen loss of ~6% by 2100
442 under SSP3-7.0 (IPCC, 2021). Notably, minimum DO levels recorded in the bays during the wet
443 season were <3 mg L^{-1} and thus occasionally came close to hypoxic levels (<2 mg L^{-1}). While the
444 temporary exposure associated with strong diel fluctuations is vastly different from an acute hypoxia
445 event (Lucey et al., 2020), these results nevertheless show a high risk for sustained hypoxia events to
446 occur inside the bays during the wet season – indeed, such events may have already occurred but gone
447 undocumented (Altieri et al., 2017). However, the strong diel DO and pH variability could also
448 enhance the resistance of resident organisms to sustained hypoxia and acidification, as has been
449 documented, for example, in corals for heat and to some degree acidification resistance (Brown et al.,
450 2022; Kapsenberg & Cyronak, 2019; Rivest et al., 2017; Safaie et al., 2018).

451 Taken together, our results show that the inland bays of Curaçao have significant potential to serve as
452 natural laboratories to study the effects of predicted future ocean conditions on resident taxa *in situ*.

453 This applies particularly within the context of strong diel fluctuations rather than shifted mean
454 conditions and with the caveat of co-occurring stressors. While a diverse range of natural laboratories
455 has been documented in recent years (Burt et al., 2020; Camp et al., 2018), true natural analogues that
456 perfectly simulate future ocean conditions rarely exist since co-occurring stressors are present at most
457 of these locations. For example, the semi-enclosed lagoon of Bouraké, New Caledonia, has among the
458 most extreme temperature, pH and DO conditions documented for a coral reef environment but also
459 has lower light and higher levels of organic matter and certain nutrients than reference reefs (Maggioni
460 et al., 2021). In Curaçao's inland bays, where coral cover and diversity are much lower than in
461 Bouraké (Figures 2, S5, Vermeij et al., 2007), co-occurring stressors include both natural and
462 anthropogenic factors, since naturally high turbidity and sedimentation rates have been exacerbated by
463 eutrophication and, in some areas, other forms of pollution (see next section). As human impacts on
464 tropical marine ecosystems continue to increase, the presence of these stressors in the inland bays can
465 offer a more realistic outlook into how global and local stressors will interact in the future but
466 comprehensive, high-resolution monitoring is essential. Overall, Curaçao's inland bays are unique
467 natural laboratories to investigate how environmental variability modulates stress tolerance to future
468 ocean conditions and could play an important role as resilience hotspots or refugia adaptation
469 (Kapsenberg & Cyronak, 2019; Rivest et al., 2017; Schoepf et al., 2023).

470 **Are Curaçao's inland bays threatened by local stressors?**

471 The inland bays generally showed elevated nutrient levels compared to the reefs, particularly during
472 the wet season, although this also depended on methodology (Fig. 2, Tables S4, S5). Given the lack of
473 major rivers in Curaçao, nutrient input into the marine environment typically occurs in the form of
474 rainfall-associated pulses during the wet season (Den Haan et al., 2016) as well as chronic seepage
475 from septic tanks (Estep et al., 2017). Thus, time-integrated monitoring via bio-indicators provides
476 more reliable estimates of nutrient levels than discrete water samples. Indeed, our seagrass samples
477 indicated that both bays should be considered eutrophic coral habitats (Govers et al., 2014a; van
478 Tussenbroek et al., 2016), with Spaanse Water Bay being slightly more eutrophied than Santa Martha
479 Bay due to high coastal development. In contrast, water samples showed nutrient concentrations
480 within the oligotrophic range (Crossland & Barnes, 1983; Tanaka et al., 2007) but are less
481 representative because they only present a snapshot in time. The water samples, however, confirmed
482 significantly higher nitrate, phosphate and ammonium levels during the wet than dry season across the
483 mangrove-seagrass-coral reef continuum, but only ammonium was 13.4% higher in the bays than on
484 reefs. Future research should consider the use of macroalgal bioindicators (Vaughan et al., 2021)
485 because, in contrast to seagrass, macroalgae are present in all habitats along this continuum (Figure 2).
486 Overall, elevated nutrient concentrations can have a wide range of effects on marine taxa (Nalley et
487 al., 2023) and may make them more susceptible to co-occurring stressors such as coastal acidification

488 (Silbiger et al., 2018), but this may not be the case for moderate nutrient concentrations in corals
489 (Dobson et al., 2021).

490 The high turbidity of the inland bays also has natural causes as low visibility and high (inorganic)
491 sediment trap collection rates were already reported ~60-100 years ago (de Kock & de Wilde, 1964;
492 van der Horst, 1924). Interestingly, in the present study, the two reef sites had higher sediment trap
493 collection rates than the two bays (Figure 5a) but this could represent a potential influx from the bays
494 that was enhanced during the wet season. In addition, the very different flow regimes make it
495 challenging to compare trap collection rates between sheltered bays and exposed reefs because there is
496 no apparent relationship between trap collection rate and turbidity in low-flow areas (Storlazzi et al.,
497 2011). Comparison with previous work (e.g. Kuenen & Debrot, 1995) is also difficult because careful
498 calibration experiments would be required to account for differences in trap design, deployment
499 parameters and locations (Storlazzi et al., 2011). While high sedimentation rates can generally have
500 negative impacts on the health of coral and other marine organisms (Fabricius, 2005; Rogers, 1990),
501 this is particularly the case when organic matter content is high (Bartley et al., 2014; Weber et al.,
502 2006). The inland bay sediment had more than twice as much organic content than reef sediment,
503 especially in Santa Martha Bay, which can influence the water clarity, as finer particles settle more
504 slowly, causing reduced light availability for longer periods, influencing the photosynthetic capability
505 of organisms and consequently the local pH and oxygen conditions (De Boer, 2007; Fabricius et al.,
506 2016; Storlazzi et al., 2015). It is therefore likely that the sediment regimes in the bays contribute to a
507 more stressful environment for sensitive organisms such as corals (Bainbridge et al., 2018).

508 Seagrass leaves collected in the two inland bays revealed the presence of certain trace metals (Table
509 S11) but it is unclear whether levels were higher than on the reefs given that no seagrass could be
510 collected there. *Halophila stipulacea* - a bioindicator for Cu and Zn (Bonanno & Raccuia, 2018) - had
511 much higher concentrations of both trace metals in Spaanse Water than in Santa Martha Bay. Given
512 that Cu is used as a biocide in antifouling paints while Zn is used to create metal alloys and pigments,
513 and as a fungicide (Nalley et al., 2021), this could well be related to the greater degree of coastal
514 development and boating activities at Spaanse Water Bay (Govers et al., 2014b). In addition,
515 *Thalassia testudinum* (collected in Spaanse Water Bay only) had Cu and Ni levels that were 20% and
516 69% higher, respectively than in the study by Govers et al., (2014b), whereas Fe and Zn
517 concentrations were ~50% lower. This suggests that trace metal concentrations in Spaanse Water Bay
518 may have changed over time but that overall trace metal pollution is ongoing. Notably, it is important
519 to acknowledge that Ni and Fe are naturally occurring elements in the marine environment. However,
520 Ni can also be present in higher concentrations as a result of industrial pollution, whereas Fe is not
521 only essential for plants and animals but is also widely utilized in various manufacturing processes
522 (Nalley et al., 2021). Several other trace metals were not detected, such as As, Cd, Co, Pb and Se, but
523 should be considered when determining the health of resident marine organisms as stress could be

524 intensified by the complex interaction between trace metals and global stressors (Kibria et al., 2021;
525 Negri & Hoogenboom, 2011).

526 **Effect-based chemical water quality assessment**

527 The combined use of passive sampling and effect-based methods represents a promising approach to
528 study chemical water quality (De Baat et al., 2020), but has so far seen limited application in tropical
529 reef ecosystems (Shaw et al., 2009) highlighting the novelty of the present study. After the comparison
530 of the bioassay responses to the respective threshold values, potential ecotoxicological risks were
531 detected using the bacterial bioluminescence inhibition assay at all four sites. This assay is both
532 indicative of general cytotoxicity in environmental samples as well as toxicity to marine bacteria.
533 Hence, the detection of ecotoxicological risks using the bacterial bioluminescence inhibition assay is
534 relevant as it indicates a concerning level of organic chemical pollution at all sites. Additionally, this is
535 of concern since bacteria dominate the ocean in abundance and support the existence of higher life
536 forms (Azam & Malfatti, 2007). Moreover, corals are holobionts, consisting of the coral itself and the
537 associated algal symbionts, bacteria and, fungi (Cleary et al., 2019; Knowlton & Rohwer, 2003), and
538 coral-associated microorganisms could be affected by the toxic compounds present in their
539 environment. This suggests that coral health may be reduced because coral-associated bacteria have
540 many beneficial roles for different coral life stages (McDevitt-Irwin et al., 2017). The interim effect-
541 based trigger value of the algal photosynthetic inhibition assay, on the other hand, was not exceeded at
542 any site, indicating that there appear to be low risks of chemicals inhibiting photosynthesis, such as
543 herbicides (Marzoni et al., 2021).

544

545 The *in vitro* CALUX assays confirmed the presence of organic chemical pollution and pointed towards
546 industrial- and wastewater effluents as sources of pollution. However, Spaanse Water Bay was the
547 only site at which the interim effect-based trigger value for the proxy for industrial pollution (PAH
548 CALUX) was exceeded. This is explainable since Spaanse Water Bay is near Caracas Bay, which was
549 previously used as an oil storage facility (1920-1986) (Debrot et al., 1998). Over the years, petroleum
550 spillage and leakage may have caused significant and persistent hydrocarbon pollution in which the
551 sediment currently serves as a source of legacy industrial pollution to the overlying water. Especially
552 in the more turbid wet season, resuspension of polluted sediments may have caused increased levels of
553 hydrocarbons in the overlying water, causing exposure of organisms to toxic chemicals, reflected in
554 the higher PAH CALUX response recorded at this location. Co-exposure to petroleum pollution and
555 ultraviolet radiation poses significant risks to the early life stages of corals (Nordborg et al., 2021).
556 The interim effect-based trigger value for the proxy for wastewater pollution (ER α CALUX) was not
557 exceeded at any of the investigated sites, but the highest response for this bioassay was also found in
558 Spaanse Water Bay. This could likely be due to the wastewater seepage that has been increasing since
559 the bay shifted towards a residential character in the 1980s (Debrot et al., 1998; Kuenen & Debrot,

560 1995). This effect can be exacerbated by the wet season but apparently did not cause ecotoxicological
561 risks at the present location and period of sampling.

562

563 The cumulative effect-based risk quotients obtained in the present study indicate that ecotoxicological
564 risks are potentially present at all the investigated locations. This pervasive mixture-toxic pressure was
565 effectively detected by combining passive sampling and a bioassay battery. This highlights the value
566 of combining these techniques, and underlines the relevance of applying effect-based methods for
567 chemical water quality assessment, also in tropical ecosystems (Caracciolo et al., 2023; De Baat et al.,
568 2020). Even the modest set of two *in vivo* and two *in vitro* bioassays, representing two relevant reef
569 organism groups and two relevant modes of action, elucidated the effects of a wide range of
570 bioavailable contaminant mixtures in the environment. In future effect-based water quality
571 assessments of coral reef environments, the set of applied bioassays can be expanded to include the
572 oxidative stress response as well as bioassays targeting integral life stages or functions of coral reef
573 biota such as scleractinian coral and sea urchin larvae (Neale et al., 2023; Shaw et al., 2009). The
574 highest cumulative effect-based risk quotient was found for Spaanse Water Bay, followed by Santa
575 Martha Bay, Santa Martha Reef and Spaanse Water Reef. This clearly illustrates that organisms in the
576 two inland bays are exposed to higher toxic pressures and the resulting ecotoxicological risks than
577 organisms inhabiting the reefs. This further confirms that inland bays can serve as sentinels for future
578 ocean conditions, also for the increasing chemical burden that threatens ecosystems worldwide
579 (Persson et al., 2022).

580 Chemical pollution can have both direct and indirect toxic effects on coral reef organisms, yet these
581 detrimental effects on tropical reef ecosystems have received little attention, especially when
582 compared with the effects of climate change like increasing sea surface temperature and ocean
583 acidification (Ouédraogo et al., 2023; Sigmund et al., 2023). In a recent study by Nalley et al., (2021),
584 a systematic review and meta-analysis provided water quality thresholds for corals. Similarly, a
585 systematic review of experimental studies by Ouédraogo et al., (2023) evaluated the toxicity of
586 chemical pollution to tropical reef-building corals, producing usable data for ecological risk
587 assessment. However, these chemical guidelines do not account for all possible chemicals that may be
588 present in water, nor do they consider the mixture effects that may arise from the numerous chemicals
589 simultaneously present in the water. Effect-based methods account for these effects of mixtures of
590 (un)known compounds, but their application in the mangrove-seagrass-coral reef continuum has, so
591 far, been limited, and unlike for freshwater systems, no well-established effect-based trigger value
592 (EBT) for coral reef systems is currently available. As a precautionary and pragmatic approach to
593 incorporate the potential sensitivity of coral reef organisms to chemical pollution, an additional safety
594 factor of 10 was applied to the freshwater EBTs of the bioassays used in the present study, to
595 extrapolate freshwater to marine water quality criteria (RIVM, 2015). However, it is presently

596 unknown if the intermediate EBTs are sufficiently protective for coral reef organisms, or whether they
597 are too strongly on the side of caution. Hence, the development of EBTs tailored to tropical reef
598 ecosystems is warranted to facilitate water quality monitoring and the protection of these vulnerable
599 and valuable ecosystems from the harmful effects of chemical pollution.

600 **Conclusion**

601 This study used a multi-disciplinary approach to assess both biotic (i.e., benthic cover and coral
602 diversity) and more than 20 abiotic parameters characterizing two inland bays and two nearby
603 reference reefs in the southern Caribbean during the cool, dry season and warm, wet season. Our
604 multi-pronged approach combined high-resolution temporal monitoring of key environmental
605 parameters with time-integrated pollution monitoring using both bio-indicators (nutrient and trace
606 metal pollution) and passive samplers and bioassays (organic chemical pollution). The inland bays of
607 Curaçao are marginal and/or extreme environments where the impacts of global stressors in
608 combination with local stressors are present. These bays can therefore act as natural laboratories to
609 examine the effects of a changing ocean on marine organisms, but a caveat may be the presence of
610 local stressors. However, local stressors have become significant threats to marine ecosystems and as
611 they will not be reduced in the near future, there is a need to understand the combined effects of global
612 and local stressors on marine organisms, which can be studied in these inland bays. In consideration of
613 the vital link between the mangrove-seagrass-coral reef continuum offered by the inland bays, it is
614 important to note that possibly the resident organisms are already at their limits in terms of their
615 capacity to persist in these multi-stressor habitats. It is therefore highly needed for future research to
616 identify their physiological mechanisms. The complexity of a multi-stressor environment such as the
617 inland bays offers great opportunities and requires prolonged monitoring, which can ultimately result
618 in the protection of these habitats as the ecological value of the inland bays in terms of marine life (e.g.
619 mangrove, seagrass beds and seagrasses) is high.

620

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