1	Acute Turbidity Exposures with Port of Miami Sediments Impact Orbicella
2	faveolata Tissue Regeneration
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27 Abstract

We evaluated acute turbidity effects on a threatened coral species (Orbicella 28 29 faveolata) under three challenge scenarios. A Port of Miami sediment homogenate was 30 used to simulate turbid conditions during dredging. We observed that low turbidity levels (≤ 4 NTU) have negative effects on *O. faveolata* tissue regeneration following a 31 32 96-h exposure. A 48-h turbidity exposure (maximum 30 NTU) followed by placement in fresh seawater had no effect on O. faveolata tissue regeneration, demonstrating that 33 short term turbidity exposures may not be detrimental to coral health. In a 13-day test, 34 treated coral fragments (maximum 30 NTU) exhibited significant delays in tissue 35 regeneration. Lytechinus variegatus embryos were used in a standard toxicity test. No 36 37 toxic effects were observed indicating pollutants in the sediment were not a factor inhibiting coral tissue regrowth. The results presented here can be used to inform 38 management decisions for proposed dredging activities proximal to coral reef habitats. 39 40 41 42 43 44 45 **Keywords:** turbidity, dredging, Orbicella faveolata, tissue regeneration, coral health, 46 sea urchin embryo development toxicity test 47

48 Introduction

Increased turbidity occurs in coastal zones and nearby watersheds due to natural 49 50 events (e.g., runoff from rainfall, wave action) and anthropogenic activities (e.g., agriculture, coastal development, dredging). Florida's seaports are some of the largest 51 in the world and generate \$117.6 billion in revenue (13.3 % of the state gross domestic 52 53 product) with marine cargo and vessel activity. (Florida Ports Council, 2016). Dredging in ports along the U.S. Florida coast is necessary for maintenance or expansion of 54 shipping channels; however, negative impacts to benthic fauna can occur from 55 increased turbidity or sediment accumulation. Elevated turbidity and increased 56 sedimentation from dredging near coral reefs may result in adverse health effects in 57 corals due to reduced light levels (Dallmeyer et al., 1982; Telesnicki and Goldberg, 58 1995), potential toxicity and/or tissue loss with surface accumulation of sediments 59 (which creates an anoxic environment), particularly if the sediment harbors significant 60 amounts of pollutants (metals, organohalide compounds, etc.) (Erftemeijer et al., 2012, 61 review). Reduced light levels negatively impact the ability of symbiotic algae to fix 62 carbon, a significant source of energy for the coral host (Muscatine and Porter, 1977). 63 64 Considering both the reduction in light and the excess mucus production that corals use to remove any accumulated sediment particles, it is reasonable to expect that 65 scleractinian corals under turbid conditions likely will have a reduced energy budget for 66 activities such as growth and reproduction. 67

68 While there have been many scientific reports on the effects of turbidity on 69 corals, there are few controlled laboratory studies linking discrete health effects from 70 increased turbidity as measured in nephelometric turbidity units, NTU (Fourney and

Figueiredo, 2017; Telesnicki and Goldberg, 1995). The Florida turbidity threshold (50 71 Jackson turbidity units) was established in 1962 and was converted to nephelometric 72 turbidity units (29 NTU) in 1983 (US Environmental Protection Agency, 1988). Since 73 the criterion was established prior to turbidity studies on shallow-water corals, it is 74 unknown whether it is sufficient for protection of ESA-listed corals such as Orbicella 75 76 faveolata. As part of a triennial review, the Florida Department of Environmental Protection is proposing the addition of a subpart (b) to the current criterion language, 77 specifically for the protection of coral reefs and hardbottom communities. Since this 78 79 addition is currently being evaluated, there is a crucial need for scientific data linking turbidity effects to coral health. The NOAA National Marine Fisheries Service (NMFS) is 80 also responsible for implementing the Essential Fish Habitat provisions of the 81 Magnuson-Stevens Fishery and Conservation Act and Section 7 of the ESA, requiring 82 the evaluation of routes of effects from dredging activities to corals, including ESA-listed 83 corals. Through these mandates, NMFS identifies measures to reduce impacts to 84 corals from dredge operations, such as development of acute and chronic water quality 85 thresholds protective of corals. 86

Growth rates (e. g., buoyant density, linear extension, etc.) have been used for decades to determine effects of turbidity on the coral animal (Bak, 1978; Dodge and Vaisnys, 1977; Goh and Chou, 1995; Hennige et al., 2008; Jokiel et al., 2014; Manzello et al., 2021; Rice and Hunter, 1992), however it is difficult to capture meaningful differences in short-term exposures (days), particularly for slower-growing coral species such as *O. faveolata*. Tissue regeneration has been used historically as an effective research tool to evaluate coral health *in situ*, with slower regeneration rates associated

with increased exposure to stressors (Dustan et al., 2008; Fisher et al., 2007;
Kramarsky-Winter and Loya, 2000; Meesters and Bak, 1993; Moses and Hallock, 2016;
Rodriguez-Villalobos et al., 2016; Traylor-Knowles, 2016). Recently we have
demonstrated its sensitivity as a laboratory method to determine acute ecotoxicological
effects to an oil-exposed, branching Pacific coral (May et al., 2020).

99 The mountainous star coral, Orbicella faveolata (formerly Montastraea faveolata, Ellis and Solander, 1786) is a massive bouldering stony coral native to the Gulf of 100 Mexico, western Atlantic Ocean and Caribbean Sea (Hoeksema and Cairns, 2020). 101 102 The relatively slow growth rate of *O. faveolata* coupled with increased loss of colonies due to disease, habitat degradation, land-based sources of pollution, and other factors 103 has resulted in its protection as a threatened species under the U.S. Endangered 104 Species Act (Federal Register, 2014). Orbicella faveolata is present in nearshore 105 waters, ranging from 0.5-40 m depths and is co-located with high-latitude corals 106 offshore from Port Everglades and Port of Miami, FL. Extensive mortality and habitat 107 loss for O. faveolata and other coral species have been associated with dredging 108 projects (Cunning et al., 2019; Miller et al., 2016). 109

The purpose of this study was to investigate how short-term (2-13 d) turbidity exposures using a simulated dredge sediment might affect tissue regeneration in *O. faveolata*. Additionally, the sea urchin embryo development assay (SUETOX), a standard method used to gauge toxicity of sediment interstitial waters to early life stages (ASTM, 2006; Chapman et al., 1995; Carr and Chapman, 1992; Carr et al., 1996), was employed to estimate potential toxicity of the sediment mixture. This assay was slightly

modified by using sediment mixed with artificial seawater at the same concentrations asthe coral exposures to gauge toxicity.

118 Methods

119 Husbandry

Colonies (>30 cm) of O. faveolata, are long-term holdings of the NOAA National 120 Centers for Coastal Ocean Science, Charleston Laboratory (NCCOS CHS) and held 121 under the Florida Keys National Marine Sanctuary, permit #FKNMS-2016-021 and 122 South Carolina Department of Natural Resources non-indigenous species permit #NI17-123 0401. One colony of O. faveolata was used to generate approximately 100 nubbins (1 124 cm long x 1 cm wide). Each nubbin was attached with cyanoacrylate gel to a custom 125 126 Teflon mounting peg. During the 12 weeks of healing, nubbins were maintained in a recirculating custom glass and Teflon aguarium system (570 L) in artificial seawater 127 (ASW, Tropic Marin, Wartemburg, Germany, 36 ppt). Biological filtration was provided 128 by a refugium compartment in the sump with a 5-inch-deep sand bed and approximately 129 80 lb of seeded live rock. A constant seawater temperature of 26 ± 1 °C was 130 maintained using a 300 W submersible glass heater (Jager TruTemp, Eheim, Deizisau, 131 Germany). Two LED light fixtures (Radion XR30 G4 Pro, Ecotech Marine, Lehigh 132 County, Pennsylvania) provided an average irradiance of 150 µmol photons m⁻² s⁻¹ with 133 a 10 h:14 h light:dark cycle. Lighting was programmed to increase or decrease slowly 134 (over 1.5 h) on each end of the light cycle. Water circulation was provided by a Teflon 135 (ETFE) lined pump (MD-55Y, Iwaki America, Inc., Holliston, MA) and 1-inch Teflon 136 137 (PTFE) piping. Calcium, alkalinity and trace element levels (e.g., Mg, K, Sr, Mo) were

regulated with custom glass calcium reactors (Greatglas, Inc., Wilmington, DE) via a
 manual drip system (total alkalinity range = 143-161 mg/L CaCO₃; pH = 8.1-8.3).

140 Sediment source and particle size analysis

Port of Miami sediment samples were collected at 20 discrete sites in April 2016 141 by the NOAA National Marine Fisheries Service, and used for mineralogy and stable 142 143 isotope analysis (organic C, organic N and inorganic C and O) (Swart, 2016). Approximately two years after analysis (samples held at room temperature), the 144 remaining samples were transferred to NCCOS CHS, for use in coral turbidity challenge 145 experiments. Sample subsets were pooled and mixed thoroughly by hand for the 146 preliminary tests. Subsequently the sediment mixture was cryomilled (SPEX Certi-Prep, 147 Metuchen, NJ) at 10 cycles per second for 9 min and dried (105 °C) for 72 h. A wet 148 sediment treatment sample in ASW (collected directly from a treatment vessel) was 149 used for particle size analysis. Samples were placed in a Malvern Mastersizer 3000 150 151 (Malvern, United Kingdom), which uses laser diffraction to determine particle size (range: $0.01 - 3500 \mu m$). Samples were circulated to ensure equal distribution in the 152 measurement cell. Data are reported as the average of 10 measurement runs. 153

154 <u>Sea urchin embryo development toxicity assay</u>

To understand potential intrinsic toxicity of the Port of Miami sediment sample, we performed a sea urchin embryo development toxicity test with *Lytechinus variegatus* (green sea urchin) using a modified standard protocol (ASTM, 2006). Sea urchin embryos were exposed to 50, 100 and 150 mg/L concentrations of the cryomilled, dried sediment homogenate mixed in artificial seawater in a static test. These were the same sediment concentrations used in the coral turbidity exposures. Assays were performed

in 20 mL pre-cleaned glass vials in a total volume of 5 mL, with approximately 200
embryos per vial. An assay negative (ASW) control was included in the experiment (4
replicate vials per treatment). Following a 48-h incubation (26 °C, no continuous
mixing), sea urchin development was halted with the addition of an equal volume of 2X
zinc-formalin fixative (Z-fix, Anatech, Poughkeepsie, NY) and embryo developmental
stage was scored and imaged (n = 100 embryos/vial).

167 Coral challenge experiments

168 Optimization of turbidity dosing system

There are several reports documenting the effect of turbid conditions on shallow 169 water coral species (Jones et al., 2016; Fourney and Figueiredo, 2017; Bessell-Browne 170 et al., 2017), however there are no cost-effective, standardized laboratory methods to 171 test turbidity effects on benthic marine organisms. Thus, we performed extensive 172 system validation tests to address this knowledge gap. In our tests, we included three 173 174 methods for sediment agitation (aguarium pump, 250 rpm stir plate, 300 rpm stir plate), three methods for suspending coral in the treatment beakers (custom-manufactured 175 polypropylene sheeting with silicone support, egg crate louver with silicone support and 176 177 egg crate louver without support) and three methods for preparing sediment (wet homogenate, wet cryomilled homogenate and dry cryomilled homogenate). A complete 178 list of materials used is detailed in Supplement 1. 179

180 96-h dose response experiment

Port of Miami sediment loads (50, 100 and 150 mg/L) were chosen based on preliminary tests to target peak turbidity measurements of approximately 5, 20 and 30 nephelometric turbidity units (NTU), respectively. These values were chosen to better

understand turbidity effects on *O. faveolata* within a broad range, not to exceed the 184 current Florida turbidity criterion (29 NTU above background) (US Environmental 185 Protection Agency, 1988), with background averaging ~1 NTU on the southeast Florida 186 coastal shelf; (Gramer and Hendee, 2018; Whitall, et al., 2019). Glass dosing beakers 187 (2.0 L) for controls and treatments (n = 4) were randomly arranged on two 15-position 188 stir plates in a temperature-controlled room (26.5 °C). The appropriate amount of dried 189 sediment homogenate was added to beakers filled with 2 L of ASW while stirring with a 190 2-inch Teflon stir bar (300 rpm). Control beakers contained no added sediment. 191 192 Sediment was mixed for 15 min prior to placing coral fragments in the center of the treatment vessels (Supplement 2). Beakers were covered with 1/8-inch glass plates 193 (which allow passage of broad spectrum light wavelengths) to reduce evaporation. 194 Lighting was provided by a single Ecotech Radion LED source (PAR = 133-168 µmol 195 photons $m^{-2} s^{-1}$) on a 10 h:14 h light:dark cycle. Turbidity and temperature 196 measurements were collected every 4 h during the course of the experiment beginning 197 at 10:30 am, except for the 2:30 am time point. Turbidity was measured using a Hach 198 model 2100P turbidimeter, calibrated with Hach StablCal® formazine standards once 199 200 daily. Salinity and pH were measured every 24 h. Total ammonia nitrogen was measured in preliminary 96 h sediment tests as previously described (May et al., 2022) 201 to ensure no toxicity occurred from that source. Measurements were below 0.07 mg/L 202 203 (< 8 µg/L ammonia) for all samples.

204 48-h turbidity exposure with recovery period

A pulse-chase experiment was devised to determine effects of an acute shortterm (48-h) exposure of coral to turbidity (100 mg/L sediment load) and to evaluate coral

recovery following the exposure. The appropriate amount of dried anthropogenic 207 sediment mixture was added to each test beaker as detailed for the dose response 208 experiment. Coral fragments were placed in the beakers 15 min following the addition 209 of sediment. Water quality measurements (turbidity, temperature, pH and salinity) were 210 performed as in the 96-h challenge experiment. Corals (controls and treatments) were 211 212 dosed for 48 h, then transferred to fresh ASW. Accumulated sediment was removed from treated fragments with gentle agitation in treatment water prior to transfer, since 213 wounds could not be imaged without sediment removal. Water changes (100 %) were 214 215 performed every 96 h thereafter, to maintain salinity. Lighting was as detailed above for the 96-h dose response experiment. Corals were imaged (see tissue regeneration 216 analysis section) at time 0 (prior to dosing), 48 h, and every 24 h following the water 217 change up to 13 d post treatment initiation. Corals were not given supplemental food 218 during the course of this experiment. 219

220 13-day turbidity exposure

A third experiment was conducted to determine the effects of a 13-day turbidity 221 exposure on coral tissue regeneration using a 100 mg/L sediment load. We based the 222 223 duration of this experiment on the wound healing process observed for the control fragments, factoring in an expected lag time for the dosed fragments. Glass dosing 224 225 beakers with ASW were randomly arranged on a single 15-position stir plate (control 226 and treatment, n = 4) and anthropogenic sediment was added and allowed to mix for 15 min before the addition of the coral fragments, as in previous experiments. Water 227 guality measurements and lighting were as described above. Water changes with either 228 fresh ASW (controls) or freshly-made treatment solution (turbid samples) were 229

performed every 96 h to keep salinity in check. Corals were imaged as described below
at time 0 and every 24 h up to 13 d post treatment initiation. Corals in the turbidity
treatment were agitated gently in the treatment water to remove accumulated sediment
prior to imaging, then immediately replaced into the treatment beakers.

234 Coral tissue regeneration analysis

235 Prior to experiment initiation, O. faveolata fragments (1 cm x 1 cm x 1 cm) were removed from the culture aquarium and placed in a Dremel workstation (Supplement 3), 236 approximately 2 cm below a Dremel rotary tool fitted with a 2.0 mm diamond hole saw 237 238 bit (Lasco Diamond Products, Los Angeles, CA). Coral support pegs were held in place manually as the rotating bit was lowered to the coral surface over the center of a polyp. 239 Nubbins were wounded to a depth of approximately 2 mm (entire polyp was removed). 240 Tissue slurry was removed by gentle application of an ASW stream using a 1000 µL 241 micropipettor. The coral was placed in a 500 mL clean glass jar filled with ASW and 242 bright field and fluorescent images (filter #U-MNV2: excitation: 400-410 nm; emission 243 (barrier filter): 455 nm; dichromatic mirror: DM400-410 nm; Chroma Technology Corp, 244 Bellows Falls, VT) were collected of the cut surface next to a centimeter ruler using a 245 246 MVX10 research macro zoom microscope with a 0.63x objective (Olympus, Melville, NY) and equipped with a DP71 digital camera (Olympus, Center Valley, PA) 247 (Supplement 4). The narrow violet wavelength excites the green fluorescent protein in 248 O. faveolata tissues, allowing for accurate measurement of tissue re-growth. At 249 experiment termination (96 h), coral wounds were imaged again as described above. 250 Skeletal area for each coral image was determined using a modified ImageJ 251 (Schneider et al., 2012) macro from the NOAA/NCCOS Coral Disease and Health 252

253 Consortium website

254	(https://cdn.coastalscience.noaa.gov/media/cdhc/Lesion_3mm_rule.txt). Pixel units
255	were calibrated to the centimeter ruler in each image. Total area of bare skeleton and
256	wound perimeter values were recorded for each fragment at time 0 and each
257	experimental time point. Percent tissue regeneration was determined from the
258	difference in skeletal area between the time 0 and the experimental time points. Linear
259	healing per day was calculated using the area and perimeter of the wound in Equation 1
260	(modified from Gorin et al., 1996).
261	Equation 1 : $(A_{t-1} - A_t)/mean(P_{t-1}, P_t)$
262	Where: At = skeletal area at given experimental time point
263	A _{t-1} = skeletal area at previous time point
264	Pt = wound perimeter at given experimental time point
265	P _{t-1} = wound perimeter at previous time point
266	Statistical analyses
267	Statistical analyses for the SUETOX test were performed using GraphPad Prism,
268	version 9.4.0. using percent normal embryo development. Data were normally
269	distributed (Shapiro-Wilk test) and residual variances were homogeneous. A one-way
270	ANOVA was performed with a Dunnett's post test using the ASW treatment as the
271	experimental control. Alpha was set to 0.05 for all tests.
272	For the 96-h dose-response test, three treatments (50, 100 and 150 mg/L) were
273	compared to the no sediment control using a one-way ANOVA followed by Dunnett's
274	post test. For the 48-h pulse-chase and 13-day turbidity challenge experiments,

- 275 differences between treatments and controls (overall and at each time point) were

evaluated using linear and (where possible) nonlinear mixed-effects models in order to
incorporate the fixed effect of treatment as well as the random effect of subject
(individual coral fragments) and any potential within-subject serial autocorrelation over
time. Models were tested under various within-subject correlation structures. The bestfitting models were selected based on significance of likelihood ratio tests, normality of
residuals, and homogeneity of variance.

282 **Results**

283 <u>Turbidity test design optimization</u>

Ten turbidity tests (with and without coral) were performed to determine the optimal method for the coral exposures (Supplement 5). The method resulting in the most stable and easily-replicated turbidity mixture included using defined weights of dry, cryomilled sediment, with continuous mixing in a 2.0 L beaker (2.0 L ASW volume) using a magnetic stir plate-Teflon stir bar combination at 300 rpm. Coral fragments on Teflon pegs were supported in the beaker on an egg crate louver, cut to fit snugly in the beaker and positioned at the 1200 mL mark.

291 Boulder coral nubbin wounding technique

292 Chisels and leather punches have been used to wound large bouldering corals in 293 the field, however when small chisels and punches were tested on the *O. faveolata* 294 nubbins, the small coral fragments shattered. Various other methods were attempted, 295 including chipping and scraping tissue using small flat tools (chisels, screwdrivers). The 296 wounds created were very uneven and difficult to measure, since tissue was not 297 removed evenly. Additionally, we could not create wounds of uniform sizes across 298 replicates. We found that removing a single polyp with a rotary drilling tool created the

299 most uniform wound sizes. The drilling process created a tissue and skeleton slurry,

which caused the remaining live tissue to bleach, if not removed. The slurry was rinsed from the live tissue with a gentle stream of ASW. This process resulted in *O. faveolata* nubbins with uniform wounds (~3 mm dia x 2 mm deep), and which healed ~60 % within four days.

304 <u>Sea urchin embryo toxicity assay</u>

Water quality parameters for the sea urchin treatments were within acceptable ranges for the test (Carr et al., 2006) (Supplement 6). Salinity ranged from 36 - 37 ppt and pH was between 8.32 and 8.40. Ammonia was at or below 13.4 µg/L for all treatments. No sediment toxicity was observed using the SUETOX test (Figure 1). A slight enhancement of normal embryo development in sediment treatments was noted (91-94 % normally developed embryos) compared to the ASW negative control (86 % normally developed embryos), but this was not statistically significant.

312 Orbicella faveolata turbidity challenge experiments

313 Water quality

General water quality parameters remained stable during the course of each 314 acute turbidity challenge experiment. Seawater temperatures ranged from 25.0-27.5 315 °C, pH ranged from 8.00-8.40 and salinity was between 36-39 ppt. Sediment loads of 316 317 100 mg/L and greater resulted in reduced pH over time in the dose-response experiment, and between water changes for the pulse-chase and 13-day experiments 318 319 (Figure 2). Turbidity results for each experiment are shown in Figure 3. Average peak 320 turbidity in the dose response experiment was 4 NTU for the 50 mg/L sediment load, 20 NTU for the 100 mg/L treatment, and 30 NTU for the highest treatment. Peak turbidity 321

was at time 0 for the low sediment load, and at 32-36 h for the two higher sediment 322 loads. No significant effects of measured water quality parameters on coral tissue 323 regeneration or linear healing rates, independent of sediment additions, were observed. 324 Particle size analysis of the wet anthropogenic sediment samples used in the coral 325 challenge experiments demonstrated that most sediment grains were less than ~100 326 327 μ m (Dv90 = 104 μ m, Supplement 7), with more than 50 % of the sediment grains classified as coarse silts or very fine sands based on the Wentworth grain size chart 328 (Wentworth, 1922). 329

Effects of 96 h dose response turbidity exposure on Orbicella faveolata tissue
 regeneration

Results of the turbidity 96-h dose response experiment for *O. faveolata* are presented in Figure 4. Turbidity from Port of Miami sediment homogenate negatively impacted coral tissue regeneration at all sediment loads (average peak turbidity = 4, 20 and 30 NTU). Percent regeneration was significantly lower for all three treatments compared to the control (p = 0.0016). Fluorescent images of representative *O. faveolata* fragments at time 0 and 96 h are shown in Figure 5.

338 Effects of acute, short-term turbidity on Orbicella faveolata tissue regeneration

A linear mixed-effects model fit to results of the 48-hr pulse-chase experiment did not detect any significant effect of turbidity on tissue regeneration and linear healing rates at any time point (Figure 6).

342 Effects of 13-day turbidity exposure on Orbicella faveolata tissue regeneration

Results of the 13-day turbidity exposure for *O. faveolata* are presented in Figure

344 7. While differences in tissue regeneration between treatment and control were

observed, the effect of treatment varied by time point (Figure 7A). Accelerated linear
healing rate between day 1 and day 2 was noted despite continued sediment exposure
(Figure 7B). The results of a nonlinear mixed-effects model incorporating a threeparameter logistic function to model the change in percent regeneration over time is
shown in Figure 8. The only parameter to vary significantly between treatment and
control was xmid (which represents the x value at the inflection point of the curve, ~50
%), with control xmid at 3.1 d versus 5.5 d for treated samples.

352 Discussion

Global commerce via shipping and the demand for deeper ports to accommodate 353 larger ships places increased pressure on nearshore benthic marine communities such 354 355 as coral reefs. Resource managers must weigh the benefits of commercial activities (ship traffic, dredging) in nearshore habitats with the health and longevity of those 356 marine species they protect. Research to understand measurable thresholds for 357 turbidity impacts is crucial for this decision-making process. To this end, we provide the 358 first report of a turbidity exposure using port sediments, resulting in negative impacts to 359 adult O. faveolata tissue regeneration after 96 hours, at turbidity levels below 5 NTU. 360

361 Dosing system

We created a relatively simple and inexpensive turbidity dosing system using a beaker and magnetic stirrer combination, taking care to eliminate potential negative effects of a vortex current within the beaker. The egg crate louver served two purposes: coral support and redirecting water flow. The egg crate provided sufficient interference and corals thrived in the microenvironment as a result. Experimental reproducibility was dependent upon the sediment particle size and dryness. We created turbid conditions

using simulated dredge sediment that mimics the particle size range of suspended 368 sediments (coarse silts to very fine sands) found to negatively affect corals during 369 dredging operations (Wang and Beck, 2017; Jones et al., 2016). Finer sediments 370 ensured more complete suspension in the water column, thus more reproducible 371 turbidity values. Evaporation was reduced with the use of glass plate beaker covers, 372 373 however, water changes were required every 96 h to maintain acceptable water quality parameters for the longer experiments. Turbidity fluctuations are expected during a 374 dredging project and depend upon weather conditions, water currents and tidal flow 375 376 (Erftemeijer et al., 2012). In our experiments, turbidity did not remain constant, but the incremental increases (as the sediment became suspended slowly) and decreases (as 377 the coral mucus trapped the sediment) mimic what might occur on the reef during 378 dredging. 379

380 <u>Sediment toxicity</u>

Potential toxicity from inner harbor sediments is possible. We did not analyze the 381 sediment for potential contaminants, however Fourney and Figueiredo (2017) reported 382 that sediment sourced from the nearby Port Everglades inner harbor contained volatile 383 384 organic compounds (0.02 mg/kg), metals (Pb and Zn) (78.02 mg/kg) and polycyclic aromatic hydrocarbons (PAH, 0.9 mg/kg) which could be toxic to corals. The Port of 385 Miami sediment homogenate we used could have contained similar levels of toxicants, 386 387 but due to the age and manipulations of the sediment (cryomilling, drying) prior to experiments, it is likely that any volatile organic compounds would be greatly reduced. 388 However, contaminants such as metals or legacy organic compounds such as 389 polychlorinated biphenyls (PCBs) would likely persist. 390

The SUETOX test is a sensitive assay that has been used to gauge toxicity of 391 marine sediment interstitial waters and industrial discharges (Balthis et al., 2018; Carr et 392 al., 2001; Carr et al., 2003; Chapman et al., 1995; May et al., 2022), Since marine 393 sediments can trap and concentrate chemical contaminants such as metals, 394 hydrocarbons, or pharmaceutical products, we exposed sea urchin embryos to the 395 396 same sediment concentrations used in the coral exposures to understand if residual sediment contaminants could have played a role in the turbidity impacts observed in O. 397 faveolata. The results of the sea urchin embryo toxicity test indicate that sediment 398 399 contaminants likely did not influence the results, since no impacts to urchin embryo development were observed in the 48-h static test. 400

401 <u>Turbidity effects on coral tissue regeneration</u>

We observed significantly decreased tissue regeneration in O. faveolata 402 fragments subjected to 50-150 mg/L of an anthropogenic sediment mixture for 96 h. 403 404 Interestingly, we did not see a correlation with dose and effect. Coral mucus was an efficient sediment trap resulting in decreased turbidity over time and sediment 405 accumulated in the wound area for all turbidity treatments. This may be a physical 406 407 factor limiting the tissue regrowth, since we observed delayed healing in the presence of sediment in the 13-d experiment compared to the no sediment controls. However, it is 408 likely that with the constant production of mucus for clearing sediment, less energy is 409 410 available to the coral for other activities, such as wound healing, feeding, respiration or growth. The results of the sea urchin embryo development test indicate that sediment 411 412 toxicity likely is not a factor in delayed tissue regeneration for these acute exposures.

Significant impacts from turbidity on *O. faveolata* tissue regeneration were not 413 observed for the shortest exposure duration (48 h), however this may be a conservative 414 result since sediment was manually removed prior to placement in fresh ASW. Tissue 415 regeneration reductions were statistically significant in the 13-day continuous exposure, 416 417 but did not directly mimic our observations in the 96-h dose response experiment (no 418 statistical significance on days 3 and 4). We speculate that the differences could be due to the daily removal of sediment from the wound site for tissue imaging in the 13-d 419 experiment, or individual variability due to innate factors. Manzello et al. (2021) have 420 421 shown that O. faveolata skeletal growth may be more resilient to turbidity impacts than other stony coral species such as *Pseudodiploria strigosa*, which indicates that O. 422 423 faveolata may have more energy available for physiological processes like tissue regeneration. While tissue healing rates of treated fragments lagged behind controls by 424 2.4 days, healing for both control and treated fragments was nearly 100 % by day 13 425 426 (Figure 7), demonstrating that O. *faveolata* may recover from initial turbidity insults with exposures of less than two weeks and below 30 NTU. However, the effects of multiple 427 stressors present during a dredging project (e.g., elevated turbidity, sediment deposition 428 429 on live tissue and light reduction) requires further testing.

We noted an accelerated linear healing rate following placement in ASW (48-72 h) in the 48-h pulse-chase experiment and also between 24-48 h in the 13-day continuous exposure experiment. Since the anthropogenic sediment likely contains a portion of organic components, it is likely that this increased availability of nutrients to the coral animal (via heterotrophy) or symbionts (increased primary production from biochemical cycling) would generate a coral 'growth spurt' while the nutrients last.

Since the availability of soluble nutrients (e.g., amino acids, fatty acids, etc) also can 436 affect growth and development of urchin embryos (Shilling and Bosch, 1994), the slight 437 increase in percent normal embryos we observed for sediment-treated samples in the 438 sea urchin toxicity test could be evidence for increased micronutrients in the sediment 439 exposures. Similar to pH observations at the Port of Miami during dredging operations 440 441 (Enochs et al., 2019), we observed a drop in pH in our challenge systems for sediment loads of >100 mg/L. This may result from the enhanced growth response of the coral 442 animal due to subsequent increased CO₂ produced from cellular respiration, or 443 decreased alkalinity from calcium carbonate deposition. However, reduced light from 444 the suspended sediment, should not be discounted as a factor in initial enhanced 445 healing rates. Increased turbidity has been shown to darken some coral tissues (i. e., 446 increase zooxanthellae numbers) for low (0-15 mg/L) suspended sediment 447 concentrations (Jones et al., 2020; Luter et al., 2021). This may be a direct result of the 448 increased organic materials in the suspended sediment, or a shift by the holobiont to 449 accommodate the reduction in photosynthetic products. This likely comes at an energy 450 cost for the coral animal, but also could result in enhanced short-term coral tissue 451 452 growth. We did not calculate symbiont numbers in these acute exposure experiments since wounding the corals would affect the outcome, however, this endpoint should be 453 considered for further work. 454

While the objective of this study was to gauge turbidity impacts from dredging, it also is reasonable to apply this knowledge to other sources of turbidity in the nearshore marine environment. Turbidity in southeast Florida may be influenced by construction in the coastal zone, or storm events which flush sediments from inlets onto the reef. Since

turbidity levels from inlets have been observed within the tested range of our study
(Whitall et al., 2019), we would expect that stony corals would be negatively impacted.
However, a better understanding of how these types of intermittent sediment fluxes may
affect the reef ecosystem, and ESA corals in particular, is needed.

463 <u>Future direction</u>

We observed impacts to O. faveolata tissue regeneration in acute turbidity 464 challenge experiments, within the range of 4-30 NTU. Our results suggest that O. 465 faveolata can recover from acute turbidity insults within a short time (days), yet 466 increased turbidity from dredging activities may last many weeks to months. However, 467 we must emphasize that in using aged, cryomilled sediments, potential sources of 468 stressors including some chemical contaminants and microbial pathogens were likely 469 greatly reduced. In addition, our laboratory studies did not mimic light attenuation 470 occurring at depth with increased turbidity. We recommend that future research 471 472 incorporate light attenuation and elevated temperatures to model seasonal or possible climate change scenarios. We also recommend longer-term (months) turbidity 473 exposures to more accurately gauge dredging effects near coral reefs. And since 474 475 dredging activities may be intermittent (depending upon working conditions), repeated exposure scenarios are important to consider, since cumulative insults could impair 476 coral health. 477

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678

Figure 1. Results of the sea urchin embryo development toxicity assay. Percent normal

680 development for each treatment was not statistically different from the artificial seawater

681 (ASW) negative control. Error bars represent the standard error.



Figure 2. pH measurements over time for the 96-h dose response experiment (panel A) and for the 48-h pulse-chase and 13-day experiments (panel B). Decreases in pH over time were noted for treatments with ≥100 mg/L sediment (panel A). For the pulse-chase experiment, both treatments were replaced with fresh seawater at 48 h (black arrow). Subsequent water changes for both pulse-chase and control treatments were performed every 96 h. White arrows indicate water and treatment changes for the 13-day experiment (every 96 h). Error bars represent the standard error.





Figure 3. Turbidity measurements over time for the 96-h dose response experiment (panel A) and for the 48-h pulse-chase and 13-day experiments (panel B). Turbidity remained stable across replicates during the dose response experiment. For the pulsechase experiment, the 100 mg/L sediment treatment was changed to fresh seawater at 48 h (black arrow). Subsequent water changes for both pulse-chase treatments were performed every 96 h. One replicate in the 13-day experiment had much higher turbidity than the other two replicates between 48 and 96 h. White arrows indicate

698 water and treatment changes for the 13-day experiment. Error bars represent standard699 error.







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Figure 5. Fluorescent images of representative *Orbicella faveolata* fragments in the 96h dose response experiment with Port of Miami sediment homogenate. Panels A-D =
time 0 images; Panels E-H = images at 96 h. Skeleton (sk) and live tissue (ts) are
indicated for controls (panels A and E), 50 mg/L sediment (panels B and F), 100 mg/L

sediment (panels C and G) and 150 mg/L sediment (panels D and H).





Figure 6. Results of the 48-h pulse-chase experiment with *Orbicella faveolata*. Panel A
= percent tissue regeneration and panel B = linear healing (mm/day). No significant

- differences were observed between the 30 NTU (100 mg/L sediment) treatment and
- control using a linear mixed-effects model. Error bars represent standard error.





Figure 7. Results of the 13-day turbidity exposure with *Orbicella faveolata*. Panel A =
percent tissue regeneration and panel B = linear healing (mm/day). Differences
between the 30 NTU (100 mg/L) treatment and control were determined using a linear
mixed-effects model. Percent tissue regeneration was significantly different on days 1,
2, 5, 6 and 7. Linear healing rate was significantly different on the first day. (Asterisks
denote significance, where p < 0.05.) Accelerated healing for treated coral fragments
was observed between days 1 and 2. Error bars represent standard error





Figure 8. Tissue regeneration results with the 13-day turbidity exposure using the
 nonlinear mixed-effects model, with percent regeneration over time modeled using a
 three-parameter logistic function. Based on likelihood ratio tests, the only parameter to

vary significantly (p < 0.05) between treatment (red line) and controls (green line) was

xmid (representing the x value at the inflection point of the curve), which was at 3.1

days for the controls and 5.5 days for coral fragments exposed to Port of Miami

sediment homogenate.

735 **Supplement 1.** Sources of supplies and equipment used in the preliminary turbidity

736 tests.

Item	Source (location)	Model or Product #				
Beaker, 2 L glass	Fisher Scientific (Waltham, MA)	02-540R				
Stir bar, 2" Teflon	Fisher Scientific (Waltham, MA)	14-512-127				
	Jeiotech Lab Companion (Billerica,					
Stir plate, 15-position	MA)	AAH332615U				
Egg crate louver	ePlastics (San Diego, CA)	W/EGG.375X2X4				
PP perforated sheeting, 3/16"	US Plastics Corp. (Lima, OH)	42562				
Silicone gel	Marineland (Blacksburg, VA)	31010				
Aquarium pump	Hydor, USA (Sacramento, CA)	Koralia Nano 240				
Light source	EcoTech Marine (Allentown, PA)	Radion XR30w Pro LED				

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738 **Supplement 2.** Side (panel A) and top (panel B) views of *Orbicella faveolata* fragment

in the test vessel. Coral is supported by an egg crate louver, cut to fit snugly in the

beaker. The wound created for the tissue regeneration assay is indicated with a yellow

arrow (panel B, shown with accumulated sediment during exposure).



Supplement 3. View of the Dremel workstation used for wounding *Orbicella faveolata*fragments and the stainless-steel tongs for holding coral fragment in place (panel A). A
close-up view of an *O. faveolata* coral fragment under the 2.0 mm diamond hole saw bit
is shown in panel B.



Supplement 4. Photomicrograph images of the Orbicella faveolata 2.0 mm diamond
hole saw wound under bright field (panel A) and near-violet fluorescent (panel B)
illumination. A centimeter ruler (with mm increments) shown in each image was used to
calculate skeletal area and wound perimeter using ImageJ.



- **Supplement 5.** Results of preliminary turbidity tests and experimental parameters
- used. Abbreviations: PP = polypropylene, PS = polystyrene, NTU = nephelometric
- turbidity units, PAR = photosynthetically active radiation, OFAV = *Orbicella faveolata*.

Date	Coral Vessel		Light	Mixing	Coral	Duration	Sediment	Results		
	species	i	source	apparatus	support					
2/13/19	none	2 L plastic	ambient	Small (40	none	5 h	Defined	Experiment termi		
		beakers,		gal/d)			sediment	after 5 h with turb		
		2.0 L		aquarium			loads (20,	out of target rang		
		seawater		pump			30, 40 g/L)	NTU). Tested 1: ²		
		volume		suspended	uspended		using wet	1:20 dilutions of 2		
				from			Port of	stock, which fell i		
				custom			Miami mix	target turbidity rai		
				PP frame						
5/6/19	OFAV	2 L glass	One	Small (40	Custom	96 h	20 g wet	Diluting sediment		
		beakers	Ecotech	gal/d)	PP		Port of	solution was time		

		with	Marine	aquarium	frame		Miami	consuming as tur
		custom	Radion	pump	with 1		mixture used	was not stable ov
		round	XR30w	suspended	cm holes		to make a	Custom PP supp
		glass	Pro LED	from	on		conc. stock	frame accumulate
		covers,	light, PAR	custom PP	silicone		for dilution to	sediment (>90 %
		1.5 L	= 125-150	frame	support		appropriate	turbidity reductior
		seawater	µmol/m²/s		in beaker		turbidity	Aquarium pump k
								sediment suspen
								but heated water
								declining coral he
5/13/19	none	2 L glass	ambient	15-position	PS egg	215 h	Defined	Turbidity more sta
		beakers		stir plate	crate		sediment	without coral, but
		with		with 2"	louver		loads (1, 3, 5	significant fluctua
		custom		Teflon stir	cut to fit		g/L) using	over time. Turbidi
		round		bar in	inside		wet Port of	ranged from 23 -2
		glass		glass	beaker,		Miami	NTU over time wi
		covers,		beaker,	held with		mixture	highest turbidity a
		2.0 L		250 rpm	silicone			for the two lower
		seawater						sediment amount

5/23/19	none	2 L glass	ambient	15-position	PS egg	120 h	Defined	Turbidity increase
		beakers		stir plate	crate		sediment	20 to >100 NTU o
		with		with 2"	louver		loads (0.5	days, but appeare
		custom		Teflon stir	cut to fit		and 0.75	stablilize after 3 d
		round		bar in	inside		g/L) using	Increased turbidit
		glass		glass	beaker,		wet Port of	to higher stir rate
		covers,		beaker,	held with		Miami	(increased from 2
		2.0 L		300 rpm	silicone		mixture	300 rpm).
		seawater						
12/2/19	none	2 L glass	ambient	15-position	PS egg	72 h	Cryomilled	Turbidity stable fo
		beakers		stir plate	crate		sediment	h for two lower se
		with		with 2"	louver		homogenate	loads (0.1 g/L = 5
		custom		Teflon stir	cut to fit		(0.1, 0.3 and	and 0.3 g/L = 40
		round		bar in	inside		0.5 g/L).	but still some sed
		glass		glass	beaker,			accumulating on
		covers,		beaker,	held in			crate and silicone
		2.0 L		300 rpm	place			supports. Turbidi
		water			with			increased for the
		volume			silicone			sediment load (>′
								NTU).
12/9/19	none	2 L glass	ambient	15-position	PS egg	144 h	Cryomilled	Sediment accum
		beakers		stir plate	crate		sediment	on silicone suppo

Iouverhomogenateto account fora stircut to fitat 0.2, 0.25differences betweeinsideand 0.3 g/Lreplicate test sambeaker,and 0.3 g/LBubbles accumulr,held inon the egg crate aomplaceand and a set and beaker,insidewithand a set and beaker,and a set and beaker,and a set and beaker,
a stircut to fitat 0.2, 0.25differences betweeinsideand 0.3 g/Lreplicate test sambeaker,beaker,Bubbles accumulr,held inon the egg crate aomplaceImage and the same and th
inside and 0.3 g/L replicate test sam beaker, beaker, Bubbles accumul r, held in on the egg crate a om place in trapped sediment with silicone in the egg crate a
beaker, Bubbles accumul r, held in on the egg crate a om place trapped sediment with silicone
r, held in on the egg crate a om place trapped sediment with silicone
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sition PS egg 96 h Cryomilled Dried cryomilled
ate crate sediment sediment homoge
" louver homogenate resulted in stable
stir cut to fit dried in oven turbidity for all se
snugly to remove loads for 96 h. T
inside liquid. for each was: 0.1
r, beaker, Tested 0.1, ~15 NTU; 0.2 g/L
no 0.2 and 0.3 NTU; 0.3 g/L = ~9
silicone g/L.
support
sition PS egg 72 h Dried and Sediment accum
ate crate cryomilled in top coral woun
" louver sediment Water quality
stir cut to fit homogenate parameters rema
r, beaker, I Tested 0.1, m no 0.2 and 0.3 silicone g/L. support 72 h Dried and te crate crate cryomilled louver into fit into the sediment

		round	XR30w	bar in	snugly		(0.2 g/L) and	stable over 72 h.
		glass	Pro LED	glass	inside		control (no	intial increase an
		covers,	light, PAR	beaker,	beaker,		sediment)	stable after the in
		2.0 L	= 125-150	300 rpm	no		tested with	h (~40 NTU).
		water	µmol/m2/s		silicone		wounded	
		volume			support		coral	
1/23/20	OFAV	2 L glass	One	15-position	PS egg	96 h	Dried and	Sediment accum
		beakers	Ecotech	stir plate	crate		cryomilled	in side coral wou
		with	Marine	with 2"	louver		sediment	Water quality
		custom	Radion	Teflon stir	cut to fit		homogenate	parameters rema
		round	XR30w	bar in	snugly		(0.2 g/L in	stable. Turbidity
		glass	Pro LED	glass	inside		duplicate)	decreased over ti
		covers,	light, PAR	beaker,	beaker,		tested with	less than 5 NTU.
		2.0 L	= 125-150	300 rpm	no		wounded	
		water	µmol/m2/s		silicone		coral to	
		volume			support		determine	
							coral effects	
							over time.	
1/27/20	OFAV	2 L glass	One	15-position	PS egg	96 h	Dried and	Experiment cond
		beakers	Ecotech	stir plate	crate		cryomilled	temperature-cont
		with	Marine	with 2"	louver		sediment	room. Compresso
		custom	Radion	Teflon stir	cut to fit		homogenate	a few hours into
		-	-	-			-	-

	round	XR30w	bar in	snugly	(0, 0.05, 0.1,	experiment, but w
	glass	Pro LED	glass	inside	0.15 g/L in	continued. Tissu
	covers,	light, PAR	beaker,	beaker,	triplicate)	regeneration for o
	2.0 L	= 125-150	300 rpm	no	tested with	was lower than e
	water	µmol/m2/s		silicone	wounded	at experiment
	volume			support	coral for 96	termination, likely
					h.	seawater tempera
						(25-29 °C).

Supplement 6. Results of the sea urchin embryo development toxicity test.

Test species =	Test species = Lytechinus variegatus. Sperm dilution used: 1:250. Sperm count (undiluted = 1.26 x 10^10 cells/mL. Ova count = 4200 eggs/mL. Fertilization = 91%.																				
Abbreviations: ASW = artificial seawater; N = normally developed embryos; U = underdeveloped embryos; A = arrested embryos; M = malformed embryos.																					
The results of	The results of four replicate vials are presented.																				
			Vial 1					Vial 2					Vial 3					Vial 4]
TREATMENT	Ν	U	Α	м	TOTAL	Ν	U	Α	м	TOTAL	Ν	U	Α	м	TOTAL	Ν	U	Α	м	TOTAL	
ASW	78	1	5	16	100	91	1	2	6	100	95	5	0	0	100	78	4	1	17	100	
50 mg/L	98	1	0	1	100	95	4	1	0	100	75	1	0	24	100	95	0	2	3	100	
100 mg/L	87	5	4	4	100	93	4	2	1	100	97	2	0	1	100	98	2	0	0	100	
150 mg/L	96	3	0	1	100	92	4	1	3	100	92	2	2	4	100	97	1	1	1	100	

Supplement 7. Particle size analysis results of the Port of Miami cryomilled sediment

⁷⁶⁰ homogenate samples used in the sea urchin and coral experiments.

NIST Biorepository Particle Size Report



Measurement Details		Measurement Details						
	Operator Name jmn1	Measurement Date Time 9/28/2022 11:18:01 AM						
	Sample Name Average of 'PortofMiami_Wet02'	Result Source Averaged						
Applysic								
Analysis	Particle Name Coment	Concentration 0.0015 %						
Partic	a Refractive Index 1.680	Concentration 0.0013 %						
Particle	Abcomption Index 0.100	Volumo Polovi (10.1) um 7.52 %						
Farucie	Dispersant Name Segurator							
Disporsan	t Pofractive Index 1 390	Dr (10) 118 um						
Dispersali	Scattering Model Mie	Dv (10) 11.0 μm						
	Analyzic Model Conoral Purpose	Dv (90) 34.5 μm						
		DV (50) 104 μm						
	Locar Observation 0.55 %	Residual 0.55 %						
Francisco								
Frequency								
Volume Density (%)								
2-								
0	0.1 1.0 [22] Average of 11:18:01 AM	10.0 100.0 1,000.0 10,000.0 Size Classes (µm) 'Portof/Miami_Wet02'-9/28/2022						



Mastersizer - v3.81 Page 1 of 2 PortofMiami_Wet

Created: 10/27/2020 Printed: 10/13/2022 11:56 AM