Original Article

Mucous sheet production in *Porites*: an effective bioindicator of sediment related pressures

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\textbf{A B S T R A C T}

Some coral species of the genus *Porites* can produce thick mucous sheets that partially or completely envelope the colony's surface. This phenomenon has been reported many times, but the cause and ecological significance remains unclear. In this study, sheet production was examined in response to elevated suspended sediment concentrations associated with a large-scale, extended dredging project on a coral reef. Approximately 400 corals at 16 locations situated from 0.2–33 km from the excavation area were examined at fortnightly intervals over the 1.5 year dredging campaign. Mucous sheets were observed on 447 occasions (from 10,600 observations), with average mucous prevalence ranging from 0–10%. Overall 74 ± 5% of the colonies <1.5 km from the dredging produced one or more sheets. High levels of mucous coverage (>95% of the colony surface) was observed on 68 occasions, and 82% of these occurred at sites close to the dredging. Approximately 50% of colonies produced ≥3 sheets over the monitoring period, and 90% of these were located close to the dredging. In contrast, at distantly located reference sites (>20 km away), mean mucous sheet prevalence was very low (0.2% ± 0.1), no colonies produced more than 1 sheet, and only 1 colony was observed with high mucous coverage. In a laboratory-based experiment, explants of *Porites* spp. exposed to fine silt also produced mucous sheets (105 sheets recorded in 1100 observations), with nearly 30% of the fragments exposed to repeated sediment deposition events of 10 and 20 mg cm\textsuperscript{-2} d\textsuperscript{-1} producing 2 new sheets over the 28 day exposure period. These multiple lines of evidence suggest a close association between mucous sheet formation and sediment load, and that sheet formation and sloughing are an additional mechanism used by massive *Porites* spp. to clear their surfaces when sediment loads become too high. These results suggest that mucous sheet formation is an effective bioindicator of sediment exposure.

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1. Introduction

Hard corals continually synthesise and secrete mucus, maintaining a near continuous mucosal barrier of several hundred-micron thickness over their surfaces (Brown and Bythell, 2005; Bythell and Wild, 2011; Jatkar et al., 2010b; Lewis, 1973). The majority of this secreted mucus dissolves in the water column and is generally not noticeable (Wild et al., 2004), although some mucus forms visible filaments and strings that eventually detach from corals producing mucus floccs in the water column (see images in Wild et al. (2004), Huettel et al. (2006) and Bythell and Wild (2011)). A more conspicuous form of mucus production occurs in corals from the genus *Porites*, resulting in complete covering of a thick mucosal layer (see Veron (2000), page 278). This phenomenon has been reported many times, but the cause and ecological significance remains unclear.

The production of a thick mucous layer in *Porites* was first described by Duerden (1906), who referred to it as a mucous felt, and since then many other terms have been used, including: sheet (Johannes, 1967), envelope (Lewis, 1973), web (Ducklow and Mitchell, 1979), sheath (Thompson, 1980), layer and covering (Marcus and Thorhaug, 1981), coat (Duerden, 1906), tunic (Edmunds and Davies, 1986), film (Coffrotch, 1988), and mat (Stafford-Smith and Ormond, 1992). The term sheet is used hereafter.

These sheets initially appear as clear mucous films, tightly covering the coral surface (Lewis, 1973), but once formed the mucus undergoes a physicochemical transformation from a fluid-
like (Ducklow and Mitchell, 1979), to gel-like consistency (Coffroth, 1985, 1988; Duverden, 1906; Stafford-Smith and Ormond, 1992). SEM imaging of sheets from Porites compressa indicate that they are made of multiple layers, which act as a physical barrier over the epidermis, but still allow the diffusion of dissolved material (Johnston and Rohwer, 2007). The biochemical composition of Porites mucous sheets has been described by Lewis (1973), and Coffroth (1990); and is likely to be composed of mucins, i.e. large glycoproteins that possess different properties of elasticity and viscosity (Jatkar et al., 2010a). The term mucus is used hereafter, and in a broad sense (see Bythell and Wild [2011]), as the sheets are likely to contain mucins along with a variety of dissolved and/or particulate organic matter excreted by the coral.

Once formed, mucous sheets can become fouled with algae, protozoa, zooplankton (Mayer and Wild, 2010), bacteria, ciliates, nauplii, crustacea (Coffroth, 1990), fecal pellets, other unidentifiable debris and sediment (Hartnoll, 1974; Johnston and Rohwer, 2007; Krupp, 1984; Lewis, 1973; Mayer and Wild, 2010). The transformation of viscous mucus to a sheet like formation has been linked to fouling by sediment (Ducklow and Mitchell, 1979). Sheets usually remain covering colonies from days to months, but eventually are shed (commonly referred to as ‘sloughed’) by water currents, leaving the colony free of fouling material (Duverden, 1906; Lewis, 1973).

Mucous sheets have been reported in Porites astreoides, P. divaricata, P. furcata, and P. porites in Florida and the Caribbean (Bak and Elgershuizen, 1976; Coffroth, 1985; Ducklow and Mitchell, 1979; Edmunds and Davies, 1986; Glasl et al., 2016; Lewis, 1973; Marcus and Thorhaug, 1981), Porites cylindrica from Japan (Kato, 1987), Porites compressa from Hawaii (Krupp, 1984; Marcus and Thorhaug, 1981), along with P. lutea, P. lobata, P. australiensis and P. murrayensis on the Great Barrier Reef of Australia (Coffroth, 1988; Coffroth, 1990; Stafford-Smith and Ormond, 1992). Similar sheet like structures have also been reported in the corals Diplosa-trea heliopora, Pachyseris speciosa, Mycedium elephantotus, Pectinia lactuca, P. poronia, Turbinaria peltata and T. mesenterina (Sofonia and Anthony, 2008; Stafford-Smith and Ormond, 1992), Heliopora coerulea (Lewis, 1973), and in other groups such as gorgonians, alcyonaceans and zoanthids (Coffroth, 1988; Rublee et al., 1980).

Fluid mucus production by corals aids feeding and self-cleaning by mucociliary transport processes (Duverden, 1906; Hubbard and Pocock, 1972; Lewis and Price, 1975; Stafford-Smith and Ormond, 1992; Stafford-Smith, 1993). A host of other functions for fluid mucus production have also been suggested, including defence against pathogens and resistance to desiccation, ultra violet radiation, pollutants and physical damage (see reviews by Brown and Bythell (2005) and Bythell and Wild (2011)). Whilst fluidic mucus production plays many important roles in corals, it is not clear why Porites spp. produce the gel-like sheets. Some studies with P. compressa, P. porites and P. cylindrica have observed a relationship between temperature, salinity and mucous sheet production (Kato, 1987; Marcus and Thorhaug, 1981). Xenobiotics such as copper and
Table 1
Summary statistics of site location, distance from dredging, depth and water quality characteristics over the dredging period, the number of colonies examined, the number of observations made and summary statistics for mucous sheet formation of Category 3 events or higher (i.e. >5–35% mucous covering) including the total number of events, and the number of new mucous sheet events.

<table>
<thead>
<tr>
<th>A</th>
<th>Site name (see Fig. 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Distance (km) from the closest location of dredging</td>
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<tr>
<td>C</td>
<td>Median water depth (m)</td>
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<td>D</td>
<td>95th percentile ($P_{95}$) of all 14 d running means (NTU)</td>
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<tr>
<td>E</td>
<td>5th percentile ($P_{5}$) of all 14 d daily light integral averages (DLI, mol photons m$^{-2}$ d$^{-1}$)</td>
</tr>
<tr>
<td>F</td>
<td>Number of surveys conducted in the dredging phase</td>
</tr>
<tr>
<td>G</td>
<td>Number of colonies at each site</td>
</tr>
<tr>
<td>H</td>
<td>Σ individual observations of a single colony (i.e. colonies × surveys × baseline and dredging period)</td>
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<tr>
<td>I</td>
<td>Σ individual observations of a single colony (i.e. colonies × surveys × dredging period)</td>
</tr>
<tr>
<td>J</td>
<td>Σ number of times a colony was observed with ≥5% mucous cover (category 3) over the dredging period</td>
</tr>
<tr>
<td>K</td>
<td>Total number of ‘new’ mucus events (≥5% mucus cover, category 3) over dredging period</td>
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<tr>
<td>L</td>
<td>% of colonies at each site observed with ≥5% mucous cover (category 3) at any stage over the dredging period</td>
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<td>M</td>
<td>Mean ± SE (Column N) incidence of mucous sheet formation (≥5% mucus cover, category 3) over the dredging period</td>
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<th>DLI$^a$</th>
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<td>11,798</td>
<td>10,600</td>
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<td>378</td>
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$^a$ A new mucous sheet was classified as one where there was no mucous sheet visible in the previous survey and therefore had to have formed between surveys.

$^b$ Nephelometric Turbidity Units.

$^c$ Daily Light Integral (DLI, mol photons m$^{-2}$ d$^{-1}$).

Crude oil can increase fluid mucous production in corals (Mitchell and Chet, 1975), and sheets have been observed in Porites lichen exposed to cyanide (Jones and Steven, 1997).

A number of studies have explored the relationship between mucous sheet formation and sediment, but with contradictory results. Bak and Elgershuizen (1976) noted that mucous sheets can be induced in the laboratory in P. astreoides on contact with fine sediment. While Coffroth (1985), also found a relationship between mucous sheet formation and sediment deposition in laboratory-based studies with P. astreoides and P. furcata. However, sheet formation also occurred following a reduction of water movement, and was only observed in response to sediment when combined with reduced water flow (Coffroth, 1985). Coffroth (1991), and Kato (1987) could not find any relationship between mucous sheet formation and sedimentation in field studies. In the most comprehensive study of the response of corals to sediments, Stafford-Smith and Ormond (1992) could not induce mucous sheet formation by fine sediment in both laboratory and in-situ manipulations, concluding that sheet formation had no apparent relationship to sediment flux.

Regular mucous sheet formation has been observed in Caribbean Porites spp. (Lewis, 1973), and cyclical mucous sheet formation linked to lunar cycles has been recorded in P. cylindrica from Japan (Kato, 1987), and P. furcata and P. astreoides from Panama (Coffroth, 1991). In Porites from Panama, the sheet formation appeared to be preceded by periods of polyp retraction (Coffroth, 1991). This observation, coupled with the response of Porites to sediments in laboratory experiments described previously, led Coffroth (1988) to suggest sheet formation is an epiphenomenon of some other coral activity pattern, and possibly the expansion, contraction or behaviour of the polyps related to suspended sediment concentrations (SSCs).

While evidence is currently equivocal, a potential link between sediment related stresses and mucous sheet production suggests that the presence of these sheets on Porites colonies may provide a potential environmental indicator of sub-lateral stress on corals. In this study, we use information from observations during a large-scale, capital dredging project to examine the relationship between mucous sheet formation in massive/sub-massive Porites spp., and dredge related sediment pressures. Specifically, we test whether temporal patterns and spatial distributions of mucous sheet formation can be explained by timing and proximity to dredging, and whether sheet formation is influenced or driven by other environmental factors such as lunar periodicity and temperature. Subsequently, we test whether mucous sheet formation can be induced in Porites spp. in a controlled, laboratory-based manipulative study, involving exposure to a range of high suspended sediment concentrations and sediment deposition rates.

2. Materials and methods

2.1. Field study

The field study was conducted before and during a large-scale capital dredging project on the reefs of Barrow Island, located ~50 km offshore of the Pilbara coast of north-west West-
ern Australia (Fig. 1). Dredging primarily occurred at 2 locations, for a turning basin near a future material offloading facility (MOF), and for an entrance channel associated with a liquefied natural gas (LNG) jetty (Fig. 1). Dredging was undertaken with a combination of trailing suction hopper, cutter suction and back hoe dredges, and typically occurred on a 24 h basis. The material dredged was predominantly unconsolidated, undisturbed carbonate sediments, which ranged from rubble to gravelly sand mixed with fine silts and clays. For further detailed description of the project see Fisher et al. (2015), and Jones et al. (2015).

As part of the state and federal regulatory approval conditions associated with the dredging, water quality and coral health monitoring was conducted at numerous sites, at different distances north and south of the primary dredging area (Fig. 1). Water quality monitoring included measurements of turbidity, light, temperature and water depth at ∼10 min intervals, and included an extended pre-dredging baseline period. For further detailed description of the water quality monitoring plan see Fisher et al. (2015), and Jones et al. (2015). Coral health monitoring involved regular photographing of individually marked (tagged) corals at each of the water quality monitoring sites. The sites, which ranged in depth from 4–11 m, were installed from June 2009 onwards in a pre-dredging (baseline period), and before dredging began on the 20 May 2010. Surveys were undertaken typically every 14 d for the duration of dredging (until 11 November 2011) (Table 1). For most sites, 27–40 surveys were undertaken over the dredging period, but for the sites closest to the dredging (LNG and LNGA) only 17 and 23 surveys were conducted respectively (Table 1).

During the dredging there was a near continuous unidirectional, southerly movement of the sediment plumes through the 1.5 y study (Evans et al., 2012; Fisher et al., 2015). Analysis of mucus sheet formation and water quality was therefore restricted to sites south of the MOF and LNG excavation areas, ranging in distance from 0.2–33.8 km from dredging activity (Fig. 1). Two reference sites (AHC and REFN), located >28 km to the north of the dredging were also examined (Fig. 1).

Photographs of tagged colonies of massive and sub-massive Porites spp. colonies were examined for the presence of mucus. Massive Porites spp. colonies can grow to more than several meters in diameter, are common throughout the Indo-Pacific, and are found from offshore clear water reefs to coastal, turbid regions. As Porites spp. are difficult to identify underwater due to their small and variable corallites (Veron, 2000), the species most likely included a mix of P. lobata and P. lutea, which are the most common massive Porites spp. in the region (Jones, 2016). Porites spp. colonies were assigned to one of seven categories based on mucus coverage, where 1 = 0%, 2 = 1–5%, 3 = 5–35%, 4 = 35–65%, 5 = 65–95%, 6 = 95–99, and 7 = 100% coverage. The number of mucous sheets produced per colony over the dredging phase was also determined where a mucous sheet event was defined as any colony classified as category 3 or greater (>5–35% mucus coverage), and where no mucous sheet was observed in the previous survey.

Water quality data were examined using the methods described in Fisher et al. (2015). Turbidity data were averaged on a daily basis (30 min). Photosynthetically active radiation (400–750 nm, PAR) data was used to calculate a daily light integrals (DLI) as mol photons m⁻² d⁻¹ and the 14 d average running mean of these variables was subsequently calculated.

2.2. Laboratory study

All laboratory testing was conducted at the Australian Institute of Marine Science (AIMS) Sea Simulator (SeaSim) at Cape Cleveland (Queensland, Australia), using 50 mm diameter cores of massive Porites spp. colonies. Cores were collected from parent colonies located at 3–10 m depth at Davies Reef, a clear-water, mid-shell reef in the central Great Barrier Reef region. The species collected included P. lobata and P. lutea, which were the two most dominant species at Davies Reef, and match those present at Barrow Island (Jones, 2016; Veron, 2000). Cores were extracted using a pneumatic drill, glued onto numbered aragonite coral plugs, and left for a 6 week recovery-period in 201 L flow-through holding tanks at the SeaSim before experimentation. Sediment used in the study was predominantly biogenic, calcium carbonate, collected from the Davies Reef lagoon. The sediment was dried and ground using a rod mill grinder until the mean grain size was ∼30 μm (range: 0.5–140 μm), as measured using laser diffraction techniques (Mastersizer 2000, Malvern instruments Ltd, UK). Total organic content of the sediment was 0.25%. All coral and sediment were collected under GBRMPA permit G13/35758.1.

Mucous sheet formation in response to sediment-exposure was examined in 10 fiberglass tanks, each holding 1000L of water. The tanks received a continuous flow of 0.4 μm filtered seawater (27 °C seawater, 33 PSU), at a rate of 400 mL min⁻¹, resulting in 0.6 turnovers of water each day. The control system for the experiment (custom control logic on Siemens S7-1500 PLC) provided the integrated management of sediment delivery, light intensity, turbidity and deposition rates (Fig. 2). Sediment was delivered to individual tanks according to scheduled simulated plumes, controlled by real time feedback from turbidity sensors (Turbinax CUS31, Endress and Hauser) (Fig. 2). Stock sediments were mixed in 500L tanks and kept in suspension by an air diaphragm pump (SandPiper S1F), while being delivered to tanks by a high velocity loop (3 m s⁻¹), and pivoting solenoid valves (Burkert, 0331), controlled by the PLC (Fig. 2). Sediment was kept in suspension by a centrifuge pump (Iwaki MX400), providing upwelling flow, and a water-movement pump (Hydrowizard ECM63, Panta Rhei), generating trains of static waves (Fig. 2). Sediment deposition events of 0, 2, 5, 10 and 20 mg cm⁻² d⁻¹ were created by generating elevated SSCs before turning off water movement pumps to allow the sediment to fall out of suspension according to sediment specific particle settlement velocities. Sediment deposition was measured in real time with a sediment deposition sensor (Ridd et al., 2001),
and using sediment pods (SedPods) (Field et al., 2012). Every few days the SedPods were capped and any trapped or accumulated sediment was determined gravimetrically. Sediment samples were filtered through pre-weighed 0.4 μm, 47 mm diameter polycarbonate filters, incubated at 60 °C for ≥24 h, and weighed to determine sediment mass. Lighting was provided by a custom designed multi-chip LED (SeaSim) controlled by the PLC, on the feedback provided by the PAR quantum sensor (Skye Instruments, Wales, UK). The control system processed the turbidity along with the time of day and virtual experiment depth (5 m), to provide light exposures similar to those experienced during a dredging project (Jones et al., 2015). Corals were exposed to a 12 h Light:Dark period which included a 3 h period of gradually increasing light levels in the morning (from 06:00–09:00 h), and a 3 h period of gradually decreasing light levels in the afternoon (from 15:00–18:00 h). The maximum light intensity in the control (sediment-free) tanks was 400 μmol quanta m−2 s−1. At 12:00 each day for 28 d the Porites spp. cores were photographed and inspected for the presence of mucous sheets.

2.3. Data analysis

Both in situ and experimental data was analysed using R (version 3.2.3, R Core Team (2015)) using a complete-subsets modelling approach where all sensible model combinations were fitted using the appropriate statistical methods and subsequently compared using Akaike Information Criterion (AICc), AICc weight values (ωi) and R2 (Burnham and Anderson, 2002).

For the field study, models were fitted using generalised additive mixed models (GAMM) using the gamm4 package, and each mucus event was modelled with a binomial distribution (Wood and Scheipl, 2013). We used GAMM rather than linear mixed models to allow for potential non-linear relationships between the response variable and the various continuous environmental predictors. Smoothing terms were fit with a cubic spline (Wood, 2006), with ‘k’ argument limited to 5 (to reduce over-fitting and ensure monotonic relationships that would be ecologically interpretable). A suite of fixed factors was examined to explain mucous sheet presence (ESM Table S1). Site, colony ID and quarter of the year
were included as random factors. Two models sets were fitted, firstly an observational level model investigated the parameters influencing mucus sheet production through time and secondly site wide metrics were assessed to identify broad scale drivers of mucus. Assumptions were evaluated using residual plots and found to be adequately met. Following standard convention, the simplest model within 2 AICc values of the model with the lowest AICc, was considered to be the “best” or optimal model. A null model including only an intercept and the random factors was also included to test if any of the included factors were indeed useful predictors.

For the laboratory study, a similar full subsets approach was used to determine the most influential parameters affecting mucus sheet production in the experimental setting. Due to the limited number of unique values for the continuous predictors (only 5 levels of sedimentation were used), GAMM was deemed inappropriate. Instead, models were fitted with generalised linear mixed effect models (GLMM), where time (week number since the start of the experiment), and deposition levels were included as fixed factors. Both colony ID and tank were included as random factors. For the full-subsets comparison (based on AIC) models were fit using the glmer function from the lme4 library (Bates et al., 2015).

3. Results

3.1. Field study

In the field study, one of the most conspicuous responses of the corals over the monitoring period was the formation of mucus sheets, occurring exclusively in massive and sub-massive Porites spp. colonies (Fig. 3b–d). The sheets initially appeared as translucent coatings covering up to 100% of the colony surface. Once the mucous sheets had formed they became fouled with sediments making them progressively opaque and easier to observe (Fig. 3b–d). Often the sheets were observed peeling off the surface, revealing relatively clean, sediment-free coral tissue (Fig. 3b–d).

In the pre-dredging baseline phase only 4 mucous sheets were observed in 1,198 observations of individual colonies (i.e. 0.33%). During the dredging phase, 441 mucous sheets were observed in 10,600 observations (4.0%, Table 1). Of the events during the dredging phase, 371 (or ~85%) were unique events, i.e. new incidences, with no sheet visible in the previous survey (Table 1).

Average mucous sheet prevalence (mean percentage of all individuals with a mucous sheet at any particular time), ranged from 0 to 10% over the dredging phase, with prevalence much higher in corals close to the primary excavation area, and decreasing with increasing distance from dredging (Table 1, Fig. 4a). The highest prevalence at any one time was 48% (10 out of 21 colonies) during December 2010 at the site MOFB, located 0.37 km from the dredging. Over the dredging period, 74 ± 5% (mean ± SE, n = 7 sites, range 55–96%) of the Porites spp. colonies <1.5 km from the dredging were observed with a mucous sheet (Table 1).

Of the 378 incidences of new mucous sheet formation (i.e. colonies that did not have a mucous sheet in the previous survey), 43% were scored as category 3 (i.e. covering 5–30% of the colony surface). Near complete and complete coverage of the colony (i.e. category 6 or 7, or >95% of the colony) was observed on 68 occasions, and of these events 82% (n = 56) occurred in the <1.5 km from the dredging area (Table 1, Fig. 4a).

Repeated mucous sheet formation during the dredging phase was also commonly observed, with ~50% of the colonies producing 3 or more mucous sheets, and ~25% producing 4 or more (Table 1). Of the colonies producing 3 or more sheets, 90% were located within <1.5 km from the dredging activity (Table 1, Fig. 4b). The maximum number of distinct mucus events for an individual colony was 9 from a colony at MOFA (located 0.4 km from the dredging).

At the four reference sites, located >20 km either north or south of the main dredging area, mean mucous sheet prevalence was 0.2 ± 0.1% (mean ± SE, n = 4 sites) (Table 1, Fig. 4). Only 1 colony was observed with mucous coverage >95% of the colony surface area (i.e. category 6), and no colonies produced more than 1 mucous sheet (Fig. 4, ESM Table S2). At one site, REFN, no mucous sheet production was ever observed in the 34 separate surveys of the 27 corals conducted over the pre-dredging and dredging phases (totalling 766 observations).

These results are confirmed by the site level analysis, which revealed the strongest predictor of mucus cover was the maximum 14-day running mean turbidity (NTU), with this parameter having the lowest ΔAIC value, a weight of 0.53 and an R² value of 0.91 (Table 2b). This relationship demonstrated an upward trend
in the probability of mucus with increasing NTU values (ESM Fig. S1b). There were other variables that also had elevated R² values and model weights, with the next 2 best parameters including the presence of sediment in the previous survey and the present of sediment on the colony currently (Table 2b).

The observational level analysis revealed the strongest predictor of mucus was the presence of sediment on a colony, which had the lowest ΔAIC value, a model weight of 1, and a R² value of 0.38 (Table 2a). The relationship between sediment cover and the probability of mucus occurring followed an upward trend, with very low occurrence when sediment cover is low, and high probability of mucus when sediment covers over 50% of the colony surface (ESM Fig. S1a). The remaining environmental variables provided poor fits to the probability of mucus event occurrence during dredging (Table 2a).

When examining site averaged relationships between mucus prevalence and distance from dredging, a logarithmic trend is observed with increased prevalence at those sites closest to the dredging activity (Fig. 5). It is apparent that the highest mucus prevalence is located within 1 km of the dredging activity (Fig. 5). The same pattern is observed with 14-day mean turbidity (NTU), with the same logarithmic pattern of decrease apparent (Fig. 5).

When comparing mean turbidity with mucus prevalence it is clear that they are correlated (Fig. 5).

3.2. Laboratory study

Over the 28 d laboratory exposure study, 1100 observations were made of individual *Porites* spp. fragments (fragment × treatment × time), and mucus sheets were recorded on
105 occasions (9.2%). Mucous sheets were only observed in those tanks with elevated sediment treatments. Sheets became quickly fouled with sediments becoming opaque, and eventually disintegrating and sloughing off the colony surface, revealing sediment-free tissue underneath (Fig. 3e and f). In some instances, the polyps underlying the mucous sheets appeared fully extended (Fig. 3e). Of the 105 observations of mucous sheets, 42% (n = 44) were unique events, i.e. new incidences, with no sheet visible on the previous day. Sheet production was initially high, with 9 of 60 explants producing a sheet in the first 24 h, decreasing to only 1 new sheet observed between days 4–8 (Fig. 6). New sheet production increased again in the second half of the study and there was a tendency towards the sheets remaining on the fragments for more than 1 d, increasing their prevalence (Fig. 6). Typically, sheets lasted for 24 h before being sloughed, and ~72% lasted 3 days or less. One colony kept a mucous sheet for 13 d. Full-subsets modelling of the experimental data suggested the best model included an additive effect of deposition and time, having an AICc of 530.3 (2.6 AICc units higher than the next best model), and weight of 0.68 (ESM Table S3).

4. Discussion

Since Duerden’s (1906) description of mucous sheet formation in Porites there have been many studies that have investigated the cause and ecological significance of the phenomenon. Several studies have examined the association between mucous sheet formation and sediments, and have produced conflicting results. In this study, there are multiple lines of convincing evidence from both field observations during a large-scale dredging project, and controlled laboratory-based studies, to suggest a close association between sediment loads and mucous sheet presence in massive Porites colonies. Mucous sheets appear to be formed in response to elevated sediment loads and subsequently trap sediment, causing them to build up on the colony’s surface. The debris laden sheet is episodically sloughed, aided by currents and possibly by polyp expansion patterns, leaving the colony surface comparatively sediment free. Stafford-Smith and Ormond (1992) noted that despite having quite poor sediment-rejection capability by mucio-ciliary transport and a morphology prone to sediment accumulation, massive Porites are often found in turbid inshore waters. They suggested that Porites must have an alternative strategy for tolerating occasional high sediment loads, with mucous sheet production potentially such a strategy.

Mucous sheet prevalence in Porites spp. was examined before and during a large-scale, extended (1.5 y) dredging program which resulted in a 10, 5 and 3-fold increase in the intensity duration and frequency of turbidity events respectively compared to baseline levels (Jones et al., 2015). Water quality showed a strong power-decay relationship with distance from dredging, with effects observed predominantly up to 3 km from dredging (Fisher et al., 2015). Close to the dredging, mucous sheet prevalence reached as high as 50%, and over the duration of the dredging ~75% of colonies were observed with a mucous sheet at some stage. In contrast, at the distantly located reference sites (>20 km away), mucous sheet prevalence was negligible (~0.2%). In addition to mucous sheet presence, there were also clear spatial patterns in the percentage of a coral’s surface covered by mucous. High levels of coverage by mucous (i.e. >95% of the colony surface) was observed on 68 occasions, and 82% of these occurred at sites close to the dredging. Furthermore, the production of multiple mucous sheets also appeared correlated with proximity to dredging. For example, over 50% of the colonies produced >3 sheets over the monitoring period and of these 90% were located close to the dredging. In contrast, at distantly located reference sites, no colonies produced more than 1 sheet, and only 1 colony was observed with a high level of mucous coverage. Overall, the strong association between mucous sheet coverage and prevalence suggests this is a sub-lethal indicator of sediment stress for corals.

Mucous sheet formation and cyclical sheet formation linked to lunar cycles have been reported previously in Porites cylindrica from Japan (Kato, 1987), and P. furcata and P. astreoides from Panama (Coffroth, 1991). We found no evidence that mucous sheet production in the massive, hemispherical Porites spp. investigated (which included Porites lobata and Porites lutea) was linked to lunar cycle or phase of the moon. No relationship between temperature and mucous sheet production was observed, which has also previously been described in some Porites species (Coffroth, 1991; Kato, 1987), despite the occurrence of a marine heat wave (Feng et al., 2013) during the dredging project. The strongest driver of mucous sheet production in our data appears to be sediment related.

The laboratory based studies paralleled the field observations, and in response to elevated SSCs and deposition of fine silt Porites spp. quickly developed mucous sheets, which became progressively fouled with sediments. As with field observations, sloughing of mucous sheets resulted in a sediment-free surface. As also observed in the field studies, the fragments were capable of producing more than 1 mucous sheets, with nearly 30% of the fragments

Table 2: Model fit statistics for the relationship between various environmental factors and the presence of mucus on massive Porites spp. colonies, at both an (a) observational level through time and (b) site level. Summaries are presented with ΔAICke information criterion (AIC) scores, model weights and R² relationships. The most influential parameter for each model set is bold.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(a) Observational level analysis</th>
<th>(b) Site level analysis</th>
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<tr>
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<tr>
<td>Maximum NTU</td>
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<td>Bleaching lag</td>
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<td>Maximum deposition</td>
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<td>Mean maximum NTU</td>
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</tr>
<tr>
<td>Null model</td>
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</table>
in the higher two sediment treatments producing two new mucous sheets over the 28 d exposure period.

These results differ from those presented by Stafford-Smith and Ormond (1992), who did not observe mucous sheet production in either the laboratory or field, even when exposing colonies to a rain of fine, silt sized particles. The results presented here suggest that mucous sheet production is an emergency response to overwhelming sediment concentrations, and therefore, the concentrations used by Stafford-Smith and Ormond (1992) may not have been sufficient to result in sheet production. Our results also differ from those presented by Coffroth (1985), who found no significant increase in mucous sheet production in Porites astreoides, related to increased sedimentation, although P. furcata was found to significantly increase mucous sheet production. These results suggest that the production of mucous sheets in response to elevated sediment exposure may be species dependent.

Massive and sub-massive Porites spp. typically use a combination of active and passive processes to remove sediments, as has been reported in numerous coral species (Stafford-Smith, 1993). Active processes include fluidic mucus production and ciliary movement (i.e. muco-ciliary transport), and Porites spp. colonies are likely to be continuously employing this mechanism to prevent the build-up of sediment (i.e. smothering) on their surfaces. Nevertheless, Porites spp. are known to be poor sediment rejecters compared to other species, having small calices, producing weak ciliary currents, and having little capacity for tissue expansion to actively shed sediments (Stafford-Smith and Ormond, 1992). Furthermore, they have a morphology that is naturally prone to sediment accumulation (in surface hollows), as opposed to finely branching species which can easily manipulate sediments to the colony edges and use passive, gravitational forces to self-clean. Considering this, Stafford-Smith and Ormond (1992) questioned why massive Porites spp. can inhabit turbid inshore waters, given their low capacity for sediment removal. We suggest the capacity for repeated mucous sheet production and sloughing is an additional mechanism for Porites spp. to self-clean under particularly heavy sediment loads.

The cue for mucous sheet formation appears to be the presence of elevated SSCs, and sediment contact with the colony surface. This is followed or accompanied by a gradual build up and incorporation of sediments into a mucous layer which becomes progressively opaque with time (Ducklow and Mitchell, 1979). Occasionally colonies were observed with transparent sheets, (suggesting sheet formation occurs before sediment deposition), although these incidents were comparatively rare. It has been suggested that polyp retraction is the ultimate cause of mucous sheet production (Coffroth, 1988), and it may also be related to a cessation in the secretion of mucus, with this hypothesis supported by the results of the laboratory investigation. In the laboratory based experiments the polyps were usually in a highly contracted state under the mucous sheet, but expansion of the polyps appeared to play a role in the peeling of the mucous sheet from the surface, suggesting an element of control by the corals in the mucous sloughing process.

Overall, the strong association between mucous sheet coverage and prevalence suggests this may be a strong sub-lethal indicator of dredging related sediment stress for corals during future dredging programs or exposure to elevated sediment loads. The massive, hemispherical Porites spp. investigated here are common in turbid inshore and clear-water offshore environments, are prominent components of reef assemblages, and significant frame-work builders in the Indo-Pacific region (Veron, 2000). Sheet formation is a sub-lethal, organism-level response and exposure bio-indicator that could provide an early warning signals of biological effects (Dodge et al., 1974; Lam and Gray, 2003). Sheet formation is rapidly identifiable by scuba divers, or by using diver-less technologies such as remotely operated vehicles (ROVs), and can provide near real time information without the need for sampling and subsequent laboratory-based analyses. False positives (Type I errors) are a significant issue for biomarkers, but mucous sheet formation in clear-water environments such as Barrow Island is typically low (<0.5% prevalence), and can increase 10-fold in response to elevated sediment loads.

Further research is required to determine the spatial extent of this mucous sheet production, and whether it is also produced in response to runoff related sediment exposure and natural turbidity events. Further investigation is also required to determine which other species of Porites produce mucous sheets in response to sediment, and whether different sediment composition, particle size, and colour impacts upon the production of these sheets. Mucous sheet formation and sloughing are likely to be an additional mechanism used episodically by massive Porites spp. to compliment known active and passive sediment rejection mechanisms, and serves to clear the colony surface when sediment loads become too high. As a protective reflex response, it is functionally analogous to a human stertation. In summary, mucous sheet prevalence appears to be a useful sub-lethal indicator of sediment related stress for coral communities and should be considered as a monitoring tool during future dredging projects and periods of naturally occurring elevated turbidity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2017.02.023.

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