



## Sediment characteristics influence the fertilisation success of the corals *Acropora tenuis* and *Acropora millepora*

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### ABSTRACT

Elevated suspended sediment concentrations (SSCs) often impact coral fertilisation success, but sediment composition can influence effect thresholds, which is problematic for accurately predicting risk. Here, we derived concentration–response thresholds and cause-effect pathways for SSCs comprising a range of realistic mineral and organic compositions on coral fertilisation success. Effect concentration thresholds (EC<sub>10</sub>: 10% fertilisation inhibition) varied markedly, with fertilisation highly sensitive to inshore organic-clay rich sediments and bentonite clay at < 5 mg L<sup>-1</sup>. Mineral clays and organic matter within these sediments likely promoted flocculation of the coral sperm, which in turn reduced fertilisation. In contrast, sediments lacking these properties bound less sperm, leading to higher SSC thresholds for coral fertilisation (EC<sub>10</sub> > 40 mg L<sup>-1</sup>). The effect thresholds for relevant sediment types were combined with *in situ* turbidity data from locations near dredging operations to assess the risks posed by dredging to coral fertilisation at these locations.

## 1. Introduction

### 1.1. Sediments and corals

Coral reefs are ecologically and economically important, providing a variety of valuable goods and services (Moberg and Folke, 1999; Stoeckl et al., 2014). However, increasing industrialisation, coastal development and overuse of marine resources has led to deterioration of coral reefs worldwide (De'ath et al., 2012; Hughes, 1994; Rogers, 1990). Sediments can impact corals by attenuating light preventing gas exchange on the coral surface (reviewed by Erftemeijer et al. (2012b); Fabricius (2005); and Jones et al. (2016)), and in some regions elevated sediment exposures have contributed to coral loss (Blakeway, 2004; EPA, 2013). Terrestrially-derived sediment, in particular, is thought to pose the most risk to organisms owing to a range of characteristics that include small grain size, high light-attenuation, and associations with microbial communities (Restrepo et al., 2016; Storlazzi et al., 2015; Weber et al., 2012; Weber et al., 2006). Many reefs have been subject to elevated suspended sediment concentrations (SSCs), including the Great Barrier Reef (GBR) which receives an estimated 5.5-fold increase

in annual sediment loads since pre-European settlement (Kroon, 2009). The excavation and disposal of sediment through dredging for the maintenance of shipping channels, expansions of ports, and instalment of pipelines contributes to local elevated turbidity and sediment exposure in the tropical coastal zone (Fisher et al., 2015; Jones et al., 2015a). Regular resuspension of inshore sediment wedges occurs through wind-driven events also exposing reefs to periodically high sediment conditions (Abdul Wahab et al., 2017; Larcombe and Woolfe, 1999; Orpin and Ridd, 2012; Whinney et al., 2017). Once suspended, fine terrestrial clays have the potential to remain in suspension for long periods (Bainbridge et al., 2012; Brodie et al., 2010; Storlazzi et al., 2015), and form nepheloid layers that may cascade towards reefs (Wolanski and Spagnol, 2000). Further, these sediments are often associated with organic matter that aggregate grains within its matrix (Bainbridge et al., 2012).

### 1.2. Sediment and fertilisation success

The early life stages of marine invertebrates are considered more sensitive than mature colonies to a range of stressors (Albright and

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Langdon, 2011; Pineda et al., 2012), with low sediment thresholds reported in some studies (Fabricius et al., 2003; Humanes et al., 2017; Ricardo et al., 2017). However, the effect of sediments on early life history stages of corals is not well understood because many sediment types and possible cause-effect pathways remain untested (Jones et al., 2015b). One of the key areas of concern is the effects of sediments on fertilisation of broadcast-spawning species, as low fertilisation success will reduce the chance of successful recruitment, population maintenance and recovery. Previous studies have reported lower fertilisation success with elevated SSCs (Erfteimeijer et al., 2012a; Gilmour, 1999; Humanes et al., 2017; Humphrey et al., 2008), but the reported effect concentrations varied widely, ranging from  $\sim 37 \text{ mg L}^{-1}$  ( $\text{EC}_{50}$ ) (Humanes et al., 2017) to no effect at  $1024 \text{ mg L}^{-1}$  (NOEC) (Humphrey et al., 2008). The considerable differences in fertilisation success thresholds are not immediately obvious. Sediments could be impacting egg viability, sperm viability, or obstructing of egg–sperm contact (Humphrey et al., 2008; Jones et al., 2015b; Ricardo et al., 2015). The source of the variability may also be related to the sediment itself, and differ with mineralogy, particle grain size and even nutrient content (Humphrey et al., 2008; Jones et al., 2015b). Recently, we demonstrated that fine siliciclastic sediments cause sediment–sperm flocs, resulting in fewer available sperm to fertilise the buoyant eggs (Ricardo et al., 2015). Further investigation is required to identify more broadly which sediment types pose the greatest risk to fertilisation: specifically, which biogeochemical properties promote adhesion and stripping of sperm from the water surface in sinking flocs.

### 1.3. Floc formation

During floc development, electrostatic charges and van der Waals forces influence the coagulation of particles including i) attraction between individual sediment grains, ii) attraction between organic particles forming polymers, and iii) the attraction between sediment grains and organic particles, a ‘polymer bridge’ (Mueller, 2015; Theng, 2012). Flocs increase the settling rate of the particles, and in marine systems can drive an important transfer of nutrients from the pelagic-zone to the benthos (Alldredge and Silver, 1988; Smith and Friedrichs, 2011). Fine sediment grains, and in particular, mineral clays (layered aluminium silicates) have a tendency to increase flocculation. Mineral clays have high specific surface areas and electrostatic charges, which promote coagulation in high ionic strength media such as seawater. Mucilaginous products (typically referred to as extracellular polymeric substances (EPS)) are also commonly found in flocs. These products are secreted in a matrix by microbes and to a lesser extent plankton, and largely composed of polysaccharides and proteins (Decho, 1990; Engel et al., 2004; Underwood et al., 1995). Flocculation of coral sperm and the subsequent reduction in fertilisation success (Ricardo et al., 2015), may be strongly influenced by the adhesive, swelling and viscous properties of mineral clays and EPS associated with sediments, but this has not been previously considered.

### 1.4. Aims

Turbidity-generating activities near coral reefs such as dredging and river runoff are often regulated by assigning turbidity (NTU) or SSC trigger values, but these do not take into account sediment composition (i.e. mineralogy, particle size and nutrient-content) that may alter thresholds at certain sites (Gordon and Palmer, 2015). This study seeks to improve decision-making and risk management by identifying concentration–response relationships and associated threshold values ( $\text{EC}_x$ ) for coral fertilisation success using several realistic sediment types of differing organic and mineral composition, in addition to common components identified within marine sediments. We then aim to quantitatively assess risk SSCs posed to coral fertilisation at a number of

elevated turbidity reef sites, by comparing threshold values with *in situ* water quality data (running-mean percentiles) recorded during dredging projects and natural resuspension events.

## 2. Materials and methods

### 2.1. Coral and gamete collection

Two common tropical broadcast-spawning coral species representative of shallow reefs from the Great Barrier Reef (GBR) to Ningaloo Reef in Western Australia (WA), *Acropora tenuis* (Dana, 1846) and *Acropora millepora* (Ehrenberg, 1834) were selected for the fertilisation experiments. Adult colonies were collected (GBRMPA Permit G12/35236.1) at < 8 m depth from two inshore reefs of the central Great Barrier Reef (Magnetic Island: 19°10'S, 146°51' E; Esk Reef: 18°46' S, 146°31' E) and two mid-shelf reefs (Davies Reef: 18°49' S, 147°39' E; Trunk Reef: 18°23' S, 146°48' E), a few days before the austral spring coral spawning events between 2014 and 2016. The colonies were transported to the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS, Townsville, Queensland) and placed in outdoor 70% shaded tanks with flow-through filtered seawater (FSW) and temperature set to the ambient reef conditions on the day of collection (between 26 and 28 °C). Colonies that showed signs of ‘setting’ (the protrusion of egg-sperm bundles from polyp mouths) were isolated in individual 60-L tanks, and egg-sperm bundles were carefully scooped from the surface following spawning. Eggs were separated from sperm using a 100  $\mu\text{m}$  mesh and rinsed in 0.4  $\mu\text{m}$  FSW to remove any remaining sperm. On each night, sperm from multiple colonies (3–6 individual colonies) were pooled and used to fertilise eggs from single colonies (Negri and Heyward, 2000).

### 2.2. Sediment preparation

Sediments were collected from sites on the GBR and WA (see Table 1 for collections sites and biogeochemical properties). These sediments were referred to as: Inshore GBR 1 (previously described in Humanes et al. (2017)); Inshore GBR 2; Inshore WA and Offshore GBR (previously referred to as ‘siliciclastic’ and ‘carbonate’ – respectively in Ricardo et al. (2015)). The two Inshore GBR sediments were kept in flowthrough FSW at 27 °C to retain their residing microbial community as much as possible, and wet-sieved to reduce the mean particle grain size. All other sediments were dried and ground with a rod mill grinder to reduce the mean particle size to silts and clays. Three commercially available high grade processed clays were also used in assays including calcium-bentonite clay (Watheroo Bentonite), mined kaolin clay (N-Essentials, Moorabbin), and laboratory-grade kaolin clay (Sigma-Aldrich). To select for fine grain sizes (Table 1) that gametes are likely to encounter on the water surface, all sediment types were settled in FSW. Sediments in suspension after 10 min were used for experiments. Suspended sediment concentrations (SSCs) and turbidity (NTU) had a linear relationship ( $r^2 > 0.97$ ) and treatment concentrations were prepared by measuring the turbidity (NTU) with a nephelometer (TPS 90FL-T). To determine the NTU–SSC relationship, SSCs were measured gravimetrically by filtering three replicate 100 mL samples onto 0.4  $\mu\text{m}$  polycarbonate filters, and dried overnight at 60 °C. Low levels of organic nutrients (< 0.5% TOC) found in Inshore WA and Offshore GBR sediments are comparable to the organic carbon content for sediments found in the Pilbara, WA and offshore reefs of both coasts (DEC, 2006). Laboratory-grade Xanthan Gum (Sigma-Aldrich) produced from the bacteria *Xanthomonas campestris* was used to mimic EPS without sediment (as a control), and concentrations were derived by mixing and sonicating a measured amount with FSW.

**Table 1**

Properties of sediments from each location including information on site location, mineral, and organic sediment analyses. PSD: particle size distribution; EPS: extracellular polymeric substance; TOC: total organic carbon.

Sediment	Inshore GBR 1	Inshore GBR 2	Inshore WA	Offshore GBR
Collection site	Orpheus Is. Reef, inshore GBR 18.60°S, 146.48°E	Pandora Reef, inshore GBR 18.81°S, 146.43°E	Onslow, inshore Pilbara 21.64°S, 114.92°E	Davies Reef, mid-shelf GBR 18.83°S, 147.63°E
Mineral analyses				
Leachate effect on fertilisation	No effect	Not tested	No effect	No effect
PSD (median $\mu\text{m}$ )	6.7	6.9	6.9	7.7
Specific surface area ( $\text{m}^2 \text{g}^{-1}$ )	1.6	1.8	1.7	1.8
Wentworth classification	Very fine silt	Very fine silt	Very fine silt	Very fine silt
Mineralogy	Terrestrial: 45% quartz, 19% kaolinite, 7% albite Carbonate: 30% calcite	Terrestrial: 43% quartz Carbonate: 32% aragonite 25% mg calcite	Terrestrial: 44% quartz, 10% magnetite Carbonate: 24% mg calcite, 12% aragonite, 9% calcite	Carbonate: 80% aragonite, 20% calcite
Organic analyses				
EPS ( $\mu\text{g}$ xanthan gum equivalent $\text{mg}^{-1}$ ) (mean $\pm$ SE)	567 $\pm$ 78	259 $\pm$ 18	66 $\pm$ 22	35 $\pm$ 8
TOC (%)	3.76	1.87	0.26	0.27

### 2.3. Sediment analysis

Sediments were analysed for dissolved metals by inductively coupled plasma optical emission spectrometry (ICP-OES). X-ray powder diffraction (XRD) was used to assess minerals and mineral clay components within sediments. Particle size distribution was determined using a Malvern Mastersizer 2000 (see Text S 1 for further details on the analyses above). Organic material was assessed for total organic carbon using a Shimadzu SSM-5000A TOC-L. To evaluate EPSs content in the sediments, Alcian Blue was used to stain acid polysaccharides that are commonly associated with EPSs (Hung et al., 2003; Passow and Alldredge, 1995). Five 20 mL replicates of  $100 \text{ mg L}^{-1}$  sediment samples were filtered through  $0.4 \mu\text{m}$  polycarbonate membranes, rinsed with 10 mL ultrapure water to remove salts, stained with aqueous 0.02% Alcian Blue solution at pH 2.5 for 2 to 5 s and immediately hydrolysed with 80% sulphuric acid. Samples were regularly agitated manually and sonicated for 2 h, and the absorbance of the hydrolysate was measured with a spectrophotometer at 787 nm. Absorbance of FSW blanks and turbidity blanks (samples not stained) were deducted from the sample absorbance. Samples were calibrated to  $\mu\text{g}$  of Xanthan Gum equivalents with the dilution series made in FSW (Hung et al., 2003). However, EPS analyses on sediment containing clays need to be interpreted with caution, as clays possibly clogged the filters leading to longer staining times ( $> 5$  s).

### 2.4. Concentration–response relationships for sediments on *A. tenuis*

Fertilisation studies have demonstrated the effect of toxicants and pollutants on fertilisation success is often dependent on the sperm concentration (Marshall, 2006; Ricardo et al., 2015). Slightly sub-optimal coral sperm concentrations increase the sensitivity of assays, while saturating sperm concentrations ( $\geq 10^6$  sperm  $\text{mL}^{-1}$ ) often mask the effect of treatments, and these high sperm concentrations are unlikely to occur for long periods *in situ* due to dilutive effects (Omori et al., 2001). To explore a range of environmentally relevant scenarios we applied sperm concentrations of  $10^4$  and  $10^5$  sperm  $\text{mL}^{-1}$  (Ricardo et al., 2015).

Each experiment included eight serially diluted suspended sediment treatments with the control defined as the treatment without sediment. For each treatment sample, 1 mL of  $\sim 100$  *A. tenuis* eggs (minimum of 30 eggs) and 1 mL of *A. tenuis* sperm were each exposed to 24 mL of the sediment treatment in separate 70 mL clear polypropylene chambers (Sarstedt) for 30 min to simulate the time taken for two compatible gametes to encounter each other *in situ* (Heyward and Babcock, 1986; Jones et al., 2015b). The sample containing eggs were then combined

with the sample containing sperm (combined volume = 50 mL) to initiate fertilisation, and placed on mechanical rollers. The rollers consisted of a series of pins rotating at  $0.3 \text{ revolutions s}^{-1}$  by an electrical motor, which maintained the sediment in suspension inside the chambers. Every 15 min the chambers were manually flipped upon their axis to ensure rotation from both directions. Water and air temperatures were controlled to the ambient water temperature where the adult colonies were collected (26–28 °C). In addition, a series of sperm dilutions were prepared in 6-well plates (10 mL, Nunc™) by adding 1 mL of each sperm dilution to 1 mL of  $\sim 100$  eggs and 8 mL of filtered seawater. This sperm dilution series was used as a control to assess gamete viability against those agitated by the rollers. Experiments were terminated when most the embryos in the controls reached the 4-cell stage, which generally occurred 150 min after the gametes were mixed for *A. tenuis*. Embryos and eggs were fixed using Z-fix (Anatech Limited) or 4% buffered formalin in FSW containing  $10 \text{ g L}^{-1}$  sodium  $\beta$ -glycerophosphate. For each replicate chamber, percent fertilisation success was determined by counting  $\sim 100$  eggs or embryos.

To explore and eliminate the possibility of other cause–effect pathways influencing the fertilisation response, the potential of toxicity caused by labile metals was assessed by testing fertilisation success in the presence of  $0.4 \mu\text{m}$  filtered aqueous fraction from a  $200 \text{ mg L}^{-1}$  sediment suspension. Inhibition by this mechanism was considered unlikely with leached metals not causing any reduction in fertilisation compared with controls (Inshore GBR 1:  $t_4 = 0.86$ ,  $p = 0.441$ ; Inshore WA:  $t_4 = 0.50$ ,  $p = 0.644$ ; Offshore GBR:  $t_4 = 0.21$ ,  $p = 0.840$ ). Pilot studies also confirmed Inshore GBR 1 SSCs did not affect the viability of the eggs ( $t$ -test:  $t_4 = 0.24$ ,  $p = 0.824$ ) as similarly found in Ricardo et al. (2015) for Inshore WA and Offshore GBR sediments. However, neither assay could be applied to Inshore GBR 2 due to time constraints.

### 2.5. Responses of coral fertilisation to sediment components

To investigate how certain components of the sediment (including clays and gums) affected coral fertilisation, and to test consistency across species, a series of smaller assays of 2–3 SSC treatments (including the control) were applied to coral gametes of *Acropora tenuis* and *A. millepora* in the same way described above. For *A. tenuis*, gametes were exposed to mined kaolin, refined kaolin, and Xanthan Gum using a sperm concentration of  $10^4$  sperm  $\text{mL}^{-1}$ . *A. millepora* gametes were exposed to Inshore GBR 1, Inshore WA, Offshore GBR, mined kaolin and bentonite clays. A pilot study revealed *A. millepora* sperm concentrations at  $10^4$  sperm  $\text{mL}^{-1}$  had inconsistent fertilisation success in this species ( $< 70\%$ ), and therefore  $5 \times 10^4$  sperm  $\text{mL}^{-1}$  was used as

the lower sperm concentration and  $10^5$  sperm  $\text{mL}^{-1}$  as the higher sperm concentration. Experiments were terminated when most the embryos in the controls reached the 4-cell stage, which generally occurred 150 min after the gametes were mixed for *A. tenuis*, and 180 min for *A. millepora*.

## 2.6. Imaging

Subsamples of the fixed sediment-gamete sample were gently washed with freshwater to remove salts and the water evaporated on a glass slide for viewing under optical compound microscopy. For scanning electron microscopy, subsamples of sediment from the bottom and middle of sediment–sperm samples were fixed in 1.25% glutaraldehyde and 0.5% paraformaldehyde in FSW. Samples were dehydrated in a microwave using a graded ethanol series (70%, 90%  $\times$  2, 100%, 100% (anhydrous)) for 40 s at 250 W and then critical point dried (Polaron KE3000, Quorum Technologies) in liquid  $\text{CO}_2$ . The dried samples were then mounted on carbon tape on aluminium stubs, coated with 3 nm platinum, and imaged using a field emission scanning electron microscope (SEM) (Zeiss 55-VP). A secondary electron detector was used for all images.

## 2.7. Risk assessment analysis to *in situ* water quality data

Risk can be defined as the magnitude of potential damage of an event multiplied by its probability of occurrence. Here, the risk that dredging may present to coral fertilisation was determined by comparing *in situ* turbidity measurements from field locations with the lowest fertilisation threshold ( $\text{EC}_{10}$ ) derived from the most relevant sediment type to that location. *In situ* turbidity (NTU) were measured with optical backscatter sensors (nephelometers) at four locations, off the coastlines of Magnetic Island in Queensland, and Onslow, Burrup Peninsula and Barrow Island in Western Australia. The coral fertilisation window only lasts for a few hours (Oliver and Babcock, 1992; Omori et al., 2001), therefore turbidity (NTU) measurements were averaged to 2-hr intervals, and the percentage of intervals exceeding each laboratory-derived threshold was determined. Magnetic Island is an inshore island of the GBR situated in a muddy embayment, regularly exposed to turbidity associated with natural resuspension events, and in proximity to a dredge disposal site located  $\sim$ 6 km away. Turbidity (NTU) was measured at 10-min intervals during coral spawning months (October to December) at two water quality sites (Nelly Bay and Horseshoe Bay) for two consecutive years between 2001 and 2006 (Macdonald et al., 2013), and converted to approximate SSC using a conversion factor of 1.1 (Larcombe et al., 1995). Similarly, Onslow waters are subject to elevated levels of turbidity associated with discharges from the Ashburton River, regular natural resuspension events, and a major capital dredging operation associated with the Wheatstone LNG project. Turbidity data before and during dredging were collected between 2011 and 2015 at five water quality monitoring sites. Two sites (ENDCH and PAROO) were within 2 km of the dredge or spoil disposal grounds, whereas three sites (ASHNEE, DIRNE, and HERALD) were located  $>$  2 km from these areas (Abdul Wahab et al., 2017). Turbidity was measured at 30-min intervals and converted to approximate SSC using a conversion factor of 1.07. Burrup Peninsula is an enclosed inshore turbid reef environment and subject to a dredging program that removed a volume of  $\sim$ 12.5  $\text{Mm}^3$  of sediment between 2007 and 2010 (Fisher et al., 2015; Jones et al., 2015a). Sediment at the location consisted of a mix of siliciclastic and carbonate sediment. *In situ* NTU data were converted to SSCs by using a conversion factor of 1.174. Five water quality sites were selected for comparison (three sites (CHC4, DPAN, and HOLD) within hundreds of metres of the dredging operation, two sites between 1 and 4 km (SUP2 and KGBY), with the turbidity measured at 60-min intervals. Dredging at Barrow Island for the Gorgon Gas Project, required the removal of  $\sim$ 7.6  $\text{Mm}^3$  of sediment, but occurred at a ‘clean water’ location primarily of carbonate

sediments with occasional terrestrial-sediment influence (Fisher et al., 2015; Jones et al., 2015a). NTUs were recorded every 10-minutes and in absence of any reported conversion factors derived from suspended sediments *in situ*, we used a conversion factor of 1.8 (mean conversion factor – see Chevron (2011)) derived from bottom sediment samples. The two nearest sites to the dredge (LNGA and LNGO) were selected for comparisons against the Offshore GBR (carbonate) sediment thresholds. This contrasted the laboratory-derived conversion factor of 0.58 highlighting potential differences in the particle size between the two sediments. For all locations, all obvious logger errors and readings attributed to cyclonic activity were removed, and instrumental drift at Burrup Peninsula was corrected. A complete description of the data handling processes, and limitations of nephelometric data are described in Jones et al. (2015a); and Macdonald et al. (2013). Additional water quality monitoring and location details are provided in Abdul Wahab et al. (2017); Fisher et al. (2015); Jones et al. (2015a); and Macdonald et al. (2013).

## 2.8. Statistical analysis

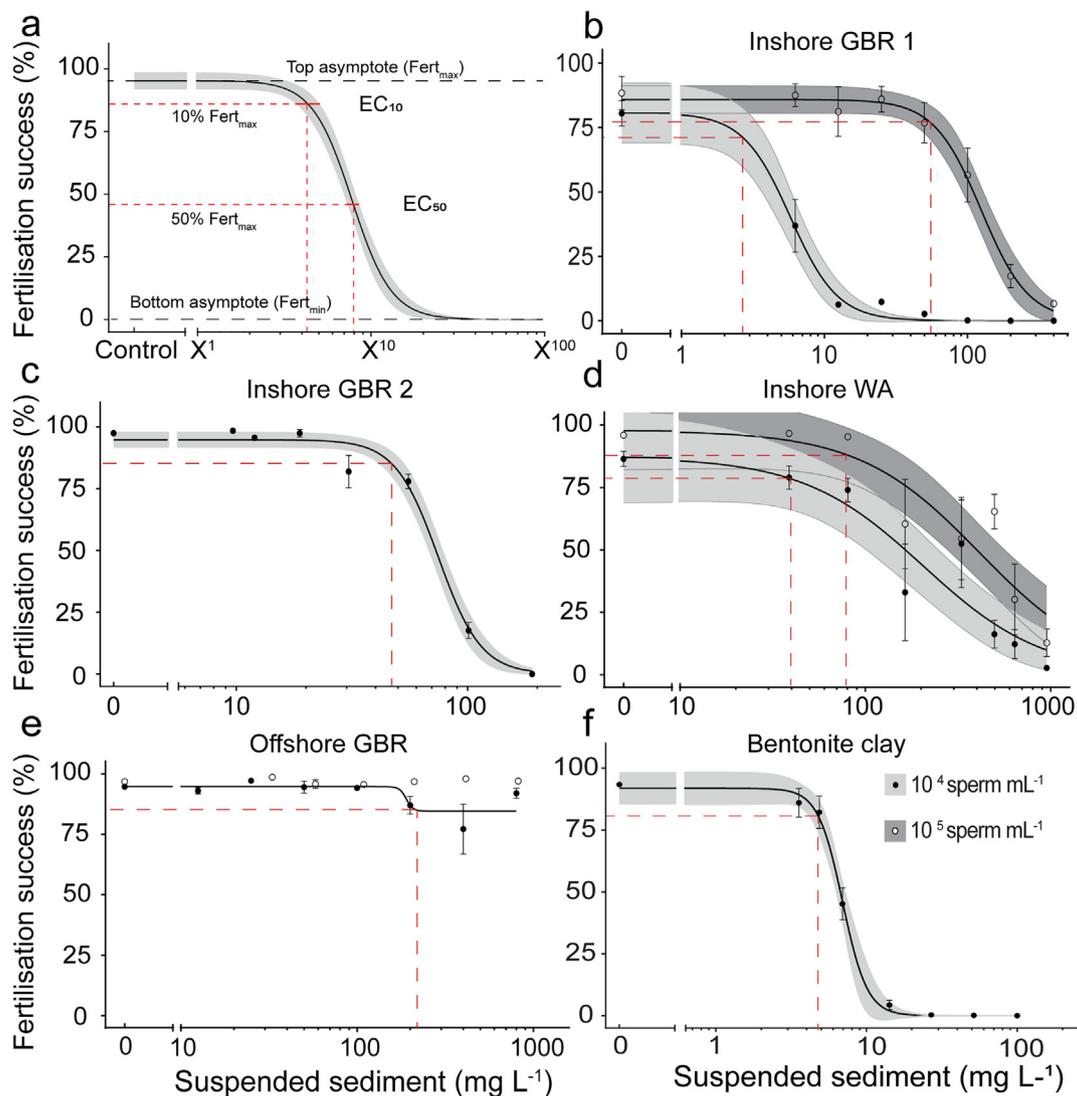
Concentration–response experiments were designed to derive effect concentrations ( $\text{EC}_x$ ), the preferred measure in ecotoxicology to determine thresholds for toxicants and pollutants (Blasco et al., 2016; Warne et al., 2014).  $\text{EC}_{10}$ s were used as thresholds which are the preferred metric for toxicants in the Australian and New Zealand for Fresh and Marine Water Quality guidelines (Warne et al., 2014).  $\text{EC}_{50}$ s were used for model comparisons, as these values are more indicative of the nonlinearity of the model and have a greater level of confidence compared with regions closer to the bounds of the curve. Specifically, SSCs that inhibited fertilisation success by 10% ( $\text{EC}_{10}$ ) or by 50% ( $\text{EC}_{50}$ ) were derived from nonlinear regression curves (four-parameter logistic models) using GraphPad Prism (v7) (Fig. 1a). Low fertilisation rates in control conditions could indicate poor gamete quality, poor sperm motility, or gamete aging, and therefore we defined the test acceptability criteria for inclusion as  $>$  70% fertilisation success in the control (sediment-free samples) (Hobbs et al., 2005). All models indicating a lower-bound at 0% were constrained. To compare  $\text{EC}_{50}$  values of two curves (e.g. two sperm concentrations), the slope parameters were first compared using global nonlinear regression and shared if there was no evidence that they were different. Experiments with only a few sediment treatment levels were fitted to binomial Generalized Linear Mixed Models (GLMM) using R (v 3.3.1), with each treatment as a categorical fixed factor, and each experimental chamber treated as random factor to account for overdispersion in the model. Each sediment type was compared against the control (no sediment), and familywise error was corrected with Dunnett's test.

To quantitatively assess risk, *in situ* turbidity data at each site were analysed using running mean percentile analysis (Jones et al., 2015a). An interval length of 2-hr (relevant to a duration of the fertilisation window) was selected. The percent of exceedances above the laboratory-derived thresholds ( $\text{EC}_{10}$ s) was then determined to produce a ‘risk percentage’. The maximum SSC where dredging-related SSCs occurred  $>$  1% above baseline SSCs was referred to as the ‘Maximum SSC for dredging risk’ – above these SSCs, dredging-related SSCs could not be confidently separated from baseline SSCs.

## 3. Results

### 3.1. Sediment composition

Inshore GBR 1 sediment was very high in total organic carbon (TOC) (3.8%) relative to the other sediments tested, and influenced by terrestrial sediment ( $\sim$ 70%), with a substantial ( $\sim$ 20%) kaolinite clay portion. Inshore GBR 2 sediment was high in TOC (1.9%) and was a mix of terrestrial ( $\sim$ 43%



**Fig. 1.** Concentration–responses relationships of suspended sediments on coral fertilisation success of *Acropora tenuis* at sperm concentrations of  $10^4$  and  $10^5$  sperm  $\text{mL}^{-1}$ . (a) Graphical representation of the concentration–response models showing  $\text{EC}_x$  values are derived from the fertilisation maximum ( $\text{Fert}_{\text{max}}$ ). (b) Inshore GBR 1. Model slopes shared and 6 replicates per SSC (c) Inshore GBR 2. 6 replicates per SSC, (d) Inshore WA. Model slopes shared, 4 replicates per SSC (e) Offshore GBR. 6 replicates per SSC (f) Bentonite clay. 6 replicates per SSC. All models except Offshore GBR were constrained to 0%. Error bars indicates SE on the raw data. Grey shading indicates the 95% CI. Note different log-scales on x-axes.

quartz) and carbonate sediment (Table 1). Inshore WA sediment was similar in mineralogy to Inshore GBR 2, but had lateritic influences (10% magnetite) and importantly, was low in TOC (0.3%). Finally, Offshore GBR sediment, was typical of offshore reef sediments with low TOC (0.3%) and composed entirely of carbonate sediments. Extracellular polymeric substances were 4-fold higher in Inshore GBR 2 sediment than Inshore WA sediment, and 7-fold higher than Offshore GBR sediment. All sediment had a similar particle size distribution and were defined as very fine silt (Wentworth scale). Sediment leachate contained low concentrations of metals (Table S 1). Only aluminium and lead were above detection limits, but their concentrations were over an order of magnitude lower than toxicity thresholds for coral fertilisation (Negri et al., 2011; Reichelt-Brushett and Harrison, 2005).

**3.2. Concentration–response relationships for sediments on *A. tenuis***

*Acropora tenuis* fertilisation was more sensitive to Inshore GBR 1 sediments than all other types, with fertilisation inhibited at a threshold concentration ( $\text{EC}_{10}$ ) of  $2.5 \text{ mg L}^{-1}$  at lower sperm concentrations of  $10^4$  sperm  $\text{mL}^{-1}$  (Table 2, Fig. 1b). This threshold increased to

**Table 2**

Summary of concentration–response thresholds for suspended sediment impacts on coral fertilisation of *Acropora tenuis*.  $\text{EC}_{10}$  and  $\text{EC}_{50}$  values reported are derived from nonlinear regression models (four-parameter logistic curves).

Sediment type	Sperm conc. (per $\text{mL}^{-1}$ )	$\text{EC}_{10}$ ( $\text{mg L}^{-1}$ )		$\text{EC}_{50}$ ( $\text{mg L}^{-1}$ )		Slope
		Best-fit	95% CI	Best-fit	95% CI	
Inshore GBR 1	$10^4$	2.5	1.5–3.6	5.8	4.5–7.1	-2.6 <sup>a</sup>
Inshore GBR 1	$10^5$	54	35–76	125	105–148	-2.6 <sup>a</sup>
Inshore GBR 2	$10^4$	47	39–54	75	70–80	-4.7
Inshore WA (siliciclastic)	$10^4$	40	12–112	205	112–364	-1.3 <sup>a</sup>
Inshore WA (siliciclastic)	$10^5$	80	26–191	414	251–651	-1.3 <sup>a</sup>
Offshore GBR (carbonate)	$10^4$	214	NA	> 800 <sup>b</sup>	NA	NA
Offshore GBR (carbonate)	$10^5$	> 820 <sup>b</sup>	NA	> 820 <sup>b</sup>	NA	NA
Bentonite clay	$10^4$	4.6	3.7–6.2	6.9	6.5–7.5	-5.3

<sup>a</sup> Shared parameter between experiments of the same sediment type.  
<sup>b</sup> Greater than the range of concentrations tested.

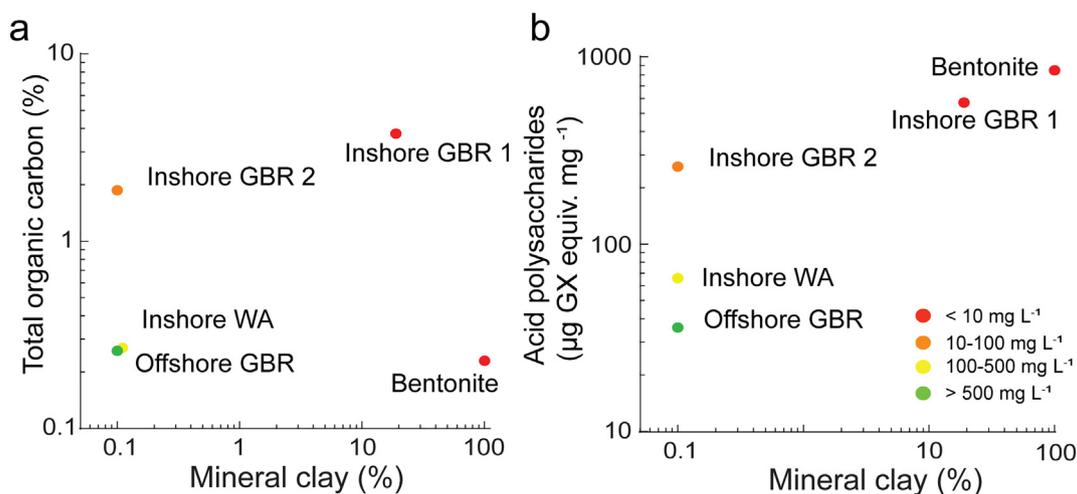


Fig. 2. Relationships between percent mineral clay and (a) total organic carbon or (b) acid polysaccharides on fertilisation inhibition ( $EC_{50}$ ) of *Acropora tenuis*. Each sediment type or clay is colour-coded based on their fertilisation inhibition threshold value.

$EC_{10} = 54 \text{ mg L}^{-1}$  at higher sperm concentrations of  $10^5 \text{ sperm mL}^{-1}$  (Table 2, Fig. 1b). Fertilisation was less sensitive to Inshore GBR 2 sediment, which was only applied to the lower sperm concentration, inhibiting coral fertilisation at  $47 \text{ mg L}^{-1} (EC_{10})$  (Table 2, Fig. 1c). Inshore WA sediment also inhibited fertilisation at similar SSCs ( $10^4 \text{ sperm mL}^{-1}$ :  $EC_{10} = 40 \text{ mg L}^{-1}$ ;  $10^5 \text{ sperm mL}^{-1}$ :  $EC_{10} = 80 \text{ mg L}^{-1}$ ) (Table 2, Fig. 1d). However, Offshore GBR sediment had only a minor impact on coral fertilisation at the lower sperm concentration ( $10^4 \text{ sperm mL}^{-1}$ :  $EC_{10} = 214 \text{ mg L}^{-1}$ ), but no obvious impact at the higher sperm concentration (Table 2, Fig. 1e). Lower sperm concentrations increased the sensitivity of the assay (Inshore GBR 1:  $F_{1,89} = 124.7$ ,  $p < 0.001$ ; Inshore WA:  $F_{1,59} = 4.13$ ,  $p = 0.047$ ; Offshore GBR: N/A), reducing the  $EC_{10}$  values by 2–21 fold. There was a stronger nonlinear effect of the Inshore GBR sediments (slope: Inshore GBR 1 =  $-2.6$ ; Inshore GBR 2 =  $-4.7$ ) compared with the Inshore WA sediment (slope:  $-1.3$ ). Bentonite clay greatly reduced fertilisation success of *A. tenuis* at the lower sperm concentration ( $EC_{10} = 4.6 \text{ mg L}^{-1}$ ) and had a strong nonlinear effect (slope:  $-5.3$ ) (Table 2, Fig. 1f). Sediments and clays with lower thresholds ( $EC_{50}$ s) were generally associated with mineral clay content and organic content (total organic carbon and acid polysaccharides) (Fig. 2a–b).

### 3.3. Responses of *A. tenuis* to clays

Mined kaolin clay caused a 13% decrease to fertilisation success at the higher SSC of  $30 \text{ mg L}^{-1}$  ( $z = 3.308$ ,  $p = 0.007$ ), but not at  $10 \text{ mg L}^{-1}$  SSCs. Refined kaolin clay caused an 83% decrease in

fertilisation success at  $30 \text{ mg L}^{-1}$  ( $z = 8.954$ ,  $p \leq 0.001$ ). Xanthan Gum caused a modest 7% decrease in fertilisation success at  $30 \text{ mg L}^{-1}$  ( $z = 2.717$ ,  $p = 0.044$ ) (Fig. S 1).

### 3.4. Responses of *A. millepora* to sediment and clays

Inshore GBR 1 sediment caused a decrease in fertilisation success of *A. millepora* ( $5 \times 10^4 \text{ sperm mL}^{-1}$ ) with 18% inhibition at  $50 \text{ mg L}^{-1}$  ( $z = 3.17$ ,  $p = 0.009$ ) and 92% inhibition at  $100 \text{ mg L}^{-1}$  ( $z = 11.63$ ,  $p < 0.001$ ) (Fig. 3). Bentonite clay, which was only exposed to a higher sperm concentration, effectively prevented coral fertilisation at  $20 \text{ mg L}^{-1}$  ( $z = 8.03$ ,  $p < 0.001$ ). There was no observable effect of Inshore WA or Offshore GBR sediments on fertilisation at either sperm concentration up to  $100 \text{ mg L}^{-1}$  SSC. Similarly, there was no effect of mined kaolin clay, which was only tested at the higher sperm concentration.

### 3.5. Imaging

The potential of each sediment type to form sperm-sediment flocs corresponded with the effect of each sediment on fertilisation success, and small sperm-sediment clumps could be observed under optical stereomicroscopes (Fig. S 2a). The Inshore GBR 1 sediment formed extensive flocs composed of sperm and  $\sim 1 \mu\text{m}$  clay particles (Fig. 4), whereas Inshore WA sediment formed more loosely-bound flocs, mostly dominated by fine silt with fewer sperm (Fig. S 2b). Offshore GBR

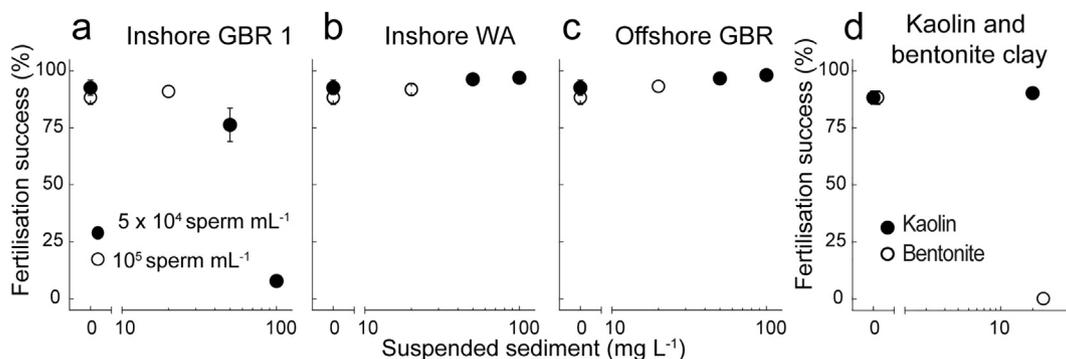
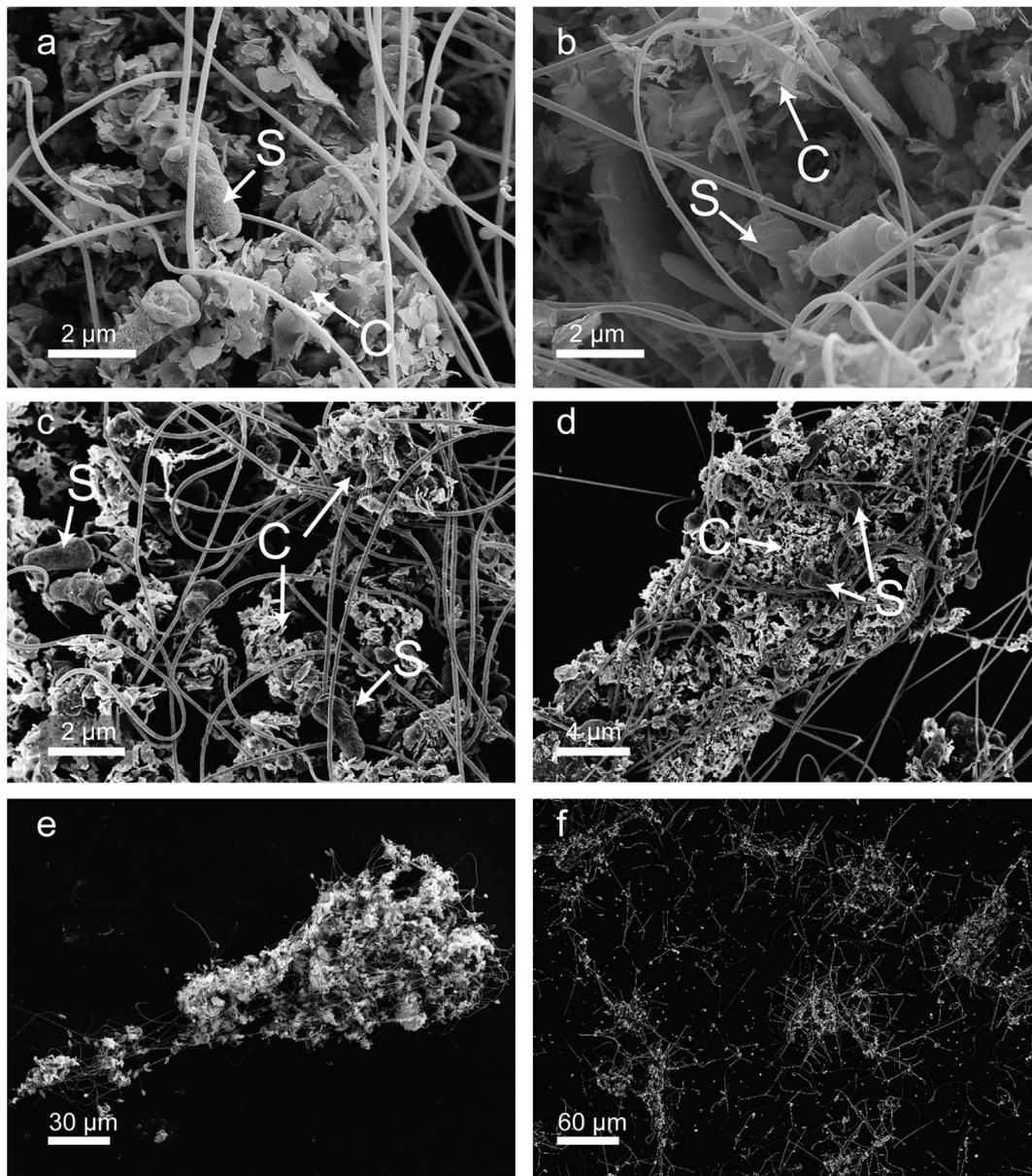


Fig. 3. Fertilisation success of *A. millepora* at lower ( $5 \times 10^4 \text{ sperm mL}^{-1}$ ) and higher ( $10^5 \text{ sperm mL}^{-1}$ ) sperm concentrations exposed to various sediment and clay types. Mean  $\pm$  SE fertilisation success following exposure to (a) Inshore GBR 1, (b) Inshore WA, (c) offshore GBR sediments, and (d) Kaolin clay and bentonite clay. There were 5 replicates per SSC. Note different scale on x-axis.



**Fig. 4.** Scanning electron microscopy images of sediment-sperm flocs of Inshore GBR 1 sediment. (a–b) High magnification images with flat platy grains observed within the floc. (c–d) Mid-magnification images of sediment-sperm flocs showing clumping of the clay around the sperm (e–f) low-magnification images of sediment-sperm flocs revealing flocs > 100  $\mu\text{m}$  in length and consisting of thousands of sperm. S, sperm; C, clay.

sediment formed few flocs, and much of the sample did not bind to the coverslip used for scanning electron microscopy. Organic matter was identified throughout Inshore GBR sediment 1, and observed within some sediment-sperm flocs (Fig. 5). Microbes were additionally observed within the Inshore GBR 1 and Inshore WA sediments (Fig. 5). Generally, no sediment was observed to bind onto the eggs (Fig. S 2c), with the exception of bentonite clay (Fig. S 2d).

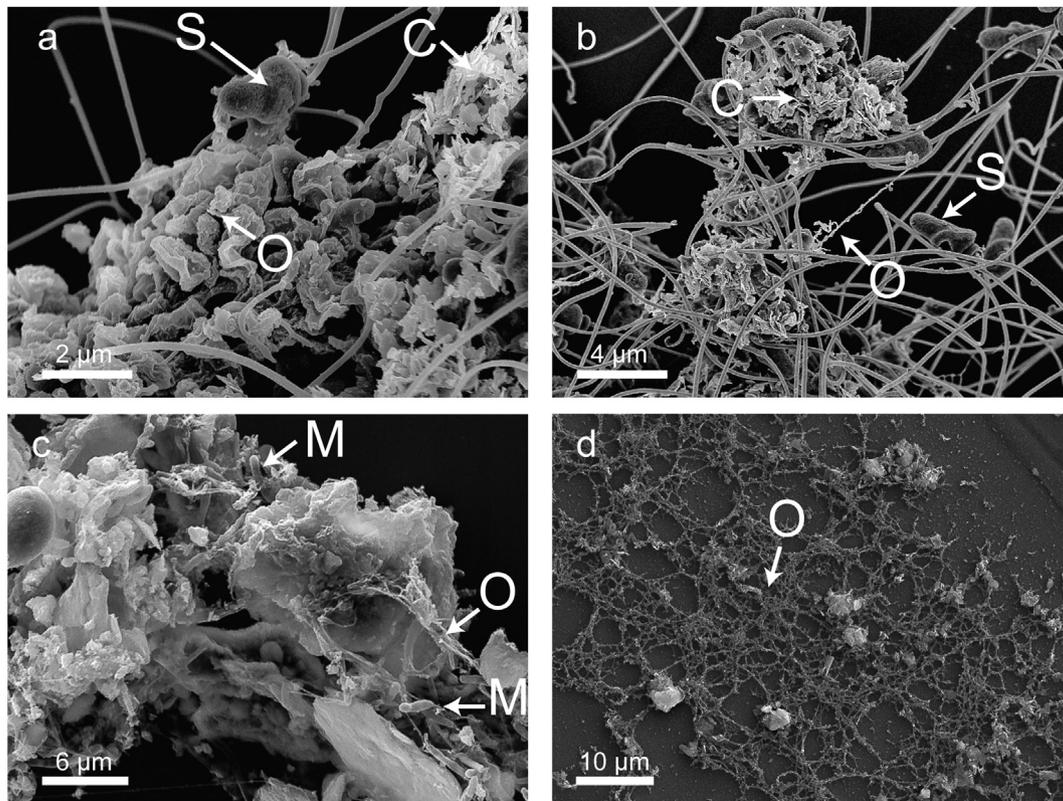
### 3.6. Risk assessment analysis of field sites

Elevated SSCs during dredging at Barrow Island occurred more frequently than background conditions until  $\sim 101 \text{ mg L}^{-1}$  but the threshold for carbonate sediment (Offshore GBR) was substantially higher ( $214 \text{ mg L}^{-1}$ ). Therefore, the risk percentages during dredging-phase were < 1% because *in situ* SSCs at this threshold were very rare (Table 3, Fig. 6).

At Magnetic Island, the  $\text{EC}_{10}$  threshold for the relevant Inshore GBR 1 sediment of  $2.5 \text{ mg L}^{-1}$  was exceeded during  $\sim 19\%$  of intervals (or a risk probability of  $\sim 0.19$ ). However, the  $\text{EC}_{10}$  ( $47 \text{ mg L}^{-1}$ ) threshold from Inshore GBR 2 was not often exceeded by the exposure observations and therefore the risk probability was close to zero for this sediment type (Table 3, Fig. 6).

At Burrup Peninsula, SSCs at sites very close to the dredge exceeded the threshold regularly during the dredging phase on 31% of the intervals compared with 0% in the pre-dredging phase (Table 3, Fig. 6). Sites further away (1–4 km) rarely exceeded the threshold during the dredging phase (0.52%) or during the pre-dredging phase (0.16%).

At Onslow, the SSCs exceeded the  $40 \text{ mg L}^{-1}$  threshold ( $\text{EC}_{10}$  for the relevant sediment, Inshore WA) on few occasions (< 1% of the time), both in the pre-dredge and during-dredge phases (Table 3). The frequencies of elevated SSCs during the pre-dredge (background) phase and the dredging phase became indiscernible above  $\sim 11 \text{ mg L}^{-1}$  (Table 3).



**Fig. 5.** Evidence of organic material (extracellular polymeric substances, EPS) that may assist with the formation of the flocs in Inshore GBR 1 sediment. (a) A sperm head coated with organic material. Clay can be observed in the corner. (b) A web of sperm around clumps of clay. Stringy material can be observed between two sperm. (c) An image of the sediment without sperm. Microbes and mucous-like material were apparent within the sample. (d) Parts of the sediment sample had a thick web of mucous-like material. S, sperm; O, organic material; C, clay; M, microbes.

## 4. Discussion

### 4.1. Summary

Sediment composition and mineralogy plays a crucial role in determining the effect of suspended sediments on coral fertilisation, with sediment rich in organic-clay complexes capable of forming a thick matrix that traps and sinks sperm. Sediment of this type had a strong effect on coral fertilisation at low SSCs, comparable to occasional background concentrations experienced on inshore reefs. Bentonite, a common mineral clay entering inshore environments through fluvial discharge, and commonly used as a drilling mud (see below), also had a striking effect on coral fertilisation. In contrast, terrigenous sediments of lower organic matter affected fertilisation only at higher SSCs relevant to concentrations close to dredging operations or produced during storm events. Carbonate-based SSCs had a very minor effect on coral fertilisation, even at low sperm concentrations. The risk of SSCs to coral fertilisation was assessed for a number of field locations, with risk depending on the sediment type, occurrences of natural resuspension events, and distances from dredging operations.

### 4.2. Inhibition of fertilisation by suspended sediments and clays

Coral sperm readily forms flocs with terrestrially-influenced sediment, and these flocs sink sperm, which reduces coral fertilisation success at the water surface (Ricardo et al., 2015). Sediments that were the most cohesive to sperm had the greatest impact on coral fertilisation. These results support other studies that identified sediment composition as being a crucial factor in determining sediment impacts on early and late stages of corals (Fabricius et al., 2003; Humphrey et al., 2008; Weber et al., 2006), yet sediment composition is largely ignored

in the vast majority of studies (Jones et al., 2016; Risk, 2014). Carbonate sediments lacking terrestrial-influences (e.g. silicates) had very little impact on fertilisation success within environmentally relevant concentrations, as observed elsewhere (Humphrey et al., 2008; Ricardo et al., 2015). However, even carbonate sediment may affect coral fertilisation indirectly; for example, by binding to ascending egg-sperm bundles, and thereby reducing egg-sperm contact rates at the water surface (Ricardo et al., 2016b).

In this study, particle grain size was highly controlled to very fine silts (all  $\sim 7 \mu\text{m}$  median), representing those sediments that would easily remain in suspension. This small grain size is very similar to the size fraction found to cause inhibition of fertilisation by Humphrey et al. (2008). However, the wide range of responses observed between sediment types here, cannot be explained by grain size alone because only one size fraction was applied. The presence of mineral clays (and other cohesive components) in the sediment may be a better indicator of fertilisation inhibition. A key finding of this study was the documentation of clay particles of  $\sim 1 \mu\text{m}$  from Inshore GBR 1 sediment clearly binding to sperm in large flocs under SEM, and this sediment had a dramatic impact on fertilisation ( $\text{EC}_{10} = 2.5 \text{ mg L}^{-1}$ ). Similarly, bentonite clay also had a striking impact on coral fertilisation. Responses from both these sediment and clay types was strongly nonlinear, signifying that only a few additional  $\text{mg L}^{-1}$  above the thresholds identified here could cause complete fertilisation failure.

### 4.3. Contribution of extracellular polymeric substances (EPS) to fertilisation inhibition

Most natural mineral particles are likely covered in organic material, which are commonly associated with flocs (Eisma, 2012).

**Table 3**

The relationships between laboratory-derived thresholds ( $EC_{10s}$ ) and *in situ* NTU derived suspended sediment concentrations (SSCs) using conversion factors. 'Maximum SSC for risk' refers to the maximum SSCs at which dredging-SSCs are > 1% than background SSCs (*i.e.* laboratory-derived thresholds above these values present a negligible risk). Details of study locations and sites can be found in Abdul Wahab et al. (2017); Fisher et al. (2015); Jones et al. (2015a); and Macdonald et al. (2013).

Site	$EC_{10}$ thresholds ( $mg L^{-1}$ ) (mean $\pm$ 95% CI)	Conversion factor used for field NTU conversions	Maximum SSC for dredging risk ( $mg L^{-1}$ )	Risk percentages (%) before dredging (mean $\pm$ 95% CI)	Risk percentages (%) during dredging (mean $\pm$ 95% CI)
<b>Dredging projects</b>					
Onslow	Inshore WA sediment: 40 (12–112)	1.07			
PAROO (< 2 km)			10.6	0 (0–0.01)	0 (0–0.77)
ASHNEE (> 2 km)			6.8	0 (0–0)	0 (0–0.37)
ENDCH (< 2 km)			15.6	0 (0–0.03)	0.05 (0–1.63)
DIRNE (> 2 km)			11.5	0 (0–0.25)	0.18 (0–1.21)
HERALD (> 2 km)			9.5	0 (0–0.05)	0.08 (0–0.74)
Average of sites			10.8	0 (0–0.07)	0.06 (0–0.94)
Burrup peninsula	Inshore WA sediment: 40 (12–112)	1.174			
CHC4 (< 1 km)			> 241.0	0 (0–0.14)	33.33 (4.46–76.45)
DPAN (< 1 km)			> 118.3	0 (0–0.14)	40.94 (1.33–78.42)
HOLD (< 1 km)			> 157.0	0 (0–0.14)	20.04 (4.50–59.03)
Average of 'near' sites			> 172.1	0 (0–0.14)	31.44 (10.29–71.30)
SUP2 (> 1 km)			23.3	0 (0–0.14)	0.35 (0.02–3.88)
KGBY (> 1 km)			22.7	0.32 (0–2.36)	0.68 (0–5.15)
Average of 'far' sites			23.0	0.16 (0–1.63)	0.52 (0.01–4.52)
Barrow island	Offshore GBR (carbonate) sediment: 214	1.8 <sup>a</sup>			
LNGA			115.2	0	0.30
LNG0			87.3	0.03	0.30
Average of sites			101.3	0.02	0.30
Barrow island <sup>b</sup>	Inshore WA sediment: 40 (12–112)	1.07			
LNGA			68.4	0 (0–0.11)	2.69 (0.43–11.27)
LNG0			62.4	0 (0–0.10)	2.49 (0.14–11.81)
Average of sites			65.4	0 (0–0.02)	2.59 (0.29–11.54)
Magnetic island	Inshore GBR 1 sediment: 2.5 (1.5–3.6)	1.1			
Nelly Bay (mean)			NA	14.48 (9.17–31.40)	NA
Horseshoe Bay (mean)			NA	23.79 (18.31–34.85)	NA
Average of sites			NA	19.14 (13.74–33.13)	NA
Magnetic island	Inshore GBR 2 sediment: 54 (35–76)	1.1			
Nelly Bay (mean)			NA	0.06 (0.03–0.07)	NA
Horseshoe Bay (mean)			NA	0 (0–0)	NA
Average of sites			NA	0.03 (0.02–0.04)	NA

<sup>a</sup> Conversion factor derived from benthic sediment rather than sediment collected from the water column.

<sup>b</sup> Assumes the suspended sediment is predominantly siliciclastic from terrestrial run-off events, rather than carbonate.

Together, our microscopy, analytical, and fertilisation results provide evidence that organic matter is involved in the formation of the sediment-sperm floc. While sediments enriched with dissolved inorganic nutrients have been shown to impact coral fertilisation inhibition (Humphrey et al., 2008; Lam et al., 2015), there is a less clear association with sediments containing particulate organic material. Particulate organic material could include a range of material in the sediment, such as seagrass fragments or algae, that do not contribute to floc formation, whereas dissolved inorganic nutrients may enrich the microbial community residing in the sediment, increasing the production of EPS and biofilms (More et al., 2014). These mucilaginous products are largely composed of mucopolysaccharides, and we propose this material can act as 'glue' to bind sediment to sperm, responsible in part for the large sediment-sperm flocs observed in Inshore GBR 1 sediment. In combination with clay particles, EPS presents a threat to coral fertilisation, particularly at inshore turbid reefs subject to increased nutrient exposure due to river discharges and runoff waters.

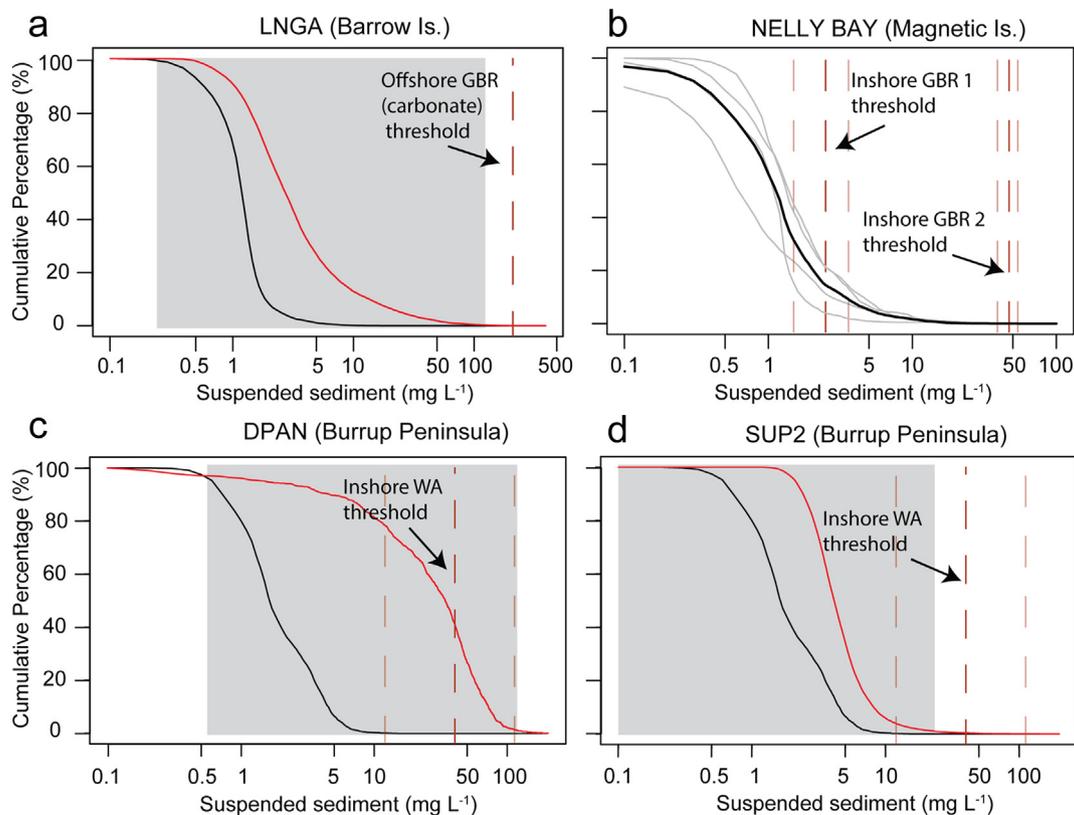
#### 4.4. Sperm limitation by sediment flocs

Suspended sediments reduce the availability of sperm to the coral eggs (sperm limitation) (Ricardo et al., 2015), and this was supported in

our experiments for all sediment types where thresholds were derived (*i.e.*  $EC_{10s}$  at the lower sperm concentration were always lower than the  $EC_{10s}$  at the higher sperm concentrations). As sediments increasingly impact coral fertilisation at lower sperm concentrations ( $10^4$  cells  $mL^{-1}$ ), the negative impacts of a sediment plume would be most apparent for less concentrated coral spawn slicks. More degraded reefs are also likely to generate lower sperm concentrations and therefore are more susceptible to sediment exposure, leading to possible Allee Effects (Birkeland, 2015; Hollows et al., 2007; Nozawa et al., 2015). As a number of pollutants and toxicants also induce sperm limitation in marine broadcast spawners (Albright and Mason, 2013; Hollows et al., 2007; Marshall, 2006), a better understanding of *in situ* sperm concentrations is needed to properly assess how resilient a reproductive event is to relevant stressors like elevated SSCs.

#### 4.5. Risk assessment

A quantitative approach was applied to analyse ecological risk (consequence  $\times$  likelihood; defined by Australia/New Zealand Standard for Risk Management (AS/NZS, 2004)) based on how frequently SSCs exceeded the standardised response threshold ( $EC_{10}$  of fertilisation failure). For the most severe scenario tested (Inshore



**Fig. 6.** Representative cumulative percentage exceedance plots of suspended sediment concentrations (SSCs) for 2-hr intervals during natural resuspension events (*i.e.* background) and dredging periods at (a) Barrow Island (site LNGA), (b) Magnetic Island (site Nelly Bay), and Burrup Peninsula at (c) site DPAN, ~0.5 km from the channel, and (d) site SUP2, ~1.8 km from the channel. The black lines indicate the natural resuspension event average. Grey lines indicate site replicate measurements. The red dashed lines indicate the laboratory-derived thresholds (light red lines = 95% CI) using a comparable sediment type for each site. The grey shading indicates the range of SSCs that are more frequent during dredging compared to background SSCs. Details of study locations, sites and conversion factors can be found in Abdul Wahab et al. (2017); Fisher et al. (2015); Jones et al. (2015a); and Macdonald et al. (2013). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

GBR 1 sediment and a low sperm concentration), natural resuspension events near Magnetic Island on the GBR exceeded the threshold ~19% of the time, or equivalent to approximately once in every 5 years during coral spawning. At this threshold, 10% or more eggs would fail to fertilise because of sediment impacts. However, when the SSCs were compared against the Inshore GBR 2 sediment threshold, there was no threshold exceedance, even during elevated suspended sediments spikes, therefore presenting a very low risk for this sediment type. Water quality data from Onslow, WA compared with the  $40 \text{ mg L}^{-1}$  threshold of Inshore WA sediment additionally produced a very low risk percentage ( $< 1\%$ ) in both the pre-dredging and dredging phases. Generally, thresholds  $< 11 \text{ mg L}^{-1}$  (beyond background effects) would be required to present a substantial risk of dredging at this location. While both pre-dredge and during-dredge phases did have SSC readings  $> 40 \text{ mg L}^{-1}$ , these events were very rare, owing mostly to large storm events. In contrast, sites very close to the dredge at Burrup Peninsula regularly exceeded the threshold ~31% of the time. But a spatial separation distance  $> 1 \text{ km}$  from the channel markedly reduced the risk percentage to  $< 1\%$ . Therefore, given both the relatively high thresholds for Inshore WA sediment, much of the risk to coral fertilisation could be alleviated with a 1 km spatial separation distance between the dredge and the coral spawn slick. The level of risk is likely to increase where cohesive sediments are present, and where coral cover and sperm concentrations are low, requiring greater spatial separation distances between the operating dredge and coral spawn slicks. The differences in risk between locations and sediment types underscore the need to carefully match laboratory-derived thresholds with *in situ* water quality data for fertilisation responses.

#### 4.6. Environmental relevance

Inshore GBR 1 sediment contained  $1\text{-}\mu\text{m}$  clay particles binding to the sperm, which were subsequently identified as kaolinite clay, a common clay found in sediments entering and residing in muddy embayments of the GBR (Bainbridge et al., 2015; Esslemont, 2000). Although there is a paucity of data of mineral clay content for WA sediment, kaolinite is presumably present in at least some locations given substantial deposits of kaolin are present near Broome on the northern WA coastline (Smolinski et al., 2016). Interestingly, mined kaolin that was low in organic content, did not have a strong effect on coral fertilisation, indicating an additional component is required to form the sediment–sperm flocs observed. In contrast, refined kaolin (while not entirely environmentally realistic) had a dramatic effect on fertilisation, indicating that kaolin with few impurities can bind to sperm. Mined bentonite clay had a powerful effect on fertilisation by binding to the sperm, and at elevated SSCs, also bound to and sank coral eggs, similar to that observed on coral embryos (Ricardo et al., 2016a). Bentonite is comprised primarily of an expandable clay montmorillonite, which has an interlayer surface area of  $700$  to  $800 \text{ m}^2 \text{ g}^{-1}$ , magnitudes greater in surface area than silt (Theng, 2012). Expandable clays are the most abundant mineral clay entering the GBR lagoon from the Burdekin catchment, Australia's largest river by discharge volume (Mitchell et al., 2006); and it is hypothesised that these terrestrial sediment fractions travel furthest offshore (Bainbridge et al., 2015). Its expandable properties also make it a common choice of drilling muds for directional drilling of wells and pipelines associated with oil and gas extraction facilities (APASA, 2005; Järnegrén et al., 2016; Sharma et al., 2006). Bentonite is sometimes used as a surrogate for sediment in assays, and

has been shown to cause negative responses in reef fish (Hess et al., 2015; Wenger and McCormick, 2013), but the extent that bentonite composes a proportion of the sediment in the inshore marine environment remains unknown.

Inshore GBR 1 sediment had a clear impact on coral fertilisation success but interestingly, organic nutrients (decaying plankton) added to sediment collected at the same location did not further increase the impact on coral fertilisation (Humanes et al., 2017), perhaps because increases in organic nutrients did not directly relate to EPS production. Close examination of the flocs in our sample under SEM revealed there was organic material present, and fibrils of EPS were observed in the raw sediment in addition to many microbes. Microbes were also observed in the Inshore WA sediment but there was little presence of EPS (and chemical analyses indicated that Inshore GBR sediments were many factors higher in EPS than the Inshore WA sediment). The impact on fertilisation using high concentrations of EPS (Xanthan Gum) generally supported the hypothesis that EPS may be involved in floc formation, although the decrease only accounted for a small reduction in fertilisation success indicating a complex association between EPS and clay particles may cause the flocculation of sperm. The binding of EPS onto clay particles has been well documented owing to the application of EPS as a flocculant in waste water remediation (Cao et al., 2011; More et al., 2014), and is well known to cause flocculation of sediments in marine waters (Eisma, 2012; Engel et al., 2004; Sutherland, 2001). More extreme examples of bio-mediated flocs are those associated with positively buoyant transparent exopolymer particles (TEP) (Fabricius et al., 2003; Petrova and Sauer, 2012; Wurl et al., 2011), which could potentially interfere with coral fertilisation because of their size and tendency to concentrate in the water surface. Further research is needed to understand the role specific microbial communities actively play in EPS production and floc formation. While Inshore GBR 1 sediment clearly showed adverse effects to coral fertilisation, the sediment had unique properties such as a very high TOC and substantial clay content (~20%), and therefore it needs to be determined how often this type of sediment occurs in inshore reef environments. Further, one such limitation of keeping 'live' sediment in aquaria is the natal microbial community gradually changing over time. Extrapolating the experimental results found here to future dredging operations is difficult because of the limited information on water quality (SSC characteristics) that occur at the water surface in close proximity to dredging operations. For example, the organic content is likely to vary substantially depending on the location, and fluctuate across both fine spatial and temporal scales.

The ability of coral populations to be able to withstand perturbations in coral fertilisation success may depend on if a demographic bottleneck occurs at the settlement/post-settlement stage. For example, if recruitment success is density-dependant (Edwards et al., 2015), larval supply will be saturated and a small reduction in fertilisation success may do little to impact overall recruitment. On the other hand, if a reef is degraded and there are no density-dependence effects, then a decline in fertilisation success may translate into lower levels of recruitment.

## 5. Conclusion

There is a growing trend to apply site-specific trigger values to effectively regulate suspended sediments or toxicants, as it becomes apparent that factors other than exposure concentration and duration can influence impacts on biota (Gordon and Palmer, 2015; Storlazzi et al., 2015; van Dam et al., 2014; Warne et al., 2014). This study indicates that sediments containing cohesive components such as those rich in mineral clays and organic matter, can affect coral fertilisation at low concentrations. However, less cohesive sediments present a far lower risk, with risk probabilities indiscernible from baseline (pre-dredging) conditions. It is therefore important that efforts to reduce mineral clays and nutrients entering tributaries are continued, and dredging projects that generate or disturb (e.g. dredge, drill or resuspend) nutrient and clay rich sediment types are regulated, especially during critical environmental periods such as multi-species synchronous coral spawning events.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2018.08.001>.

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