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Effects of sediments on the reproductive cycle of corals:

Experimental studies

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WAMSI Dredging Science Node

Theme 7 Report

Projects 7.3, 7.4 & 7.5

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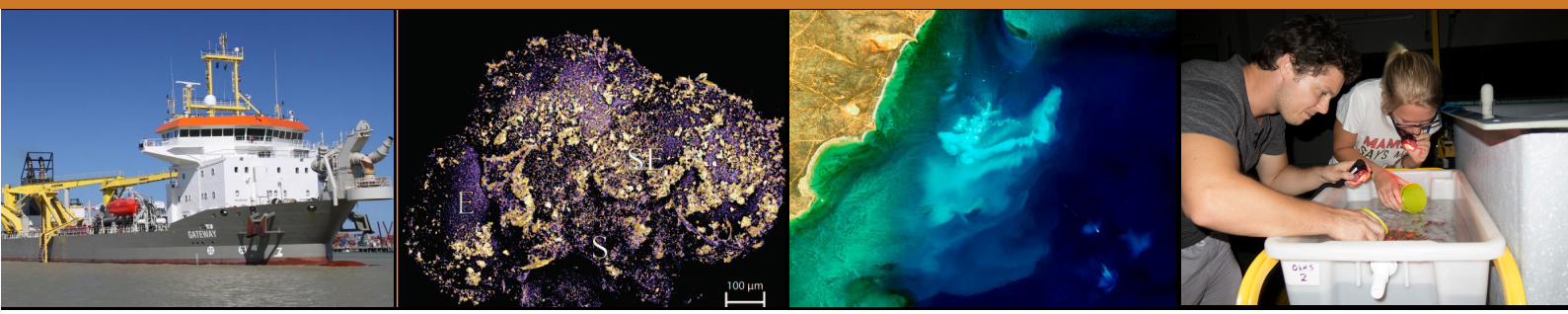

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Front cover images (L-R)

Image 1: Trailing Suction Hopper Dredge *Gateway* in operation during the Fremantle Port Inner Harbour and Channel Deepening Project. (Source: OEPA)

Image 2: Microscopy image of a coral egg-sperm bundle after ascent through elevated concentrations of suspended sediments, revealing considerable attachment of sediment grains (yellow) to the bundles (purple). (Source: AIMS)

Image 3: Dredge Plume at Barrow Island. Image produced with data from the Japan Aerospace Exploration Agency (JAXA) Advanced Land Observing Satellite (ALOS) taken on 29 August 2010.

Image 4: WAMSI researcher Gerard Ricardo (UWA) collecting freshly spawned eggs for laboratory experimentation. (Source: AIMS)

WAMSI Dredging Science Node

The WAMSI Dredging Science Node is a strategic research initiative that evolved in response to uncertainties in the environmental impact assessment and management of large-scale dredging operations and coastal infrastructure developments. Its goal is to enhance capacity within government and the private sector to predict and manage the environmental impacts of dredging in Western Australia, delivered through a combination of reviews, field studies, laboratory experimentation, relationship testing and development of standardised protocols and guidance for impact prediction, monitoring and management.

Funding Sources

The $20 million Dredging Science Node is delivering one of the largest single issue environmental research programs in Australia. This applied research is funded by **Woodside Energy, Chevron Australia, BHP Billiton and the WAMSI Partners** and designed to provide a significant and meaningful improvement in the certainty around the effects, and management, of dredging operations in Western Australia. Although focussed on port and coastal development in Western Australia, the outputs will also be broadly applicable across Australia and globally.

This remarkable **collaboration between industry, government and research** extends beyond the classical funder-provider model. End-users of science in regulator and conservation agencies, and consultant and industry groups are actively involved in the governance of the node, to ensure ongoing focus on applicable science and converting the outputs into fit-for-purpose and usable products. The governance structure includes clear delineation between end-user focussed scoping and the arms-length research activity to ensure it is independent, unbiased and defensible.

And critically, the trusted across-sector collaboration developed through the WAMSI model has allowed the sharing of hundreds of millions of dollars worth of environmental monitoring data, much of it collected by environmental consultants on behalf of industry. By providing access to this usually **confidential data,** the **Industry Partners** are substantially enhancing WAMSI researchers' ability to determine the real-world impacts of dredging projects, and how they can best be managed. Rio Tinto's voluntary data contribution is particularly noteworthy, as it is not one of the funding contributors to the Node.

Funding and critical data **Critical data**

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Contents

Executive Summary

Background and approach

In recognition of the risk posed by dredging, the Western Australian EPA has produced generic guidance documents which articulate a framework for proponents and consultants to use for all dredging activities and provides a common approach to setting environmental outcomes. The latest version was released in 2016[1]. A key component of this framework relevant to designing dredging proposals and predictions of environmental impacts, is that proponents should consider *critical windows of environmental sensitivity* (CWES). CWES include times of the year, or particular sites, where key species or ecological communities or critical processes may be particularly vulnerable to pressures from dredging[1]. CWES are often associated with reproduction and recruitment process which underpin the maintenance and resilience of communities. The most well-known CWES concerns the annual (sometimes biannual) synchronous, multi-specific mass release of gametes by many broadcasting spawning corals. Since 1993, dredging proponents in WA have been required to recognize the increased risk of dredging during this coral spawning window, and since 1999 were typically required to stop all turbidity generating activities for an appropriate time. The coral spawning CWES has evolved over time as new information became available (see 7.1). Most recently proponents have been required to cease dredging for 10 d: i.e. 3 d before and for 7 d after the predicted first night of mass spawning but with a caveat whereby proponents could continue to engage in turbidity generating activities if they could provide '...*peer-reviewed scientific evidence that if those turbidity generating activities were to continue during coral mass spawning events, any effect, if it were to occur, would not significantly impact the functional ecology of local and regional reefs*...'. When the window was first introduced comparatively little was known about the effects of sediments on the early life-history stages and the approach was precautionary. Since then there has been some further information from a number of different studies, but it is fair to say that the precautionary approach is still being applied. There is a critical lack of information required to improve the effectiveness of the coral spawning CWES and the suite of experiments described in this report was designed to address this issue (Ricardo 2017).

The life-cycle of broadcast spawning corals is complex (Fig. 1), involving: (1) synchronised release of gametes packaged in positively buoyant egg-sperm bundles which rise to the surface and then dissociate allowing fertilization to occur; (2) fertilization; (3) embryo and larval development (which occurs at the surface and later in the water column) and (4) larval settlement and metamorphosis into a sessile polyp (on the reef).

[1] EPA (2016) Environmental Protection Authority 2016, Technical Guidance – Environmental Impact Assessment of Marine Dredging Proposals, EPA, Western Australia

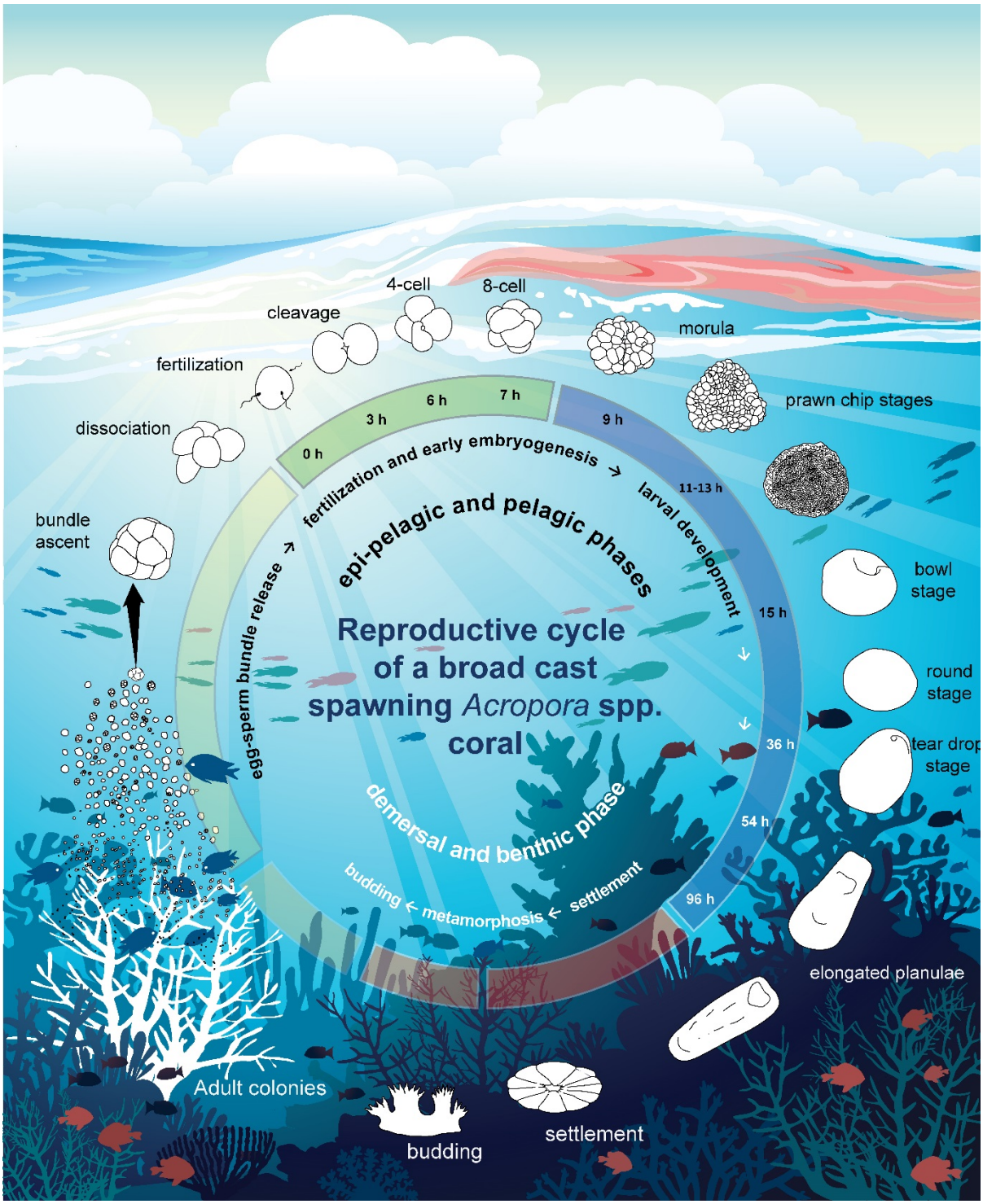

Figure 1. A stylised depiction of the reproductive cycle of a broadcast spawning *Acropora spp.* (see text)

We identified all biologically plausible[2] cause–effect pathways whereby sediment could affect these four broad life phases, and then designed and conducted a series of experiments to validate the pathways and determine pressure-response relationships. All experiments were conducted under controlled laboratory conditions (aquaria) with environmentally relevant exposure scenarios determined from analyses of water quality conditions occurring during dredging programs in Western Australia (Fisher et al. 2015, Jones et al. 2015a, Jones et al. 2015b). Experiments were conducted with a range of broadcast spawning species including *Acropora tenuis*, *A. millepora, Montipora digitata*, in addition to a brooding species, *Pocillopora acuta*, for the larval experiments. Pressure-response experiments were designed to deliver thresholds for relevant sediment-related stressors (i.e. suspended sediment, deposited sediments and light intensity) that impact on each life stage. The approach was based on ecotoxicological criteria, where a 10% effect on an ecologically-relevant process (EC_{10}) is considered the threshold applicable to guideline derivation and risk assessments (Warne et al. 2015).

Spawning (egg-sperm bundle rise)

Optical microscopy and backscatter scanning electron microscopy revealed a novel threat to reproductive success, whereby sediments adhered to the mucous membrane of the egg-sperm bundles, reducing their ascent or preventing them from reaching the water surface (Fig. 2a). This was referred to as the *ballasting* effect. Other than directly decreasing the number of eggs that could be fertilised, ballasting would also lead to a subsequent reduction in egg–sperm encounters at the surface, a proxy for fertilisation success. Our experimental observations (from sediment column assays) of this mechanism were successfully captured by a mathematical model that quantified the reduction in ascent probability and egg–sperm encounters as a function of suspended sediment concentration (SSC), particle grain size, and depth of the adult colony. Larger grain sizes and adult colonies spawning in deeper waters reduced the SSC–ballasting threshold. For colonies spawning from 15 m depth, coarse-silt SSCs of 35 mg L^{-1} and 87 mg L^{-1} resulted in a 10% and 50% decrease in egg–sperm encounters. From shallower (5 m deep), the EC_{10} occurred at an SSC of 106 mg L^{-1}. These SSCs and grain sizes can occur during dredging programs but are commonly associated with upper-percentiles of sediment plumes from dredging or natural resuspension events and occur relatively close (kms) to dredging activities compared to the potential extent of dredging-generated plumes (10's kms see below).

Fertilisation

The second series of experiments investigated how elevated SSCs may directly impact the fertilisation stage of coral gametes at the water's surface. Fertilisation success in corals increases non-linearly with sperm concentration because of an increase in egg-sperm encounters, resulting in fertilisation success increasing markedly between 10^3 and 10^5 sperm mL^{-1}, however in presence of sediment, higher concentrations of sperm were required to achieve a comparable level of fertilisation success.

A range of microscopy techniques revealed that some sediments adhered to sperm, forming sediment–sperm flocs, and this co-occurred with an overall decrease in sperm numbers at the water surface – determined using flow cytometry (Fig. 2b,c). This flocculating caused the sperm concentration to fall below the threshold required for fertilisation. This was referred to as the sperm limitation effect. Despite sticking to the egg-sperm bundles the sediments did not appear to stick to the eggs (which lack a mucous coating), suggesting the effects on fertilization success were more associated with the sperm. Sperm limitation was identified in the presence of siliciclastic sediment (230 and ~700 mg L^{-1}), with 2–37 fold more sperm required to achieve maximum fertilisation rates, when compared with sediment-free treatments. Considerable (>45%) decreases in sperm concentration at the water's surface was recorded in the presence of siliciclastic sediment and a >20% decrease for carbonate sediment.

Concentration–response thresholds were then assessed for a range of sediment types. Fertilisation was found to be more sensitive to organic-clay rich sediments which inhibited fertilisation by 10% (EC_{10}) at an SSC as low as 2.5 mg L^{-1}, with clear binding of the mineral clay (identified as kaolinite) to sperm. While kaolinite clay did not cause

[2] in epidemiology, biologically plausible mechanisms are those where there is a credible or reasonable biological and/or toxicological basis linking the proposed cause and effect

a direct effect by itself, bentonite clay had a pronounced effect on fertilisation at SSCs as low as 4.6 mg L^{-1} (EC_{10}). Sediments with lower organic content, or mineral clay content, had a much lower impact on coral fertilisation (EC_{10} >40 mg L^{-1}). For all sediment types, the effect was more pronounced at sub-optimal sperm concentrations (because of sperm limitation). Collectively, these findings demonstrate that high SSCs can remove sperm from the water's surface during coral spawning events, reducing the window for fertilisation with potential flow-on effects for recruitment.

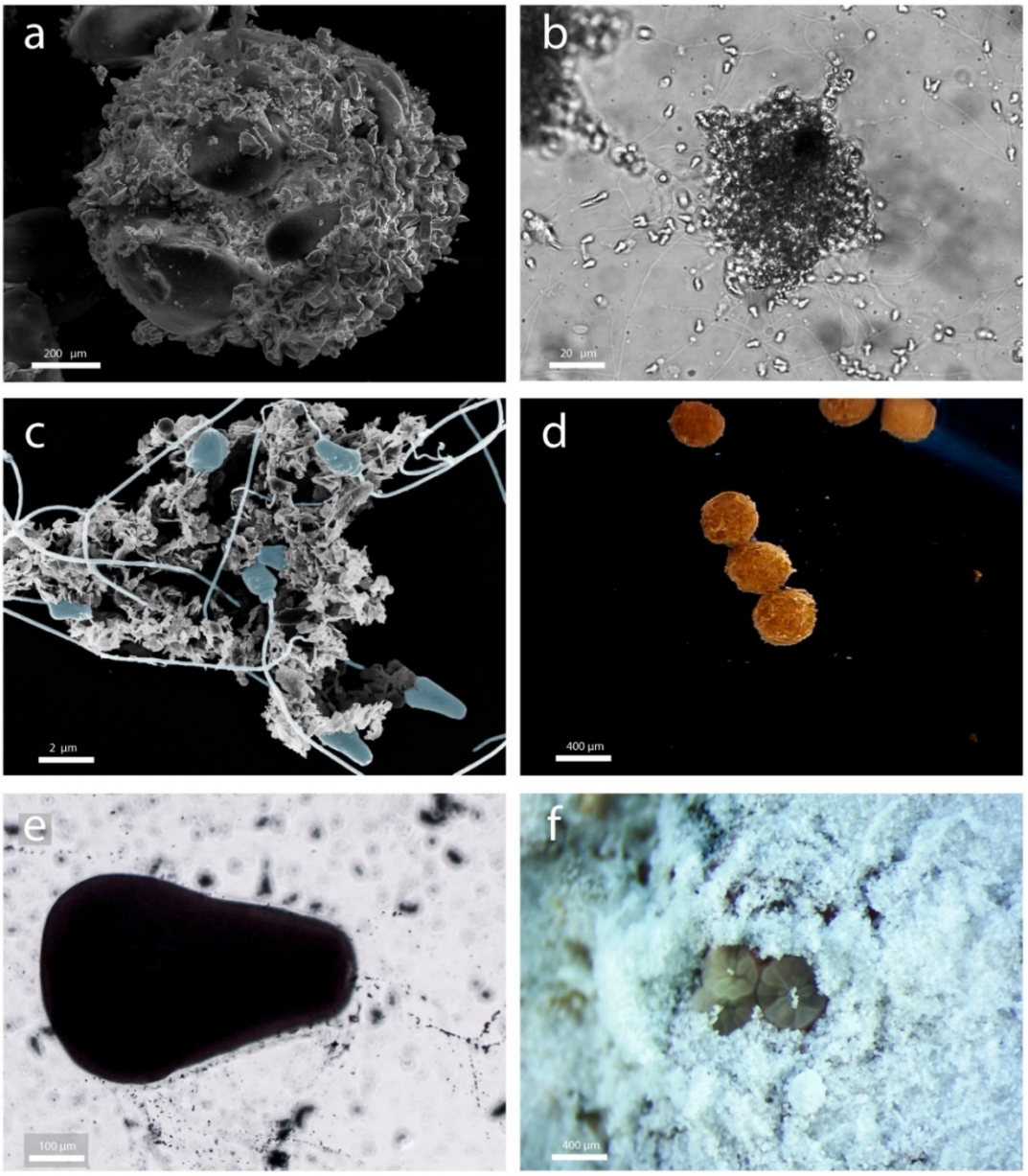

Figure 2. Sediment impacting corals across various developmental stages. (a) An egg-sperm bundle covered in coarse-silt sediment. Approximately 50 grains of coarse-silt are capable of sinking an egg-sperm bundle. (b) Sperm aggregating on a siliciclastic sediment floc. (c) A high magnification false-coloured image of a sediment–sperm floc. Sperm coloured in blue. Thin ~1 µm plate-like grains (mineral clays) can be observed coating sperm within the floc. (d) Embryos developing inside mucous cocoons after being exposed to elevated siliciclastic suspended sediments. (e) A larva clearing sediment with mucous strands and ciliary beating. (f) A few larvae that successfully managed to burrow and undergo metamorphosis on sediment-covered surfaces.

Embryo and larval development

Elevated SSCs (rather than sediment deposition and light attenuation) was considered the most relevant dredging-related hazard for the pelagic stages of the larvae involving early embryogenesis at the surface and larval development at the surface and in the water column. Embryos were subjected to short (12 h) suspended sediment exposures commencing from ages of 3, 6 and12 hours old, or a long (30 h) exposure commencing at 6 hours old. Embryo survivorship and subsequent metamorphosis were not affected across the range of SSCs tested, in some assays as high as ~1000 mg L^{-1}. A novel adaption was identified in which embryos form a mucous coating which protects the developing embryos until they are capable of swimming, at which point they use cilia beating to break free. This was referred to as the *cocooning* effect and was common in embryos but not in larvae (Fig. 2d). Embryo cocooning occurred at SSCs ≥35 mg L^{-1} (EC_{10}) after exposure to siliciclastic sediment, but were only observed in the presence of carbonate sediments at much higher SSCs. Once transferred into sediment-free seawater, functional ~36-h-old embryos began emerging from the cocoons, coinciding with ciliary development, and these continued to develop normally and undergo settlement. Ciliated (>36-h-old) larvae exposed to suspended sediments for 60 h were also observed to secrete mucus, but were unaffected by suspended sediment concentrations up to ~800 mg L^{-1} (Fig. 2e). These results show that embryogenesis and larval development is comparatively insensitive to elevated SSCs (of the sediment types used), and mucous secretion and cilia beating effectively protect coral embryos and larvae from elevated SSCs.

Larval settlement

The next series of experiments revealed a strong effect of sediments that deposit or accumulate on substrates, preventing larval settlement. Choice experiments were conducted where larvae were offered settlement options of crustose red algae (CRA) attached to differentially orientated surfaces with different levels of deposited sediment. Larvae preferentially settled on upward facing surfaces, but very low levels of deposited sediment, equivalent to a thin veneer of sediment (<150 µm), deterred settlement and caused a change in larval settlement preference to sediment-free, downward facing surfaces. Low settlement was observed on vertical surfaces, regardless of sediment treatment. The newly settled larvae have limited energy reserves that need to be augmented by photosynthetic symbionts for calcification, division and to begin forming the three-dimensional skeleton that effectively lifts them clear of the substrate. The poorly illuminated undersides may be sub-optimal for longer-term post-settlement survival. When only upward-facing surfaces were presented, 10% of settlement was inhibited as deposition levels as low as 0.9 to 16 mg cm^{-2} (EC_{10}), regardless of sediment type (carbonate and siliciclastic) or particle grain size (fine and coarse silt). Grooves within the settlement surfaces slightly improved options for settlement (EC_{10} = 29 mg cm^{-2}), but were quickly infilled at higher deposited sediment levels.

We found that light attenuation typical of dredging plume conditions did not affect settlement success on healthy CRA surfaces. In another experiment CRA surfaces were temporarily smothered with sediment for 6 d, the sediment was then washed off, and larvae were allowed to settle. A 6 d smothering of ~7 mg cm^{-2} caused the CRA to bleach by ~50%, and the larvae were very reluctant settle on these previously sediment-covered surfaces. This is the first direct link between the degradation of CRA by sediment smothering, and a decrease in larval settlement. This was referred to as the *substrate health* effect.

The study shows that even a thin veneer of sediment can have consequences for larval settlement due to a reduction of optimal substrate and that sediment smothering can impact the substrate health also affecting settlement. While grooves and overhangs may provide alternative settlement sites, these may not be optimal and recruits settling at these sites may also be subject to constant stress from turbidity and sediment infilling.

Summary

The sequence of experiments have provided much needed information on cause–effect pathways and empirical data on pressure–response relationships for several of the key early life history stages of corals (Ricardo 2017). The *ballasting*, *sperm limitation*, *cocooning* and *substrate health* effects outlined above have not been described previously in the scientific literature, indicating just how little is really known for risk assessment purposes. The sediment type and the sperm concentration used in tests were found to substantially affect fertilization success.

This probably explains the wide range of fertilisation threshold values (<50 mg L^{-1} to >1000 mg L^{-1}) reported in previous studies. Some stages were very sensitive (i.e. settlement) and others were very insensitive (embryogenesis and larval development), and the thresholds identified can be used for impact prediction purposes and inform management, regulatory and operational decisions around dredging to improve protection during the coral spawning *critical windows of environmental sensitivity*.

Considerations for predicting and managing the impacts of dredging

Key overall findings

Environmental risk is associated with the probability or likelihood of an event causing an undesirable effect. For the coral spawning *CWES*, the information that is needed is what are the likely cause-effect pathways (the mechanisms) and the associated key pressures parameters, and the period of time over which these mechanisms operate (i.e. for fertilization a few hours and for larval development several days etc). Once pressure-response relationships are derived and the relationship characterized, then the probability of this occurring *in situ* can be assessed using pressure-field predictions (from plume modelling). Projects 7.3–7.5 examined the mechanisms and well established ecotoxicological criteria were used to quantify the thresholds as EC_{10}s for application in risk assessments for impact prediction. Although beyond the scope of the study – which was designed to produce numbers to parameterize risk assessment models – for contextual purposes the EC_{10} values are briefly compared to water quality data collected during several large scale dredging programs in Western Australia where spatial and temporal patterns in water quality were examined in detail (see Fisher et al. (2015), Jones et al. (2015a)).

The three key physical stressors associated with turbidity generating activities are elevated suspended sediment concentrations, light attenuation and sediment deposition, and these stressors had very different effects on the different early life history phases:

- Elevated SSCs primarily impacted on the pelagic processes including bundle ascent, fertilisation and temporarily caused cocoon development in embryos;
- Light attenuation did not affect the early life phases apart from having a weak influence on larval settlement under some circumstances;
- Deposited sediments had a very strong *direct* impact on settlement – driving larval settlement to the sediment-free under-sides of substrata which may be less optimal for post-settlement survival (see below).
- Deposited sediments also had *indirect* impacts on settlement by smothering and causing deterioration in the quality of crustose red algae, which is a powerful settlement inducer for corals, and therefore could have effects on coral settlement *in situ*.

Impact prediction

Bundle ballasting. The lowest threshold for effects of suspended sediments on the egg-sperm encounter rates was 35 mg L^{-1} (EC_{10} see Table 1). Effects on bundle ascent and egg-sperm contact take place over a few hours and suspended sediments can increasingly exceed this level within 2 km of dredging activities (Fisher et al. 2015, Jones et al. 2015a). Although this cause–effect pathway is possible, it is probably restricted to sites close to dredging operations. These thresholds should be considered lenient because sediments more typical of inshore dredging operations (richer in organic nutrients and clays) are more cohesive and likely to bind and accumulate more readily onto ascending bundles (see further below).

Table 1. Concentration–response thresholds for suspended sediment impacts on the ascent of *Montipora digitata* egg-sperm bundle and egg-sperm encounter rates. All sediments were coarse-silt* carbonates.

Experiment	Water depth (m)	EC_{10} (mg L^{-1})	EC_{50} (mg L^{-1})
Bundle ascent	10	71	211
Egg-sperm contact		53	131
Bundle ascent	15	47	141
Egg-sperm contact		35	87

**Wentworth classification for grain size*

Fertilisation (Project 7.3). The fertilization stage is a critical process occurring over a period of a few hours on the night of spawning. With one exception (see below) the EC_{10} values tested over optimal and slightly sub-optimal sperm concentrations were in the range of 10s of mg L^{-1}, and as with effect on the bundles, this cause–effect pathway is possible but probably restricted to sites close to dredging operations (Table 2).

The lowest threshold observed (EC_{10} = 2.5 mg L^{-1}) was observed for lower sperm concentrations (10^4 sperm mL^{-1}) and a sediment rich in organic material and mineral clays, collected from a mud-flat that borders a fringing coral reef (Table 2). This is a low value, and such SSCs are possible in the far-field (kms away from dredging activities) and during natural storms and resuspension events. The sediment used was one of 2 surficial samples collected from the inshore GBR and the second sample, collected ~20 km from the first silty-sand benthos also close to a fringing coral reef, returned a much higher EC_{10} values of ~47 mg L^{-1} similar to other fine sediments. The main contrast between the two sediments owed to the mineral clay content, with the sediment that caused a strong response containing ~20% kaolinite. For this sediment, clay particles (~1 µm) were also clearly observed in the large sediment-sperm flocs. However, kaolinite by itself did not elicit the same response, indicating other cohesive components (such as mucopolysaccharides), were further required for floc formation in this sediment Commercially available bentonite clay, which is known to be very cohesive, had similarly low EC_{10} values (4.6 mg L^{-1}) and formed large flocs with coral sperm. Overall, the conclusions from this study was that the effects of sediment on fertilisation success is highly dependent on the nature of the sediment, in particular the type and proportion of mineral clay content, and mucopolysaccharides content. There is less risk of suspended sediments to fertilisation success when exposed to comparatively clean sediment low in mineral clays and organic content.

For future hazard assessment purposes consideration should be given to assessing the cohesiveness of the sediments with coral sperm, which could be estimated by characterizing the mineral clay, organic carbon and extracellular polymeric substances (EPS) content of the sediments which are being dredged[3]. These analyses should be performed on the sediments that the gametes are likely to come into contact with i.e. sediments released by the drag head or cutter head, overflowing from a hopper barge or released at the dredge material placement sites from barges, which may be quite different from the consolidated seabed sediments (especially the surficial few cms collected before dredging).

[3] this is especially relevant when relocating inshore, typically muddy sediments to offshore dredge material placement sites.

Table 2. Concentration–response thresholds for suspended sediment impacts on coral fertilisation of *Acropora tenuis*. EC_{10} and EC_{50} values reported are derived from nonlinear regression models at different sperm concentrations (see text).

Experiment	Sediment type	PSD*	Sperm mL^{-1} concentration	EC_{10} ($mg\ L^{-1}$)	EC_{50} ($mg\ L^{-1}$)
Fertilisation success	Inshore GBR 1	Very fine silt	10^4	2.5	5.8
			10^5	54	125
	Inshore GBR 2		10^4	47	75
	Inshore WA (siliciclastic)		10^4	40	205
			10^5	80	414
	Offshore (carbonate)		10^4	214	>800**
			10^5	>820**	>820**
	Bentonite	Clay	10^4	4.6	6.9

* Wentworth classification for grain size

** Greater than the range of concentrations tested

***Commercially available high grade processed calcium-bentonite clay (Watheroo Bentonite)

Embryo and larval development (Project 7.4): The larval development phases are the least sensitive to suspended sediment, with sediment grains easily removed by ciliary beating and mucous production. There were no lethal effects at SSCs greater than 100s mg L^{-1} (see Table 3). There were also no clear legacy effects of sediments i.e. exposure to suspended sediments did not affect the ability of larvae to subsequently settle on suitable substrates. The environmental significance of the cocooning effect in these experiments is difficult to evaluate. Cocooning could be a survival mechanism to temporarily endure high SSCs, but given the high SSCs (>35 mg L^{-1}) required, the effect would occur in close proximity to dredging.

Gilmour (1999) reported significant effects of a 50 mg L^{-1} SSC on coral larvae, but as discussed in Jones et al. (2015b), there were methodological issues associated with this experiment such as a lack of water exchange in the test containers, high larval densities (15 mL^{-1}) and a build-up of dead material which would have influenced the results. These limitations were recognized by Gilmour (1999), but the 50 mg L^{-1} value has nevertheless been widely cited (as discussed in Jones et al. (2015b)) and the result is not supported in these studies.

Table 3. Concentration–response thresholds for very-fine-silt* suspended sediment impacts on coral embryos and larvae. EC_{10} and EC_{50} values reported are derived from nonlinear regression models.

Experiment	Sediment type	Exposure duration (h)	Species	EC_{10} (mg L^{-1})	EC_{50} (mg L^{-1})
Embryo survival	Siliciclastic	30	*Acropora millepora*	>75**	
Settlement (pre-exposed embryos)					
Embryo survival	Carbonate			>78**	
Settlement (pre-exposed embryos)					
Cocoon formation (pre-exposed embryos)	Siliciclastic	12		35	134
Larval survival		60		>747**	
Settlement (pre-exposed larvae)					
Larval survival	Carbonate			>802**	
Settlement (pre-exposed larvae)					
Larval survival	Siliciclastic		*Acropora tenuis*	>747**	
Settlement (pre-exposed larvae)				300	>747**
Larval survival	Carbonate			>802**	
Settlement (pre-exposed larvae)					
Larval survival			*Pocillopora acuta*	>915**	

* Wentworth classification for grain size

** Within the range of concentrations tested

Larval settlement (Project 7.5): The experiments in Project 7.5 showed that whilst the larvae of *Acropora millepora* are not averse to contact with deposited sediment, probing it and temporarily resting on the sediment covered surfaces (see video), they will not settle if the substrate is covered in only a thin veneer of sediment. *In situ*, there are many surfaces that will be sediment-free but these will typically be cryptic surfaces i.e. overhangs or on downward facing surfaces were light levels will be intrinsically lower.

The aversion of coral larvae to settling on sediment-covered surfaces has been known for a while, but this study revealed just how low the deposited sediment levels have to be to cause the effects: i.e. as low as a few mg cm^{-2} (Table 4). Settling in shaded (more cryptic) locations could reduce pressure from predation, but they will receive less light (needed for rapid growth) than if they settled on an upward facing, sunlight exposed surface. Deposited sediments also have a strong effect on the health and function of the settlement inducer crustose red algae (CRA). A few days of smothering of CRA[4] by a thin veneer of sediment caused a marked decrease in the health of CRA and a subsequent decrease in settlement success (Table 4). Deposited sediment levels that cause shifts in the settlement distribution, and or reduce effectiveness of CRA to induce settlement, occur within common natural background levels, indicating these shifts are already likely to operate to some degree on most reefs. These subtle shifts of the recruiting population to undersides may eventually lead to a slower growth rate of the coral population in the light-limited overhangs and crevices until they eventually grow outwards into light. Further studies are needed to evaluate the consequences of this, nevertheless, we propose that the settlement life history stage is the most sensitive to sediment of the five stages tested and currently one of the most difficult to evaluate.

[4] a mixed community dominated by *Titanoderma prototypum* and *Peyssonnelia* spp.

Table 4. Concentration–response thresholds for deposited sediment impacts on coral larval settlement of *A. millepora*. EC_{10} and EC_{50} values reported are derived from nonlinear regression models.

Experiment	Sediment type	Sediment treatment	PSD*	Surface		Cue	EC_{10} (mg cm^{-2})	EC_{50} (mg cm^{-2})
				Structure	Aspect			
Settlement (sediment)	Carbonate	Deposited sediment	Fine silt	Flat	Upper	CRA	1.3	11
			Coarse silt				2.9, 16**	11, 48**
	Siliciclastic						4.2	13
	Carbonate						0.9	7.1
Settlement (sediment on multi-surface prism)				Grooves			29	88
Settlement (post-smothered CCA)				Flat			69	104
							7.2	33

*Wentworth classification for grain size.

**This experiment was repeated on a following year.

Impact prediction across life phases

Thresholds (10% effect levels, EC_{10}) for each of the early life phases and processes from bundle ascent to larval settlement were quantified and can be applied for management and risk predictions. **The intention is that these thresholds can be coupled with sediment transport/hydrodynamic models to estimate the probability that plumes of suspended sediments of various concentrations from dredging activities encounter various phases of the life cycle, from egg-sperm bundles, gametes, embryos and larvae.**

For example, gametes released from corals in close proximity to dredging activities and encountering SSCs of tens of mg L^{-1} (see Fisher et al. (2015), Jones et al. (2015a), (Jones et al. 2015b)) could affect the egg–sperm bundles and cause *sperm limitation* effects (see Table 1 and 2) – clearly validating, under some circumstances, the use of the coral spawning *critical window of environmental sensitivity* (CWES). However, where coral spawning occurs at a distance from the dredging activities, and developing 1–2 d old pelagic embryos and larvae drift into a turbid plume of similar SSCs, there is comparatively little risk of negative effects on survivorship, or subsequent metamorphosis of the embryos (see Table 3).

The proximity of corals to dredging activities, and dredging activities occurring at the time of coral spawning, will be site specific and highly variable, but the information needed to assess the risk is now available from the laboratory based studies (see *residual knowledge gaps* below).

For suspended sediments the relative sensitivities of the life history stages were fertilisation > bundle rise > embryo and larval development. Fertilisation was clearly the most sensitive and the sensitivity was highly dependent on sediment type with sediments containing clays and high organic carbon representing the greatest hazard to reproductive success. Conversely, larval settlement was relatively unaffected by sediment type and size but only a thin veneer of sediment on the benthic substratum can influence the settlement preference of the larvae.

A hypothetical representation of some of the threshold values guided by the fertilisation, larval, and settlement sensitivities are presented (Figure 3). Generally, the fertilisation stage thresholds are dependent on sediment type. The larval stage is relatively robust to elevated SSCs and thresholds consistently sit above what might occur during dredging operations. Settlement thresholds (on up-ward facing surfaces) are very low, and sit within background levels. Counter-intuitively, crevices and grooves that increase the settlement threshold put the most sediment-tolerant larvae at risk from dredging. When applying the results of these studies, the reader is reminded that although the effects of sediment-related stress have been investigated for the key life history stages, this represents a subset of the range of factors each life history stage is subject to in both space and time.

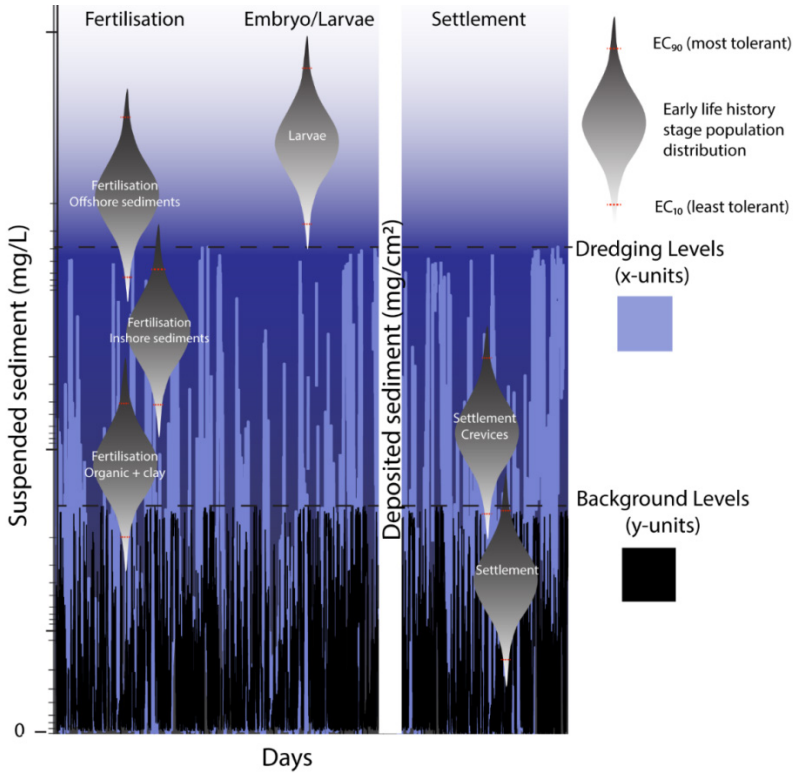

Figure 3. A simplified conceptual diagram on how threshold values for early life history stages of corals can relate to natural (background) and dredging sediment levels5. All or part of an affected early life history stage population (represented by the violin-shaped symbol) can be impacted by natural and dredging conditions, and can fall into one of three categories. Background sediment levels range from 0 to x-units, dredging sediment levels from 0 to y-units, and sediment levels above natural and dredging level are represented as > y-units. If all or a portion of an affected population falls within the Background range, dredging does not cause any further impact and thus represents no additional risk. If all or a portion of an affected population fall between the Background and Dredging conditions, efforts to decrease y-units by managing dredging would be of benefit. Finally, if all or a portion of an affected population falls above the dredging conditions (y-units) then it is both unaffected by Background and Dredging conditions and thus is at no risk of being affected by dredging. Note: the axes of this conceptual diagram are intentionally unitless.

Pre-development Surveys

Given the considerations above, several pre-development issues should be considered to effectively model risk to help protect reproductive processes and coral recruitment from the impacts of dredging including:

- Quantifying the community composition of corals likely to be exposed to sediment plumes, the relative abundance of spawning corals to assess the significance of the spawning events and assessing the timing of predicted spawning through appropriate literature and reproductive surveys (these issue are covered in Project 7.2);
- The locations and proximity of coral reef habitats to the excavation and dredge material placement sites should be mapped;
- Sediment type could have an influence on thresholds for SSC affecting the egg-sperm bundles and fertilisation (Project 7.3). For hazard assessment purposes particular attention should be given to components of sediment that increase its cohesiveness, often characterized by mineral clay, organic carbon and extracellular polymeric substances (EPS) content of the sediments which are being dredged[5]. These analyses should be performed on the sediments the gametes are likely to come into contact with i.e. sediments released by the drag or cutter head, overflowing from a hopper barge or dredge or released

[5] Ricardo GF (2017) The impacts of dredging on the early life history stages of coral. PhD Thesis The University of Western Australia (submitted February 2017)

at the dredge material placement sites from barges, which may be quite different from the consolidated seabed sediments (especially the surficial few cms) before dredging. Sampling sediments after dredging would confirm exposures and may identify further "enrichment" with fines.

The Coral Spawning CWES

The CWES has primarily been associated with protecting the pelagic, planktonic stages when the gametes and embryos/larvae are in the water column. From a wider perspective, the success of the coral spawning CWES as a management instrument should ultimately be evaluated in terms of the successful recruitment of juveniles to the next generation. This requires a greater appreciation of the effects of dredging activities on the rest of the life-cycle, including the settlement and post-settlement phases. These are very important phases where the sub-millimeter sized planula settle, metamorphose into polyps, often gain algal symbionts, and start heterotrophic feeding and producing polyps (budding). High post-settlement mortality rates (~90%) are well known in most free-spawning marine invertebrates and the small size of the new coral recruits with often limited energy reserves makes them particularly vulnerable to a range of factors including sediment smothering and light attenuation (see Fig 2F).

Currently, the coral spawning CWES is typically set as 3 d before the predicted night of spawning (to allow for suspended sediment to settle out of the water column) and 7-d afterwards (to account for some asynchrony or uncertainty in spawning dates and to allow at least some time for the larvae to settle). **This period is not long enough to fully cover the 'peak' settlement period for some species** (see Project 7.1 and Jones et al. (2015b). **An obvious step would be to extend the window to fully cover the settlement period, accommodating both the major (autumn) and minor (spring) spawning periods in WA, as well split spawning events which occur every 2–3 years.** Irrespective of whether this is reasonably practicable, the wider issue – for the successful recruitment of juveniles into the next generation – is the consequences of (1) changes to the environment that may have already occurred from activities preceding the window, and (2) resuming dredging activities at the end of the window.

Dredging activities create sediment deposition zones of loose unconsolidated sediment on coral habitat (see Figure 4), which even before coral spawning could ultimately result in the need for settlement in sub-optimal locations. Similarly high levels of sediment deposition could already have caused damage to crustose coralline algae which is a strong settlement inducer. Even if larvae have managed to settle close to dredging activities (i.e. within sediment deposition footprints) resuming dredging after a window will almost certainly impose physiological challenges to any recently settled corals from sediment smothering and light attenuation. These factors collectively diminish the usefulness of the Coral Spawning CWES.

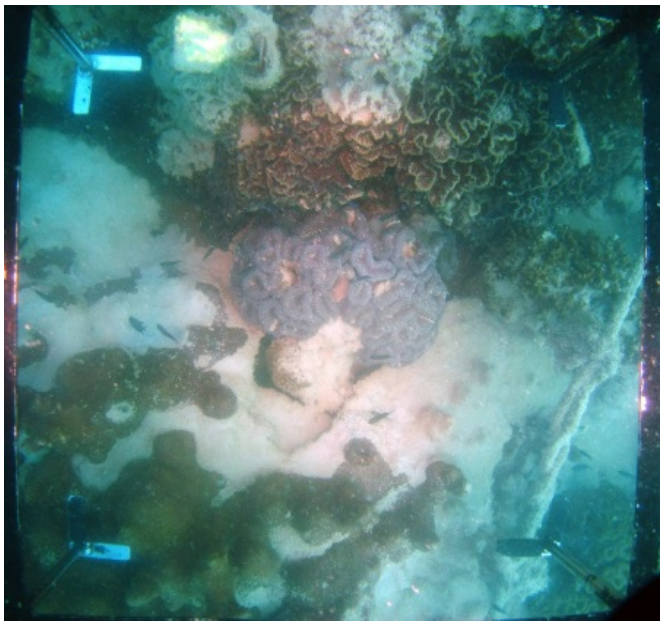

Figure 4. Coral reef covered in deposited sediment at a coral and water quality monitoring site within a few hundred metres of a capital dredge operation in the Pilbara region of WA. Deposited sediment observed here on upper-surfaces is magnitudes greater than all derived thresholds in settlement assays.

These issue are not easily resolved from a policy standpoint, but evidence from these studies clearly support the introduction of the Coral Spawning CWES 25 years ago under the precautionary principle. The studies have also provided numbers that can be used to manage water quality during coral spawning periods using spatially based plans, which take into account the geographical separation of dredging plumes and spawning corals and the likelihood of interaction at different stages of the pelagic phase. Dredge management plans (for maintenance and capital dredging programs) should continue to address the issue, and how to minimize the chances of plumes from turbidity generating activities (including dredge material placement) encountering the early life-history phases. Options include deciding when to start dredging (for maintenance dredging) or, depending on the project in question, dredging areas or using dredge material placement sites that are furthest away (down current) from reefs. Other commonly used turbidity minimization techniques could be considered such as restricting or shortening overflow periods, reducing production rates and/or using different types of dredges during the coral spawning CWES.

Residual Knowledge Gaps

The experiments in Theme 7.3–7.5 provide management, regulators and industry with a greatly improved understanding of the mechanisms and thresholds by which sediments from dredging can affect the early life stages and processes of coral. While the thresholds derived here for impacts on egg-sperm bundle ascent, fertilisation, embryo and larval development and settlement can be combined with modelled and measured SSCs and deposited sediments to assess risk, there are several outstanding residual knowledge gaps relating to: (i) other early life stage events and processes not tested; (ii) limited species and sediment conditions tested, (iii) limitations of field data; and (iv) cumulative and interacting pressures.

Other early life stage events and processes not tested: Our experiments were limited to a sub-set of early life stages from bundle ascent to settlement. In Theme 7.1 we identified over 30 potential cause-effect pathways by which dredging could affect the early life stages of coral. Earlier stages potentially at risk include fecundity and spawning synchrony which could be affected by light attenuation or the physical interaction with elevated SSCs or deposited sediments. Larval dispersal could be affected by energy depletion from removal and avoidance of sediment particles by cilia and mucous production. Latter stages potentially at risk include juvenile survival over the first 12 months and this remains to be tested, especially where larvae are driven to potentially less optimal sites of attachment due to deposited sediments. Currently the spawning EW is designed only to protect spawning through to early settlement and would need to be significantly extended if fecundity and/or juvenile survival

were found to be sensitive to turbidity generation from dredging.

Limited species and sediments types tested: The experiments in Theme 7.3-7.5 focussed on *Acropora*. spp. as one of the dominant taxa across northern Australia. It is expected that the differences in the sensitivity of gametes, embryos and larvae to sediments between species may be narrower than the sensitivity between species of adult corals that exhibit a far wider range of sizes and morphologies; however, the sensitivities of gametes, embryos and larvae different species, including more work on Australian brooded corals should be investigated in future studies. The sediment types had a large effect on fertilisation thresholds due to differences in the formation of sperm-sediment flocs. Future experimental studies should assess the impacts of sediment type on all early life history stages.

Limitations in field data: Fertilisation success in the presence of sediment is highly dependent on sperm concentrations, and more *in situ* research is needed on likely sperm concentrations during spawning events to further evaluate the significance of the cause-effect pathway. Sediment type also had a large effect on fertilisation success and the selection of suitable thresholds for application in risk assessments clearly need to take sediment type (at the dredging site) into account. Large-scale dredging projects are known to create sediment deposition zones around the excavation and dredge material placement sites, as temporarily resuspended sediments settle back to the seabed. The results of this study show that low levels of loose unconsolidated sediments can alter larval settlement preferences either, by directly acting on the coral larvae, or indirectly, by reducing the health of crustose algae covered surfaces. The significance of the low thresholds for settlement due to deposited sediments are uncertain as *in situ* data on the scale and duration of deposited sediments and the structure of benthic habitats that influence the proportion of substrate affected are generally unknown.

Cumulative and interacting pressures: To further complicate the comparison between the generated thresholds and conditions *in situ*, many of the dredging stressors (i.e. SSCs, deposited sediment and light intensity) may act alone or more likely in combination. Cause–effect pathways are thus not mutually exclusive, and together represent a source of cumulative effects. Likewise, many of the cause–effect pathways identified through experimental studies may be affected or altered by changes in environmental and biological conditions (e.g. thermal stress, nutrient enrichment, completion with other benthic biota).

Characterization of the benthic habitat in relation to topographic complexity: Benthic habitats with low complexity will be more likely suffer from blanketing of upper settlement surfaces with films of sediments that reduce settlement directly or through impacting the health and effectiveness of preferred settlement substrata (CRA) (Theme 7.5).

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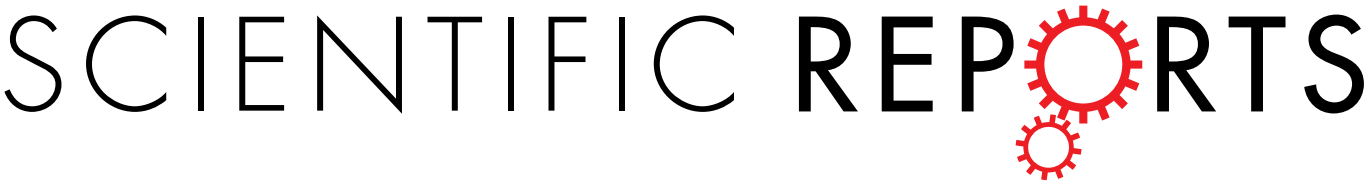

OPEN

That sinking feeling: Suspended sediments can prevent the ascent of coral egg bundles

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Spawning synchrony represents a common reproductive strategy in sessile marine organisms and for broadcast spawning corals, buoyancy of egg-sperm bundles is critical to maximise fertilisation at the ocean surface. Here we demonstrate a novel threat to coral reproduction whereby buoyant egg-sperm bundles intercept and are "ballasted" by sediment grains on their journey to the ocean surface, preventing them from reaching the ocean surface and greatly reducing egg-sperm encounter rates. Empirical observations of this mechanism are successfully captured by a mathematical model that predicts the reduction in ascent probability and egg-sperm encounters as a function of sediment load. When applied to 15 m deep reefs, the model predicts that 10% and 50% reductions in egg-sperm encounters occur at 35 mg L^{-1} and 87 mg L^{-1} suspended sediment concentrations, respectively, and for a 5 m deep reef a 10% reduction occurs at 106 mg L^{-1}. These concentrations are commonly associated with sediment plumes from dredging or natural resuspension events. The potential for sediments to sink coral gametes highlights the need to carefully manage the timing of turbidity-generating human activities near reefs during spawning periods.

Declining water quality is a major threat to coral reefs. Natural resuspension events[1], river runoff[2] and sediment plumes associated with human activities including dredging operations[3,4] elevate the concentrations of suspended solids (SS) in marine waters. Elevated SS can attenuate light availability required for primary production, reduce feeding efficiency in filter feeders, and settle onto sessile invertebrates such as corals, reducing solute exchange and causing partial or complete mortality[5]. Elevated SS can also negatively impact the early life history stages of coral including fertilisation, larval development, settlement and post-settlement survival[6,7]. However, the vulnerability to suspended solids of reproductive stages prior to fertilisation has not been considered[8].

The coordinated release of coral gametes, packaged as buoyant egg-sperm bundles within a mucous sheath, is the culmination of months of gametogenic synchronization in broadcast spawning species[9]. The ascent through the water column, timely arrival at the surface, and release of gametes from the bundles are critical for increasing egg-sperm encounter rates, and subsequent fertilisation. We hypothesized that, during ascent, the bundle can intercept suspended sediment grains that stick to its mucous coating. Here we demonstrate this mechanism through experimental observations and mathematical modelling and show that the ballasting effect of intercepted sediments is often sufficient to reverse the ascent, causing a sizeable fraction of bundles to sink. The detrimental impact of this loss of bundles reaching the water surface on egg-sperm encounters is nonlinear because the bundles carry both eggs and sperm, and the encounter rate is proportional to the product of their respective concentrations at the surface. Even for bundles that remain positively buoyant, reaching the surface might be delayed, further reducing egg-sperm encounter rates. This is the first study to examine the effects of environmental pressures on the success of gamete ascent, a critical step in recruitment success, which serves to replenish and facilitate recovery of coral reefs[10].

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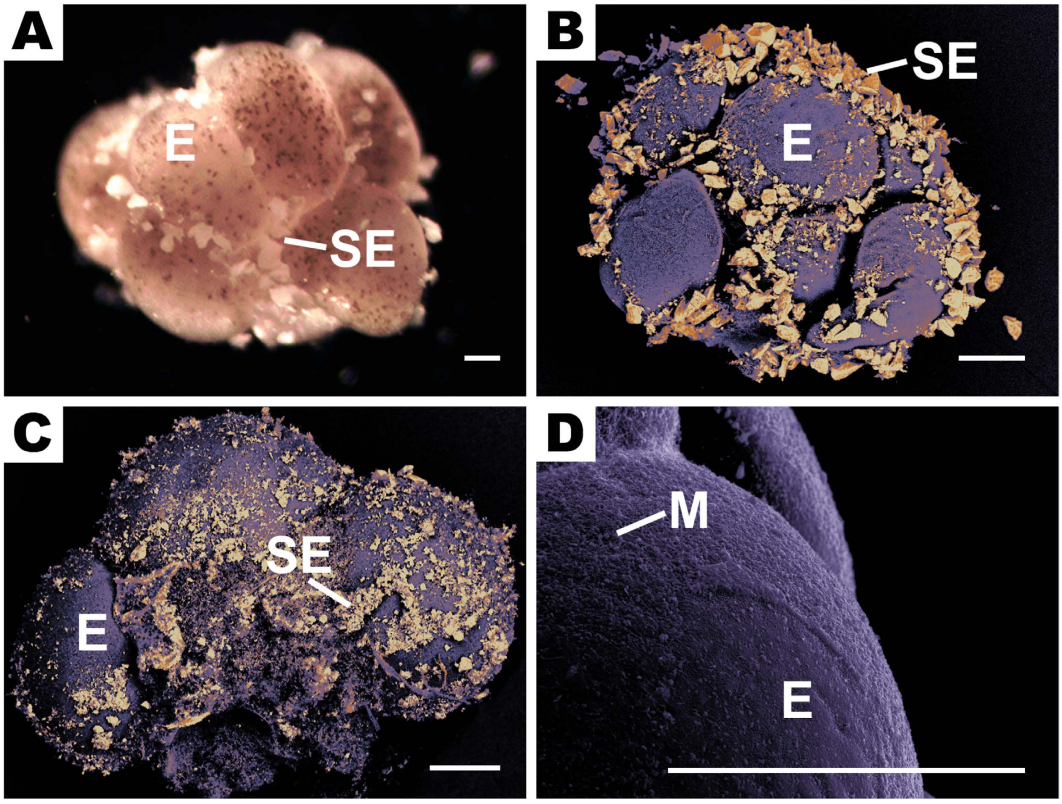

Figure 1. Microscopy images of coral egg-sperm bundles after failed ascent through elevated concentrations of suspended sediments, revealing considerable attachment of sediment grains to the bundles. Attached sediments hampered the ascent of these otherwise positively buoyant bundles. (**A**) Optical microscopy image showing sediment grains attached to a *M. digitata* egg-sperm bundle. (**B–D**) Colored backscatter scanning electron microscopy micrographs, showing sediment grains in yellow and biotic matter in purple. Shown are coral bundles of (**B**) *A. nasuta* and (**C**) *M. digitata*. Panel (**D**) shows the sticky mucous membrane, which thickens where the oocytes contact each other. All scale bars = 200 μm. E, egg; SE, sediment; M, mucous membrane.

Results and Discussion

Imaging of individual bundles revealed strong sediment attachment (Fig. 1). Light microscopy demonstrated preferential accumulation of sediment grains on the mucous coating between oocytes (Fig. 1A). Scanning electron microscopy yielded high-contrast images showing bundles covered in a tangle of sperm, sediment and mucus (Fig. 1B,C), clearly distinguishing sediments (yellow) from biotic matter (purple). The mucous coating was thickest at the junctures between oocytes (Fig. 1D).

The fraction of ascending bundles decreased nonlinearly with increasing SS load (Fig. 2), with 50% of bundles failing to ascend at a SS load of $EC_{50,A} = 3262\,mg\,L^{-1}$ (95% c.i.: 2523–4218 $mg\,L^{-1}$). Taken together with the visual evidence of sediment attachment (Fig. 1A–C), these results support the proposed mechanism of sediment ballasting.

Ascent failure predictions from the model are in excellent agreement with our laboratory observations over the full range of SS loads tested (Fig. 2). For the 77 cm tall water column used in experiments, the model's prediction of $EC_{50,A} = 2768\,mg\,L^{-1}$ is within 20% of the experimental value of 3262 $mg\,L^{-1}$ (95% c.i.: 2523 – 4218 $mg\,L^{-1}$), and the predicted and observed dependence of ascent failure on SS load were statistically indistinguishable ($F = 1.853$, $p = 0.1655$). This agreement further supports our hypothesis that the ballasting from sediments intercepted by a rising bundle can terminate its ascent, and validates the use of the model to predict sediment ballasting under natural conditions.

When applied to typical water depths at which corals live, the model shows that considerable reductions in the fraction of ascending bundles occur even at modest sediment loads (Fig. 3A). For example, the model predicts $EC_{10,A} = 236\,mg\,L^{-1}$ for $h = 3$ m and $EC_{10,A} = 47\,mg\,L^{-1}$ for $h = 15$ m (Table 1). The ascent failure increases with SS load and with water depth, in both cases due to the larger number of sediment grains encountered by a bundle before reaching the surface (Fig. 3A, Table 1).

Because bundles carry both sperm and eggs, ascent failure has a quadratic effect on egg-sperm encounter rates at the surface, with direct repercussions on fertilization rates. The effect is quadratic because encounter rates are proportional to the product of egg and sperm concentrations at the surface and each bundle that fails to ascend negatively impacts both. This is evident in the SS loads causing a certain (*e.g.*, 10%) reduction in encounters ($EC_{10,E}$) being lower than the SS loads causing the same reduction in ascents ($EC_{10,A}$): for example, $EC_{10,E} =$

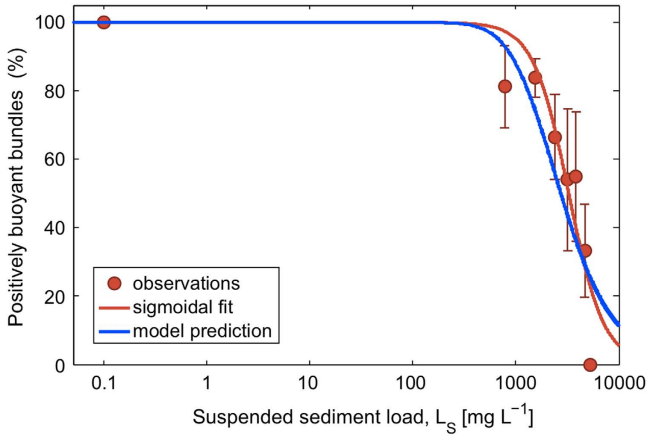

Figure 2. The fraction of positively buoyant egg-sperm bundles decreases with increasing sediment load. Red circles denote laboratory observations of the fraction of *M. digitata* bundles successfully rising through a 77 cm tall water column, for different values of the total sediment load, L_S. Error bars are standard errors (s.e.m.) computed over 4 runs containing replicates of at least 3 bundles each. The red curve is a sigmoidal fit to the observations, given by $y = 100\ \{1 + 10\text{^}[2.537\ (\log_{10}L_S - 3.514)]\}^{-1}$. Also shown is the prediction from a physically-based Monte Carlo model (blue line) that quantifies the ballasting of rising bundles by sediment grains encountered by direct interception, for the same conditions as in the experiments (bundle radius $r_B = 489.6 \pm 54.6\,\mu m$ (mean ± s.d.); bundle rising speed $v_B = 6.35 \pm 1.37\,mm\,s^{-1}$; sediment grain radius $r_s = 25.6 \pm 8.8\,\mu m$; sediment density $\rho_S = 2500\,kg\,m^{-3}$).

$179\,mg\,L^{-1} < EC_{10,A} = 236\,mg\,L^{-1}$ for $h = 3\,m$ and $EC_{10,E} = 35\,mg\,L^{-1} < EC_{10,A} = 47\,mg\,L^{-1}$ for $h = 15\,m$ (Fig. 3B, Table 1).

Bundles ascending through suspensions of smaller sediments are less sensitive to ascent failure (Fig. 3B), because the grain radius r_S strongly impacts bundle-grain encounters ($\beta_{INT} \sim r_S^2$, Eq. 1). For instance, sediments with $r_S = 16 \pm 5\,\mu m$ (mean ± s.d.) result in $EC_{10,A} = 891\,mg\,L^{-1}$ for $h = 3\,m$ and $EC_{10,A} = 177\,mg\,L^{-1}$ for $h = 15\,m$, ~4-fold greater than corresponding values for $r_S = 25.6 \pm 8.8\,\mu m$ grains (Fig. 3B).

Sediment ballasting can be detrimental even when ascent succeeds, because decreased buoyancy reduces rising speed and thus increases time to surface. A quantification of the ascent time obtained by dynamically tracking the bundle's buoyancy yields delays of <10 min for corals at 3 m depth, but up to 40 min for corals at 15 m depth at high SS loads (Fig. 4). Bundles spawned from deeper colonies are thus at greatest risk of missing adequate gamete concentrations for fertilization at the surface.

The ascent failure of coral egg-sperm bundles is a previously unrecognised mechanism threatening coral recruitment if spawning overlaps with conditions of elevated SS loads, such as those associated with dredging activities or natural resuspension events. The ballasting of coral gametes during ascent adds to the cumulative risk that sediments pose on early life history stages including reduced fertilisation, larval development and settlement[7,11], and could contribute to recruitment failure on nearby reefs. Our model predicts that bundles released from deeper corals have the greatest chance of sinking because of accumulation of sediment grains and that medium-coarse silt sediments (~$25 \pm 10\,\mu m$ radius) result in a strong ballasting effect. Additionally, our work shows that finer silt-sized sediments (~$16 \pm 5\,\mu m$ radius), which remain in suspension longer, can also sink bundles at elevated SS loads (~$180\,mg\,L^{-1}$) at deeper water sites. Importantly, in all cases, ascent failure not only results in less reproductive material reaching the water surface, it also translates to a quadratic decrease in egg-sperm encounters, which will directly impact fertilisation success (Fig. 3B). As recruitment is necessary to sustain populations and facilitate recovery from disturbances, recruitment failure may have a long-lasting negative legacy on a given reef[10].

Sediment ballasting is not strongly dependent on the bundle's rising speed and may thus be relatively insensitive to variations in rising speed among bundles from different species. This is due to the dual effect of a faster rising speed, which enhances encounters with grains per unit time (Eq. 1) but reduces the ascent time (h/v_B, Eq. 2). Faster rising speeds are in fact predicted to somewhat reduce the number of sediment grains encountered (Eq. 2). More consequential for ascent success are the bundle's density at release, ρ_B, and size, r_B, with larger and more buoyant bundles withstanding greater sediment ballasts (Eq. 3). A smaller bundle density is intuitively beneficial. A larger size is beneficial because it increases buoyancy while, counter intuitively, not increasing the encounter rate with sediment grains (r_B does not appear in Eqs 1 and 2; Methods). Coral species with larger egg-sperm bundles are thus predicted to fare better under heavy SS load conditions.

Sediment ballasting can be aggravated by additional encounter mechanisms beyond direct interception. Whereas Brownian motion is typically negligible (see Methods), relative fluid motion in highly turbulent environments (turbulent kinetic energy $>10^{-6}\,W\,kg^{-1}$) could contribute several percent to direct interception (Methods), rendering actual encounter rates somewhat higher than predicted here. On the other hand, not every encounter between a bundle and a sediment grain will result in attachment. Based on the high stickiness of the mucus enveloping egg-sperm bundles[12,13], we have assumed 100% capture efficiency of encountered sediment grains; the strong agreement between experimental data and modelling outcomes supports this approach for

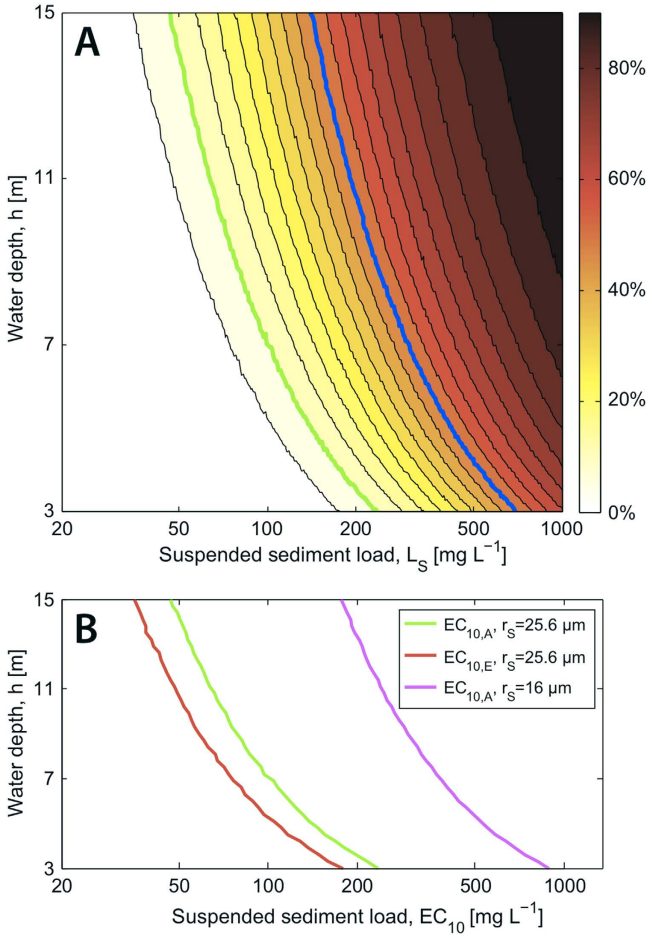

Figure 3. Reduction in the number of coral egg-sperm bundles reaching the water surface due to sediment ballasting, predicted by a model of sediment ballasting as a function of water depth, *h*, and sediment load, L_S. (**A**) Percent reduction. The green and blue contours denote the SS loads that reduce successful ascents by 10% ($EC_{10,A}$) and 50% ($EC_{50,A}$), respectively. (**B**) EC_{10} values for ascent failure ($EC_{10,A}$; green and purple lines, referring to two different sediment grain radii r_S) and encounter failure ($EC_{10,E}$, red line), as a function of water depth, *h*. Note that $EC_{10,E} < EC_{10,A}$ because the decrease in egg-sperm encounters is quadratic in the decrease in ascents. For both panels, model parameter values are as in Fig. 2, except for sediment grain radii r_S in panel B, indicated in the legend.

	Ascent failure		Encounter failure	
Water depth [m]	$EC_{10,A}$ [mg L^{-1}]	$EC_{50,A}$ [mg L^{-1}]	$EC_{10,E}$ [mg L^{-1}]	$EC_{50,E}$ [mg L^{-1}]
15	47	141	35	87
10	71	211	53	131
5	141	422	106	262
3	236	699	179	436
0.77 (Model)	911	2726	690	1676
0.77 (Expt)	—	3262	—	—

Table 1. Concentrations of suspended solids predicted to cause a 10% (EC_{10}) and a 50% (EC_{50}) reduction in bundle ascent (subscript A) and gamete encounter (subscript E) for coral bundles of *M. digitata*. Model parameters as in Fig. 2.

M. digitata. Any reduction in the capture efficiency due to lower adhesion between sediments and bundle or to limited mucous coverage of the bundle, will result in a proportional decrease in the encounter rate and thus an increase in EC_{10} values.

Even when sediment loads are insufficient to sink bundles, they cause ascent delays that may reduce reproductive success. Spawning synchrony is crucial for achieving an adequate sperm concentration at the water surface[14],

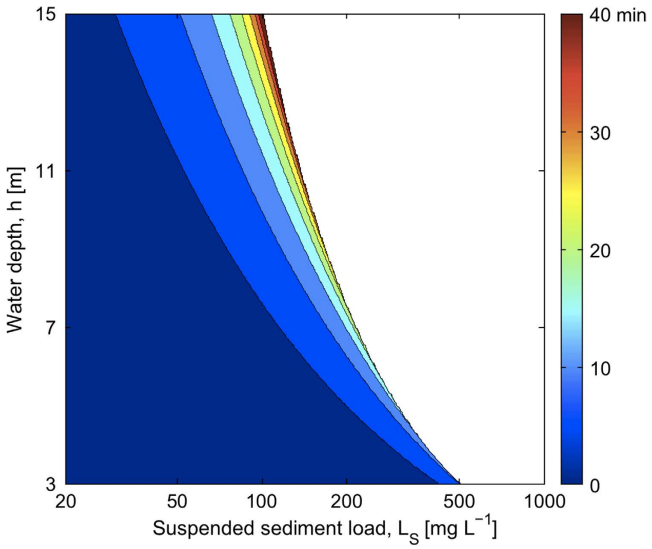

Figure 4. Time delay in the ascent of those coral egg-sperm bundles that successfully reach the surface, as a function of water depth, *h*, and suspended sediment load, L_S. Model parameter values as in Fig. 2.

and mixing significantly reduces fertilisation probabilities as little as 1 h after spawning[15]. With such a narrow fertilization window, the ~40 min ascent delay predicted here for bundles from the deepest corals (Fig. 4) would be ecologically significant. Subtle but consistent differences in the spawning times of closely-related species is also thought to be a mechanism for reproductive isolation, preventing or reducing hybridization[16]. Loss of punctuality and blurring of the fertilization window could reduce the efficacy of that important prezygotic isolation barrier.

The laboratory observations and mathematical simulations presented here indicate that ascent failure is probable for bundles from all but shallow-water colonies if spawning occurs in proximity to turbidity-generating processes. This is apparent when comparing the EC_{10} and EC_{50} values computed here (Table 1) with reported SS loads from the environment. The latter include values of 50–840 mg L^{-1} measured within a few kilometres from dredging and disposal operations[17–19] and values >100 mg L^{-1} caused by natural resuspension events in shallow lagoonal areas[1,20]. Furthermore, river discharge loads over inshore reefs can exceed 1200 mg L^{-1} [21], indicating that sediment ballasting could be particularly relevant to coral spawning in fringing habitats.

To predict the risks posed by turbidity-generating activities such as dredging to the spawning of corals, and possibly of other species relying on buoyant eggs for fertilization[22–24], we propose that sediment ballasting be integrated in ocean circulation and sediment transport models. This will couple sediment resuspension, advection and settling with sediment interception by rising bundles, so that the SS loads at any given site can be used to produce local risk maps for coral fertilization. These findings describe a novel threat to coral reefs, yet they also present a practical approach for improving management of sediment-generating activities during sensitive coral spawning events.

Methods

To test the effect of different SS loads on the ascent of egg-sperm bundles, we quantified the fraction of bundles from the digitate coral *Montipora digitata* successfully ascending through a 77 cm tall acrylic column containing different loads of carbonate sediments (radius $r_S = 25.6 \pm 8.8\,\mu$m, mean $\pm$ s.d.) suspended in 0.4 μm-filtered seawater at 28 °C. After sediment-laden water was added to the column, 3–5 freshly collected and intact egg-sperm bundles were transferred to the top of the column and the column was inverted, allowing bundles to rise. The number of bundles ascending was assessed after 164 s (the time by which 95% of bundles surfaced in sediment-free water). Four replicate runs, for a total of ≥ 12 bundles, were performed for each SS load. Bundles from *M. digitata* and *Acropora nasuta* were also exposed to elevated SS loads and examined by light and scanning electron microscopy to determine attachment of sediment grains (SI Methods).

To further test the mechanistic basis of sediment ballasting and predict its consequences in natural conditions, we developed a mathematical model based on encounter rate theory for differentially settling particles: the bundle of radius r_B rising with speed v_B and the sediment grains of radius r_S sinking with speed v_S. The dominant encounter mechanism is 'direct interception' (SI Methods), which occurs when the center of a sediment grain comes within one grain radius of the bundle. The encounter kernel β_{INT}, given by[25]

$$\beta_{INT} = 1.5\pi (v_B - v_S) r_S^2, \quad (1)$$

represents the equivalent volume of seawater from which all sediment grains are 'captured' by a rising bundle per unit time. This yielded the number of sediment grains encountered by the bundle during its ascent time h/v_B (where h is the water depth),

$$N_S = \beta_{INT}\, C_S \frac{h}{v_B} = \frac{9}{8}\left(1 - \frac{v_S}{v_B}\right)\frac{h}{r_S}\frac{L_S}{\rho_S}, \quad (2)$$

where $C_S = L_S/M$ is the concentration of sediment grains, L_S the SS load, $M = (4/3)\pi\rho_S r_S^3$ the mass of one grain, and $\rho_S = 2500\,kg/m^3$ the sediment density. These grains ballast the bundle, whose density, ρ, becomes

$$\rho = \frac{r_B^3\rho_B + N_S r_S^3 \rho_S}{r_B^3 + N_S r_S^3}, \quad (3)$$

where ρ_B is the sediment-free bundle density (SI Methods). When ρ exceeds the seawater density, the bundle sinks. For the sizes and densities of sediments and bundles used in this study (SI Methods), approximately 50 sediment grains are sufficient to sink a bundle.

For each scenario, 50,000 bundle ascents were modelled using a Monte Carlo approach to take into account variability in key parameters. Parameter values and their distributions were obtained from our experimental observations and literature (SI Methods). This allowed us to quantify the fraction of failed ascents and the critical SS loads $EC_{10,A}$ and $EC_{50,A}$ (the SS loads causing 10% and 50% ascent failure).

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Author Contributions

G.F.R., A.P.N. and R.S. designed and performed research; all authors wrote the paper.

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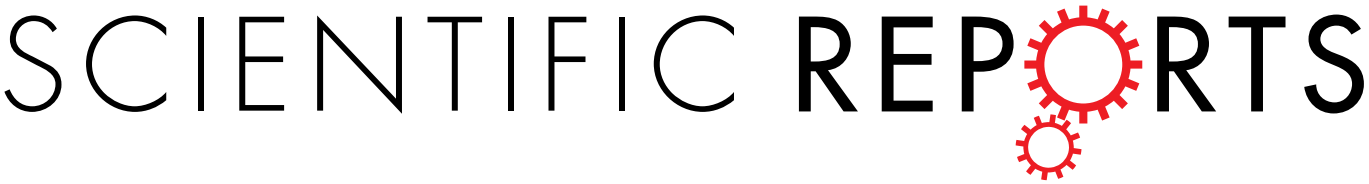

OPEN

Suspended sediments limit coral sperm availability

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Suspended sediment from dredging activities and natural resuspension events represent a risk to the reproductive processes of coral, and therefore the ongoing maintenance of reefal populations. To investigate the underlying mechanisms that could reduce the fertilisation success in turbid water, we conducted several experiments exposing gametes of the corals *Acropora tenuis* and *A. millepora* to two sediment types. Sperm limitation was identified in the presence of siliciclastic sediment (230 and ~700 mg L^{-1}), with 2–37 fold more sperm required to achieve maximum fertilisation rates, when compared with sediment-free treatments. This effect was more pronounced at sub-optimum sperm concentrations. Considerable (>45%) decreases in sperm concentration at the water's surface was recorded in the presence of siliciclastic sediment and a >20% decrease for carbonate sediment. Electron microscopy then confirmed sediment entangled sperm and we propose entrapment and sinking is the primary mechanism reducing sperm available to the egg. Longer exposure to suspended sediments and gamete aging further decreased fertilisation success when compared with a shorter exposure. Collectively, these findings demonstrate that high concentrations of suspended sediments effectively remove sperm from the water's surface during coral spawning events, reducing the window for fertilisation with potential subsequent flow-on effects for recruitment.

Dredging to create or widen shipping channels is a largely unavoidable component of most port and coastal infrastructure developments[1]. Sediments released into the water column by dredging and natural resuspension events can migrate over nearby benthic communities where they can have pronounced effects on organisms such as coral[2–4], seagrass[5], fish and invertebrates[6,7]. Understanding the potential environmental effects of dredging is now particularly important in tropical Australia where a resources boom has increased demand for further development of large-scale coastal infrastructure facilities and ports[8]. For example, 200 M m^3 of sediments along the Western Australian coastline and an estimated 85 M m^3 of sediment in or around the Great Barrier Reef Marine Park are proposed to be dredged over the next 25 years to allow ship access to these facilities[9,10].

Dredging and natural resuspension events release sediments into the water column and once disturbed, fine silts and clays can remain in suspension for extended periods and travel distances of 100s of km[11,12]. The effect on adult coral colonies can be significant, with sediments causing decreased productivity and growth rates[13–15], and subsequent sediment smothering causing tissue damage and mortality[16–18]. Consequently, coral loss can occur when dredging occurs in the proximity of reefs[19,20].

The early life-history stages of corals are also known to be susceptible to elevated suspended sediments (SS) (see review by Jones, *et al.*[21]), and there is a great deal of attention to these effects, as reproduction and recruitment underpins the maintenance and resilience of reef communities, and poor water quality conditions during spawning periods could lead to loss of the entire reproductive output for the year[22–24]. Most scleractinian corals are broadcast spawners, releasing gametes 1–2 times a year and often over a few nights in synchronous, multispecific mass spawning events[25–27]. Gametes are released packaged in a positively buoyant bundle that dissociate within an hour upon reaching the water's surface, releasing sperm and eggs[28,29]. Mixing of gametes by wind, waves, and currents occurs at or just under the water surface promoting out-crossing but also causing sperm concentrations to rapidly dilute. Sperm dilution combined with the deleterious aging of the gametes, limits the opportunity for successful fertilisation to a comparatively short (<2 h) window following spawning[30,31].

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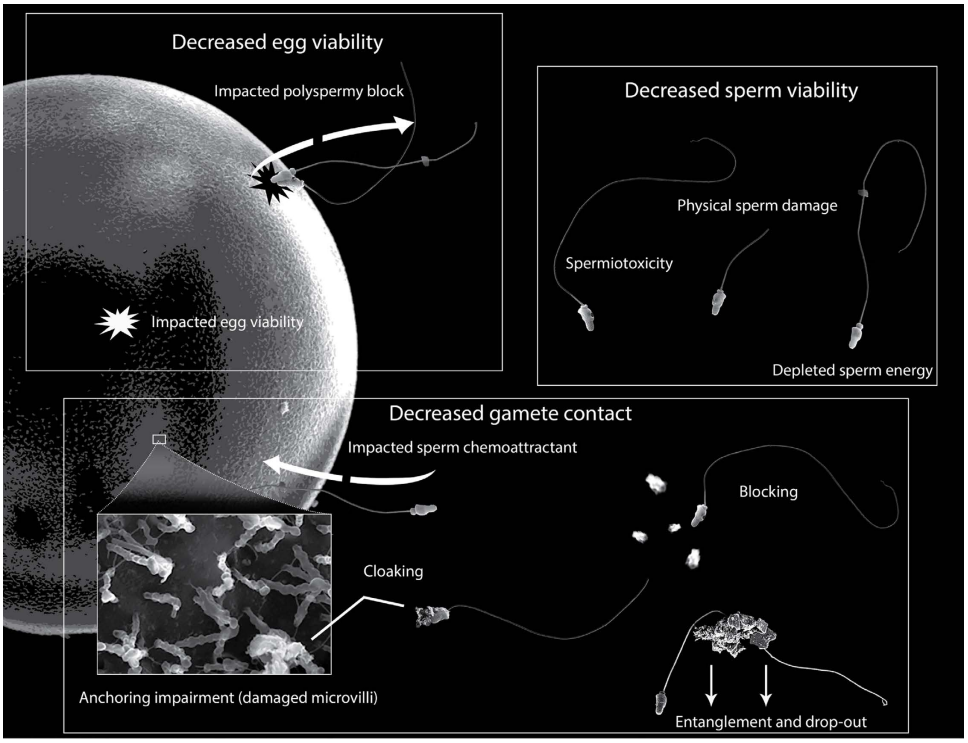

Figure 1. Possible cause-effect pathways in which suspended solids (SS) can reduce fertilisation rates in broadcast spawning corals. Clockwise from top left, SS could impact egg viability through damage to the egg or through impacting the polyspermy block. Similarly, SS could impact sperm viability from toxic substances leached from the sediment, physically through abrasion, or by depleting energy reserves via increased flagellum beating. SS could also decrease egg-sperm contacts and sperm penetration by damage to anchoring protrusions on the egg (microvilli), by impairing the sperm chemoattractant, by cloaking of the gametes, slowing and blocking sperm, and by entanglement and sinking of the sperm.

As a framework for evaluating the effects of sediment on coral reproduction, Jones, *et al.*[21] recently developed a conceptual model of known and biologically plausible cause-effect pathways whereby suspended sediments could affect all aspects of the reproductive processes, from gametogenesis though spawning, embryogenesis, settlement and post-settlement survival. Of the 30+ proposed known and putative cause-effect pathways, many were associated with the fertilisation stage. Three main groups consisting of nine pathways were postulated in which elevated suspended sediment could prevent coral fertilisation. These were: (i) impacts on egg viability, (ii) impacts on sperm viability, and (iii) reduced egg-sperm encounters and sperm penetration into the egg. These potential mechanisms are depicted in the conceptual diagram of Fig. 1.

Several studies have reported that SS can reduce fertilisation success at concentrations as low as $\geq$50 mg L^{-1} [32–34], i.e. within an environmentally relevant range associated with dredging projects[21]. These studies included species from the genus *Acropora*, which are typical of clear water as well as *Pectinia lactuca*, which is common in turbid water habitats. However, some experiments have not detected any effects at SS concentrations as high as ~1000 mg L^{-1} [34]. The reason for these marked differences in sensitivity is not known, but could be linked with sediment geochemical parameters (including effects of sediment-bound contaminants), particle grain size and nutrient content[21,34]. Further, differences in responses could be species-specific, or to differences in the experimental conditions under which the tests were conducted[21]. For example, one likely source of the variation between studies is the use of different sperm concentrations, which can significantly influence the outcome of fertilisation experiments[35,36]. Fertilisation success in corals increases non-linearly with sperm concentration because of an increase in egg-sperm encounters, resulting in maximum fertilisation success at 10^5–10^7 sperm mL^{-1} [31,37,38]. In fertilisation assays, the effect of a treatment is usually more pronounced at lower sperm concentrations as inhibitory effects can be masked at higher, saturating sperm concentrations[36]. For corals, few toxicology studies have attempted to match the range of ecologically relevant sperm concentrations that occur *in situ*[39,40]. A number of approaches have been proposed to address this issue including use of a number of sperm concentrations in assays, and a calculation of two metrics which describe maximum fertilisation (F_{max}) and the sperm concentration required to reach this maximum $[Sperm]_{max}$[35]. Further, determination of the EC_{50} (sperm concentration required for half the maximum fertilisation, F_{max}) has been used when data are non-linear and asymptotic[39,41]. Another equally important source of variability between studies could be gamete viability and quality which can vary between spawning nights and decrease quickly following release from the bundle[31,42].

To improve risk assessments of the effects of dredging and turbidity-generation near reefs during spawning events, and to better understand the source of the variability between past studies, there needs to be an improved understanding of the mechanism(s) by which sediment reduces fertilisation success. In this study, we examine

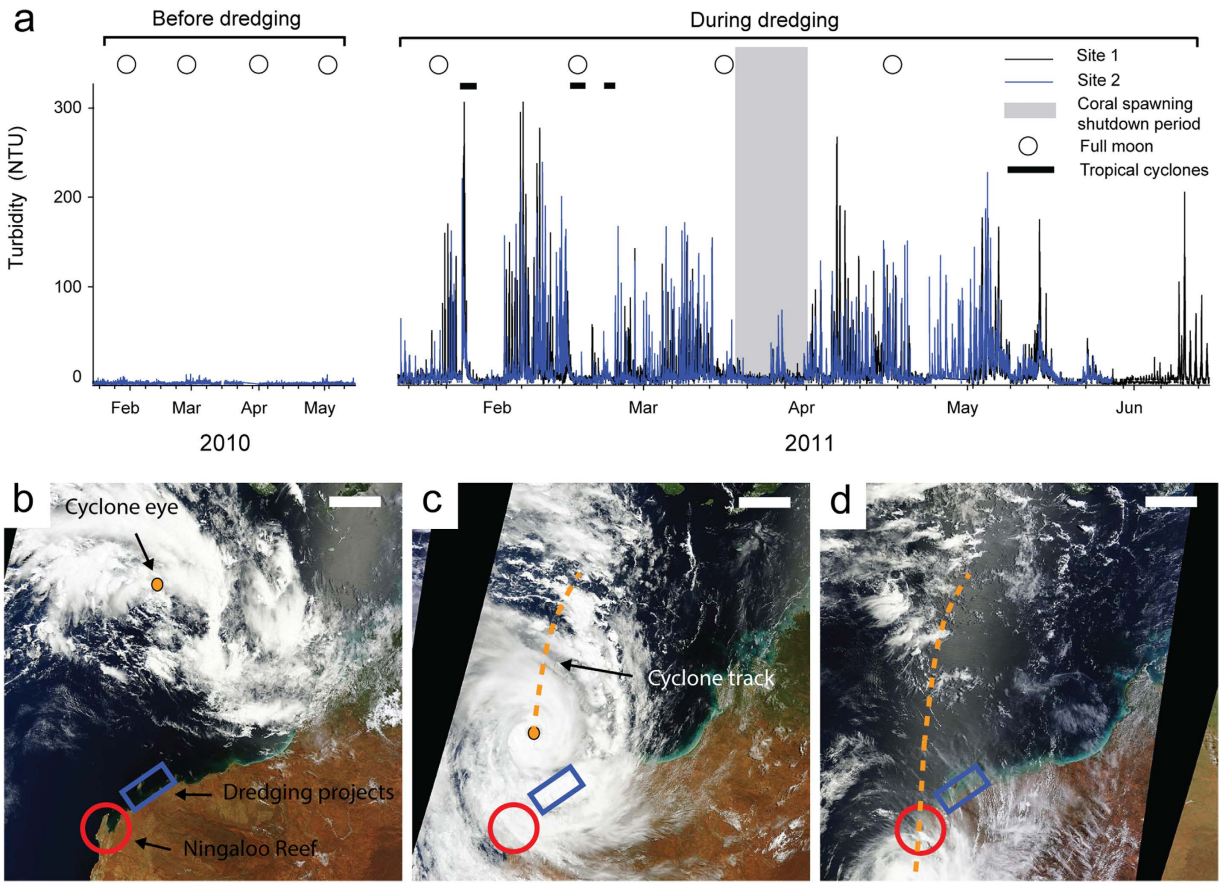

Figure 2. Turbidity-generating events during coral spawning periods in north-west Australia. (a) Turbidity (NTU) readings at two water quality monitoring sites ~300 m north (Site 1) and south (Site 2) during the autumn (coral spawning) months in 2010 (before dredging) and 2011 during dredging a major capital dredging program (~7.6 M m^3 of sediment dredged over 530 d) at Barrow Island. The autumn full moons are indicated and the shaded area indicates the major predicted autumn coral spawning period 8 days after the full moon. Dredging operations were suspended for 12 days from 20–31 March 2011 for the coral spawning environmental window and for a few days associated with the close proximity of cyclones Bianca, Dianne and Carlos. **(b–d)** Moderate Resolution Imaging Spectroradiometer (MODIS) images from the Terra Satellite of Category 3 Tropical Cyclone Olwyn passing through Western Australian coastline from 10–13 March. Cyclone Olwyn developed in the Indian Ocean approximately 800 km from the Kimberley and Pilbara and hit the Ningaloo coastline on 12–13 March 2015, coinciding with the March coral spawning event. Scale bars = 200 km. We acknowledge the use of Rapid Response imagery from the Land, Atmosphere Near real-time Capability for EOS (LANCE) system operated by the NASA/GSFC/Earth Science Data and Information System (ESDIS) with funding provided by NASA/HQ.

cause-effect pathways for the effect of sediments (relevant to dredging and natural resuspension events) on coral gametes through a series of manipulative experiments. We test the hypothesis that greater sperm concentrations would be required to attain maximum fertilisation under the presence of environmentally realistic suspended sediment concentrations and test how prolonged exposure of gametes to suspended sediment concentrations before fertilisation may amplify failure as gametes aged or were reduced in number. The principle finding, that sediments primarily impact sperm rather than eggs, is then discussed with respect to water quality conditions that can occur during dredging projects and natural turbidity events such as wind and wave resuspension from storms and cyclones.

Results

SS generation during dredging operations and cyclones. In order to select dredging-relevant SS concentrations for the series of experimental exposures, turbidity data from instantaneous nephelometer measurements at two sites during the Barrow Island dredging (see Methods) were assessed. SS concentrations derived from the turbidity measurements during dredging activities regularly exceeded ~100 mg L^{-1} and on occasions >300 mg L^{-1} (Fig. 2a). Similar SS concentrations were measured during a natural resuspension event (category 4 Cyclone Bianca (2011)) which passed approximately 100 km to the west of the dredging location. Dredging was temporarily suspended for a few days during the cyclone and when cyclones Dianne (2011) and Carlos (2011) were in close enough proximity to have affected safety. All dredging activities were stopped for a further 12 day period

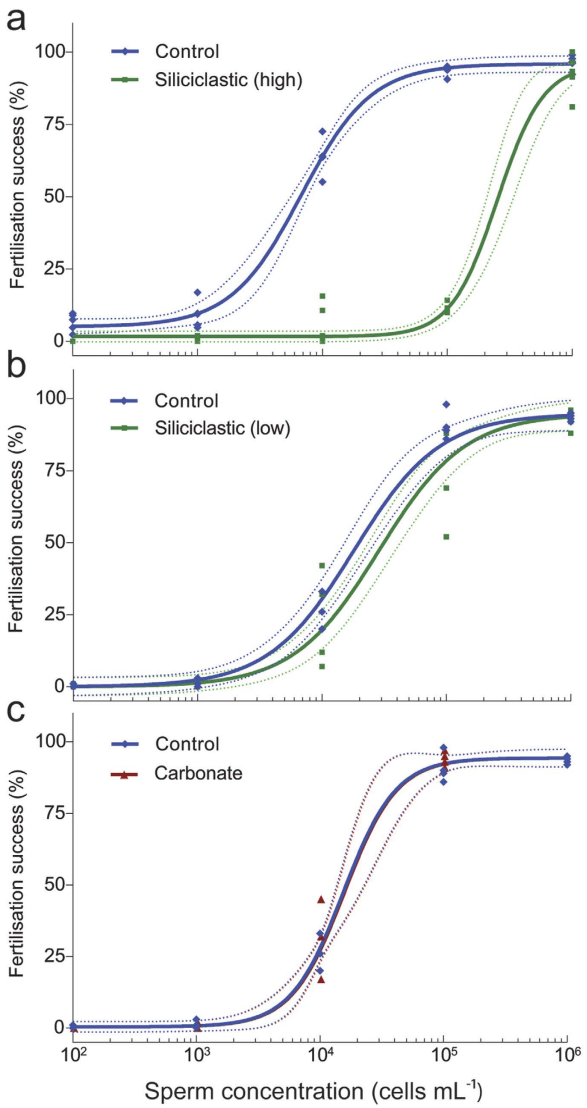

Figure 3. ***Acropora tenuis*** **fertilisation success (%) curves fitted to four-parameter logistic models plotted over a range of sperm concentrations (10^2–10^6 sperm mL^{-1}).** (**a**) Siliciclastic (high): 705 mg L^{-1} , green; control: 0 mg L^{-1}, blue and (**b**) siliciclastic (low): 230 mg L^{-1}, green; control: 0 mg L^{-1}, blue) and (**c**) carbonate: 230 mg L^{-1}, red; control: 0 mg L^{-1}, blue) suspended solid sediment concentrations. Dashed lines represent 95% confidence bands.

(20 March to 31 March 2011) during the predicted autumn spawning period to comply with the mandatory coral spawning impact minimization window[21,43]. Over these shutdown periods suspended sediment concentrations were reduced dramatically, largely to baseline (pre-dredging) levels (Fig. 2a). The maximum turbidity peaks were also similar to those recorded during cyclones and notably a category 3 Tropical Cyclone (Olwyn) which passed by the Pilbara coast of Western Australia during the first night of the coral spawning period (12–15 March 2015) causing turbidity levels to exceed 275 NTU for 24 h (pers. comm. - Travis Elsdon) (Fig. 2b–d).

Influence of SS on fertilisation at multiple sperm concentrations. Fertilisation success increased non-linearly with sperm concentration (Fig. 3a–c). For siliciclastic ($\leq$10 μm) sediment, fertilisation curves shifted to the right in the presence of both high and low sediment concentrations. The difference in the EC_{50} values (sperm concentration required for half the maximum fertilisation, F_{max}) was statistically significant and ranged from ~2 fold greater at the low sediment treatment ($F_{1, 43} = 5.55$, P=0.023) to 37 fold greater in the high sediment treatment (high: $F_{1, 53} = 363.90$, P = <0.001) (Table 1). No significant difference was detected between the EC_{50} for the carbonate ($\leq$10 μm) sediment and control curves ($F_{1, 43} = 0.13, 0.721$). Maximum fertilisation (F_{max}) for all sediment treatments were high (>90% fertilisation) and there was no significant difference in comparison with the controls (siliciclastic (high): $t_8 = 1.16$, P = 0.281, siliciclastic (low): $t_6 = 0.71$, P = 0.507, carbonate: $t_6 = 1.19$, P = 0.278), indicating that sediment did not affect egg viability. There was no difference in fertilisation success between the agitated controls on the rollers and the non-agitated controls in the 6-well plates ($F_{1, 41} = 0.005$, P = 0.946).

	Control (0 mg L^{-1})	Siliciclastic (high) (~700 mg L^{-1})
Best-fit parameters (95% CI)		
EC_{50}	6.76×10^3 (5.74×10^3–7.96×10^3)	2.51×10^5 (1.92×10^5–3.28×10^5)
*Top/F_{max} (%)	95.88 (93.03–98.72)	95.88 (93.03–98.72)
Bottom (%)	5.06 (2.38–7.74)	1.65 (0.00–3.51)
Slope	1.547 (1.09–2.00)	2.36 (1.74–2.99)
	Control (0 mg mL^{-1})	**Siliciclastic (low) (~230 mg L^{-1})**
Best-fit parameters (95% CI)		
EC_{50}	1.81×10^4 (1.31×10^4–2.50×10^4)	2.87×10^4 (2.05×10^4–4.06×10^4)
*Top/F_{max} (%)	92.72 (88.89–100.00)	92.72 (88.89–100.00)
*Bottom (%)	~0.00 (0.00–3.22)	~0.00 (0.00–3.22)
*Slope	1.26 (0.95–1.58)	1.26 (0.95–1.58)
	Control (0 mg L^{-1})	**Carbonate (~230 mg L^{-1})**
Best-fit parameters (95% CI)		
EC_{50}	1.54×10^4 (1.23×10^4–1.93×10^4)	1.54×10^4 (1.23×10^4–1.93×10^4)
*Top/F_{max} (%)	94.33 (91.19–97.48)	94.33 (91.19–97.48)
*Bottom (%)	0.54 (0.00–3.02)	0.54 (0.00–3.02)
*Slope	2.04 (1.09–3.00)	2.04 (1.09–3.00)

Table 1. Results of data fitted to a four-parameter logistic function (Fig. 2). The curves were constrained between 0 and 100%. *Indicates sharing of parameters (bottom, top/F_{max}, and slope).

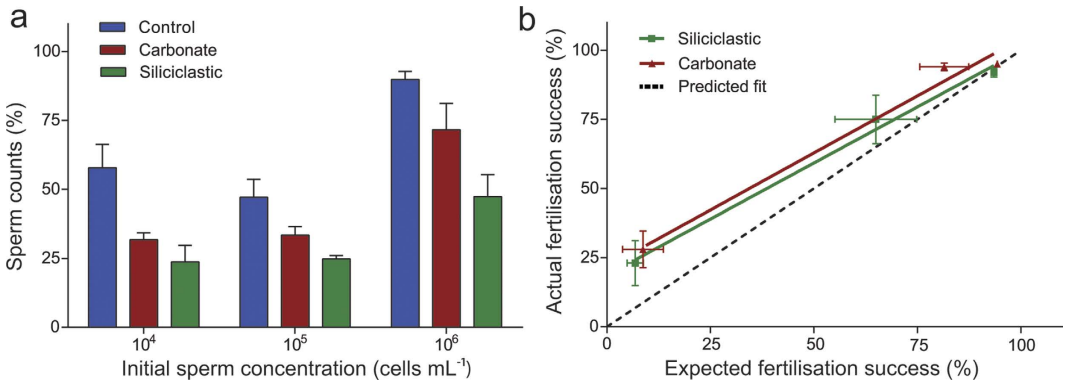

Figure 4. (**a**) Bar chart of *Acropora tenuis* sperm counts (means ± SE) in the water's surface (top 1 cm) as a proportion of the initial sperm concentration after 30 minutes exposure to suspended sediments (230 mg L^{-1}). $n = 3$. (**b**) Means (SE) of interpolated sperm counts (expected) with fertilisation success data (actual).

Sperm counting in the water's surface with SS. Flow cytometry revealed a ~10% (at 10^6 sperm mL^{-1}) to 50% (at 10^4 and 10^5 sperm mL^{-1}) decrease in sperm numbers in the absence of sediment following 30 min of agitation, probably caused by the negative buoyancy and some clustering and flocculation of the sperm. There were further significant (22%–43%) decreases in surface sperm counts across the range of initial sperm concentrations for siliciclastic SS treatments (Fig. 4a and Table 2). A decrease of 14%–26% sperm counts was observed with carbonate sediment but this was only statistically significant at the low sperm concentration (10^4 sperm mL^{-1}).

To assess whether reductions in sperm at the surface caused by suspended sediment quantitatively accounts for observed effects on fertilisation, the expected rates of fertilisation caused by sperm dropout (Fig. 4a) were calculated by interpolation of reduced sperm concentrations into the control fertilisation curves in Fig. 3. The observed fertilisation in the presence of SS was then plotted against the fertilisation expected from sperm dropout (Fig. 4b). Once all samples were standardised to their initial sperm concentration, the reduction of sperm was generally proportional to the drop in the fertilisation. The exceptions were with carbonate sediment at initial sperm concentrations of 10^4 and 10^5 sperm mL^{-1}, where greater fertilisation occurred than expected for the reduction in sperm observed.

When sperm were added to suspended sediments, small flocs appeared on the bottom of the chambers (Fig. 5a). Scanning electron microscopy images revealed *A. tenuis* sperm tangled, coated, buried, and damaged in aggregations of fine sediment grains (Fig. 5b). Similar inspection of the eggs revealed very few sediment grains bound to the outer surface (Fig. 5c).

Initial sperm concentration (sperm mL^{-1})	Sediment	Difference from control (%)	t-ratio ($n = 3$)	P value
10^4	Carbonate	26	2.938	0.0425
10^4	Siliciclastic	34	3.279	0.0305
10^5	Carbonate	14	1.913	0.1283
10^5	Siliciclastic	22	3.396	0.0273
10^6	Carbonate	18	1.822	0.1425
10^6	Siliciclastic	43	5.008	0.0074

Table 2. Difference (%) in sperm counts at the water's surface compared with the control.

Gamete exposure duration of SS on fertilisation. There was a significant effect of siliciclastic sediment on fertilisation success of *A. millepora* at both sperm concentrations (5×10^4 sperm mL^{-1}: $F = 9.18$, $P = < 0.007$; 10^5 sperm mL^{-1}: $F = 38.12$, $P = < 0.001$) but the effect was more pronounced when the gametes had been exposed for longer durations (120 min), resulting in a 19% greater decrease at 5×10^4 sperm mL^{-1} and a 46% greater decrease at 10^5 sperm mL^{-1} (Fig. 6a). In the absence of SS, fertilisation success halved (51%) for gametes at the high sperm concentration but decreased by 76% at the lower sperm concentration. This decrease in fertilisation success at the low sperm concentration in the absence of sediments meant the impact of sediment was not as pronounced, because fertilisation success was already low (21%). After a long exposure to sediments, both sperm concentration triggered almost no fertilisation success (3%).

The contraction of the fertilisation window with suspended sediments and gamete aging is described schematically in Fig. 6b. Under sediment-free conditions, fertilisation peaks and gradually decreases over time as the slick dilutes and gametes age. The effects of SS are amplified under decreasing sperm concentrations and gamete viability, mostly impacting the tail of the window and causing a contraction to the left. Under extreme sediment concentrations, the maximum fertilisation rate is heavily impacted in addition to leftward contraction of the fertilisation window.

Discussion

By applying several lines of evidence we propose sperm coagulates with sediment particles, contributing to reduced numbers of sperm at the water surface, and subsequently to sperm limitation and reduced fertilisation. These effects are more pronounced (i) at lower sperm concentrations, (ii) for siliciclastic sediments and (iii) following prolonged exposures of aging gametes to suspended sediments, and each of these factors in combination can impact fertilisation success and can contract the fertilisation window.

Elevated siliciclastic sediments had marked effects on fertilisation success at moderate sperm concentrations ($< 10^6$ sperm mL^{-1}), increasing the EC_{50} (half the maximum fertilisation success) 2–37 fold, yet eggs were still capable of being fertilised, even at high sediment concentrations if the eggs were saturated with high sperm concentrations (10^6 sperm mL^{-1}). This suggests the mechanism affecting fertilisation success by sediment was not related to egg viability[35]. Furthermore, when examined under SEM, few sediment grains were bound or attached to the egg surface, suggesting egg cloaking was not a major cause of reduced fertilisation success (Fig. 1). Instead, the strong dependence of the fertilisation success rate on the sperm concentration suggests an occurrence of sperm limitation with sediment suspensions physically impeding the movement of the sperm or attaching to sperm and causing them to sink, therefore reducing opportunities for egg-sperm contact. The observed reduction in sperm concentration at the surface of the water in the presence of sediments is consistent with sediments vertically "stripping" sperm from upper surface (and away from the eggs). This mechanism is further supported by the microscopy showing sperm cells highly entangled with the sediment particles. The current study applied continual agitation to maintain the sediments in suspension (and mimicking water movement *in situ*), but the density of the sperm-sediment flocs was still great enough to overcome the replenishment of sperm to the surface and largely accounted for the observed reductions in fertilisation.

In addition to the physical effects of sediment on sperm there may be an additional mechanism associated with decreasing gamete viability. Eggs of the corals *Montipora digitata* and *Platygyra sinensis* remain viable for fertilisation 2 h after spawning but begin to show signs of reduced viability > 2 h[31]. Here, after a short (15 min) exposure period to SS, there was a small yet significant decrease in *A. millepora* fertilisation even at high sperm concentrations; however, the effects of SS exposure on fertilisation was much more pronounced following 2 h SS exposures. However, gametes exposed for 2 h almost completely failed to fertilise, regardless of the sperm concentration. The mechanism for this further reduction in fertilisation success after prolonged exposure to SS is not known but could include damage through abrasion, impact to the polyspermy block, or a depletion of energy reserves in the sperm.

The net effect of the decrease in sperm availability and impact on aging gametes could have a significant impact on the overall fertilisation window by reducing the opportunity for successful fertilisation (Fig. 6b), similar to dilutive forces associated with wind, waves and currents. Although the impact of SS is masked at saturating sperm concentrations ($> 10^6$ sperm mL^{-1}), these sperm concentrations *in situ* are likely fleeting because of dilutive effects and an often patchy distribution of spawning conspecifics. Only one study has provided insight into *in situ* coral sperm concentrations of a single species, *Montipora digitata*[31]. Washed eggs were fertilised with sperm samples collected from a spawning slick in relatively calm weather conditions. During the main night of spawning, high fertilisation success was recorded in a few samples indicating saturating sperm concentrations, but more often the fertilisation success was low indicating sub-optimal sperm concentrations. Further, high fertilisation rates were recorded with sperm taken after 1 h following spawning from 1 m below the water's surface, suggesting that sperm

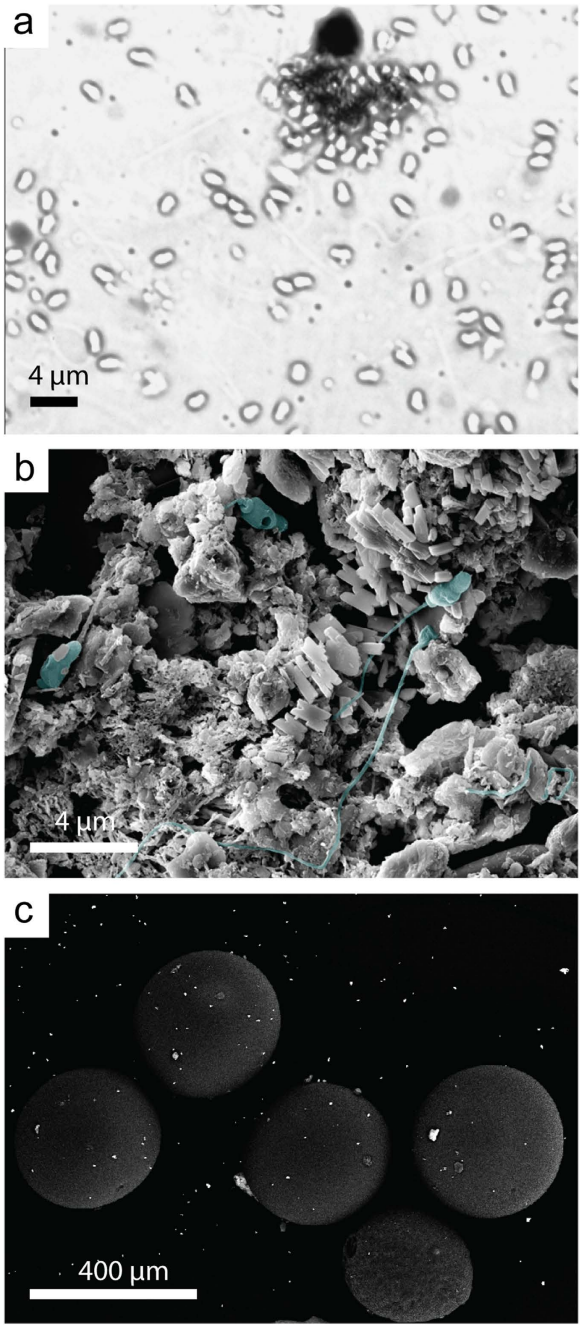

Figure 5. Optical and scanning electron microscopy images showing *Acropora tenuis* sperm and eggs after 30 min exposure to suspended solids. (**a**) optical microscopy image of sperm aggregating around a sediment floc, (**b**) secondary electron image of sperm (artificially coloured blue) tangled in sediment clumps, (**c**) back-scattered electron image of eggs, showing fine particles of scattered sediment, but the eggs are largely sediment free.

rapidly sank. Given the rapid dilution of sperm and the aging of gametes *in situ*, the fertilisation window is limited to less than 2 h[30,31]. For degraded reefs with low numbers of fecund adult conspecifics, the fertilisation window is likely constrained by sperm limitation and further exacerbated by the impacts of SS.

A number of studies have stressed the importance of suitable sperm concentrations in determining effects on fertilisation in marine invertebrates, with greater sensitivity to pollutants and climate stressors reported at sub-saturating sperm concentrations[35,39,41]. The reported sensitivity of coral fertilisation to SS has varied considerably between past studies and part of this variation is likely due to differences in sperm concentration. For example, Gilmour[33] reported significant decreases in fertilisation at just 50 mg L^{-1} SS using a low sperm concentration of 10^4 sperm mL^{-1}, whereas Humphrey, *et al.*[34] reported no effects for some sediment types up to 1000 mg L^{-1} but using saturating sperm concentrations of 2×10^6 sperm mL^{-1}.

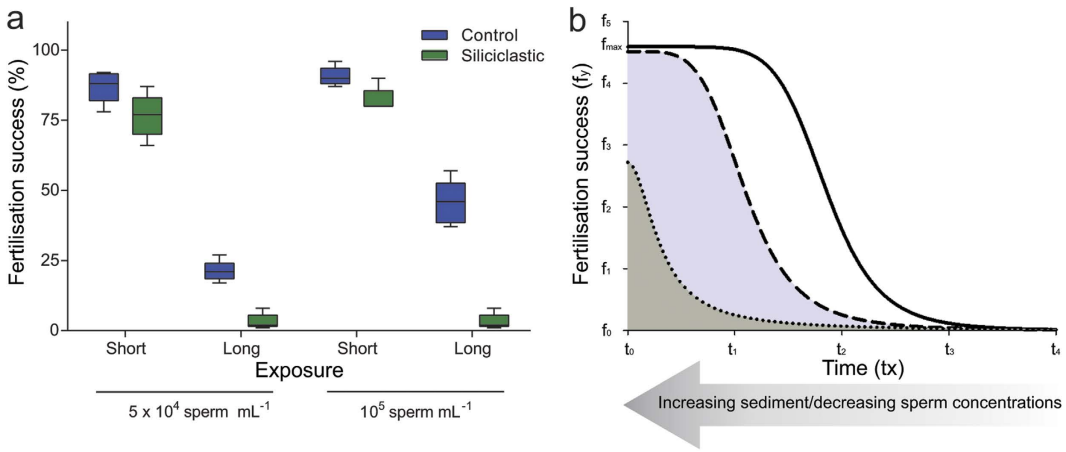

Figure 6. (**a**) Boxplots of *Acropora millepora* fertilisation success after short (15 min) and long exposure (120 min) periods to siliciclastic suspended solids (230 mg L^{-1}) and control (0 mg L^{-1}) treatments at two sperm concentrations (5×10^4 sperm mL^{-1} and 10^5 sperm mL^{-1}). (**b**) A schematic diagram of changes to the fertilisation window (fertilisation success over time) with increasing sediment concentrations and decreasing sperm concentrations. The relative changes of the fertilisation window are the represented by the areas under the curves. The unbroken line (–) is typical of a normal fertilisation window, with fertilisation decreasing with gamete viability over time. As sediment concentration increases, which has the same effect as decreasing sperm concentration, the curve is moved to the left, shown by the dashed line (––). If sperm numbers are very limited, through high sediment concentrations or sperm dilution, the curve (–––) moves further left and the maximum fertilisation success (F_{max}) decreases.

The purpose of the present study was to identify mechanisms by which sediments can affect fertilisation and we applied relatively high concentrations of SS to ensure well-defined impacts on gametes. Nevertheless, SS concentrations were guided by water quality analyses during major capital dredging projects in the Pilbara region of Western Australia in addition to other instances of SS recorded during natural and anthropogenic sediment plumes[21,44–46]. In Australia, one management strategy to reduce coral spawning slicks encountering sediment plumes is a mandatory shutdown period of dredging operations during coral spawning periods ("environmental window") employed by regulatory authorities of Western Australia and the Great Barrier Reef Marine Park[21,43,47]. The rapid improvement in water quality associated with such a shutdown period is clearly evident in Fig. 2; however, given the costs associated with shut-down periods, it is important to consider whether partial shutdowns guided by conservative water quality guidelines might be equally protective of coral spawning success. Answering this question and effectively quantifying the risk associated with elevated sediment requires further studies determining (and spatially modelling) statistical metrics such as EC_{10} and EC_{50} for fertilisation across a range of sediment types. For example, carbonate sediments tend to have far less impact on coral fertilisation than nearshore terrigenous sediment types (this study and Humphrey, *et al.*[34]). The present study highlights the need for these future studies to carefully consider the appropriate choice of sperm concentration(s) and use a wide range of sediment concentrations, otherwise the effect of the treatment could be masked by sperm-saturation effects. Fertilisation experiments with sediments are challenging due to difficulties in simultaneously maintaining sediments and fragile gametes in suspension over the duration of experiment. Irrespective of the methods used, both the initial and final suspended sediment concentrations should be measured, as some flocculation is likely given sperm-sediment interactions. Moreover, selection of a suitable particle grain size for the experiment is fundamental and sediment leachate, contaminant and associated nutrient concentrations should be assessed as part of normal ecotoxicological procedures[48]. This approach would also benefit assessments of the potential environmental effects of wind-driven wave resuspension of terrestrially-derived sediments on the fertilisation success of corals[49]. Optimizing the coral fertilisation assays using the techniques described here should improve assessments of the environmental implications of these natural turbidity events.

In summary, sperm limitation through physical effects such as entanglement with sediment particles and stripping from the surface was identified as the key mechanism preventing and reducing successful fertilisation of coral eggs exposed to elevated suspended sediments. This finding may also have implications for other broadcast spawning marine organisms, such as fish, molluscs and echinoderms[50]. When combined with natural factors such as decreasing gamete viability and dilutive effects, the opportunity for fertilisation may be substantially reduced in the presence of sediment. As fertilisation is a critical step in recruitment, a decrease in fertilisation success may result in demographic bottleneck, reducing ongoing population maintenance or replenishment following disturbances. Furthermore, as coral spawning is ephemeral and usually confined to just a few nights, it is important that these fertilisation and recruitment processes are not inhibited.

Materials and Methods

Choice of suspended sediment (exposure) concentrations. SS in a shallow coral habitat during a major capital dredging program (~7.6 M m^3 of sediment dredged over 530 d) at Barrow Island Western Australia was

monitored indirectly by submerged nephelometers taking turbidity (NTU) readings every 10 minutes, positioned 1–2 m above the seabed at two water quality monitoring sites ~300 m north (site 1, 20.829 °S, 115.509 °E) and south (site 2, 20.822 °S, 115.511 °E) during the autumn (coral spawning) months in 2010 (before dredging) and 2011 (during dredging) (see Ministerial statement 800 searchable on the WA EPA website www.epa.wa.gov.au for more details) (Fig. 2). NTU recordings were made with a sideways mounted optical backscatter device (nephelometer) that can be used to estimate suspended sediment concentrations (as mg L^{-1}) by applying site specific algorithms (conversion factors) based on gravimetrically determined TSS levels versus nephelometer readings of 1.3–1.6 NTU = 1 mg L^{-1} [51]. Benthic sediments before dredging were predominantly unconsolidated, undisturbed carbonate with a low total organic content of $<0.8\%$ (w/w), which formed a thin veneer (0.5–3 m thick) overlying limestone pavements ranging from rubble to typically gravelly sand mixed with fine silts and clays.

Sediment preparation and analysis. Experiments were conducted with two different types of marine sediments, a terrestrially-influenced siliciclastic (~50% quartz) sediment that we describe as "siliciclastic", with relatively high concentrations of iron and aluminum (collected from 10 km offshore in the Pilbara region of Western Australia and in an area subject to the influence of the Ashburton River). The second type of sediment was primarily biogenic calcium carbonate (~80% aragonite), typical of offshore reefal sediment of the Great Barrier Reef (collected from Davies Reef on the Great Barrier Reef, Queensland, see Supplementary Table S1 for chemical analyses and collections locations). The sediments, hereafter referred to as siliciclastic and carbonate, were screened and milled to $<63\,\mu m$ then mixed with 0.4 μm filtered seawater (FSW) to create a 5 g L^{-1} suspension. To remove larger particles, the sediment was allowed to settle for 10 min and the top three-quarters of the suspension was siphoned off for use in the experiments. This reduced the modal peak of the particle size distribution to $\leq 10\,\mu m$, which is a typical grain size likely to be found at the water's surface. To further concentrate the stock solution, the sediment was left to settle overnight and the supernatant removed. Sediment concentrations were measured gravimetrically by taking three 100 mL replicate samples using 0.4 μm polycarbonate filters and dried overnight at 60 °C. Turbidity was measured with a nephelometer (TPS 90FL-T) and a linear NTU-SS relationship was determined. During experiments, desired sediment suspensions were created by mixing the sediment stock with FSW. The final concentration of each experimental chamber was calculated by taking the average of turbidity readings before and after the experiment. The mean SS exposures for each treatment were determined using the NTU-SS relationships. Turbidity generated by the gametes (~5 NTU) was deducted from the final turbidity reading. Salinity (35.5 ppt) and pH (8.1) did not deviate throughout the experiment and dissolved oxygen remained above $>95\%$ saturation.

Coral collection and gamete preparation. Colonies of *Acropora tenuis* (Dana, 1846) >20 cm were collected from 3–5 m depth on 21 October 2013 and 9 October 2014 from Magnetic Island (central inshore Great Barrier Reef region, 19.157 °S, 146.861 °E). Colonies of *A. tenuis* (Dana, 1846) and *Acropora millepora* (Ehrenberg, 1834) >20 cm were collected from 3–5 m depth on the 14–17 November 2013 and 3–6 November 2014 from Trunk Reef (18.329 °S, 146.846 °E) and Davies Reefs (18.832 °S, 147.633 °E), both which are located in the central, mid-shelf region of the Great Barrier Reef. The gravid colonies were then transported to the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS) and placed in flow-through tanks at 27–29 °C according to the temperature of their natal reef. Following spawning the egg-sperm bundles were gently scooped from the surface and the eggs separated from sperm using a 100 μm mesh filter and washed five times in FSW as described by Negri and Heyward [37]. Eggs of each species were selected from a single colony, while sperm from multiple colonies (3–5) were pooled and the resultant stock was counted using a hemocytometer under a compound microscope. The sperm stock was then diluted to achieve a working stock concentration of 1×10^8 sperm mL^{-1} from which further dilutions were made.

Influence of SS on fertilisation at multiple sperm concentrations. Sperm concentration-response experiments were conducted using siliciclastic SS at ~230 and 700 mg L^{-1}, carbonate SS at 230 mg L^{-1} and FSW (control). For the fertilisation assays sediment concentrations of 230 mg L^{-1} and 700 mg L^{-1} were used together with a FSW (control). For each of six sperm concentrations (10^1–10^6 sperm cells mL^{-1}) of *A. tenuis*, 48 mL of the SS treatment was transferred into ten replicate 180 mL clear polystyrene chambers. Eggs were added to half the chambers (1 mL of ~200 eggs) and the desired sperm concentration (1 mL) was added to the other half. Gametes were independently exposed to the suspended sediment treatments for 30 min, simulating the time taken for the eggs to become viable and for compatible gametes to encounter each other *in situ* [52]. Sperm were then mixed with their corresponding eggs to initiate fertilisation and placed on mechanical rollers at 0.3 revolutions s^{-1} (see Supplementary Fig. S2) located at a constant temperature room set to the temperature of their parent's natal reef (27–29 °C). The rollers consisted of a series of 3 cm diameter cylinders rotated by an electrical motor and use of the rollers maintained a constant suspension of sediment throughout the fertilisation period. The chambers were removed from the rollers when the majority of the embryos in the controls had developed to the 4-cell stage, which for *A. tenuis*, was typically 150 min after mixing sperm with eggs. Turbidity was immediately measured using a nephelometer and then embryos and eggs were fixed using Z-fix fixative (Anatech Limited). Eggs and embryos were transferred to counting trays and the first 100 embryos or eggs in each experimental chamber were assessed for fertilisation success. To control for the possible effects of mechanical agitation, similar fertilisation experiments were conducted under stationary conditions in 6-well cell culture plates (Nunclon, Thermo Scientific) on three separate occasions. In these experiments, 1 mL of the desired sperm concentration was added to 1 mL of ~200 eggs and 8 mL of FSW. Final sperm concentrations were over the range 10^1–10^6 sperm mL^{-1}.

Sperm counting in the water's surface with SS. Changes in sperm concentration of *A. tenuis* were measured using a C6 Flow Cytometer and Cflow Sampler software (Accuri Cytometers Inc.). One mL of either

5×10^4, 5×10^5 and 5×10^6 sperm mL^{-1} were added to 50 mL of 230 mg L^{-1} siliciclastic or carbonate sediment, or FSW (control) making a sperm concentration of 10^4–10^6 sperm mL^{-1}. Sperm-only and sediment-only controls at these concentrations were also prepared. All chambers were mechanically agitated for 30 min. From each chamber, a 1 mL subsample was taken from ~1 cm below the surface for flow cytometry. Using forward and side scatter, the regions for gating were determined by running the controls separately. Values from forward scattering are proportional to particle size and the threshold was set at 20,000 signal intensity units using a calibration of 1 μm florescent beads; side scattering is proportional to their complexity and was set at the minimum signal intensity threshold of 10 units. Two clear cluster distributions were observed for each of the sediment particles and the sperm in the sample. Three replicates of each treatment or control were measured. The flow cytometer was run to count at least 100,000 particles or 2 min.

Microscopic examination. Subsamples from the bottom and middle of sediment-sperm samples were fixed in 1.25% glutaraldehyde and 0.5% paraformaldehyde in FSW and stored at 4 °C. Samples were dehydrated in a microwave using a graded ethanol series (70%, 90% × 2, 100%, 100% (anhydrous)) for 40 s at 250 W and then critical point dried (Polaron KE3000, Quorum Technologies) in liquid 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 SEM (Zeiss 55-VP).

Gamete exposure duration of SS on fertilisation. To determine if a decline in gamete viability with age compounded the effect of sediment on fertilisation success, gametes (90 min age) were exposed to a suspension (230 mg L^{-1}) at two sperm concentrations for two time periods (15 and 120 min). A pilot study revealed low rates of fertilisation at sperm concentrations of 10^4 sperm mL^{-1} for *A. millepora*; therefore, 5×10^4 sperm mL^{-1} was used in addition to 10^5 sperm mL^{-1} for the experiment. Similar to the first experiment, 1 mL of ~200 eggs and 1 mL sperm was added to 10 replicates chambers each containing 48 mL of the SS treatment or control (FSW) and gently agitated on mechanical rollers. After 15 min, half the gamete exposure chambers were mixed with their counterpart to initiate fertilisation and placed back on the mechanical rollers. The remainder were mixed at 120 min and placed on the rollers. The chambers were removed from the rollers when the majority of the embryos in the controls reached the 4-cell stage, which for *A. millepora*, was generally 180 min after mixing sperm with eggs. Turbidity measurement and fixation of the embryos and eggs were conducted as described previously.

Design and statistical analysis. We used a similar assay technique proposed by Marshall[35] to calculate the fertilisation maximum (F_{max}) over a range of sperm concentrations because the effect of polyspermy in acroporid corals appears to be minor (if at all)[37,39]. We also calculated a second metric, the EC_{50} - the concentration that yields the half maximum fertilisation response.

Fertilisation success rates were fitted to nonlinear regression curves (four-parameter logistic models) using the program GraphPad Prism (v5, San Diego, USA). The model was constrained between 0 and 100%. All curves were tested for normality of the residuals and a replicate test was applied to assess goodness of fit. For the treatment data sets, the model and the inflection point i.e. EC_{50} values were determined with greater confidence by global fitting the parameters top, slope and bottom of the curves if there was no significant difference with the controls ($p > 0.05$)[53]. The fertilisation maximum (F_{max}) was compared between the sediment treatments and the controls with an unpaired t-test. Agitated assays were compared with non-agitated assays to test for any effect of the mechanical rollers on coral fertilisation.

To assess changes in sperm numbers at the water surface in the presence of sediments, the estimated number of sediment particles incorrectly occurring in the gate was deducted from the total sperm count. Changes in sperm numbers between sediment treatments and the controls were analysed with unpaired t-test using GraphPad Prism (v5, San Diego, USA). Sperm counts were compared with fertilisation success curves by first standardising the data to the initial sperm concentration and then interpolating the values onto the fertilisation success curve. To test gamete exposure durations on fertilisation success, each sperm concentration was analysed separately and fit to Generalized Linear Models (GLM) using packages lattice in R (R Development Core Team, 2014). Data exploration was done by methods described in Zuur, *et al.*[54], with the presence of outliers investigated using Cleveland dotplots. Quassi-binomial errors and the log link function were used because the data were over-dispersed.

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Author Contributions

G.F.R. and A.P.N. designed the experiments. G.F.R. conducted the experiments. G.F.R, A.H. and A.P.N. analysed the data. R.J.J. analysed the seawater quality data. G.F.R. and P.L.C. conducted the microscopy analysis. G.F.R. wrote the manuscript and all authors made comments on the manuscript (G.F.R., R.J.J., P.L.C., A.H. and A.P.N.).

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RESEARCH ARTICLE

Mucous Secretion and Cilia Beating Defend Developing Coral Larvae from Suspended Sediments

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Abstract

Suspended sediments produced from dredging activities, or added to the sediment budget via river runoff, are a concern for marine resource managers. Understanding the impact of suspended sediments on critical life history stages of keystone species like corals is fundamental to effective management of coastlines and reefs. Coral embryos (*Acropora tenuis* and *A. millepora*) and larvae (*A. tenuis*, *A. millepora* and *Pocillopora acuta*) were subjected to a range of suspended sediment concentrations of different sediment types (siliciclastic and carbonate) to assess concentration-response relationships on ecologically relevant endpoints, including survivorship and ability to metamorphose. Embryos were subjected to short (12 h) suspended sediment exposures from ages of 3–12 hours old or a long (30 h) exposure at 6 hours old. Neither the survivorship nor metamorphosis function of embryos were significantly affected by realistic sediment exposures to ~1000 mg L^{-1}. However, some embryos exhibited a previously undescribed response to dynamically suspended sediments, which saw 10% of the embryos form negatively buoyant cocoons at siliciclastic suspended sediment concentrations $\geq$35 mg L^{-1}. Scanning electron and optical microscopy confirmed the presence of a coating on these embryos, possibly mucus with incorporated sediment particles. Cocoon formation was common in embryos but not in larvae, and occurred more often after exposure to siliciclastic rather than carbonate sediments. Once transferred into sediment-free seawater, functional ~36-h-old embryos began emerging from the cocoons, coinciding with cilia development. Ciliated (> 36-h-old) larvae exposed to suspended sediments for 60 h were also observed to secrete mucus and were similarly unaffected by suspended sediment concentrations to ~800 mg L^{-1}. This study provides evidence that mucous secretion and cilia beating effectively protect coral embryos and larvae from suspended sediment and that these mechanisms may enhance their chances of successful recruitment.

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Introduction

Coral reefs provide a range of benefits to coastal communities through tourism, fishing and coastal protection, and have been collectively valued at US $9.9 trillion/yr. globally [1]. However, coral reefs are considered to be in decline due to the impacts of both global (e.g. climate change) and regional (e.g. declining water quality) disturbances [2]. Successful coral reproduction underpins the maintenance of communities and their resilience to disturbance [3, 4]. Of ongoing concern is the increased supply of terrestrial sediment near coral reefs [5], the release of sediments into the water column from dredging activities [6], and the resuspension of sediments from natural wind and wave events [7, 8], and how these stressors may impact coral reproduction and recruitment processes [9–11].

Sediments resuspended from dredging operations can remain elevated for several kilometers, occasionally reaching hundreds of mg L^{-1} (but often <10 mg L^{-1}) [12–14]. Similarly, inshore reefs are frequently exposed to suspended sediment concentrations (SSCs) < 5 mg L^{-1}, but subject to spikes of >100 mg L^{-1} usually associated with cyclonic activity [15, 16]. These particles have the potential to affect both existing populations of key reef-building taxa, as well as reproduction processes and recruitment of new individuals to these populations[17, 18]. Particularly relevant to managing the risk of dredging projects around coral reefs is the potential for sediment plumes to interact with coral spawning slicks, produced from synchronous, multi-specific release of gametes by broadcasting spawning coral species. For example, since the early 1990s, dredging projects in Western Australia have been required to shut-down all turbidity generating activities (i.e. dredging and disposal of dredge material at sea) shortly before and after synchronous spawning periods [17]. Similar shutdown periods have been implemented on the Great Barrier Reef [19], and have recently been suggested for coral reefs in Singapore [20]. The shutdown policy was introduced under a precautionary principle, which still remains in place, as there are many possible cause-effect pathways whereby suspended sediments (SS) can interact with the reproductive cycle of corals, and few of these have ever been quantified [17]. In particular, the impact of sediment on the planktonic stage remains poorly explored compared with the fertilisation [9–11, 20, 21] and settlement stages, both which often show susceptibility to low sediment levels [22, 23].

For broadcast spawning corals, the planktonic stage begins following fertilisation of coral gametes at the water's surface [17]. The first visible signs of embryogenesis generally occur a few hours after fertilisation with the zygote undergoing holoblastic cleavage until four blastomeres are formed [24] (Fig 1). During these initial stages of cleavage, the embryo is increasingly vulnerable to physical disturbance, including fragmentation in turbulent conditions; however, embryonic cells can continue re-dividing resulting in functional, albeit smaller, embryos [25]. Further division of the embryo results in the morula stage, followed in many coral species by a flattened, concave bilayer dish (prawn-chip stage) ~7–9 h after insemination, and then a blastopore formation (bowl stage, Fig 1) [26, 27]. The term 'embryo' is used here to denote these developmental stages from fertilisation until blastopore closure. At this stage ciliation and movement occurs [24, 28] and the term 'larvae' is used to denote the motile planktonic stage [29]. Like most benthic marine organisms, corals undergo a planktonic larval phase following fertilisation generally lasting 4–10 days (reviewed by Jones et al. [17]), although larger larvae and those that acquire algal symbionts, *Symbiodinium* spp., have the greatest potential to disperse long distances through energy derived from lipids or supplied via photosynthesis [30, 31]. The larval stage ends when the larvae permanently attach to a substratum and undergo metamorphosis [29]. At a behavioural level, coral exhibit a sensory capacity to identify sites that are suitable for settlement, but are limited in their ability to navigate towards reefs and therefore are considered mostly planktonic [32–35].

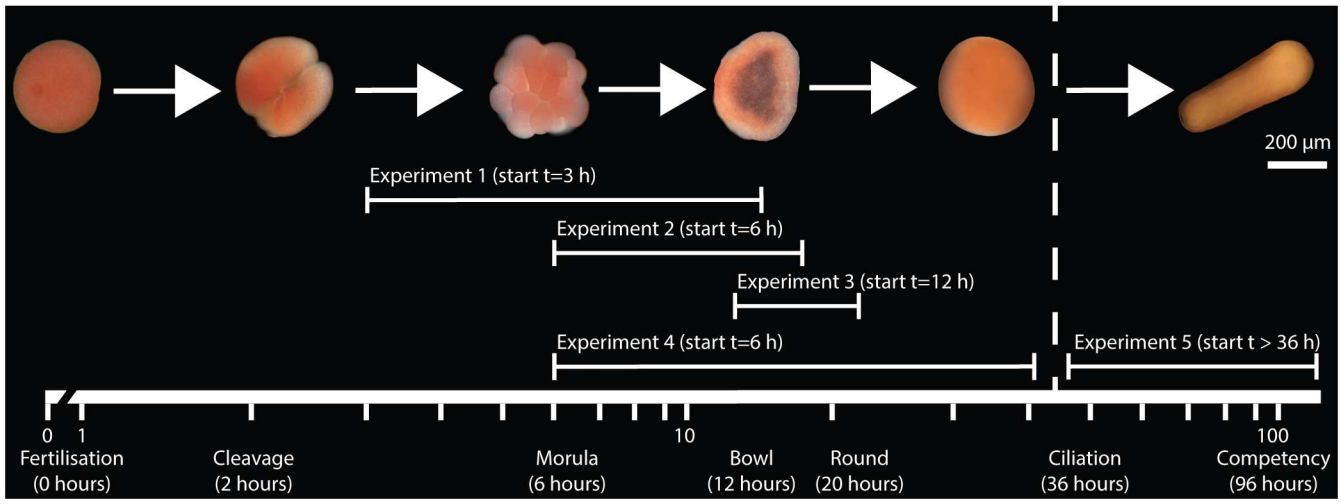

Fig 1. Diagram illustrating the sequence of experiments throughout the embryonic and larval developmental stages of the genus *Acropora*. The experimental design included three experiments (experiment 1, experiment 2 and experiment 3) where embryos were subjected to three separate 12-hour sediment exposures commencing at 3, 6, and 12 hours after fertilisation. In addition, two long sediment exposure experiments (experiment 4 and experiment 5) were conducted covering most of embryogenesis (6–36-h-old embryos) and a 60-hour period following ciliation using larvae at 3–6 days old.

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A range of responses of planktonic stages to SS have been reported. Humphrey et al. [21] found no difference in developmental abnormalities in very early stage embryos (~four-cell stage) of 3-h-old *Acropora millepora* after sediment exposures of 200 mg L^{-1}. Similarly, Gilmour [10] found no clear trends in survivorship of 3–18 h-old embryos of *A. digitifera* exposed to ~100 mg L^{-1}. At the larval stage, Te [36] did not observe any larval mortality of the brooding coral *Pocillopora damicornis* subjected to 1000 mg L^{-1} SSC, but Gilmour [10] found significant mortality of *A. digitata* larvae at much lower SSC of ~50 mg L^{-1}. More recently, in a pilot study by Larsson et al. [37] observed a decrease in larval survivorship of *Lophelia pertusa* at ~ 25 mg L^{-1} SSC.

Some of the variability in the outcome of these studies could be methodological, with some approaches unlikely to achieve a uniform, consistent suspension of sediments throughout the course of the experiment [17]. Gilmour [10] used coarse silt to fine-grained sediments, whereas Larsson et al. [37] removed coarse-grain sediment through a two day settlement process to select only for very fine-grained particles for testing. The broadcast spawning *Lophelia pertusa* larvae are much smaller (~20%) in length than the brooding *P. damicornis* larvae, and therefore likely to contain comparatively less energy reserves that may be drawn upon to overcome sediment encounters. *L. pertusa* larvae used by Larsson et al. [37] is also a cold water species, as opposed to the tropical species used by Gilmour [10], Humphrey et al. [21], and Te [36].

As part of an experimental sequence to investigate the effects of sediments on the early life-history stages of corals and understand the risk associated with turbidity-generating events during coral spawning periods [9, 11, 17], we examined the survivorship and metamorphosis response of embryo and larvae of several tropical coral species after exposure to different sediment types and concentrations. The quantitative approach used in this study, and derivation of concentration–response relationships, will allow more informed assessment of the risk, as opposed to hazard (sensu Harris et al. [38]), of SS on the planktonic phase of corals.

Materials and Methods

Sediment collection and preparation

Experiments were conducted with two types of marine sediments: predominantly siliciclastic sediments collected from Onslow Reef (Pilbara region, Western Australia: 21°38'32 S, 114° 55'27 E), and predominantly carbonate sediments collected from Davies Reef, (Great Barrier Reef, Queensland: 18°49'12 S, 147°39'21 E). Collection and preparation of the field-collected sediment have been described in Ricardo et al. [9]. Briefly, the sediments were screened, milled and settled until the modal grain size was <10 μm as measured using laser diffraction techniques (Mastersizer 2000, Malvern instruments Ltd). Each type of sediment had relatively low proportions of total organic carbon (0.26%). Suspended sediment treatments were created by making a serial dilution of the concentrated stock with 0.4 μm filtered seawater (FSW), and the resultant turbidity (NTU) measured with a nephelometer (TPS 90FL-T) and spectrophotometer (Shimadzu, UV-1800). Sediment concentrations in the samples were determined by spectrophotometry at the start and end of the experiments, or when a water change was conducted (typically every 12 h). For each treatment, 3.5 mL of the sample was measured for absorbance at 820 nm and the mean of the initial and final absorbance readings calculated. Turbidity and absorbance values both correlated linearly with SSCs ($R^2 > 0.98$) and were therefore used to derive total SSCs in the chambers. To confirm SSCs for each experiment, 3 × 100 mL replicate samples of the highest concentration were filtered through 0.4 μm polycarbonate filters (Advantec), which were dried overnight in an oven at 60°C and the sediments weighed on an analytical balance to 0.0001 g. During the experiments, salinity (35.5 ppt) and pH (8.1) remained constant. Dissolved oxygen was measured in the highest sediment treatment and remained above 95% saturation. All experiments were carried out in a temperature controlled room set at the same temperature as the outdoor aquaria (27–29°C depending on the month of spawning), and the water temperature within the chambers did not deviate from this range.

Coral collection and larval culture

Colonies of *Acropora millepora* (Ehrenberg, 1834), *A. tenuis* (Dana, 1846), and *Pocillopora acuta* (Lamarck, 1816) were collected from <10 m depth 3–5 days before the predicted spawning events from 2013–2015 in the central Great Barrier Reef from inshore and mid-shelf reefs (19°10'13 S, 146°51'53 E; 18°22'53 S, 146°47'43 E; 18°49'12 S, 147°39'21 E; 18°48'48 S, 147° 39'26 E; 18°46'25 S, 146°31'07 E) (S1 Table). All corals were collected under the Great Barrier Reef Marine Park Authority Permit G12/35236.1.

Gravid colonies were transported to the National Sea Simulator at the Australian Institute of Marine Science (AIMS), and placed in outdoor flow-through seawater tanks of 27–29°C (equivalent to the water temperature at their collection site). At the 'setting stage' just prior to spawning (see Babcock and Heyward [39]), colonies were isolated in individual tanks and egg-sperm bundles gently scooped from the water surface after spawning. The embryo and larval culture procedures were conducted following methods described in Negri and Heyward [40]. Briefly, gametes were cross-fertilised for ~1.5 h in 20 L of 0.4 μm FSW in a 50 L container. The embryos were then washed free of sperm by gently transferring them into another 50 L container also containing FSW. This process was repeated three times. Embryos were then transferred into 500 L fiberglass tanks filled with FSW, where they were left to develop for 12 h, after which time gentle aeration and water flow was introduced to provide adequate water circulation and maintain sufficient dissolved oxygen levels.

For the brooder *P. acuta*, larval traps were placed on tanks containing adult colonies on the night of the new moon in April 2014. Over the following seven mornings, larvae were collected

from the traps and transferred to 5 L glass chambers containing FSW, with gentle aeration and water flow.

Embryo concentration–response experiments

Embryos of *A*. *millepora* were exposed to a range of SSCs (up to ~1,000 mg L^{-1}) for 12 h, in 3 separate experiments, starting with embryos 3 h after fertilisation (3–15 h, experiment 1), 6 h after (6–18 h, experiment 2) and 12 h after (12–24 h, experiment 3) (Fig 1). A longer-term (30-h) experiment was also conducted at a range of lower SSCs (<100 mg L^{-1}) starting with embryos 6 h after fertilisation (6–36 h, experiment 4). The control chambers contained no sediment but all other conditions were identical to the treatment chambers. We assessed embryo survivorship in all experiments, and in experiment 2 and 3 we assessed numbers of embryos forming cocoons, and in experiment 4 we assessed the ability of larvae to settle following the exposure of embryos to sediments. The SSC ranges were selected to span the maximum running-mean values embryos could encounter for a given exposure duration based on the analyses of water quality conditions during three major capital dredging projects [13] and for peaks in instantaneous turbidity measurements during one capital dredging project (see below). Specifically, we covered the maximum SSCs for the relevant durations of exposure. For example, shorter exposure durations (i.e. 12 h) result in higher 100% running means (i.e. hundreds of mg L^{-1}), whereas longer exposures (1–3 days) result in lower 100% running means (tens of mg L^{-1}). Between 10–20 embryos were added to chambers containing 150 mL of each SS treatment and placed on mechanical rollers at 0.3 revolutions s^{-1} to maintain the sediments in suspension. Every few hours the chambers were gently inverted a few times and then placed back on the mechanical rollers. After exposure, the total number of surviving embryos was counted, with damaged, missing, and inert embryos defined as dead. To test the ability of larvae to undergo normal metamorphosis, embryos previously subjected to SS were transferred into FSW for a further 5-day recovery period, and after this time the larvae were considered competent to settle (competency generally commences after 4 days [17]). These 6-day-old larvae were then exposed to a 2 × 2 mm chip of live crustose coralline algae (CCA, *Hydrolithon onkodes*) to assess their ability to settle and undergo metamorphosis [29]. The experiment was repeated using *A*. *tenuis* embryos of the same age to examine replicability between species.

Larval survivorship and metamorphosis concentration–response experiments

Larval survivorship was examined in >3-day old *A*. *millepora*, *A*. *tenuis* and *P*. *acuta* larvae subjected to sediment suspensions to ~800 mg L^{-1} over a period of 60 h (Fig 1). As with the embryo assays, sediment concentrations used in the larval experiments spanned the range of environmentally relevant concentrations expected for 1–3 day exposures. The control chambers contained no sediment but all other conditions were identical to the treatment chambers. For each sediment concentration, 20 larvae were added to each of 4 × 180 mL chambers containing 150 mL of the sediment suspension. Sediments were kept in suspension by rotating the chambers at 0.3 revolutions s^{-1} using mechanical rollers, and the resuspension was assisted by the use of three 6 × 6 × 75 mm rods attached to the inner-surface of the chambers (as baffles) to disturb the water movement. Every few hours the chambers were gently inverted a few times and then placed back on the mechanical rollers. Every 12 h, the sediment suspensions were changed, and the larvae assessed for survivorship at the end of the experiment. Surviving larvae were then gently washed and transferred to 6-well tissue culture plates containing 10 mL of FSW, and at 6-day old were assessed for their ability to undergo attachment and metamorphosis using CCA

chips, as described previously (except for *P. acuta* where metamorphosis rates were poor in the controls—see 'test acceptability criteria' below).

Optical and scanning electron microscopy

Embryos and larvae at the end of the experiments were examined using light microscopy, and by scanning electron microscopy (SEM) using samples fixed in 1.25% glutaraldehyde and 0.5% paraformaldehyde in FSW. The SEM samples were subsequently dehydrated in a microwave using a graded ethanol series for 40 s at 250 W and then critical point dried (Polaron KE3000, Quorum Technologies) in liquid CO_2. The dried samples were mounted on carbon tape on aluminium stubs, coated with 3 nm platinum, and imaged using a field emission SEM (Zeiss 55-VP). Backscattered signals are proportional to atomic composition and therefore these images were used to identify sediments on the larval samples. Elements of greater atomic number, such as calcium, iron, and silicon appeared bright relative to the sample [41] and the biological material are primarily composed of elements with low atomic numbers (carbon, oxygen and hydrogen), and appeared dark in the sample.

Water quality during turbidity-generating events

A year of water quality turbidity readings (1/6/2010 to 31/5/2011) were examined to determine the ephemeral nature of SS pulses and brief reprieves from SS exposure. Turbidity readings were collected with sideways mounted optical backscatter devices (nephelometers) at two water quality coral reef sites Site 1 (LNGA: 20° 49.322' S, 115° 30.665' E) and Site 2 (LNGO: 20° 49.713' S, 115° 30.507' E) subjected to periodic cyclone events and ~300 m from a major capital dredging program at Barrow Island, Western Australia. For further water quality collection and sites details see Ricardo et al. [9], and Jones et al. [13].

Statistical Analysis

We defined the test acceptability criteria as experiments that had a high rate of survivorship (>80%) and metamorphosis (>50%) in the controls, and only these were included for analysis [42]. Suspended sediment concentrations that resulted in a 10% (EC_{10}) or greater response in absolute terms were calculated where possible, by fitting the data to non-linear regression curves (four-parameter logistic models) with 95% confidence bounds using the software GraphPad Prism (v7), and by fitting binomial Generalized Linear Models (GLM) using a probit link using the statistics program R (v3.1.2). Non-linear regression (NLR) models were only fitted under the criteria that they passed normality of residuals and the replicates test (a measure of deviation from the model) [43]. GLM models were corrected for overdispersion using quasi-likelihood estimations [44] and EC_X values extracted using the package dose.p [45]. For easier interpretation, GLM models were only plotted if NLR models could not be fitted. Four chambers leaked during experiment 5 leading to a loss of larvae and therefore these data were not included in the analyses. All sample size analyses were conducted *a priori* for binomial GLM using G*Power (v3.1.9.2). Pilot experiments indicated high survivorship (>90%) in the controls and low cocoon formation (0%), and therefore because the response could only be unidirectional e.g. survivorship cannot be >100% [46]), survivorship and cocoon formation were run as one-tailed hypotheses with $\alpha = 0.05$, $\beta = 0.8$. Post-hoc analysis confirmed our sample sizes were sufficient. For settlement assays, which were considered two-tailed hypotheses, a lower settlement rate in the control than expected in the *A. millepora* embryo and *A. tenuis* larval experiments meant that our minimum detection effect was 13 and 16% respectively at $\alpha = 0.05$, $\beta = 0.8$.

In situ 10-min turbidity data were converted to approximate SSCs using the conversion factor of 1.3 NTU: SSC [9]. The data were analysed using Matlab (v8.6) for sediment pulse durations above 35 mg L^{-1} (a SSC required for the formation of embryo cocooning to occur—see Results).

Results

Impacts of suspended sediments on embryogenesis

The 3-h old embryos exposed for 12-h to ~800 mg L^{-1} SSC (experiment 1) fragmented upon agitation (used to keep the sediment suspended) and so it was not possible to quantify survivorship. There was no effect of exposure of 6-h-old embryos to either siliciclastic or carbonate sediments at concentrations up to ~80 mg L^{-1} for 30 h (experiment 4, Fig 2a). The SS exposure did not have any significant effect (GLM: b = -0.1761, t = -1.209, p = 0.204) on the subsequent ability of the larvae to metamorphose following a 4.5-day recovery period, despite a 29% decrease in settlement rates (S2 Table, Fig 2a). The experiment was repeated using 6-h-old embryos subjected to SSCs of ~900 mg L^{-1} for 12 h, and there was also no effect on survivorship in either sediment type (experiment 2, Fig 2b). Exposure of 12-h-old embryos for 12 h to elevated SSCs (siliciclastic: ~1200 mg L^{-1}; carbonate: ~800 mg L^{-1}) had no effect on survivorship for either sediment type (experiment 3, Fig 2c), although in the 10 mg L^{-1} exposures, embryos sometimes clumped with mucus, causing a decrease in survivorship in some chambers (Fig 2c). The high SSC exposures of ~1000 mg L^{-1} had no impact on subsequent metamorphosis (S1 Fig). In *A. tenuis*, a single exposure experiment on 12-h-old embryos at siliciclastic SSCs to ~1000 mg L^{-1} had no impact on survivorship (S1 Fig).

Although there was no effect of SS on survivorship, embryos often formed 'cocoons' that quickly became negatively buoyant and resulted in the embryos sinking (Fig 3a). The color of the cocoon reflected the colour of the sediment grains (Fig 3a and 3f), and under SEM the embryo cocoon appeared to be a casing composed of sediment grains incorporated in mucus (Figs 3b–3d and 4a–4e). Once transferred to FSW, the larvae were able to free themselves from the cocoon, with the movement generated by newly developed cilia (Fig 3g and 3h and S1 File). For 6-h-old embryos, cocooning was observed in high proportions at the high SSC (Fig 2b). Cocoon formation was observed in 12-h-old embryos exposed to the siliciclastic sediments with 10% (EC_{10}) of the embryos forming cocoons at 35 mg L^{-1} (95% C.I.; 20–60) (S2 Table, Fig 2c), but embryos of the same age showed less sensitivity to carbonate sediment with mucous cocooning (23 ± 7%, mean ± SEM) only observed in the highest (~800 mg L^{-1}) sediment concentration (Fig 2c). In sediment-free FSW, the entrapped larvae were first observed emerging from the cocoon after 12 h and by 48 h, >75% had emerged (Fig 2d). After emerging from the cocoon, the larvae were capable of swimming and undergoing normal metamorphosis (S1 Fig).

Cocoon formation was observed as late as 72 h after fertilisation in the development sequence in *A. millepora*, but only in a few individuals (data not shown). Exposure to siliciclastic SS for a brief 1-h period elicited some cocooning in embryos. Cocoon formation was also observed for *A. tenuis* embryos subjected to siliciclastic SSC at ~600 mg L^{-1}, with all emerging from the cocoon within 4 days (S1 Fig). However, embryos of either species did not create the cocoons without water movement and attempts to recreate mucous cocoons by inverting the chambers every 5 min (a less effective method for sediment resuspension) were largely unsuccessful. As a final examination of the cocoon formation, commercially available high grade processed calcium-bentonite clay (Watheroo Bentonite) was tested and caused all embryos to form cocoons at SSC as low as 20 mg L^{-1} and inverting the chambers every 5 min was capable of inducing cocoon formation at bentonite treatment of ~100 mg L^{-1}.

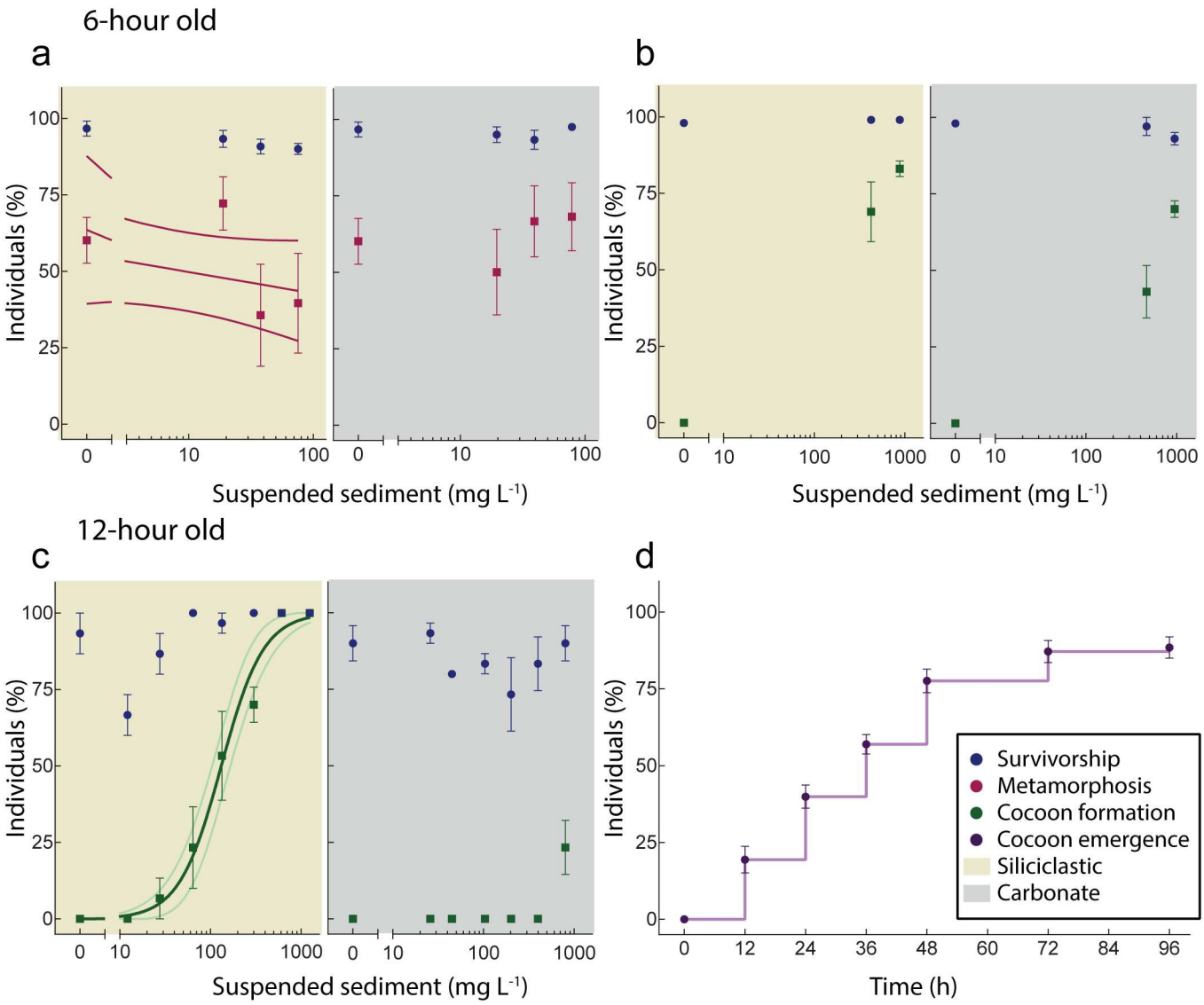

Fig 2. Concentration-response relationships for *A. millepora* embryos. a) Survivorship and ability to metamorphose after prolonged exposure to siliciclastic (yellow shade) and carbonate (grey shade) suspended sediment (SS) from 6–36 h age, n = 6 per concentration. b) Survivorship and cocoon formation after exposure to a 12-h sediment exposure of siliciclastic (yellow shade) and carbonate (grey shade) SS from 6–18 h age. c) Survivorship and cocoon formation after exposure to a 12-h sediment exposure of siliciclastic (yellow shade) and carbonate (grey shade) SS from 12–24 h age. d) Larval emergence from the cocoon after exposure to 12-h sediment exposure (siliciclastic sediment only, n = 12 per time interval). Data points staggered for visualization. Each replicate contains 10–20 embryos.

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Impacts of suspended sediments on larval development and metamorphosis

There was no effect of either sediment type on survivorship or ability to metamorphose of >3-day-old larvae of *A. millepora*, even at very elevated SSC (~800 mg L^{-1}), and for extended exposure durations (~60 h) (Fig 5a). Similarly, no effect on survivorship was observed for *A. tenuis* using either sediment type (Fig 5b). Upon transferring to clean FSW, siliciclastic sediments at high concentrations caused a non-significant decrease in the ability of larvae to metamorphose (GLM: b = -0.182, t = -1.780, p = 0.085), and a similar non-significant trend was observed for larvae exposed to carbonate sediment (GLM: b = -0.1581, t = -1.540, p = 0.134)

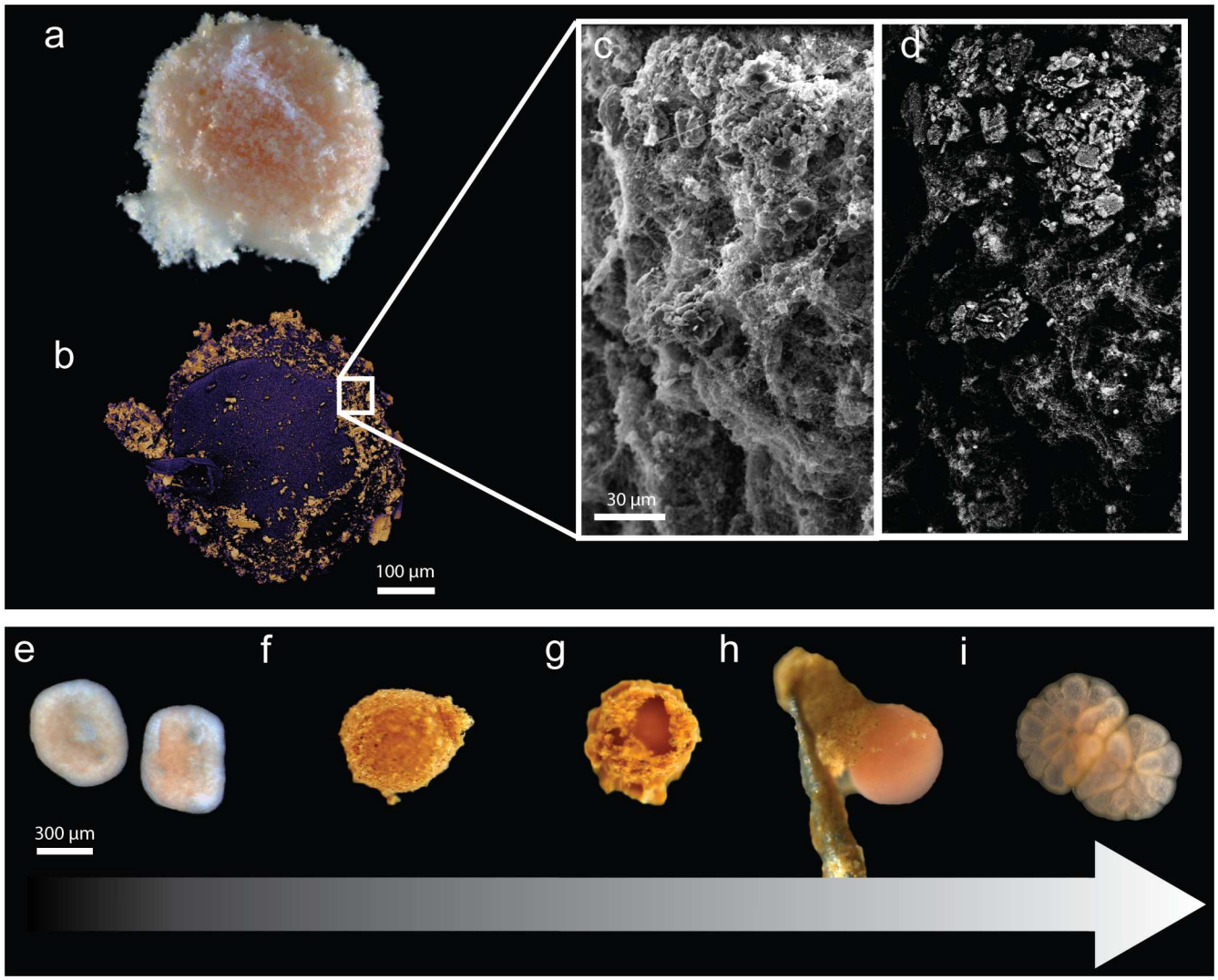

Fig 3. Microscopy of *A. millepora* embryos in mucous cocoons. a) a mucous cocoon under optical microscopy following exposure to carbonate sediment, b) a false-colored backscatter electron image of a scrapped mucous cocoon showing sediment (yellow) bound the embryo (purple), c) secondary electron image showing the mucous coating (high contrast) and d) backscatter electron image showing sediment grains (high contrast). Progression of mucous cocoons through development, e) early developmental stages (i.e. bowl stage at 12 h old) embryos before sediment exposure, f) mucous cocoons during sediment exposure (the orange color of the cocoon reflects the orange color of the siliciclastic sediment used), g) ciliated larva spinning and tearing open the cocoon, h) larva emerging from the cocoon (with assistance using a dissection probe for photograph), i) larvae (6 days old) were capable of metamorphosis once competent.

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(S2 Table, Fig 5b). The survivorship of *Pocillopora acuta* larvae was not affected by exposure of carbonate sediments up to ~900 mg L^{-1} (Fig 5c). Optical microscopy revealed larvae actively cleared sediment grains and florescent beads through cilia beating (Fig 6a and 6b) in addition to some mucous secretion (Fig 6b and S2 File) and scanning electron images revealed few sediment grains adhered to larvae (Fig 6c).

Water quality during turbidity-generating events

Turbidity peaks during dredging and natural events are very episodic, with turbidity remaining above 35 mg L^{-1} (the concentration required to form mucous cocoons) for a mean of 49 min (average of both sites) (S2 Fig). The time at these elevated concentrations was highly skewed to

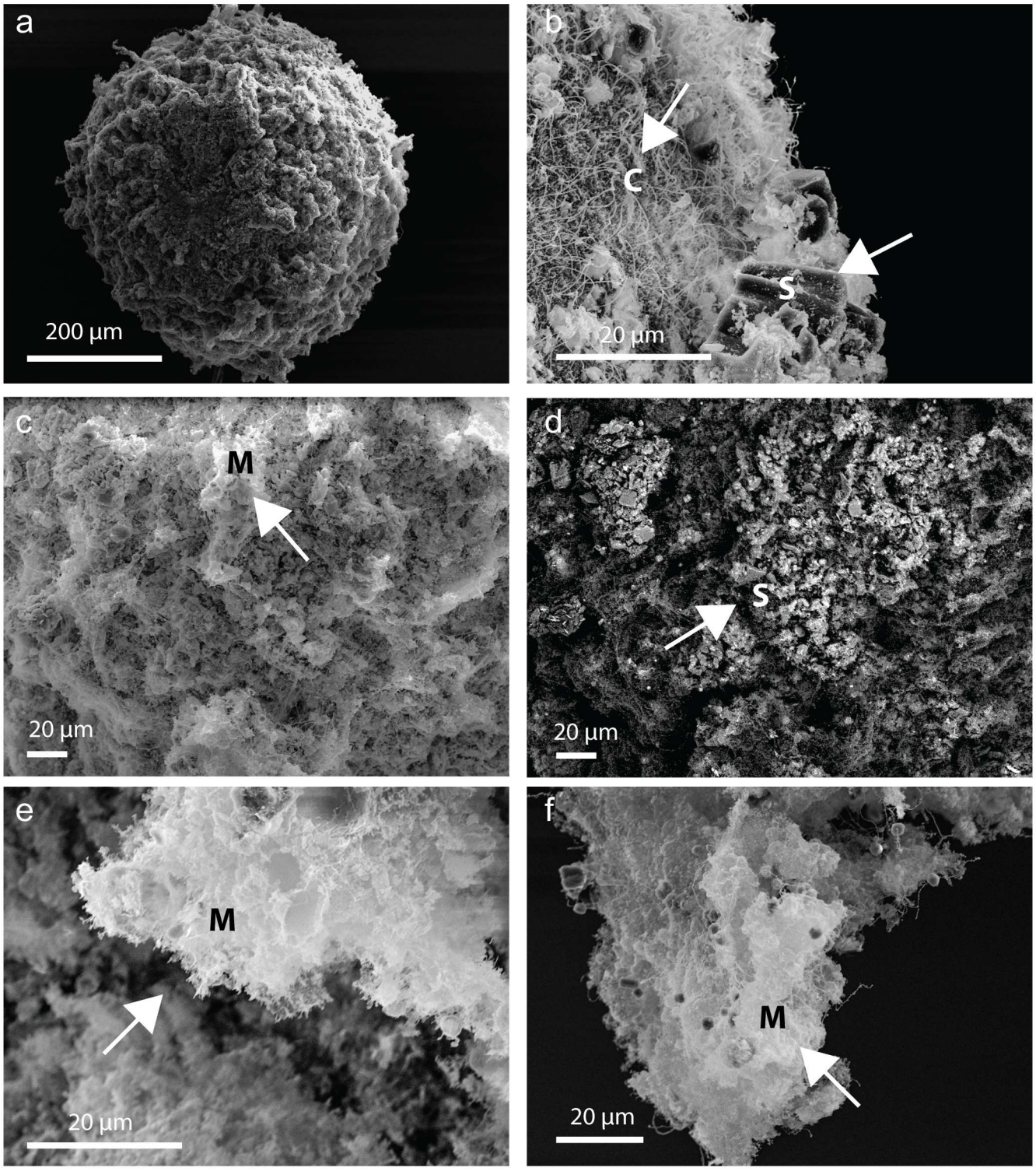

Fig 4. Scanning electron microscopy images of the mucous cocoon around an embryo of *Acropora millepora*. a) Image of the cocoon showing a thick mesh enveloping the embryo. b) With part of the cocoon removed, cilia can be observed developing underneath. A closer inspection of the cocoon showing c) a stringy web interpreted as mucus and d) bound sediment grains clearly revealed under backscatter electron microscopy. e-f) Thick protrusions of mucus could be seen throughout parts of the cocoon.

doi:10.1371/journal.pone.0162743.g004

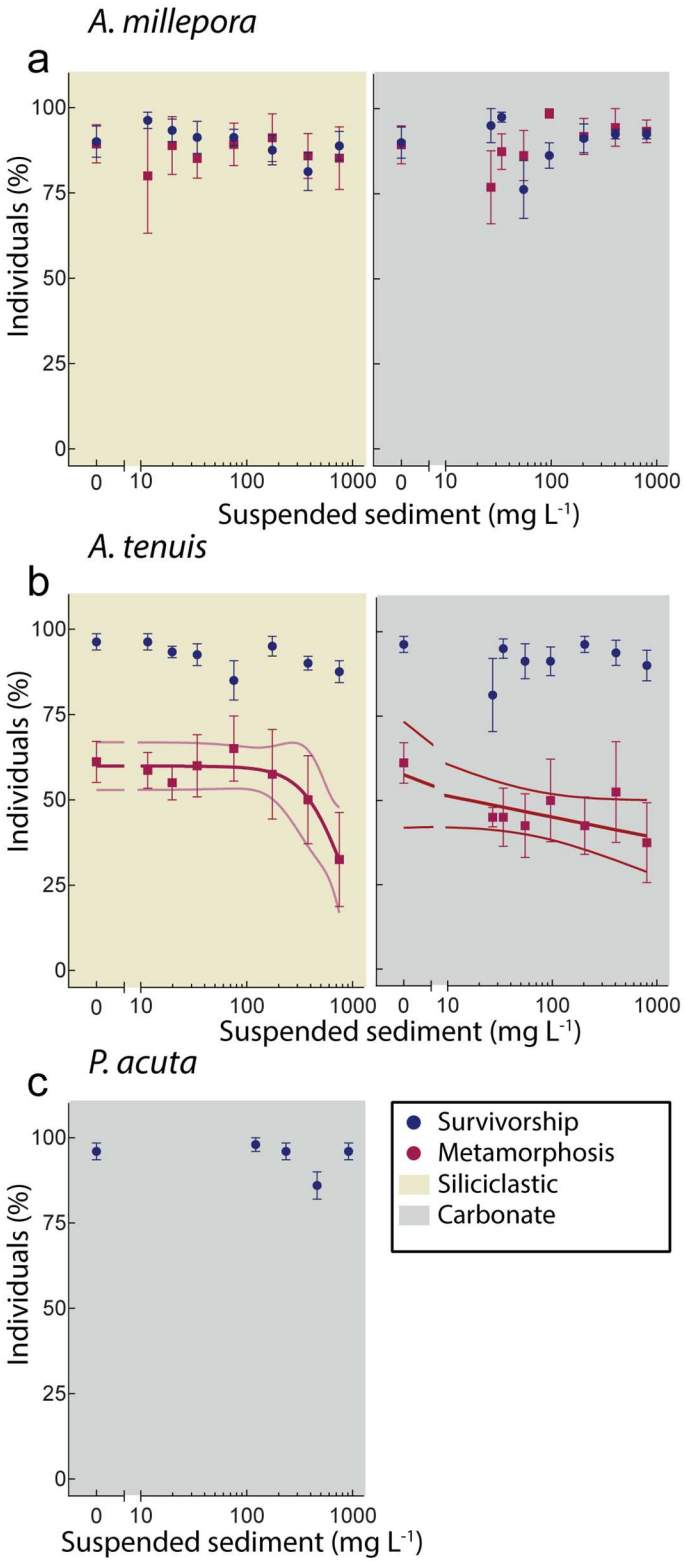

Fig 5. Concentration-response relationships between suspended sediments (SS) and larval survivorship and ability to undergo metamorphosis. a) Survivorship and metamorphosis of > 3-day-old larvae *A. millepora* following sediment exposure to siliciclastic (yellow shade) and carbonate (grey shade) sediment. c) Survivorship and metamorphosis of > 3-day-old larvae *A. tenuis* following sediment exposure to

siliciclastic (yellow shade) and carbonate (grey shade) sediment. e) Survivorship of *P. acuta* following sediment exposure to carbonate sediment. Data points staggered for visualization. Each replicate contained 10–20 larvae, with n = 4–5 per concentration.

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brief peaks lasting <1.5 h (~80%). In total, there were 595 turbidity events above 35 mg L^{-1} at Site 1 and 456 turbidity events at Site 2 over the course of the year.

Discussion

Coral embryos and larvae are relatively resilient to elevated concentrations of SS, employing impressive protective strategies such as mucous production and cilia beating to assist in the removal or avoidance of sediments. A novel mechanism was observed which involved the encapsulation of embryos in a sediment–mucous layer under dynamic resuspension conditions, with the planula shedding the cocoon once ciliated. There was no obvious legacy of damage impacting further larval development and metamorphosis under realistic sediment exposure concentrations and durations.

Suspended sediments in dredge plumes constitute a hazard to developing embryos and coral larvae, but whether they constitute a risk depends on the SSCs generated, the probability of encountering those conditions and the sensitivity of embryos and larvae to the sediments [17, 38]. Recent analyses of temporal and spatial patterns in water quality during several large-scale capital dredging programs have emphasized the very high variability in SSCs and the transient natures of plumes (S2 Fig) [13, 14]. Close to dredging, concentrations can reach hundreds of mg L^{-1}, but these high values are typically short-lived events (i.e. a few hours). Over time periods which are more relevant for the planktonic phase of coral larvae (i.e. days), the upper percentiles of SSCs are typically a few tens of mg L^{-1} [6]. These water quality values were derived from fixed optical backscatter nephelometers, measuring turbidity as a proxy for SSCs in passing plumes. Coral embryos and larvae could encounter and drift with highly turbid plumes and may therefore be subject to high concentrations for longer durations than recorded by fixed devices. Taking a conservative approach, embryos and larvae were exposed to very high SSCs (up to ~800 mg L^{-1}) over periods of several days. Even under these high conditions, and in response to two very different sediment types, there was no obvious effect on survivorship of embryos and ciliated larvae of two broadcast spawning *Acropora* spp. and mature planulae of the brooding species *Pocillopora acuta*. Importantly, there was also no subsequent impact of the sediments on the ability of the larvae to metamorphose when transferred to sediment-free clean seawater.

For the brooded larvae, these results support the earlier study of Te [36], who also did not see any effects on survivorship in larvae of *P. damicornis* at SSCs of up to 1,000 mg L^{-1}. However, the results differ from the study of Gilmour [10], who found effects on survivorship as low as ~50 mg L^{-1} in the broadcast spawning species *Acropora digitifera*. As discussed in Jones et al. [17], there may have been a range of water quality issues associated with the incubation chambers used in the study, in particular the possibility of stagnation and reduced water exchange caused by the suspended sediment and high larval concentrations. Preliminary studies with the *Acropora* species used here, indicated that if water was not exchanged regularly with FSW (i.e. every 12 h), mortality of a few larvae quickly resulted in the loss of all remaining larvae in the chamber (Ricardo personal observation). Larsson et al. [37], who reported effects on larval survivorship of the deep-water coral *Lophelia pertusa* at low concentrations (25 mg L^{-1}), also reported issues with their methodology including handling stress and low sample size. In their opportunistic study (from an unexpected spawning event), many larvae disappeared on the first day of the study and their analysis was based on changes in larval survivorship from one day

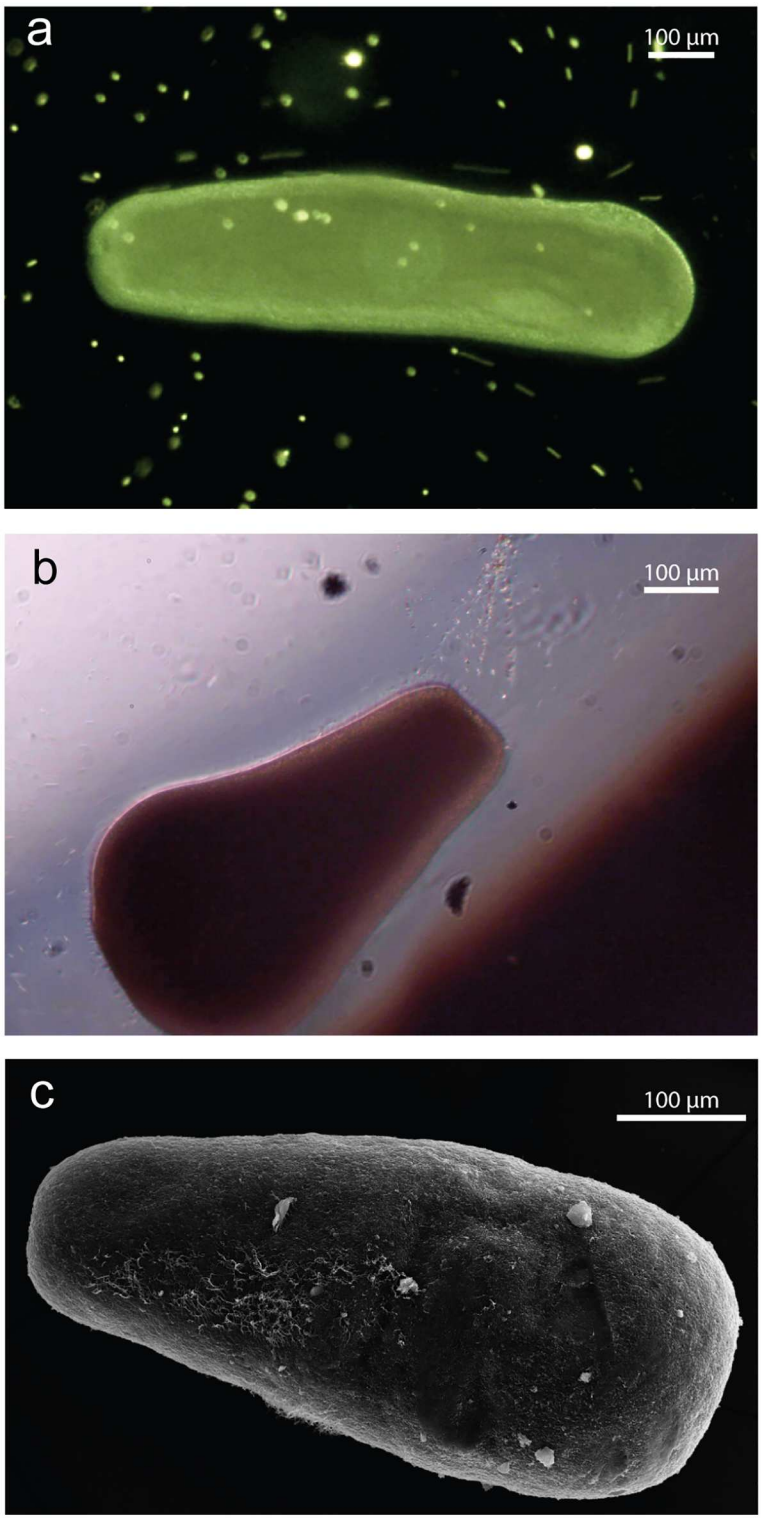

Fig 6. Observations of sediment removal mechanisms of *A. millepora* larvae under optical and scanning electron microscopy. a) florescence microscopy of a larva deflecting florescent beads through cilia beating b) optical microscopy of a larva clearing sediment through mucous production, and c) scanning electron microscopy of a larva after being exposed to elevated SS for 60 h showing few grains adhering to its surface.

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after adding the larvae until the end of the 5-day experiment. The authors emphasized the need to conduct more extensive assessment of the effects of sediment exposure to examine the reproducibility of the pilot study. We suggest that conditions listed above may have led to an overestimation of the sensitivity of the larvae to SS. However, the sediments used in the current study were relatively nutrient-poor, relevant to those found along the Western Australian coastline or beneath the substrate biofilm layer. Sediments occurring in nutrient-rich waters, or in combination with other stressors, may have a greater impact on the pelagic stages. Further, differences between species in terms of energy reserves or ability to remove sediment grains may also lead to species-specific responses.

Our results indicate that coral embryos and larvae may be less sensitive to elevated SSCs than other early life stages and processes. Here, there was no obvious decline in survivorship or larval metamorphosis after sediment exposure within realistic environmental concentrations. We previously demonstrated a potential 10% impact on coral bundle ascent at concentrations as low as 47 mg L^{-1} [11], whereas effects on fertilisation have been reported at SSCs as low as 35–100 mg L^{-1} [10, 11, 21]. Coral embryos and larvae appear well equipped to deal with elevated sediment particles, either repelling them by beating cilia, or removing them by mucous secretion, which represents a novel protection mechanism in coral larvae. Secretion of a mucosubstance was commonly observed throughout embryogenesis and larval stages during exposure to SS. In adult corals, mucous production is associated with feeding processes (i.e. mucous entrapment), and in conjunction with ciliary movement, for self-cleaning and manipulating sediments that have settled on the coral's surfaces [47, 48]. Both mucous production and ciliary movement have a latent, sub-lethal energetic cost [49] although the cost of cilia beating in corals is believed to be negligible compared with the energetic cost of respiration [50]. Overall the ciliary beating and mucous production did not translate to any obvious impairment of larval development or the ability of the larvae to metamorphose once transferred to sediment-free seawater.

Under elevated SSCs, the early developing embryos became covered in a layer of the mucosubstance that completely cocooned the embryo. The accumulation of sediment grains on the cocoon quickly sunk the embryo, typically within 2 h of exposure. Within the cocoon, the early-stage embryos underwent normal larval development, eventually forming cilia which resulted in their movement and spinning inside the casing. After the larvae were removed from sediment exposure, they were capable of shedding the cocoon and completing development into functional larvae and undergoing metamorphosis. Thus, cocoon formation around embryos appeared to act as a mechanism for protection, and removal of sediment in the absence of cilia. The formation and shedding of the cocoons adds to a number of ways mucus is utilised in marine organisms. Mucous cocooning has previously been observed in some fish of the suborder Labroidei, as a means to protect against parasites and predators [51, 52], and mucous secretion is a common physiological process in adult corals as a response to stress including exposure to sediment [6, 53]. Some adult colonies of the genus *Porites* can occasionally form thick, viscid 'sheets' of mucus on their surfaces, which can ultimately envelope the whole colony [54, 55], and capture sediments, algae and debris [56]. The mucous sheet eventually sloughs off the colony's surface by water movement, thereby removing the sediment [53, 55]. However, the use of mucus during early life history stages is less understood. During broadcast spawning events, coral egg-sperm bundles are wrapped in a mucous membrane that packages the gametes and it is hypothesized the mucus is secreted from the eggs [57]. Previously, we demonstrated that sediment grains can bind to the bundle membrane and coarse grains may sink and delay the egg-sperm bundle from reaching the surface where fertilisation takes place [11]. In some corals, embryogenesis occurs close to the surface of the adult coral, usually trapped within a mucous matrix [24, 58, 59]. The matrix was described as adhesive and

adhered to objects it contacted [58]. At the other end of the pelagic life-history stage, mucous secretion is hypothesized to aid in the attachment of the larva to substrata during settlement [53].

In this study, cocooning was commonly observed during the embryo stage but few, if any, were observed once the larvae were ciliated and neither was cocooning observed earlier on eggs exposed for 3-h to SS (S3 Fig). Therefore, it is unlikely the cocoon is a remnant part of the bundle mucous membrane. Recently, a hyaline layer (which assists in cellular orientation of embryos during development) has been proposed for one species of coral *Tubastraea coccinea*, but this has yet been identified in other species [60]. At these very early stages of development, it is unlikely mucous-producing cells have been formed, and in *Acropora millepora* these tend to increase in numbers typically after ~170 h [26]. An attempt to stain the cocoon with Alcian Blue (which stains acidic polysaccharides) was unsuccessful but may have failed because coral mucous composition can vary in carbohydrate, proteins and lipids [53, 61]. Another possibility is that the mucosubstance secretes directly from the ectoderm.

Mucous cocoons formed on 10% of embryos in the presence of siliciclastic SSCs as low as ~35 mg L^{-1}. Despite the average sediment concentration near dredging operations remaining elevated over longer periods [13], SS pulses >35 mg L^{-1} were usually short in duration, often lasting < 1h. Sharp decreases in SSCs between sediment pulses may offer larvae in mucous cocoons a brief reprieve to split and emerge from the cocoon. If, however, the sediment concentration remains elevated or sediment deposition rate is high, the embryos or larvae could remain entrapped and smothered. Cocoons created in response to carbonate sediments required greater concentrations of SS, probably owing to these sediments being less abrasive and sticky in nature. In contrast, only 20 mg L^{-1} of highly sticky bentonite clay caused all embryos to form mucous cocoons. With the exception of bentonite clay, embryo mucous cocoons only formed under constant agitation, and especially under unidirectional movement. Therefore, naturally suspended particles in the absence of water movement is unlikely to create mucous cocooning and locations with high agitation such as inshore wave-swept shorelines that have abrasive or sticky components in the sediment may be necessary to activate this response.

The formation of sediment-mucous cocoons has implications for the larval dispersal stage of the coral lifecycle. Most dispersal between reefs is through self-recruitment (philopatric), but larvae are capable of travelling considerable distances (teleplanic), which may increase genetic diversity and assist in the transition of coral populations to higher latitudes as water temperatures increase [30, 62, 63]. Many larvae of broadcast spawning corals become competent and recruit between 4–10 days [17], and the sinking of the embryos in sediment-mucous cocoons in conditions of high turbidity may reduce the pelagic phase by 1–2 days, restricting dispersal, and therefore may limit the ability of distant reefs to recover from disturbances. Further, these larvae may be restricted to settlement in unfavorable areas near their natal reef, which may be subject to ongoing elevated sediment levels. Other cause-effect pathways may affect embryo and larval dispersal phases that were not investigated in the study. Reductions in light associated with SS may confuse phototactic responses of larvae, entrainment of circadian rhythms [64] and the combined impact of downward sediment flux and increased cilia beating (to deflect sediment grains) may interfere with vertical positioning in the water column, ultimately impacting on dispersal and settlement [17]. Moreover, larval exposure to sediment may carry a legacy of impact on life-history stages beyond settlement. However, assessing the consequences of these impacts in both the laboratory and the field remains a challenge.

Conclusion

Newly developing embryos and ciliated larvae were robust to high SSCs used in this study, with no impact on larval survivorship or their ability to metamorphose observed. Combined, both life-history stages demonstrated an ability to remove sediment grains and tolerate high sediment loads. Therefore, SS related risks to the embryo and larval stages should be considered of lower concern when compared with more sediment-sensitive life-history stages such as fertilisation and settlement [11, 22, 23], which bracket the pelagic stages.

Supporting Information

S1 Fig. Survivorship, cocoon formation and settlement of 12-h-old embryos after 12-h exposure to siliciclastic sediment. Survivorship, cocoon formation and settlement of a) *Acropora millepora* and b) *Acropora tenuis* embryos. Settlement was assessed after the ciliated larvae emerged from the cocoon and had developed until competency. No settlement data were presented for *A*. *tenuis* because of insufficient rates in the control.
(TIF)

S2 Fig. The duration of NTU-derived suspended sediment concentrations remaining above 35 mg L^{-1} at two water quality monitoring sites ~300 m from a major capital dredging program (~7.6 M m^3 of sediment dredged over 530 d at Barrow Island. a) Site 1 was located 300 m north and b) Site 2 located 300 m south. Dredging operations were suspended for 12 days from 20–31 March 2011 for the coral spawning environmental window and for a few days associated with the close proximity of cyclones Bianca, Dianne and Carlos.
(TIF)

S3 Fig. Microscopic examination of the *Acropora tenuis* eggs after 3-h sediment exposure.
(TIF)

S1 File. Larva of *A*. *millepora* spinning in a mucous cocoon shortly before emerging.
(MP4)

S2 File. Larva of *A*. *millepora* deflecting sediment grains and producing mucus.
(MP4)

S3 File. Raw data for experiments conducted in the study.
(XLSX)

S1 Table. Sites and dates of coral collections.
(DOCX)

S2 Table. Summary table of experiments with a >10% decline in response compared to the control.
(DOCX)

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Supervision: APN RJJ PLC.

Validation: GFR.

Visualization: GFR PLC.

Writing – original draft: GFR.

Writing – review & editing: GFR RJJ APN PLC.

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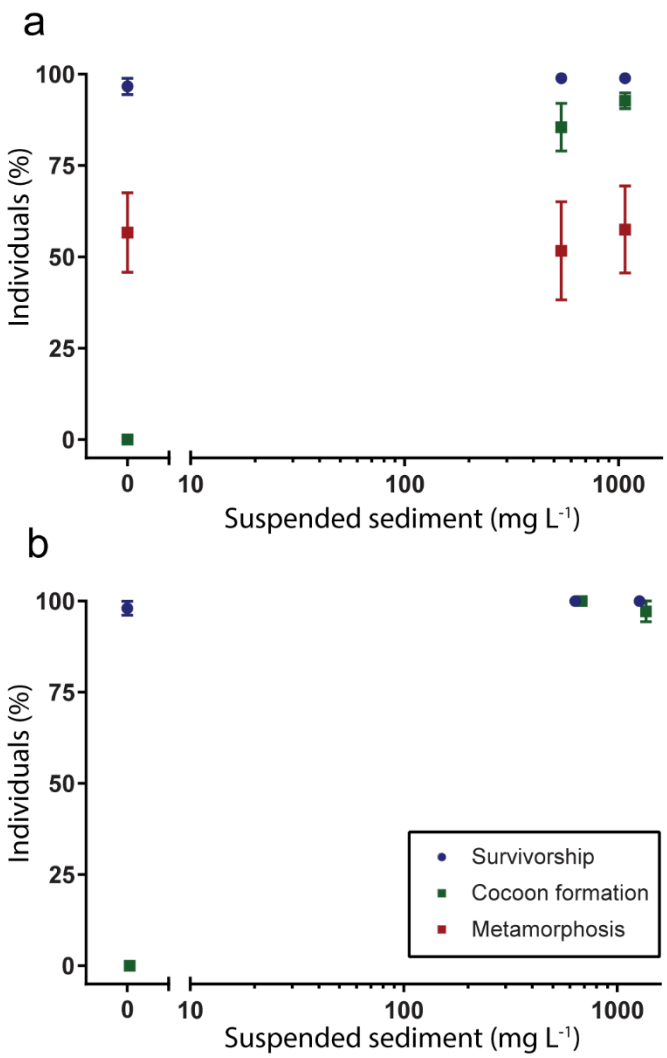
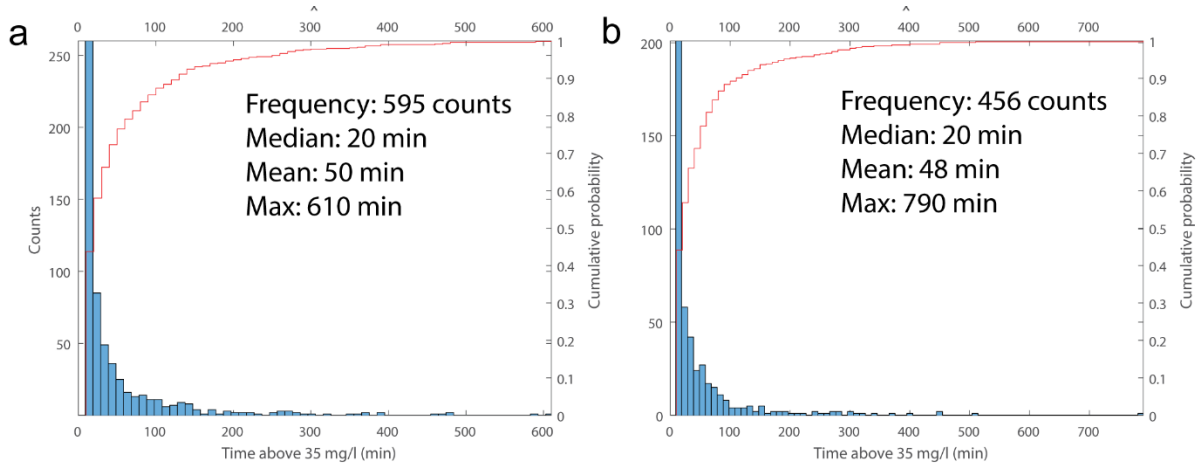
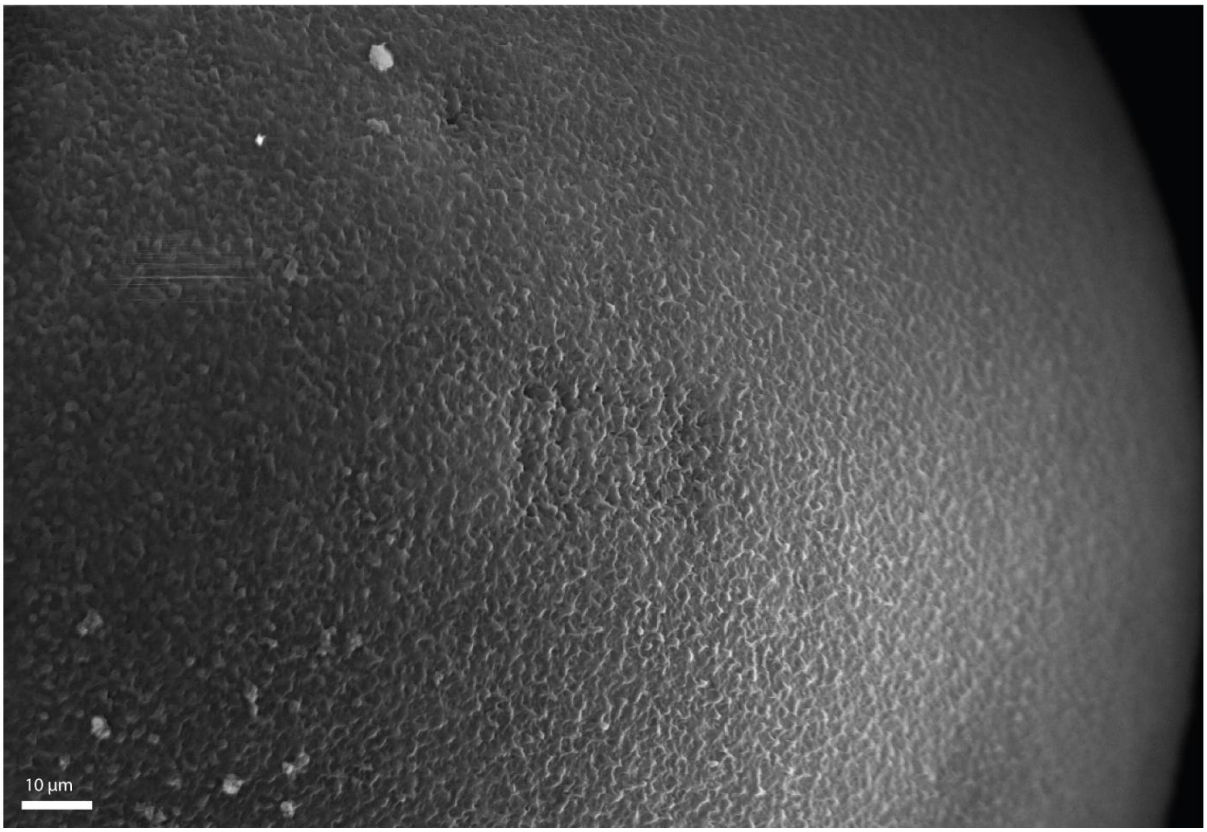
Supporting Information



S1 Fig. Survivorship, cocoon formation and settlement of 12-h-old embryos after 12-h exposure to siliciclastic sediment. Survivorship, cocoon formation and settlement of a) *Acropora millepora* and b) *Acropora tenuis* embryos. Settlement was assessed after the ciliated larvae emerged from the cocoon and had developed until competency. No settlement data were presented for *A. tenuis* because of insufficient rates in the control.



S2 Fig. The duration of NTU-derived suspended sediment concentrations remaining above 35 mg L^{-1} at two water quality monitoring sites ~300 m from a major capital dredging program (~7.6 M m^3 of sediment dredged over 530 d at Barrow Island. a) Site 1 was located 300 m north and b) Site 2 located 300 m south. Dredging operations were suspended for 12 days from 20–31 March 2011 for the coral spawning environmental window and for a few days associated with the close proximity of cyclones Bianca, Dianne and Carlos.



S3 Fig. Microscopic examination of the *Acropora tenuis* eggs after 3-h sediment exposure.

S1 Table. Sites and dates of coral collections.

Collection sites	Latitude/Longitude	Location	Species collected	Dates
Magnetic Island	(19°10'13 S, 146°51'53 E)	Central, inshore	*Acropora tenuis*	21 October 2013 6 October 2014
Trunk Reef	(18°22'53 S, 146°47'43 E)	Central, mid-shelf	*Acropora tenuis*, *Acropora millepora*	14–17 November 2013, 3 November 2014, 10 December 2014, 19–21 November 2015
Davies Reef	(18°49'12 S, 147°39'21 E), (18°48'48 S, 147°39'26 E)	Central, mid-shelf	*Acropora tenuis*, *Acropora millepora*, *Pocillopora acuta*	12 February 2014 (*P. acuta* only), 10 December 2014, 19–21 November 2015
Esk Reef	(18°46'25 S, 146°31'07 E)	Central, mid-shelf	*Acropora tenuis*, *Acropora millepora*	26–27 October 2015

S2 Table. Summary table of experiments with a >10% decline in response compared to the control.

Experiment	EC10 ± 95% CI (mg L^{-1})	EC20 ± 95% CI (mg L^{-1})	EC50 ± 95% CI (mg L^{-1})	P-value
Ability of *A. millepora* to metamorphose after embryos exposed to siliciclastic SS (Fig 2a)	NLR: N/A	NLR: N/A	NLR: N/A	N/A
	GLM: 0.87 (0.10 – 246)*,**	GLM: 7.0 (0.10 – 476)*	GLM: N/A	0.204
Cocoon formation of *A. millepora* after embryos exposed to siliciclastic SS (Fig 2c)	NLR: 35 (20 –55)	NLR: 57 (38 – 81)	NLR: 134 (104 – 173)	N/A
	GLM: 40 (28–57)	GLM: 59 (44 – 80)	GLM: 129 (103 – 162)	<0.001
Ability of *A. tenuis* to metamorphose after larvae exposed to siliciclastic SS (Fig 4b)	NLR: 300 (1 – N/A)*	NLR: 431 (1 – N/A)	NLR: N/A	N/A
	GLM: 9.6 (1.0 – 163)*,**	GLM: 83 (9.0 – 764)*	GLM: N/A	0.085
Ability of *A. tenuis* to metamorphose after larvae exposed to carbonate SS (Fig 4b)	NLR: N/A	NLR: N/A	NLR: N/A	N/A
	GLM: 8.3 (0.2 – 298)*,**	GLM: 69 (6 – 793)*	GLM: N/A	0.134

*Denotes when EC_x value occurs within 95% CI of the control. ** Denotes an effect size within the minimum detection limit. N/A Denotes when a model could not be fitted, or the EC_x value occurs outside of the range of the concentrations tested.

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Settlement patterns of the coral *Acropora millepora* on sediment-laden surfaces

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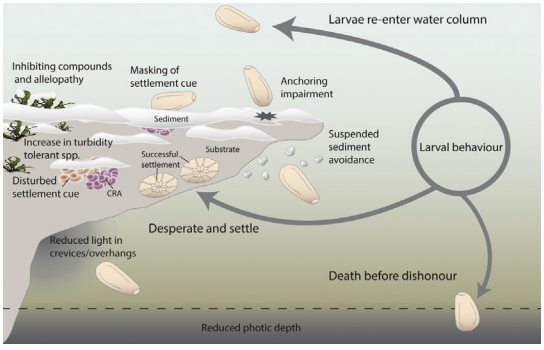
HIGHLIGHTS

- Very low levels of deposited sediment impact coral settlement behaviour.
- Larvae avoid sediment-covered substrates, but will settle nearby (e.g. downward facing surfaces).
- Deposited sediment also decreases the effectiveness of crustose red algae to induce settlement.
- There was no evidence of light intensity impacting settlement under realistic conditions.
- These results have implications for natural and anthropogenic sediment deposition events.

GRAPHICAL ABSTRACT

A conceptual diagram showing possible coral settlement behaviour and cause–effect pathways in response to sediment stressors, such as suspended sediments, deposited sediment and reduced light.



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ABSTRACT

Successful recruitment in corals is important for the sustenance of coral reefs, and is considered a demographic bottleneck in the recovery of reef populations following disturbance events. Yet several factors influence larval settlement behaviour, and here we quantified thresholds associated with light attenuation and accumulated sediments on settlement substrates. Sediments deposited on calcareous red algae (CRA) directly and indirectly impacted coral settlement patterns. Although not avoiding direct contact, *Acropora millepora* larvae were very reluctant to settle on surfaces layered with sediments, progressively shifting their settlement preference from upward to downward facing (sediment-free) surfaces under increasing levels of deposited sediment. When only upward-facing surfaces were presented, 10% of settlement was inhibited at thresholds from 0.9 to 16 mg cm^{-2} (EC_{10}), regardless of sediment type (carbonate and siliciclastic) or particle size (fine and coarse silt). These levels equate to a very thin (<150 μm) veneer of sediment that occurs within background levels on reefs. Grooves within settlement surfaces slightly improved options for settlement on sediment-coated surfaces (EC_{10}: 29 mg cm^{-2}), but were quickly infilled at higher deposited sediment levels. CRA that was temporarily smothered by sediment for 6 d became bleached (53% surface area), and inhibited settlement at ~7 mg cm^{-2} (EC_{10}). A minor decrease in settlement was observed at high and very low light intensities when using suboptimal concentrations of a settlement inducer (CRA extract); however, no inhibition was observed when natural CRA surfaces along with more realistic diel-light patterns were applied. The low deposited sediment thresholds indicate that even a thin veneer of sediment can have consequences for larval settlement due to a reduction of optimal substrate. And while grooves and overhangs provide more settlement options in high deposition

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areas, recruits settling at these locations may be subject to ongoing stress from shading, competition, and sediment infilling.

1. Introduction

1.1. Sedimentation on coral reefs

Sedimentation in tropical ecosystems poses a threat to the persistence of coral reefs through direct impacts on existing populations and by reducing recovery following disturbance events (Hodgson, 1990; Weber et al., 2012). Sediments can be released into the water column by terrestrial run-off (Kroon et al., 2012; Fabricius et al., 2016), natural resuspension events (Orpin and Ridd, 2012), and a range of anthropogenic activities including dredging and dredge spoil disposal (Jones et al., 2016). Once released into the water column, fine sediments can remain in suspension for extended periods and travel considerable distances via advection (Wolanski and Spagnol, 2000; Bainbridge et al., 2012; Fisher et al., 2015). Sediments will also settle out of suspension depending on suspended sediment concentration (SSC), grain size, density, ability to flocculate, and hydrodynamics of the water column (Smith and Friedrichs, 2011). Compaction and consolidation of recently settled sediments takes days to weeks (Wolanski et al., 1992), and until consolidated, sediments are prone to successive resuspension and deposition and further lateral dispersion. While the impact of sediment deposition on adult corals has been reasonably well studied (see reviews Erftemeijer et al., 2012, and Jones et al. (2016)), comparatively less is known about how sediment accumulating onto substrates may interfere with coral settlement, and the recolonisation of local populations in turbid environments.

1.2. Coral recruitment

The coral recruitment process is a complex sequence that involves larval supply, settlement behaviour, successful attachment and metamorphosis, and post-settlement survival (Harrison and Wallace, 1990). Successful recruitment is dependent on a suite of physical and biological factors (Ritson-Williams et al., 2009; Doropoulos et al., 2016), and referred to by Gleason and Hofmann (2011) as a '…dizzying array of abiotic and biotic factors, both positive and negative, that can determine whether a coral larva ultimately ends up on the reef…'. The larvae are weak swimmers predominantly relying on currents for dispersal (Baird et al., 2014). When they reach developmental competence (the ability to settle), they descend in the water column and temporarily enter a demersal phase, when they actively and repeatedly test, probe and explore the substrate presumably searching for some characteristic properties to indicate a favourable settlement location (Müller and Leitz, 2002). Settlement for many species is induced by chemical cues, often associated with calcareous red algae (CRA — which includes crustose coralline algae (CCA) and non-coralline red algae) (Heyward and Negri, 1999; Tebben et al., 2015), and/or microbial biofilms (Webster et al., 2004). For most larvae of broadcast spawning corals, competency occurs after only a few days development (Connolly and Baird, 2010), with settlement peaking between 4 and 10 d after spawning (Jones et al., 2015). Observations of attachment through mucous production and firing of spirocysts or nematocysts have been reported (Paruntu et al., 2000; Harii and Kayanne, 2002; Okubo et al., 2008; Larsson et al., 2014), and once attached the larvae metamorphose by flattening into disc-shaped structures with septal mesenteries radiating from the central mouth region (Heyward and Negri, 1999). Early recruits are small, initially <1 mm in diameter, and vulnerable to grazing, overgrowth and smothering from sediment for the ensuing 12 months (Rogers, 1990; McCook et al., 2001; Jones et al., 2015; Moeller et al., 2016).

1.3. Cause-effect pathways

Many studies have shown correlations between low recruitment success and sediment in situ although the specific mechanism(s) or cause-effect pathway(s) underlying this correlation is not known (Wittenberg and Hunte, 1992; Dikou and Van Woesik, 2006; Salinas-de-León et al., 2013; Jokiel et al., 2014; Bauman et al., 2015). A range of established and also biologically plausible mechanisms (i.e. where there is a credible or reasonable biological and/or toxicological basis linking the proposed cause and effect) has recently been described in Jones et al. (2015). These mechanisms, which are based on larval behaviour, chemotaxis, and physical characteristics of the substrate have been expanded upon in Fig. 1, and include: 1) avoidance of small non-consolidated grains that prevent attachment or access to suitable underlying substrates (Harrigan, 1972; Perez et al., 2014); 2) masking, obscuring or deterioration of chemical settlement cues by sediment (Harrington et al., 2005); 3) the production of inhibitory chemicals from sediment-tolerant organisms (Quéré and Nugues, 2015; Morrow et al., 2017); and 4) changes in the quality and quantity of light (Mundy and Babcock, 1998; Fabricius et al., 2016).

In the presence of these inhibitory factors, or absence of cues to stimulate settlement, coral larvae may either continue to seek a more suitable substrate until lipid reserves become depleted and death occurs (colloquially referred to as '*death before dishonour*' – see Fig. 1) (Raimondi and Morse, 2000; Bishop et al., 2006), or possibly re-enter the plankton to seek a more suitable reef. The larvae may also settle onto sub-optimal microhabitats (colloquially referred to as '*desperate larva hypothesis*'), which may have subsequent consequences for the recruits, juveniles and/or adult stages, including increased competition, light-limitation or sediment smothering (Baird and Hughes, 2000; Doropoulos et al., 2016; Moeller et al., 2016).

1.4. Concentration–response thresholds

The effects of sediments on coral settlement have been investigated in several different ways, including examining responses to suspended sediment i.e. sediments kept in suspension expressed as mg L^{-1} (Te, 1992; Gilmour, 1999), accumulating sediments i.e. to a continual downward flux (deposition) of sediment expressed in mg cm^{-2} d^{-1} (Babcock and Davies, 1991; Babcock and Smith, 2002), or accumulated sediment i.e. sediments that have settled on surfaces and expressed as mg cm^{-2} (Hodgson, 1985; Perez et al., 2014). Often only one of these measurements is reported when several cause–effect pathways could be co-occurring, complicating the interpretation of the reported thresholds (Jones et al., 2015). Despite this, there is clear evidence that SSCs have a limited impact larval settlement (Babcock and Davies, 1991; Te, 1992; Humanes et al., 2017), indicating that sediment depositing and accumulating on surfaces may represent more significant cause–effect pathways (Babcock and Davies, 1991).

The aim of the present study was to experimentally determine and quantify the effects of accumulated sediment that result directly and indirectly (via impact to the CRA) in changes to larval settlement patterns, and whether these thresholds were influenced by additional factors such as light intensity, surface structure and surface aspect. These thresholds may assist regulatory agencies assign improved guideline

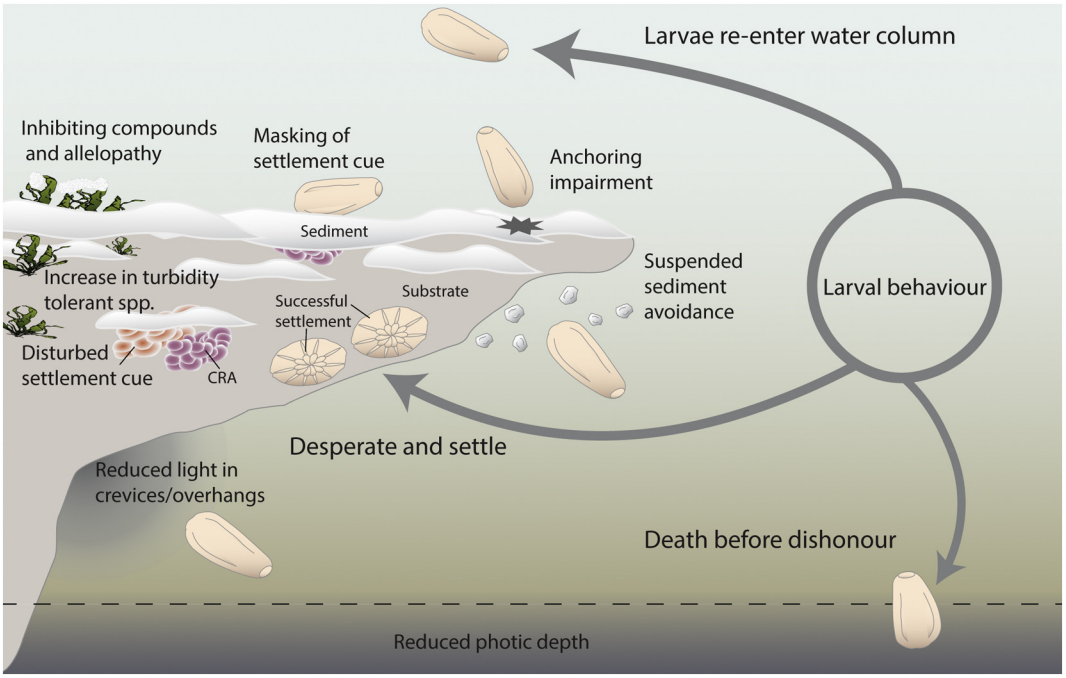

Fig. 1. A conceptual diagram showing possible coral settlement behaviour and cause–effect pathways in response to sediment stressors, such as suspended sediments, deposited sediment and reduced light. Potential mechanisms proposed in Babcock and Davies (1991), Babcock and Mundy (1996), Raimondi and Morse (2000), Birrell et al. (2005), Bishop et al. (2006), Doropoulos et al. (2016), and Morrow et al. (2017).

values around turbidity-generating activities such as dredging and land run-off near coral reefs, and are also crucial to interpret coral recruitment patterns in naturally turbid reefs.

2. Materials and methods

2.1. Coral collection and larval culture

All experiments were conducted at the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS, Queensland) using larvae cultured from colonies of *Acropora millepora* (Ehrenberg, 1834). Adult colonies were collected from <8 m depth from an inshore reef of the central Great Barrier Reef (Esk Reef: 18°46′ S, 146°31′ E) and from mid-shelf reefs (Davies Reef: 18°49′ S, 147°39′ E; Trunk Reef: 18°23′ S, 146°48′ E), GBRMPA Permit G12/35236.1. The gravid colonies were collected 3 to 5 days before the predicted night of spawning, transported to the SeaSim, and placed in outdoor flow-through filtered seawater (FSW) tanks (at temperatures equivalent to the collection sites, 27–29 °C). Setting colonies were isolated in individual tanks and spawning occurred ~2 h after sunset. Egg-sperm bundles from all colonies were gently scooped from the surface and cross fertilised for ~45 min in 20 L of 1 μm FSW. The embryos were washed free of sperm by transferring them into 20 L of new FSW. This process was repeated three times and the embryos then transferred into 500 L flow-through fiberglass tanks to undergo embryogenesis under static conditions. The following afternoon, gentle aeration and water flow was introduced. Water in the larval culture tanks and used in experiments were controlled to temperatures between 27 and 29 °C (equivalent to the current temperatures at the collection sites), salinity at 36 ppt, pH at 8.16, and dissolved oxygen remained above 90% saturation.

2.2. Substrate types and conditioning

Round aragonite and PVC settlement plugs (20 mm diameter discs) were used in the experiments. Aragonite plugs are commonly used in settlement assays, but PVC plugs can easily be modified to create surface structures that represent microhabitat complexity. Each PVC plug had

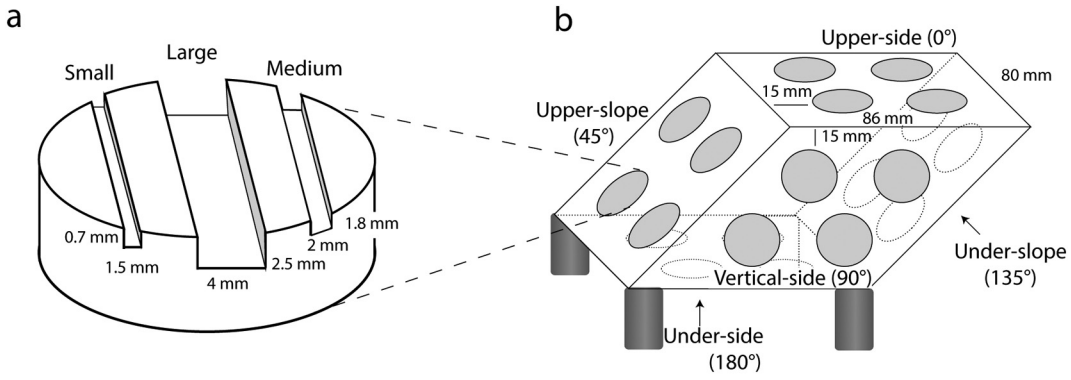

Fig. 2. Design of the PVC settlement plug and the settlement prism. a) geometry and dimensions of the settlement plug containing three parallel grooves (referred to as small, medium and large grooves), b) the settlement prism which holds the settlement plugs at five different aspects referred to as upper-side (0°), upper-slope (45°), vertical-side (90°), under-slope (135°) and under-side (180°). Grey circles are slots where the settlement plugs are positioned.

three horizontal grooves cut parallel across the surface, including a small groove (1.5 mm wide × 0.7 mm deep), a medium groove (2 × 1.8 mm) and a large groove (4 × 2.5 mm) (Fig. 2). Before use, the plugs were conditioned for ~3 months in seawater and developed a thick assemblage of calcareous red algae (CRA) dominated by the species *Titanoderma prototypum* (70–100% cover) and a smaller cover of *Peyssonnelia* sp. (0–30%). Both CRA types are effective settlement inducers for *A. millepora* larvae (Heyward and Negri, 1999; Harrington et al., 2004). While there was no obvious change in CRA composition between aragonite and PVC plugs, there was a small reduction in *Peyssonnelia* sp. cover across the three spawning seasons. In addition, a sub-optimal inducer for settlement was needed to test potentially subtle effects of light on settlement. Ethanol extracts of the crustose coralline algae *Porolithon onkodes* have been shown to induce settlement, and we applied sub-optimal concentrations of CCA extract (Heyward and Negri, 1999) in some tests to increase the sensitivity of the assay for this purpose (0.08% v/v of extract in the final test volume).

An oblique rectangular prism (two parallelogram-faces and four rectangular sides) was designed to assess settlement patterns on various surface aspects (Fig. 2 b). The PVC prism had five differently orientated surface planes; an upper-side (0°, relative to a horizontal plane), upper-slope (45°), vertical-side (90°), under-slope (135°), and underside (180°). Four plugs could be positioned within each plane.

2.3. Sediment types

Two marine sediment types were used in this study. Carbonate sediment, composed almost entirely of aragonite and calcite, was collected from Davies Reef, Great Barrier Reef, Queensland. Carbonate sediments are typical of reefs away from the influence of river systems. Siliciclastic sediment, composed mostly of silicates but also with notable fractions of iron (5% w/w) and aluminium (3% w/w), were collected from near Onslow, Pilbara, Western Australia. Mineral characteristics of the sediment can be found in Ricardo et al. (2015). Both sediments were milled, screened and dried to <63 μm. The sediments were then separated into two size classes, and the particle size distribution measured for each class using laser diffraction techniques (Mastersizer 2000, Malvern Instruments Ltd), yielding a final modal size of 16 μm and 48 μm. These values correspond to 'fine silt' and 'coarse silt' respectively (Udden–Wentworth scale). Each type of sediment had relatively low concentrations of total organic carbon (~0.3% w/w), comparable to that found in Western Australia (DEC, 2006), although sediment processing likely reduced the active microbial community.

River sand was additionally used in experiments to prevent the larvae from settling on the sides of settlement plugs. The river sand was sieved to >2 mm, autoclaved and washed in 0.4 μm FSW for a minimum of six days to ensure the sand did not affect water quality in settlement experiments.

2.4. Light intensity

Two light regimes were applied in the experiments; either a constant exposure of light, or a variable exposure that matches the change in light intensity throughout a day. *Light regime 1* (constant) had six light levels supplied for 12 h at a constant irradiance of 0.5, 5, 14, 47, 140, 470 photosynthetically active radiation (PAR, μmol photons m^{-2} s^{-1}). The 12-h period is relevant to the diurnal (day-time) period larvae may encounter upon approaching a reef. *Light regime 2* (variable) also had six light levels, but light levels increased from darkness to a midday peak (0.5, 5, 14, 47, 140, 470 μmol photons m^{-2} s^{-1}) then decreased linearly to darkness, corresponding to daily light integrals (DLI) of 0.01, 0.1, 0.3, 1, 3, 11 mol photons m^{-2} d^{-1}, typical of the DLI range found during autumn spawning in Western Australia (Jones et al., 2016). Light was applied using LED aquarium lights (Hydra, AquaIllumination), and ~12 h light/dark with light set to start and finish at local sunrise and sunset times. PAR was measured with an Apogee Quantum light sensor (MQ-100), and the values were corrected for LED blue-light, which made up most of the wavelengths (Fig. A.1). The lower light intensities were measured with a Jaz Spectrometer (Jaz-EL200).

2.5. Experiment 1 — deposited sediment and surface aspect

To assess whether coral settlement patterns on several differently oriented surfaces changed with increasing levels of deposited sediment, larvae were provided CRA-covered plugs embedded in different planes of the prism. Larvae only settled on the conditioned plugs, allowing settlement patterns to be clearly quantified. Different amounts of coarse carbonate silt (None, Very Low, Low, Medium, High, Very High) were added to the different 5-L aquaria and thoroughly mixed with FSW to create different SSCs. The sediment was left to settle overnight (~12 h), resulting in various deposited sediment levels on the upward-facing surfaces (0° and 45°) of prism. The deposited sediment levels on the upper-side horizontal surface (0°) corresponded to ~0, 5, 15, 30, 90, and 180 mg cm^{-2}. Five replicate aquaria were used per treatment in addition to a variable light regime that resulted in a DLI of 3 mol photons m^{-2} d^{-1} on the upper horizontal surface. Approximately 100 larvae were then added to each aquarium, and left for 24 h to choose a settlement site. The proportion of larvae settling on each surface aspect or not settled was then recorded, with the data for the four plugs per surface pooled. Sediment from all plugs were carefully removed (pooled per surface), filtered onto a 0.4 μm filter, dried at 60 °C for 24 h, and weighed to determine the level of deposited sediment for each surface.

2.6. Experiment 2 — deposited sediment on upward-facing surfaces

Settlement inhibition thresholds for deposited sediments of two sediment types (carbonate and siliciclastic) and two grain size fractions (fine and coarse silt) on the upper-surfaces were determined in 60 mL glass jars (Plasdene Glass-pak). Forty jars were used for each sediment type and grain size combination (i.e. carbonate fine, carbonate coarse, siliciclastic fine, siliciclastic coarse). For each trial, five replicates across eight deposited sediment treatments (0, 0.3, 1, 3, 10, 30, 100, 300 mg cm^{-2}) were used, with the control defined as plugs without deposited sediment (Fig. 3 a). These deposited sediment levels generally cover the range of amounts recorded in situ (Goatley and Bellwood, 2010; Tebbett et al., 2017).

Within each jar, a flat CRA-covered aragonite plug was embedded into ~1 cm river sand until the surface of the plug was flush against the sand surface. Pre-weighed sediments were mixed with 0.4 μm filtered seawater (FSW), added to each jar, and left to deposit on the plugs for ~6 h until the water appeared clear. Ten larvae were then transferred to each jar using disposable 1 mL plastic pipettes with care taken not to disturb the settled sediment. All jars were covered to prevent evaporation of water. After 24 h, the proportion of settlement was determined using the definition of metamorphosis described in Heyward and Negri (1999). To visually assess larval behaviour in the presence of sediment, larvae were photographed at 10 s intervals for ~2.5 h, and the images combined into a time-lapse. To compare flat plugs (as used above) with plugs containing grooves, the above experiment was repeated the following year using PVC plugs, in addition to coarse carbonate silt.

2.7. Experiment 3 — smothered CRA

A variation on Experiment 2 enabled us to assess whether temporary smothering of CRA impacted settlement (Fig. 3 b). Similar to methods described above, conditioned plugs with a complete surface-film of CRA were covered in 0, 0.3, 1, 3, 10, 30, 100, 300 mg cm^{-2} of coarse carbonate silt in 60 mL glass jars. Once the sediment had completely settled, the jars were placed in a 12-cm deep plastic tray with free-flow

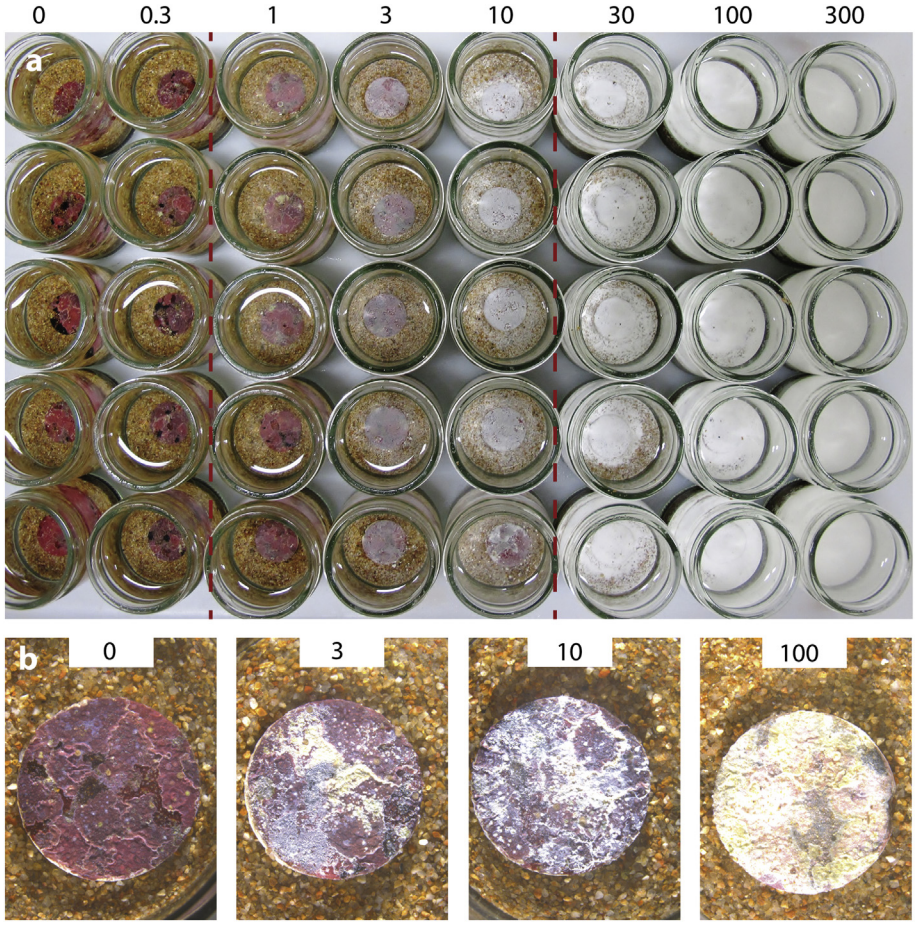

Fig. 3. Calcareous red algae settlement plugs used in the settlement assays. a) Experimental set-up of Experiment 2 for coarse-silt carbonate sediment at 0, 0.3, 1, 3, 10, 30, 100, 300 mg cm^{-2}. Red-lines indicate the approximate range of the EC_{10} among sediment types. b) Settlement plugs used in Experiment 3 after coarse-silt carbonate sediment was removed following 6-d exposure at 0, 3, 10, 100 mg cm^{-2}. White tissue indicates pigment loss.

of FSW added in such a way as to not agitate the sediment within the jars. The tray was kept under a DLI of 3 mol photons m^{-2} d^{-1}. After 6 days, sediments were washed off the plugs using FSW, then embedding in river-sand (as described previously) and settlement success tested using 10 larvae per jar.

During the assessment of settlement, each CRA-covered plug was photographed at a constant exposure and magnification. The proportions of healthy and bleached (white) CRA in 32-bit RGB images (TIFF) were measured using ImageJ (v1.49) (Schneider et al., 2012). The background edge-effects of the plug and areas not containing CRA (such as other algae, sand grains or coral recruits) were manually removed prior to analysis in all images. Images were converted to a 32-bit floating-point greyscale containing 255 pixel-intensity units. The threshold for 'healthy CRA' and 'bleached CRA' was set manually based on the control (not sediment exposed) plugs. Healthy CRA was defined as ranging between 1 and 170 intensity units (darker pixels), whereas bleached CRA ranged in pixel units between 170 and 255 intensity units (lighter pixels).

2.8. Experiment 4 — light intensity and surface structure

To determine if light intensity and surface structure affects larval settlement patterns, larvae were exposed to a realistic *Light regime 2* in addition to conditioned (CRA covered) PVC plugs containing grooves. Four plugs were placed in the upper-side (0°) of a prism (Fig. 2 b) in 5 L aquaria with four replicate aquaria per light treatment. Fifty larvae were then added to each aquarium and after 24 h the number of larvae that settled within each groove type was assessed. All aquaria were assessed to confirm larvae had only settled on the plugs.

2.9. Experiment 5 — light intensity

To determine if light intensity impacts larval settlement, 10 competent larvae (>4-d-old) were added to each well of 6-well tissue culture plates containing a sub-optimal settlement cue concentration (8 μL of crustose coralline alga extract) and 10 mL of FSW. Two 6-well plates (total of 12 replicate wells) were added to each light level of *Light regime 1* and settled larvae were counted after 12 h. This experiment was repeated with a separate culture of genetically different larvae using 18 replicate wells per light level (total number of replicate wells per treatment = 30).

2.10. Statistical analysis

In Experiment 1 and 4, data were initially fitted with a Poisson log-linear generalized linear mixed model (GLMM), which can be used as a surrogate model for multinomial data (Venables and Ripley, 2013), using R (v. 3.3.1). Overdispersion was then accounted for by refitting the data to a negative binomial mixed model (Zuur et al., 2009). Models were compared by adding and removing predictor variables, and assessed using likelihood ratio tests. Adding 'aquaria' as a random factor did not improve either model, so ultimately a negative binomial GLM was selected for both experiments. Experiment 1 was also fitted with Poisson generalized additive models (GAM) for easier visual interpretation by treating the predictor variable (the surface aspect) as

continuous. Additionally in Experiment 1, the overall decrease in settlement success in each sediment treatment scenario was compared with the control (no sediment), by combining the data for coral settlement at each surface aspect, and then analysed as a proportion of the total larvae added, using a logit-link binomial GLMM with 'aquaria' as a random factor (Zuur et al., 2009). The proportion of settlement success on the upward-facing surfaces in Experiments 1–3 were fitted with nonlinear regression four-parameter logistic models using GraphPad Prism (v.7). For each model, the bottom of the curve was constrained to 0% as best-fit line approached 100% inhibition. EC_{10} values (the deposited sediment level that causes 10% inhibition of settlement) were determined for each assay. EC_{50} values for each sediment type (carbonate, siliciclastic) and silt size (fine, coarse) were compared using global nonlinear regression with parameters shared if there was no evidence that they were different (Motulsky and Christopoulos, 2004). The assay comparing flat plugs with grooved plugs was analysed in the same way but one replicate sample was removed from the analysis because the CRA became rapidly diseased (identified as bright orange regions). In Experiment 5 (light intensity), the data from the two assays were combined and fitted with a quadratic-polynomial model.

3. Results

3.1. Experiment 1 — deposited sediment and surface aspect

In the control (no sediment) treatment, larvae preferentially settled on the two upward facing surfaces i.e. upper-side (0°): 38% (95% CI: 27–53%) and upper-slope (45°): 20% (14–29%) (Table A.1), compared to the downward facing surfaces. This settlement pattern progressively reversed under increasing deposited sediment levels with larvae generally changing their settlement preference from the upward-facing to the downward-facing surfaces (Fig. 4 a–f). No or very little sediment was observed on the vertical (or downward facing surfaces) and <10% of the larvae settled on the vertical sides regardless of sediment treatment. On the upper-side, deposited sediment at 69 (95% CI: 17–N/A) mg cm^{-2} was associated with a 10% decrease in settlement (EC_{10}), representing a change in settlement preference to less sediment covered sides (Fig. 5 b). Overall, there was no difference in settlement between the different sediment treatments when all larvae from all surfaces were counted (Fig. 5 a).

3.2. Experiment 2 — deposited sediment on upward-facing surfaces

There was rapid settlement directly onto the CRA covered plugs, with most larvae attaching and metamorphosing within 1 to 2 h (File B.1). Settlement on the upward-facing surfaces of the CRA-covered plugs was high (controls: 89 ± 1%). EC_{10} values (10% inhibition of settlement compared with the control) occurred in response to deposited sediments between 0.9 and 4.2 mg cm^{-2} (carbonate fine silt: 1.3 mg cm^{-2} (95% CI: 0.35–3.3); carbonate coarse silt: 2.9 mg cm^{-2} (1.2–5.8); siliciclastic fine silt: 4.2 mg cm^{-2} (2.4–6.4); siliciclastic coarse silt: 0.9 mg cm^{-2} (0.1–3.6)) (Fig. 6 a–d). These values equate to a fine film or veneer of sediment over the plug (Fig. 3 a). There were no significant differences between the EC_{50} values of any sediment type or particle size fraction ($F_{3,148} = 1.38$, $p = 0.251$). Larvae were observed avoiding areas high in sediment when there was sediment-free substrate nearby (File B.1).

When plugs with grooves were compared to flat plugs, there was a significant difference in sensitivity to deposited sediments (EC_{50}: $F_{1,91} = 9.86$, $p = 0.002$), and the EC_{10} increased (from 16 mg cm^{-2} to 29 mg cm^{-2}) because larvae could settle on the sides of the grooves until they were completely infilled with sediment (Fig. 6 e). Considering all sediment types, the mean of the EC_{10} values on the flat plugs was 5.1 mg cm^{-2}.

3.3. Experiment 3 — smothered CRA

The threshold for settlement inhibition (EC_{10}) on CRA plugs that were previously smothered by sediment for 6 d was 7.2 mg cm^{-2} (95% CI: 0.94–46) (Fig. 7 a), and CRA smothered to this extent was 53% (95% CI: 47–60%) bleached (Figs. 3 b, 7 b). The correlation between CRA bleaching and larval settlement was statically significant ($F_{1,38} = 28.01$, $p < 0.001$), and CRA bleaching explained 42% of the settlement variation.

3.4. Experiment 4 — light intensity and surface structure

Very few larvae settled outside of the parallel grooves (3 ± 1, mean ± SE), with the greatest number of larvae settling in the largest groove (13 ± 1). However, the largest groove had >3-fold larger surface area compared to the smallest groove, and when each groove was normalized by surface area, the greatest density of settled larvae were found to be on the smallest groove (21 ± 2 settlers cm^{-2}) (Fig. 8 a). Based on AICs, there was no effect of light-intensity ($\chi^2 = 0.841$, $p = 0.359$) under a realistic diurnal cycle (*Light regime 2*), and therefore the final model only contained surface structure as a factor.

3.5. Experiment 5 — light intensity

Settlement success peaked (70%) at 6.9 μmol photons m^{-2} s^{-1} (95% CI: 1.6–27) following settlement induction by sub-optimal concentrations of CCA extract. A 10% settlement decrease (EC_{10}) occurred at low light 1.4 μmol photons m^{-2} s^{-1} (95% CI: 0.7–3.4) and high light 33 μmol photons m^{-2} s^{-1} (95% CI: 12–67) (Fig. 8 b).

4. Discussion

4.1. Summary

Previous research has suggested that sediment impacts coral recruitment more than adult stages (Fabricius, 2005; Erftemeijer et al., 2012; Jones et al., 2015), and this study formally quantified the impacts of deposited sediment and light intensity on larval settlement. Larvae preferred to settle on surfaces that are virtually free of sediments, and that deposited sediment loads of ~5 mg cm^2, which is equivalent to only a thin (~<150 μm) veneer of silt-sized sediment, can influence settlement preferences. The study also shows that settlement of *A. millepora* larvae is reduced if surfaces that are attractive for settlement (calcareous red algae, CRA) have recently been covered by sediment, regardless of whether there was any sediment present at the time of settling. These results have a range of implications for managing the effects of sediment on coral reefs, and notably within the context of dredging activities during environmentally sensitive period such as coral spawning (Jones et al., 2015).

4.2. Accumulated sediment and surface aspect

The majority of *A. millepora* larvae preferred to settle on upward facing-surfaces, but if these surfaces were covered in silt, larvae instead chose downward facing, sediment-free surfaces to settle on. Despite these pronounced changes in settlement preferences there was no overall reduction in the number of larvae settling on the prisms in the different sediment treatments; that is, larvae ultimately found a surface to settle on. This result is consistent with other studies (Birkeland, 1977; Babcock and Davies, 1991), perhaps owing to adequate conditions associated with these under-surfaces including a strong settlement inducer, sufficient light levels and an absence of competition. Since the larvae were introduced to the tanks after all the sediment had fallen out of suspension, and the overlying water lacked any turbidity, this switch in settlement preference was exclusively related to the presence and quantity of unconsolidated sediment accumulated on the plugs. While a

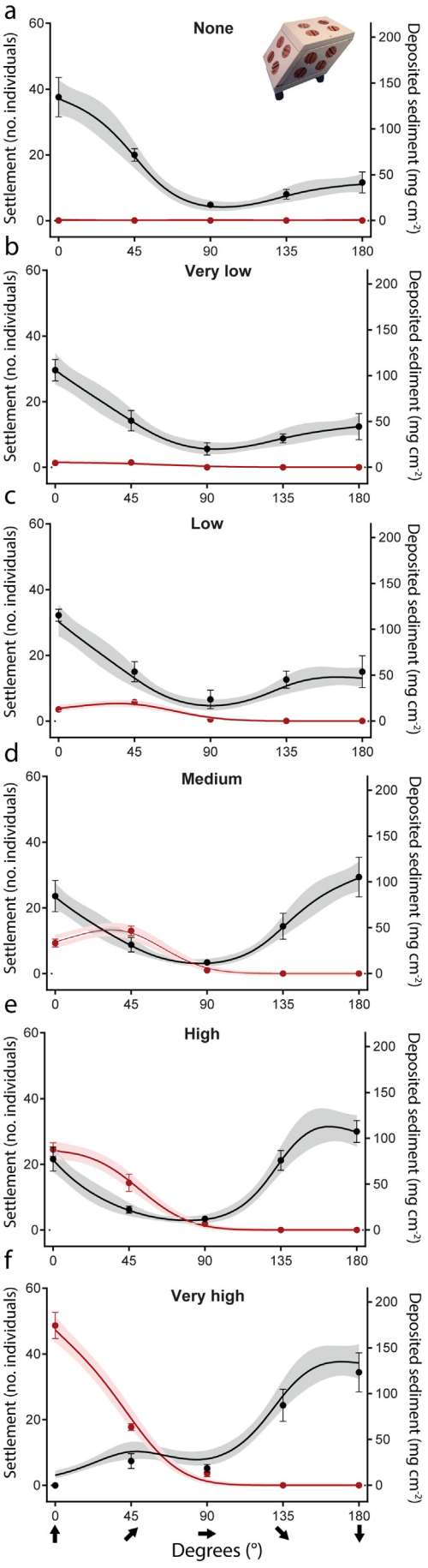

Fig. 4. Experiment 1. Numbers of settled larvae across different surface aspects (0–180°) (black lines) and deposited sediment levels (mg cm^{-2}) (red lines). Each scenario was titled based on the deposited sediment on the upper surface. There were five replicate aquaria per sediment treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

proportion of the *A. millepora* larvae appear to prefer settlement on sediment-free upward-facing surfaces (see also Babcock and Davies, 1991), these trends have not been observed in situ (Babcock and Smith, 2002). This conflict may be in part explained by low levels of sediment accumulating in the controls/references of the field study (reported as 0.76 to 1.32 mg cm^{-2} d^{-1} over the 4 to 8 d exposure period), with the sediment possibly accumulating to within the very low thresholds found here that affect larval settlement on flat horizontal surfaces. Additionally, preferences of natural settlement inducers and competitors to colonise certain surface orientations, as well as predation of newly settled recruits on exposed surfaces may also determine responses observed in situ (Raimondi and Morse, 2000; Baird et al., 2003; Doropoulos et al., 2016).

When the larvae were only offered a single horizontal flat surface for settlement, they showed a strong aversion to a very thin layer of sediment, regardless of sediment type or grain size. The settlement inhibition thresholds for each sediment type and grain size were similar although settlement on coarse-grained carbonate sediment was slightly higher in one spawning year, likely due to slight differences in larvae competency and CRA assemblages, or a change in plug material (grooves could only be manufactured on PVC plugs). It is possible that particle grain size could produce differences in settlement had a wider range of particle sizes been compared i.e. silts (<63 μm) versus sands (>63–2000 μm); however, the silt-sized sediments used in this study are typically of those associated with sediment plumes from natural resuspension events, dredging, and dredge spoil disposal (Bainbridge et al., 2012; Jones et al., 2016). Grooved plugs offered some minor refuge to deposited sediment in comparison with flat surfaces because the vertical wall of the groove did not accumulate sediment at low deposition levels. However, as the deposited sediment levels increased, the grooves became infilled, causing complete inhibition of settlement.

Thin tracks were regularly observed on the sediment-covered plugs where larvae had searched across the surface for suitable attachment sites (see also Perez et al., 2014). In preliminary experiments larvae were regularly observed settling on sediment-free surface just a few mm away from thick deposits of sediments (File B.1). Collectively these observations suggest the larvae could detect a settlement cue and were not averse to contact with the loose sediment. The larvae searched the substrate for a place of settlement with their aboral end as described by Harrigan (1972), and at the time of attachment, we observed mucous production between the aboral end and the substrate, consistent with previous observations of Harii and Kayanne (2002). It is not known if the mucus is produced to provide initial attachment to the substrate or to clean the site before metamorphosis. At high accumulated sediment levels, long strands of mucus were often observed, which could be a stress response (sediment clearing) or an increased effort to prepare surfaces for attachment. When the sediment film was reasonably thin or not continuous, the larvae were capable of burrowing into the sediment to find the substrate but this behaviour was drastically reduced with thicker sediment films. Minor gregarious settlement groupings of 3 or 4 individuals were also observed, which may assist the larvae to burrow into the settlement or create some elevation above the sediment layer.

4.3. Smothered CRA

Larvae avoided settling on the CRA-encrusted plugs that had previously been smothered with sediment, even though the sediment had been removed from the plugs at the time of settlement. The smothering of the CRA by sediment caused it to discolour or 'bleach', and this is the

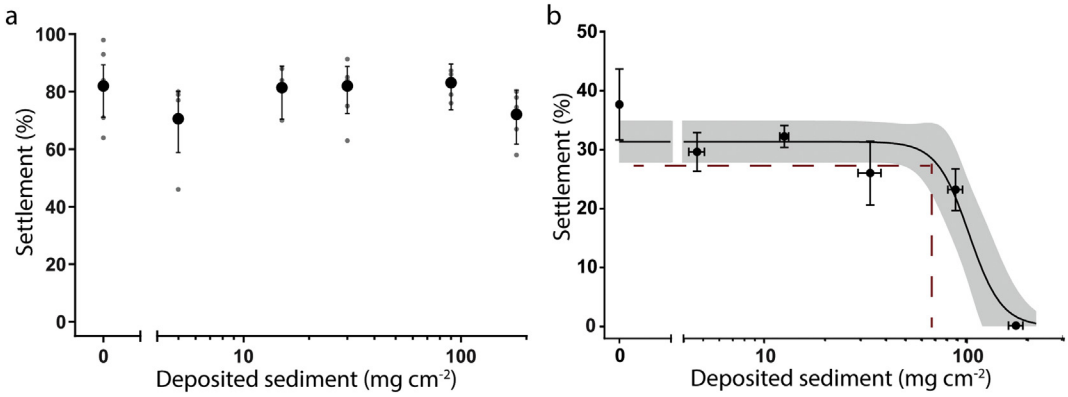

Fig. 5. Experiment 1. Total settlement responses on the settlement prism. a) Percent of total settlement in each sediment treatment regardless of surface aspect. b) Concentration–response relationships between sediment deposition levels and percent settlement success for only the upper surface (0°). Black: model predicted mean ± 95% CI. Grey: raw data. Red dashed line indicates the EC_{10} value. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

first study to describe a direct link between decrease in larval settlement and changes in the health of the CRA from sediment smothering. Only a temporary 6-d sediment smothering of low-level deposited sediment (7 mg cm^{-2}) caused a decrease in larval settlement, which corresponded with ~50% bleaching (increased whiteness) of the CRA plugs. Harrington et al. (2005) described similar pigment loss and reduced photosynthetic efficiency in CRA-smothered settlement tiles, albeit following smothering at ~100 mg cm^{-2} of sediment over a shorter time-period. However, pigment loss on CRA plugs only explained 42% of the variation in larval settlement success, indicating that tissue bleaching and the immediate loss of the colour red (see Mason et al., 2011) may not be a reliable indicator to predict quality changes of the CRA as a settlement inducer. Sediment-smothering may reduce light available to the CRA, and could possibly cause anoxic conditions that inhibit settlement due to a deterioration of settlement cues or a release of chemicals by the algae related to cellular stress. Although the plugs were colonised predominantly with *T. prototypum* and *Peyssonnelia* spp., variability in settlement between plugs may indicate subtle differences in coverage, species assemblage, or susceptibility to sediment-smothering (Harrington et al., 2005).

Coral larvae show a settlement preference to only a few species of CRA — including *T. prototypum* and *Peyssonnelia* spp. identified on the settlement plugs (Heyward and Negri, 1999; Price, 2010; Doropoulos et al., 2012), and decrease in abundance of these ecologically important species could have a disproportionate effect on recruitment success (Harrington et al., 2004; Price, 2010; Doropoulos et al., 2012). Corals may have adapted to seek substrates covered in such pioneering species as a guide to select an area of the reef with low competition, adequate light levels and low in sedimentation (Fabricius and De'Ath, 2001; Vermeij and Sandin, 2008; Price, 2010; Smith et al., 2016). Continued smothering of CRA in areas of high sediment deposition, in addition to sediment-related light attenuation (Riul et al., 2008), may lead to long-term loss of the crustose algae community, impacting future recolonisation of disturbed reefs. More work is needed to determine if the trends observed here are consistent across other CRA species and communities.

4.4. Light intensity and surface structure

Surprisingly, there was no evidence of light intensity affecting larval settlement under environmentally realistic conditions (Experiment 4). Responses to light intensity may be species-specific (Morse et al., 1988; Babcock and Mundy, 1996; Mundy and Babcock, 1998), and other factors associated with light could play an important role in site selection for larval settlement including spectral changes and substrate colour (Morse et al., 1988; Mundy and Babcock, 1998; Mason et al., 2011), but were not tested here. Nevertheless, larvae readily settled in near darkness, suggesting that either phototactic behaviour is comparatively weak for settlement in *A. millepora* and/or chemotaxic cues from the CRA settlement inducer overwhelmed the influence of light intensity. For example, in the presence of a subtle settlement inducer and a constant light regime (Experiment 5), there was some settlement inhibition at very low, or medium–high light intensities, suggesting that larvae may, under some circumstances, defer settlement on sun-exposed or very low light surfaces (Jokiel et al., 1985). Such very low light intensities may occur during extreme turbidity, that substantially reduce the light at depth and within crevices and overhangs (Bak, 1978; Jones et al., 2016). However, light measurements presented in this study should be considered with some caution, as spectral wavelengths and intensities can occur outside of the detection limits of our light-meter, and the reduction in light within grooves, which could not be measured, is expected to be substantial (Doropoulos et al., 2016).

4.5. Ecological and management significance

Sediments released into the water column can affect many different stages of the reproductive cycle including the egg sperm bundles and fertilisation (Ricardo et al., 2015; Ricardo et al., 2016b), embryogenesis (Ricardo et al., 2016a; Humanes et al., 2017) and settlement (Gilmour, 1999; Perez et al., 2014). This study demonstrated that very low levels of accumulated sediment can alter larvae settlement preferences of a common broadcast spawner. There is scarcity of in situ information on enduring deposited sediment levels under ordinary background conditions or during and after dredging events (Jones et al., 2016), but estimates indicate background levels could settle and accumulate to tens of mg cm^{-2} (Goatley and Bellwood, 2010; Tebbett et al., 2017; Whinney et al., 2017), before being resuspended by storm events (Wolanski et al., 2005; Storlazzi et al., 2009). The threshold values we report for settlement are likely to occur even within background conditions and this may in part explain why recruits are often found attached to under-sides in situ (Babcock and Davies, 1991; Maida et al., 1994; Babcock and Smith, 2002). Inhibition responses may be further complicated in reef systems where competition and allelopathy further limit access to suitable areas for settlement and growth (Birrell et al., 2005; Doropoulos et al., 2016; Morrow et al., 2017). Dredging activities result in much higher accumulated sediment loads on the seabed, as sediments are typically released into a relatively calm water column (compared to natural resuspension events) and the hydrodynamics are insufficient to keep the sediments in suspension (Bak, 1978, Jones et al., 2016). In recognizing this risk, development proponents in Australia are usually required to avoid dredging activities for ~2 weeks when corals are spawning (Jones et al., 2015; EPA, 2016). Large-scale dredging projects create sediment deposition zones around the excavation and dredge material placement sites, as temporarily resuspended sediments

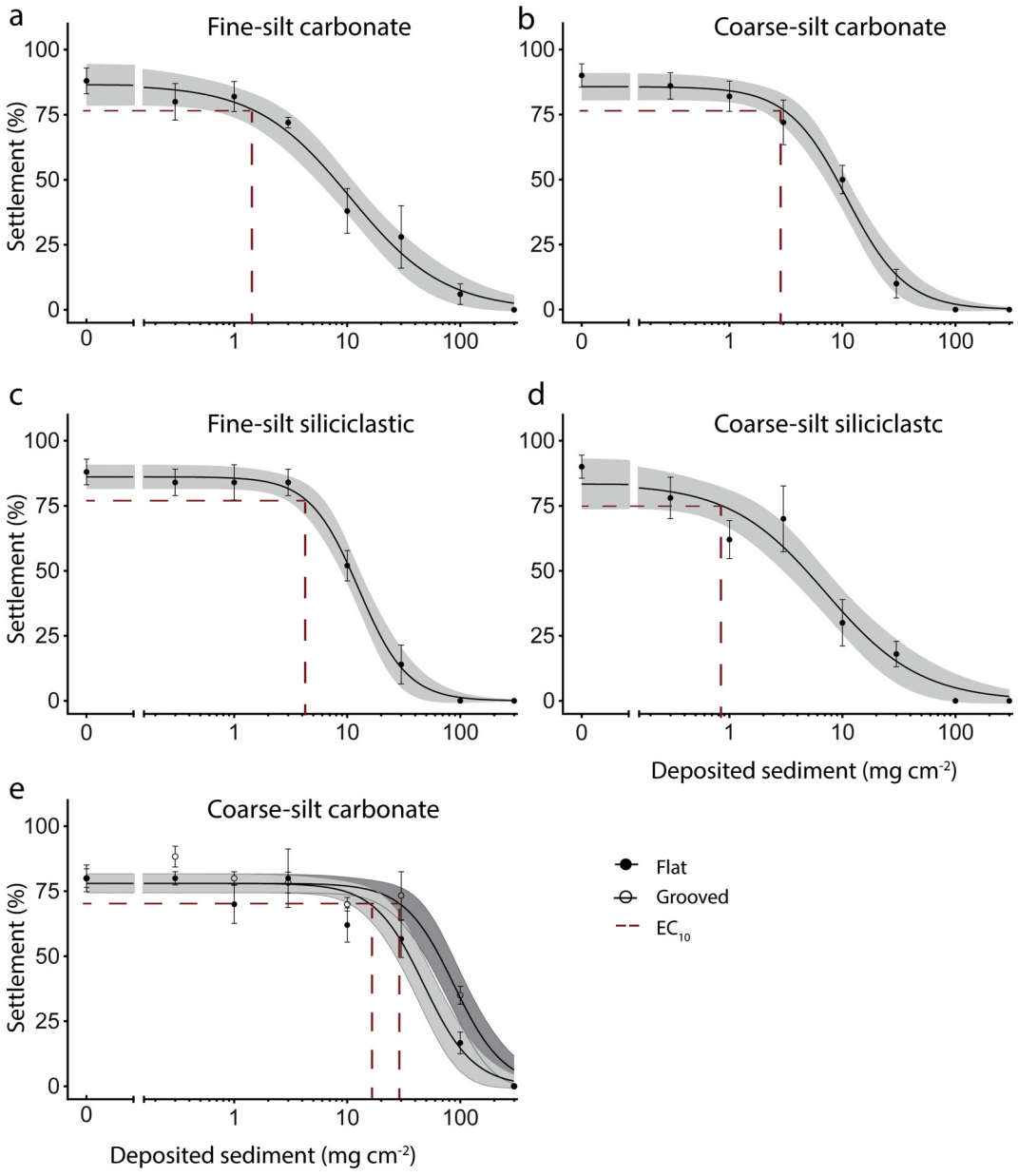

Fig. 6. Experiment 2. Inhibition of settlement of *A. millepora* on upward-facing sediment-covered CRA plugs. Deposited sediment–response relationships with plugs covered in sediments of a) fine-silt carbonates, b) coarse-silt carbonates, c) fine-silt siliciclastics and, d) coarse-silt siliciclastics. Each deposited sediment treatment had 5 replicate samples with 10 larvae per sample. e) Deposited sediment–response relationships with flat and grooved CRA plugs covered in coarse-silt carbonates. Top and Slope parameters of the models were shared. Each deposited sediment treatment had 6 replicate samples with 10 larvae per sample. For all models, grey shaded areas represent the 95% confidence bands and symbols represent the raw mean ± SE of the data. Red dashed lines indicate EC_{10} threshold values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

settle back to the seabed. The results of this study show that low levels of loose unconsolidated sediments can alter larval settlement preferences either, by directly acting on the coral larvae, or indirectly by reducing the health and ecological function of CRA-covered surfaces. Therefore, while the shut-down period may improve conditions for the fertilisation and larval life-history stages (Humphrey et al., 2008; Ricardo et al., 2015; Humanes et al., 2016), the shutdown period may be less effective for addressing the impact of deposited sediments on recruitment. To improve predictions of likely impacts of dredging on larval settlement, further research is needed to better define the background/dredging deposited sediment interface, and document how this interface changes during the ~2 week shut-down period.

It is difficult to evaluate the ecological significance of the change in the settlement preference away from upwards facing (i.e. light–exposed but sediment influenced) surfaces to more downward facing (and hence more poorly illuminated but sediment-free) surfaces. Although there was no difference in the total number of larvae that settled between treatments in this study, that was only because alternative, sediment-free surfaces (downfacing planes) with suitable CRA settlement cues were provided (cf the difference between experiments 1 and 2). The question then becomes what is the availability of alternative sediment-free substrates for larvae to settle on in the field, are they limiting, and what are consequences of settling there for post settlement survival? Recruits growing in shaded areas may suffer greater mortality and impaired growth rates compared to those in unshaded areas (Baird and Hughes, 2000; Box and Mumby, 2007) and this is likely relevant for larvae settling on downward-facing surfaces. On the other hand, larvae that settle in exposed areas may be subject to continued sedimentation (Fabricius et al., 2003; Moeller et al., 2016). Cryptic microhabitats, such as crevices, holes, grooves, nooks and crannies which larvae tend

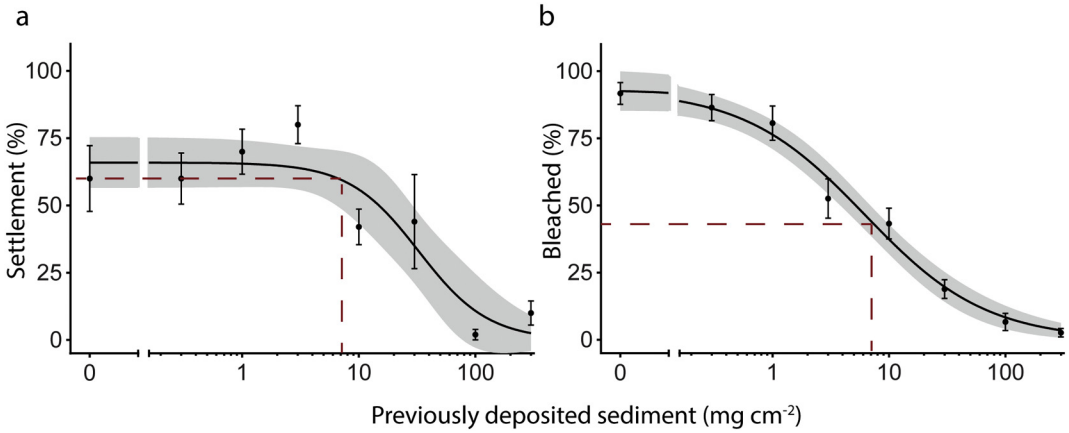

Fig. 7. Experiment 3. Percent settlement success and bleaching on CRA plugs previously smothered for 6 d with coarse silt carbonate. a) Inhibition of settlement of *A. millepora* on CRA plugs after sediments were removed. There were five replicate jars per sediment treatment, with 10 larvae used per jar to assess settlement after sediment smothering. Red dashed lines indicate the EC_{10} value. b) Percent of pigment loss (bleaching) on CRA plugs. Red dashed line indicates the corresponding bleaching response at the EC_{10} derived from the settlement assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to prefer, also may not provide refuge, because they are likely to trap more sediment compared to exposed surfaces. Regardless of the issues with sub-optimal settlement sites, this study highlights the importance of a three-dimensional coral reef framework in providing alternative settlement options. Difficulties in evaluating the ecological significance of settlement preference changes also remains challenging because many studies on the effects of sediments on coral recruitment appear to conflict. But apparent disagreement may be explained by limited reporting of sediment characteristics (i.e. particle size, composition, organic content, deposition levels, resuspension rates and toxicants etc. sensu Jones et al., 2016), and differences in the methodologies used to assess settlement success (Abelson and Gaines, 2005).

5. Conclusion

Deposited sediment impacts the larval behaviour at low levels on upward facing surfaces, but uncertainty remains on how thresholds determined here relate to in situ conditions, and how changes in settlement patterns translate to post-settlement impacts. Given these gaps in knowledge, a precautionary approach to managing turbidity-generating events such as dredging in the weeks following coral spawning should continue.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.07.153.

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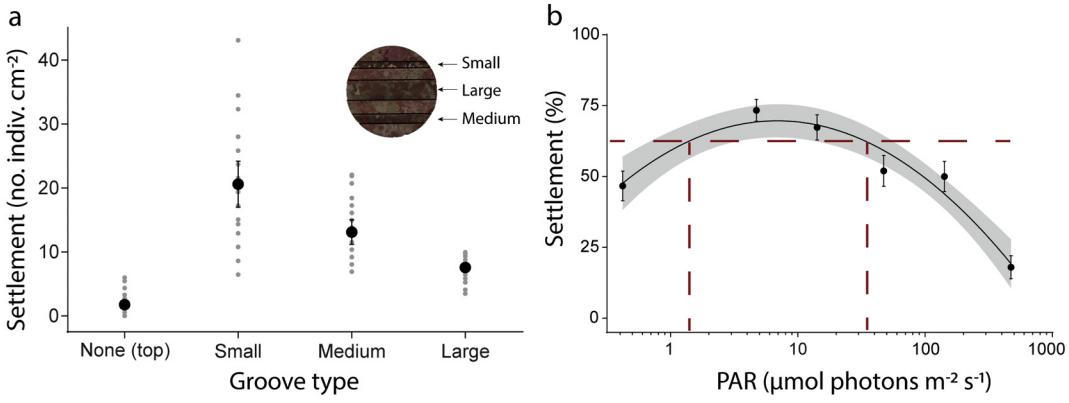

Fig. 8. Experiments 4 and 5. Settlement success of *A. millepora* larvae across various light intensities and surface structures. a) Number of settlers across various surface structures (groove types) adjusted for surface area over a 24-h-period using a strong settlement inducer (condition plugs in CRA). There were 24 replicates of each groove type. b) Settlement success at constant light intensity steps (over the range between ~0.5 and 473 µmol photons m^{-2} s^{-1}) for 12 h. Data were pooled from two days using two cultures with 30 replicate wells per light treatment with 10 larvae per replicate well. Red dashed lines indicate EC_{10} values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Appendix A

Table A.1 Summary of Tukey pairwise comparisons at each interaction from a negative binomial to assess differences in settlement choice among surface orientations and treatment scenarios (Experiment 1).

None			**Very Low**		
Surface	**Z ratio**	**p value**	**Surface**	**Z ratio**	**p value**
Upper-side – Upper-slope	2.612	0.0682	Upper-side – Upper-slope	2.905	0.0302
Upper-side – Vertical-side	6.858	<.0001	Upper-side – Vertical-side	5.692	<.0001
Upper-side – Under-slope	5.712	<.0001	Upper-side – Under-slope	4.503	0.0001
Upper-side – Under-side	4.589	<.0001	Upper-side – Under-side	3.387	0.0063
Upper-slope – Vertical-side	4.636	<.0001	Upper-slope – Vertical-side	3.053	0.0192
Upper-slope – Under-slope	3.279	0.0092	Upper-slope – Under-slope	1.693	0.4384
Upper-slope – Under-side	2.054	0.2404	Upper-slope – Under-side	0.501	0.9873
Vertical-side – Under-slope	-1.542	0.5351	Vertical-side – Under-slope	-1.418	0.6157
Vertical-side – Under-side	-2.763	0.0454	Vertical-side – Under-side	-2.580	0.0741
Under-slope – Under-side	-1.272	0.7085	Under-slope – Under-side	-1.198	0.7526
Low			**Medium**		
Surface	**Z ratio**	**p value**	**Surface**	**Z ratio**	**p value**
Upper-side – Upper-slope	3.052	0.0193	Upper-side – Upper-slope	3.619	0.0027
Upper-side – Vertical-side	5.617	<.0001	Upper-side – Vertical-side	5.831	<.0001
Upper-side – Under-slope	3.675	0.0022	Upper-side – Under-slope	1.931	0.3008
Upper-side – Under-side	3.052	0.0193	Upper-side – Under-side	-0.91	0.8934
Upper-slope – Vertical-side	2.788	0.0423	Upper-slope – Vertical-side	2.694	0.0549
Upper-slope – Under-slope	0.648	0.967	Upper-slope – Under-slope	-1.744	0.4067
Upper-slope – Under-side	0.000	1.0000	Upper-slope – Under-side	-4.476	0.0001
Vertical-side – Under-slope	-2.164	0.1934	Vertical-side – Under-slope	-4.242	0.0002
Vertical-side – Under-side	-2.788	0.0423	Vertical-side – Under-side	-6.542	<.0001
Under-slope – Under-side	-0.648	0.9670	Under-slope – Under-side	-2.826	0.0380
High			**Very High**		
Surface	**Z ratio**	**p value**	**Surface**	**Z ratio**	**p value**
Upper-side – Upper-slope	4.291	0.0002	Upper-side – Upper-slope	-3.491	0.0044
Upper-side – Vertical-side	5.545	<.0001	Upper-side – Vertical-side	-3.133	0.0149
Upper-side – Under-slope	0.075	1.0000	Upper-side – Under-slope	-4.685	<.0001
Upper-side – Under-side	-1.352	0.6584	Upper-side – Under-side	-5.026	<.0001
Upper-slope – Vertical-side	1.640	0.4716	Upper-slope – Vertical-side	1.071	0.8216
Upper-slope – Under-slope	-4.222	0.0002	Upper-slope – Under-slope	-4.263	0.0002
Upper-slope – Under-side	-5.505	<.0001	Upper-slope – Under-side	-5.576	<.0001
Vertical-side – Under-slope	-5.485	<.0001	Vertical-side – Under-slope	-5.16	<.0001
Vertical-side – Under-side	-6.608	<.0001	Vertical-side – Under-side	-6.392	<.0001
Under-slope – Under-side	-1.427	0.6101	Under-slope – Under-side	-1.437	0.6034

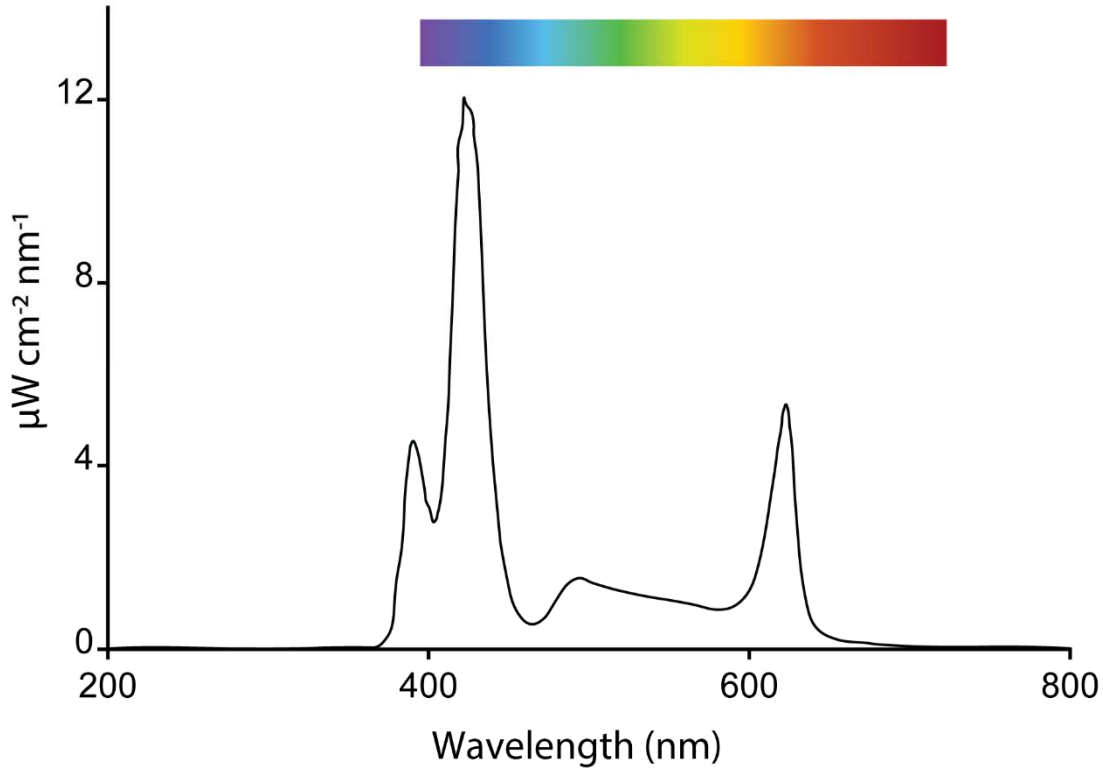

Figure A.1 Light spectra used in light experiments.