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# Effects of coal contamination on early life history processes of a reef-building coral, *Acropora tenuis*

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ABSTRACT

Successful reproduction and larval dispersal are important for the persistence of marine invertebrate populations, and these early life history processes can be sensitive to marine pollution. Coal is emerging as a contaminant of interest due to the proximity of ports and shipping lanes to coral reefs. To assess the potential hazard of this contaminant, gametes, newly developed embryos, larvae and juveniles of the coral *Acropora tenuis* were exposed to a range of coal leachate, suspended coal, and coal smothering treatments. Fertilisation was the most sensitive reproductive process tested. Embryo survivorship decreased with increasing suspended coal concentrations and exposure duration, effects on larval settlement varied between treatments, while effects on juvenile survivorship were minimal. Leachate exposures had negligible effects on fertilisation and larval settlement. These results indicate that coral recruitment could be affected by spills that produce plumes of suspended coal particles which interact with gametes and embryos soon after spawning.



## 1. Introduction

Land and marine-based activities are major contributors to the deterioration of water quality within near-shore ecosystems, including coral reefs (Brodie and Mitchell, 2005; Fabricius, 2005; Foley et al., 2005). Unburnt coal is a contaminant of emerging interest in tropical marine regions because the world's top two coal exporters, Australia (392 Mt in 2015) and Indonesia (368 Mt in 2015) (IEA, 2016), are also home to some of the world's most extensive and diverse coral reef communities. Consequently, large quantities of coal are transported through Australasia and the Indo-Pacific each year (IEA, 2014) in proximity to coral reef ecosystems. Chronic inputs of coal to the marine environment occur during storage and ship loading/off-loading processes (Ahrens and Morrisey, 2005); however, ship groundings represent more severe coal contamination scenarios. While there is limited documentation of suspended coal concentrations in seawater during a spill event, past events have released 17,000–100,000 t of coal into the marine environment (Alcaro et al., 2002; Arbex, 2003; DEARSA, 2013). Salvage of grounded vessels may require cargo to be discarded overboard, as was the case in a recent salvage operation in South Africa where 10,000 t of coal was purposely released into the coastal marine environment (DEARSA, 2013). Examples of relevant scenarios where large spills onto reefs were narrowly avoided include groundings of the *MV Double Prosperity* (Philippines) and *Shen Neng 1* (Great Barrier Reef) coal carriers that were transporting 65,000 and 68,000 tons, respectively.

The spatial extent of contamination by coal, including from a localised point source like a ship grounding, is dependent on factors such as particle size, the density of the coal, and hydrodynamic drivers at the input area (Johnson and Bustin, 2006). Larger particles (>1 mm) generally sink close to the input source, with sediments collected in close proximity to coal terminals reported to contain 1–45% (w/w) coal (Allen, 1987; Goldberg et al., 1977; Hamilton et al., 1979; Johnson and Bustin, 2006; Toki et al., 2012). Coal spill simulations have shown that coal particles 1–2 mm and 2–10 mm in size can be carried away from the accident site along the seafloor, while particles >10 mm will remain close to the ship (Jaffrennou et al., 2007). Environmental consequences include smothering and abrasion of benthic flora and fauna (Alcaro et al., 2002). These sunken coal particles can erode over time, acting as a secondary source for new fine suspended particles in the water column (Jaffrennou et al., 2007). Previously settled coal particles can become re-suspended and hydrocarbon marker analyses indicates that coal particles may be transported offshore (up to 40 nautical miles) (Burns and Brinkman, 2011). In contrast, small coal particles can remain on the surface forming a thin film, or become suspended

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in the water column allowing dispersal from the input source via wind and water currents (Jaffrennou et al., 2007; Johnson and Bustin, 2006). However, fine suspended coal particles have also been observed to settle in experimental conditions over time (Berry et al., 2016). Based on estimates of coal particle size distribution in coal ships and flotation tests, it has been suggested that approximately 15% of coal cargo may be lost to ocean currents in a spill event (Lucas and Planner, 2012). Coal chunks and small particles can thus contaminate bottom sediments, the water column and the ocean surface, environments inhabited by the early life-history stages of many marine invertebrates. The early pelagic stages of marine invertebrates have limited capacity to avoid water-borne contaminants and are often more sensitive to particulates, pollution and climate stressors than adult stages (Byrne, 2011; Fabricius, 2005; Humanes et al., 2016; Jones et al., 2015).

There are specific features of coal fines that may result in harmful effects to marine organisms such as negative interactions caused by physical proximity of particles (abrasion, adhesion and smothering), and the release (leaching) of trace elements and polycyclic aromatic compounds (PACs), which include polycyclic aromatic hydrocarbons (PAHs), into the water (Ahrens and Morrisey, 2005; Lucas and Planner, 2012). Both trace elements and PACs pose toxic threats to marine organisms when threshold levels are exceeded; however, this is also dependent on their bioavailability (Kennish, 1998). To date, experimental investigations into the toxicity of coal on reproduction and early life histories of marine organisms have been limited, with more research investigating freshwater fauna. One study showed that exposure to PAC extracts from certain coal types increased mortality of zebra fish (*Danio rerio*) embryos (Meyer et al., 2013); however, exposure of embryos to deposited coal did not cause negative effects. Similarly, some doses of coal leachate reduced sperm production by mummichog fish (*Fundulus heteroclitus*) after 6 weeks exposure, but sperm production was not substantially affected in field populations sampled close to coal-fired power plants (Cochran, 1987). Exposure of fathead minnows to coal leachate reduced spawning success to 36% in comparison to 90% success in control treatments; however, spawning still occurred in all leachate concentrations tested (Carlson, 1979). A field experiment conducted in a river containing suspended solids from coal washeries found 98–100% mortality of rainbow trout (*Salmo gairdneri*) eggs during incubation in river gravels due to reduced dissolved oxygen supply (Turnpenny and Williams, 1980). While these studies have provided insight into the potential toxic and indirect effects of coal on early life histories, only one early development stage was investigated per species and none investigated alternate stress-pathways associated with coal contamination such as physical contact with particulate matter (suspended or deposited) or trace element leachate (e.g., copper and zinc), which are both known to negatively impact early life-history processes of fish (Johnston and Wildish, 1982; Wenger et al., 2014) and corals (Jones et al., 2015; Negri et al., 2011; Reichelt-Brushett and Harrison, 2000, 2005; Victor and Richmond, 2005).

The potential for coal contamination, including its physical characteristics and the possible toxicity of leachates, suggest that unburnt coal from large spills may pose a risk to marine invertebrate populations. Corals are the foundation species of tropical reefs and they inhabit environments that could be impacted by coal contamination. The majority of reef-building corals reproduce by broadcast-spawning (Harrison and Wallace, 1990), as do many other marine invertebrate taxa. Fertilisation, embryogenesis and larval development take place at the water surface and in the water column for ~4–5 days (Babcock and Heyward, 1986; Richmond, 1997) before competent larvae begin to settle onto suitable benthic substrata (mainly crustose coralline algae, CCA) and metamorphose into single-polyp juvenile corals (Heyward and Negri, 1999). Successful reproduction and recruitment is essential for coral population maintenance and growth (Harrison and Wallace, 1990), and decreased water quality and substratum quality can affect these critical processes (Richmond, 1993). Previously, we demonstrated that contamination by coal can cause mortality of adult corals (along with reduced growth rates of seagrass and fish) (Berry et al., 2016); however, we currently lack data to assess risks posed by coal related stressors across the various early life history stages. Moreover, the quantity of coal eliciting negative effects, and the relative sensitivities of early life stages of coral remains unknown.

To address these knowledge gaps, we experimentally tested the effects of suspended coal, coal leachate and direct coal smothering on gamete fertilisation, embryo survivorship, larval settlement and juvenile survivorship of the common reef-building coral *Acropora tenuis*. The lack of available environmental data necessitated the application of a wide range of coal concentrations that could result from chronic inputs and acute spill scenarios. In addition, we considered multiple exposure durations because spatial variation in hydrodynamic conditions means that there is likely to be variation in the residence time of coal contamination in different coastal areas. This information will provide insight into the effects of coal on the early life stages and processes of coral that have the potential to influence coral reef recruitment and population maintenance following coal spill events.

## 2. Materials and methods

### 2.1. Coral collection and gamete preparation

Experiments were conducted at the National Sea Simulator, Australian Institute of Marine Science (AIMS), during the 2013 and 2014 coral spawning events on the Great Barrier Reef (GBR) (outlined in Table 1). Experiments were designed to assess the effects of: 1) coal on gamete fertilisation; 2) coal on survivorship during early embryo development; 3) exposure to coal during embryo development on subsequent larval settlement; 4) coal deposition onto CCA on subsequent larval settlement; 5) coal encapsulation on larval settlement and survival; 6) coal deposition on juvenile survivorship; 7) coal leachate on gamete fertilisation and larval settlement (Table 1).

Colonies of *Acropora tenuis* were collected from Magnetic Island (19.199°S, 146.792°E) and Trunk Reef (18.329°S, 146.846°E) prior to the October and November full moons. Corals were acclimated under natural light conditions in 1000 l flow-through holding tanks (27–29 °C, 35.8 ± 0.03 PSU). After corals spawned, bundle collection and gamete separation was conducted in accordance with methods described in Negri and Heyward (2000). For fertilisation and embryogenesis experiments, eggs from a single colony were combined with pooled sperm samples from up to four different colonies. Fertilisation experiments involving suspended coal were conducted on multiple spawning nights to increase the sample size of coral colonies and eggs ($n = 3$ nights, 12 coral colonies). Remaining gametes from the spawned colonies were fertilised and larvae cultured in 500 l flow-through tanks which were gently aerated after 36 h development (Negri and Heyward, 2000). Water temperatures in the rearing tanks were consistent with reef temperatures (27–29 °C).

### 2.2. Coal preparation

Thermal coal (sourced from central Queensland, Australia) was crushed, milled and sieved to isolate particles <63 μm. For experiments involving suspended coal (hereafter referred to as coal), coal-seawater suspensions were pre-mixed with seawater using a blender followed by continuous mixing on a magnetic stirring plate for 3 h. Past experimental studies on temperate marine organisms have investigated the effects of suspended coal concentrations ranging from 1 to 13,500 mg coal $l^{-1}$ (Bender et al., 1987; Pearce and McBride, 1977). Because we wanted to incorporate potential coal concentrations resulting from low chronic inputs and acute spills, and since fine coal particles will become diluted as they are dispersed from an input source, we chose to investigate the suspended coal concentrations: 12.5, 25, 50, 100, 200, 400, 500, 600, 700, 800 mg $l^{-1}$. For leachate experiments, stock suspensions (10,000 mg coal $l^{-1}$) of the fine thermal coal particles were mixed for

**Table 1**
Outline of experiments conducted during 2013 and 2014 spawning events.

| Developmental process/stage | Nature of coal exposure | Duration of coal exposure (h) | Response variable | Methods section |
|---|---|---|---|---|
| Fertilisation | Sperm (5 concentrations) and eggs exposed to coal: 0, 50 and 200 mg $l^{-1}$ | 2.5 | Fertilisation success | Section 2.3 |
| | Gametes exposed to coal: 0–800 mg $l^{-1}$ | 2.5 | | Section 2.3 |
| | Gametes exposed to dilutions of a leachate from a 10,000 mg $l^{-1}$ coal suspension | 2.5 | | Section 2.7.1 |
| Embryo development | 3 h, and 12 h old embryos exposed to coal: 0–800 mg $l^{-1}$ | 1, 12, 24, 72 | Survivorship & settlement | Section 2.4, Section 2.5.1 |
| Larvae | Pre-competent larvae (72 h old) exposed to coal: 0–800 mg $l^{-1}$ | 1, 12, 24, 72 | | Section 2.5.1 |
| | Competent larvae exposed to coal: 800 mg $l^{-1}$ | 12, 24 | | Section 2.5.2 |
| | Competent larvae exposed to CCA smothered with coal: Pre-smothered and washed off, light dusting (12 mg $cm^{-2}$) of entire CCA, full coverage (22 mg $cm^{-2}$) | 48 | Settlement success | Section 2.5.3 |
| | Competent larvae exposed to dilutions of a leachate from a10,000 mg $l^{-1}$ coal suspension | 48 | | Section 2.7.2 |
| Juvenile | 6-week old juveniles exposed to coal and carbonate sediment (fully smothered) | 96 | Clearance rates and survivorship | Section 2.6 |

24 h on magnetic stirring plates at 27 °C. Suspensions were vacuum filtered through pre-combusted solvent rinsed filters (Whatman GF/F, 0.7 μm). Coal leachate solutions were then diluted with filtered (0.45 μm) seawater (FSW) to five concentrations (100, 50, 25, 12.5 and 6.25% v/v of leachate from the original suspension).

### 2.3. Effects of suspended coal particles on coral fertilisation

The primary mechanism for inhibition of fertilisation by suspended solids is sperm-limitation due to the removal of sperm from the water column by sperm-particle interactions (Ricardo et al., 2015); therefore, sensitivity of the fertilisation is highly dependent on initial sperm concentrations. In the first fertilisation experiment we exposed coral eggs to five sperm concentrations ($10^2$, $10^3$, $10^4$, $10^5$, and $10^6$) and three suspended coal treatments: control (0 mg coal $l^{-1}$), low (50 mg coal $l^{-1}$), and high (200 mg coal $l^{-1}$). Eggs and sperm were added to 200 ml jars each containing 180 ml coal treatments and were placed on custom designed mechanical rollers to keep particles suspended and achieve constant water circulation within jars. Fertilisation was assessed after 2.5 h (SOM Fig. S1). We observed sperm-saturation at $10^5$ sperm cells $ml^{-1}$ and $10^6$ sperm cells $ml^{-1}$ for the low and high coal concentrations, respectively. The sperm concentration that gave the half-maximal fertilisation response ($EC_{50}$) at 50 mg coal $l^{-1}$ was $1.2 \times 10^4$ sperm cells $ml^{-1}$. Since we were investigating coal concentrations lower than 50 mg $l^{-1}$, we applied $2 \times 10^4$ sperm cells $ml^{-1}$ in the subsequent fertilisation experiments that included coal suspension concentrations of 12.5–800 mg $l^{-1}$, $n = 5$ replicate jars per concentration.

Fertilisation experiments were repeated on 3 spawning nights (eggs from $n = 1$ colony, sperm from $n = 2$–4 colonies on each night). In each experiment, sperm and ~100 eggs were added to pre-mixed coal or leachate treatments separately for 30 min. Sperm were then added to egg treatments and left to fertilize for 2.5 h. The control and blank treatments in each experiment consisted of filtered seawater (FSW). Specimens were then fixed with *Z*-fix concentrate (zinc-formalin solution, Anatech Ltd., diluted 1:4 parts seawater) and assessed for successful fertilisation, which was identified by the onset of embryogenesis (2–4 cell divisions). Irregularly shaped embryos were recorded for the coal concentrations: 0, 50, 100, 200, 400 and 800 mg $l^{-1}$ (on one night only).

### 2.4. Effects of coal on early development stage survivorship

Three development stages: 3 h old (2–4 cell embryos); 12 h old (prawn chip stage embryos); and 72 h old (pre-competent larvae), were transferred into 180 ml jars ($n = 20$ per jar) containing 25, 50, 100, 200, 400 and 800 mg coal $l^{-1}$ and left for 4 different exposure durations (1 h, 12 h, 24 h, and 72 h, $n = 5$ replicate jars per concentration and exposure duration). At the end of each exposure period, survivorship was calculated. An additional set of each development stage was exposed to each coal concentration for 24 h and 72 h, after which surviving larvae were gently transferred into new jars containing FSW and were left to develop into competent planula (until 6 d old) for settlement experiments. Water exchanges were made daily in each jar.

### 2.5. Effects of coal on competent larvae settlement

#### 2.5.1. Effects of exposure to coal during embryo development on larval settlement

Once competency was reached (6 d post fertilisation), the larvae ($n = 60$ per treatment) that had been exposed to coal as embryos were gently transferred into 6-well plates containing 9 ml coal-free FSW and a small piece (approximately 3 $mm^2$) of live crustose coralline algae *Hydrolithon onkodes* (CCA), which is a natural cue for larval settlement at reefs (Heyward and Negri, 1999). Settlement was quantified after 72 h by counting the number of swimming compared with settled/metamorphosed larvae.

#### 2.5.2. Effects of coal encapsulation on larval settlement and survival

Observations from pilot studies and embryo exposures revealed that the majority of larvae (days to weeks old) exposed to concentrations > 400 mg coal $l^{-1}$ were completely encapsulated by coal. Microscopic examination (Leica MZ16) revealed that larvae continuously gyrate within their "coal ball" in an effort to break free (Supplementary Video 1). Unsuccessful escapees eventually died within the coal ball (personal observation, K. Berry). To assess the effect of complete coal encapsulation on larval survivorship and settlement, ten day old larvae ($n = 20$ per jar) were exposed to 800 mg suspended coal $l^{-1}$ under non-static conditions for 12 and 24 h in 50 ml jars ($n = 5$ jars per exposure time). The jars were then left static for 24 h so that the coal could settle and the larvae could break free from encapsulation. The larvae (both escaped and encapsulated) were then gently transferred into 6-well plates and were cued to settle using small pieces of CCA as per Section 2.5.1. The numbers of settled, unsettled, encapsulated and dead larvae were counted after 48 h.

#### 2.5.3. Effects of coal smothered CCA on subsequent larval settlement

Small pieces of live CCA were cut to a consistent size (approximately 3 $mm^2$) and placed into 6-well plates ($n = 6$ per treatment) containing 9 ml FSW. Since larvae tend to settle in cryptic areas, rather than exposed horizontal surfaces (Harrison and Wallace, 1990), the aragonite beneath the CCA surface layer was left exposed as a settlement option. Four treatments were examined in this experiment: 1) a light dusting ($12.5 \pm 0.9$ mg $cm^{-2}$ d/wt) of pre-wetted coal was deposited on top of CCA chips; 2) a full layer ($22 \pm 1.5$ mg $cm^{-2}$ d/wt) of pre-wetted coal was deposited onto the upper surface of CCA chips; 3) CCA chips were fully smothered with coal for 8 h and were then rinsed clean; and 4) an experimental control consisted of coal settled onto the plate surrounding the CCA. Competent larvae ($n = 10$) not previously exposed to coal were gently added to the surface water of the wells ($n = 6$). Settlement was quantified after 48 h as per Section 2.5.1.

### 2.6. Effects of coal deposition on juvenile survivorship

Ten day old larvae, that had not previously been exposed to coal, were transferred into 6-well plates ($n = 14$ larvae per well and 40 plates) and cued to settle (as per Section 2.5.1). Following settlement (~24 h), plates were placed into 1000 l flow-through aquaria and the recently metamorphosed corals were left to develop for 6 weeks at a light intensity of ~60 μmol photons $m^{-2}$ $s^{-1}$. Symbiont (zooxanthellae) uptake occurred through horizontal transmission from adult *A. tenuis* colonies in the tank. Survivorship of 6-week old juveniles was assessed, after which plates were divided into 3 groups of coral polyps (mean $n = 273 \pm 25$ per treatment) and were then randomly placed into 55 l flow through aquaria ($n = 3$ aquaria, $n = 18$ plates per aquarium). A treatment that consisted of smothering juveniles with clean carbonate sediment was implemented to help distinguish between physical and chemical effects. Stock suspensions of coal and a clean carbonate sediment (both <63 μm) were mixed for 3 h using magnetic stirring plates and were added individually ($70 \pm 1.7$ mg $cm^{-2}$ d/wt) into wells of randomly-selected plates within the three replicate aquaria until the recruits were completely smothered. Control plates contained only FSW and, to avoid cross-contamination between treatments, plates were covered with a lid when treatments were being applied. The coal and sediment were allowed to stabilise for 3 h, after which, lids were carefully removed from all plates to allow water exchange in the large aquaria. After 96 h, the number of recruits that had cleared off coal and sediments were counted. The plates were then gently agitated to wash particles away from all recruits. Survivorship was assessed under dissecting microscope. The percentage of cleared juveniles was calculated relative to the total number of juveniles that survived in each respective treatment.

### 2.7. Effects of coal leachate on coral reproduction

#### 2.7.1. Effects of leachate on gamete fertilisation

This experiment was conducted during one spawning night using eggs from $n = 1$ colony, sperm from $n = 5$ colonies. 15 ml of each leachate concentration was added to separate glass scintillation vials ($n = 6$ per concentration) and eggs and sperm were separately exposed to dilutions of leachate from a 10,000 mg coal $l^{-1}$ suspension (as per Section 2.2) for 30 min. Sperm were then added to egg treatments and left to fertilize for 2.5 h. Irregularly shaped embryos were recorded.

#### 2.7.2. Effects of coal leachate on larval settlement

Competent larvae (6 d old) were exposed to dilutions of leachate from a 10,000 mg coal $l^{-1}$ suspension ($n = 10$ larvae per jar, 5 jars per concentration) as described in Section 2.2. Exposure lasted for 48 h at 27 °C. Larvae were then gently rinsed in large volumes of FSW and transferred to 6-well plates containing 9 ml uncontaminated FSW and a small piece (approximately 3 $mm^2$) of CCA. Settlement was quantified after 48 h as per Section 2.5.1.

#### 2.7.3. Trace metal analysis

Water samples were taken from each suspended coal treatment concentration and the highest leachate dilution (100%). Suspended coal solutions were syringe filtered through 0.45 μm filters into 250 ml acid-washed bottles. Acid preservative (1% mixture of nitric acid (34.5%) and hydrochloric acid (0.16%)) was added to each sample. Samples were analysed for trace metals (Co, As, Cd, Cu, Pb, Mn, Mo, Se, Ni) at Charles Darwin University, Australia (coal suspension treatments) and The University of Sydney, Australia (leachate treatments) using inductively coupled plasma-mass spectrometry.

#### 2.7.4. Polycyclic aromatic hydrocarbon (PAH) analysis

Coal suspensions (800 and 10,000 mg $l^{-1}$) and a seawater control ($n = 1$) were prepared as previously described and vacuum filtered through pre-combusted solvent-rinsed filters (Whatman GF/F, 0.7 μm). All glassware was solvent-rinsed and dried prior to use. Triplicate leachate samples (1 l) were transferred to amber bottles and refrigerated. PAH analyses were performed at ChemCentre (Perth, Western Australia). Briefly, leachate samples were extracted three times with dichloromethane, the combined extracts (80 ml) were dried with sodium sulphate and 8 ml aliquots were concentrated to 1 ml under nitrogen gas. Surrogate standards (2-fluorobiphenyl, nitrobenzene-d5, and p-terphenyl-d14) were added to the samples before extraction, and internal standards (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12) were added to the extracts before analysis. A method blank (filtered seawater) and a spiked control (filtered seawater with a known amount of acenaphthene and pyrene added) were also prepared and analysed with the sample batch. PAHs were analysed based on USEPA method 8270 using gas chromatography–mass spectrometry (GC–MS) in selected ion monitoring (SIM) mode.

### 2.8. Statistical analyses

Concentration-response data are generally modelled to calculate inhibition concentration ($IC_{xx}$) values, which provide information on the concentration needed to inhibit a biological or biochemical function by a certain percentage ($_{xx}$). However, only data from the fertilisation experiment were suitable for fitting standard non-linear regressions to estimate $IC_{XX}$ values and effects on other life stages/processes were analysed using general linear models (GLM) and generalized linear mixed-effects models (GLMER, package = lme4) in R (version 3.2.3, R Core Team 2015) and PERMANOVA in Primer.

#### 2.8.1. Effects of coal on coral fertilisation

To test for differences in mean fertilisation success between coal treatments, results from three nights of spawning were analysed using a GLMER with a binomial distribution in R. The effect of spawning night was included as a random effect and a random observation component was also included to account for overdispersion. To determine the concentration-response relationship between suspended coal and gamete fertilisation, a four-parameter sigmoidal curve (constrained between 0 and 100%) was fitted to gamete fertilisation success data to estimate the non-lethal concentration, and the concentrations which inhibit fertilisation by 10 and 50% ($IC_{10}$ and $IC_{50}$ values) were estimated using GraphPad Prism (Version 6.0, San Diego, USA). Abnormal embryo data were analysed with a GLM using a quasibinomial distribution and chi-square test in R.

#### 2.8.2. Effects of coal on early development stage survivorship

Each age group was analysed independently using the factors: exposure duration and concentration. Survivorship data were analysed using GLMER with a binomial distribution and Chi square test, run in R. The effect of jar replicates was included as a random effect and a random observation component was also included to account for overdispersion when necessary. GLM with a binomial distribution were used to determine the lowest observed effect concentration (LOEC) of each respective exposure time for each age group.

#### 2.8.3. Effects of exposure to coal during embryo development on larval settlement

Each age group was analysed independently using the factors: exposure duration and concentration. Analysis of variance was implemented based on permutations using the PERMANOVA routine of PRIMER (Version 6.0), respectively. Euclidean distance was used as the similarity measure (with 9999 permutations) and pair-wise comparisons were made with the Student *t*-test with Monte Carlo simulations considered when unique permutations were <1000. Factors included: coal concentration (7 levels, fixed) and exposure duration (2 levels, fixed). LOECs for each development stage were determined for each respective exposure time using the factor: coal concentration (7 levels, fixed).

*2.8.4. Effects of coal encapsulation on larval settlement and survival*

Comparisons in proportions of settled, unsettled, smothered and dead larvae after 12 and 24 h exposure to extreme (800 mg coal $l^{-1}$) coal concentrations was assessed by Chi-square test in GraphPad Prism (Version 6).

*2.8.5. Effects of coal smothered CCA on subsequent larval settlement*

Comparisons in settlement onto the four coal smothered CCA treatments were analysed with a GLM using a binomial distribution and chi-square test, run in R.

*2.8.6. Effect of coal deposition on juvenile survivorship*

Comparisons in survivorship between control, coal smothered and sediment smothered juveniles were analysed with a GLMER using a binomial distribution in R. The effect of the 6 well plate and tank were included as random effects. Coal and sediment removal was compared using a linear model and chi-square test, run in R.

*2.8.7. Effects of coal leachate on coral reproduction*

Fertilisation, settlement success and abnormal embryo data were analysed with GLMs using a quasibinomial distribution in R, respectively.

## 3. Results

*3.1. Effects of coal on coral fertilisation*

Fertilisation success was high in uncontaminated water (96 ± 1%) and ranged between 95 ± 1% down to 0% in coal treatments 12.5–800 mg coal $l^{-1}$ (Fig. 1A, Table 2). Coal particles did not coat the eggs but instead appeared to form flocs, possibly with sperm (Fig. 1B). The magnitude of fertilisation varied with coal concentration and fitting a four-parameter sigmoidal curve ($r^2 = 0.91$) to the data revealed concentrations that inhibit fertilisation by 10% and 50% ($IC_{10}$ and $IC_{50}$) of 47 (95% confidence limits = 39–56) mg coal $l^{-1}$ and 107 (95% confidence limits = 98–116) mg coal $l^{-1}$, respectively. There were strong reductions in mean fertilisation success at concentrations ≥50 mg coal $l^{-1}$ and <1% success was measured at concentrations ≥400 mg coal $l^{-1}$. The LOEC was 50 mg coal $l^{-1}$ ($Z_{11,189} = -3.4$, $P < 0.001$). Mean embryonic abnormalities ranged between 2 ± 1% in the static control treatments, 7 ± 2% in the rolled control treatments and 19 ± 3% in suspended coal treatments. Although significant ($P < 0.05$) the increased proportion of abnormal embryos was not dose-dependent.

*3.2. Effects of coal on early development stage survivorship*

Early development stages exposed to coal exhibited substantial mortality and these effects were highly dependent on development age and duration of exposure (Fig. 2A–C). Maximum mortality was 26 ± 3%, 26 ± 11%, and 17 ± 3%, for 3 h and 12 h embryos and 72 h old larvae, respectively, and maxima always occurred after 72 h exposure to the highest concentrations of coal (either 400 or 800 mg coal $l^{-1}$). The 3 h old embryos exposed to concentrations ≥400 mg $l^{-1}$ for 72 h developed into visibly smaller larvae than those exposed to the lower concentrations; however, larval sizes were not specifically quantified. Although mortality was lowest in 72 h old larvae exposed to coal, treated larvae were less mobile than control larvae after 72 h coal exposure, the latter of which were all swimming actively. Additionally, larvae exposed to ≥ 400 mg coal $l^{-1}$ ingested coal particles (Fig. 3C).

Survivorship of 3 h and 12 h old embryos and 72 h old larvae were all significantly affected by coal concentration and exposure duration at some level. For 3 h old embryos, exposure durations ≥24 h resulted in significantly lower survivorship than shorter exposures of ≤12 h ($Z_{3,128} = -4.4$, $P < 0.001$). LOECs for 1 h, 12 h, 24 h and 12 h exposure durations were: N/A, 100 mg $l^{-1}$, 50 mg $l^{-1}$ and 50 mg $l^{-1}$, respectively (Fig. 2B, Table 2). For 72 h old larvae exposure durations ≥24 h resulted in significantly lower survivorship than shorter exposures of ≤12 h ($Z_{3,128} = -3.5$, $P < 0.001$). The LOEC for 24 h exposure duration was 200 mg $l^{-1}$ (Fig. 2C, Table 2). No significant differences were found for survivorship between concentrations at 1 h, 12 h and 72 h. Overall, mortality was higher for early development stages, and the longer the exposure lasted (Fig. 2A–C).

*3.3. Effects of exposure to coal during embryo development on larval settlement*

Settlement success ranged from 60 ± 8% to 95 ± 3% across treatments and varied with coal concentration (0–800 mg $l^{-1}$), exposure duration (24 or 72 h) and development age (3 h, 12 h, 72 h) (Fig. 2D–F, Table 2). Lowest mean settlement values were 63 ± 6%, 60 ± 8% and 60 ± 5% for 3 h, 12 h and 72 h olds after 72 h exposure to 50, 400 and 800 mg coal $l^{-1}$, respectively. Larvae that had been exposed to coal for 72 h as 3 h and 12 h old embryos exhibited apparent decreases in settlement success at high concentrations; however, these were not found to be statistically different. Exposure time significantly affected settlement of both 3 h (Permanova Pseudo-$F_{1,70} = 20.8$, $P < 0.001$) and 12 h old embryos (Permanova Pseudo-$F_{1,70} = 25.2$, $P < 0.001$), with lower settlement success observed after 72 h coal exposure. For larvae exposed to coal at 72 h-old, settlement was significantly different between coal treatments (Permanova Pseudo-$F_{1,70} = 8.1$, $P < 0.001$) but not exposure durations. The LOEC for both exposure durations was 800 mg $l^{-1}$ for this development stage.

*3.4. Effects of coal encapsulation on larval settlement and survival*

Coal encapsulation occurred in the majority of larvae exposed to 800 mg coal $l^{-1}$ for 12 and 24 h (92 ± 5% and 99 ± 1% respectively) (Fig. 3). Survivorship following coal encapsulation was 87 ± 1% and 93 ± 3% for 12 and 24 h exposures, respectively, while 100% survivorship was observed in control larvae treatments. After an additional 24 h, 100% and 84 ± 6% of 12 h and 24 h exposed larvae had escaped from coal balls,

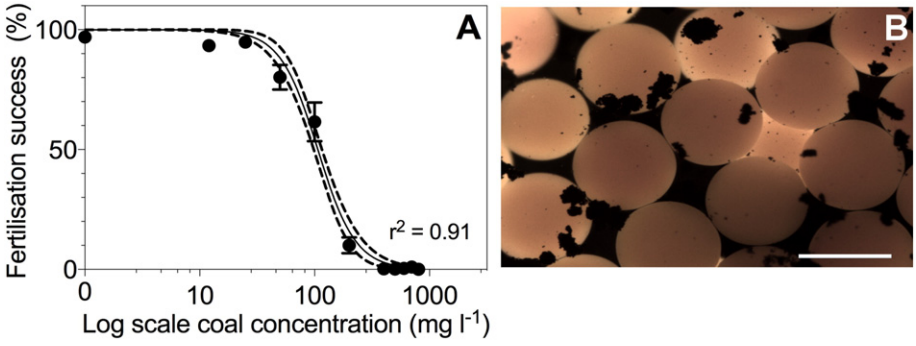


**Fig. 1.** Concentration-response relationship. (A) Fertilisation success (mean % ± SE) with increasing suspended coal (log scale) and (B) unfertilized eggs and what is likely sperm/coal flocs at 400 mg coal $l^{-1}$. Scale bar = 500 μm.

**Table 2**
Results summary of concentration-response experiments. Abbreviations: NOEC = no observed effect concentration, LOEC = lowest observed effect concentration, $IC_{10}$ = coal concentration required for 10% inhibition of response, $IC_{50}$ = coal concentration required for half-maximal response, - = not applicable, * = alternative unit as specified. Images adapted from Jones et al., 2015.

| Development stage | Coal exposure type | Response | Exposure duration (h) | NOEC (mg $l^{-1}$)* | LOEC (mg $l^{-1}$)* | $IC_{10}$ (mg $l^{-1}$) | $IC_{50}$ (mg $l^{-1}$) | *P*-value | Results section |
|---|---|---|---|---|---|---|---|---|---|
| Gametes | Suspension | FertilisationFigs. 1 & 6A | 2.5 | 25 | 50 | 47 | 107 | <0.001 | 3.1 |
| | Leachate | | 2.5 | 100% | – | – | – | >0.05 | 3.7 |
| 3 h (2–4 cell) embryo | Suspension | Survival Fig. 2A | 1 | 50 | 100 | – | – | 0.021 | 3.2 |
| | | | 12 | 400 | 800 | – | – | 0.010 | |
| | | | 24 | 25 | 50 | – | – | 0.031 | |
| | | | 72 | 25 | 50 | – | – | 0.019 | |
| | | Settlement Fig. 2D | 24 | 800 | – | – | – | >0.05 | 3.3 |
| | | | 72 | 800 | – | – | – | >0.05 | |
| 12 h (Prawn chip) embryo | Suspension | Survival Fig. 2B | 1 | 800 | – | – | – | >0.05 | 3.2 |
| | | | 12 | 50 | 100 | – | – | 0.032 | |
| | | | 24 | 25 | 50 | – | – | 0.018 | |
| | | | 72 | 25 | 50 | – | – | 0.044 | |
| | | Settlement Fig. 2E | 24 | 800 | – | – | – | >0.05 | 3.3 |
| | | | 72 | 800 | – | – | – | >0.05 | |
| 72 h (tear drop) Larvae | Suspension | Survival Fig. 2C | 1 | 800 | – | – | – | >0.05 | 3.2 |
| | | | 12 | 800 | – | – | – | >0.05 | |
| | | | 24 | 100 | 200 | – | – | 0.032 | |
| | | | 72 | 800 | – | – | – | >0.05 | |
| | | Settlement Fig. 2F | 24 | 400 | 800 | – | – | <0.001 | 3.3 |
| | | | 72 | 400 | 800 | – | – | <0.001 | |
| Larvae | Leachate | Settlement Fig. 4 & 6B | 48 | 100% | – | – | – | >0.05 | 3.7 |
| | Smothered CCA | | 48 | Washed CCA | 12.5 mg $cm^{-2}$ | – | – | <0.001 | 3.5 |
| Adult | Suspension | Survival | 14 d | 70 | 200 | 29 | 87 | 0.01 | Berry et al., 2016 |
| | Suspension | Survival | 28 d | 0 | 40 | 34 | 36 | 0.001 | |

respectively. Settlement of control larvae ranged between 94 ± 2% and 96 ± 2%, while settlement of previously encapsulated larvae were reduced to 30 ± 9% and 37 ± 7% for 12 and 24 h exposure durations, respectively (Fig. 3). Chi-square goodness of fit test showed a significant difference in the measured proportions of settled, unsettled, smothered and dead larvae ($X^2_9 = 206.6$, $P < 0.001$) across exposure treatments.

### 3.5. Effects of coal smothered CCA on subsequent larval settlement

Settlement success was 95 ± 3% on CCA that had not been exposed to coal or that had been initially smothered but then cleared of coal after 8 h (Fig. 4). Larval settlement was significantly lower on CCA that had 12.5 mg $cm^{-2}$ ($Z_{3,20} = -4.6$, $P < 0.001$) and 22 mg $cm^{-2}$

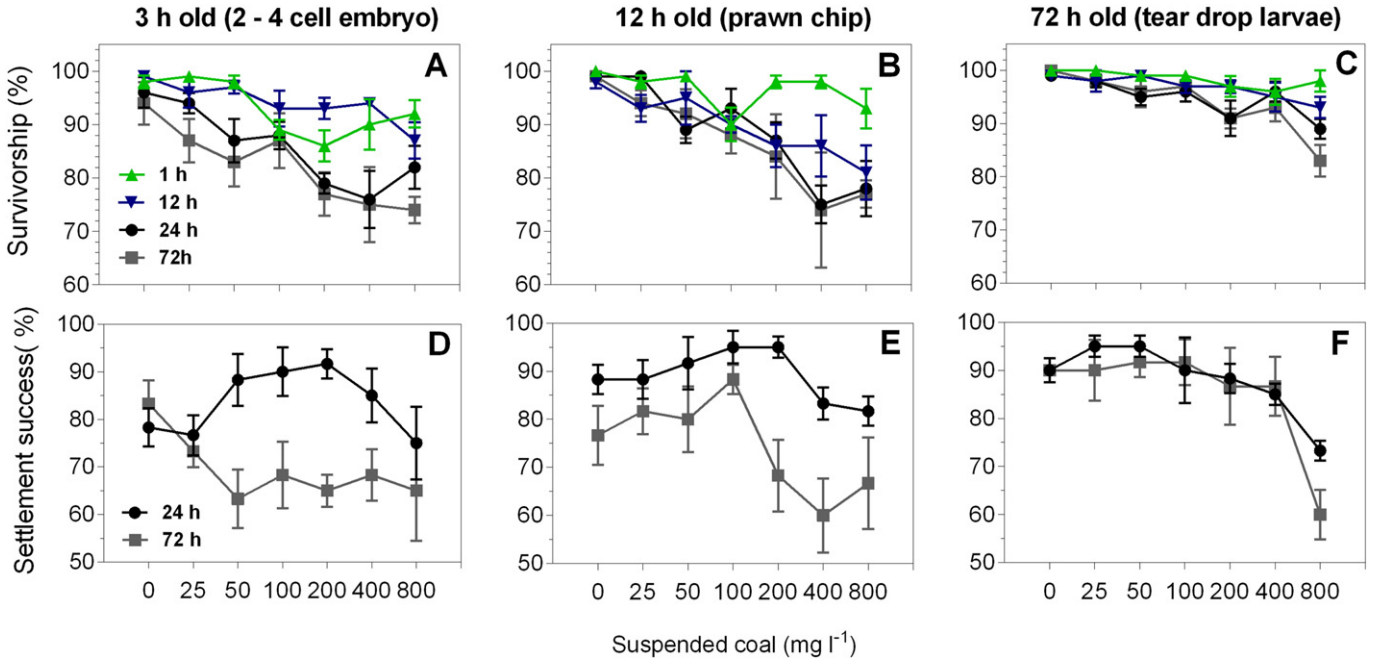


**Fig. 2.** Effects of coal on survivorship and early development. Survivorship and settlement success (mean % ± SE) in 3 h old (2–4 cell stage) (A, D), 12 h old (prawn chip stage) (B, E) and 72 h old (tear drop larvae) (C, F) early development stages after exposure to a range of suspended coal concentrations over 4 exposure durations (1 h, 12 h, 24 h, 72 h, please refer to figure legend). The 3 development stages were also exposed to coal for 24 h and 72 h, and grown out to larvae in clean seawater. Settlement success was measured once specimens reached competency (6 d) (D–F).

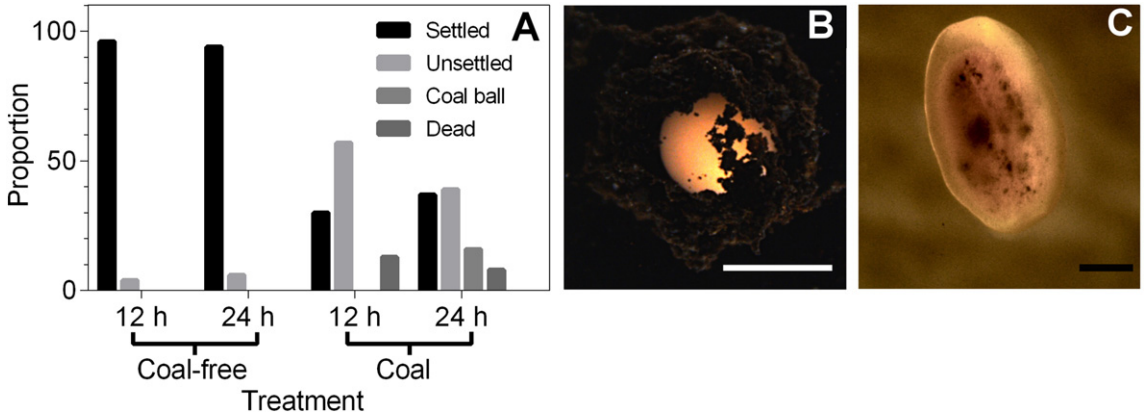


**Fig. 3.** Effects of coal encapsulation on coral larvae. (A) proportion of settled, unsettled, coal coated and dead larvae after 12 and 24 h exposure to coal-free seawater and 800 mg coal $l^{-1}$. Images depict (B) a larva in a coal ball and (C) a larva that has ingested coal. Scale bars = 500 μm.

($Z_{3,20} = -4.6$, $P < 0.001$) coatings of coal, decreasing to 50 ± 15% and 50 ± 8% respectively (Fig. 4).

### 3.6. Effects of coal deposition on juvenile survivorship

After 96 h of smothering, a significantly larger number of juvenile corals smothered in sediment (60 ± 7%) had cleared themselves off compared with recruits smothered in coal (33 ± 5%) ($F_{1,63} = 7.8$, $P = 0.007$, Fig. 5A). Mean survivorship was 94 ± 3%, 90 ± 4% and 83 ± 4% for control, coal smothered and sediment smothered juveniles, respectively (Fig. 5B). No significant ($P > 0.05$) difference in survivorship was found between coal and control treatments or coal and sediment treatments; however, mortality in sediment smothered juveniles was significantly different than juveniles in the clean seawater controls ($Z_{2,96} = -14.4$, $P < 0.001$).

### 3.7. Effects of coal leachate on coral reproduction

There was no effect of coal leachate on coral fertilisation or metamorphosis ($P > 0.05$). Fertilisation success ranged between 94 ± 3% and 97 ± 1% in all treatments (Fig. 6A), while larval settlement success ranged between 83 ± 5% and 100 ± 0% in all treatments (Fig. 6B, Table 2). Embryonic abnormalities were minimal (ranging between 1 ± 0.5% and 1.4 ± 0.6%) and did not differ substantially between leachate treatments.

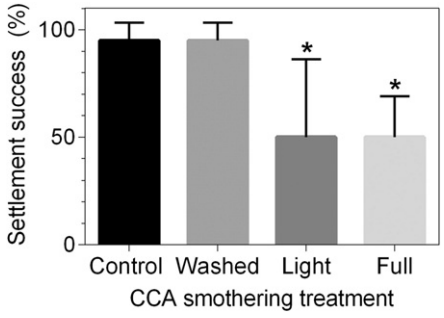


**Fig. 4.** Coal deposition onto CCA: effects on larval settlement across CCA smothered treatments (mean % ± SE). Washed treatments included the smothering of CCA with coal for 8 h, after which CCA was washed with FSW. The light and full coal treatments consisted of the deposition of 12.5 ± 0.9 mg $cm^{-2}$ d/wt and 22 ± 1.5 mg $cm^{-2}$ d/wt of pre-wetted coal onto CCA.* depict significant differences relative to control treatments.

### 3.8. Water quality analyses

Elevated concentrations of certain trace metals (maximum: Mn = 1.0 μg $l^{-1}$, Co = 0.35 μg $l^{-1}$, Zn = 6.8 μg $l^{-1}$, Cu = 0.81 μg $l^{-1}$) leached from coal suspensions during 3 h and 72 h exposures (Table S1, Supplementary material). The maximum magnitude of change in dissolved metal concentrations leached from coal suspensions, in relation to control seawater, was generally minimal: As = 0.14 μg $l^{-1}$; Co = 0.33 μg $l^{-1}$; Cu = 0.44 μg $l^{-1}$; Pb = 0.1 μg $l^{-1}$; Mn = 0.77 μg $l^{-1}$; Mo = 0.4 μg $l^{-1}$; Ni = 0.08 μg $l^{-1}$; Zn = 4.43 μg $l^{-1}$. However, concentrations of Co and Cu exceeded 99% levels of protection (% species) outlined by the ANZECC marine water quality guidelines (ANZECC, 2000). Cobalt and Cu did not exceed the 95% species protection guideline of 1 μg $l^{-1}$ and 1.3 μg $l^{-1}$, respectively. In the 100% leachate treatment water, Co, Cu and Zn concentrations were equal to, or above, 99% guideline levels (Table S1, Supplementary material). Additionally, only trace concentrations of polycyclic aromatic hydrocarbons were detected from extractions of leachate from 800 mg coal $l^{-1}$ (suspension experiments) or 10,000 mg coal $l^{-1}$ (leachate experiments) suspensions (maximum of total PAH = 0.61 μg $l^{-1}$) (Table S2, Supplementary material). Only naphthalene has an Australian trigger value (99% protection level = 50 μg $l^{-1}$) (ANZECC, 2000).

## 4. Discussion

### 4.1. Experiment overview

Our results indicate that suspended and settled coal particles have the potential to affect early life history processes of the reef building coral *A. tenuis*. Gamete fertilisation, embryo survival, larval survival and larval settlement were all significantly reduced over a range of coal concentrations and exposure scenarios. Earlier development stages (gametes and embryos) were most sensitive to coal, being affected at lower concentrations and over shorter exposure durations compared with larvae and juveniles. Development age, coal concentration, and exposure duration are therefore important factors affecting the severity of coal impacts on early life history stages and processes of corals.

Coal represents a potential physical and chemical hazard to marine organisms through smothering and abrasion or the leaching of inorganic/organic constituents of the coal, respectively (Ahrens and Morrisey, 2005). In our study, there was no apparent toxic effect of dissolved leachate from coal on fertilisation, larval settlement or juvenile survival. While coal contains PAHs and some minerals, only low concentrations of PAHs and trace metals were detected in the leachate. With the exception of Co, Cu and Zn, the dissolved concentrations of PAHs and metals that were detected were at least an order of magnitude lower than

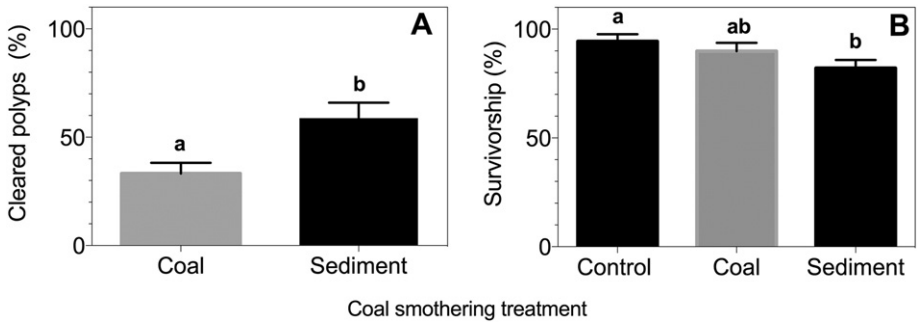


**Fig. 5.** Effects of coal smothering on coral juveniles. (A) Ability of smothered juveniles to clear coal and sediments and (B) juvenile survivorship (mean % ± SE) after 96 h smothering by coal and sediment. Significant ($P < 0.05$) differences between treatments are depicted by different letters.

trigger guidelines (where they exist) ANZECC (2000). PAHs and metals from all coal treatments were lower than concentrations previously found to inhibit fertilisation and metamorphosis in corals (Heyward, 1988; Negri et al., 2016; Negri and Heyward, 2000; Negri and Heyward, 2001; Reichelt-Brushett and Hudspith, 2016; Reichelt-Brushett and Harrison, 1999). This limited leaching of contaminants from coal is consistent with some previous studies (Bender et al., 1987; Jaffrennou et al., 2007). Although it is possible that PAHs and trace metals may have been available to the coral through direct contact with the fine coal, our experimental results coupled with water quality analysis suggests that the coal used in our study did not pose a toxic threat to early life histories of *A. tenuis* and that the measured effects were likely caused by physical interactions. This interpretation is consistent with our previous study, which attributed mortality of adult corals exposed to suspended coal to physical effects including reduced light and feeding, as well as smothering (Berry et al., 2016).

### 4.2. Effects of suspended coal on early life histories of A. tenuis

Gamete fertilisation was the most sensitive early life history process to suspended coal exposures (summarised in Table 2), with complete inhibition observed at the highest concentrations. When coral gametes are released into the water column, positively buoyant eggs float upon the surface (Arai et al., 1993) and sperm swim at and just below the water surface before eventually sinking (Padilla-Gamino et al., 2011). The probability that coal particles directly affect eggs is low, as the sperm dilution experiment indicated egg viability was not affected even at high concentrations of coal (Fig. S1). While eggs appeared to have limited interaction with coal at the surface (i.e. coal did not stick to eggs), the very small particles of floating coal may have interacted directly with the sperm. Unfertilised eggs in the present experiment were surrounded by flocs of coal, which may have formed through these interactions between sperm and coal (Fig. 1B). Sperm entanglement and coating with suspended sediments has been shown to reduce the number of sperm available for fertilisation, rather than affecting egg viability (Ricardo et al., 2015), and the same mechanism seems likely for fine coal particles. However, the coal flocs were often in close proximity to the coral eggs and this may have also prevented some sperm-egg interactions. The impacts of suspended sediments on coral fertilisation are variable, with LOECs ranging from 50 to 169 mg $l^{-1}$ in past studies (Erftemeijer et al., 2012; Gilmour, 1999; Humphrey et al., 2008). Differences in sensitivity could be due to many factors such as the particle type and composition, particle size, angularity, stickiness, sperm concentration and experimental methods used (Jones et al., 2015). A recent study has shown that suspended sediments can also adhere to and sink egg-sperm bundles during their ascent to the surface, further reducing fertilisation potential (Ricardo et al., 2016), and this mechanism may likewise be relevant for suspended coal.

A wide range of coal concentrations (50–800 mg coal $l^{-1}$) resulted in significant reductions in survivorship of 3 h old (2–4 cell stage) and 12 h old (prawn chip) embryos, and 72 h old larvae. We found that 3 h and 12 h old embryos were more sensitive to suspended coal than 72 h larvae, exhibiting the lowest LOECs and highest mean mortality. Many coral embryos lack a protective embryonic envelope, making them more susceptible to disruption by natural forces (Heyward and Negri, 2012), potentially contributing to the sensitivity of these early development phases. Embryos fragmented due to moderately turbulent ocean conditions continued to develop into proportionally smaller larvae, yet still metamorphosed into juvenile corals (Heyward and Negri, 2012). Exposure to some types of sediments can cause increased embryo abnormalities (Humphrey et al., 2008; Erftemeijer et al., 2012). *Acropora millepora* embryos exposed to 5 types of sediments exhibited a maximum abnormality level of 45% when exposed to 16 mg $l^{-1}$ of sediments (Humphrey et al., 2008). Our highest level of abnormal development (19%) was similar to the lowest level (21%) measured in *A. millepora* (Humphrey et al., 2008). It has been suggested that abnormal development of embryos may lead to a reduction of viable larvae (Bassim et al., 2002). In the present study larvae were considerably more tolerant than embryos, possibly due to their mobility and the action of their cilia which would help protect them from close contact with particles, and to break free from coal encapsulation. This age-related trend of increasing tolerance to suspended coal may continue into

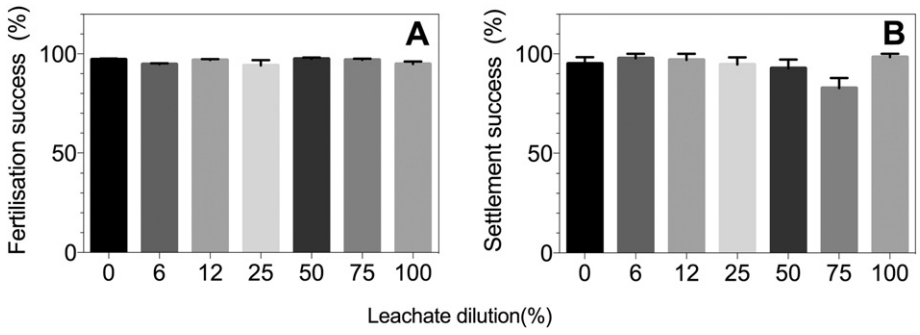


**Fig. 6.** Effects of coal leachate on coral reproduction (A) fertilisation and (B) settlement success (mean % ± SE) in relation to increasing leachate concentrations (0–100%).

adulthood with LOECs for adult *A. tenuis* being 200 mg coal $l^{-1}$ ($EC_{10}$ = 29 mg coal $l^{-1}$) over the shortest 14 d exposure (Table 2) (Berry et al., 2016). The observation that coal particles can be ingested by larvae (Fig. 3C), indicates that this is a potential pathway for uptake that should be investigated further.

The effects of coal on larval settlement varied across the tested experimental scenarios (i.e. settlement after exposure as embryos and pre-competency, settlement after coal encapsulation and settlement onto coal smothered CCA), with maximum reductions in settlement ranging from 30 to 50%. Larvae that had been exposed to suspended coal during their early development stages exhibited lower settlement success with increased coal concentration; however, significant effects were only apparent for the highest coal treatment over the longest exposure duration (800 mg coal $l^{-1}$ for 72 h larvae). These results suggest that if corals in their early development stages passed quickly through a site contaminated with low-moderate coal concentrations, subsequent development and settlement would not be significantly affected. Our results are consistent with three previous studies conducted with dredge spoil sediments and coastal marine sediments, which found no significant effects of suspended sediments on post-fertilisation embryonic development (Erftemeijer et al., 2012; Humphrey et al., 2008) and survival (Gilmour, 1999). However, the reduction in larval settlement (~50%) in the presence of coal-smothered CCA highlighted that indirect effects of coal contamination, such as deposition onto reef substrata could have implications for the success of coral recruitment since larvae can disperse widely from their natal reef via currents (Harrison et al., 1984). Numerous studies have investigated the effects of various types, amounts and scenarios of sediment deposition on larval settlement with variable results (Babcock and Davies, 1991; Babcock and Smith, 2000; Gilmour, 1999; Hodgson, 1990; McClanahan and Obura, 1997; Te, 1992)

For example, sedimentation rates of 0.5–325 mg $cm^{-2}$ $d^{-1}$ did not result in substantial reductions in settlement of *Acropora millepora* larvae in aquaria (Babcock and Davies, 1991). However, sedimentation rates of 1.88–11.7 mg $cm^{-2}$ $d^{-1}$ caused significant declines in settlement of the same species in situ (Babcock and Smith, 2000), highlighting that many biotic and abiotic factors are not taken into consideration in laboratory experiments that could influence settlement success in the natural environment and the effects of sediment deposition are potentially underestimated in controlled experimental conditions. It has been suggested that sediment deposition can reduce coral settlement by masking the settlement cues of CCA or by reducing the total area of suitable substratum for attachment (Jones et al., 2015). Sediment deposition can also change settlement preferences of larvae to the undersides of settlement surfaces; however, such orientation could be less optimal for juvenile growth due to light limitation (Babcock and Davies, 1991).

The physical similarities between suspended sediments and coal particles mean that many of the mechanisms associated with reduced coral settlement and juvenile health are likely to apply to coal. Stress pathways associated with coral smothering can include increased energy expenditure on particle clearance, reduced heterotrophic feeding, reduced light levels (impairment of autotrophy) and the reduction of gas/metabolite exchange (Peters and Pilson, 1985; Rogers, 1990). The high survivorship rate of coal-smothered symbiotic juveniles was surprising since 67% remained smothered after 96 h and therefore experienced very low light levels that would impede photosynthetic energy acquisition by symbiotic dinoflagellates. This finding could be related to the exposure duration, or the procedure where coal was mixed with filtered, rather than raw seawater. Investigations into the effects of muddy coastal sediment deposition (43 h) on *Acropora willisae* recruits found no or minimal mortality; however, mortality increased up to >80% when transparent exopolymer particles (TEP, marine snow) were added to the sediment (Fabricius et al., 2003). TEP concentrations are high within 10 km of the Queensland coast during the months when spawning takes place (Fabricius et al., 2003), suggesting TEP could aggregate with coal in the water column. It is therefore possible that our results underestimate the effects of coal smothering that might occur under natural organic-rich seawater conditions.

### 4.3. Implications and conclusions

Fertilisation, embryo survivorship and larval settlement of the coral *Acropora tenuis* were all significantly inhibited by a range of coal particle exposure scenarios. As the early life stages of corals are planktonic and dispersed over a wide area, a coal spill, especially if it involves a plume of suspended coal particles, is likely to affect a larger area than just a reef location where coral spawning is occurring at the time of the spill. Other broadcast spawners, such as fish and many other tropical reef invertebrates may be similarly vulnerable to coal spills. Although we are beginning to appreciate the potential impacts of coal on tropical marine organisms (this study and Berry et al., 2016), the likelihood of spills and the level of exposure remains uncertain. There is insufficient information on ship groundings to estimate the risk of a coal spill with any certainty, but we assume that the likelihood of a major coal carrier grounding and subsequent spill taking place during a spawning event is low. Nevertheless, the results from the present study indicate that such an acute event could have deleterious effects on coral reproduction and recruitment and these potential effects can now be factored into risk assessments. Further studies are required to evaluate the responses of nearshore species to long-term, low level, chronic exposures to coal, which are more likely to occur at sites in close proximity to ports.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2016.10.011.

## Author contributions

K.L.E.B., A.N. and M.H. designed the study, K.L.E.B. and A.N. performed the study, K.L.E.B. analysed the data with input from M.H. and A.N. D.B., and K.A.B. analysed organic chemistry data. K.L.E.B., M.H., A.N. and D.B. wrote the manuscript. All authors reviewed the manuscript. There are no competing financial interests.

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