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### Effects of Coal Contamination on Tropical Marine Organisms

Thesis submitted by Kathryn Laura Elizabeth Berry, MSc In February 2017

For the degree of Doctor of Philosophy College of Science and Engineering James Cook University

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In memory of John N. Berry and Shannon H. Matthews, two ocean explorers that were lost too soon



#### Abstract

Australia exports more coal by sea than any other nation. A series of new mines and port expansions are currently proposed that will lead to an estimated four-fold increase in coal exports through the Great Barrier Reef (GBR) World Heritage Area over the next decade. Increased shipping presents a greater risk of shipping incidents that can potentially release large quantities of coal into the environment. Despite local and international concern related to the shipment of coal through the GBR, there are currently large knowledge gaps pertaining to the risks associated with coal contamination, particularly in tropical marine environments. The overarching objective of this thesis was to quantify the levels at which coal particles become a threat to the health of tropical marine organisms. I placed specific focus on reef-building corals and seagrass because they provide essential ecosystem services. I also investigated juvenile-reef fish as they represent higher level taxa that inhabit coral reefs and seagrass ecosystems. I used an experimental approach to assess the threats posed by both acute and chronic coal particle contamination that is either currently taking place (i.e. fugitive losses from ports), or that could occur in the future (i.e., ship spill scenarios). Additionally, both the physical and chemical effects of coal contamination were considered.

The maintenance of coral reef populations is dependent on the success of early life history processes, such as fertilisation, and larval recruitment onto the reef. A coal spill may contaminate the ocean surface through to the seabed, potentially posing a threat to the early life history stages of corals that develop in the water column. In Chapter 2 I established threshold values for short-term coal exposures to coal suspensions and leachate dilutions, for coral gametes, embryos and larvae. Moderate concentrations ( $\geq$  50 mg l<sup>-1</sup>) of suspended coal particles significantly inhibited fertilisation and reduced embryo survivorship, which could lead to lower larval densities. Low levels of coal deposition (12.5 mg cm<sup>-2</sup>) onto surfaces significantly reduced larval settlement. While a large coal spill is unlikely to occur during a mass spawning event, this chapter highlighted that even low to moderate coal concentrations can impair coral recruitment.

Another potential contamination scenario from a shipping incident involves the chronic release of coal suspensions. The hypothesis that chronic coal contamination will negatively affect key demographic rates (growth and mortality) of a reef-building coral (*Acropora tenuis*), seagrass (*Halodule uninervis*) and reef-fish (*Acanthochromis polyacanthus*) was tested in Chapter 3. The aim was to establish threshold values that elicit lethal and sub-lethal responses. This was investigated by employing a concentration-response experimental design that exposed the organisms to a range of coal concentrations (0 to 275 mg coal l<sup>-1</sup>) over 14 and 28 d. Low to moderate levels of suspended coal caused extreme light attenuation and coal particles were extremely sticky, adhering to the

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tissue surfaces of all test organisms. Coral survivorship and seagrass growth rates declined with increasing coal concentrations, and effects were larger after 28 compared with 14 d of exposure. Fish growth rates were similarly depressed in all coal treatment intensities but survivorship was high (98% survivorship). Reduced growth can have serious consequences on reproductive success and survivorship, which could alter population growth in reefs and seagrass meadows subjected to chronic coal contamination.

The mechanisms underlying sub-lethal effects of coal suspensions on the reef fish, *Acanthochromis polyacanthus*, focusing on aerobic respiration and gill morphology, was investigated in Chapter 4. Acute exposure (5 d) to 38 or 73 mg coal  $\Gamma^1$  temporarily reduced fish standard oxygen consumption rates (M<sub>02</sub>) by 17 %; however, prolonged exposures resulted in significant elevations in M<sub>02</sub>, by 30-47% compared with control fish. After 31 d exposure to suspended coal concentrations (0, 38, 73 and 275 mg coal  $\Gamma^1$ ), coal particles had adhered to fish gills, most notably in the highest coal treatments. In response, the fish exposed to 275 mg coal  $\Gamma^1$  shed parts of their filament epithelium, which increased their respiratory surface area, thus enhancing their oxygen uptake efficiency. This chapter emphasizes that low-moderate concentrations of chronic coal exposure can elicit energetically costly stress responses (increased metabolism, gill remodelling) in fish. However, changes in gill structure potentially mitigate negative effects of coal exposure and this may have contributed to the low fish mortality measured in Chapter 3.

In Chapter 5 I investigated the effects of chronic coal deposition and acute coal suspensions on the physiological performance (photosynthesis, respiration and calcification) of three morphologically distinct coral species, and compared these responses with those elicited by carbonate sediments. Corals were exposed to chronic deposition (2 x 30 mg cm<sup>-2</sup> exposures per week for 4 weeks) and acute exposure to a high concentration (1250 mg l<sup>-1</sup>) of suspended particles. Trace metal and polycyclic aromatic hydrocarbon (PAH) leachate was measured from each particle type. Corals were generally less efficient at clearing coal particles from their tissues compared to sediments, and coal exposure lead to greater reductions in physiological performance than sediment. Elevated dissolved concentrations of certain trace metals (AI, Mn, Co, Ni) in coal suspensions may have contributed to the negative impacts of coal. This chapter emphasizes the differences in the response of corals to coal and the need for management agencies to consider contamination by coal particles independently of- and in addition to sediment exposures.

This thesis provides important evidence that tropical marine organisms can be directly and indirectly affected by a range of coal contamination scenarios; including decreased coral fertilisation and settlement, reduced physiological performance and survivorship of adult corals, and decreased seagrass and fish growth rates. The results underscore the importance of adequately assessing the

risks posed by increased shipping through ecologically sensitive areas, such as coral reefs and seagrass meadows. The research identified threshold estimates for sub-lethal and lethal effects that can be implemented to improve environmental impact assessments and risk modelling. By addressing critical knowledge gaps that presently impede adequate assessment of the risks posed by coal contamination and spills to coral reef and coastal ecosystems, this thesis contributes to the management of sustainable coal transportation through the Great Barrier Reef Marine Park, and other coastal environments.

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#### 1.1 Coal: importance, production and trade by sea

Coal is a fossil fuel that plays an important role in heavy industry and electricity generation in many countries (Ahrens and Morrisey, 2005). Coal is used for steel production, cement manufacturing, chemical and pharmaceutical industries and paper manufacturing (World Coal Association, 2013), and approximately 29.3% of the world's electricity is generated by coal (International Energy Agency, 2016). Traded since the Roman times, the long-term use of coal and its trade by sea has made coal one of the oldest and most widespread forms of contamination in estuarine and marine environments (Ahrens and Morrisey, 2005). Coal remains the second largest dry bulk commodity transported by sea (UNCTAD, 2015). Over 800 million tonnes - Mt - of the total 1,300 Mt of coal shipped in 2015 was transported via shipping routes in tropical marine environments (e.g. in Australia, Indonesia, and Colombia (International Energy Agency 2016)) and, since 2010, there have been five documented collier (i.e. coal ship) incidents in tropical regions (e.g. in the Philippines, Madagascar, Hawaii, India and Australia). While the quantity of coal released during such incidents is often not recorded, large collier spills have resulted in up to 60,000 tonnes of coal release into the marine environment (MV Smart, South Africa 2013). Moreover, smaller, yet substantial spills (~600 tonnes) have been documented during trans-ship loading incidents (Sanchez, 2014). Despite such large quantities of coal being shipped globally, and re-occurring coal ship incidents and accidental spillages into the ocean, there are currently large knowledge gaps regarding the ecological effects of unburnt coal on tropical marine organisms. As such, environmental regulations for coal are generally considered poor or non-existent, and environmental risk assessments inadequate in many countries (e.g. PGM, 2012). This is particularly concerning since the world's top two coal exporters, Australia (392.3 Mt in 2015) and Indonesia (368.4 Mt in 2015) (International Energy Agency, 2016), are also home to some of the world's most extensive and diverse coral reef ecosystems, including the Great Barrier Reef World Heritage Area.

#### 1.2 Coal in the marine environment: sources and behaviour

Unburnt coal enters the global marine environment via a range of pathways including the natural erosion of coal seams, and anthropogenic inputs during various stages of coal processing (Ahrens and Morrisey, 2005). For example, coal may enter the marine environment during coal washing, transport, storage, and ship loading/off-loading processes (Figure 1.1). Ports are well documented sites for chronic coal contamination into the marine environment (Ahrens and Morrisey, 2005; Johnson and Bustin, 2006; Sydor and Storts, 1980; Toki, 2012). Ports often include coal storage areas that are situated in close proximity to the coast and can hold stockpiles of up to 3.5 Mt of coal (Australian Coal Association 2004 as in Ahrens and Morrisey, 2005) and coal stockpiles and ship loading conveyor belts are generally not covered. Consequently, at coal terminals like the Duluth-Superior Harbour (USA), up to twenty metric tonnes of coal particles can be deposited into the marine environment annually due to wind action on storage piles, grooming of coal piles and ship loading operations (Sydor and Storts, 1980). The Duluth-Superior estimate was made ~40 years ago when coal loading volumes were small in comparison to current operations; ports can now load up to 495,000 tonnes of coal in a single day (Port Waratah, Australia (Kirkwood, 2015). Many coal ports operate best management practices to reduce fugitive losses; however, environmental impact assessments maintain that the potential for coal dust generation during loading and accidental spillage during transfer activities is "almost certain" with a probability of 95-100% to occur throughout a year, and the potential for cumulative impacts to marine water quality considered possible (GHD, 2012).

Collier groundings represent the most severe coal contamination scenario. Collier incidents have the potential to cause both acute and chronic contamination depending on the extent of structural damage, whether the ship sinks or is salvaged, degradation of the cargo hold over time, and the hydrodynamics at the incident site. While there is limited documentation of suspended coal concentrations in seawater during a spill event, past events have released 17,000-60,000 tonnes of coal into the marine environment (DOEARSA, 2013; Jaffrennou et al., 2007). The recent sinking of the *MV Mykonos* off the coast of Madagascar in 2016 could result in up to 160,000 tonnes of coal being released into the marine environment (World Maritime News, 2016). In cases where incidents do not result in direct spillage, vessel salvage operations may require cargo to be discarded overboard, as was the case in a recent salvage operation in South Africa where 10,000 tonnes of coal was purposely released into the coastal marine environment (DOEARSA, 2013). In addition to incidents involving large ships, nine barges, each carrying ~600 tonnes of coal, reportedly sunk in Colombian ports between 2006-2010 (Patino, 2013; as in Sanchez, 2014).



**Figure 1.1** Coal sources to the aquatic environment (Achten and Hofmann, 2009; modified from Ahrens and Morrisey, 2005).

The spatial extent of contamination by coal, whether it involves coal release from a localised point source, from trans-shipping or from 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/wt) coal (Allen, 1987; Goldberg et al., 1977; Hamilton et al., 1979; Johnson and Bustin, 2006; Toki, 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 source, smothering benthic flora and fauna (Jaffrennou et al., 2007). 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 by currents and waves and hydrocarbon marker analyses indicate that coal particles can 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 in the water column allowing dispersal from the input source via wind and water currents (Jaffrennou et al., 2007; Johnson and Bustin, 2006). Thin coal films have been documented on the sea surface up to 200 metres away from coal port infrastructure during calm weather conditions (Johnson and Bustin, 2006). 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, potentially posing a threat to marine organisms with a range of life histories.

#### **1.3** Effects of coal on aquatic organisms and environments

Coal is a combustible, sedimentary rock that is a heterogeneous mixture of carbon, organic compounds, and inorganic material in the form of moisture and mineral impurities (Ward, 1984). The assortment of inorganic constituents influences the behaviour of coal, its interactions with the environment and potential biological effects (Ahrens and Morrisey, 2005). Coal contamination has the potential to cause a variety of impacts to aquatic flora and fauna (Figure 1.2) with at least four main features of coal that may result in adverse biological effects to marine organisms (Hyslop et al., 1997): 1) the attenuation of light through the water column by particles or from smothering, 2) the physical presence of solid material, 3) the release of inorganic substances, and 4) the release of organic substances. All of these features can cause sub-lethal and/or lethal consequences on marine organisms (Berry et al., 2003; Erftemeijer et al., 2012b; Jones et al., 2016; Peters et al., 1997); however, the severity of these features are highly dependent on the exposure duration, concentrations and types of coal. Altered light regimes can directly impact primary productivity, behaviour and feeding efficiency, while the physical presence of solid material can lead to tissue abrasion, clogging of feeding and respiratory organs and smothering of sessile benthic organisms (Berry et al., 2003). Such impacts have the potential for flow on effects such as reduced growth and reproductive outputs.



**Figure 1.2** The potential biological effects of suspended and deposited coal on marine organisms. Modified from what is known about sediments (Berry et al. 2003) and information on coal reviewed in Ahrens and Morrisey (2005). All images were available for reuse with modification. Links to image sources are provided in Appendix B.

The physical presence of coal particles in water can have multiple different effects on aquatic organisms (Table 1.1). For example, coal particles cause abrasive damage to leaves of aquatic moss (Eurhynchium riparioides) (Lewis, 1973) and macroalgae (Hyslop and Davies, 1998), inhibition of algal growth (Shelton, 1973), enhancement of algal growth (Hyslop and Davies, 1998), as well as reductions in photosynthesis in mangrove leaves that have had coal dust settle onto them (Naidoo and Chirkoot, 2004). Attenuation of light through the water column caused by suspended coal particles could impair vision and modify predation and or competition processes, as well as reduce growth and abundance of plants (Ahrens and Morrisey, 2005). Effects aren't just limited to sessile organisms, with exposure of Coho salmon (Oncorhynchus kisutch) to a range of coal concentrations resulting in a 96 hour LC<sub>50</sub> (the concentration at which 50% of the test population is killed) of 7,000 mg coal  $I^{-1}$ , with mortalities occurring at coal concentrations greater than 3,000 mg  $I^{-1}$  (Pearce and McBride, 1977). Coal mixed with sand influenced the burrowing behaviour in Dungeness crabs (Cancer magister) (Pearce and McBride, 1977). However, a later study found no effect of coal mixed with sand on crab oxygen consumption or gill ventilation rates (Hillaby, 1981); although technical issues with the experimental design of the latter study mean those effects of coal on these organisms remain unclear.

Colliery waste has also been found to have detrimental effects on species richness and diversity in a range of biological communities. For example, the annual dumping of 6.2 million metric tonnes of fine coal, fly ash and other colliery wastes off the north coast of England influenced local rocky and sandy shore ecosystems (Hyslop et al., 1997; Shelton, 1973). This included inhibition of algal growth, and marked declines in lobster and crab catches in local fisheries, which was associated with active avoidance of the contaminated environment (Shelton, 1973). The magnitude of dumping at this site was greater than that which occurs in the majority of dumping practices; however, the findings demonstrated that large amounts of colliery waste can reduce the productivity of fish populations and alter the biodiversity of ecosystems.

Coal contamination of marine ecosystems could lead to an alteration of the substratum composition. Coal has a lower density (1.2-2.9 g cm<sup>-3</sup>) (Alpern, 1977) than most other typical particulates found in sediment, such as quartz and therefore larger coal particles may settle onto substrates that are composed of smaller sized particulates (e.g. sand and silt), which can alter the stability of the substrate, the suitability of the substrate for recruitment and colonization, and/or interfere with bottom dwelling organisms (reviewed in Ahrens and Morrisey 2005). For example, large colliery waste inputs into England's coastal region resulted in coal contamination within sediments and only low numbers of polychaetes (*Nephtys spp.* and *Nereis diversicolour*), amphipods, and the occasional brittle starfish (*Ophiura spp.*) were found (Shelton, 1973). Secondary effects of coal deposition also include reduced larval survival, changes to population dynamics and,

in addition, potential flow-on effects to higher order consumers have been hypothesized (Johnson and Bustin, 2006).

**Table 1.1** Examples of studies conducted on the physical impacts of coal to aquatic flora and fauna (Reviewed in Ahrens and Morrisey, 2005).

Study organism	Coal treatment	Observed effect	Source
Coho salmon	13,500, 8,000, 3,500 and 3,000	96 hour $LC_{50}$ of 7,000 mg l <sup>-1</sup> ; mortalities	Pearce and
(Oncorhynchus	mg l <sup>-1</sup> .	occurred at concentrations > 3,000 mg $l^{-1}$ .	McBride
kisutch)			(1977)
Dungeness crabs	Coal was mixed with sand for a	Deposition of coal on the gill lamellae of	Hillaby (1981)
(Metacarcinus	21 day exposure experiment to	crabs, but oxygen consumption and gill	,
magister)	examine effect on oxygen	ventilation were not impacted.	
	consumption and gill		
	ventilation.	1	
Aquatic moss	3 week exposure to range of	At 5000 mg l <sup>-1</sup> , Chl <i>a</i> production was	Lewis (1973)
(Eurhynchium	coal concentrations.	limited; however it was never entirely	
riparioides)		prevented at any concentration.	
Macroalgae (Ulva	Algae were exposed to a range	U. lactuca lost weight in the presence of	Hyslop and
lactuca)	of particle sizes: < 500 µm, 500-	contery waste and gained weight in the	Davies (1998)
	2000 μm, and 0-2000 μm under	absence of collery waste, with the	
	conditions for 8 days	2000 um of colliery waste	
Mangroves	Coal dust was found to	Dust reduced carbon dioxide exchange and	Naidoo and
(Avicennia marina)	accumulate on leaf surfaces,	chlorophyll fluorescence by 17-39%,	Chirkoot
,	branches, and trunks of	decreasing photosynthetic performance.	(2004)
	mangroves.		· · /
Juvenile chinook	Fish were exposed to coal dust	A significant increase in CYP1A1 expression	Campbell and
salmon	concentrations of 60, 200 or	was measured in fish exposed to coal dust.	Devlin (1997)
(Oncorhynchus	500 mg l <sup>-1</sup> for 8 days.	CYP1A1 plays an important role in the	
tshawytscha)		activation of PAHs to carcinogenic and	
		mutagenic metabolites.	
Rainbow trout	Front were exposed to 100-600 $m_{\rm e}$ l <sup>-1</sup> of angle	Coughing rates increased twofold in coal	Hughes
(Saimo gairaneri)	mg i of coal.	exposed fish but the coughing frequency	(1975)
		11 out of 14 cases the mean coughing rate	
		decreased once fish were transferred into	
		particle-free water. Coughing rate was also	
		found to decrease with coal exposure.	
Fathead minnows	Exposed to suspended coal	100% mortality was measured after 96 h.	Carlson et al.
(Pimephales	concentrations of 25,000 mg $l^{-1}$ .		(1979)
promelas)			
Young steelheads	Field studies: Fish were	Field studies: steelhead ventilation rates	Pautzke
(Oncorhynchus	exposed to water polluted and	increased and mortality occurred within	(1937)
mykiss gairaneri,	not polluted by coal mine	1.5 h exposure, with 100% mortality in fish	
cited as Suimo	ware enclosed in cases at each	within 2.5 h. High lovels of mucus covered	
gununennj	experimental site. No replicates	the fish gills and skin and solid masses of	
and	were used for any treatment.	coal particles were adhered to the mucus.	
		Discolouration was observed in the gills	
juvenile cutthroats	Validation study: Fish were left	colour.	
(Oncorhynchus	overnight in water pumped		
clarkii, cited as	directly from the mine.	Juvenile cutthroats were more sensitive to	
Salmo clarkii)		the polluted water and 100% mortality was	
		observed within 30 minutes exposure.	
		Validation study: no mortality was	
		observed.	

Coals are classified into four ranks (lignite, sub-bituminous, bituminous and anthracite) based on their maturity and each rank varies in chemical composition and, thus, differs in energy content, use, and the potential for biological effects (Ahrens and Morrisey 2005). Different coal types, i.e. coking coal (used for steel production and cement manufacturing) and thermal coal (used for energy production) can vary in their rank, making generalisations about the chemical effects of coal difficult. Both polycyclic aromatic hydrocarbons (PAHs) and trace elements are present in variable amounts in coal depending on coal rank and coal type, and both can be leached from unburnt coal upon contact with water (Cheam, 2000).

One class of hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), are potentially hazardous, and the consequential effects on aquatic organisms are dependent on numerous factors including the type of coal, its mineral impurities, bioavailability, and concentration (Ahrens and Morrisey, 2005; Davis and Boegly, 1981; Querol et al., 1996). Commonly occurring PAHs in coal leachates include naphthalene, phenanthrene, chrysene, fluoranthene and pyrene (see Ahrens and Morrisey 2005). Consistent to metal leaching, PAHs have been found to leach more from sulphur-rich bituminous coals than from low-sulphur sub-bituminous coal or lignite (Stahl and Davis, 1984).

Certain studies have revealed that metals like Cu and Cd can be leached from coals, metals that are known to be toxic to corals (e.g. Brown, 1987; Negri and Heyward, 2001; Reichelt-Brushett and Hudspith, 2016; Reichelt-Brushett and Harrison, 2005), seagrasses (Macinnis-Ng and Ralph, 2002, 2004), and other marine organisms when they exceed particular threshold levels. Although many of the detected levels of leached metals from coal have been within acceptable sediment quality or water quality guidelines (Hyslop et al., 1997; Lucas and Planner, 2012), there were exceptions such as Cu (Lucas and Planner 2012), and contaminants can also be bio-accumulated through the food chain and exported to other areas by mobile animals (Blackmore and Wang, 2004; Wang, 2002). Metal toxicity is also dependent on the speciation (dissolved form) of the metal, specifically the free metal ion concentration (Hall and Anderson, 1995). Specific trace elements within coal, and their ability to leach are highly dependent on the mineral impurities present in the coal (such as sulphur) (see Ward, 2002). Leaching of metals can be accelerated in the presence of oxygen or oxidizing agents or if coal remains wet between leaching events (Davis and Boegly, 1981; Querol et al., 1996). To date, there is no consensus in the literature as to whether PAH leachate concentrations will exceed water quality guidelines or pose toxic threats (mutagenic or narcotic toxicity) to marine invertebrates (Ahrens and Morrisey, 2005). Numerous coal leaching experiments have been conducted using a range of techniques (Table 1.2) as there is currently no standard leaching protocol that is widely applied.

# **Table 1.2** Studies on the leaching of trace metals and polycyclic aromatic hydrocarbons from coal (References depicted by a \* are reviewed in Ahrens and Morrisey, 2005).

Study organism	Coal treatment	Observed effect	Source
N/A	Investigated leaching with a number	Increases in temperature and	Coward et
	of variables such as temperature, pH,	decreases in pH generally resulted in	al. (1978)*
	particle size, oxygen saturation,	increased leachate concentrations of	
	contact time and flow.	most metals analysed; Sulphur rich	
		coal had a lower leachate pH and	
		generally leached higher amounts of	
		metals such as Cd, Co, Cu, Mn, Ni and	
		Zn.	
Marine gastropod	Gastropods were exposed to coal	Bioaccumulation of Cd from coal was	Siboni et al.
(Hexaplex trunculus)	particles in the substrate.	detected in the hepatopancreas.	(2004)*
N/A	Coal (10 kg) from the Bowen Basin,	Leaching of metals from coal to	Lucas and
	Queensland shaken (in 20L water)	seawater over this short period was	Planner
	using a sieve shaker for 2 hours. The	detected for Cd, Cu, Pb, Mn, Mo, Ni	(2012)
	coal was then left for 24 h before the	and Zn, with Cu and Mn exceeding	
	leaching solution was sampled.	water quality guidelines.	
N/A	Environmental monitoring of metals	Detected lower metal concentrations	Hyslop et al.
	in colliery waste washed up on	(many below guidelines levels) in	(1997)*
	beaches in comparison to that	colliery waste washed up on a beach	
	measured in coal and waste prior to	compared to that measured in coal	
	dumping.	and waste prior to dumping.	
Mummichogs	Fish were dosed in static exposure	Significant reductions in sperm and	Cochran
(Fundulus	tanks with water	gamete production after exposure to	(1987)*
heteroclitus)	extracts of coal (leachates).	some coal leachates; however this	
		result was dose dependent.	
Eastern oysters	Exposure to coal dust plus leachate	After 7, 14, and 28 days exposure,	Bender et
(Crassostrea	[10 mg l <sup>-</sup> ] for 15 days, and 1 mg l <sup>-</sup>	polynuclear aromatic hydrocarbon	al. (1987)*
virginica)	coal dust for 28 days in a flow	body burden had not increased,	
	through system.	provided coal particles were first	
		purged from their guts. Additionally,	
		high coal exposure did not exhibit any	
		effect on shell growth.	
N/A	Leaching potential of metals from 3	Few trace elements leached into both	Cabon et al.
	South African coals was examined in	freshwater and seawater at a pH of "8.	(2007)
	freshwater and seawater.	win was an exception with high	
	Fish were surges at to 10 ms l <sup>-1</sup> and	leachate levels in both water types.	Carbart at
Fathead minnows	Fish were exposed to 10 mg i coal	No damage to gill surfaces of skin was	Gernart et
(Pimephales	particles with phenanthrene	observed. Ingested coal particles	al. (1981)*
prometas	for 14 days	caused mucous secretion by gut	
	10f 14 days.	mucosa. Phenanthrene uptake was not	
Painhow travit	Fish wore expected to $200 \text{ mg}^{-1}$ cool	Growth was inhibited; however each	Horbortand
	rish were exposed to 200 mg i Coal.	norticulates were not toyis to reinhous	Disbards
(Uncornynchus mykics)		trout No mortality was absorved	
ттукіззу		trout. No mortality was observed.	(1973)", citod in
			Corbort of
			aı. (1901)

#### 1.4 Coal in the tropical marine environment: Australia as a case study

Coal is Australia's second highest export commodity (Australian Government 2016) and the Queensland commodity market is dominated (>80%) by coal trades (AMSA 2014). Over the last three decades there has been a strong growth in Australia's mining sector and this growth has stimulated increases in coastal development, particularly related to port infrastructure and shipping activities (Grech et al., 2013). Australia is currently ranked first in global seaborne coal exports (International Energy Agency, 2016), with the country's largest reserves (~34 billion tonnes) found within the state of Queensland (217.8 Mt exported in 2014-2015, Queensland Government, 2016). Currently, there are more than 50 operating mines in Queensland, and further large-scale thermal coal mines are proposed in the Surat and Galilee Basins (Queensland Government, 2016), including the recently approved Carmichael mine, which is forecast to produce 60 Mt of coal per year for 60 years (Queensland Government, 2017).

The majority of coal in Queensland is transported by rail to the ports of Hay Point, Gladstone and Abbot Point, all situated adjacent to the Great Barrier Reef (GBR) World Heritage Area (GBRWHA) (Figure 1.3). The GBR consists of ~3000 coral reefs that span along 2300 km of the Queensland coastline (GBRMPA, 2014). This region is ecologically significant as habitat to many hundreds of fish species, endangered turtles, dolphins, dugongs and whales, a high diversity of hard and soft coral species, and seabirds (GBRMPA, 2013 a). Large scale human impacts combined with climate change could greatly impact the ecological function and biodiversity of this region; therefore, coastal development and port expansions are major management issues for the GBR (Great Barrier Reef Marine Park Authority, 2014).

Coal is carried by bulk carriers and over 1300 coal vessels transited through the GBR region in 2013-2014 (Queensland Government, 2015). The average annual growth in ship traffic is forecast at ~ 3-6% if demand continues and proposed large-scale mines and associated infrastructure progress (Braemer Seascope, 2013; Bureau of Resources and Energy Economics, 2012). It has been forecast that 2450 coal ships will sail through Queensland in 2020 (AMSA, 2014); however, larger ship sizes (i.e., higher volumes of cargo per vessel) and global demand will influence these estimates. Since the introduction of live traffic imaging systems shipping incidents have fallen from an average of one per year to one in ten years (PGM, 2012), however, the forecast rise in shipping traffic through the complex GBR ecosystem is considered to increase the risk of shipping and pollution incidences such as spills and groundings (AMSA, 2013; GBRMPA, 2013 b). Relevant examples of scenarios where large coal spills onto coral reefs were narrowly avoided include groundings of the *MV Prosperity* (Philippines, 65,000 tonnes) while transporting coal from Australia to India in 2011 and the *Shen Neng 1* (GBR, 68,000 tonnes) which grounded on the southern GBR in 2010 (GBRMPA, 2011).



**Figure 1.3** Ports, ships and shipping lanes within the Great Barrier Reef World Heritage Area. Black dots represent vessels tracked at 1 hour intervals over 107 days. Coal ports are depicted by blue squares (from North to South: Abbot Point, Hay Point and Gladstone). Note: Coal is shipped from Brisbane port, situated south of the Great Barrier Reef World Heritage Area (GBRWHA). Ships loaded in Brisbane may still transit through the GBRWHA. SOURCE: e-Atlas and AMSA 2013 (Lawrey, 2013). While shipping incidents may result in coal spills of over hundreds of thousands of tonnes, coastal habitats are already chronically exposed to particulate coal. The risk of chronic coal exposure in coastal marine systems is greatest near the large coal export ports (Hay Point, Gladstone, and Abbot Point). Coal stockpiles along the GBR are not covered and dust can also enter the marine environment directly from stockpiles during windy conditions (GHD, 2012) (Figure 1.4 A), during loading operations (Figure 1.4 B) and/or heavy monsoonal rainfall can transport fine coal particles and leachate towards the ocean, potentially overflowing settlement ponds and sumps designed to minimize particle transport.



**Figure 1.4** Images of chronic coal dust emissions into the environment. Chronic coal dust emissions occur where coal is stored, from action of wind on stockpiles and whenever coal is moved. For example, fugitive losses occur during maintenance of stockpiles (**A**) and ship loading operations (**B**). Unknown quantities of these emissions are deposited into the ocean. These photos were taken from the Port of Gladstone, Queensland, Australia (Photo: K. Berry, 2013).

#### 1.5 Problems and knowledge gaps

The UNESCO World Heritage Commission was recently poised to downgrade the World Heritage status of the Great Barrier Reef to 'World Heritage in Danger', based on ongoing concerns about coastal development, development of industrial ports, grounding of ships and declining water quality (UNESCO, 2012). This high-profile international recognition that increased industrial activity along the Queensland coast poses a threat to the health of the GBR clearly has implications for decisions regarding the management of coal exportation/transportation through the GBR. Despite local and international concern related to the shipment of coal through the GBR, there are currently large knowledge gaps pertaining to the risks posed by coal contamination, including the absence of any rigorous quantification of the levels at which coal contamination becomes a threat to the health of tropical marine organisms. It is therefore a timely challenge to assess the threat that chronic and acute coal exposures pose to the marine environment as a result of terrestrial run-off, loading of coal

at coastal ports, ship-to-ship transfer at sea (trans-shipping), or large shipping spills. Because of these knowledge gaps, the threats and risks associated with a coal spill on the GBR are not currently included in the "Environmental Impact Assessment of coal shipments through the GBR" (PGM, 2012), which seems incongruous for a World Heritage Area. It is therefore imperative that new research is initiated.

#### 1.6 Thesis aims and objectives

The overarching aim of my research was to quantify and understand the sub-lethal and lethal effects of coal contamination on three tropical marine taxa commonly found on the GBR: reefbuilding corals, reef-fish and seagrass. I placed particular focus on reef-building corals because they provide the main structural component of coral reefs, and because coral reefs have been impacted by collier groundings in recent years. Thus, different species of corals were investigated and compared and a whole of life-cycle approach was used to assess the potential effects on adult corals, as well as the effects on early life history processes and stages that are essential for coral recruitment and reef maintenance. I also investigated seagrasses because they are key habitat-forming species found in proximity to coal ports in Queensland, as well as juvenile-reef fish as they represent higher-level taxa that inhabit coral and seagrass ecosystems during their potentially sensitive early development stages. To accomplish these goals, I addressed the following four specific objectives:

- 1. Identify the early life history stages and processes of reef-building corals most at risk from coal contamination and determine the exposure scenarios that cause the greatest threat.
- 2. Identify the concentrations and exposure durations of coal (suspended and deposited) required to negatively impact key demographic rates (growth and mortality) of a reefbuilding coral (*Acropora tenuis*), reef-fish (*Acanthochromis polyacanthus*, spiny chromis) and seagrass (*Halodule uninervis*).
- 3. Identify sub-lethal impacts of suspended coal particles on a juvenile planktivorous coral reef damselfish (*Acanthochromis polyacanthus*).
- 4. Determine how different coral species are impacted by chronic, low level coal deposition and suspended particles. Understand whether and how these effects differ from the effects of calcium carbonate sediments that are commonly re-suspended on coral reefs.

#### 1.7 Overview of research methods

My research used an experimental approach; with large scale aquarium experiments conducted using state of the art aquarium facilities at the National Sea Simulator (at the Australian Institute of Marine Science). Experiments were designed to identify biological responses of key marine organisms to coal exposures that simulated the effects of accidental coal particulate loss during storage and loading procedures, as well as major coal spills associated with ship groundings. Custom designed flow-through aquaria suitable for short and long-term coal particle exposures were used to maintain water quality and the continued suspension of coal particles at desired concentrations. Throughout each experiment, water quality parameters such as light, pH, total suspended solids (TSS), coal deposition, and temperature were measured. Each experiment was followed by water quality analysis to quantify leaching of metals (using ICP-MS) and polycyclic aromatic hydrocarbons (PAH, using GC-MS). Such analyses helped to determine whether the observed responses were due to physical or chemical effects of coal, which is important for determining the most influential stress-pathways of coal contamination.

Test organisms were investigated for a range of response variables that included physiological (e.g. oxygen consumption and photosynthesis) and biochemical responses (e.g. pigment content), and demographic processes such as growth and mortality. Observational indicators of stress such as mucus release, behavioural changes, and tissue discolouration were monitored throughout experimental durations.

#### **1.8** Significance of research

Corals and seagrasses are the foundation species of diverse tropical coastal ecosystems like coral reefs and seagrass meadows, which are situated in proximity to coal ports and shipment routes in the GBR. These environments of high ecological value provide essential habitat for a plethora of marine invertebrates and vertebrates such as fish. These habitats are at risk of increased exposure to coal as loading volumes and shipping increase within the GBR; however, the biological effects, behaviour and distribution of particulate coal in tropical waters are largely unknown. Targeted research on the effects of coal on tropical marine organisms is essential to improve assessments of the environmental risks posed by coal loading and transport in the vicinity of these highly valued ecosystems.

This thesis presents the first targeted research on the potential impacts of coal contamination on tropical marine organisms, apart from one study in South Africa on mangroves (Naidoo and Chirkoot). My research will provide marine park managers and regulators, as well as industry, with scientifically rigorous information to improve impact assessments and risk modelling. By addressing critical knowledge gaps that presently impede competent assessment of the risks that coal exportation poses to coral reef and coastal ecosystems, my project will contribute primary information for inclusion in

more sustainable management plans of coal shipments through the GBR.

#### 1.9 Thesis structure

This thesis is presented in a series of research chapters formatted for journal publication. Because the thesis chapters have been written for journal submission, unavoidable repetition of some sections occurs. Supplementary figures and tables relevant for each chapter as well as supporting publications are provided as appendices. The four objectives listed in section 1.6 (above) are addressed in Chapters 2 to 5:

**Chapter 2.** The effects of suspended coal, coal leachate and direct coal smothering on early life history stages and processes of the common reef-building coral *Acropora tenuis* are examined. The early life processes investigated include gamete fertilisation, embryo survivorship, larval settlement and juvenile survivorship after exposure to a wide range of coal concentrations that could result from chronic inputs and acute spill scenarios. Where possible, I evaluated exposure-response relationships to generate threshold levels of coal that cause impairment.

**Chapter 3.** The potential impacts of a large collier spill were examined by measuring the effects of suspended coal particles and coal deposition on key demographic rates (growth and mortality) of a reef-building coral (*Acropora tenuis*), reef-fish (*Acanthochromis polyacanthus,* spiny chromis) and seagrass (*Halodule uninervis*) species. The resulting information was used to generate exposure-response relationships to identify threshold levels of coal that are harmful to corals (adults), reef-fish and seagrasses (lethal and sub-lethal). Additionally, coal toxicity via leaching was investigated to help identify key stress pathways associated with coal contamination.

**Chapter 4.** Aerobic metabolism and gill morphology were measured to elucidate the sub-lethal effects of suspended coal particles on a planktivorous coral reef damselfish, *Acanthochromis polyacanthus*. Using intermittent respirometry techniques and standard histological techniques I identify some of the acute and chronic physiological responses and morphological adaptations in a juvenile reef-fish during exposure to suspended coal particles.

**Chapter 5.** The sub-lethal effects of chronic, low level coal deposition and acute suspended sediment exposure on three species of reef-building corals was investigated. Reef-building corals exhibit distinct morphologies that are recognized as having variable tolerances to deposition of particulate matter. In this study I compare how 3 morphologically distinct coral species tolerate (e.g. by assessing survival and tissue health), and respond (e.g. impacts to productivity and calcification, clearance rates) to chronic coal particle exposure and carbonate sediment deposition.

**Chapter 6.** A summary of the key findings of each research chapter is presented in the context of the outlined research objectives. I discuss the results in an ecological context and synthesize findings from the complete thesis into a conceptual framework of stress-pathways and biological effects of coal contamination in the tropical marine environment. I also address how this research can be implemented to improve policy and management of coal shipments through highly valued ecosystems.

### 2. Effects of coal contamination on early life history processes of a reefbuilding coral, *Acropora tenuis*

The content of this chapter has been published as:

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#### 2.1 Abstract

Successful reproduction and larval dispersal are important for the existence of healthy marine invertebrate populations, and these early life history processes can be sensitive to marine pollution. Coal is increasingly 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.

#### 2.2 Introduction

There are specific features of coal that may result in harmful effects to marine organisms such as negative interactions caused by physical proximity of fine 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. The authors suggested the potentially toxic PACs were not bioavailable and independent from PAH content (Meyer et al., 2013). Similarly, some doses of coal leachate caused 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 freshwater fathead minnows to coal leachate (from 6.3 g l<sup>-1</sup> centrifuged; 25 g l<sup>-1</sup> uncentrifuged) 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. Cu and Zn), which are both known to negatively impact early life-history processes of fish (e.g. Johnston and Wildish, 1982; Wenger et al., 2014) and corals (e.g. Jones et al., 2015b; Negri et al., 2011b; Reichelt-Brushett and Harrison, 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; Peters et al., 1997). 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, I 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, I 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.

# 2.3 Materials and Methods

# 2.3.1 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 2.1). 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 2.1).

Developmental process/stage	Nature of coal exposure	Duration of coal exposure (h)	Response variable	Methods section
	Gametes (5 sperm concentrations) exposed to coal: 0, 50 and 200 $$ mg l $^{-1}$	2.5	Fortilization	2.3.3
	Gametes exposed to coal: 0-800 mg l <sup>-1</sup>	2.5	Fertilisation	2.3.3
Fertilisation	Gametes exposed to dilutions of a leachate from a 10,000 mg l <sup>-1</sup> coal suspension	2.5	Success	2.3.7
Embryo	3 h, and 12 h old embryos exposed to coal: 0-800 mg l <sup>-1</sup>	1, 12, 24, 72		2.3.4, 2.3.5.1
	Pre-competent larvae (72 h old) exposed to coal: 0-800 mg l <sup>-1</sup>	1, 12, 24, 72	Survivorship & settlement	2.3.4 2.3.5.1
	Competent larvae exposed to coal: 800 mg $\Gamma^1$	12, 24		2.3.5.2
Larvae	Competent larvae exposed to CCA smothered with coal: Pre-smothered and washed off, light dusting (12.5 mg cm <sup>-2</sup> ) of entire CCA, full coverage (22 mg cm <sup>-2</sup> )	48	Settlement success	2.3.5.3
	Competent larvae exposed to dilutions of a leachate from a 10,000 mg l <sup>-1</sup> coal suspension	48		2.3.8
Juvenile	6-week old juveniles exposed to coal and carbonate sediment (fully smothered)	96	Clearance rates and survivorship	2.3.6

# Table 2.1 Outline of experiments conducted during 2013 and 2014 coral spawning events.

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 I 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, 13 coral colonies). Remaining gametes from the spawned colonies were fertilised and larvae cultured in 500 I 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).

#### 2.3.2 Coal preparation

Thermal coal (sourced from central Queensland, Australia) was crushed, milled and sieved to isolate particles < 63  $\mu$ m. 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 - 13,500 mg coal  $\Gamma^1$  (Bender et al., 1987; Pearce and McBride, 1977). Because I 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, I chose to investigate the suspended coal concentrations: 12.5, 25, 50, 100, 200, 400, 500, 600, 700, 800 mg  $\Gamma^1$ . For leachate experiments, stock suspensions (10,000 mg coal  $\Gamma^1$ ) of the fine thermal coal particles were mixed for 24 h on magnetic stirring plates at 27°C. This high concentration (10,000 mg coal  $\Gamma^1$ ) was tested because I wanted to investigate a wide range of leachate dilutions that could be possible during a major coal spill event. Suspensions were vacuum filtered through pre-combusted solvent rinsed filters (Whatman GF/F, 0.7  $\mu$ m). Coal leachate solutions were then diluted with filtered (0.45  $\mu$ m) seawater (FSW) to five concentrations (100, 50, 25, 12.5 and 6.25% v/v of leachate from the original suspension).

# 2.3.3 Effects of suspended coal particles on coral 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).

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 I exposed coral eggs to five sperm concentrations ( $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ , and  $10^6$  sperm cells ml<sup>-1</sup>) and three suspended coal treatments: control (0 mg coal l<sup>-1</sup>), low (50 mg coal l<sup>-1</sup>), and high (200 mg coal l<sup>-1</sup>). 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 (Appendix A Figure A2.1). I observed sperm-saturation at  $10^5$  sperm cells ml<sup>-1</sup> and  $10^6$  sperm cells ml<sup>-1</sup> for the low and high coal concentrations, respectively. The sperm concentration that gave the half-maximal fertilisation response (EC<sub>50</sub>) at 50 mg coal l<sup>-1</sup> was  $1.2x10^4$  sperm cells ml<sup>-1</sup>. Since I was investigating coal concentrations lower than 50 mg l<sup>-1</sup>, I applied 2 x  $10^4$  sperm cells ml<sup>-1</sup> in the subsequent fertilisation experiments that included coal suspension concentrations of 12.5-800 mg l<sup>-1</sup>, n = 5 replicate jars per concentration.

# 2.3.4 Effects of coal on early development stage survivorship

Three development stages: 3 h old (2-4 cell embryos); 12 h old embryos (convex-concave cellular bi-layer stage, referred to as the "prawn chip" stage, Hayashibara et al., 1997); 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<sup>-1</sup> 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.

## 2.3.5 Effects of coal on competent larvae settlement

# 2.3.5.1 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<sup>2</sup>) 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.

## 2.3.5.2 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  $I^{-1}$  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 online with publication). 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  $I^{-1}$  under non-static conditions for 12 and 24 hours in 50 ml jars (n = 5 jars per exposure time). The jars were then left static (i.e. no water movement) 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 2.3.5.1. The numbers of settled, unsettled, encapsulated and dead larvae were counted after 48 h.

#### 2.3.5.3 Effects of coal smothered CCA on subsequent larval settlement

Small pieces of live CCA were cut to a consistent size (approximately 3 mm<sup>2</sup>) and placed into 6well 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<sup>-2</sup> d/wt) of pre-wetted coal was deposited on top of CCA chips; 2) a full layer (22  $\pm$  1.5 mg cm<sup>-2</sup> 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 *2.3.5.1*.

# 2.3.6 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 2.3.5.1). 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  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>. Symbiont (*Symbiodinium spp.*) 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 = 10 plates per aquarium). 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<sup>-2</sup> 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.

## 2.3.7 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. Fifteen 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 2.3.2) for 30 minutes. Sperm were then added to egg treatments and left to fertilize for 2.5 h. Irregularly shaped embryos were recorded.

# 2.3.8 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 2.3.2. 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<sup>2</sup>) of CCA. Settlement was quantified after 48 h as per 2.3.5.1.

## 2.3.9 Trace element 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 elements (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.

# 2.3.10 Polycyclic aromatic hydrocarbon (PAH) analysis

Coal suspensions (800 and 10,000 mg  $\Gamma^{1}$ ) 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 I) 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, nitrobenzened5, 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.

#### 2.3.11 Statistics

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 linear models (LMs), general linear models (GLMs) and generalized linear mixed effects models (GLMER, package = Ime4) in R.

#### 2.3.11.1 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 (version 3.2.3, R Core Team 2015). The effect of spawning night was included as a random effect and a random observation component was also included to account for over-dispersion. 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<sub>10</sub> and IC<sub>50</sub> values) were estimated using GraphPad Prism (Version 6.0,

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San Diego, USA). Abnormal embryo data were analysed with a GLM using a quasibinomial distribution and chi-square test in R.

## 2.3.11.2 Effects of coal on early development stage survivorship

Each age group of embryos (i.e. 3 h, 12 h, and 72 h old) was analysed independently using the factors: exposure duration and concentration. Survivorship data were analysed using GLMERS 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. GLMs with a binomial distribution were used to determine the lowest observed effect concentration (LOEC) of each respective exposure time for each age group.

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

Each age group of embryos (i.e. 3 h, 12 h, and 72 h old) 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).

# 2.3.11.4 Effects of coal smothering and deposition on larval settlement and juvenile survivorship. Effects of coal leachate on fertilisation and settlement

Comparisons in proportions of settled, unsettled, smothered and dead larvae after 12 and 24 h exposure to extreme (800 mg coal  $I^{-1}$ ) coal concentrations were assessed by Chi-square test in GraphPad Prism (Version 6). 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. 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. Fertilisation, settlement success and abnormal embryo data were analysed with GLMs using a quasibinomial distribution in R, respectively.

# 2.4 Results

# 2.4.1 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  $\Gamma^1$  (Figure 2.1 A, Table 2.2). Coal particles did not coat the eggs but instead appeared to form flocs, possibly with sperm (Figure 2.1 B). 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<sub>10</sub> and IC<sub>50</sub>) of 47 (95% confidence limits = 39-56) mg coal  $\Gamma^1$  and 107 (95% confidence limits = 98-116) mg coal  $\Gamma^1$ , respectively. There were strong reductions in mean fertilisation success at concentrations > 50 mg coal  $\Gamma^1$  and < 1% success was measured at concentrations ≥ 400 mg coal  $\Gamma^1$ . The LOEC was 50 mg coal  $\Gamma^1$  ( $Z_{11,189} = -3.4$ , 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.

**Table 2.2** Results summary of concentration-response experiments that tested the effects of coal on early life history stages of *Acropora tenuis*. Abbreviations: NOEC = no observed effect concentration, LOEC = lowest observed effect concentration, IC10 = coal concentration required for 10% inhibition of response, IC50 = coal concentration required for half-maximal response, - = not applicable, CCA = crustose coralline algae, \* = alternative unit as specified.

Development stage	Coal exposure type	Response	Exposure duration (h)	NOEC (mg l <sup>-1</sup> )*	LOEC (mg l <sup>-1</sup> )*	IC <sub>10</sub> (mg l <sup>-1</sup> )	IC <sub>50</sub> (mg l <sup>-1</sup> )	P-value	Results section
Gametes	Suspension	Fertilisation Figs. 2.1 &	2.5	25	50	47	107	<0.001	2.4.1
3	Leachate	2.6A	2.5	100%	-	-	-	> 0.05	2.4.7
2 h			1	50	100	-	-	0.021	
3 n (2.4 coll)		Survival	12	400	800	-	-	0.010	242
(2-4 cell)	Suspension	Fig. 2.2A	24	25	50	-	-	0.031	2.4.2
			72	25	50	-	-	0.019	
y w		Settlement Fig. 2.2D	24	800	-	-	-	> 0.05	2.4.3
Ŭ			72	800	-	-	-	> 0.05	
12 h		Survival Fig. 2.2B Settlement Fig. 2.2E	1	800	-	-	-	> 0.05	2.4.2
(Prawn chip)			12	50	100	-	-	0.032	
embryo	Suspension		24	25	50	-	-	0.018	
(ASA)			72	25	50	-	-	0.044	
			24	800	-	-	-	> 0.05	2.4.3
			72	800	-	-	-	> 0.05	
72 h	Suspension	n Survival Fig. 2.2C Settlement Fig. 2.2F	1	800	-	-	-	> 0.05	
(toor drop)			12	800	-	-	-	> 0.05	2.4.2
Larvae			24	100	200	-	-	0.032	
			72	800	-	-	-	> 0.05	
			24	400	800	-	-	< 0.001	2.4.3
			72	400	800	-	-	< 0.001	
Larvae	Leachate	Settlement	48	100%	-	-	-	> 0.05	2.4.7
	Smothered CCA	Fig. 2.4 & 2.6B	48	Washed CCA	12.5 mg cm <sup>-2</sup>	-	-	<0.001	2.4.5



**Figure 2.1** Concentration-response relationships of *Acropora tenuis* gametes. (**A**) Fertilisation success (mean % ± S.E.) 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.

# 2.4.2 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 (Figure 2.2 A-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 the highest mortality always occurred after 72 h exposure to the highest concentrations of coal (either 400 or 800 mg coal l<sup>-1</sup>). The 3 h old embryos exposed to concentrations  $\geq$  400 mg l<sup>-1</sup> 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  $\geq$  400 mg coal l<sup>-1</sup> ingested coal particles (Figure 2.3 C).

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  $\geq$  24 h resulted in significantly lower survivorship than shorter exposures of  $\leq$  12 h ( $Z_{3,128} = -4.4$ , P < 0.001). LOEC's for 1 h, 12 h, 24 h and 72 h exposure durations were: 100 mg l<sup>-1</sup>, 800 mg l<sup>-1</sup>, 50 mg l<sup>-1</sup> and 50 mg l<sup>-1</sup>, respectively (Figure 2.2 A, Table 2.2). For 12 h old embryos, exposure durations  $\geq$  12 h resulted in significantly lower survivorship than shorter exposures of  $\leq$  1 h ( $Z_{3,128} = -4.1$ , P < 0.001). LOEC's for 1 h, 12 h, 24 h and 72 h exposure durations were: 100 mg l<sup>-1</sup>, 800 mg l<sup>-1</sup>, 50 mg l<sup>-1</sup> and 50 mg l<sup>-1</sup>, respectively (Figure 2.2 A, Table 2.2). For 12 h old embryos, exposure durations  $\geq$  12 h resulted in significantly lower survivorship than shorter exposures of  $\leq$  1 h ( $Z_{3,128} = -4.1$ , P < 0.001). LOEC's for 1 h, 12 h, 24 h and 12 h exposure durations were: N/A, 100 mg l<sup>-1</sup>, 50 mg l<sup>-1</sup> and 50 mg l<sup>-1</sup>, respectively (Figure 2.2 B, Table 2.2). For 72 h old larvae exposure durations  $\geq$  24 h resulted in significantly lower survivorship than shorter exposures of  $\leq$  12 h ( $Z_{3,128} = -3.5$ , P < 0.001). The LOEC for 24 h exposure duration was 200 mg l<sup>-1</sup> (Figure 2.2 C, Table 2.2). 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 (Figure 2.2 A-C).

# 2.4.3 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  $\Gamma^1$ ), exposure duration (24 or 72 h) and development age (3 h, 12 h, 72 h) (Figure 2.2 D-F, Table 2.2). 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  $\Gamma^1$ , respectively. Larvae that had been exposed to coal as 3 h and 12 h old embryos exhibited apparent decreases in settlement success with increasing concentration; however, these were not found to be statistically different. Exposure time significantly affected settlement of both 3 h (Permanova Pseudo-F<sub>1,70</sub> = 20.8, *P* < 0.001) and 12 h old embryos (Permanova Pseudo-F<sub>1,70</sub> = 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<sub>1,70</sub> = 8.1, *P* < 0.001) but not exposure durations. The LOEC for both exposure durations was 800 mg  $\Gamma^1$  for this development stage.



**Figure 2.2** Effects of coal on survivorship and early development of *Acropora tenuis* embryos and larvae. Survivorship and settlement success (mean  $\% \pm S.E.$ ) 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 larvae reached competency (~ 6 d) (**D**-**F**).

# 2.4.4 Effects of coal encapsulation on larval settlement and survival

Coal encapsulation occurred in the majority of 6-day old larvae exposed to 800 mg coal l<sup>-1</sup> for 12 and 24 h (92 ± 5% and 99 ± 1% respectively) (Figure 2.3 A). 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 48 h, 100% and 84 ± 6% of 12 h and 24 h exposed larvae had escaped from coal balls, 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 (Figure 2.3 A). 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.



**Figure 2.3** Effects of coal encapsulation on *Acropora tenuis* 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**) coal coated larva and (**C**) a larva that has ingested coal. Scale bars = 500 µm.

# 2.4.5 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 (Figure 2.4). Larval settlement was significantly lower on CCA that had 12.5 mg cm<sup>-2</sup> ( $Z_{3,20} = -4.6$ , P < 0.001) and 22 mg cm<sup>-2</sup> ( $Z_{3,20} = -4.6$ , P < 0.001) coatings of coal, decreasing to 50 ± 15% and 50 ± 8% respectively (Figure 2.4).



**Figure 2.4** Coal deposition onto CCA: effects on larval settlement across CCA smothered treatments (mean %  $\pm$  S.E.). Washed treatments included the smothering of CCA with coal for 8 hours, after which CCA was washed with FSW. The light and full coal treatments consisted of the deposition of 12.5  $\pm$  0.9 mg cm<sup>-2</sup> d/wt and 22  $\pm$  1.5 mg cm<sup>-2</sup> d/wt of prewetted coal onto CCA.\* depict significant differences relative to control treatments.

# 2.4.6 Effects of coal deposition on juvenile survivorship

After 96 h of smothering, a significantly larger number of juvenile corals smothered in sediment  $(60 \pm 7\%)$  had cleared themselves off compared with recruits smothered in coal  $(33 \pm 5\%)$  (F<sub>1,63</sub> = 7.8, P = 0.007, Figure 2.5 A). Mean survivorship was 94 ± 3%, 90 ± 4% and 83 ± 4% for control, coal smothered and sediment smothered juveniles, respectively (Figure 2.5 B). 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).



**Figure 2.5** Effects of coal smothering on *Acropora tenuis* juveniles. (**A**) ability of smothered juveniles to clear coal and sediments and (**B**) juvenile survivorship (mean  $\% \pm$  S.E.) after 96 h smothering by coal and sediment. Significant (P < 0.05) differences between treatments are depicted by different letters.

# 2.4.7 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 (Figure 2.6 A), while larval settlement success ranged between 83 ± 5% and 100 ± 0% in all treatments (Figure 2.6 B, Table 2.2 and 2.3). Embryonic abnormalities were minimal (ranging between 1 ± 0.5% and 1.4 ± 0.6%) and did not differ substantially between leachate treatments.



**Figure 2.6** Effects of coal leachate on *Acropora tenuis* reproduction (**A**) fertilisation and (**B**) settlement success (mean  $\% \pm$  S.E.) in relation to increasing leachate concentrations (0-100%).

# 2.4.8 Water quality analyses

Elevated concentrations of certain trace elements (maximum: Mn = 1.0, Co = 0.35, Zn = 6.8, and Cu = 0.81  $\mu$ g l<sup>-1</sup>) leached from coal suspensions during 3 h and 72 h exposures (Table 2.3). The maximum magnitude of change in dissolved metal concentrations leached from coal suspensions, in relation to control seawater, was generally minimal: As = 0.14; Co = 0.33; Cu = 0.44; Pb = 0.1; Mn = 0.77; Mo = 0.4; Ni = 0.08; and Zn = 4.43  $\mu$ g l<sup>-1</sup>. However, concentrations of Co and Cu exceeded 99% levels of protection (% species) outlined by the ANZECC & ARMCANZ marine water quality guidelines (ANZECC & ARMCANZ, 2000). Co and Cu did not exceed the 95% species protection guideline of 1  $\mu$ g l<sup>-1</sup> and 1.3  $\mu$ g l<sup>-1</sup>, respectively. In the 100% leachate treatment water, Co, Cu and Zn concentrations of polycyclic aromatic hydrocarbons were detected from extractions of leachate from 800 mg coal l<sup>-1</sup> (suspension experiments) or 10,000 mg coal l<sup>-1</sup> (leachate experiments) suspensions (maximum of total PAH = 0.61  $\mu$ g l<sup>-1</sup>) (APpendix A Table A2.1). Only naphthalene has an Australian trigger value (99% protection level = 50  $\mu$ g l<sup>-1</sup>) (ANZECC & ARMCANZ, 2000).

**Table 2.3** Trace element concentrations ( $\mu$ g l<sup>-1</sup>) in coal leachate assays. Abbreviations: ELHS = early life history stage, ID = no reliable trigger value, < = below reporting limit. Bolded concentrations exceed ANZECC & ARMCANZ guidelines for 99% species level of protection. Cd, Pb and Se were all below reporting limits.

ELHS exposed	Exposure Type	Coal (mg l <sup>-1</sup> )	Exposure time (h)	Mn	Со	Ni	Cu	Zn	As	Мо
		Guideline		ID	0.005	7	0.3	7	ID	ID
		Reporting								
		limit		0.09	0.010	0.10	0.10	0.50	0.40	1.20
		0	3	<	0.014	0.50	0.39	2.41	1.20	11.0
	Suspended	12	3	<	0.017	0.42	0.79	6.84	1.29	11.2
		25	3	<	0.024	0.51	0.43	5.64	1.24	11.0
Comotos		50	3	<	0.027	0.55	0.40	5.26	1.27	11.3
Gametes & embryos		100	3	0.10	0.033	0.54	0.49	5.56	1.34	11.1
		200	3	0.20	0.060	0.53	0.51	4.00	1.24	11.2
		400	3	0.27	0.12	0.49	0.61	3.27	1.25	11.2
		800	3	0.58	0.23	0.54	0.72	3.75	1.19	11.4
Embryos & larvae	Coal suspension	0	72	0.26	0.019	0.51	0.37	5.22	1.20	11.2
		25	72	0.49	0.036	0.59	0.81	4.84	1.20	11.2
		50	72	0.10	0.047	0.50	0.42	4.95	1.32	11.2
		100	72	0.12	0.065	0.52	0.44	4.35	1.18	11.3
		200	72	0.22	0.11	0.50	0.46	4.10	1.18	11.3
		400	72	0.70	0.18	0.55	0.68	5.10	1.23	11.2
		800	72	1.03	0.35	0.53	0.70	5.19	1.17	11.3
Gametes	Lassbata	0	24	0.14	<	<	1.7	5.5	2.35	10.9
& larvae	& larvae		24	9.57	3.73	2.00	1.35	12.5	2.42	12.0

# 2.5 Discussion

# 2.5.1 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 *Acropora 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 trigger guidelines (where they exist) (ANZECC & ARMCANZ, 2000). PAHs and metals from all coal treatments were lower than concentrations previously found to inhibit fertilisation and metamorphosis in corals (e.g. Heyward, 1988; Negri et al., 2016; Negri and Heyward, 2000; 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.

# 2.5.2 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 2.2), with complete inhibition observed at the highest coal concentrations ( $\geq$  400 mg coal I<sup>-1</sup>). 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 will directly affect eggs is low, as the sperm dilution experiment indicated egg viability was

not affected even at high concentrations of coal (Appendix A Figure A2.1). 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 (Figure 2.1 B). 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 – 169 mg  $l^{-1}$  in past studies (Erftemeijer et al., 2012a; 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., 2015b). 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., 2016b), and this mechanism may likewise be relevant for suspended coal.

A wide range of coal concentrations (50-800 mg coal  $1^{-1}$ ) 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 of Acropora tenuis. I 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). This characteristic potentially contributed to the sensitivity of the embryos examined in this chapter. Exposure to some types of sediments can cause increased embryo abnormalities (Erftemeijer et al., 2012a; Humphrey et al., 2008). Acropora millepora embryos exposed to 5 types of sediments exhibited a maximum abnormality level of 45% when exposed to 16 mg l<sup>-1</sup> 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 (Ricardo et al., 2016a). Whether this age-related trend of increasing tolerance to suspended coal continues into adulthood is investigated in *Chapter* 3.

The effects of coal on larval settlement of *A. tenuis* 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 30-50%. 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 I<sup>-1</sup> 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., 2012a; 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). For example, sedimentation rates of 0.5 - 325 mg cm<sup>-2</sup> d<sup>-1</sup> 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<sup> $^2$ </sup> d<sup> $^1$ </sup> 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., 2015b). 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 related to suspended sediments 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, which removed organic and inorganic particulates > than 0.45 µm. Investigations into the

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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 may occur under natural organic-rich seawater conditions.

# 2.5.3 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 this study provides insight into the potential impacts of coal contamination on coral populations, 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 I 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.

# 3. Simulated coal spill causes mortality and growth inhibition in tropical marine organisms

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## 3.1 Abstract

Coal is a principal fossil fuel driving economic and social development, and increases in global coal shipments have paralleled expansion of the industry. To identify the potential harm associated with chronic marine coal contamination, three taxa abundant in tropical marine ecosystems (the coral *Acropora tenuis*, the reef fish *Acanthochromis polyacanthus* and the seagrass *Halodule uninervis*) were exposed to five concentrations ( $0 - 275 \text{ mg coal } \Gamma^1$ ) of suspended coal dust (< 63 µm) over 28 d. Results demonstrate that chronic coal exposure can cause considerable lethal effects on corals, and reductions in seagrass and fish growth rates. Coral survivorship and seagrass growth rates were inversely related to increasing coal concentrations ( $\geq 38 \text{ mg coal } \Gamma^1$ ) and effects increased between 14 and 28 d, whereas fish growth rates were similarly depressed at all coal concentrations tested. This investigation provides novel insights into direct coal impacts on key tropical taxa for application in the assessment of risks posed by increasing coal shipments in globally threatened marine ecosystems.

## 3.2 Introduction

The international trade of coal is highly dependent on transportation by sea, and coal shipments continue to increase from Australia (International Energy Agency, 2016). Recent scientific, political and public opinion has raised concerns regarding the increase in coal mining and shipping adjacent to sensitive tropical coastal environments, including World Heritage listed sites such as the Great Barrier Reef (GBR) (Hughes et al., 2015). To date, major concerns about these activities have pertained to increased dredging to facilitate port access for coal vessels, and the burning of coal increasing greenhouse gas emissions. However, growth in seaborne coal trade has also been accompanied by increased shipping accidents that have potential to cause widespread damage to marine ecosystems. For instance, the groundings of the bulk coal carriers *Castillo de Salas* (Spain, 1986), *Eurobulker IV* (Italy, 2000) and *MV Smart* (South Africa, 2013) released between 17,000 and 60,000 tonnes of unburnt coal into the marine environment (DOEARSA, 2013; Jaffrennou et al., 2007). Calm weather conditions helped prevent 68,000 tonnes of coal on board the grounded *Shen Neng I* from spilling onto the GBR in 2010 (ATSB, 2010).

Despite the occurrence of these large-scale incidents over a period of several decades, there is currently no scientific consensus on the levels at which unburnt coal becomes a threat to the health of tropical marine organisms. Although seaborne coal trade is highest in tropical regions (Indonesia and Australia) (International Energy Agency, 2016), studies of coal impacts on marine environments have generally been conducted in temperate regions where, for example, long-term colliery waste contamination was linked with declines in species richness and diversity (Hyslop et al., 1997). The paucity of knowledge on coal impacts in tropical environments means that we are unable to competently assess the potential threats of accidental release of coal into tropical marine environments.

Other forms of particulate matter contamination in seawater (e.g. sediment) can reduce the growth and survival of tropical marine organisms by reducing light penetration into water and by smothering tissues through direct deposition of particles onto organisms (Fabricius, 2005; Rogers, 1990). Similarly, direct pathways for organism harm by coal are likely to include suspended particles, increased light attenuation from turbidity, and smothering of sessile benthic organisms, leading to reduced photosynthesis and feeding. In addition, coal may contain contaminants such as polycyclic aromatic hydrocarbons (PAHs) and trace metals, and a fraction of these contaminants can be released from coal into the surrounding seawater (Ahrens and Morrisey, 2005; Cabon et al., 2007; Lucas and Planner, 2012). Metals can be toxic to marine species by disrupting enzyme activity and membrane structure, but the effects of metals are highly dependent on speciation and bioavailability (Bryan, 1971). PAHs affect organisms via non-specific narcosis (Di Toro and McGrath, 2000) and can be carcinogenic and mutagenic to marine life (Eisler, 1987). Sub-lethal chronic effects may include reduced growth, decreased fecundity and reproductive failure; however, the response to PAHs also varies greatly with bioavailability and the capacity of organisms to detoxify during metabolism (Kennish, 1998).

I evaluated lethal and sub-lethal coal concentrations by quantifying the effects of suspended fine coal particles and coal particle deposition on key demographic rates (growth, mortality) of coral (*Acropora tenuis*), fish (*Acanthochromis polyacanthus*, spiny chromis) and seagrass (*Halodule uninervis*). The potential effects of coal on key representative species from the tropics, is critical for the development of appropriate risk assessments and policy development associated with the safe and sustainable shipment of coal through the GBR and other tropical ecosystems of high ecological value.

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#### 3.3 Materials and Methods

#### 3.3.1 Conceptual basis for experimental design

This experiment was conducted at the indoor facilities of the National Sea Simulator at the Australian Institute of Marine Science (AIMS) in June - July 2014. Thermal coal (sourced from central Queensland, Australia) was crushed, milled and sieved to isolate particles < 63  $\mu$ m. Organisms were exposed to five coal treatment levels (0 - 275 mg coal  $l^{-1}$ ) in custom 55 l flow-through tanks (n = 3 per treatment). The flow-through coal delivery system was based on that described in Flores et al. (2012) (Figure 3.1). The base of each aquarium sloped (36°) towards the front of the tank to reduce particles accumulating on the bottom. An external pump (Eheim Compact+ 3000 at 3000 l h<sup>-1</sup>: Eheim GmbH, Germany) suctioned particles from the lowest point at the base of each aquarium and resuspended the particles at the back of the tank. Re-suspension and dissolved oxygen saturation, was further maintained with an air stone situated at the rear of each tank. Fresh seawater (filtered to  $<1 \,\mu$ m) was added at a rate of 4 I  $h^{-1}$  to each tank by irrigation dripper (2 water turnovers per tank per day). Experimental coal concentrations were maintained by pulsing coal solutions from highly concentrated stocks (120 - 1200 mg l<sup>-1</sup>) of suspended coal (in 238 l fibreglass tanks) into each experimental tank (10 pulses of 80 ml stock suspension per h<sup>-1</sup>, Figure 3.1). The coal stock suspension was maintained using an external pump (Eheim Compact 1260 at 2400 l h<sup>-1</sup>: Eheim GmbH, Germany) that suctioned coal at the stock tank base and delivered it via PVC pipe back to the top of the stock tank, thus maintaining coal particles in suspension (Figure 3.1).

Each treatment tank contained 9 coral (*A. tenuis*) colonies (5 cm length), 10 fish (*A. polyacanthus*, ~11 weeks old) and 3 pots of seagrass (*H. uninervis*, average 33 shoots per pot growing in sediment), with the exception of the 202 mg coal  $l^{-1}$  treatments which contained no fish.





# 3.3.2 Study species and sampling design

Acropora tenuis is a common Indo-Pacific branching coral species frequently found at both inshore and offshore reefs of the GBR. Three *A. tenuis* colonies were collected from Davies Reef in the GBR lagoon (-18.789°S, 147.734°E) at a depth of 4 - 6 m. Coral colonies were maintained in 1000 l indoor flow-through holding tanks (27°C, salinity 35.8  $\pm$  0.03 PSU, 12h light:dark photoperiod at ~200 µmol photons m<sup>-1</sup> s<sup>-1</sup>) at the National Sea Simulator (Australian Institute of Marine Science, AIMS, Townsville) prior to the experiment. After 1 week of acclimation to indoor conditions, coral colonies were cut into smaller colony fragments (hereafter colony) approximately 5 cm in length. Colonies were glued to calcium carbonate pegs and left for a 6 week recovery/acclimation period.

The seagrass species *Halodule uninervis* is commonly found in coastal Queensland environments, including port areas. *H. uninervis* was collected at Cockle Bay, Magnetic Island (-19.199°S, 146.792°E). Cores of intact seagrass were placed in plant pots lined with a plastic bag which was filled with seawater and sealed during transportation to AIMS. Seagrasses were re-potted within 24 hours and maintained in 150 l indoor flow-through holding tanks (27°C, salinity 35.8 ± 0.03 PSU, 12h light:dark photoperiod at ~200 µmol photons m<sup>-1</sup> s<sup>-1</sup>) at the National Sea Simulator. Seagrasses were acclimated to the laboratory conditions 4 weeks prior to the commencement of the experiment.

Eight-week-old *Acanthochromis polyacanthus* were sourced from a captive breeding program at the Marine and Aquaculture Research Facilities Unit, James Cook University, Townsville. Upon arrival at AIMS, fish were acclimated for 2 weeks to temperature-controlled (27°C, salinity 35.8  $\pm$  0.03 PSU, 12h light:dark photoperiod at ~200 µmol photons m<sup>-1</sup> s<sup>-1</sup>) laboratory conditions. Each fish was then tagged with an individual fluorescent marker by subcutaneous injection of an elastomer dye with an insulin needle and were left to recover for one week. Fish were randomly assigned to experimental tanks (n = 10 per tank) and were fed once per day with 4 mg of crushed INVE 5/8 enriched food per fish (Wenger et al., 2012) and also had access to the *Artemia salina* nauplii provided to the corals. Food was also added to the treatment without fish so that corals were exposed to the same food regime.

#### 3.3.3 Response variables monitored for experimental organisms during coal exposure

Growth and mortality were assessed for the study species in each experimental treatment and at two time points during the experiment (14 and 28 d). For seagrass, leaf elongation was used as a proxy for growth. Between each sampling point 5 leaves per pot (n = 45 per treatment) were randomly chosen and pierced 2 times with an insulin needle at the top of the sheath. The distance between the sheath holes and needle scars in the leaf were measured using callipers. New holes were made approximately 1 week prior to each sampling period. Measurements were converted into growth rate per day (mm d<sup>-1</sup>). For fish, the standard length was measured in each fish 5 d prior to the commencement of the experiment and again after 14 and 28 d of coal exposure. Fish length was measured in seawater-filled zip lock bags with hand held callipers. Seagrass and fish growth inhibition values were calculated relative to the mean control growth rates at 14 and 28 d, respectively.

Coral mortality was measured at each sampling interval. Nine random coral colonies were sacrificed by snap freezing with liquid nitrogen then photographed at 2 different (non-overlapping) angles next to a scale bar. To avoid confounding irregularities at the bases of colonies due to fragmentation and gluing, the bottom 3 mm of each branch was omitted from the measurement. ImageJ software (U.S. NIH, MD, USA http://rsb.info.nih.gov/ij/) was used to analyse the proportion of dead tissue on each coral fragment. Tissue was categorized as 1) alive = presence of pigmented or bleached tissue; 2) dead = sloughed tissue (visible skeletal structure) or coal smothered skeleton. For the seagrass, loss of above ground shoot density was used as a proxy for mortality. Prior to the commencement of the experiment and at each sampling interval individual seagrass shoots were counted in each pot. Change in shoot density was calculated by subtracting the shoot count of each pot at a time point from the initial (time 0) shoot count of the same pot. These values were converted into percentage change in shoot density relative to time 0 for each respective treatment level. Finally, fish mortality was assessed at each sampling interval by counting the number of live fish in each tank and subtracting that number by the starting number (i.e. n=10). All experimental protocols involving fish were approved by James Cook University and the methods were carried out in accordance with the approved James Cook University animal ethics guidelines (A2038).

#### 3.3.4 Water quality parameters

Water quality parameters were measured in each treatment tank throughout the 28 d exposure period. Total suspended solid (TSS) sampling was performed 6-7 times per fortnight on 500 ml aliquots from each tank (n = 1 per tank per sampling point) during the experimental period. Water was shaken and filtered through pre-weighed filters (0.7 µm glass microfibre) that were then rinsed with deionized water and oven dried (60°C) until a constant weight was maintained. The gain in weight of each filter was multiplied by 2 to express the TSS in mg  $l^{-1}$ . Since other organic materials, such as algae, faecal matter and uneaten fish food, were present in all of the experimental tanks including the control treatment, the mean TSS measured in control tanks was subtracted from coal treatments to derive measurements of total suspended coal (TSC; Appendix A Figure A3.1 a). Temperature was measured 5 times per fortnight (n = 1 per tank) with a thermometer and light attenuation, expressed as photosynthetically active radiation (PAR) was measured weekly (n = 1 per tank) with a Li-250A light meter (Li-cor, Lincoln NE, USA) at the height of the corals and seagrass in the experimental tanks (approximately 25 cm below the water surface). Dissolved oxygen saturation was measured at the start of the experiment followed by twice per week (n = 1 per tank) using a Hach Probe (HQ 40d) and pH was measured on 3 occasions (n = 3 per tank) using a potentiometric pH probe (console: OAKTON, USA; pH probe: EUTECH, USA).

Coal deposition rates were measured in each tank using 2 methods (Appendix A Figure A3.1 b and c). The first involved small sediment traps (n = 3 per tank) (20 ml glass vials, 15 mm opening diameter, 58 mm height) with the top at a height similar to the corals (Flores et al., 2012). The second method used flat-surfaced sediment pods (n = 1 per tank), which allow for re-suspension of particles (Field et al., 2013). Both traps (sampled weekly) and pods (sampled 4-5 times per fortnight) were collected 24 h after deployment and contents were filtered through pre-weighed filters (0.7  $\mu$ m glass microfibre) that were rinsed with deionized water and oven dried (60°C) until a constant weight was maintained for determination of deposition rate. Similar to TSC measurements, the mean weight of organic material deposited onto control filters was subtracted from the mean coal deposition values in each treatment in order to present a measurement of coal deposition only.

Water samples were taken at 28 d to assess the potential contamination by trace metals (Co, As, Cd, Cu, Pb, Mn, Mo, Ni, and Zn). Metal analysis was conducted at Charles Darwin University (Australia) using inductively coupled plasma mass spectrometry (ICP-MS). Water samples (0.45  $\mu$ m syringe filtered leachate, 150 ml) were taken from each treatment (n = 3; 3x50ml per tank, which was pooled for each treatment replicate). PAHs were not detected in *Chapter 2* and were not bioavailable in previous coal seawater leaching studies (Bender et al., 1987; Jaffrennou et al., 2007) and were therefore not analysed here.

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## 3.3.5 Statistical analyses

To evaluate organism responses to coal particles a multifactor analysis of variance between 14 and 28 d was implemented based on permutations using the PERMANOVA routine of PRIMER (Version 6.0). 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 used when unique permutations were < 1000. Coral mortality (%) data was arcsin square root transformed prior to analysis. The n for each taxa in the PERMANOVA analysis were as follows: coral (n = 9 per treatment per time point), fish (n = 23 - 29), and seagrass (growth n = 45 leaves per treatment per time point; change in shoot density n = 9 pots per treatment per time point). The factors analysed were: coal concentration (5 fixed), exposure time (2 fixed), tank (3 random: nested within concentration), with the addition of replicates (e.g. replicated seagrass pots, random: nested within tank) where appropriate (Appendix A Table A3.1). Four-parameter sigmoidal curves were fitted to coral mortality data to estimate lethal concentration (LC<sub>10</sub> and LC<sub>50</sub>) values for mortality using GraphPad Prism (Version 6.0). Concentrations resulting in mean inhibition of growth (IC<sub>10</sub> and IC<sub>50</sub>) were estimated for fish and seagrass using linear interpolation in SigmaPlot (Version 11.0). Analysis of variance (one-way ANOVA) was used to compare means of trace elements between coal treatments in SigmaPlot (Version 11.0). Elements (Co and Pb) that did not meet the assumptions of normality (based on Shapiro-Wilk normality test) were log-transformed.

#### 3.4 Results and discussion

#### 3.4.1 Water quality in experimental treatments

Experimental treatments of suspended and settled coal particles mimicked five broad pulse intensities (ranging from 0 - 275 mg coal  $\Gamma^1$ , Table 3.1) lasting 28 d. Attenuation of light in coal treatments ranged from 44 - 99%, relative to control values (Table 3.1). Coal deposition rates ranged from 11 - 241 mg cm<sup>-2</sup> d<sup>-1</sup> in sediment traps and 2 - 46 mg cm<sup>-2</sup> d<sup>-1</sup> on flat surfaces (pods) (Table 3.1, Appendix A Figure A3.1 b and c). Trace metal analysis of experiment treatment water (filtered leachate) sampled at 28 d showed significantly (*P* < 0.05) higher concentrations of Co, As and Ni in certain coal treatments in comparison with control water (Table 3.2). However, the highest metal concentrations were not always measured in the highest coal treatments. The magnitude of change in dissolved metal concentrations in relation to control seawater was minimal: As varied by 0.3 µg  $\Gamma^1$ ; Cd = 0.1 µg  $\Gamma^1$ ; Co = 0.2 µg  $\Gamma^1$ ; Cu = 0.2 µg  $\Gamma^1$ ; Pb = 0.1 µg  $\Gamma^1$ ; Mn = 0.3 µg  $\Gamma^1$ ; Mo = 0.8 µg  $\Gamma^1$ ; Ni = 2.4 µg  $\Gamma^1$ ; Zn = 0.9 µg  $\Gamma^1$ . These findings suggest that metals were not likely contributing to the observed effects.

Although the tanks were moderately turbulent (water flow of 5 - 10 cm sec<sup>-1</sup>) due to the presence of pumps, the coal particles attached to many surfaces within the tanks, contributing to lower total suspended coal (TSC) exposures in the latter half of the four week pulse (Appendix A Figure A3.1 a). While there is limited evidence documenting the concentrations of suspended coal present in seawater during a spill event, our high coal treatment (275 mg l<sup>-1</sup>) was lower than the concentrations applied to temperate species in other experimental studies (500 - 13,500 mg coal l<sup>-1</sup>) (Lewis, 1973; Pearce and McBride, 1977). Moreover, the results of the present experiment may be considered conservative in relation to the broader effects of coal during a spill event as I only investigated the effects of fine coal particles (< 63  $\mu$ m) which are likely to remain in suspension for long periods (Jaffrennou et al., 2007; Johnson and Bustin, 2006). A large spill scenario at sea would also release larger particles that settle more rapidly, posing further risks of physical damage, including smothering (Jaffrennou et al., 2007).

**Table 3.1** Summary of water quality parameters for long-term exposure experiments. Mean ( $\pm$  S.E.) total suspended coal (TSC) (mg l<sup>-1</sup>), light (PAR, µmol photons m<sup>-2</sup> s<sup>-1</sup>), light attenuation (% rel. to 0 mg coal l<sup>-1</sup>), coal deposition rates (mg cm<sup>-2</sup> day<sup>-1</sup>) in glass vials and deposition pods, temperature (°C), dissolved oxygen (mg l<sup>-1</sup>) and pH. Note: Mean deposition of particulate matter in control treatments (7.3 mg l<sup>-1</sup> for TSC, 5.2 and 0.7 mg cm<sup>-2</sup> day<sup>-1</sup> for vials and pods, respectively) was subtracted from all coal treatments to depict only coal suspension and deposition. Variation in TSC and deposition over time are presented in Appendix A Figure A3.1. n = total replicates per treatment over the experiment duration.

Treatment	TSC	Light	Light	Coal	Coal	Temperature	Dissolved	рН
			attenuation	deposition	deposition		Oxygen	
			(%)	(vials)	(pods)			
	n = 35-	n = 12	n = 12	n = 36	n = 22-27	n = 30	n = 27	n = 9
	39							
Control	0	177±8.59	-	0	0	26±0.15	8.5±0.02	8.1±0.02
Low	38±6	99±9.39	44	11±1.99	2.3±0.24	26±0.13	8.3±0.03	8.0±0.00
Moderate	73±11	21±3.76	88	38±4.94	11±1.48	26±0.10	8.3±0.02	8.0±0.00
Medium	202±32	1.9±0.70	99	126±24	25±3.16	27±0.09	8.2±0.02	8.0±0.00
High	275±36	1.1±0.32	99	241 ±37	46±4.25	26±0.10	8.3±0.02	8.0±0.00

**Table 3.2** Trace element concentrations ( $\mu$ g l<sup>-1</sup>) from water samples (n = 3) in each treatment of the long-term exposure experiment (mean ± S.E.). Coal treatments where levels were significantly different from the control treatment (ANOVA, one-way analysis of variance) are depicted with a \*.

Element	Leach test ( $\mu g \Gamma^1$ ) from each coal treatment.									
	Control	Low	Low Moderate		High					
	(0 mg l⁻¹)	(38 mg l⁻¹)	(73 mg l⁻¹)	(202mg l <sup>⁻¹</sup> )	(275 mg l⁻¹)					
As	1.2±0.1	1.4±0.1	1.5±0.1*	1.4±0.1	1.4±0.0					
Cd	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0					
Со	0.0±0.0	0.1±0.0*	0.1±0.0*	0.2±0.0*	0.1±0.0*					
Cu	0.3±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.3					
Pb	0.0±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0					
Mn	0.3±0.1	0.3±0.1	0.4±0.1	0.6±0.1	0.6±0.2					
Mo	11.3±0.2	11.0±0.5	11.4±0.5	12.0±0.2	12.1±0.1					
Ni	0.5±0.1	1.1±0.2	1.8±0.4	2.9±0.8*	2.6±0.5					
Zn	2.1±0.7	2.9±1.1	3.0±0.9	2.5±0.3	2.9±0.8					

#### 3.4.2 Responses of tropical organisms to coal exposure

## 3.4.2.1 Corals

In all coal treatments, particles settled directly onto coral polyps and connecting tissue (i.e., coenosarc, Figure 3.2 B and 3.2 C) and the initial response of corals to coal exposure was the release of fine mucus strands, which trapped coal particles and removed them from the tissue surface, similar to the response of corals exposed to sediments (Peters and Pilson, 1985; Rogers, 1990; Stafford-Smith and Ormond, 1992). Branching corals, such as A. tenuis, are often considered among the most resistant morphologies to sedimentation due to their vertical growth (Stafford-Smith and Ormond, 1992), yet despite their active mechanisms for coal removal; some coral tissue died and sloughed off the skeleton within 14 d in all treatments  $\geq$  38 mg coal  $I^{-1}$  (Figure 3.2 B, C and 3.3 A). The extent of tissue mortality on coral branches differed significantly among coal treatments (Permanova, Pseudo- $F_{4,10}$  = 43.4, P = 0.0001), and over time (Permanova, Pseudo- $F_{1,10}$  = 20.5, P = 0.0009) (Figure 3.3 A, see statistical outputs in Appendix A Tables A3.1 and A3.2). After 14 d of exposure, control and low (38 mg coal l<sup>-1</sup>) coal treatments exhibited significantly lower coral mortality than treatments  $\geq$  202 mg coal l<sup>-1</sup> (Student-t post hoc, Monte Carlo simulation, P < 0.05). After 28 d, mortality in all coal treatments  $\geq$  38 mg coal  $I^{-1}$  was significantly higher than the controls (Student-t post hoc, Monte Carlo simulation, P < 0.05). Corals in the control treatment exhibited less than 3% mortality, while 100% tissue mortality occurred in all branches in the three highest coal exposures (Figure 3.2 C and 3.3 A). Corals in the 38 mg coal I<sup>-1</sup> treatment exhibited significantly lower mortality than corals in treatments  $\geq$  73 mg coal  $l^{-1}$  (Student-t post hoc, Monte Carlo simulation, P < 0.05). Pair-wise comparisons between treatments revealed lowest observed effect concentrations (LOEC) of 202 mg coal  $I^{-1}$  and 38 mg coal  $I^{-1}$  at 14 and 28 d, respectively. Fitting four-parameter sigmoidal curves to the data revealed lethal concentrations (LC<sub>10</sub> and LC<sub>50</sub>) of 29 mg coal  $I^{-1}$  and 87 mg coal  $I^{-1}$  at 14 d, respectively, and 34 mg coal  $I^{-1}$  and 36 mg coal  $I^{-1}$  at 28 d, respectively (Appendix A Figure A3.2 a).

Coral mortality in response to fine coal particle exposure may have a number of causes. The accumulation of coal particles on the vertical tissue could have caused anoxia at the coral-coal interface (Weber et al., 2012). Similar surface accumulation of particles was not observed after a month in comparable exposures of *Acropora millepora* branches to fine carbonate sediments (Flores et al., 2012) and could indicate either greater adhesion by coal or reduced fitness in corals exposed to coal rather than sediments. The energetic costs of removing deposited particles (including mucus production) may be further exacerbated in the presence of coal by the strong attenuation of light over 14 and 28 d, which would reduce primary production rates by the symbiotic dinoflagellates (*Symbiodinium spp.*). Although the corals were fed once per week with *Artemia* nauplii, heterotrophic feeding behaviour may have been altered in smothered sections of coral colonies (Peters and Pilson, 1985).

#### 3.4.2.2 Fish

The health of coal-exposed fish was compromised in all coal treatments and differences in fish size and colour were observed over the course of the experiment (Figure 3.2 D). Fish growth rates varied significantly between coal treatments and controls (Permanova, Pseudo- $F_{3,8.1} = 21.7$ , P = 0.01), and over time (Permanova, Pseudo- $F_{1,8.5} = 141.4$ , P = 0.0002) (Figure 3.3 B, see statistical outputs in Appendix A Tables A3.1 and A3.2). Significant differences in growth occurred within the first 14 d of the experiment, with fish exposed to coal levels  $\geq 38$  mg coal  $\Gamma^1$  showing significantly lower growth rates than control fish, irrespective of coal treatment levels (Student-t post hoc, Monte Carlo simulation, P < 0.05, Figure 3.3 B). Growth inhibition, relative to control fish, at 14 d ranged from  $36.1\pm4.6\% - 40.2\pm4.5\%$  and  $42.2\pm6.0\% - 52.3\pm4.3\%$  at 28 d (Appendix A Figure A3.2 b). The LOEC on fish growth rates was 38 mg coal  $\Gamma^1$  at both time points and linear interpolation of the growth data revealed inhibition concentration (IC<sub>10</sub>) estimates of 11 and 9 mg coal  $\Gamma^1$  at 14 and 28 d, respectively, and IC<sub>50</sub> estimates of 73 mg coal  $\Gamma^1$  at 28 d. IC<sub>50</sub> estimates were not possible at 14 d because growth inhibition was below 50%.

The negative impact of coal on fish growth is consistent with the response of marine fish to increased suspended sediments which is thought to be caused by visual impairment leading to reduced prey capture success and increased foraging time and energy expenditure (Sigler et al., 1984; Wenger et al., 2012). A preliminary post mortem investigation on the coal-exposed fish in this experiment revealed coal in the alimentary tracts, which was mistakenly ingested and could have

physically blocked normal feeding and digestion contributing to starvation and debilitation. In addition, it is possible that suspended coal affected fish respiration (Hughes, 1976; Sutherland and Meyer, 2007), an effect that may have been consistent across all coal treatments (see *Chapter 4* for a detailed investigation of these effects).

Despite the considerable effects on fish growth, all coal-exposed fish survived except for two individuals that were exposed to the highest coal treatment of 275 mg coal  $\Gamma^1$ . The lethal effects of suspended sediments on fish are dependent on the particle size, angularity, exposure duration, and are typically observed when concentrations reach  $\geq$  hundreds of mg  $\Gamma^1$  (Birtwell, 1999; Lake and Hinch, 1999; Wenger et al., 2012). The survival of fish in the current study, along with the very high LC<sub>50</sub> (7000 mg coal  $\Gamma^1$ ) reported for 8 d coal exposures of juvenile coho salmon (Pearce and McBride, 1977), support the notion that coal spills are not likely to cause direct mortality in fish under most coal spill scenarios. However, suspended sediment can prolong reef fish larvae development (Wenger et al., 2014), negatively influence gill morphology and increase pathogenic bacterial communities on larval gills (Hess et al., 2015), suggesting further studies are required to investigate the vulnerability of early life stages of fish to suspended coal. Although mortality was low, certain post-settlement processes are size dependent for reef fishes (Perez and Munch, 2010), suggesting that lower growth rates *in situ* may have later implications on individual survivorship (Wenger et al., 2012). Moreover, as fecundity of fish is size dependent (Hislop, 1988; Perez III et al., 2014), suppressed growth can lower lifetime reproductive output.

# 3.4.2.3 Seagrass

Coal particles were observed to attach to the seagrass leaves less than 24 h after exposure commenced, and many leaves were completely coated in a film of coal throughout the experiment (Figure 3.2 E). Coal also accumulated onto the sediment surface in seagrass pots where new shoots develop (Figure 3.2 E). Significant differences were measured for leaf elongation (Permanova, Pseudo-F<sub>4,10</sub> = 35.9, P = 0.0002) and shoot density (Permanova, Pseudo-F<sub>4,10</sub> = 9.8, P = 0.0002) between experimental treatments. Leaf elongation rates differed significantly over time (Permanova, Pseudo-F<sub>1,10</sub> = 67.5, P = 0.0001) (see statistical outputs in Appendix A Tables A3.1 and A3.2). Leaf elongation was more sensitive than shoot density and was significantly affected (Student-t post hoc, Monte Carlo simulation, P < 0.05) in treatments  $\geq$  73 mg coal  $\Gamma^1$  (LOEC) at both 14 d and 28 d (Figure 3.3 C). The magnitude of the effect of coal exposure on leaf elongation rates was large, with overall growth inhibited by  $6.7\pm5.0\% - 45.2\pm3.8\%$  and  $31.1\pm4.5\% - 49.5\pm3.1\%$  relative to controls at 14 and 28 d, respectively (Appendix A Figure A3.2 c). The estimated threshold for impact for this parameter (IC<sub>10</sub>) was 42 mg coal  $\Gamma^1$  at 14 d and 12 mg coal  $\Gamma^1$  at 28 d, while the IC<sub>50</sub> was 275 mg coal  $\Gamma^1$  at 28 d. IC<sub>50</sub> estimates were not possible at 14 d because growth inhibition was below 50%. Shoot density continued to increase in control and 38 mg coal  $\Gamma^1$  treatments throughout the experiment duration,

however, it was significantly reduced (Student-t post hoc, Monte Carlo simulation, P < 0.05) at 28 d in coal treatments  $\geq$  73 mg coal l<sup>-1</sup> (28 d LOEC), with a mean net loss of 1.3±3.4% - 4.6±1.4% of shoots at this time point (Figure 3.3 D).

The coal exposures may have impacted the seagrass in multiple ways. Seagrass requires light to conduct photosynthesis and the light environment was greatly affected by attenuation through the water column (Table 3.1). Irradiance intensity is a principal factor regulating seagrass growth and shading of surface irradiance to low levels (0.2 - 4.4 mol m<sup>-2</sup> d<sup>-1</sup>) has contributed to reduced leaf elongation and shoot loss in the same species (Collier et al., 2012). In addition, the direct coating of leaves with a layer of coal particles is likely to further reduce light penetration (Naidoo and Chirkoot, 2004). Both types of shading and reduced transport of  $CO_2$  into the leaves through the coal barrier will limit photosynthetic carbon fixation, chlorophyll *a* production (Naidoo and Chirkoot, 2004) and inhibit growth (Ralph et al., 2007). Seagrasses maintain a store of carbohydrates within the root-rhizome complex and this is likely to have enabled slight positive leaf extension over the experimental exposures (Alcoverro et al., 2001). Although not directly measured in this experiment, coal exposure can also cause abrasive damage to aquatic plants (Hyslop and Davies, 1998; Lewis, 1973).



**Figure 3.2** Images of *Acropora tenuis, Acanthochromis polyacanthus* and *Halodule uninervis* after coal exposure. Stages of coral health degradation after 14 d exposure to 0 mg coal  $I^{-1}$  (**A**), 73 mg coal  $I^{-1}$  (**B**) and 275 mg coal  $I^{-1}$  (**C**). Mucus strands were used to actively remove settled coal (**B**) and coal deposition that exceeded removal efforts resulted in colony mortality (**C**). Fish from control vs. coal exposed treatments (0 mg coal  $I^{-1}$  - 275 mg coal  $I^{-1}$ ) after 28 d exposure (**D**). Coal settled onto seagrass leaves and substrate (**E**). Note: No fish were present in the 202 mg coal  $I^{-1}$  treatment. All scale bars = 5 mm.



**Figure 3.3** The Differences in measures of key demographic rates (growth and survival) in relation to coal concentration and exposure duration. Differences in the mean ( $\pm$  S.E.) survival of corals (*A. tenuis*) (**A**), growth rates of fish (*A. polyacanthus*) (**B**) and seagrass (*H. uninervis*) (**C**), and percentage change in seagrass shoot density (**D**) at 14 d (closed circle) and 28 d (open circle) exposure. Asterisks depict a significant difference (P < 0.05) between the mean coal treatment and control values. Note: mean change in seagrass shoot density (**D**) is relative to time 0 values at each treatment level in each replicate seagrass pot. Mean values above 0 suggest growth, while values below 0 suggest mortality. No fish were present in the 202 mg coal l<sup>-1</sup> treatment.

## 3.5 Conclusions

While there were differences in sensitivity between the taxa tested here, both sessile and mobile organisms were affected by similar concentrations of coal particles. In most cases the impacts increased with suspended coal concentration and exposure duration. Although this study did not specifically investigate the stress-response pathways, it was clear that coal particles affect corals, fish and seagrass in ways that are similar to the effects of other suspended solids, including: light limitation, direct smothering and reduced feeding efficiency. Despite these similarities, coal particles appear to have more severe effects on corals than other suspended solids. For instance, chronic exposure of corals to fine carbonate sediment in a similar experimental setup resulted in only 11% mortality in branching coral Acropora millepora after 12 weeks exposure to 100 mg l<sup>-1</sup> (83 mg cm<sup>-2</sup> d<sup>-1</sup> deposition) (Flores et al., 2012), while only high levels of acute bottom sand deposition (200 mg  $cm^{-2}$  $d^{-1}$ ) caused mortality in branching coral Acropora palmate (Rogers, 1983). These differences may be due to coal particles attenuating light more strongly and adhering to coral to a greater extent than inorganic particles. While several metals were elevated in coal treatments in comparison with controls, the magnitude of this increase due to leaching was minimal and only the relatively low toxicity element Co was detected at concentrations greater than ANZECC & ARMCANZ guidelines (ANZECC & ARMCANZ, 2000). Our results are consistent with Chapter 2 and previous studies that showed coal generally does not leach toxic levels of trace metals (Cabon et al., 2007; Lucas and Planner, 2012), however, since ANZECC & ARMCANZ guidelines were exceeded for Co there is scope for toxic effects and further studies are warranted on this topic. Though not measured in the present study, leaching of PAHs from coal is also generally considered low and to have very low bioavailability (Ahrens and Morrisey, 2005; Bender et al., 1987; Jaffrennou et al., 2007), suggesting that the measured adverse effects in the present study were consistent with Chapter 2, in that they were primarily due to physical mechanisms.

As global demand for, and marine transport of, coal continues to increase, specific information is needed to effectively manage risks to areas of high conservation value, such as coral reefs and seagrass meadows, that may be impacted by unburnt coal from terrestrial sources and accidental spills. This first study to examine the effects of fine coal particles on tropical marine organisms demonstrates that moderate to high levels of coal contamination can substantially decrease growth and increase mortality of important reef-building coral species, reef fish and seagrass. Further research is warranted to measure the effects of coal contamination on reproduction and early life histories of fish and invertebrates, as well as the effects of ingestion and smothering on sessile benthic organisms. Considering that hydrocarbon markers for coal have been identified up to 40 nautical miles offshore in the World Heritage listed GBR (Burns and Brinkman, 2011), understanding the risks posed by unburnt coal also requires an improved understanding of *in situ* chronic exposures

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from coastal operations (Ahrens and Morrisey, 2005) and potential transport into reef and seagrass systems by wind and currents. The experimental scenario applied in the present study is particularly relevant to shipping accidents, where high concentrations of unburnt coal can be present in water adjacent to globally threatened habitats. The effect thresholds of coal to coral, fish and seagrass, such as those identified here, are critically important for marine park managers, regulators, industry and shipping operators as a basis to improve risk assessments and policy development associated with safer and more sustainable shipment of coal.
# 4. Suspended coal particles cause gill remodelling and elevated metabolism in a coral reef fish

## 4.1 Abstract

Global coal exports are highest in tropical regions, with millions of tonnes of coal per year shipped through and around highly-valued coral reef ecosystems. Collier incidents, such as the sinking of the MV Mykonos off the coast of Madagascar in 2016, can result in hundreds of thousands of tonnes of coal being released into the marine environment. While high concentrations of coal contamination can be lethal to marine organisms, little is known of the sub-lethal effects that may reduce the performance and fitness of tropical species including fish. Here, I used measurements of aerobic metabolism and gill morphology to elucidate the sub-lethal effects of suspended coal particles on a planktivorous coral reef damselfish, Acanthochromis polyacanthus. Five days of exposure to 38 or 73 mg coal I<sup>-1</sup> temporarily reduced standard oxygen consumption rates by 17 %  $(M_{02})$ ; however, sustained exposures for 21 and 31 days resulted in a significant 30-47% elevation in  $M_{02}$  compared with control fish in coal-free seawater. Consequences of elevated  $M_{02}$  include increased basic metabolic costs, which reduces the available energy for growth and other activities. Fish exposed to coal for 31 days exhibited shorter gill lamellae, indicating that coal particles damaged lamellae tips. Histological analysis also revealed the gill lamellae of A. polyacanthus as being naturally covered by an extraordinarily thick filament epithelium, restricting the surface area available for gas exchange. Fish exposed to 275 mg coal l<sup>-1</sup> shed parts of this filament epithelium, thereby exposing more respiratory surface area and compensating for the observed gill damage at the tips. Gill remodelling to this extent has only previously been reported in a few air-breathing and hypoxiatolerant fish. This study suggests that coral reef fish may employ gill remodelling to regulate gas exchange and to potentially offset some of the adverse effects of coal particles on gill morphology and oxygen uptake.

#### 4.2 Introduction

Global coal production reached 7,708 MT in 2015, with 1,300 MT exported by sea (International Energy Agency, 2016). Negative environmental effects from coal are usually considered in the context of CO<sub>2</sub> emissions generated from coal burning and associated climate change; however, coal mining operations and transport can also directly alter the condition and use of marine habitats (Levings, 1985) and contaminate surrounding environments through accidental inputs into aquatic systems (Campbell and Devlin, 1997). Sources contributing to coal contamination include natural erosion of coal seams, coal mine drainage and washings, spillage during transport and loading/offloading processes, and major spills associated with collier

grounding incidents (Ahrens and Morrisey, 2005). Coal has a low specific gravity (1.2-2.9 g cm<sup>-3</sup>) and small coal particles (~0.5 mm and smaller) spilt at sea are likely to become suspended in the water column, with dispersal and dilution dependent on the hydrodynamics in the area (Jaffrennou et al., 2007; Johnson and Bustin, 2006).

Australia and Indonesia possess some of the world's most diverse coral reef ecosystems and these countries currently lead global coal trade (International Energy Agency, 2016). Despite large quantities of coal being stored and transported in close proximity to highly valued coral reefs, including transport within World Heritage Areas, little is known of how coal contamination may impact tropical reef organisms. Large collier grounding incidents have taken place on or near coral reefs in recent years (e.g. MV New Mykonos, Madagascar in 2016; MV Prosperity, Philippines in 2011; Vogetrader, Hawaii, U.S.A. in 2010; and Shen Neng 1, Great Barrier Reef, Australia in 2010), with spillage of large quantities of coal (up to 160,000 tonnes) representing a so far unquantified risk. Anecdotal evidence provided by fisherman in Colombia and Indonesia reported reduced coral cover and fish abundance in waters exposed to chronic inputs and barge spills from coal ports (Cohen, 2014) and coal mine washings (Ambarini and Septaria, 2014), highlighting the need for further scientific investigation. In Chapter 3 of this thesis, I demonstrated that 14 d of exposure to coal concentrations less than 275 mg coal l<sup>-1</sup> can significantly reduce growth rates and shoot density of seagrass, as well as decrease coral survivorship (Berry et al., 2016). Similarly, in Chapter 2 I showed that lower coal concentrations (50 mg coal I<sup>-1</sup>) can substantially inhibit coral fertilisation and coal deposition (12.5 mg cm<sup>-2</sup> d/wt) onto reef substrates can reduce coral larval settlement by up to 50% (Berry et al., 2017). I revealed in Chapter 3 that chronic exposure to coal also causes reduced growth rates in juvenile damsel fish (see below) and this was the first investigation into the effects of coal on tropical marine fishes.

Tropical fish species inhabit environments in close proximity to potential sources of coal spillage and coal dust, making them ideal model organisms for testing the potential impacts of coal contamination due to large spills. Unlike corals and seagrass, fish can potentially relocate as a strategy to avoid adverse environments; e.g. during high turbidity events (Newcombe and Jensen, 1996; Williams and Harcup, 1974). However, many reef fish are strongly site-attached and thus inhabit specific home ranges following their settlement on the reef (Wilson et al., 2006). Indeed, lemon damselfish (*Pomacentrus moluccensis*) exhibited an 81% reduction in post-settlement movement between habitat types (i.e. different coral colonies), relative to control fish, in response to elevated suspended sediments (30 mg  $l^{-1}$ ) (Wenger and McCormick, 2013), suggesting that relocation may not be a viable strategy for many coral reef fish during a coal spill event.

Existing knowledge of the lethal and sub-lethal effects of coal particle contamination on fish has stemmed primarily from temperate freshwater ecosystems. In general, large quantities of suspended particles, including coal, are required to elicit direct lethal effects on fishes. For example, 100% mortality was observed in fathead minnows (*Pimephales promelas*) after acute 96 h exposure to 25,000 mg coal  $I^{-1}$  (Carlson, 1979) and the 96 h LC<sub>50</sub> of juvenile coho salmon (Oncorhynchus kisutch) exposed to suspended coal was 7,000 mg coal 1<sup>-1</sup> (Pearce and McBride, 1977). Results in *Chapter 3* of this thesis showed that chronic exposure to 275 mg coal<sup>-1</sup> resulted in mortality of only 2 (of 90 fish in coal treatments) tropical marine juvenile damsel fish (Acanthochromis polyacanthus) after 28 d exposure (Berry et al., 2016). Although there are numerous factors contributing to lethality such as fish age, exposure duration, particle characteristics such as particle type, angularity and chemical composition (Lake and Hinch, 1999), the overall cause of death from contact with suspended solids is usually attributed to oxygen starvation from damage or disruption to respiratory tissue (Appleby and Scarratt, 1989; Servizi and Martens, 1992). In addition to physical effects of particle exposure, potentially toxic trace metals and polycyclic aromatic hydrocarbons are constituents of coal and could leach from particles. Although many studies have found low bioavailability of leachate from coal particles (Bender et al., 1987; Gerhart et al., 1981; Herbert and Richards, 1963), 100% mortality was reported in fathead minnows (Pimephales promelas) within 96 h exposure to non-centrifuged leachate, and chronic exposure (3-24 weeks) to centrifuged leachate delayed fish maturation and lowered spawning success (Carlson et al., 1979).

In *Chapter 3* of this thesis, I demonstrated that 14 d of exposure to coal concentrations of 38, 73 and 275 mg coal l<sup>-1</sup> can significantly reduce growth rates of juvenile reef fish (Berry et al., 2016) but the mechanism underlying this effect is unknown. Direct physical interactions between fish and coal include ingestion of coal particles (Berry et al., 2016; Gerhart et al., 1981; Pautzke, 1938; Pearce and McBride, 1977), which can lead to extensive mucus secretion by gut mucosa (Gerhart et al., 1981) and may also contribute to lower growth rates (Berry et al., 2016; Herbert and Richards, 1963; Williams and Harcup, 1974). Suspended coal particles may also interact directly with fish gills. The gill is a multifunctional organ responsible for a broad range of processes such as gas exchange, osmoregulation and hormone metabolism (Evans et al., 2005). Small reductions in gill efficiency can impair respiration, ion exchange and nitrogen excretion (reviewed in Wenger et al., in press). Coal exposure has elicited increased coughing (Hughes, 1975) and ventilation frequency (Pautzke, 1938) in freshwater fish, suggesting that coal causes respiratory distress (Heath, 1995). Histological analyses and observations of fish and oyster gills revealed that solid masses of coal particles can adhere to gills (smothering) and clog lamellae (Carlson et al., 1979; Hillaby, 1981; Pautzke, 1938; Pearce and McBride, 1977), which are the primary sites of gas exchange (Hughes and Morgan, 1973; Hughes, 1984). Adherence of suspended solids to lamellae is expected to increase the resistance to gas transfer across gills (Hughes, 1973), yet the consequences of continuous coal exposure on gill morphology and oxygen uptake rates have not been studied. In addition, various

pollutants can cause cell proliferation in fish gills (Hess et al., 2015; Mallatt, 1985; Mueller et al., 1991). This structural modification can protect gills from abrasive damage and/or reduce the permeability of gills to toxins (Mallatt, 1985); however, it also reduces the permeability of the gills to oxygen and can affect respiratory function (Heath, 1995). In contrast, environmental challenges such as hypoxia can elicit a remodelling of gill structure to enhance respiratory surface area, suggesting that gill remodelling plays an important role in the regulation of oxygen uptake (reviewed in Nilsson, 2007; Nilsson et al., 2012). In the absence of empirical data, it remains unclear how gill morphology and oxygen uptake of fish may be influenced during sustained exposure to coal particles.

Here, I studied a common tropical reef fish species, *Acanthochromis polyacanthus*, to resolve the sub-lethal impacts of suspended coal particles on fish performance, and to gain insight into the mechanisms underlying the reduced fish growth under coal exposure observed in *Chapter 3* of this thesis. First, I examined the effects of three suspended coal concentrations (0, 38, 73 mg coal  $I^{-1}$ ) on oxygen consumption rates ( $M_{o2}$ ) after three exposure durations (5, 21 and 31 d). Following 31 days of exposure, I assessed structural changes and damage to gills in four suspended coal concentrations (0, 38, 73 and 275 mg coal  $I^{-1}$ ) that may underlie changes in gas exchange and metabolism, and potentially other vital processes like osmoregulation. I aimed to identify the coal concentrations that elicit sub-lethal responses in fish, as well as the structural and physiological mechanisms that contribute to those responses.

## 4.3 Materials and Methods

#### 4.3.1 Fish husbandry

Juvenile *Acanthochromis polyacanthus* were sourced from a captive breeding program at the Marine and Aquaculture Research Facilities Unit (MARFU), James Cook University, Townsville. Fish were moved to the indoor facilities of the National Sea Simulator, Australian Institute of Marine Science (AIMS) and acclimated for 2 weeks to temperature-controlled (27°C, salinity 35.8 ± 0.03 PSU, 12 h light:dark photoperiod at ~200 µmol photons m<sup>-2</sup> s<sup>-1</sup>) laboratory conditions. Fish were individually tagged with a subcutaneous injection of elastomer dye and left to recover for one week. Fish were randomly assigned to experimental tanks (n = 10 per tank) and were fed once per day with 4 mg of crushed INVE 5/8 enriched food per fish (Wenger et al., 2012) and supplemented by provision of *Artemia salina* nauplii once per week.

## 4.3.2 Experimental design

This research was conducted as part of a larger experiment that is described in *Chapter 3* (Berry et al., 2016). Four suspended coal concentrations (0, 38, 73, 275 mg coal l<sup>-1</sup>) were maintained in 3 replicate flow-through tanks (55 l) for each concentration. Each replicate tank contained 10 fish with a coal delivery system that was based on that described in Flores et al. (2012). An air stone was situated at the rear of each tank to maintain dissolved oxygen saturation and coal suspension. Filtered seawater (<1  $\mu$ m) was added to each tank (4 l h<sup>-1</sup>) by irrigation dripper and coal concentrations were maintained by pulsing highly concentrated coal solutions (120 - 1200 mg l<sup>-1</sup>) into each experimental tank (10 pulses of 80 ml stock suspension h<sup>-1</sup>). All experimental protocols involving fish were approved by James Cook University and the methods were carried out in accordance with the approved James Cook University animal ethics guidelines (A2038).

#### 4.3.3 Water quality parameters

Water temperature was controlled with in-room air conditioning and was maintained between 26-27°C. Irradiance, expressed as photosynthetically active radiation (PAR) and light attenuation were measured weekly with a Li-250A light meter (Li-cor, Lincoln, NE, USA). Dissolved oxygen saturation was measured twice weekly with a Hach Probe (HQ 40d) while pH was measured a total of three times using a potentiometric pH probe (console: OAKTON, USA; pH probe: EUTECH, USA). Total suspended coal (TSC) was measured 2-3 times per week. Water samples (n = 3; 3x50 ml per tank, pooled for each treatment replicate) were collected from each tank at the end of the experiment for trace metal analyses (Cu, Zn, Co, Mg, Mo, As, Se, Pb, Ni) that were conducted using inductively coupled plasma mass spectroscopy (ICP-MS) at Charles Darwin University (Darwin, Australia). Hydrocarbon analyses (n = 1 per treatment) were conducted using gas chromatography mass spectrometry (GC-MS) at ChemCentre (Perth, Australia).

#### 4.3.4 Respirometry

Standard oxygen consumption rates (standard  $M_{o2}$ ), representing a measure of the minimum aerobic metabolic rates required to sustain life, were measured following best practices in aquatic respirometry (Clark et al., 2013). A total of 63 fish (0.43 ± 0.02 g) were used in respirometry trials, divided evenly into three of the same treatment groups as detailed above (0, 38 and 73 mg coal I<sup>-1</sup>; n=7). Fish respirometry was conducted after 5 d, 20 d (73 mg coal I<sup>-1</sup>), 23 d (38 mg coal I<sup>-1</sup>), 30 d (73 mg coal I<sup>-1</sup>) and 33 d (38 mg coal I<sup>-1</sup>) exposure to the coal treatments. The mid- and latter time points needed to be split over days due to time constraints, but were close together and from here referred to as the 21 and 31 d sampling points. The highest coal treatment (275 mg coal I<sup>-1</sup>) could not be tested due to coal interference with the oxygen sensor system. Fish were fasted for 24 h prior to respirometry, and each trial lasted an average of 26 h.

Briefly, eight respirometers were constructed using transparent containers (diameter 57 mm, height 78 mm) covered largely with opaque tape to minimise external disturbances while allowing some penetration of light. Each respirometer contained a cylindrical magnetic stir bar (40 x 8 mm) that was housed under a perforated Perspex screen. All eight respirometers were placed in a reservoir bath (L x W x H = 490 x 390 x 310 mm; water depth 90 mm) mounted on a custom-built table containing eight magnetic drive plates. The stir bars rotated at all times, however the Perspex screen was designed to ensure that the water in each respirometer remained well-mixed without requiring the fish to swim to maintain position. The respirometers were submerged in a reservoir bath and flushed intermittently (15:15 min flush:seal cycles) with water containing either the coal treatments or control water (26.9±0.01°C). Oxygen levels in each respirometer were recorded continuously at 0.5 Hz using contactless sensor spots connected via fibre-optic cables to a Firesting  $O_2$  system (PyroScience, Aachen, Germany). Oxygen consumption rates (mgO<sub>2</sub> min<sup>-1</sup> kg<sup>-1</sup>) of the fish were subsequently calculated in LabChart v. 7 (ADInstruments, Sydney, NSW, Australia) from the decline in oxygen concentration in the respirometers between flush cycles. Measurements of background (microbial) respiration were conducted in each trial by leaving one respirometer without a fish. Background respiration was minor on all occasions (< 5% of fish  $M_{o2}$ ) but, nevertheless, was accounted in the fish  $M_{02}$  calculations. Upon removal from respirometers, fish were measured for standard length and weight prior to being returned to the holding tank. Fish tags were checked to ensure new fish were measured at each time point. Between each trial all equipment was rinsed with chlorinated freshwater, followed by filtered seawater. The mean of the lowest 10% of fish  $M_{02}$  values (from a total of ~50 measurements per individual) was calculated for each individual and then outliers ( $\pm$  2 S.D. from the mean) were excluded to generate the standard  $M_{O2}$  (Clark et al., 2013).

#### 4.3.5 Histological preparation

Euthanized fish (n = 12 per treatment) were fixed in Bouin's solution for 24 hours, and washed and stored in 70% ethanol. Fish were serially dehydrated (Shandon Southern Duplex Processor BS5) and embedded in paraffin wax blocks (Kiernan, 2008). Fish were sectioned longitudinally at 5  $\mu$ m thickness, and five sections per fish were either stained with Meyer's hematoxylin and eosin (H & E) or with Periodic Acid Schiff (PAS) and Alcian blue (pH 2.5), and counterstained with Mayer's hematoxylin (Kiernan, 2008). For each section, one filament carrying several secondary lamellae was photographed (Olympus DP12 Microscope Digital Camera System), resulting in n = 5 micrographs per stain and per fish.

## 4.3.6 Gill analyses

The gill arches of juvenile *A. polyacanthus*, as for fish in general (Wilson and Laurent, 2002), consisted of primary gill filaments carrying two rows of secondary lamellae (Figure 4.1). Lamellae had a total length of 50 to 100 µm (base to tip), and were partly covered by the filament epithelium, which is sometimes also referred to as 'interlamellar cell mass' (Sollid et al., 2003; Sollid and Nilsson, 2006). The lamellar epithelium above the filament epithelium consisted of one to several cell layers, and is assumed to be the primary site of gas exchange since it is in direct contact with the surrounding water (Evans et al., 2005; Wilson and Laurent, 2002). Accordingly, the 'functional lamellar length' is defined as the section of the lamellae that is not covered by the filament epithelium (Figure 4.1). The gill diffusion distance is defined as the average distance between the water and the blood space of the functional lamellar length, and includes the thickness of the lamellar epithelium and non-tissue space caused by epithelial lifting (Mallatt, 1985) (Figure 4.1). Mucus cells resided in the filament epithelium, and would occasionally appear on the lamellar epithelium. Mucus adhered to the lamellar epithelium and the filament epithelium.



**Figure 4.1** Micrographs of gill lamellae of *Acanthochromis polyacanthus* stained with Haematoxylin and Eosin (H&E). (**A**) lamella protruding from the gill filament. 1) blood cell, 2) pillar cells supporting the 3) gill epithelium, 4) thickening of the epithelium (hyperplasia), 5) filament epithelium. (**B**) measurements taken in ImageJ to derive the diffusion distance. 1) area of the functional lamella (area between the black and yellow outlines), 2) area of the pillar cell system (area within the yellow outline), 3) length of the functional lamella, i.e. part of the lamella above the 4) filament epithelium, 5) thickness of the filament epithelium. Scale bars: 5  $\mu$ m.

Changes in gill morphology were quantified using two different methods. For the first method, lamellae were rated on a scale from 0 to 3 based on the thickness of their lamellar and filament epithelium (Figure 4.2). The categories were defined as follows; 0 (reference category) – The filament epithelium of lamellae in this category covered < 15% of the total lamellar length, while the lamellar epithelium consisted of one or two cell layers, without major irregularities in epithelial thickness. 1 – The lamellar epithelium showed irregularities in thickness compared to the reference category, such as noticeable cell growth at the base of lamellae. Overall, between 15 and 25% of the total lamellar length was either covered by the filament epithelium and/or the lamellar epithelium was considerably thicker compared to the epithelium of the reference category. 2 – The thickness of the lamellar and/or filament epithelium of lamellae was increased relative to the reference category, covering between 25 and 50% of the total lamellar length. 3 – Over 50% of the total lamellar length

was either covered by the filament epithelium and/or showed a thicker lamellar epithelium compared to the reference category. Similar approaches to the assessment of gill morphology have been used in previous studies (e.g. Bernet et al., 1999; Oliva et al., 2013). All lamellae in H & E micrographs that were free of sectioning errors were assessed (n = 20 to 35 per fish).



**Figure 4.2** Micrographs of gill lamellae of *Acanthochromis polyacanthus* showing different degrees of lamellar thickness, which were rated on a scale from 0 to 3 based on the thickness of the lamellar and filament epithelium. The categories were defined as follows; (**A**) 0 (reference category) – The filament epithelium of lamellae in this category covered < 15% of the total lamellar length, while the lamellar epithelium consisted of one or two cell layers, without major irregularities in epithelial thickness. (**B**) 1 – The lamellar epithelium showed irregularities in thickness compared to the reference category, such as noticeable cell growth at the base of lamellae. Overall, between 15 and 25% of the total lamellar length was either covered by the filament epithelium of the reference category. (**C**) 2 – The thickness of the lamellar and/or filament epithelium of lamellae was noticeably increased relative to the reference category, covering between 25 and 50% of the total lamellar length. (**D**) 3 – Over 50% of the total lamellar epithelium compared to the reference category. H & E stain. Scale bars: 5 µm.

For the second method, several morphological parameters were measured in ImageJ (Version 1.48, National Institute of Health, USA) (outlined in Figure 4.1). Total lamellar length was measured from the base to the tip of the lamellae in H & E micrographs. The thickness of the filament epithelium was measured on both sides of the lamellae, and the average of these two measurements was calculated and standardized to the lamellar length. Functional lamellar length was determined by subtracting the average filament thickness from the total lamellar length. To determine the gill diffusion distance, the total area of the functional part of the lamellae visible in micrographs was determined by tracing its outline (see Hess et al., 2015 for more details). The area of the pillar cell system and blood space was measured the same way, subtracted from the total functional area and divided by twice the functional length to obtain the average diffusion distance (Hess et al., 2015). To determine the amount of mucus present in the space between two lamellae, the area covered by mucus in PAS/alcian blue micrographs was traced by adjusting the colour threshold of the image (Clark, 2013; Hess et al., 2015). Mucus cells present in and on the filament and lamellar epithelium were counted. Filament thickness was measured for 20 to 35 lamellae per fish, diffusion distance was determined for 12, and mucus cover and number of mucus cells were determined for 5 lamellae per fish. Lamellae were selected randomly, and all analyses were performed blindly with respect to treatments.

#### 4.3.7 Statistical analyses

All analyses were carried out in R (version 3.2.3, R Core Team 2015). Model assumptions were assessed using diagnostic plots and all response variables except  $M_{02}$  and lamellar length were logtransformed to meet assumptions. ANCOVA was used to compare absolute oxygen consumption rates across groups, with coal concentration and time as factors and mean-centered fish mass as the covariate. Multiple comparisons were made using Tukey test and the Bonferroni correction. The frequency distribution of scores for lamellae (i.e. categories 0 - 3) were compared between treatments using proportional odds logistic regression (package 'ordinal', (Christensen, 2015), with scores as response variable, treatment as fixed factor, and fish identity as a random factor (random intercepts) to account for multiple scores per individual. The proportional odds assumption was assessed using a mosaic plot. General linear mixed models (GLMM) (package 'Ime4', Bates et al., 2015) with a gamma distribution and log link function were used to compare gill diffusion distance, total lamellar length, functional lamellar length, filament thickness, filament thickness relative to lamellar length, mucus cover, and mucus cover relative to the interlamellar space between treatments, using treatment as fixed factor, and fish identity as random factor allowing for random intercepts. A GLMM with a poisson distribution, appropriate for use with count data, and log link function was used to compare the number of counted mucus cells between treatments. Model

parameters were estimated with Laplace approximation, and *P*-values were estimated with Wald Z (Bolker et al., 2009).

## 4.4 Results

### 4.4.1 Standard oxygen consumption

Exposure of juvenile *A. polyacanthus* to suspended coal particles significantly impacted their standard oxygen consumption rates ( $M_{O2}$ ) after prolonged exposure to coal (i.e. > 21 d). A significant interaction was found between coal treatments and duration of exposure. Acute (5 d) exposure to suspended coal particles elicited reductions in  $M_{O2}$  in both coal treatments by 17 % relative to controls. This reduction was not statistically significantly (P > 0.05). After 21 d,  $M_{O2}$  in coal exposed fish was 47% and 18% greater than control fish in 38 mg coal  $\Gamma^1$  and 73 mg coal  $\Gamma^1$ , respectively. The increased  $M_{O2}$  measured in the 38 mg coal  $\Gamma^1$  fish was significantly different than control fish  $M_{O2}$  (Figure 4.3; Linear model<sub>2,18</sub>, t = 4.0, P < 0.01). After 31 d the  $M_{O2}$  in coal exposed fish was higher than control fish, with significant increases of 30% in 38 mg coal  $\Gamma^1$  (Linear model<sub>2,18</sub>, t = 3.2, P = 0.05) and 38% in 73 mg coal  $\Gamma^1$  (Figure 4.3; Linear model<sub>2,18</sub>, t = 3.4, P = 0.03).



**Figure 4.3** Standard metabolic rates (mg O<sub>2</sub> min<sup>-1</sup> kg<sup>-1</sup>) for *Acanthochromis polyacanthus* (n = 7 for each treatment) after 5, 21 and 31 d of exposure to 0, 38 or 73 mg coal l<sup>-1</sup> at 27°C. Fish exposed to 275 mg coal l<sup>-1</sup> were not measured due to oxygen sensor interference by coal.

#### 4.4.2 Gill analyses

Visual inspection of fish following 31 d of exposure revealed coal particles adhered to the skin and gill surfaces, with higher quantities of particles and discolouration (darkening) of gills in the medium- and high-coal treatments (73 and 275 mg coal l<sup>-1</sup>, Figure 4.4 A-B). Overall, gill structure was altered in coal exposed fish relative to controls (Figure 4.5 A-F), with the most severe alterations observed in the highest-coal treatment fish. Across treatments, the thickness of lamellar tissue decreased with increasing coal concentration (Figure 4.5 A). The percentage of lamellar thickness scores in categories 0 and 1 (i.e. lamellae with a relatively thin filament and lamellar epithelium) significantly increased with increasing coal concentration; while only 54% of lamellae were scored 0 or 1 in control treatments, the proportion of lamellae in these two categories increased to 90% in the highest coal concentration (Figure 4.4 C and D, Figure 4.5 A). Significant differences were found between control fish gills and fish exposed to the medium (z = -2.3, P = 0.02) and high coal treatments (z = -3.4, P = 0.0006). Lamellar length was generally smaller in fish gills exposed to coal, with significant differences in the high-coal treatment (Figure 4.5 B;  $GLMM_{3.44}$ , t = -2.1, P = 0.03), yet the functional length of lamellae was consistent across treatments (Figure 4.5 C; P > 0.05). Mean gill diffusion distance (i.e. the thickness of the respiratory epithelium on lamellae) was reduced by 6-17% across coal treatments relative to control fish, however this reduction was not statistically significant (Figure 4.5 D; P > 0.05). The mean gill filament thickness (relative to lamellar length) was 5 - 21% greater in fish exposed to low- and medium-coal treatments and 29% smaller in fish gills exposed to the high-coal treatment, relative to the control fish. A significant difference was found between filament thickness in the control and high-coal treatment fish (Figure 4.5 E;  $GLMM_{1.46}$ , t = -4.2, P < 0.0001). The percentage of interlamellar space covered by mucus decreased by 19-60% in coal exposed fish, relative to control gills, although the difference was only significant in the high-coal treatment (Figure 4.5 F; GLMM<sub>3,44</sub>, t = -4.2, P < 0.0001, see statistical outputs in Appendix A Table A4.1).



**Figure 4.4** Images of gills of Acanthochromis polyacanthus exposed to (**A**) the control treatment (0 mg coal  $I^{-1}$ ), (**B**) the high-coal treatment (275 mg coal  $I^{-1}$ ) and micrographs of gill filaments from fish exposed to (**C**) the control (0 mg coal  $I^{-1}$ ) and (**D**) the high-coal treatment (275 mg coal  $I^{-1}$ ). In (**B**) the gills are discoloured black from coal and coal particles adhered to various parts of the gill structure. In (**C**) the lamellar epithelium is several cell layers thick due to extensive tissue growth. The filament epithelium covers a significant part of the lamellae, as was typical for fish from the control treatment (Rank 0 for tissue thickness, Figure 4.2 and 4.5 A). In (**D**), the epithelium of most coal-exposed lamellae is only 1 to 2 cell layers thick, reducing the diffusion distance of oxygen between water and blood. The filament epithelium covers a small portion of the lamellae, increasing the surface area available for gas exchange (Rank 3 for tissue thickness, Figure 4.2 and 4.5 A). H & E stain, scale bars: 10 µm.



Figure 4.5 Alterations in Acanthochromis polyacanthus gill morphology after 31 d coal exposure. Coal exposed fish exhibited differences in the mean (± S.E.) (A) lamellar thickness (% per defined category<sup>+</sup>), (**B**) total lamellar length ( $\mu$ m), (**C**) functional lamellar length ( $\mu$ m), (D) diffusion distance (µm), (E) filament thickness (µm) and (F) total mucus coverage (% relative to interlamellar space) across coal treatments (38, 73 and 275 mg coal  $1^{-1}$ ). \* represent a significant difference between control and coal treatments, using P < 0.05. <sup>+</sup> The categories were defined as follows; 0 (reference category) - The filament epithelium covered < 15% of the total lamellar length and lamellar epithelium consisted of one or two cell layers, without major irregularities in epithelial thickness. 1 – The lamellar epithelium showed irregularities in thickness compared to category 0, between 15 and 25% of the total lamellar length was covered by the filament epithelium and/or the lamellar epithelium was considerably thicker compared to the epithelium of category 0. 2 – The thickness of the lamellar and/or filament epithelium of lamellae was noticeably increased relative to category 0, covering between 25 and 50% of the total lamellar length. 3 - Over 50% of the total lamellar length was either covered by the filament epithelium and/or showed a thicker lamellar epithelium compared to category 0. See the methods and Figure 4.2 for further details.

Dissolved oxygen levels in the treatment tanks were 8.3-8.5 mg l<sup>-1</sup> throughout the experimental duration and light attenuation increased (by 44-99%) with coal concentration, relative to control values (Table 4.1). The magnitude of change in dissolved metal concentrations (filtered leachate) in relation to control seawater was minimal (Table 3.2, *Chapter 3*). These findings suggest that metals were not likely contributing to the observed deleterious effects. Water from certain experimental treatments contained significantly (P < 0.05) higher concentrations of Co, As, and Ni in comparison with control water; however, these results were not coal concentration dependent. In *Chapter 2* I measured only trace concentrations of polycyclic aromatic hydrocarbons (PAH) from extractions of leachate from 800 mg coal l<sup>-1</sup> or 10,000 mg coal l<sup>-1</sup> suspensions (maximum of total PAH = 0.61 µg l<sup>-1</sup>) (Berry et al., 2017). Since I used the same type of coal in both experiments I assume that PAH leachate from 275 mg coal l<sup>-1</sup> would be lower than 0.61 µg l<sup>-1</sup> and unlikely to impact on organism health (Berry et al., 2017).

**Table 4.1** Summary of water quality parameters in experimental treatments assessing coal effects on *Acanthochromis polyacanthus*. Mean ( $\pm$  S.E.) total suspended coal (TSC) (mg l<sup>-1</sup>), light (PAR, µmol photons m<sup>-2</sup> s<sup>-1</sup>), light attenuation (% rel. to 0 mg coal l<sup>-1</sup>), temperature (°C), dissolved oxygen (mg l<sup>-1</sup>) and pH. Table adapted from *Chapter 3* (Berry et al., 2016).

Treatment	TSC	Light	Light attenuation (%)	Temperature	Dissolved Oxygen	рН
	n = 35-39	n = 12	n = 12	n = 30	n = 27	n = 9
Control	0	177 ± 8.6	-	26.3 ± 0.2	8.50 ± 0.02	8.17 ± 0.01
Low	37.6 ± 5.7	99.3 ± 9.4	43.8 ± 5.3	26.3 ± 0.1	8.35 ± 0.03	8.05 ± 0.01
Moderate	72.5 ± 11.3	20.7 ± 3.8	88.3 ± 2.1	26.4 ± 0.1	8.30 ± 0.02	8.04 ± 0.00
High	275 ± 35.9	$1.1 \pm 0.32$	99.4 ± 0.2	26.4 ± 0.1	8.32 ± 0.02	8.05 ± 0.00

#### 4.5 Discussion

Tropical marine environments are increasingly exposed to a range of pollutants that can have direct negative effects on fishes (Kroon et al., 2014; Wenger et al., 2015). Here I show that acute (5 d) exposure to suspended coal particles (38 and 73 mg coal  $\Gamma^1$ ) can reduce standard oxygen consumption ( $M_{02}$ ) rates. However longer-term exposure resulted in significant increases in  $M_{02}$ , which likely contributed to the reductions in growth rates measured in *Chapter 3* within 14 d (Berry et al., 2016). Exposure to 275 mg coal  $\Gamma^1$  elicited gill modifications that were likely induced by coal adherence and clogging of gills reducing the capacity for oxygen uptake. These findings contribute to our very limited understanding of the potential impacts of coal on tropical reef species, a situation that needs to be addressed in order to better manage and/or respond to coal spills in these sensitive habitats of high ecological value.

Standard oxygen consumption  $(M_{02})$  is an estimate for an individual's standard metabolic rate and the substantial (18-47%) elevation in  $M_{O2}$  measured in coal exposed fish after 21 d exposure suggests that coal acted as a stressor which disturbed homeostasis (Heath, 1995; Wendelaar Bonga, 1997). In fishes, increased oxygen consumption is often stimulated by the release of stress hormones (e.g. catecholamines) in response to physical or chemical stressors (Wendelaar Bonga, 1997). Stress responses can suppress immune function and reallocate energy away from activity, reproduction, and growth (Schreck et al., 2016). Indeed, reduced or negative growth is commonly observed in fish during or after a stress event (Wendelaar Bonga, 1997). For instance, a 10% and 20% increase in the metabolic rate of largemouth bass (Micropterus salmonids) reduced growth rates by 22% and 42%, respectively (Rice, 1990). In coho salmon, a 20% increase in respiratory rate was associated with a decline in growth rate by 8-16% (Vaughan et al., 1982). A. polyacanthus from the present study exhibited reduced growth rates that ranged from 36-46% and 42-52% relative to controls at 14 and 28 d respectively (reported in Chapter 3, Berry et al., 2016). In addition to the effects of increased Mo2 on growth, A. polyacanthus in the current experiment also likely experienced reduced food intake due to coal particle ingestion, with dissected alimentary tracts of fish from all coal treatments containing large quantities of coal (personal observation, K Berry). The elevated  $M_{o2}$  combined with lower food intake would increase demand on body energy stores, such as liver glycogen (Appleby and Scarratt, 1989; Stern and Stickle, 1978), and likely contributed to the reduced growth rates observed for fish in coal treatments in *Chapter 3*.

The morphology of the gills is a plastic rather than a fixed trait. For instance, some species can adapt to function better under changed oxygen conditions by reversibly remodelling their gill morphology (e.g. Heath, 1995; Nilsson, 2007; Nilsson et al., 2012). This adaptation has been observed in a few species of Cyprinids and air-breathing fish, e.g. the super hypoxia tolerant crucian carp (reviewed in Nilsson, 2007; Nilsson et al., 2012). Substantial interlamellar cell mass (ILCM) is common

in crucian carp (Carassius carassius) and goldfish (Carassius auratus) under normoxic conditions and these fish can shed their ILCM when exposed to hypoxic conditions, thus increasing their respiratory surface area and capacity of oxygen uptake (Sollid et al., 2003; Sollid and Nilsson, 2006). Gill remodelling has also been observed in response to increased temperature (Sollid et al., 2005), exercise (Brauner et al., 2011; Fu et al., 2011; Perry et al., 2012), air exposure (killifish) (Ong et al., 2007) and high environmental ammonia levels (Sinha et al., 2014). Previous studies have revealed that gills can grow protective tissues when exposed to pollutants, either to minimize contaminant uptake or to reduce abrasive damage (Mallatt, 1985). Fish exposed to 275 mg coal l<sup>-1</sup> exhibited significant alterations to their gill structure, however, the structural changes I observed were not consistent with gill modifications commonly reported after exposure to other forms of suspended solids for example: increased mucus production (Hess et al., 2015; O'Connor et al., 1977); lamellar fusion (Lowe et al., 2015; Wong et al., 2013); and epithelial lifting (Au et al., 2004; Cumming and Herbert, 2016). These differences are likely due to the 'stickiness' of coal and its adherence to the gill surfaces and clogging of lamellae. The adherence of particles to secondary lamellae interferes with gas transfer by: 1) reducing the surface area across which gas transfer occurs, 2) increasing the distance between water and blood, and 3) interfering with the convective processes between secondary lamellae (Hughes, 1973). All of these processes can reduce oxygen uptake efficiency, requiring energy-demanding compensatory mechanisms to be initiated (Hughes, 1973). Such mechanisms include increased ventilation rates and hematological responses (e.g. blood cell volume, hemoglobin concentration and blood cell counts) to increase the effectiveness of oxygen transfer to the blood (O'Connor et al., 1977). This basic response is observed in oxygen deprived fish (Hughes, 1964; Hughes, 1973) and has been measured in white perch, hogchokers and striped killifish exposed to 650 mg  $l^{-1}$ , 1240 mg  $l^{-1}$  and 960 mg  $l^{-1}$  suspended solids, respectively (O'Connor et al., 1977). Similar effects from coal exposure are likely, but further studies are required to test this hypothesis.

Similar to Cyprinids, *A. polyacanthus* gills exhibited a thick filament epithelium and lamellar epithelium under control condition, which contradicts the common perception that fish gills ideally have a large surface area and a thin lamellar epithelium. Almost half of all lamellae (48%) in control gills were scored in categories 2 or 3 (i.e. thick lamellar and filament epithelium), while only 9% of lamellae were scored in these categories in the highest coal-treatment. These observed changes in gill morphology (i.e. reduced filament thickness, lamellar thickness, and oxygen diffusion distance) strongly suggest that *A. polyacanthus* in the high-coal treatment remodeled their gills to enhance oxygen uptake capacity, and to compensate for coal particle adherence and clogging of lamellae. In contrast, abrasive damage was likely the cause of reductions in lamellar length (Lake and Hinch, 1999). This study provides the first evidence of active gill remodelling to regulate oxygen uptake in a coral reef fish. My results also show that different types of suspended particles can lead to opposing

types of gill modifications (e.g. lamellar fusion under sediment exposure vs. reduced lamellar thickness under coal exposure) highlighting the need to consider the physical properties of suspended particles when predicting their physiological and ecological effects.

Over 800 Mt of the total 1,300 MT of coal exported by sea in 2015 was transported via shipment routes in tropical marine environments (e.g. in Australia, Indonesia, and Colombia; International Energy Agency 2016) and collier incidents and/or spillage have been reported in each of these countries since 2010 (Caballero-Gallardo et al., 2015; DOEARSA, 2013; GBRMPA, 2011). Transport with Cape-sized colliers that can carry up to 180,000 tonnes of coal is predicted to increase, replacing smaller (Panamax) colliers that carry ~70,000 tonnes (AMSA, 2014). This doubling in coal cargo means the potential scale of spills associated with collier incidents could also increase. It is therefore critical to develop adequate risk assessments of coal spillage, which requires a far better understanding of sub-lethal and lethal effects of coal contamination on tropical organisms. Our results represent the first time that direct, sub-lethal effects of coal particles have been documented in coral reef fish, and underscore the importance of considering these consequences during the risk assessment phases of new coal developments, including ports, and for the management of existing and future coal transport operations in tropical marine waters.

## 5. Chronic and acute coal exposure reduces the physiological performance of corals more than reef sediment

## 5.1 Abstract

Coal is the second largest dry bulk commodity shipped by sea and undocumented quantities of coal particles enter the tropical marine environment during ship loading. Similar to suspended sediments, coal particles could negatively affect habitat-forming species such as corals through light attenuation and smothering. Here, I experimentally investigated the stress-response pathways for coal and sediment deposition on three morphologically distinct reef-building corals, Acropora tenuis (branching), Porites spp. (massive) and Montipora spp. (foliose). Coal or sediment particles were deposited (30 mg cm<sup>-2</sup>) onto corals twice per week for 28 d (chronic deposition), after which rates of oxygen production, respiration and calcification were measured in particulate-free seawater. In addition, I measured the acute effects of exposure to suspended coal and sediment on both corals exposed to chronic deposition and corals that had been kept under control conditions. Finally, I measured particle rejection behavior, particle clearance efficiency, tissue discolouration, symbiont density and chlorophyll a content in corals treated with coal and sediment. This study revealed that light attenuation by coal suspensions has a far greater effect on corals in comparison to carbonate sediment suspensions, and corals are generally less efficient at removing deposited coal than sediments. Consequently, chronic deposition caused significantly greater tissue discoloration in coalsmothered Porites and Montipora spp. Moreover, chronic coal deposition significantly decreased respiration rates in A. tenuis (-60%) and Porites spp. (-38%) and deposition of both particle types significantly reduced dark calcification rates in all species. All species exhibited substantially reduced oxygen production and calcification rates during acute exposure to coal suspensions, while respiration rates were variable. During acute exposure, corals previously exposed to chronic coal deposition exhibited CaCO<sub>3</sub> dissolution rather than calcification, which decreased by 223-399%, relative to control corals. The findings from this chapter demonstrate that chronic and acute coal exposure lowers the physiological performance of corals more severely than the tested sediment. Differences between the responses of corals to coal vs sediment deposition should be further tested and accounted for when assessing risks associated with coal contamination in proximity to coral reefs.

### 5.2 Introduction

Reduced water clarity from suspended solids is an important stressor in coastal marine environments, particularly for photosynthetic sessile organisms such as corals. The presence of suspended particulates can negatively affect corals, and thereby affect reef ecosystem functioning,

through three pathways; smothering, abrasion and light attenuation (Jones et al., 2016). To date, research in this field has focused primarily on sediments sourced from river-runoff, coastal developments and dredging operations (Erftemeijer et al., 2012b; Fabricius, 2005; Jones et al., 2016; Jones et al., 2015b), while other particulate contaminants, such as unburnt coal particles, have received less attention. Although fine coal particles and sediments are generally considered to elicit similar responses by marine organisms (Ahrens and Morrisey, 2005), this has not been formally tested with tropical species. Potential differences in the effects of coal versus sediment, for instance due to differences in the physical properties and chemical composition of these particulates, could mean that management strategies developed for sediments are inappropriate for the management of coal.

Concentrations of suspended particulates are spatially and temporally variable in coastal marine habitats. For example, on the Great Barrier Reef (GBR), sediments from terrestrial sources enter the marine environment during periods of high-rainfall (Devlin et al., 2001; Jones and Berkelmans, 2014). During dredging operations, high concentrations of suspended solids have been measured near the operation for several hours (100-500 mg  $l^{-1}$ ), with low turbidity conditions (avg.  $<10 \text{ mg } \text{I}^{-1}$ ) that are higher than baseline levels persisting over longer periods (weeks to months) (Jones et al., 2015a). Carbonate sediment concentrations on coral reefs vary depending on windand wave-driven resuspension of bottom sediments (Larcombe et al., 2001). Concentrations of suspended coal particles are also likely to fluctuate over time depending on sources. Unburnt coal particles can enter the aquatic environment naturally through erosion of coal seams or during mining operations (e.g. washing processes), transport to ports, storage, and loading of ships (Campbell and Devlin, 1997; Sydor and Storts, 1980). Information on the quantities of coal particles entering the environment during these processes is limited, however fugitive loss from port stockpiles and during loading processes are certain to occur (GHD, 2012). Inputs of coal from such sources are likely to occur in pulses due to timing of ship loading, or when stockpiles are 'refilled' and could be acute or chronic.

Another potential source of acute and chronic coal contamination is collier incidents that involve the direct release of coal into the marine environment, either immediately following a shipping incident or in pulses over time e.g. as a ship's hull breaks up. Since 2010, there have been 4 documented incidents of collier groundings onto- or a ship sinking in proximity to- coral reefs in the Philippines, Madagascar, Hawaii and Australia. Large collier spills have resulted in the release of up to 60,000 tonnes of coal into the marine environment (*MV Smart*, South Africa 2013) and smaller spills (~600 tonnes) have been documented during trans-ship loading incidents (Sanchez, 2014). Indeed, nine barges, each carrying ~600 tonnes of coal, reportedly sunk in Colombian ports between 2006-2010 (Patino, 2013; Sanchez, 2014). Anecdotal evidence from fishers have reported

that coal barge spills/sinking's at Colombian coal ports have contributed to the degradation of nearby coral reefs (Cohen, 2014). Moreover, scientific investigations after the *Eurobulker IV* sunk in Italy, revealed smothering of benthic flora and fauna by coal and abrasive damage to seagrass leaves (Alcaro et al., 2002; Jaffrennou et al., 2007).

Suspended particulates from natural and anthropogenic activities can both harm corals directly through physical interaction and indirectly thorough light attenuation. For example, suspended particulates can cause physical abrasion of coral tissues (Johannes, 1975; Loya, 1976; Rogers, 1983) and increased turbidity, which is directly linked to the presence of increased suspended particulate matter, decreases the quantity and spectral quality of light available for photosynthesis by the endosymbiotic algae (*Symbiodinium* sp.) that live within coral tissues (Jones et al., 2016). Exclusion of light from corals can alter coral reef structure and function by decreasing net primary productivity, respiration and calcification (Bak, 1978; Rogers, 1979), leading to bleaching and mortality following chronic exposures (Bessell-Browne et al., 2017).

In addition to the negative impacts of suspended particulates, over time these particles sink through the water column and are deposited on the substratum. This particle deposition further reduces the amount of light (and its spectral quality) reaching the coral tissue, resulting in impaired photosynthesis and reduced translocation of metabolites to the coral animal, and/or creating an anoxic layer adjacent to tissue (Muscatine et al., 1981; Weber et al., 2012). High quantities of deposition can prevent coral polyps from expanding their tissues and feeding (Anthony, 2000; Mills and Sebens, 1997). The combination of reduced photosynthetic capacity and heterotrophic feeding capabilities, coupled with increased energy expenditure for particle clearance (e.g. ciliary or tentacle movements, entrapment of particle by mucus) can lead to increased metabolic rates, reductions in growth, as well as reductions in tissue biomass and lipid content (Anthony and Fabricius, 2000; Flores et al., 2012; Philipp and Fabricius, 2003; Rogers, 1983; Telesnicki and Goldberg, 1995). Additional stress can be elicited by chemical pollutants, such as trace metals, which can adsorb to sediments (e.g. Berry et al., 2013; Wang et al., 1999) and come into contact with corals via feeding, direct attachment to coral mucus and via direct uptake from the water through the tissue (Esslemont, 2000; Peters et al., 1997).

The impacts of coal on corals, fish and seagrass that I have reported so far (Berry et al., 2017; Berry et al., 2016; *Chapters 2-4*) indicate that some of the cause-effect pathways for coal may differ from the cause-effect pathways previously documented for sediments. In *Chapter 3*, I determined that chronic exposure (14 and 28 d) of corals to suspended coal particles caused significant increases in coral mortality as well as reductions in seagrass and reef-fish growth rates (Berry et al., 2016). Compared with sediments, coal particles are far darker in colour and suspensions of coal resulted in

very high light attenuation, up to 99% over a short light path (25 cm) (*Chapter 3*, Berry et al., 2016). Moreover, coal particles adhered to organisms, including corals, seagrass and fish gills and skin (*Chapter 3*) to a greater extent than has been reported in similar experimental studies using suspended sediments, and caused mortailty in branching *Acropora sp.* corals at far lower concentrations than prevously reported for sediments (e.g. Flores et al., 2012). Finally, in juvenile corals, only 33% of polyps smothered by coal cleared themselves in comparison to 60% of carbonate sediment-smothered polyps after 96 h (*Chapter 3*, Berry et al., 2017), potentially reflecting an inability of corals to clear the more sticky coal particles.

An improved understanding of the physiological responses of corals to acute and chronic exposure to both coal and carbonate sediments is required to establish whether risks posed by coal particles should be managed differently to those posed by sediments. The aims of this study were to understand the physiological responses of corals to: 1) chronic coal deposition and 2) suspended particles; and 3) investigate mechanisms underlying the effects measured in aims 1 and 2. I hypothesized that, compared with the effects of suspended carbonate sediments, coal exposure would elicit greater impacts on coral metabolism due to more rapid light attenuation in coal compared with sediment suspensions, and greater energy requirements to effectively clear particles from tissues. I used an experimental approach to address these aims. Corals were exposed to different treatments designed to: 1) measure the effects of successive 'chronic' coal or sediment depositions (30 mg cm<sup>-2</sup>) on coral health as indicated by colony photosynthesis, respiration and calcification as well as tissue pigmentation and symbiont density (see Figure 5.1); and 2) measure the effects of 'acute' exposure to suspended coal or sediment on coral photosynthesis, respiration and calcification rates (see Figure 5.1). Aim 3 was addressed by: (a) assessing rates of sediment removal from coral tissues; (b) using microsensors to measure light and oxygen concentration at the tissue surface beneath the deposited layer of coal or sediment; and (c) quantifying physical and chemical properties of seawater containing different particulates.

#### 5.3 Materials and methods

#### 5.3.1 Coral collection and sediment preparation

Three morphologically distinct scleractinian coral species commonly found on the Great Barrier Reef (GBR) were examined in this study: *Acropora tenuis* (branching), *Montipora spp.* (foliose) and *Porites spp.* (massive). Colonies of *Acropora tenuis* and cores of massive *Porites spp.* were collected from Davies Reef (-18.789067°, 147.733951°) and *Montipora* colonies were collected from Pelorus Island (-18.5486°, 146.4972°) on the GBR in April 2015. Corals were maintained in 1000-I flow-through holding tanks under natural, 70% neutrally shaded light conditions at the Australian Institute

of Marine Science for one week to recover and then fragmented using an electric band saw. Colony sizes averaged 16 cm<sup>2</sup> for *A. tenuis,* 32 cm<sup>2</sup> for *Montipora spp.* and 22 cm<sup>2</sup> for *Porites spp..* Coral colonies were attached to individually labelled aragonite pegs using super glue and were left to recover for 4 weeks in flow-through seawater in the large temperature controlled aquarium system at the National Sea Simulator (Australian Institute of Marine Science). Corals were fed twice weekly with *Artemia* naupili.

Coking coal (sourced from central Queensland, Australia) was crushed, milled and sieved to isolate particles 63-125  $\mu$ m. Carbonate sediment from Davies Reef of the same size fraction was used to compare differences in effects caused by the different particle types. Due to differences in particle density between coal (~1.2 - 1.9 mg cm<sup>-3</sup>) and sediment (~2.7 mg cm<sup>-3</sup>) pilot studies were conducted to determine the quantity of each particle type required to achieve a consistent deposition layer of 30 mg cm<sup>2</sup>, and this was 100 g of coal and 75 g of sediment respectively in the 60 l tanks.

#### 5.3.2 Aim 1: measure effects of chronic deposition on coral health

## 5.3.2.1 Experimental set-up

The successive deposition experiment mimics chronic particle input and/or resuspension and was conducted in 60 | flow-through  $(0.8 | h^{-1})$  rectangular experimental tanks (n = 2 tanks per treatment) that were maintained at 27°C, salinity 35.8 ± 0.03 PSU, 12h light:dark photoperiod at 350  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>, n = 7 coral colonies per species and per tank. Repeated particle depositions of 30 mg cm<sup>-2</sup>, within each replicate experimental aquarium, were conducted twice weekly for a total of eight depositions over the course of the experiment. Prior to the commencement of particle addition, glass petri dishes (6 cm diameter on average, 1.2 cm high) were placed randomly throughout experimental tanks (n = 6 per tank) to quantify relative-deposition. Particles were added to 1 l of 1  $\mu$ m-filtered seawater and sonicated for 40 min to ensure wetting before being added to experimental aquaria. The tank water flow and aeration were all stopped during addition of the 1 l particle suspensions. Immediately after addition, fan pumps were used to distribute the particles evenly throughout the tanks. When the particles were evenly mixed, based on visual assessment, pumps were turned off and particles were allowed to settle. Water flow was turned on 8 h postdeposition to avoid reductions in water quality due to stagnation and the petri dishes were covered and removed 24 h post-deposition prior to re-commencement of aeration. Particle deposition was measured gravimetrically by filtering the content of the petri dish contents onto pre-weighed filters (GFF, 0.7 µm glass microfibre) that were then rinsed with deionized water and oven dried (60°C). The gain in weight of each filter was divided by the surface area of the petri dish to calculate relative deposition per unit surface area of the substratum. Relative deposition rates of coal and sediments in chronic exposures averaged 28.7 ( $\pm$  S.E. 1.3) and 31.1 ( $\pm$  S.E. 0.97), respectively. Deposited sediments were siphoned from aquarium before subsequent additions.

#### 5.3.2.2 Coral health measurements

To determine rates of oxygen production (i.e. photosynthesis), respiration and calcification I used incubation chamber techniques described in Strahl et al. (2015). In brief, after 4 weeks of successive particle depositions, 7 replicate colonies per species were gently rinsed to remove particles adhering to tissue and were incubated in the light and in the dark (see Figure 5.1). The lids of custom-made, inverted clear acrylic incubation chambers (height: 10.5 cm, radius 4.5 cm) were placed into respective treatment tanks and corals were secured to the lid, above a magnetic stir bar. The chambers (600 ml) were then filled with treatment water and all air bubbles were removed before sealing the chambers with the corals inside. Chambers were placed into a black flow-through water bath (27°C) and a battery operated pulley system rotated the magnetic stir bars at a constant speed (202 rpm) to mix seawater inside the chamber. Light incubations were run for 1.5 h under controlled light conditions (350 mol photons  $m^{-2} s^{-1}$ ). At the end of each trial the oxygen concentration was measured in each chamber using a hand-held dissolved oxygen probe (HQ 40 d Hach Probe). Corals were transferred back into their treatment tanks and a subsample of water (50 ml) was taken from each chamber for alkalinity measurements and fixed with 25  $\mu$ l of mercuric chloride to prevent biological activity altering alkalinity prior to measurement. After 1.5 h of dark adaptation, the incubations were repeated in the dark. To account for the biological oxygen demand of the coal and microorganisms in the water, one chamber in the water bath contained no coral for the duration of the incubation. After measurement, corals were then snap-frozen in liquid nitrogen for determination of tissue pigment and surface area. The alkalinity anomaly technique was used to measure light and dark calcification rates (Chisholm and Gattuso, 1991).

Chlorophyll *a* (Chl *a*) and symbiont (*Symbiodinium* spp.) density were measured from frozen corals. Coral tissue was air stripped from the skeleton using 14 ml of chilled filtered seawater. The blastate was homogenized for 30-60 s (30 for *A. tenuis* and 60 for *Porites* and *Montipora*) and centrifuged at 1500 x *g* for 3 minutes at 4°C. The samples were then separated into host and symbiont. The symbiont pellet was resuspended in 5 ml FSW with the vortex and centrifuged at 1500 x *g* for 3 min at 4°C. This step was repeated for *Porites* and *Montipora*. The pellet was then resuspended, vortexed and divided into subsamples for symbiont density (0.5 ml placed into 0.5ml of 3% formalin) and pigment analysis (0.75 ml). Pigments were extracted twice from coral colonies using 95% ethanol and quantified spectroscopically using the equations of (Ritchie, 2008) and (Lichtenthaler, 1987). Endolithic algae were not visible in the samples and assumed not to contribute any significant amount of Chl *a* to the samples. Chl *a* concentrations were standardised to coral surface area, which was measured using the wax dipping method (Stimson and Kinzie, 1991).

*Symbiodinium spp.* were counted using a heamocytometer. Coral health was also measured by visually assessing tissue discolouration over time. Corals were photographed before the commencement of particle deposition (T0) and upon completion of the experiment (28 d). ImageJ software (U.S. NIH, MD, USA http://rsb.info.nih.gov/ij/) was used to calculate the proportion of coral tissue that had changed colour (e.g. paled or bleached).

# 5.3.3 Aim 2: effects of suspended coal or sediment on coral photosynthesis, respiration and calcification rates

To determine the differences in response of corals during acute exposure to high concentrations of suspended coal and sediments over short periods of time I repeated the incubations described above (5.3.2.2) with new corals (n = 7 per species per treatment) that were either (a) previously exposed to the chronic deposition experiment (5.3.2.1) or (b) not previously exposed to either type of particulate matter, i.e. acute response. The protocol outlined in section 5.3.2.2 Coral Health measurements was followed except that rather than corals being incubated in particle-free seawater, corals were exposed to 1250 mg  $\Gamma^1$  of either coal or sediment during short 1.5 hour incubations. This high concentration was chosen because it was considered likely to cause impacts on the coral based on the results of *Chapter 2* and *Chapter 3*.



**Figure 5.1** Diagrammatic representation of experiments associated with aims 1 and 2 (outlined in 5.3.2 and 5.3.3). 30 mg cm<sup>-2</sup> was deposited onto corals twice weekly for four weeks. Twenty-four hours after the final deposition, corals not previously exposed to particulates (T I), previously deposited with coal (T II) and previously deposited with sediments (T III), were cleared of remaining particles and incubated in particle-free seawater for measurements of photosynthesis, respiration, light and dark calcification rates. A second set of incubations was then run with corals from treatments T I, T II and T III with suspended coal particles (corals from treatments TI + SC and TII + SC) and suspended sediments (corals from treatments T I + SS and TIII + SS).

#### 5.3.4 Aim 3: mechanisms of effect

#### 5.3.4.1 Clearance behaviour and rates of sediment removal

The suspended particles present in the aquaria used for the deposition experiments prevented visual observation of the degree of coral tissue expansion under the different particle suspensions. A separate, smaller scale, experiment was therefore conducted to observe the response of each species to each particle type, as well as to measure the efficiency of each species to clear their tissue of coal and sediment. Coral colonies of each species (n = 4 per species) were placed into clear Perspex chambers (600 ml) and exposed to 30 mg cm<sup>-2</sup> depositions of suspended coal or carbonate sediment. Particles were left to settle for 1 h after which colonies were photographed (from directly overhead, perpendicular to the tissue surface) every 30 minutes for 6 hours. The coral surface area covered in coal or sediment was measured at each time point using ImageJ software (U.S. NIH, MD, USA http://rsb.info.nih.gov/ij/) and particle rejection efficiency was calculated as the proportion of the tissue surface that was cleared of particles between the initial and final time points (6 h post-deposition). Particle rejection behavior, such as visual mucus excretion, tentacle contraction and expansion was also recorded.

## 5.3.4.2 Microsensor analysis of light and oxygen at the tissue surface

Microelectrodes were used to measure the depletion of oxygen and light at the tissue surface, below the layer of deposited particles, and these values were compared to control (particle free) conditions. Measurements were only conducted for massive Porites spp. for logistical reasons, but similar effects would be expected for Montipora spp. (because it has a similar corallite size to Porites spp. and its tissue/skeleton surface is relatively smooth) but different effects are likely for Acropora due to its branching morphology and larger corallites. Colonies were measured under each of four exposure scenarios: 1) 1 µm-filtered seawater, 2) coal deposition, 3) calcium carbonate deposition, and 4) activated carbon deposition, which acted as a toxicity control for the coal, and also accounted for the difference in particle colour/reflectance between coal and calcium carbonate. Measurements were performed on n = 3 Porites cores, with each exposure regime applied to each core on different days (after an 18 h recovery). Each Porites core was placed in a custom-built flow chamber (432 ml), through which filtered seawater was circulated (~1 cm s<sup>-1</sup>). A light intensity of 350  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> was used. O<sub>2</sub> microelectrodes (tip size: 10  $\mu$ m in diameter; 90% response time (t<sub>90</sub>) <1 s) were built as described in Revsbech (1989) and calibrated with air-saturated water (100% air-saturation) and N<sub>2</sub>-bubbled water (0% air saturation). Microsensors were fixed to a motor-driven micromanipulator (MM3, Märzhäuser, Wetzlar, Germany) controlled by custom-written profiling software (µ-Profiler, http://www.microsen-wiki.net/). The microelectrode was placed into direct contact with the coenosarc (interconnective tissue between polyps). To evaluate the response of corals to particle deposition, water flow was turned off and 90 mg  $\Gamma^1$  pre-sonicated and aerated coal, calcium carbonate or activated carbon was added to the chamber. The respective particles were allowed to settle for 15 min and then the flow was re-commenced. Oxygen concentration profiles (n = 5) were measured from the tissue surface upwards into the water column in vertical steps of 50 µm. The coral was allowed to recover overnight (~ 18 h). The experiment was then repeated the next day with the next particle type. The above steps were repeated using a light microelectrode using vertical steps of 100 µm. After profiles were completed in particle free filtered seawater, the coral was smothered in coal or sediment. Downward movements were then noted until the bulb was completely submerged in the particles to determine the distance required for absolute light attenuation.

## 5.3.4.3 Physical and chemical properties of seawater

Comparison of depth-related light attenuation between coal and sediments was conducted by suspending 1250 mg l<sup>-1</sup> of coal and sediment particles in 60 l tanks (n = 1 per treatment). A third tank contained particle-free filtered seawater. Once particles were well mixed light measurements were taken using a Li-cor sensor at the water surface followed by measurements at 1, 5, 10 and 15 cm depth. Measurements of attenuation with depth (0, 10, 20, 30 and 40 cm depth) were repeated for different concentrations of suspended coal particles (0, 10, 30, 100, 300 mg coal l<sup>-1</sup>) using the same method in a deeper tank.

Water samples were taken for trace element analysis (n=3 per treatment) to detect leaching from coal and carbonate sediments both 8 h post deposition (after particle settlement and before water flow was turned on) and 16 h post re-commencement of water flow. Trace metals measured included: Al, Mn, Co, Ni, Cu, Zn, As, Mo, Cd, and Pb. Trace metal analysis was conducted at Charles Darwin University, Australia, using inductively coupled plasma mass spectrometry (ICP-MS). Water samples (0.45  $\mu$ m syringe filtered leachate, 150 ml) were taken from each treatment (n = 3; 3x50 ml per tank, which was pooled for each treatment replicate). Coal suspensions (n = 3) and a seawater control (n = 1) were additionally sampled after the deposition period (8 h) for PAH analysis, which was conducted at ChemCentre (Perth, Western Australia) using GC-MS. Samples were prepared and analysed as previously described in section *2.3.10 Chapter 2*.

## 5.3.5 Statistical analyses

Oxygen production, respiration, calcification and response variables such as symbiont density and Chl *a* content were compared between treatments using general linear models (GLM) with R software (version 3.2.3, R Core Team 2015). Data were analyzed separately for each species and responses of corals exposed to coal and sediment treatments were compared with those of controls. Residuals were inspected for normal distribution and heteroscedasticity by visually inspecting QQ plots and frequency distributions. The proportion of healthy tissue was arcsin square root transformed and normality was tested using the Shapiro-Wilk test. Not all treatments met the assumption of a normal distribution so non-parametric tests were run using Kruskal-Wallis and Dunn's multiple comparisons test in GraphPad Prism.

#### 5.4 Results

## 5.4.1 Aim 1: effects of chronic deposition on coral health

#### 5.4.1.1 Physiological performance

Rates of oxygen production were significantly lower (50%) in *A. tenuis* corals previously smothered by sediments, in comparison with control corals (Table 5.1 and see Appendix A Table A5.1 for statistics). Respiration rates of *A. tenuis* colonies previously smothered by coal were significantly lower, by 60%, in comparison to controls (Table 5.1). Light and dark calcification rates were significantly reduced in corals previously smothered by coal and sediments, resulting in CaCO<sub>3</sub> dissolution in the dark. The reductions in calcification were more pronounced in coal smothered corals (light and dark calcification rates declined by 146% and 294%, respectively, relative to control corals), than sediment smothered corals (light and dark calcification declined by 99% and 139%, respectively, relative to control corals).

In massive *Porites spp.* previously smothered by coal, rates of oxygen production were significantly higher, (+ 36%), and respiration rates were significantly reduced (-38%), in comparison to control corals (Table 5.1). Corals previously smothered by sediments did not exhibit differences in rates of oxygen production or respiration. *Porites spp.* previously smothered by coal exhibited significantly reduced light and dark calcification rates, by 82% and 197%, relative to control corals, resulting in CaCO<sub>3</sub> dissolution in the dark. *Porites spp.* previously smothered by sediments also exhibited significant reductions in dark calcification rates, by 185%, relative to control corals, and CaCO<sub>3</sub> dissolution.

In *Montipora spp.*, mean rates of oxygen production and respiration were not affected in corals previously smothered by coal or sediments. *Montipora spp.* previously smothered by coal exhibited significant declines in light and dark calcification rates in comparison to control corals, by 61% and 71%, respectively, while corals previously smothered by sediments exhibited declines in dark calcification rates, by 76%, relative to control corals (Table 5.1).

**Table 5.1** Oxygen production, respiration and calcification of corals in experiments 5.4.1 and 5.4.2. Percentage difference (mean  $\pm$  S.E.) in oxygen production, respiration, and calcification rates (measured in the light and the dark) in corals (a) exposed to chronic coal and sediment deposition, and (b) corals exposed to coal and sediment suspensions, relative to corals not previously exposed to particulates (controls). The values shown depict treatments that were significantly different than control values (P < 0.05), while "-" depict treatments that were not significantly different (P > 0.05). A summary of statistics is provided in Appendix A (Table A5.1). Abbreviations: A = Acropora tenuis, M = Montipora spp., P = Porites spp., sed = sediment, SW = particle free seawater, SC = suspended coal, SS = suspended sediments.

Treatment		Species	Oxygen production	Respiration	Light calcification	Dark calcification				
			$(mgO_2 cm^{-2} min^{-1})$		$(\mu mol CaCO_3 cm^{-2} min^{-1})$					
Controls not exposed to particulates										
Control	-	А	0.26 (0.06)%	-0.13 (0.02)%	5.20 (1.1)%	5.10 (1.1)%				
(т I)		Ρ	0.44 (0.07)%	-0.34 (0.03)%	8.49 (1.4)%	4.29 (1.4)%				
		М	0.37 (0.04)%	-0.28 (0.05)%	7.17 (1.1)%	2.65 (0.6)%				
				% change relat	ive to control					
a) Reponses to chronic deposition (Results section 5.4.1.1, Appendix A Figure A5.1)										
Coal pre-exposed +SW (T II)	Coal	А	-	-60 (8)%	-146 (9)%	-294 (30)%				
		Ρ	+36 (13)%	-38 (6)%	-82 (9)%	-197 (17)%				
		М	-	-	-61 (6)%	-71 (14)%				
Sed pre-exposed +SW	Sed	А	-50 (9)%	-	-99 (6)%	-139 (4)%				
		Р	-	-	-	-185 (16)%				
(T III)		М	-	-	-	-76 (5)%				
b) Responses to suspended particles (Results section 5.4.2, Appendix Figure A5.3)										
Coal pre-exposed +SC	Coal	А	-99 (2)%	-	-72 (6)%	-399 (37)%				
		Р	-104 (4)%	-55 (4)%	-79 (6)%	-325 (20)%				
(T II + SC, chronic)		м	-68 (5)%	+228 (50)%	-	-223 (40)%				
Sed pre-exposed +SS	Sed	А	-	-	-	-93 (10)				
		Р	-	-	-	-99 (27)				
(T III + SS,chronic)		М	-50 (13)%	-	-36 (5)%	-				
Non-exposed +SC (T I + SC, acute)	-	А	-112 (4)%	-	-149 (7)%	-119 (8)%				
		Р	-135 (3)%	-26 (5)%	-138 (7)%	-192 (6)%				
		м	-124 (10)%	-	-58 (6)%	-88 (14)%				
Non-exposed +SS (T I + SS, acute)	-	А	-	-	-	-119 (17) %				
		Р	-	-	-	-103 (13)%				
		м	-	-	-	-				

## 5.4.1.2 Tissue pigmentation, symbiont density and Chl a content

At the end of the 4 week deposition experiment there was a significant difference in *Porites* (ANOVA: F = 11.8, P = 0.0028) and *Montipora* (ANOVA: F = 7.2, P = 0.0272) tissue health (based on changes in tissue coloration) between coal deposited corals and control corals (Figure 5.2, Appendix A Figure A5.2). Relative to control values, tissue health was degraded by 17% and 0.9% in massive *Porites*, and 19% and 15% in *Montipora spp.* for coal and sediment treatments, respectively. *A. tenuis* exposed to coal and sediment deposition remained healthy (> 99% healthy tissue). There was no significant difference in symbiont density or Chl *a* content in any coral species between experimental treatments (P > 0.05) (Appendix A Figure A5.2).



**Figure 5.2** Tissue discolouration in *Montipora* (**A** and **B**) and *Porites* spp. (**C** and **D**) after 4 weeks of successive coal depositions. **A** and **C** show areas of the coral tissue surface where coal accumulated after deposition pulses and was not removed by coral clearance efforts. **B** and **D** show tissue discolouration that occurred in areas where coal and sediments accumulated. The particles were cleared from corals in **B** and **D** manually via gentle washing.

## 5.4.2 Aim 2: effects of exposure to suspended coal or sediment on coral photosynthesis, respiration and calcification rates

The rates of oxygen production in *A. tenuis* declined significantly in the high coal suspension treatments in comparison to control corals (Table 5.1). Corals previously smothered in coal exhibited significant declines in oxygen production by 99%, relative to control corals, while corals experiencing acute suspensions (i.e. fresh corals, not previously exposed to deposition) by coal also exhibited significant declines in oxygen production by 112% (Table 5.1), relative to control corals. Light calcification rates were also significantly reduced in these treatments by 72% and 149%, relative to control corals, with dissolution of CaCO<sub>3</sub> in the treatments containing corals during acute suspensions. Dark calcification was significantly reduced in all incubations containing suspended coal and sediments, in comparison to control corals. This decline was greatest in corals previously exposed to coal (-399%), followed by corals experiencing acute suspensions to coal and sediments (both reduced by -119%) and corals previously smothered by sediments (-93%). Dissolution of CaCO<sub>3</sub> occurred in all treatments with the exception of corals previously smothered by sediments.

In massive *Porites spp*. incubated with suspended coal particles, rates of oxygen production, respiration and light and dark calcification were significantly lower in corals previously smothered by coal and corals experiencing acute suspensions (Table 5.1), in comparison to control corals. Significant reductions in dark calcification rates were also measured in both suspended sediment treatments, which were reduced by 99% in previously smothered corals and 103% in corals experiencing acute suspensions, relative to control corals (i.e. corals in particle-free incubations).

In *Montipora spp*. incubated with suspended particles, a significant reduction was measured in oxygen production rates in corals previously smothered by coal (-68%) and sediments (-50%), as well as corals experiencing acute coal suspensions (-124%), relative to control corals (Table 5.1). Respiration rates were significantly higher in coal suspensions by corals previously smothered by coal particles, by +228%, relative to control corals. Suspended coal and sediments significantly reduced light calcification rates in corals experiencing acute suspensions (by 58%) and those previously smothered by sediments (by -36%), relative to control corals. Dark calcification rates were significantly lower in sediment suspensions by corals experiencing acute suspensions, by -88%, relative to control corals. Significant reductions, by -223% including CaCO<sub>3</sub> dissolution, was measured in coal suspensions by previously coal smothered corals, relative to control corals.

#### 5.4.3.1 Particle removal efficiency

In the chronic deposition experiment, visual observations revealed that 29% and 50% of *Porites spp.* colonies had failed to remove all sediments and coal respectively between successive depositions in the first week of the experiment, while 100% and 93% of *Montipora spp.* colonies retained sediment and coal, respectively. Branching *A. tenuis* on the other hand was able to clear all sediments and coal between depositions. Over a shorter time frame (6 h), corals also required a longer time period to remove coal particles from their tissues compared with sediments and this was particularly apparent during the first few hours (Figure 5.3). By six hours post-deposition; *A. tenuis* had cleared 76 ± 0.08%, mean ±S.E. of coal and 85 ± 0.05% of the sediments from its tissue; *Porites spp.* had cleared 90 ± 0.02% of the coal and 91 ± 0.03% of the sediments from its tissue. The proportion of *Montipora spp.* tissue that was cleared of sediments declined over time because the continued movement of cilia and mucus spread sediments that had previously accumulated in tissue crevices.



**Figure 5.3** Relative proportions (mean  $\% \pm$  S.E.) of coral tissue that had been cleared of coal or sediment at 0.5 h intervals after deposition of 30 mg cm<sup>-2</sup>. Corals (n = 4 per treatment) had not previously been smothered by coal or sediment. Corals were fully smothered and measurements commenced 1 h post deposition.

#### 5.4.3.2 Microsensor analysis of light and oxygen at the tissue surface

Oxygen production at the coral tissue surface ceased when corals were smothered with coal, sediment or activated carbon, when profiles were conducted in the light (Figure 5.4 A-C) demonstrating that smothering prevented photosynthetic oxygen production. Moreover, tissue surface oxygen concentration was even more depleted under particle smothering than under normal dark respiration (Figure 5.4, A-C) indicating total depletion of oxygen at the tissue layer due to respiratory oxygen demands. Oxygen production re-commenced when polyps began to clear particles and light and oxygen could penetrate through the sediments to the tissue surface (Appendix A Figure A5.4). When smothered with coal or sediment, the corals exhibited absolute light attenuation at the tissue surface (Figure 5.5). At a wavelength of 550, light levels just (100  $\mu$ m) above and below the coal were attenuated by coal far more than the carbonate sediments (53 ± 1% more above and 415 ± 4% below).



Oxygen concentration (µmol I<sup>-1</sup>)

**Figure 5.4** Oxygen microprofiles in the light and dark at the surface of *Porites* before and after the addition of (**A**) coal, (**B**) sediment and (**C**) activated carbon. Points represent mean  $\pm$  S.D. of 4-5 replicates per treatment. Symbol legend: open circles = light profiles, open squares = light profile + particles, closed circles = dark profiles, closed squares = dark profile + particles, black arrow = estimated height of the deposited layer. The deposition of particles suppressed oxygen production during photosynthesis (seen by the difference between open circles and squares) and impeded respiration (seen by the difference between closed circles and squares). Under normal respiration, ciliary action/tentacle movements stir the water so that oxygen is not zero at the tissue surface and, indeed, the coral has some oxygen to respire. The respiration has depleted all O<sub>2</sub> available for use. Additional profiles are provided in Appendix A Figure A5.5.



**Figure 5.5** Light spectra measured at different particle deposition depths. Measurements were taken 100  $\mu$ m above and below the surface of the coal or sediment layer, as well as at the coral tissue surface. Particle deposition resulted in absolute light attenuation at the coral surface for both coal and sediments.

## 5.4.3.3 Clearance behaviour

Both *A. tenuis* and *Montipora spp.* responded to deposition by immediately releasing fine mucus sheets entrapping coal and sediments (Figure 5.6 A, B), although coal and sediment were also observed to accumulate in corallites of *A. tenuis* (Figure 5.6 A), and on flat areas and in crevices of *Montipora spp.* (Figure 5.6 E, F). Polyps of *A. tenuis* retracted in both coal and sediment deposition treatments, however polyps remained retracted for longer in coal exposures (only 2 of the 4 colonies exhibited extended polyps in coal deposition treatments 4 h post deposition). *Porites* rapidly moved their polyps to remove sediments. When smothered by coal *Porites* polyps initially contracted before commencement of removal. Mucus sheets were used by *Porites spp.* to remove coal particles but not sediments.



**Figure 5.6** Acropora tenuis released mucus sheets in response to coal (**A**) and sediment (**B**) deposition that efficiently bound particles and were subsequently shed from the tissue surface. Massive *Porites* initially contracted their polyps in response to coal deposition but then commenced moving their polyps (**C**) and released mucus sheets (not pictured) to remove coal. *Porites* were more efficient at clearing sediment and commenced clearance immediately after deposition (**D**). *Montipora spp.* accumulated both coal (**E**) and sediments (**F**) on flat surfaces and crevices of the tissue surface.
# 5.4.3.4 Physical properties of seawater

The coal suspensions applied in incubations caused substantially greater light attenuation than suspended sediments (Figure 5.7 A). In the very high coal suspensions (1250 mg l<sup>-1</sup>) light was attenuated by 41% at 1 cm depth in coal treatments, relative to controls, while light in the sediment treatment was not attenuated. Between 5 - 15 cm depth (the depth of corals in the incubations), light attenuation ranged between 98 - 100% in coal suspensions and 42 - 64% in sediment suspensions (Figure 5.7 A). Based on these measurements, coral colonies in the coal and sediment incubations were exposed to 0.09 and 75 mol photons m<sup>-2</sup> s<sup>-1</sup>, respectively, while particles were in suspension prior to settlement. Substantially lower quantities of coal, 10 mg coal l<sup>-1</sup>, reduced light levels by 100 mol photons m<sup>-2</sup> s<sup>-1</sup> within a 10 cm depth increment and absolute light attenuation was measured for 100 mg coal l<sup>-1</sup> suspensions at 10 cm depth (Figure 5.7 B).



**Figure 5.7** Comparison of experimental depth-related light attenuation in (**A**) filtered seawater (FSW) and 1250 mg  $\Gamma^1$  suspensions of coal and calcium carbonate sediments, and (**B**) a dose and depth-related light attenuation relationship for coal suspensions. Note the different scales on the x-axis. 'FSW' is equivalent to 0 mg  $\Gamma^1$ .

# 5.4.3.5 Chemical properties of seawater

The maximum magnitude of change in dissolved metal concentrations leached from coal suspensions during deposition, in relation to control seawater, were: Al = 33; As = < 0.40; Co = 0.56; Cu = 0.18; Pb = < 0.01; Mn = 2.2; Mo = 0.2; Ni = 0.44; Zn = 0.7  $\mu$ g l<sup>-1</sup>. The maximum magnitude for sediment leachates, in relation to control seawater were: Al = 0.4, As = 0.3; Co = < 0.01; Cu = 1.53; Pb = < 0.01; Mn = 0.04; Mo = 0.2; Ni = 0.12; Zn = 0.41  $\mu$ g l<sup>-1</sup>. Elevated concentrations of certain trace elements (maximum: Al = 36, Mn = 2.3, Co = 0.58, and Ni = 0.78  $\mu$ g l<sup>-1</sup>) leached from coal suspensions

during the 8 h deposition period during zero water flow, however leachate concentrations were diluted to concentrations similar to the particle free FSW within 16 h after water flow recommenced (Table 5.2). Comparatively, concentrations of Al, Cu and Zn were higher in sediment treatments 16 h post re-commencement of water flow, with a magnitude of change of: Al = 1.38; Cu = 1.88; Zn = 0.68  $\mu$ g l<sup>-1</sup>, respectively, in relation to control seawater (Table 5.2). Low concentrations of PAHs leached from coal suspensions, with total PAHs measured at 0.04 ± 0.01  $\mu$ g l<sup>-1</sup>, mean ± S.E., during the 8 h deposition period with no water flow (Appendix A Table A5.2). Leachate from the sediment used in this experiment was previously analysed (Flores et al., 2012) and no PAHs were detected. With the exception of Co and Cu, the dissolved concentrations of metals and PAHs that were detected were lower than trigger guidelines (where they exist) (ANZECC & ARMCANZ, 2000).

**Table 5.2** Trace element concentrations ( $\mu$ g l<sup>-1</sup>) in experimental treatments. Water samples were taken from particle treatments after the 8 hour deposition period when water flow was off, and again 16 hours post –recommencement of water flow through the tanks. Abbreviations: P.F. = post –recommencement of water flow, < = below detection limit, NA = value not available. Values in bold are above ANZECC & ARMCANZ trigger values.

Treatment	Al	Mn	Со	Ni	Cu	Zn	As	Мо	Cd	Pb
Particle free FSW	3.63 (0.16)	0.12 (0.01)	0.02 (0.00)	0.34 (0.03)	0.47 (0.04)	1.43 (0.39)	1.16 (0.04)	10.93 (0.03)	<	0.07 (0.03)
Coal deposition 8h	36.30 (0.40)	2.29 (0.16)	0.58 (0.04)	0.78 (0.05)	0.65 (0.04)	2.13 (0.16)	1.04 (0.07)	11.13 (0.03)	0.02 (0.00)	0.06 (0.04)
Coal 16 h P.F.	5.09 (0.28)	<	0.02 (0.00)	0.38 (0.02)	0.31 (0.01)	1.83 (0.69)	1.41 (0.04)	11.20 (0.06)	<	0.09 (0.01)
Sediment deposition 8h	4.04 (0.42)	0.16 (0.01)	0.02 (0.00)	0.46 (0.06)	2.00 (0.03)	1.84 (0.45)	1.45 (0.03)	11.10 (0.06)	<	0.05 (0.02)
Sediment 16 h P.F.	5.01 (0.67)	0.10 (0.00)	0.020 (0.00)	0.40 (0.05)	2.35 (1.99)	2.11 (1.38)	1.34 (0.01)	11.00 (0.06)	0.03 (0.00)	0.10 (0.05)
Detection Limit	0.50	0.090	0.010	0.10	0.10	0.50	0.40	1.20	0.020	0.010
ANZECC & ARMCANZ trigger values	NA	NA	0.005	7	0.3	7	NA	NA	0.7	2.2

#### 5.5 Discussion

This chapter demonstrates that the physiological responses of corals to coal particulates are different to their responses to carbonate sediments, but these differences are species specific. In particular, for the chronic deposition exposures, respiration and light calcification were substantially reduced after coal deposition but not sediments, with the exception of *A. tenuis*. Acute exposures to suspended coal resulted in significant reductions in oxygen production and light calcification, which were not observed in corals exposed to suspended sediments. Both chronic and acute exposures to suspended coal and sediments substantially impacted dark calcification; and the effect was most severe in corals exposed to coal suspensions when they had previously been exposed to chronic coal deposition. The likely primary mechanisms underlying the effects of coal on corals are: 1) light attenuation via suspended and deposited particles; 2) reduced gas exchange during smothering; 3) increased (energetically costly) surface clearance activity; and 4) potential chemical effects from increased exposure to trace metal leachate measured during particle deposition.

The symbiotic relationship between the animal host and symbiotic algae within coral tissue generates a dynamic internal carbon cycle that results in metabolic energy production (Al-Horani et al., 2003) and up to 95% of a coral's metabolic requirements are met by photosynthesis (Falkowski et al., 1984; Muscatine et al., 1981). Thus, prolonged periods of light limitation may substantially impair a coral's metabolism because respirations rates are higher in the light compared to in the dark (i.e. ATP production is greater in the light, Al-Horani et al., 2003). Respiration rates were significantly lower in response to chronic coal exposures. The decline in respiration measured was likely a direct function of decreased photosynthetically fixed carbon availability due to light limitation during smothering periods and a decline in the net fixed carbon translocated from algal symbionts (Symbiodinium spp.) to the host, as has been measured in corals at depth that receive less irradiance (McCloskey and Muscatine, 1984). Despite cessation of measured oxygen production after the deposition of both coal and sediments onto Porites in the microsensor analyses, the reduction in respiration rates were only observed in coal treatments. These findings indicate coal exposure elicits more and/or additional stress on corals than sediments due to more severe and prolonged light attenuation, prevention of gas exchange and increased metabolic costs associated with the lower clearance efficiencies observed. Under more extreme sedimentation conditions of 200 mg cm<sup>-2</sup>, four reef-building corals (Favia favus, Favites pentagona, Platygyra daedalea and Gyrosmilia interrupta) exhibited similar severely diminished productivity and respiration (Riegl and Branch, 1995). It was determined that despite the overall respiration dropping, the demand upon remaining photosynthetically produced carbon increased due to increased mucus production, resulting in a shift of the energy balance of the corals (Riegl and Branch, 1995) and similar processes are likely to occur under less severe coal deposition scenarios.

In the acute suspension exposures, reduced respiration was only observed in coal treatments for *Porites spp.*, while *Montipora spp*. exhibited an increase in respiration rates by 228%. This variability in physiological responses might be attributed to the difference in particle clearance efficiencies between the two species. *Porites spp*. exhibited the least efficient clearance of coal and responded to coal deposition by contracting its polyps, thereby experiencing prolonged exposure to light attenuation and anoxic conditions, as was observed using microsensor techniques. In comparison, *Montipora spp*. responded immediately to particle deposition by moving cilia and releasing mucus sheets, thus increasing their respiration rates due to increased energy expenditure (Dallmeyer et al., 1982; Riegl and Branch, 1995). Both species accumulated coal and sediment on flat surfaces and in crevices, contributing to tissue discolouration in those areas. In comparison *A. tenuis* accumulated fewer particles due to its upright branches and exhibited the least amount of tissue discoloration. However, this species suffered high mortality rates in *Chapter 2* when exposed to continuous exposure to coal suspensions for 14 d. It can therefore be speculated that a coal spill scenario resulting in chronic constant exposure to a reef could result in high mortality levels in coral species such as *Porites* and *Montipora*.

Overall, the effects of coal and sediment exposures on calcification rates were similar for chronic and acute exposure scenarios. Coal significantly reduced both light and dark calcification rates, while only dark calcification rates were reduced in sediment treatments (Montipora spp. were the exception). Although only chronically coal exposed corals exhibited significant declines in respiration rates, corals in both chronic deposition treatments would have experienced sustained reductions in primary productivity in the smothered regions as well as increased energy expenditure for particle clearance over the 28 d experiment. These cause-effect pathways can impair calcification because a) calcification is enhanced in the light (Al-Horani et al., 2003; Goreau, 1959; Pearse and Muscatine, 1971) and b) calcification is an energy consuming process (Al-Horani et al., 2003). Reduced calcification has been measured in corals living in naturally turbid environments (Dodge et al., 1974) and those affected by dredging activities (Bak, 1978). Madracis mirabilis and Agaricia agaricites exposed to dredge plumes exhibited acute decreases in calcification rates by 33%, and calcification was suppressed after the period of environmental disturbance, suggesting that recovery from sedimentation can be protracted. My findings of CaCO<sub>3</sub> dissolution for both A. tenuis and Porites colonies previously smothered by coal and sediments incubated in FSW support this notion of longterm calcification suppression post-deposition, at least for a 24 h interval after deposition exposure. Further research is required to examine recovery from coal exposures over longer time periods.

The effects of acute coal suspensions on light calcification were directly related to the attenuation of light, which led to a decrease in oxygen production. Decreased oxygen production has also been observed in *Montastraea annularis* during exposure of up to 525 mg l<sup>-1</sup> of peat for 1 h

(Dallmeyer et al., 1982). A single exposure to peat suspensions resulted in decreased oxygen production the following day in ambient conditions, once again highlighting that recovery from suspended particle exposure can be slow, and that there is a need for testing on multiple species. In the present study, the reduced light calcification in coal vs. sediment suspensions was likely attributed to the stronger and longer attenuation of light due to the dark colour of coal particles and the longer suspension time due to the lower specific gravity of coal (Ahrens and Morrisey, 2005). The extreme attenuation of light by coal could have major implications during a large spill event. Corals were situated at a depth of 25 cm in experimental tanks and absolute light attenuation was measured within 5 cm depth with 100 mg coal l<sup>-1</sup>. Therefore, in a reef environment where corals are likely to be deeper than 0.5 m, far less coal would be required to cause significant light attenuation than the concentrations tested in this chapter. These findings also highlight that even if the particles remain in suspension (i.e., do not directly smother tissues) the corals may still experience metabolic impairment. Indeed, a recent study comparing the chronic effects of light limitation and the physical effects of suspended sediments relevant to dredging concluded that light limitation is a driving mechanism behind coral health deterioration (Bessell-Browne et al., 2017).

In addition to the substantial light attenuation and reduced clearance efficiency, increased concentrations of certain trace metals (Al, Mn, Co, Ni) from coal suspensions may represent an additional stressor in the coal treatments. Relatively low concentrations of elevated metals and PAHs were found in the coal leachate. However, Cu and Co exceeded the 99% species protection guidelines (ANZECC & ARMCANZ, 2000) in each treatment, including the control water. Co was higher in the coal deposition leachate while Cu was higher in the sediment leachate. In addition to the dissolved contaminants, corals are exposed to metals through feeding and attachment of particles to mucus, which can effectively bind trace metals (Esslemont, 2000; Peters et al., 1997). Metal exposure can cause long term impacts to biological processes of corals such as respiration (Howard and Brown, 1984), reproduction and larvae settlement (e.g. Negri and Heyward, 2001; Reichelt-Brushett and Harrison, 2000). Impacts to adult corals include physiological stress and bleaching response (Jones, 1997), and reduced growth (Howard and Brown, 1984). The mechanisms by which PAHs affect corals are less understood and most studies have examined petroleum hydrocarbons (Haapkylä et al., 2007). Hydrocarbons can cause histological anomalies (Peters et al., 1981) and affect coral growth (Guzmán et al., 1994), especially by decreasing calcium deposition (Dodge et al., 1985), suggesting that high PAH levels in some leachates could contribute to reduced calcification rates. Petroleum hydrocarbons can also decrease coral reproduction (e.g. Negri et al., 2016; Negri and Heyward, 2000). Despite the detection of low elevated levels of some metals and PAHs in the seawater associated with coal depositions, further work is needed to determine the bioavailability of metals and PAHs from direct contact by coal particles onto coral tissue to better understand the risk this mechanism poses in addition to smothering and light attenuation.

The information acquired in this chapter provides insights into the effects of multiple stresspathways (deposition, suspension, light limitation) associated with deposited and suspended coal and sediment particles (63 -125 µm) on sessile corals that are commonly found in tropical regions where large quantities of coal are mined, processed, stored and shipped. The mechanism behind high coral tissue mortality previously observed under suspended coal exposure in Chapter 3 (Berry et al., 2016) was shown here to be most likely due to prolonged or frequent reductions in primary productivity from light attenuation (from both suspended and deposited coal particles) and smothering (reduced respiration and anoxia). This study highlights that although stress-pathways of coal and sediments are likely to be similar, particle characteristics (e.g. colour and stickiness) and the alterations to the abiotic environment caused by coal contamination (with regards to light attenuation and possibly trace metal leachate), contributed to the more severe impacts measured in coal exposed corals versus sediment exposed corals. The observation that corals were impacted to a greater extent by coal than by sediments following both acute and chronic coal exposures indicates that changes in coral communities may be induced by large coal spills more rapidly and/or severely than expected for other turbidity generating events of a similar scale, such as dredging activities. These findings highlight the need for thorough monitoring efforts for the quantification of coal contamination in coastal environments adjacent to coral reefs, especially in world heritage areas, such as the Great Barrier Reef.

## 6.1 Overview

Large quantities of coal are loaded and shipped in close proximity to ecologically and economically valuable coastal marine environments such as coral reefs and seagrass meadows. In any situation, when a known contaminant is entering the environment, inputs should be monitored and appropriate environmental impact assessments conducted. Such assessments require experimental testing to determine the concentrations and exposure durations required for a known contaminant to elicit detrimental effects, information that is essential to appropriately identify the threats associated with contaminant inputs and risk management.

Research presented in this thesis significantly advances the current understanding of the potential effects of coal contamination on tropical marine organisms commonly found within the Great Barrier Reef World Heritage Area (GBRWHA), where coal represents the largest export commodity (AMSA, 2014). In this concluding chapter, I synthesize the results presented in the preceding chapters which identify the stress-response pathways and quantify hazards associated with acute and chronic coal contamination to corals, seagrass and fish. I also highlight potential flow-on effects of these measured responses on coral reef benthic communities and the reef ecosystem more broadly and conclude by identifying future research priorities, and recommending management actions where the results of this thesis can be implemented for improved conservation.

# 6.2 Effects of coal on tropical marine organisms

#### 6.2.1 Stress pathways associated with coal contamination

Laboratory experiments conducted in *Chapters 2-5*, revealed that the main direct stressresponse pathways associated with small coal particles (<  $63-125 \mu$ m) are physical interactions with 1) suspended coal particles (*Chapter 2-5*), 2) smothering by deposited coal particles (*Chapter 2-5*), and 3) chemical effects from leaching of inorganic (trace metal) constituents (*Chapter 5* and references cited in *Chapter 1*) (Ahrens and Morrisey, 2005; Hyslop et al., 1997). Indirect stresspathways include the attenuation of light by suspended (*Chapter 3* and 5) and deposited particles (*Chapter 2, 3* and 5), as well as habitat alteration by coal deposition onto benthic substrata (*Chapter 2*). Each of the above mentioned stress-pathways impacted each test organism differently due to variability in traits and life stages (Figure 6.1, Table 6.1), demonstrating that each feature must be taken into consideration when assessing the overall impacts of both chronic coal contamination and/or a large contamination event.



**Figure 6.1** A conceptual model of the effects of coal contamination on tropical marine organisms identified in this thesis. All examined stress pathways and cause–effect pathways, as well as biological and physiological responses measured in *Chapters 2-5* are outlined. Examples of potential broad-scale impacts associated with the measured effects are also provided. The numbers next to the measured effects correspond with the associated stress-pathway (listed above).

**Table 6.1** Results summary of thresholds established in coal exposure experiments (*Chapters* 2, 3, 4). 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, ELHS = early life history stage, CCA = crustose coralline algae.

Organism or life stage	Coal exposure type	Response	Exposure duration (h)	NOEC (mg l <sup>-1</sup> )*	LOEC (mg l <sup>-1</sup> )*	IC <sub>10</sub> (mg l <sup>-1</sup> )	IC <sub>50</sub> (mg l <sup>-1</sup> )	Chapter
Coral (A. tenuis)	Suspension	Fertilisation Figs. 2.1 & 2.6A	2.5	25	50	47	107	2
Gametes	Leachate		2.5	100%	-	-	-	
3 h	Suspension	Survival Fig. 2.2A	1	50	100	-	-	
(2-4 cell)			12	400	800	_	-	
embryo			24	25	50	-	-	
m m			72	25	50	-	-	
a a		Settlement	24	800	-	_	-	
		Fig. 2.2D	72	800	-	-	-	
12 h	Suspension	Survival Fig. 2.2B	1	800	-	-	-	
(Prawn chip)			12	50	100	-	-	
embryo			24	25	50	-	-	
A B			72	25	50	-	-	
668863		Settlement	24	800	-	-	-	
CORRECTOR OF		Fig. 2.2E	72	800	-	-	-	
72 h			1	800	-	-	-	
(tear drop)		Survival	12	800	-	_	-	
Larvae	Suspension	Fig. 2.2C	24	100	200	_	-	-
$\bigcap$			72	800	-	-	-	
		Settlement Fig. 2.2F	24	400	800	_	-	
$\sim$			72	400	800			
			72	400	800	-	-	
Larvae	Leachate	Settlement Fig. 2.6B & 2.4	48	100%	-	-	-	
	Smothered		48	Washed	12.5	-	-	
	CCA		·	CCA	mgem			L
Adult corals	Constant	Survival Fig. 3.3A & A3.2a	14 d	73	202	29	87	3
(A. tenuis)	deposition		28 d	0	38	34	36	
Seagrass	Suspension & deposition	Leaf growth rate Fig. 3.3C & A3.2c	14 d	38	73	42	-	3
(H. uninervis)			28 d	38	73	12	275	
SY.		Shoot density Fig. 3.3D	14 d	0	-	-	-	
TUTTIT			28 d	38	73	-	-	
				1			•	
Fish		Growth rate Fig. 3.3B &	14 d	0	38	11	-	- 3
(A. polyacanthus)			20.1	6	20	0	70	
	Suspension	A3.2b	28 d	0	38	9	/3	
	Juspension	Oxygen consumption Fig. 4.3	5 d	0	38	-	-	4

# 6.2.2.1 Early life history stages of corals (Acropora tenuis)

This thesis demonstrates that the early life history stages ('ELHS') of corals (gametes – precompetent larvae) were affected by acute exposure to coal suspensions. The vulnerability was greatest for fertilisation, with significant reductions in gamete fertilisation at concentrations  $\geq$  50 mg coal l<sup>-1</sup>, followed by significant mortality observed in early embryo development stages (e.g. 2-4 cell stage, prawn chip and early larvae, Figure 6.1, Table 6.1). The sensitivity to suspended coal decreased as development progressed, with negligible effects of the tested exposure scenarios measured in motile larvae (at concentrations < 400 mg l<sup>-1</sup>) or juvenile corals (Table 6.1). The variability between life stages is likely due to the more complex tissue structures present in the later-stage larvae, including their ability to draw upon lipid reserves, as well as the ability to avoid or remove particles via mucus production and cilia beating (larvae), or by cilia and/or tentacles (juveniles) (Harrison and Wallace, 1990; Ricardo et al., 2016a).

Coal deposition onto the reef substratum, including onto crustose coralline algae (CCA) poses the greatest direct threat to coral recruitment, as was evidenced by the reduction in larval settlement at deposited coal levels of 12.5 and 22 mg d/wt cm<sup>-2</sup>. Larvae react to biological films and rely on chemical cues for settlement site selection (Harrington et al., 2004; Heyward and Negri, 1999; Richmond, 1993) and this thin layer of coal particles either (i) changed the substrate texture (through the presence of unconsolidated particles) or (ii) masked the chemical signature of the CCA, reducing settlement success (Babcock and Davies, 1991; Babcock and Smith, 2000; Birrell et al., 2005; Richmond, 1993). Although larval settlement on CCA surfaces that had been temporarily (8 h) covered in coal particles was not different than settlement onto clean CCA, over longer periods coal particles, like sediments (Harrington et al., 2005), may affect CCA health and its ability to induce settlement and recruitment. The effects on coral recruitment due to longer-term deposition of coal onto CCA remain unknown; however, lower survivorship has been reported for early recruits onto sediment covered surfaces (Babcock and Smith, 2000). If a reef is contaminated by coal, larvae may avoid upper coal-affected surfaces and instead settle in less optimal locations such as on the undersides of crevices that receive less light irradiance (Babcock and Davies, 1991). This, in turn, may reduce juvenile growth rates and suppress fitness in the long term.

Considering their relatively high sensitivity to suspended coal particles over short time periods, and the potential for coral ELHS to spend substantial periods of time in the water column (days to months) (reviewed in Jones et al., 2015b), coal suspensions could strongly decrease larval survival and subsequent recruitment. However, the likelihood of a large coal spill coinciding with a mass spawning event is low. A more likely scenario is that the deposition of coal particles on reef substrata

from a large spill would lower the quality, or availability, of substrata (CCA) for settlement and recruitment. Chronic coal inputs from ports also occur regularly (GHD, 2012), and although corals are not typically found in high abundance in the vicinity of ports, the recruitment of other nearshore invertebrates may be sensitive to coal deposition onto the benthos. Previous studies have revealed effects of coal contamination on the early life history stages (ELHS) of nearshore temperate marine vertebrates (Carlson, 1979; Cochran, 1987; Meyer et al., 2013). The relative risks posed by chronic inputs and spills in the tropics can only be ascertained after comprehensive monitoring of coal contamination in the environment in conjunction with modelling of coal dispersal from accidental spill locations and from chronic sources.

## 6.2.2.2 Effects of coal on corals (Acropora tenuis, massive Porites spp. & Montipora spp.)

Corals are sessile benthic organisms that can obtain energy phototrophically and heterotrophically, thus making them vulnerable to increased turbidity, particle deposition and light limitation (Jones et al., 2016). The potential for large coal spills to negatively affect marine biota was exemplified by the 2000 grounding of the *Eurobulker IV* in Italy, smothering sessile flora and fauna with coal within the vicinity of the ship (Alcaro et al., 2002). When similar scenarios were simulated experimentally in this thesis, the coral species investigated (*Acropora tenuis*, massive *Porites spp., Montipora spp.*) were negatively influenced by both the direct and indirect suspended and deposited coal particles (*Chapter 3 and 5*), light attenuation (*Chapter 3 and 5*), and potential chemical effects from coking coal leachate (*Chapter 5*). While it is difficult to distinguish direct cause-effect relationships between these factors my results clearly demonstrate that acute and chronic coal contamination can cause sub-lethal and lethal effects in corals, and that those responses are variable between coral species.

Chronic exposure to both suspended and deposited coal particles caused significant mortality in *A. tenuis* in *Chapter 3* within 14 d (Table 6.1). Follow up work in *Chapter* 5 provided evidence that coral tissue mortality was likely caused by a combination of stress-pathways, including the smothering of tissue by sticky coal particles (see section 5.4), which reduced gas exchange at the coral tissue boundary layer and created anoxic conditions, as has been observed after deposition of organic-rich sediments onto corals (Weber et al., 2012). In addition, smothering stimulated particle clearance efforts (e.g. mucus, tentacle and ciliary movements) which are known to increase metabolic costs and draw upon energy reserves (Aller and Dodge, 1974; Bak, 1978; Dallmeyer et al., 1982; Flores et al., 2012). Adding to the increased energy costs from active removal of particles, exposing corals to suspended and deposited coal would impede heterotrophic feeding and further reduce energy intake of the colony (Stafford-Smith and Ormond, 1992). Finally, suspended and deposited coal particles lowered the light intensity reaching the coral tissue surface, thereby decreasing primary productivity and respiration, which may have contributed to the observed reduction in calcification rates in *Chapter 5* (Bak, 1978). Recent studies have demonstrated that chronic light limitation has detrimental effects on coral health even in the absence of contact abrasion by particles (Bessell-Browne et al., 2017); however, that study assessed carbonate sediments which do not adhere to coral tissues to the same extent as coal particles (*Chapter 5*). The sub-lethal effects reported here could lead to reduced growth and increased vulnerability to additional stressors which could, in turn lower the local abundances of susceptible species. In the long term such effects could lower coral diversity, and potentially lead to a shift in species composition of reef communities (Dodge and Vaisnys, 1977).

# 6.2.3 Effects of coal on seagrass (Halodule uninervis)

Seagrass shoot density and leaf growth represent effective bioindicators for light limited environments (McMahon et al., 2013) since depressed photosynthetic carbon fixation reduces carbon allocation to growth (Alcoverro et al., 2001; Ralph et al., 2007). In the coal exposure simulation investigated in *Chapter 3*, light was attenuated by suspended coal particles (by an average of 44-99% for 4 weeks), but also by particles sticking to seagrass leaves and forming a permanent coating. Although I did not measure seagrass photosynthetic production rates directly, reductions due to coal exposure seem likely as studies on mangroves reported 17-39% declines in photosynthesis in leaves coated with coal dust near a coal terminal in South Africa (Naidoo and Chirkoot, 2004).

The reduced seagrass shoot density and growth rates observed in *Chapter 3* are consistent with effects of light limitation reported in other studies (Chartrand et al., 2016; Collier et al., 2016; Gordon et al., 1994). In *Chapter 3*, seagrass shoot density took longer to respond to coal-induced light limitation than leaf growth rates, supporting that seagrasses suspend growth and use energy reserves to maintain existing blades when under light limitation (e.g. Alcoverro et al., 2001). Other studies have reported that seagrasses can survive under such conditions for one month (e.g. *Halophila ovalis*, (Longstaff et al., 1999) to several months (e.g. *Posidonia sinuosa* in Gordon et al., 1994; *Thalassia testudinum* in Lee and Dunton, 1997; *Halodule pinifolia* in Longstaff and Dennison, 1999) by drawing upon energy reserves stored as starch in roots and rhizomes. Although the experimental coal exposures applied in this study were not high enough to completely smother older/taller seagrass leaves; coal deposited in the pot base could have smothered new shoots, potentially contributing to the depression of shoot density in the higher coal concentrations.

In addition to the effects of smothering, suspended and deposited coal may have caused abrasive damage to seagrass blades. Although not directly measured in this thesis, abrasive damage due to coal exposure has been observed in other aquatic plants (moss and macroalgae) (Hyslop and Davies, 1998; Lewis, 1973). Previous research investigating the effects of sedimentation on seagrass

plants show that sedimentation effects are dependent on various factors such as the depth and duration of burial, and life history of the species, with critical thresholds ranging from 2-13 cm yr<sup>-1</sup> (Erftemeijer and Robin Lewis III, 2006; Marba and Duarte, 1994; Vermaat, 1997). No previous studies have tested the effects of coal exposure on tropical seagrasses and extrapolating the results of this thesis to apply to seagrasses more generally is difficult as experiments were focused on a single seagrass species. Nevertheless, in a four week shading experiment, *Z. muelleri* and *H. ovalis* were found to have a greater morphological sensitivity to short-term light deprivation than *C. serrulata* and *H. uninervis* (Collier et al., 2016), suggesting that the declines in shoot density measured in this thesis may be conservative in terms of the potential responses of other common tropical seagrass species to coal exposures.

#### 6.2.4 Effects of coal on juvenile reef fish (Acanthochromis polyacanthus)

Fish exposed to coal were impacted by numerous stress-pathways (Chapters 3 and 4) including direct physical contact by suspended coal particles, which lead to ingestion and gill smothering, as well as indirect effects of particle feeding and behaviour due to light attenuation. All but 2 of 90 fish survived the coal treatments; however growth rates in all coal treatments were inhibited by suspended coal particles (Chapter 3) and this suppression of growth was not dose-dependent. There are numerous factors that potentially contributed to the consistent reductions in fish growth rates across the tested coal concentrations. Firstly, visual impairment in fish can lead to lower foraging success and feeding efficiencies (Gardner, 1981; Johnston and Wildish, 1982; Sweka and Hartman, 2001; Wenger et al., 2012) and the light attenuation and black particles in suspension may have led to A. polyacanthus ingesting coal rather than, or in addition to, the food supplied. The ingested coal (identified in *Chapter 3*) provided no nutritional value and likely caused starvation and debilitation (Boehlert and Morgan, 1985). Second, the significant increases in standard metabolic rates measured in fish exposed to 38 and 73 mg coal  $l^{-1}$  in *Chapter 4* provides evidence that coal contamination elicited stress in the coal exposed fish even at relatively low concentrations. Stress adaptation involves the reallocation of metabolic energy away from investment activities (including growth and reproduction) toward activities to restore homeostasis (i.e. respiration and tissue repair) (Wendelaar Bonga, 1997).

Oxygen requirements for metabolism place critical limits on fish physiology, behaviour and growth (Hughes and Morgan, 1973; Hughes, 1973). The energetically costly gill remodelling (Nilsson, 2007; Nilsson et al., 2012) measured in *Chapter 4* is consistent with remodelling observed in fish exposed to oxygen limited environments (i.e. hypoxia) (Sollid et al., 2003; Sollid et al., 2005), suggesting that the adherence of coal particles to the gills limited gas exchange between the water and respiratory surface area. In this thesis, similar effects were observed even though dissolved

oxygen levels in the treatment tanks were normoxic (see Table 3.1, *Chapter 3*). The substantial reduction in the epithelium thickness and oxygen diffusion distance would have increased the functional respiratory surface area and the oxygen carrying capacity of the gills, thus increasing respiratory regulation (Heath, 1995). That growth was decreased in coal-treated fish despite gill remodelling (*Chapter 3 and 4*) suggests that changing gill structure may have promoted oxygen exchange sufficiently to ensure fish survival but was not sufficient to support normal growth.

Despite the significant sub-lethal effects of coal on fish measured in *Chapters 3 and 4*, only 2 of 90 juvenile damsel fish died after 28 d exposure to a mean coal concentration of 275 mg coal I<sup>-1</sup> (*Chapter 3*, Berry et al., 2016), providing further support that large quantities of suspended coal are required to elicit lethal effects on fishes, as has been observed in numerous laboratory and field investigations (Carlson, 1979; Gerhart et al., 1981; Pearce and McBride, 1977). However, the coal-exposed fish were clearly in poor body condition (e.g. increased metabolic rates and coal ingestion) and the observed growth suppression may prolong development as has been observed in fish larvae exposed to suspended sediments (Wenger et al., 2014). Certain post-settlement processes are size dependent for reef fishes (Perez and Munch, 2010), suggesting that lower growth rates from coal exposure may have later implications on individual survivorship (Wenger et al., 2012) and fecundity (Hislop, 1988; Perez and Munch, 2010).

#### 6.3 Chemical effects of coal

In addition to the physical impacts on marine organisms discussed above, coal has the potential to leach inorganic and organic constituents, such as trace metals and polycyclic aromatic hydrocarbons (PAHs), both of which can harm tropical marine organisms above threshold levels (van Dam et al., 2011). PAHs that are characteristic of coal, rather than petroleum biomarkers, have been detected in sediment samples up to 40 nautical miles off the coast of Mackay in the GBR in decreasing concentrations from the coast to offshore (Burns and Brinkman, 2011), indicating widespread transport of coal particles and potentially chronic low-level exposure to coral reef organisms. Metal concentrations in coal stockpile run-off can be high enough to threaten groundwater quality, and storm water run-off from coal piles have been shown to contain elevated levels of metals such as Al, Cu, Cd, Pb, Fe and Zn (Cook and Fritz, 2002; Curran et al., 2000). An investigation into coal leaching under a ship grounding scenario using coal mined in Queensland Australia, found copper concentrations could potentially exceed Queensland water quality guidelines (Lucas and Planner, 2012).

The effects of two coal types were investigated in this thesis, thermal coal (*Chapters 2-4*) and coking (or metallurgical) coal (*Chapter 5*). Elevated metal and PAH concentrations in thermal coal leachate were very low in water samples analysed from *Chapters 2-4*. Although some elevated trace

elements leached from the thermal coal assays in *Chapters 2* and *3*, there were no effects of leachate on coral larvae (*Chapter 2*) and the concentrations were below those reported to affect the sensitive fertilisation and metamorphosis processes in coral (Heyward, 1988; Negri and Heyward, 2001; Reichelt-Brushett and Hudspith, 2016; Reichelt-Brushett and Harrison, 1999, 2004, 2005). Our experimental results (low toxicity of leachate), coupled with water quality analyses (low concentrations of metals and PAHs) suggest that the coal used in *Chapters 2-4* did not pose a toxic threat to the tested organisms. These results are consistent with previous studies that showed limited leaching of toxic levels of trace metals (Cabon et al., 2007; Lucas and Planner, 2012) or PAHs (Ahrens and Morrisey, 2005; Bender et al., 1987; Jaffrennou et al., 2007), suggesting that the measured adverse effects in *Chapters 2-4* were primarily due to physical mechanisms.

Elevated concentrations of the trace metals Al, Mn, Co, and Ni were observed to leach from the coking coal used in *Chapter 5*. The highest concentrations were detected during the 8 h deposition periods when water flow was temporarily ceased in experimental tanks to allow particle settlement. Little is known of the toxicity of these metals to adult corals; however Al, Co and Ni toxicity has been assessed on coral fertilisation and/or settlement. Both Al and Ni were only toxic to coral larvae and gametes at concentrations over 100-fold greater than the concentrations detected here, respectively (Negri et al., 2011b; Reichelt-Brushett and Hudspith, 2016), while up to 2500  $\mu$ g l<sup>-1</sup> of Co did not inhibit coral fertilisation (Reichelt-Brushett and Hudspith, 2016). PAH concentrations were also only detected at very low concentrations in coking coal leachate and unlikely to have contributed to observed impacts on coral.

Since the experimental design in Chapter 5 did not specifically distinguish between physical versus chemical stress-pathways it remains unclear whether the leachate or contaminated coal particles impacted coral health. Metal accumulation may be a useful marker for coal exposure but was not tested in this thesis. Incorporation of metals into corals can occur via a variety of mechanisms including uptake of dissolved metals into the tissue and feeding on particulate matter (reviewed in Peters et al., 1997). Metals can transfer into the skeleton from the tissue or through direct adsorption from seawater or particulates (Howard and Brown, 1984). Cellular components such as the symbiotic algae (Symbiodinium spp.) accumulate certain metals (Al, Fe, As, Mn, Ni, Cu, Zn, Cd, Pb) at higher concentrations than host tissue (Esslemont, 2000; Harland and Nganro, 1990; Reichelt-Brushett and McOrist, 2003) and processes such as spawning and bleaching, or loss of Symbiodinium spp. can act as depuration mechanisms for trace metals and other contaminants (Hardefeldt and Reichelt-Brushett, 2015). The inorganic and organic minerals present in each coal type affect the release of trace metals and PAHs (Ward, 2002) making it difficult to generalise and predict the potential toxicity of coal on marine organisms (Chapter 1, Table 1.2, reviewed extensively in Ahrens and Morrisey, 2005). Furthermore, there is substantial variability in leaching levels reported among and between coal types along with inconsistent reports on bioavailability and subsequent effects on a range of species (Ahrens and Morrisey, 2005). Further research is warranted to investigate leaching and the potential toxicity of PAHs and metals from a range of coal types and under ecologically relevant experimental scenarios.

# 6.4 Comparisons between coal and sediment

The effects of particulates on marine organisms depend on particle characteristics such as size, angularity, chemical composition (Lake and Hinch, 1999), the microbial community present on the particles, and organic matter content (Weber et al., 2012). While the broad physical stress-pathways associated with coal and sediments in the water column may be similar, this thesis provides evidence that the effects of coal are distinct from other forms of particulates in several ways. Firstly, substantially less coal is required to cause absolute attenuation of light than carbonate (or other types of) sediments that are commonly suspended in coastal coral reef environments (Chapter 5, Bessell-Browne et al., 2017; Flores et al., 2012; Jones et al., 2015a). Therefore autotrophic organisms that rely on sunlight for energy production could be more severely impacted by lower concentrations of coal suspensions. Secondly, a re-occurring observation from each experimental chapter was the stickiness of the coal particles and the immediate (and seemingly irreversible) adherence of particles to organisms and tank surfaces (Figure 6.2). The inorganic fraction of coal affects its stickiness (Ward, 2002) and this strong adherence of coal particles to tissues would reduce gas exchange between water and tissues as well as require energy demanding clearance processes to clear coal from tissues and gills. In Chapter 3, coral colony mortality was first observed after two weeks of exposure to 73 mg coal  $I^{-1}$  (38 mg cm<sup>-2</sup> d<sup>-1</sup>). In contrast, exposure of the branching coral Acropora millepora to fine grained carbonate sediment resulted in (very minor) coral colony mortality only after 12 weeks exposure to 100 mg l<sup>-1</sup> TSS (83 mg cm<sup>-2</sup> day<sup>-1</sup>) (Flores et al., 2012). Furthermore, the changes in gill morphology of fish measured in Chapter 4 appeared to be adaptations associated with increased resistance to gas transfer due to the adherence of coal particles onto the gill surfaces (Hughes, 1976). While studies on the effects of suspended solids on fish generally report gill changes associated with damage and/or gill protection, such as epithelial lifting, increased mucus production, and decreased oxygen diffusion distance (Au et al., 2004; Cumming and Herbert, 2016; Hess et al., 2015; Wong et al., 2013). Another difference between coal and sediments was the nature and concentrations of potentially toxic leachate (see 6.3 above). This thesis suggests that suspended and deposited coal may be more detrimental for marine organisms than other forms of sediments. Nevertheless, additional studies that directly compare the effects of a wider range of sediment types are needed.



Figure 6.2 Coal adhered to coral (A), seagrass (B) and fish tissue (C) (Chapters 3, 4, 5).

# 6.5 Potential flow-on effects of coal impacts on tropical marine ecosystems

In this thesis I examined the effects of coal exposure on three taxa commonly found in tropical marine environments, including fish, reef-building corals and seagrass. Both reef-building corals and seagrass provide essential nursery grounds and habitat for invertebrates and vertebrates, such as fish (Nagelkerken et al., 2000; Orth et al., 2006). During a coal spill event and/or chronic spillage, however, organisms not directly impacted may still be indirectly affected, with potential flow-on effects at a community or ecosystem level. In addition, other environmental variables, such as temperature (which may cause thermal stress in corals and subsequent bleaching) and water flow (which affects particle dispersal and tissue abrasion) are likely to influence the severity of effects of coal contamination.

#### 6.5.1 Likelihood of exposures

The risks posed by coal to tropical marine communities and ecosystems depend on both the level and duration of exposure, and the sensitivity of key organisms. This thesis has documented the sensitivity of corals, seagrass and fish to suspended and deposited coal particles but, to date, environmental monitoring of coal presence in the marine environment has been inadequate to effectively quantify the current level of exposure *in situ*.

Coal enters the environment through chronic inputs and major spillage events such as coal ship grounding incidents (Ahrens and Morrisey, 2005; Xuan and Robins, 1994). Coal contamination can be from unburnt coal, but can also comprise other forms of colliery waste such as coal fines and fly ash. In general, the quantities of chronic inputs are lower than major spill events but they can be substantial (Sydor and Storts, 1980) and occur at greater frequencies (i.e., daily, whenever a boat is loaded or stockpiles are disturbed) (GHD, 2012). Major spillage events can potentially result in contamination by 160,000 Mt, which is the maximum coal ship cargo size involved in a shipping incident to date (*MV New Mykonos*). Despite the risk of an acute shipping incident being categorized as "unlikely to impossible" in the 2014 Great Barrier Reef Outlook Report (GBRMPA, 2014), exports continue to increase from the GBR and; therefore, there remains a risk that an incident may occur. Indeed, fifty-four major shipping incidents were reported in the GBR region between 1987 and 2009 (GBRMPA, 2009). Additionally the grounding of a coal-carrying ship on a reef in the southern GBR occurred in 2010 (GBRMPA, 2011). The low specific gravity of small coal particles in comparison to sediments means that dispersal of a fine coal plume and subsequent resuspension may increase the zones of impact.

# 6.5.2 Chronic impacts of colliery waste and ecosystem recovery

While little is known regarding the long-term effects of chronic coal inputs into the GBR coastline, this subject has been studied extensively in north-east England (reviewed in Ahrens and Morrisey, 2005). Large quantities of colliery waste (over 2x10<sup>6</sup> tonnes yr<sup>-1</sup> on occasion) were dumped into the coastal environment in the U.K. for almost a century (Eagle et al., 1979; Hyslop et al., 1997). Field investigations revealed reduced abundance and biodiversity in coastal ecosystems where colliery waste was previously disposed (Barnes and Frid, 1999; Hyslop et al., 1997; Johnson and Frid, 1995; Shelton, 1973). Such impacts included reduced macro-invertebrate and macro-algal diversity (Hyslop et al., 1997), and infilling of crab and lobster habitats (Shelton, 1973). In certain regions coal was prevalent within sediments and only low numbers of mobile polychaetes, amphipods, and the occasional brittle star were found (Shelton, 1973). The results of environmental monitoring of long-term marine coal-disposal sites in the UK highlight the importance of establishing appropriate regulatory guidelines and monitoring schemes to conserve coastal environments at risk from chronic coal contamination.

# 6.5.3 Food safety and food security

Direct impacts of coal contamination include increased turbidity, smothering of the seabed and water contamination, as discussed above. However, such effects can result in secondary impacts such as changes to habitats and ecosystems, lowered productivity, altered food chains, and decreased fisheries yields due to fish mortality and/or biological contamination (Johnson and Frid, 1995). The results described in this thesis, including the immediate effects of smothering of seagrass and the ingestion of coal by fish and coral larvae (Figure 6.3) could have implications that reverberate up the food chain. Water flow did not facilitate coal removal from seagrass leaves in *Chapter 3*, nor was coal dust that had settled onto mangrove leaves near a South African port removed by wind, rain or physical washing (Naidoo and Chirkoot, 2004). Collectively, these results indicate that flora smothered by coal may stay smothered for an extended period of time. Seagrasses in the GBR support fisheries productivity and are a food source for threatened dugongs and green turtles

(Schaffelke et al., 2005) and any reduction in seagrasses may impact these populations. In addition, the adherence of coal particles to seagrass blades may result in the uptake of contaminants from the coal, a potential source of metal and PAH contamination; however, it is currently unclear whether ingestion of coal-smothered seagrass would be detrimental for dugongs and turtles. Evidence of bioaccumulation exists for temperate invertebrates; bivalves inhabiting a coal contaminated site in Alaska contained an array of hydrocarbons that were characteristic of the surrounding coal contaminated sediment (Shaw and Wiggs, 1980).

Ultimately, coal contamination in the marine environment could also result in contamination of human food sources, which could have implications for food safety and food security. Similar to the discolouration of fish skin I observed in Chapters 3 and 4, fishers in Canada have reported discoloured, darker crabs near the Roberts Bank Coal Terminal in British Columbia, which were difficult to market (Johnson and Bustin, 2006). Subsistence fisheries in the vicinity of busy coalshipping lanes are critical for Aboriginal communities in northern Australia (Greiner et al., 2006) and Indonesia. Indeed, colliery waste contamination is reportedly impacting local fisheries in the central Bengkulu district of Indonesia (Ambarini and Septaria, 2014). Certain fish stocks are transient and comanaged between Australia, Indonesia and Papua New Guinea (FAO, 2002). It is therefore possible that a major coal incident in one region could impact subsistence fisheries in another country; however, this risk needs further investigation. In addition, socio-economic conflicts related to natural resource use can develop due to losses of livelihood (e.g. fishing industry) or sustenance (Ellis, 1989). Over 80% of the cargo shipped through the GBR is coal (AMSA, 2014) and impacts associated with a collier incident in this region could have detrimental implications for important food sources and should be incorporated into risk assessments. Indeed, all levels of impact should be taken into consideration when designing and implementing regulations for coal transportation and waste/spillage management.



**Figure 6.3** Coal-organism interactions observed in *Chapters 2, 3* and *4*. Coal particles adhered to seagrass leaves (**A**) and coral tissues (not pictured), while fish (not pictured) and coral larvae (**B**) ingested coal particles. Coal particles were observed inside coral tissue post metamorphosis (**C**). It is possible that ingestion of coal contaminated organisms could have implications that reverberate up the food chain and further investigations are warranted.

#### 6.5.4 Turbidity

Increased turbidity associated with a coal spill could influence additional aspects of fish behaviour, not investigated in this thesis such as predator-prey interactions; however the magnitude of this influence would be dependent on the concentration of suspended coal and level of light attenuation, and types of predators and prey (Nurminen et al., 2010). Short-term reductions in water clarity (i.e. turbidity) can be advantageous for fish when hiding from predators or when suspended particles increase visual contrast of prey (Boehlert and Morgan, 1985). However, increased turbidity has also been found to decrease anti-predatory behaviour, making prey easier to target (e.g. in pike larvae (*Esox lucius*) (Lehtiniemi et al., 2005). Long-term exposure to increased turbidity levels could have major implications on individual performance and demography as slow growing juvenile fish can be at greater risk from increased competition and predation, and will also require increased time to reach sexual maturity (Wenger et al., 2012).

# 6.6 Threshold levels and management implications

A wide range of concentrations and exposure durations were tested in this thesis in order to identify a coal exposure threshold for impacts on several representative tropical marine species of high ecological importance (summarised in Table 6.1). Lowest observed effect concentrations (LOEC) are also reported but these values do not necessarily represent the lowest stress thresholds - rather they represent the lowest concentration tested in my thesis that elicited a statistically significant response. Where possible the threshold values ( $EC_{10}$  and  $EC_{50}$ ) were derived from non-

linear regressions, and these data are preferred for application in environmental impact and risk assessments (Warne, 2002) and should be applied to coal shipments and spillage. An additional factor for consideration is that the threshold values were established from controlled laboratory experiments in filtered seawater. However, coastal seawater in the tropics may have high concentrations of nutrients and a large biological load that can affect the behavior of coal and modify its effects. For example, biologically-mediated flocculation enhances the stickiness of floc aggregates and the deposition rates of sediments (Bainbridge et al., 2012). Such flocculation affects microbial communities and nutrients bound to particulates (Bainbridge et al., 2012), which can greatly influence the severity of impacts on organisms once deposited, such as increased mortality in juvenile corals (Fabricius et al., 2003; Fabricius and Wolanski, 2000) and coral tissue necrosis (Weber et al., 2012). The impacts of coal particles on marine biota may be similarly enhanced in the coastal environment.

Additionally, many chronic inputs of coal take place in coastal environments and the inshore coral reefs of the GBR have higher background turbidity levels than offshore reefs due to landbased run-off, tidal forces (Larcombe et al., 2001; Wolanski et al., 2008; Wolanski and Spagnol, 2000) and dredging (Ports Australia, 2014). Coral communities in near shore environments of the GBR are likely to be better adapted to function and survive in turbid conditions (Anthony and Larcombe, 2000; Anthony, 2000), however the combined effects of coal and inshore sediments is unknown. The greater tolerance of inshore *Montipora spp*. (in terms of physiological performance) to suspended and deposited coal and sediments (in comparison to the offshore species) described in Chapter 4 indicates that different thresholds and guidelines should be developed that take into account local conditions and community tolerance. Finally, the thresholds established in this thesis do not take cumulative pressures into account. Simultaneous stressors such as elevated sea surface temperatures can impact the sensitivity of marine organisms (Noyes et al., 2009); especially corals which live close to their thermal tolerance limits (Negri et al., 2011a; Negri and Hoogenboom, 2011). Management options that consider cumulative impacts include temporarily reducing turbidity guidelines during coral bleaching events, as was applied at Magnetic Island in Queensland (Chin & Marshall 2003). Targeted management and reductions of additional anthropogenic stressors, such as contaminant inputs (including coal), will contribute to mitigation of natural and/or cumulative stress on marine organisms including vulnerable coral and seagrass habitats (Ban et al., 2014; Grech et al., 2016).

#### 6.7 Future directions

It is currently problematic to compare the harmful thresholds identified in this thesis with levels of coal contamination in the environment, because there are no data available on the concentrations of suspended coal particles and deposition levels associated with large spills or with chronic inputs from ports and trans-ship loading. While thresholds identified in this thesis address both concentration and duration components, more information is required on the frequency and scale of chronic coal spillage into the coastal marine environment before risk to the environment can be adequately assessed. Information on the locations and magnitudes of coal sources should be combined with hydrodynamic modelling to determine the fate and dispersal trajectories of coal in marine systems. The dispersal of coal particles is dependent on factors such as particle size, the density of the coal particles, and hydrodynamic drivers such as currents (Jaffrennou et al., 2007; Johnson and Bustin, 2006). In comparison to sand particles, coal will also be transported over longer distances due to its low specific gravity (Ahrens and Morrisey 2005).

The SLIM oceanographic model predicted that a coal plume emanating from the large Abbot Point coal terminal in Queensland would reach the coral reefs ~200 km south within three months under the influence of wind, tides and the East Australian Current (Andutta and Wolanski, 2012). However, this modelling scenario only considered surface particles and further modelling scenarios are necessary to account for suspended particles, deposition and resuspension. Such analyses could be readily conducted from known point sources such as coal ports; however, accurately modelling coal dispersal from large collier spills is far more complex. The frequency of coal release and the quantity of coal released during a large collier incident is highly uncertain, so efforts instead could be made to model a few broadly representative cases studies, and to monitor coal in the environment to cross-validate model outputs. For example, spills from trans-shipping of coal occur regularly in Colombia where this method of vessel loading is common practice (Sanchez, 2014). Another useful scenario to address is the planned dumping of coal at sea as part of a ship reclamation process such as occurred during reclamation of the MV Smart (DOEARSA, 2013). In such an event, hydrodynamic, sediment transport and/or oil spill models could be used to predict particle dispersal and environments most at risk from contamination. Environmental monitoring should be used to assess the accuracy of dispersal model predictions in the first instance, and where proven to be accurate, the models could then be used to prioritise locations for clean-up efforts and long-term environmental impact monitoring.

Currently the only data on amounts and details of coal released into the environment after a spill are occasional reports of total tonnage released. Therefore the experimental exposures applied here (*Chapters 2, 3* and 4) were designed to cover a very broad concentration range and a

moderately long duration to ensure the thresholds of harm could be identified. The highest concentration (275 mg l<sup>-1</sup>) of coal investigated in *Chapter 3* was low compared with previous studies investigating spill scenarios in temperate regions that have applied concentrations ranging from 500 mg l<sup>-1</sup> – 25,000 mg l<sup>-1</sup> (Carlson, 1979; Gerhart et al., 1981; Lewis, 1973; Pearce and McBride, 1977). In some respects the experimental design in *Chapter 3* was conservative as it only considered the potential effects of fine coal particles in the absence of other likely co-stressors (cumulative stressors discussed above) and without the additional potential impacts of larger particles that are likely to be released during spills. These larger coal particles are far more likely to settle rapidly and be swept along the seafloor (Jaffrennou et al., 2007; Johnson and Bustin, 2006), posing increased risks of physical damage. More research is required in order to assess the risk of larger coal particles, as well as the combined effects of various coal particles sizes, and a greater range of exposure frequencies.

The three coral species used in this study exhibited different tolerances to coal, reflecting the variable tolerance of coral species to sediments due to differences in morphology, polyp size and feeding behaviour (Stafford-Smith, 1993; Stafford-Smith and Ormond, 1992). While three species of coral, and single species of seagrass and fish, were examined in this thesis; clearly further work is required to assess the applicability of the identified thresholds to other species and taxa. Additionally, contaminant uptake into organism tissues, either from solubilised metals or PAHs in the water, or via direct contact with coal particles post-deposition was beyond the scope of this thesis and further research is warranted to verify that physical properties of coal play a greater role than associated chemical contaminants in impacting corals, fish and seagrass. The potential impacts of contamination. Natural biotic (e.g. predation and competition) and abiotic factors (e.g. temperature and salinity) influence species productivity and development of ecosystems, including post-settlement survivorship and recruitment patterns (Chadwick and Morrow, 2011; Humanes and Bastidas, 2015; Nozawa and Harrison, 2007; Vermeij, 2006). Any additional stressors, such as coal, could be detrimental to recruitment and population maintenance.

### 6.8 Concluding remarks

This thesis highlights the potential hazards of acute and chronic coal contamination and shows that direct and indirect physical effects of exposure to coal fines can significantly affect the condition and survival of several life stages of corals, growth rates and survival of seagrass, and growth rates and condition in juvenile fish. This information is critical considering recent large coal mine approvals in Queensland, one of which is forecast to become one of the largest coal mines in the world and contribute considerably to increased shipping in the GBR region (Queensland Government, 2017). The number of coal ships that transit within the GBR is forecast to increase by 150% (GBRMPA, 2013)

b) and account for 65% of all vessel traffic through the GBR by 2032 (PGM, 2012). Important knowledge acquired from this research includes the identification of threshold levels and exposure durations required to elicit harm in the tested organisms, information that can now be considered during the risk assessment phases of new coal port developments, expanding ports, and for the management of existing and future coal transport operations in Australia or anywhere coal is shipped in bulk. Inclusion of outputs from this thesis could be taken up in, for example, future Great Barrier Reef Strategic Assessments (GBRMPA, 2013 b) and Environmental Impact Assessments (EIAs) examining the potential impacts of coal dust on the marine environment from ports situated within the GBRWHA (such as Abbot Point, Hay Point and the Port of Gladstone) and EIAs for coal shipments through the GBRWHA (PGM, 2012).

There are still major knowledge gaps pertaining to the quantities, frequency and sites contaminated by chronic coal inputs from coastal ports. The findings of this thesis highlight the need for widespread and effective monitoring of chronic inputs, and increased collaboration between port authorities, management agencies, ecologists and hydrodynamic modellers to understand the fate of coal contamination in the environment. Results of this thesis also support further examination of the physical and chemical effects of low-level chronic coal deposition onto species, such as seagrasses, in close proximity to ports and potential effects on coastal recreational fisheries.

Coal contamination is a global issue and coastal environments are impacted wherever coal is loaded or offloaded (Ahrens and Morrisey 2005). While my thesis focused on the GBR in Australia, through which an estimated 218 Mt of coal was shipped in 2015, coal is also loaded and transported in bulk in other locations such as Indonesia and Colombia which exported 368 Mt and 82 Mt in 2015, respectively (International Energy Agency, 2016). An additional application for this research is to improve risk assessments for trans-ship transfer methods, which are used heavily in Indonesia and Colombia and are considered to pose a high risk to the environment due to barge sinking incidents in rough weather conditions (Sanchez, 2014). Despite coal being one of the oldest and most widespread forms of marine and estuarine contamination, and regardless of knowledge of long-lasting environmental impacts of chronic coal contamination (Johnson and Frid, 1995), environmental regulations are generally considered poor or non-existent and environmental risk assessments inadequate in many countries (e.g. PGM, 2012). My research will provide marine park managers and regulators, as well as industry, with scientifically rigorous information to improve impact assessments and risk modelling, as well as prioritize aspects of major coal spill mitigation and clean-up efforts. By addressing critical knowledge gaps that impeded competent assessment of the risks that coal exportation poses to coral reef and coastal ecosystems, this thesis contributes important information that can help improve the sustainable management of coal shipping in areas of high biodiversity and conservation value.

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# Appendix A: Supplementary Figures and Tables



**Figure A2.1** Concentration-response relationship. Fertilisation success (%) at high (200 mg coal  $I^{-1}$ , triangle), low (50 mg coal  $I^{-1}$ , square) and control (0 mg coal  $I^{-1}$ , circle) suspended coal concentrations with increasing sperm concentrations. Under suspended coal treatments, fertilisation curves shifted to the right for both low and high coal concentrations and drastic differences in fertilisation of coal exposed gametes was observed across sperm concentrations; while conversely, a treatment will become more obvious at a lower sperm concentration (Dinnel et al., 1987). It is therefore important to choose appropriate sperm concentrations (Ricardo et al., 2015). The saturating-sperm concentration in our experiment was observed at  $10^6$  cells ml<sup>-1</sup> for the high coal concentration and closer to  $10^5$  for the low coal concentration. In order to avoid masking effects in the low coal treatments I selected a sperm concentration of  $2x10^4$  for application in subsequent fertilisation experiments.

**Table A2.1** Polycyclic aromatic hydrocarbon (PAH) leachate analysis ( $\mu g l^{-1}$ ) from the suspended coal (800 mg  $l^{-1}$ ) and leachate (10,000 mg  $l^{-1}$ ) experiments. Numbers 1-3 represent replicates.

РАН	Reporting Limit	Seawater	800 - 1	800 - 2	800 - 3	10,000 - 1	10,000 - 2	10,000 - 3
Naphthalene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthylene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluorene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Phenanthrene	0.01	0.01	0.06	<0.010	<0.010	0.03	0.01	<0.010
Anthracene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluoranthene	0.01	<0.010	0.04	0.02	0.02	0.02	0.01	0.01
Pyrene	0.01	<0.010	0.01	0.01	0.01	0.01	0.01	<0.010
Benz(a)anthracene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Chrysene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(b)fluoranthene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(k)fluoranthene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(a)pyrene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Indeno(1,2,3-cd)pyrene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Dibenzo(a,h)anthracene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(g,h,i)perylene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
1-methylnaphthalene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
2-Methylnaphthalene	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
C2-alkylnaphthalenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C3-alkylnaphthalenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C4-alkylnaphthalenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Methylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C2-alkylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C3-alkylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C4-alkylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Dibenzothiophene	0.01	<0.010	0.04	<0.010	<0.010	<0.010	<0.010	<0.010
Methyldibenzothiophene	0.05	<0.050	0.05	<0.050	<0.050	<0.050	<0.050	<0.050
C2-alkyldibenzothiophenes	0.05	<0.050	0.15	<0.050	<0.050	<0.050	<0.050	<0.050
C3-alkyldibenzothiophenes	0.05	<0.050	0.26	<0.050	<0.050	<0.050	<0.050	<0.050
Methylpyrenes/fluoranthene	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C2-alkylpyrenes/fluoranthene	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C3-alkylpyrenes/fluoranthene	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Methylchrysenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C2-alkylchrysenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Methylbenzopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C2-alkylbenzopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Methylindenopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
C2-alkylindenopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Total PAHs		0.01	0.61	0.03	0.03	0.06	0.03	0.01



**Figure A3.1** (related to Table 3.1). Levels of suspended and deposited coal measured in experimental coal treatments. Mean (± S.E.) total suspended coal (TSC, **a**) and coal deposition using sediment vials (**b**) and pods (**c**) in all treatments over the experiment duration. Variation of TSC within tanks ranged from 33 - 120% (based on coefficient of variation) and was approximately equivalent to the variation in mean values among tanks within treatments (40 - 99%). TSC values in the coal treatments peaked between 4 - 14 days, after which concentrations stabilised. In general, TSC values gradually declined (by an average of 28 - 78%) between 14 d and 28 d of the experiment due to increased adherence of coal onto sides of aquaria and organisms, as well as flocculation and settlement.



**Figure A3.2** Estimates of lethal and sub-lethal coal concentrations to *Acropora tenuis*, *Acanthochromis polyacanthus* and *Halodule uninervis*. Mean ( $\pm$  S.E.) concentration-response relationship of coral tissue mortality (**a**) with coal exposure and estimates of growth inhibition in fish (**b**) and seagrass (**c**) at 14 d (closed circles) and 28 d (open circles). Lethal concentration (LC<sub>10</sub> and LC<sub>50</sub>) values for corals (**a**) and concentrations resulting in growth inhibition of 10% and 50% of the tested population (IC<sub>10</sub> and IC<sub>50</sub>) for fish (**b**) and seagrass (**c**) were estimated using linear interpolation.

**Table A3.1** Statistical outputs of organism response variables. Abbreviations: df = degrees of freedom, SS = sum of squares, MS = mean squares, Co = colony, Fi = fish, Po = pot, Ta = tank, Tr = treatment, Ti = time (14 d and 28d), () = nested factors, x = interaction, P = *P*-value, perm = Permanova, Perms = permutations, CV = coefficient of variation, \* = significant *P*-value.

Variable	Source	df	SS	MS	Pseudo-F	P(perm)	Unique	% CV
Corol	T.	4	20.2	7.6	42.6	0.0001*		61.0
Corai		4	30.3	7.0	43.0	0.0001	9464	01.8
mortality	11	1	3.5	3.5	20.5	0.0009*	9835	11.2
	Ta(Tr)	10	1.7	0.2	1.4	0.212	9949	1.3
	Tr x Ti	4	2.0	0.5	3.0	0.0738	9952	5.7
	Co(Ta(Tr))	30	3.7	0.1	1.4	0.2008	9926	2.4
	Ti x Ta(Tr)	10	1.7	0.2	1.9	0.086	9944	4.1
Fish growth	Tr	3	0.2	0.07	21.7	0.01*	9959	23.4
	Ti	1	0.1	0.1	141.4	0.0002*	9803	24.9
	Ta(Tr)	8	0.02	0.003	1.1	0.3521	9934	0.3
	Tr x Ti	3	0.002	0.0005	0.5	0.6688	9959	0
	Ti x Ta(Tr)	8	0.007	0.0009	0.3	0.9446	9945	0
Seagrass	Tr	4	116.1	29.0	35.9	0.0002*	9925	19.6
growth	Ti	1	110.8	110.8	67.5	0.0001*	9830	30.4
	Ta(Tr)	10	8.1	0.8	0.6	0.7978	9940	0
	Po x Ti	4	11.0	2.7	1.7	0.2363	9964	1.5
	Po(Ta(Tr))	30	39.8	1.3	2.5	0.0003*	9914	5.0
	Ti x Ta(Tr)	10	16.4	1.6	1.4	0.2479	9939	1.8
	Ti x Po(Ta(Tr))	30	36.1	1.2	2.3	0.0003*	9896	8.4
Seagrass	Tr	4	341.4	85.4	9.8	0.0002*	2215	28.9
shoot	Ti	1	4.4	4.4	0.9	0.3755	9691	0
density	Ta(Tr)	10	87.2	8.7	0.6	0.7759	9926	0
	Tr x Ti	4	44.8	11.2	2.2	0.1447	9952	4.6
	Po(Ta(Tr))	30	413.3	13.8	2.4	0.0097*	9909	27.1
	Ta(Tr) x Ti	10	50.8	5.1	0.9	0.5572	9938	0

**Table A3.2** Statistical outputs of pair-wise comparisons (Student-t post hoc analysis) between treatment levels. Abbreviations:  $A = 0 \text{ mg coal } I^{-1}$ ,  $B = 38 \text{ mg coal } I^{-1}$ ,  $C = 73 \text{ mg coal } I^{-1}$ ,  $D = 202 \text{ mg coal } I^{-1}$ ,  $E = 275 \text{ mg coal } I^{-1}$ , Treat. = treatment, d = day, P = P-value, perm = Permanova, Perms = permutations, MC = Monte Carlo, Den 0 = denominator equals 0, \* = significant *P*-value.

			14 d			28 d				
Variable	Treat.	t	P(perm)	Perms	P(MC)	Treat.	t	P(perm)	Perms	P(MC)
	А, В	1.4	0.4031	2	0.2285	А, В	9.0	0.1009	10	0.001*
	A, C	2.5	0.0978	4	0.0655	A, C	20.7	0.1004	4	0.0001*
	A, D	4.6	0.0972	4	0.0114*	A, D	20.7	0.1001	4	0.0001*
	Α, Ε	Den 0				Α, Ε	20.7	0.1021	4	0.0001*
Coral	В, С	2.3	0.1009	10	0.0824	В, С	19.8	0.1035	4	0.0001*
mortairty	B, D	4.2	0.0988	10	0.0133*	B, D	19.8	0.0989	4	0.0001*
	B, E	29.0	0.1010	4	0.0001*	В, Е	19.8	0.0961	4	0.0001*
	C, D	0.5	0.8032	10	0.6561	C, D	Den 0			
	С, Е	1.9	0.3998	2	0.1268	С, Е	Den 0			
	D, E	1.9	0.0934	4	0.1309	D, E	Den 0			
	А, В	4.6	0.0447	358	0.0099*	А, В	5.3	0.0183	716	0.0057*
	A, C	5.6	0.0338	360	0.0044*	A, C	11.9	0.1009	719	0.0001*
Fish	Α, Ε	4.0	0.0892	692	0.0138*	Α, Ε	12.0	0.0959	719	0.0004*
growth	В, С	0.8	0.4634	90	0.4488	В, С	0.9	0.4848	720	0.4382
	В, Е	0.3	0.7736	357	0.7690	В, Е	1.3	0.1547	719	0.2673
	С, Е	0.2	0.8788	356	0.8546	С, Е	0.7	0.4967	719	0.5223
	А, В	0.8	0.5052	10	0.4541	А, В	2.8	0.1011	10	0.0549
	A, C	4.1	0.0992	10	0.0151*	A, C	8.2	0.1034	10	0.001*
	A, D	6.1	0.0997	10	0.0032*	A, D	5.6	0.0986	10	0.0059*
	Α, Ε	5.8	0.1007	10	0.0044*	Α, Ε	17.0	0.1034	10	0.0002*
Seagrass	В, С	3.5	0.1010	10	0.0257*	В, С	0.9	0.6009	10	0.4261
growth	B, D	5.4	0.0989	9	0.0053*	B, D	1.2	0.2968	10	0.2856
	B, E	5.1	0.1038	10	0.0066*	В, Е	1.7	0.0991	10	0.1680
	C, D	0.7	0.7016	10	0.5352	C, D	0.7	0.5998	10	0.5530
	C, E	1.2	0.3895	10	0.3138	С, Е	1.6	0.2991	10	0.1809
	D, E	0.8	0.6999	10	0.4739	D, E	0.2	0.8973	10	0.8574
	А, В	0.9	0.5022	10	0.4317	А, В	2.1	0.0940	10	0.1097
	A, C	2.3	0.1986	6	0.0824	A, C	3.2	0.1042	9	0.0289*
	A, D	2.6	0.0992	9	0.0544	A, D	4.3	0.0990	10	0.0131*
Seagrass	Α, Ε	1.9	0.1961	8	0.1323	Α, Ε	4.2	0.1049	7	0.0133*
density	В, С	1.8	0.1880	8	0.1466	В, С	2.1	0.1953	8	0.1016
	B, D	2.3	0.1959	5	0.0786	B, D	5.5	0.1042	10	0.0052*
	В, Е	1.3	0.2947	5	0.2529	B, E	5.9	0.1036	7	0.0062*
	C, D	0.1	1.0000	5	0.9109	C, D	0.7	0.6989	8	0.5047
	С, Е	0.5	0.6977	7	0.6742	С, Е	0.6	0.7021	7	0.5711
	D, E	0.5	0.8041	7	0.6661	D, E	0.5	1.0000	2	0.6381

**Table A4.1** Parameter estimates of GLMMs (transformed by a log link function) used to test for differences in *Acanthochromis polyacanthus* gill condition between coal treatments. Fish identity was included as random factor in all models. Bold numbers represent sig. effects ( $\alpha = 0.05$ ).

Response variable	Fixed effect	Parameter	Standard	z-value	P-value
	(treatment)	estimate	Error		
Lamellar scores	$30 \text{ mg coal I}^{-1}$	-0.36	0.54	-0.67	0.50
	73 mg coal l <sup>-1</sup>	-1.23	0.54	-2.27	0.02
	275 mg coal l <sup>-1</sup>	-1.86	0.54	-3.42	0.0006
Response variable	Fixed effect	Parameter	Standard	t-value	P-value
	(treatment)	estimate	Error		
Gill diffusion	Intercept	0.81	0.10	7.50	<0.0001
distance (µm)	$(0 \text{ mg coal } \text{I}^1)$				
	30 mg coal l <sup>-1</sup>	-0.15	0.15	-1.01	0.31
	73 mg coal $I^{-1}$	-0.08	0.15	-0.05	0.57
	275 mg coal l <sup>-1</sup>	-0.21	0.15	-1.41	0.15
Total lamellar length	Intercept	4.25	0.04	108.73	<0.0001
(μm)	(0 mg coal l <sup>-+</sup> )				
	30 mg coal l <sup>-1</sup>	0.03	0.05	0.59	0.55
	73 mg coal l <sup>-1</sup>	-0.04	0.05	-0.76	0.44
	275 mg coal l <sup></sup>	-0.11	0.05	-2.10	0.03
Functional lamellar	Intercept	4.04	0.05	74.46	<0.0001
length (µm)	(0 mg coal I <sup>-1</sup> )				
	30 mg coal l <sup>-1</sup>	-0.00	0.07	-0.12	0.90
	73 mg coal I	-0.06	0.07	-0.81	0.41
	275 mg coal I	-0.03	0.07	-0.43	0.66
Etherne and the failure are	Internet	2.42	0.10	24.47	10.0001
Filament thickness	Intercept	2.43	0.10	24.17	<0.0001
(μm)	$(0 \text{ mg coal } I^{-1})$	0.27	0.14	1.01	0.05
	30 mg coal I <sup>-1</sup>	0.27	0.14	1.91	0.05
	$75 \text{ mg coal } \text{I}^{-1}$	0.04	0.14	2.24	0.74
	275 mg coarr	-0.40	0.14	-3.24	0.001
Filament thickness	Intercent	2.81	0.05	/9.01	<0.0001
(% lamellar length)	$(0 \text{ mg coal } 1^{-1})$	2.01	0.05	45.01	0.0001
(// idinenial length/	$30 \text{ mg coal l}^{-1}$	0.19	0.08	2.37	0.01
	73 mg coal l <sup>-1</sup>	0.05	0.08	0.72	0.47
	275 mg coal l <sup>-1</sup>	-0.34	0.08	-4.23	<0.0001
Total mucus cover	Intercept	5.28	0.002	2643.10	< 0.0001
(μm²)	$(0 \text{ mg coal I}^{-1})$				
•	30 mg coal l <sup>-1</sup>	-0.56	0.002	-282.70	<0.0001
	73 mg coal l <sup>-1</sup>	-0.36	0.001	-181.70	<0.0001
	275 mg coal l <sup>-1</sup>	-1.01	0.001	-507.20	<0.0001
Mucus cover	Intercept	3.60	0.15	23.39	<0.0001
(% interlamellar	$(0 \text{ mg coal } \text{I}^{-1})$				
space)	1				
	30 mg coal l <sup>-1</sup>	-0.37	0.21	-1.69	0.08
	73 mg coal l <sup>-+</sup>	-0.21	0.21	-0.97	0.33
	275 mg coal l <sup>-1</sup>	-0.92	0.21	-4.22	<0.0001
Number of mucus	Intercept	3.24	0.08	36.74	<0.0001
cells	$(U mg coal I^{-})$	0.20	0.12	2.20	0.02
	$30 \text{ mg coal I}^{-1}$	-0.28	0.12	-2.26	0.02
	73 mg coal I	-0.40	0.12	-3.23	0.001
1	Z/SING COALL	-0.07	0.12	-0.32	<0.0001

**Table A5.1** Statistical outputs of GLMs outlined in Table 5.1. Comparisons were made between control corals and corals exposed to coal and sediments (P < 0.05). Abbreviations: A = *Acropora tenuis*, M = *Montipora spp.*, P = *Porites spp.*, sed = sediment, SW = particle free seawater, SC = suspended coal, SS = suspended sediments, df = degrees of freedom (null deviance, residual deviance), - = no significant difference.

Treatment		Species	Oxygen production	Respiration	Light calcification	Dark calcification		
a) Reponses to chronic deposition (Results section 5.4.1.1)								
Coal pre- exposed +SW	t value (df) <i>P</i> -value	A	-	3.0 (62, 54) 0.005	-7.7 (59,51) <0.0001	-10.7 (60,52) <0.0001		
	t value (df) <i>P</i> -value	Р	2.0 (60,51)3.6 (60,51)0.0470.0007		-4.6 (60,51) <0.0001	-6.1 (60,51) <0.0001		
	t value (df) <i>P</i> -value	м	-	-	-4.4 (61,53) (P <0.0001)	-2.4 (59,51) (P =0.02)		
Sed pre-	t value (df) <i>P</i> -value	A	-2.9 (62,54) 0.005	-	-5.2 (59,51) <0.0001	-5.1 (60,52) <0.0001		
exposed +SW	t value (df) <i>P</i> -value	Ρ	-	-	-	-5.5 (60,51) <0.0001		
	t value (df) <i>P</i> -value	М	-	-	-	-2.5 (59,51) 0.01		
b) Responses	to suspended	d particles (I	Results section 5	.4.2)	1			
Coal pre- exposed +SC (chronic)	t value (df) <i>P</i> -value	A	-5.8 (62,54) <0.0001	-	-3.4 (59,51) 0.0004	-14.5 (60,52) <0.0001		
	t value (df) <i>P</i> -value	Ρ	-5.9 (60,51) <0.0001	4.9 (60,51) <0.0001	-4.3 (60,51) <0.0001	-9.8 (60,51) <0.0001		
	t value (df) <i>P</i> -value	м	-2.9 (62,54) 0.006	-8.5 (62,54) <0.0001	-	-7.5 (59,51) <0.0001		
Sed pre- exposed +SS	t value(df) <i>P</i> -value	A	-	-	-	-3.2 (60,52) 0.002		
	t value (df) <i>P</i> -value	Ρ	-	-	-	-3.4 (60,51) 0.001		
(chronic)	t value (df) <i>P</i> -value	М	-2.1 (62,54) 0.04	-	-2.5 (61,53) 0.02	-		
Non- exposed	t value (df) <i>P</i> -value	A	-6.5 (62,54) <0.0001	-	-7.9 (59, 51) <0.0001	-4.3 (60,52) <0.0001		
+SC (acute)	t value (df) <i>P</i> -value	Ρ	8.0 (60,51) <0.0001	2.4 (60,51) -7.8 (60,51) 0.02 <0.0001		-6.0 (60,51) <0.0001		
	t value (df) <i>P</i> -value	М	-5.1 (62,54) <0.0001	-	-4.2 (61,53) 0.0001	-3.0 (59,51) 0.0048		
Non- exposed	t value (df) <i>P</i> -value	A	-	-	-	-4.1 (60,52) 0.0001		
+SS (acute)	t value (df) <i>P</i> -value	Р	-	-	-	-3.2 (60,51) 0.002		



**Figure A5.1** Oxygen production and dark respiration rates (mgO<sub>2</sub> cm<sup>-1</sup> min<sup>-1</sup>) for each coral species (n=7) across exposure treatments: T I = corals not previously deposited with particles incubated in particle free seawater; T II = corals previously deposited by coal incubated in particle free seawater; T III = corals previously deposited by sediment incubated in particle free seawater; T III = corals previously deposited by coal incubated in particle free seawater; T III = corals previously deposited by coal incubated with coal suspensions (SC); T III + SC = corals previously deposited by sediments incubated with suspended sediments (SS); TI + SC or SS = corals previously not exposed to particulates incubated with either SC or SS. \* = significant differences between particle treatment corals and corals not previously exposed to particles (TI, controls).



**Figure A5.2** Measurements of coral tissue health (based on changes in tissue colour), symbiont density (cells cm<sup>-2</sup> of tissue) and chlorophyll *a* content ( $\mu$ g cm<sup>-2</sup>) in each species after 28 d exposure to particle free seawater (TI), coal (TII) and sediment (TIII) deposition. n = 14 per species for tissue health and n = 7 per species for symbiot density and chlorophyll a content. \* = significant differences between particle treatments and particle-free treatments (controls).



**Figure A5.3** Light and dark calcification rates ( $\mu$ mol CaCO3 cm<sup>-2</sup> min<sup>-1</sup>) for each coral species (n=7) across exposure treatments: T I = corals not previously deposited with particles incubated in particle free seawater; T II = corals previously deposited by coal incubated in particle free seawater; T III = corals previously deposited by sediment incubated in particle free seawater; T III = corals previously deposited by coal incubated with coal suspensions (SC); T III + SC = corals previously deposited by sediments incubated with suspended sediments (SS); TI + SC or SS = corals previously not exposed to particulates incubated with either SC or SS. \* = significant differences between particle treatment corals and corals not previously exposed to particles (TI, controls).



**Figure A5.4** Oxygen microprofiles in the light showing the change in oxygen at the *Porites spp.* tissue surface over time after being deposited by sediment. Increasing oxygen levels at the coral surface over time (x axis) depict the removal of sediment by *Porites spp.* As particles are cleared, gas exchange is not impaired and light can reach the coral tissue enabling their symbiotic algae to photosynthesize.



**Figure A5.5** Oxygen microprofiles in light and dark at the surface of massive *Porites spp.* before and after the addition of coal, sediment and activated carbon. Activated carbon was used as a toxicity control with similar light reflectance to coal particles. Points represent mean  $\pm$  SD of 4-5 profile replicates per treatment.

**Table A5.2** PAH concentrations ( $\mu g l^{-1}$ ) in the coal treatment water samples. Water was only sampled after the 8 hour deposition period when water flow was off. PAH leachate from this sediment type was previously investigated and no PAH leachate was detected (Flores et al., 2012). Abbreviations: R.L. = reporting limit, R1 = replicate 1, R2 = replicate 2, R3 = replicate 3.

Parent and Alkyl PAHs (µg l <sup>-1</sup> )	R.L.	Control	Coal R1	Coal R2	Coal R3	Sediment
Naphthalene	0.01	<0.010	< 0.010	<0.010	<0.010	< R.L.
Acenaphthylene	0.01	<0.010	< 0.010	<0.010	<0.010	< R.L.
Acenaphthene	0.01	<0.010	< 0.010	<0.010	<0.010	< R.L.
Fluorene	0.01	<0.010	< 0.010	<0.010	<0.010	< R.L.
Phenanthrene	0.01	0.01	0.07	0.03	<0.010	< R.L.
Anthracene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
Fluoranthene	0.01	<0.010	0.05	0.02	0.02	< R.L.
Pyrene	0.01	<0.010	0.02	0.01	0.01	< R.L.
Benz(a)anthracene	0.01	<0.010	0.01	<0.010	<0.010	< R.L.
Chrysene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
Benzo(b)fluoranthene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
Benzo(k)fluoranthene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
Benzo(a)pyrene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
Indeno(1,2,3-cd)pyrene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
Dibenzo(a,h)anthracene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
Benzo(g,h,i)perylene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
1-methylnaphthalene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
2-Methylnaphthalene	0.01	<0.010	<0.010	<0.010	<0.010	< R.L.
C2-alkyInaphthalenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C3-alkyInaphthalenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C4-alkylnaphthalenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
Methylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C2-alkylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C3-alkylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C4-alkylphenanthrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
Dibenzothiophene	0.01	<0.010	0.04	<0.010	<0.010	< R.L.
Methyldibenzothiophene	0.05	<0.050	0.05	<0.050	<0.050	< R.L.
C2-alkyldibenzothiophenes	0.05	<0.050	0.14	<0.050	<0.050	< R.L.
C3-alkyldibenzothiophenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
Methylpyrenes/fluoranthenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C2-alkylpyrenes/fluoranthenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C3-alkylpyrenes/fluoranthenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
Methylchrysenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C2-alkylchrysenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
Methylbenzopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C2-alkylbenzopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
Methylindenopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
C2-alkylindenopyrenes	0.05	<0.050	<0.050	<0.050	<0.050	< R.L.
Total PAHs		0.01	0.38	0.06	0.03	< R.L.
Total PAHs (Average of reps)				0.039 ± 0.0	1	

### **Appendix B: Image Links**

All images not drawn in Figure 1.1 were labelled as available for reuse with modification. Links are provided below:

#### Coal mine

https://pixabay.com/en/photos/?image\_type=&cat=&min\_width=&min\_height=&q=Coal+Mine%2C+ Mine%2C+Cave%2C+Lorry%2C+Coal&order=popular

#### Cargo Ship

https://pixabay.com/en/photos/?image\_type=&cat=&min\_width=&min\_height=&q=Cargo+Ship%2C +Shipment%2C+Freighter%2C+Cargo%2C+Courier&order=popular

Bird

http://www.publicdomainpictures.net/view-image.php?image=76495&picture=oiseau-apercu-dessin-2

Fisher https://pixabay.com/en/fish-fishing-fisherman-hobby-546943/

Fish

https://pixabay.com/en/tarpon-fish-mounted-taxidermy-1652063/ https://pixabay.com/en/photos/?image\_type=&cat=&min\_width=&min\_height=&q=Trout%2C+Fish %2C+Rainbow+Trout%2C+Animal&order=popular

Worm

https://pixabay.com/en/photos/?image\_type=&cat=&min\_width=&min\_height=&q=Worm%2C+Ani mal%2C+Insect&order=popular

Copepod

https://commons.wikimedia.org/wiki/File:Copepodkils.jpg

Fish larvae

https://commons.wikimedia.org/wiki/File:Sebastes\_goodei\_larvae\_drawing.gif

Clownfish

https://pixabay.com/en/photos/?image\_type=&cat=&min\_width=&min\_height=&q=Fish%2C+Nemo %2C+Animal%2C+Sea%2C+Clown+Fish&order=popular

Filter feeder

https://commons.wikimedia.org/wiki/File:Spirobranchus\_giganteus,\_Koh\_Phangan.jpg

Seagrass

https://commons.wikimedia.org/wiki/File:Thalassia\_hemprichii.jpg

Coral

https://pixabay.com/en/coral-collage-vintage-plant-blue-1653752/

## **Appendix C: Publications**

- 1. Berry KLE, Hoogenboom MO, Brinkman D, Burns K, Negri AP. (2017) Effects of coal contamination on early life history stages of a reef-building coral, Acropora tenuis. Marine Pollution Bulletin, http://dx.doi.org/10.1016/j.marpolbul.2016.10.011
- 2. Berry KLE, Hoogenboom MO, Flores F, Negri AP (2016). Simulated coal spill causes mortality and growth inhibition in a coral, seagrass and reef fish species. Nature Scientific Reports, DOI:10.1038/srep25894
- **3.** Kroon, F, Berry KLE, Brinkman D, et al. (**2016**) Identification, impacts, and prioritisation of emerging contaminants present in the GBR and Torres Strait marine environments. Tropical Water Quality Hub, National Environmental Science Programme.