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REPORT

Sedimentation rapidly induces an immune response and depletes energy stores in a hard coral

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Abstract High sedimentation rates have been linked to reduced coral health within multiple systems; however, whether this is a direct result of compromised coral immunity has not been previously investigated. The potential effects of sedimentation on immunity of the hard coral *Montipora patula* were examined by comparing physiological responses of coral fragments inoculated with sterilized marine sediments and those under control conditions. Sediments were collected from terrestrial runoff-affected reefs in SW Madagascar and applied cyclically for a total of 24 h at a rate observed during precipitation-induced sedimentation events. Coral health was determined 24 h after the onset of the sedimentation stress through measuring metabolic proxies of O_2 budget and lipid ratios. Immune response of the melanin synthesis pathway was measured by quantifying phenoloxidase activity and melanin deposits. Sedimentation induced both immune and metabolic responses in *M. patula*. Both phenoloxidase activity and melanin deposition were significantly higher in the sediment treatment compared to controls, indicating an induced immune response. Sediment-treated corals also showed a tendency towards increased respiration (during the night) and decreased photosynthesis (during the day) and a significant depletion of energy reserves as compared to controls. These data highlight that short-term (24 h) sedimentation, free of live microorganisms, compromises the health of *M. patula*. The energetically costly immune response, potentially elicited by residual endotoxins and other inflammatory particles associated with the sterile sediments, likely contributes to the energy depletion. Overall, exposure to sedimentation adversely affects coral health and continued exposure may lead to resource depletion and an increased susceptibility to disease.

Keywords Coral immunity · Sedimentation · Environmental stress · Phenoloxidase · Lipid storage · Melanin

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Introduction

Coral reefs are continuing to undergo major global declines (Hughes et al. 2003; Burke et al. 2011) due to a diversity of factors including increasing sea surface temperatures and sediment loads, which all ultimately compromise coral

health and lead to increased susceptibility to disease (Harvell et al. 1999, 2007; Hughes et al. 2003; Raymundo et al. 2005; De'ath and Fabricius 2010). While nutrient loads and terrestrial runoff have been suggested as promoters of coral disease (Fabricius 2005; Kline et al. 2006; Voss and Richardson 2006; Harvell et al. 2007; Haapkylä et al. 2011), the potential influence of sedimentation on coral immunity, the metabolic cost involved in immune response upregulation and the speed at which these processes can be affected remain important gaps in our current understanding of coral health and disease dynamics.

Immunity is the ability of an organism to resist infection (Stedman 2000) and is one of the key life history traits (along with reproduction and growth for example) among which energy must be shared (Schmid-Hempel 2003). As such, immunity plays a determining role in disease resistance within all organisms, including corals, with species investing less in constituent immunity being most at risk of infection (Palmer et al. 2010). Investigating how coral immunity is affected by factors such as environmental stressors is therefore a key to strengthening our understanding of coral disease dynamics and reef decline (Palmer and Traylor-Knowles 2012). The coral immune repertoire includes a variety of components ranging from mucus production and melanin synthesis to the activation of antimicrobial peptides and immune cells (e.g., phagocytosis; Reed et al. 2010; Palmer and Traylor-Knowles 2012). In particular, the melanin synthesis cascade is a key component of invertebrate immunity and is initiated when the activating enzyme phenoloxidase (PO) is converted from its precursor, prophenoloxidase (PPO), upon the detection of pathogens or damage-associated molecular patterns (PAMPs or DAMPs; Cerenius and Söderhäll 2004; Palmer et al. 2012). While PAMPs are typically associated with pathogens (e.g., lipopolysaccharide from gram negative bacterial cell walls), DAMPs are released as a result of cellular damage (Rock et al. 2010). These molecular patterns are recognised by pattern recognition receptors (e.g., Toll-like receptors) which initiate appropriate signalling pathways and induce relevant responses (Palmer and Traylor-Knowles 2012). In the case of the melanin synthesis pathway, initiated responses help the organism to resist infection. These include the production of cytotoxic quinones (melanin precursors) and metabolites (reactive oxygen species), as well as the protection of compromised tissues and physical lesions by the formation of a melanin barrier (the end product of the cascade; Palmer et al. 2011a) isolating tissues from pathogens and other potential sources of cellular damage (Sugumaran 2002; Palmer et al. 2008; Cerenius et al. 2010). Measuring phenoloxidase (PO) activity allows the level of active immune responses to be quantified (whereas PPO, the inactive form of PO, provides information on constituent immune potential). However, upregulating and investing in environmental stress resistance (e.g., mucus production) and immunity is energetically costly (Armitage et al. 2003; Schmid-Hempel 2003). Additionally, corals investing heavily in immunity appear to do so at the expense of other life history traits such as growth and reproductive output (Palmer et al. 2010). As such, investment in immunity is species-specific and depends on their life history strategy, resulting in certain species being more susceptible to disease than others (Palmer et al. 2010).

Lipid content is a commonly used proxy for quantifying the effects of environmental stress on coral energy reserves (e.g., Ward 1995; Anthony 2006; Fitt et al. 2009; Seeman et al. 2012) and can take from weeks to months to reflect changes (Anthony et al. 2009; Cooper et al. 2009). However, lipids are not the sole energy source available to corals, with other sources including proteins, carbohydrates, and contributions from symbionts (Lesser 2013) and should therefore not be used to compare energy reserves across species. While the use of extensive modelling of coral energy budgets (Anthony et al. 2009) are ideal, differences in energy reserves can be efficiently estimated between clonal coral fragments using recent methods such as nonpolar to polar lipid ratios (Saunders et al. 2005). Because coral tolerance to subsequent stress depends in part on available energy resources, this parameter may also provide insight into the future potential stress tolerance (e.g., susceptibility to severe bleaching) of a coral (Anthony et al. 2009).

Sedimentation, as a result of terrestrial runoff, can affect corals both directly (e.g., physical damage by abrasion) and indirectly (e.g., reduction of light availability through increased turbidity), at both ecological and individual scales (Rogers 1990; Fabricius 2005; Erftemeijer et al. 2012). Sediment deposits on corals have been suggested to act as a reservoir of coral pathogens (Richardson 1997) and can lead to tissue bleaching and necrosis (Philipp and Fabricius 2003), primarily through microbial activity (Weber et al. 2012). In turn, this might trigger changes in coral-associated microbial communities and increases in disease susceptibility (Harvell et al. 2007; Williams et al. 2010). However, different coral species display varying levels of sensitivity to sedimentation (Erftemeijer et al. 2012), with *Montipora* spp. being among the more susceptible group, similar to the patterns in immune levels observed among coral genera (Palmer et al. 2010). The diversity in sensitivity to sedimentation is in part due to the variety in sediment removal efficiency, which is dependent on both species and growth form (Erftemeijer et al. 2012).

Sediment-shedding mechanisms can be either passive (e.g., use of water currents) or active (e.g., polyp inflation, mucus production, and ciliary beating; Stafford-Smith and Ormond 1992; Erftemeijer et al. 2012), the latter of which

is energetically costly. The considerable metabolic costs of active shedding can cause a shift of the energy budget away from remaining metabolic requirements (e.g., growth, reproduction, and immunity; Riegl and Branch 1995). For example, in *Acropora acuminata*, energy expenditures through mucus exudation following environmental stress can account for up to 40 % of the net carbon fixed by photosynthesis (Crossland et al. 1980). Such investment in mucus shedding could therefore lead to energetic depletion, increasing the risk of adverse effects from subsequent exposure to environmental stressors (Anthony et al. 2009). Sediment deposition on corals could further deplete energetic resources through inducing an energetically costly immune upregulation (Armitage et al. 2003)—such as in response to pathogens or damage-associated molecular patterns (PAMPs or DAMPs) released by sedimentation-induced cellular damage (Rock et al. 2010) or associated with the sediments. In the long term, no matter the cause, energetic depletion would likely lead to compromised immunity (Feder et al. 1997; Seppälä et al. 2008), both at the baseline level and during an immune response, resulting in increased susceptibility to infection (Palmer et al. 2010).

In this research, under controlled experimental conditions, we investigated the influence of sterile sedimentation on coral health. Sterile sediments were used to rule out confounding factors such as microbial activity, the presence of opportunistic pathogens, and significant modifications of the bacterial communities during transport from the field. In particular, we addressed the following questions: (1) Does short-term (24 h) sedimentation, using sterilised natural sediments applied at ecologically relevant levels, induce an immune response in corals? (2) What are the effects of sedimentation on coral metabolic costs and energy reserves?

Materials and methods

Experimental design and sampling

Samples

Eight $\sim$6 cm^2 *Montipora patula* from a stable mesocosm (self-sustaining since 2005) at the Numerical Ecology of Aquatic Systems laboratory at the University of Mons (Belgium) were fragmented into three pieces of $\sim$2 cm^2 so as to avoid stress during sampling. *M. patula* was chosen for this experiment as it is considered to be susceptible to sedimentation (Erftemeijer et al. 2012). All fragments were mounted so as to avoid contact onto a single polycarbonate support using nylon string and left under ambient conditions to recover and heal completely for 3 weeks. Each coral (all three fragments) was then transferred to one of eight 1L respirometer chambers (Duran bottle; four controls and four to receive sediments) and acclimated there for 3 d. Each chamber was connected in a flow-through manner to the mesocosm, and the water [salinity = 34.6 ± 0.1 ppt, pH = 8.05 ± 0.05, temperature = 25.2 ± 0.4 °C, alkalinity = 2.33 ± 0.01 mmol kg^{-1}, N ($NO_3 + NO_2 + NH_4$) $\approx$ 1 µmol l^{-1}, $PO_4 < 1$ µmol l^{-1}] was agitated by magnetic stirring. Lighting of 250 ± 10 µmol cm^{-2} s^{-1} was provided by 2 × 24 LED's (3 W; 50 % cold white, 37.5 % royal blue, 12.5 % cyan blue; LUMIRIUM) with a 12-12 h light/dark cycle.

Sediment and inoculation

Once acclimated, the four treatment chambers were subjected to sedimentation in eight 3-h cycles (Fig. 1) for a total of 24 h in order to simulate chronic, but short-term sedimentation, while controls underwent the same cycles without sediment addition. Water exchange was switched off at the start of each cycle, O_2 concentration was recorded for 2 min ($O_{2\ start}$), and then sediments were added for 3 min (h + 02 min–h + 05 min; in treatment tanks only). The magnetic stirring was turned off 1 min after the end of the sediment input (h + 06 min), thereby allowing thorough mixing of the sediment over the entire surface of the respirometer chamber (63.58 cm^2). After 2 h of settling time (h + 2 h), magnetic stirring was switched back on and, after 3 min, O_2 concentration was measured for another 2 min ($O_{2\ end}$; h + 2:03–h + 2:05). Finally, at h + 2h05, water exchange was turned back on until the start of the next cycle, completely renewing the water and clearing all suspended sediments from the chamber. In order to improve on the ecological relevance of the data, the sediments used consisted of wet-sieved (250 µm) and autoclaved marine sediments collected one month before the experiment from terrestrial runoff-affected reefs in SW Madagascar using sediment traps (following Storlazzi et al. 2011) and kept suspended in sea water from the mesocosm (concentration = 18.73 mg ml^{-1}). These were added in short pulses (Fig. 1) into treatment chambers using a multi-channel peristaltic pump (Gilson Minipuls 2, Gilson, Middleton, WI, USA) at a rate of 8.75 ml min^{-1}, simulating over 24 h a sedimentation rate of $\sim$62 mg cm^2 d^{-1}, consistent with observed rates on reefs in SW Madagascar affected by sediment plumes during the rainy season (C Sheridan pers. obs.). The sediment composition (with method used for determination) was as follows: (1) size of grains (thin sections): <63 µm: 97.62 ± 0.26 %; 63–125 µm: 2.08 ± 0.19 %; 125–250 µm: 0.30 ± 0.09 %; (2) organic content (combustion) = 13.26 ± 0.01 %; (3) mineral content (X-ray diffraction): quartz = 5.3 ± 1.5 %, clays = 30.1 ± 2.7 %,

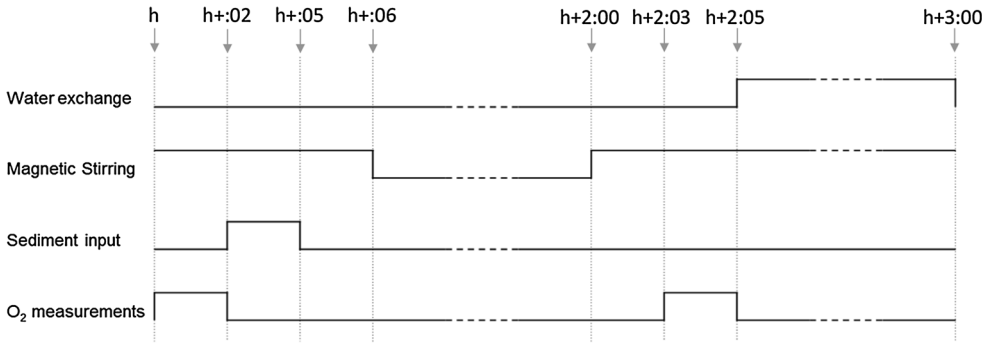


Fig. 1 Schedule of environmental parameters for experimental and control tanks. Automated parameters for each 3 h cycle of sediment inoculation. Cycle started at 12:00 and finished at 12:00 the next day (time not to scale). *Raised bars* signify that parameter is ON, otherwise parameter is OFF. "h" = start time of sediment inoculation cycle; time expressed as h:mm. *Dashed lines* denote a continuation of the ongoing process without change

calcite = 5.2 ± 0.9 %, calcite-Mg = 31.4 ± 2.0 %, aragonite = 28.0 ± 1.7 %.

At the end of the inoculation run, the three fragments from each coral were collected and two fragments were flash frozen in liquid nitrogen then stored at −80 °C while the final fragment was fixed in 4 % paraformaldehyde (PAF) for 12 h. Flash frozen samples were analysed as detailed below for lipid fraction ratios and quantification of melanin synthesis activity while PAF-fixed samples were used for melanin deposits quantification in coral tissues.

Phenoloxidase assay

Four flash frozen coral fragments from both the sediment-stressed and control treatments were extracted following a protocol adapted from Mydlarz and Palmer (2011). Briefly, samples were airbrushed (Paasche H202S) with extraction buffer (50 mM phosphate buffered saline pH 7.8 with 0.05 mM dithithreitol) over ice. The tissue slurry was homogenised by two cycles of vortexing with 212–300 µm glass beads (3:1, respectively, vol:vol; Sigma G9143-100G) for 20 s and standing over ice for 5 min, then centrifuged at 4 °C at 2,400×*g*. Aliquots of the supernatant were stored at −80 °C.

Phenoloxidase (PO) activity was measured on a FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany) at 490 nm every 5 min for 45 min according to (Palmer et al. 2011b) using dopamine hydrochloride as substrate. All samples were run in triplicate on a 96-well plate and data were normalised to protein content per sample (as determined using a Bradford assay using Bovine Gamma Globulin as standard) and to any change in absorbance over time of control wells containing 45 µl MQ H2O (instead of coral extract), 30 µl dopamine hydrochloride (10 mM), and 40 µl phosphate buffer. Mean PO activity was quantified as change in absorbance over time.

Melanin deposit quantification

The eight fragments of *M. patula* (four sediment-stressed, four controls) fixed in 4 % paraformaldehyde in phosphate buffered saline for 12 h were decalcified progressively in 3–10 % formic acid. Samples were then processed sequentially through a graded ethanol series (30, 50, 70, 90, 100 %), two changes of isobutanol (1 h at room temperature then 12 h at 60 °C), three changes of paraffin (Paraplast Plus, Sigma-Aldrich) at 60 °C (12, 1, 4 h) prior to the paraffin embedding. Five-micrometre transverse sections were stained with Fontana-Masson melanin stain. Fifteen randomly selected photographs from a minimum of three histological sections of the free body wall region were taken using a Carl Zeiss AxioCam Icc 3 camera fitted onto a Carl Zeiss Axio Scope.A1 microscope. Following Palmer et al. (2010), the mean volume fraction (V_f) of melanin present in coral tissue layers was determined using the ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA).

Lipid fraction ratio determination

Lipid extraction was performed following a method adapted from Folch et al. (1957). The remaining eight flash frozen samples were crushed under liquid nitrogen using a sterile mortar and pestle and weighed then transferred to a solution of 20-fold the volume of tissue sample of chloroform:methanol (2:1, v/v), placed on a stirring plate for 1 h then filtered (MN 615, Macherey–Nagel, Duren, Germany) into a separatory funnel. The filtered extract was rinsed with 20 % of its volume of NaCl 0.58 % and left to separate into two phases. The lower phase was finally filtered through anhydrous sodium sulphate into a pre-weighed conical flask, and evaporated using a rotary evaporator (Laborata 4001, Heidolf, Schwabach, Germany). Dry weights were recorded

to determine total lipid content, then the dried extracts were re-dissolved in chloroform:methanol (2:1, v/v) using a dilution of 1:50 (w/v) and the dissolved lipids were stored at −20 °C until further analysis. In order to determine the ratio of storage (neutral/nonpolar) lipids to structural (polar) lipids, lipid fractions were separated using thin-layer chromatography following Saunders et al. (2005), a method allowing the separation of even small lipid quantities (Vanderplanck et al. 2011). For each sample, five replicate chromatograms were obtained and scanned (HP Photosmart Premium 309 g, Hewlett Packard, Washington, USA; 600dpi) within 5 min of removal from the oven to generate grey scale images. Lipid fractions were quantified by measuring peak intensities (ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA) of the bands corresponding to cholesterol and cholesteryl oleate standards (Saunders et al. 2005) and average nonpolar to polar lipid ratios were calculated.

Respirometry

To examine how sedimentation stress may affect the metabolism of *M. patula,* we examined the net O_2 flux ratio (change of O_2 concentration between the end of the acclimation phase and the experimental phase) during both day and night. Net oxygen concentration ($[O_2]$; O_2 produced by photosynthesis − O_2 consumed by respiration) was measured for a duration of 2 min in each respirometer chamber at the start of each cycle ($[O_{2\ start}]$) and 3 min after the end of the 2 h sediment settling phase ($[O_{2\ end}]$; Fig. 1), and this was carried out during both acclimation and experiment phases. The variation in net $[O_2]$ (obtained as net $[O_{2\ end}]$ − net $[O_{2\ start}]$) normalised by the buoyant weight (of each coral fragment) was measured for both day and night as the change in mean $[O_2]$ between the end of the acclimation phase and the sediment inoculation phase and is expressed as net O_2 flux ratio. Photosynthesis/Respiration (*P/R*) ratios were also determined from the $[O_2]$ of sediment-stressed and control samples as the quotient of mean daytime overnight time net O_2 flux ratios.

Statistical analysis

All statistical analyses were performed using the R v2.15.3 (R Core Team 2013). Kruskal–Wallis rank sum nonparametric tests were used to examine differences between sediment-treated and control samples for each of the physiological parameters as the assumptions of normality and homoscedasticity of parametric tests were not satisfied.

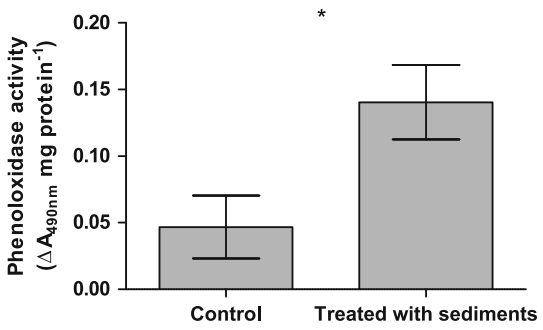


Fig. 2 Phenoloxidase (PO) activity. Mean PO activity (±SE) for *Montipora patula* treated with sediments ($n = 4$) and controls ($n = 4$). "*" denotes <0.05 significance level

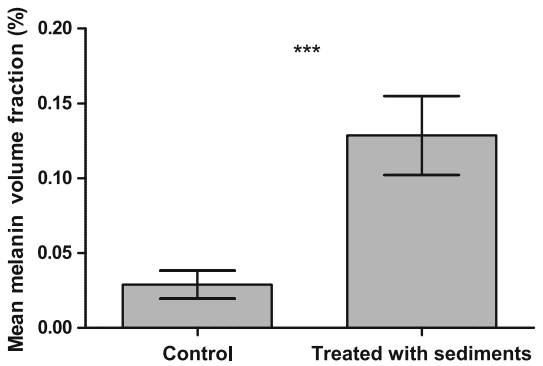


Fig. 3 Melanin quantification in histological sections. Mean melanin volume fraction (V_f) (±SE) expressed as the relative percentage of melanin deposits present within the tissue of sediment-treated corals ($n = 4$) and controls ($n = 4$). "***" denotes < 0.001 significance level

Results

Immunity: quantification of phenoloxidase and melanin deposits

Following coral exposure to sterile sedimentation, phenoloxidase (PO) activity (Fig. 2) was nearly threefold higher in sediment-stressed *M. patula* as compared to control fragments (Kruskal–Wallis; $H_{(1)} = 4.08$, $p < 0.05$). Similarly, the mean volume fraction (V_f) of melanin in the tissue layers was more than fourfold higher in sediment-stressed corals when compared to controls (Kruskal–Wallis; $H_{(1)} = 21.93$, $p < 0.001$; Fig. 3). Melanin deposits were found predominantly in the epidermis (Fig. 4).

Metabolism: lipid ratio analysis and respirometry

The ratio of nonpolar (storage) to polar (structural) lipids was sixfold lower in sediment-treated samples as compared

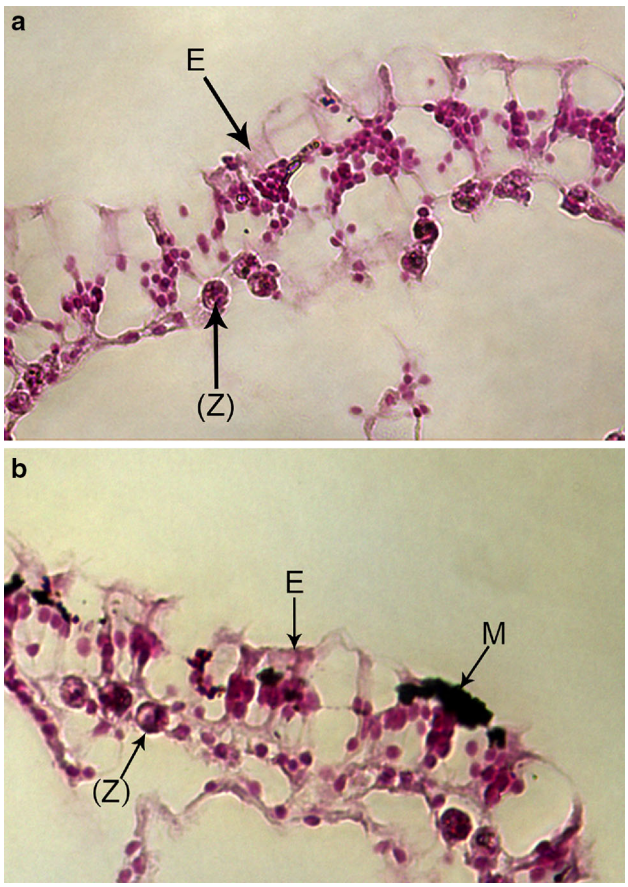


Fig. 4 Melanin deposits on *Montipora patula.* Fontana-Masson stained transverse sections of a control *M. patula* fragment **(a)**, and one treated with sediments **(b)** showing melanin deposits (M) on the outer layer of the epidermis (E). *Z* Zooxanthellae

to controls, with an average ratio of just 2.2 %, as opposed to 13.5 % in controls (Kruskal–Wallis $H_{(1)} = 28.64$, $p < 0.001$; Fig. 5). There was a trend of lower O_2 flux ratios in sediment-treated corals than in controls (Fig. 6), though this was not statistically significant for either day

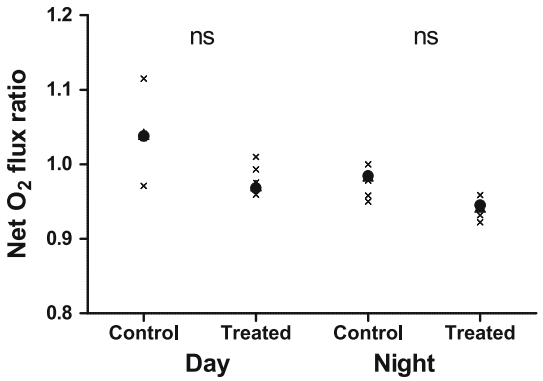


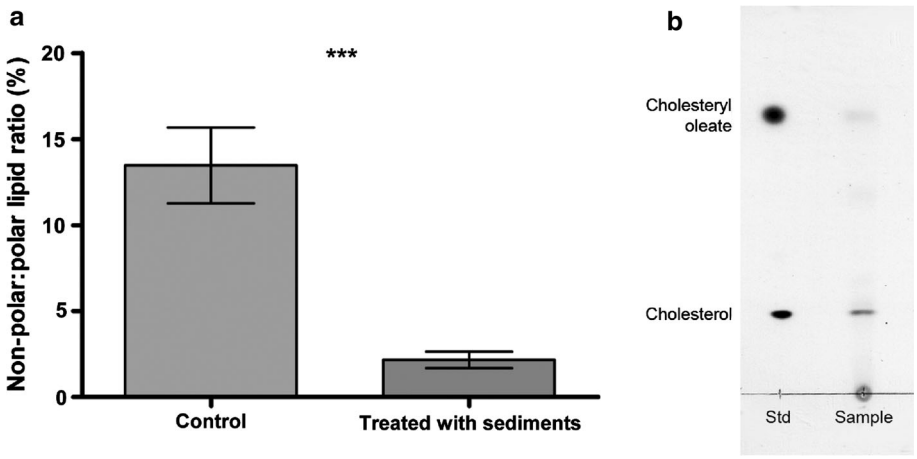
Fig. 6 Repirometric assay—O_2-flux ratios. Net O_2 flux ratio of *Montipora patula* corals treated with sediments ($n = 4$) or controls ($n = 4$). "x" are mean net [O_2] flux ratios per sample, and *black circles* are overall medians. "ns" denotes lack of significance

($p > 0.05$) or night ($p > 0.05$). Similarly, sediment-treated *M. patula* fragments showed no significant difference in their *P/R* ratios (Fig. 7) when compared to controls ($p > 0.05$).

Discussion

Acute exposure to sterile sedimentation induced a significant immune response and a reduction in storage lipids within the hard coral *M. patula*. These findings demonstrate a direct link between sedimentation (microbe-free) and compromised coral health, which helps distinguish between sediment-induced impacts and tissue degradation resulting from microbial activity (Rogers 1990; Weber et al. 2012), and has implications on a variety of scales. Short-term investment in immunity will reduce immediate infection risk, but may lead to longer-term energy depletion and increased disease and bleaching susceptibility

Fig. 5 Lipid ratio analyses. **a** Nonpolar:polar lipid ratios (±SE) of *Montipora patula* samples treated with sediments ($n = 4$) and controls ($n = 4$). "***" denotes <0.001 significance level. **b** Thin-layer chromatography plate showing the separation of polar (cholesterol) and nonpolar (cholesteryl oleate) lipid fractions for one of the *M. patula* fragments used in the experiment



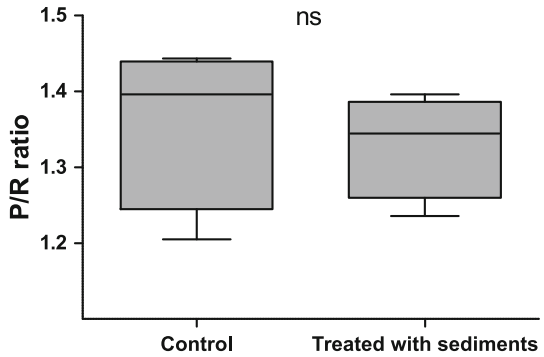


Fig. 7 Repirometric assay—photosynthesis/respiration (*P/R*) ratios. *P/R* ratios (±SE) of *Montipora patula* corals treated with sediments ($n = 4$) or controls ($n = 4$) as obtained from net O_2 measurements. "ns" denotes lack of significance

(Anthony et al. 2009), which has vast ecological implications in areas of high sedimentation. These effects may also be relatively long lasting, as depressed coral energy reserves following environmental stress may take time to recover (Fitt et al. 2000; Grottoli et al. 2004).

Immunity

Short-term sedimentation, at ecologically relevant levels, induced a significant immune response in the coral *M. patula*. Melanin synthesis pathway activation is a key response to immune challenge in invertebrates (Cerenius and Söderhäll 2004). The upregulation of this pathway in response to sedimentation provides both antimicrobial defence to prevent infection and melanin deposition to form a physical barrier against sediment deposits (Cotter and Wilson 2002; Dubovskii et al. 2010). The melanin synthesis pathway is activated upon the detection of "nonself", for example in the presence of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) as well as other non-infectious stimuli (Rock et al. 2010). As such, the higher PO activity and quantity of melanin deposits in sediment-treated corals suggests that, although the sediments had been sterilised, they were still recognised as "nonself" by the coral. Although PAMPs might have remained despite the sterilisation process (Rossi et al. 2012), sediment-associated microbes had been inactivated and, in this case, could not be implicated in coral damage as showed by Weber et al. (2012). Damage-associated molecular patterns (DAMPs), potentially released through sediment-induced cellular damage (abrasion), could be an alternative explanation for the observed response. General immune responses can be elicited by the release of DAMPs, such as intracellular molecules (e.g., ATP and DNA), into the extracellular environment when tissue becomes compromised, thereby protecting remaining tissue and lowering risk of infection (Heil 2012). Additionally, the sterile sediment could have been detected as "nonself" as a variety of organic and inorganic sterile particulates can stimulate an inflammatory response (Rock et al. 2010). Although the molecules involved in the activation of the melanin synthesis pathway could not be determined, the sedimentation-induced immune response (melanin synthesis) in *M. patula* highlights the role that this stressor may play in coral physiology.

Energetic reserves

Sediment-treated *M. patula* showed a sixfold decrease in the ratio of storage to structural lipids when compared to controls, indicating a severe depletion of energy reserves in just 24 h. This was not influenced by changes in *Symbiodinium* density and/or contributions as illustrated by the absence of change in zooxanthellae density or PAM-estimated photosynthetic efficiency (data not shown). This contrasts with results obtained by Philipp and Fabricius (2003) (having applied double the amount of sediments and allowed longer settling periods), and it would therefore be interesting to see whether such changes would occur over longer stress periods using similar sediment types and loadings. In this case, the observed energy depletion was likely the result of tremendous energy expenditures for sediment removal (such as through mucus production) and immune upregulation (Riegl and Branch 1995; Armitage et al. 2003). Energy depletion is, in turn, likely to limit resources available for other key life history traits, potentially resulting in reduced spawning success (Szmant and Gassman 1990) and growth (Suzuki et al. 2000). These results are in agreement with existing long-term data, (Saunders et al. 2005; Flores et al. 2012), but suggest that such changes in energy storage may happen within a matter of hours (24 h) as opposed to weeks as previously thought (Saunders et al. 2005; Cooper et al. 2009). Assessing the ratios of neutral to polar lipids could therefore be used as a proxy for measuring the effects of both short and long-term sub-lethal sedimentation stress on corals. While measuring solely these lipid ratios does not represent a complete estimate of the energetic budget and reserves (Lesser 2013), it nevertheless provides evidence of recruitment of the lipid fraction of energy reserves.

Metabolic effects

Sediment-stressed corals showed a tendency towards slight, though not significant, increases in respiration (during the day) and lower photosynthesis (during the night) when compared to controls, as displayed by reduced

net O_2 flux and *P/R* ratios. This could be because unaffected zooxanthellae (*cfr.* Energy reserves section) kept photosynthesising efficiently, and though the coral host may have been consuming more oxygen, the effect on photosynthesis/respiration remained nonsignificant. However, the literature suggests that this lack of significance is a result of the short duration and/or insufficient intensity of the sediment stress applied. Existing data show that much higher sediment dosage and/or stress duration than those used in the present study are required to significantly affect photosynthesis and respiration (Philipp and Fabricius 2003; Piniak 2007), highlighting that sedimentation stress on corals is both dose and time dependent (Philipp and Fabricius 2003). Alternatively, it could be that under sediment stress, gas exchange between the water column and the coral surface is reduced (through sediment deposition); this could in turn lead to anaerobic conditions within the coral tissue (Weber et al. 2012). As a result, anaerobic pathways of energy recruitment could be promoted. Although carbohydrates tend to be used preferentially as an energy source under such conditions, anaerobic lipid metabolism pathways have also been described (Monroig et al. 2013).

Ecological implications and conclusions

Our findings show that corals mount a significant immune response when subjected to ecologically relevant levels of sedimentation. However the upregulation of immunity, together with that of sediment-shedding mechanisms, incurs significant energetic costs, which can cause the depletion of coral energy reserves over both the short (this study) and long (Flores et al. 2012) term. As can happen following salinity stress (Lirman and Manzello 2009), the resulting energy depletion could impair sediment-shedding mechanisms, resulting in sediment accumulation, hypo/anoxic stress, and eventually bleaching and mortality (Weber et al. 2006, 2012). Similarly, energy depletion following sedimentation stress could result in a weakening of the immune system, reducing the capacity of corals to resist infections, and increasing disease prevalence. This would be in agreement with Anthony et al. (2009) who suggested that energy depletion following stress may increase the intensity of the effects resulting from subsequent stress (e.g., bleaching). Future research should therefore focus on the relationship between energy depletion following sedimentation stress and the impact that this may have on subsequent coral infection rates. While in the present study, we describe fast responding markers of sedimentation stress on a susceptible coral species, comparing the difference in tolerance mechanisms between susceptible and resistant species would also be an important future direction to explore. In particular, adaptive mechanisms such as shifts towards heterotrophy during sedimentation plumes, the rapid replenishment of energy reserves once conditions improve, and temporary upregulation of photosynthetic efficiency (Anthony and Larcombe 2000) should be priority research objectives to better our understanding of coral tolerance to environmental stress.

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