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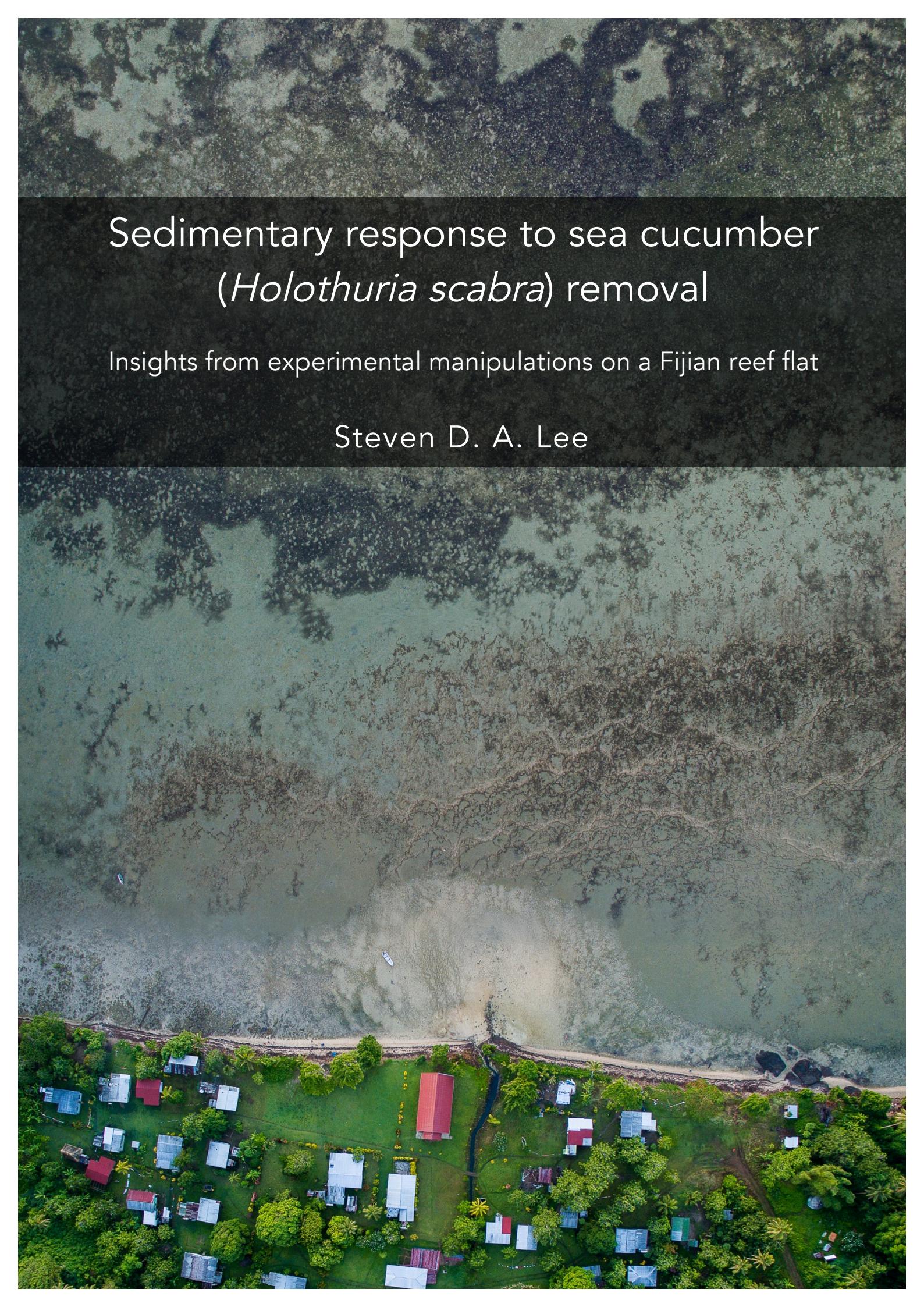


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The background image is an aerial photograph of a coastal landscape. At the top, a dark, textured area represents a reef flat or seagrass bed. Below it, a lighter green area shows a sandy beach and shallow water. In the bottom foreground, there is a cluster of small houses with red roofs, surrounded by lush green vegetation and trees. A narrow path or road leads from the village towards the coast.

Sedimentary response to sea cucumber (*Holothuria scabra*) removal

Insights from experimental manipulations on a Fijian reef flat

Steven D. A. Lee



Master of
Science Course

International Studies in
Aquatic Tropical Ecology

Sedimentary response to sea cucumber (*Holothuria scabra*) removal

Insights from experimental manipulations on a Fijian reef

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Presented to the University of Bremen, Faculty for Biology and Chemistry

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Bremen, August 2016

GEFÖRDERT VOM



Statement according to §10 (11) Allgemeiner Teil der
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1 ABSTRACT

Bioturbation through reef ecosystem engineers such as sea cucumbers can potentially enhance recycling of organic matter (OM) in reef sands. Benthic-pelagic coupling demonstrates that changes to the water column influence sediment properties as well as that sedimentary processes can affect the quality of overlying water. Coastal ecosystems are increasingly stressed by OM enrichment from human activities such as farming and sewage discharge. Thus the ability of sediment to degrade OM efficiently is vital to the buffering capacity of coastal ecosystems. However, growing demand from Asian markets is driving the overexploitation of sea cucumbers. Consequently, there is a need to understand the impact of their removal on reef ecosystems. This thesis aims to develop an understanding of how the removal of sea cucumbers affects sedimentary function in a biocatalytical recycling system. *Holothuria scabra* was historically found in high densities and exhibit a natural burrowing cycle thus playing a key role in bioturbation. Densities of *H. scabra* were manipulated in enclosures *in situ* on a reef flat at Natuvu Village on Vanua Levu, Fiji to simulate different fishing intensities. Two treatments ($n=4$ treatment $^{-1}$) were used; high density (350 g m^{-2}) and exclusion (0 g m^{-2}), and two controls accounted for cage effects; cage control (no cage walls) and natural (60 g m^{-2}). Sedimentary oxygen consumption (SOC), grain size distribution, porosity, and oxygen (O_2) penetration depth were recorded. Sediment reworked through ingestion by *H. scabra* was quantified over the course of 2 weeks. After incorporating spatial surveys of *H. scabra*, our calculations show that the current population on Natuvu's reef flat has the potential to rework ca. $7850 \text{ kg sediment dry wt year}^{-1} 1000 \text{ m}^{-2}$. SOC rates were consistently higher in exclusion than high-density enclosures. O_2 penetration depth decreased significantly when the stressors of both sea cucumber removal and elevated sea temperatures (likely caused by the 2016 El Niño) were combined. Thus removal of sea cucumbers may reduce the capacity of reef sediment to buffer negative effects of OM pulses, impeding the function and productivity of reef ecosystems. Consequently Pacific island communities are left more vulnerable as the ecosystem services that they rely heavily on for their livelihood may be compromised as a result of the extensive overfishing of sea cucumbers.



INTRODUCTION

2 INTRODUCTION

2.1 Holothuroidea biology and ecology

Holothuroidea (sea cucumbers) are benthic invertebrates that belong to the phylum Echinodermata. There are 1693 species belonging to the class Holothuroidea (WoRMS 2016), of which about 300 species inhabit shallow waters (<20 m) of the Indian and Western Pacific oceans (Preston 1993). Sea cucumbers are found throughout the world's oceans and seas, from polar to tropical latitudes, and from the intertidal zone to deep-sea trenches. Most species of sea cucumbers inhabit soft bottom sediment, living on or buried in the sediment (Purcell et al. 2012). The majority of commercial sea cucumbers exist as separate sexes, however a few species are hermaphroditic. Sea cucumbers are predominantly broadcast spawners i.e. they release their gametes directly into the water column. Accordingly the Allee effect influences their fertilization success (i.e. a higher density of sea cucumbers spawning is positively associated with a higher recruitment rate). Spawning is normally triggered by environmental and chemical cues from the same species (Purcell 2010). Sea cucumbers can be categorized based on their predominant feeding strategies: suspension or benthic feeders. The majority of sea cucumbers are deposit feeders, which consume benthic detritus including decaying organic matter as well as microalgae, and microorganisms (Yingst 1976; Hickmann et al. 2006; Plotieau et al. 2013). Some sea cucumbers use their tentacles to capture suspended particles such as *Psolus chitinoides* or move sediment into their mouth such as *Stichopus chloronotus* (Graham and Thompson 2009). Historically sea cucumbers were found in relatively high densities on reef flats e.g. Papua New Guinea mean density of *Holothuria scabra* 2900 individuals ha⁻¹ and *Actinopyga echinates* 1800 individuals ha⁻¹ (Shelley 1981). Thus sea cucumbers were a dominant component of the benthic fauna on marine soft bottom habitats.

2.2 Commercial use of sea cucumbers

Sea cucumbers have been fished since the 1800s from Pacific islands (Ward 1972; Hair et al. 2016). The majority of fished sea cucumbers are processed into a product known as

bêche-de-mer or trepang (Kinch et al. 2008), by a simple process involving salting and drying (Ram et al. 2008). The largest consumers of sea cucumbers by far are the Chinese, followed by other south east Asians such as Singaporeans, Vietnamese, Koreans, Malaysians, and Japanese (Purcell 2014b). Sea cucumbers are a delicacy of Chinese fine cuisine (Fabinyi 2011) and traditional Chinese medicine claims sea cucumbers have special healing properties (Bordbar et al. 2011).

If properly processed, bêche-de-mer is a dry, non-perishable commodity (Ram et al. 2008). As a result of its non-perishability, ease of transport, and high market value (Kinch et al. 2008), the bêche-de-mer trade forms the backbone of many remote coastal communities, providing a critically important economical contribution for Pacific island communities (Kinch et al. 2008). For example bêche-de-mer is the second-most valuable marine resource, after tuna, to the national economy of the Solomon Islands (Ramofafia 2004).

2.3 Overexploitation of sea cucumbers for the bêche-de-mer trade

The sea cucumber trade in the Pacific is characterized as having a high value product that is high in demand and centered around a poorly regulated fishery (Kinch et al. 2008; Carleton et al. 2013 Purcell et al. 2014; Purcell 2014b). Consequently, this has led to intense exploitation throughout the Pacific for many decades, depleting stocks and even forcing the closure of fisheries (Kinch et al. 2008; Anderson et al. 2011; Purcell 2014b). Several high value species such as *Apostichopus japonicus*, *Isostichopus fuscus*, *Holothuria lessoni*, and *Holothuria scabra* are already endangered, and a considerable number of species that are commercially harvested are data deficient (Purcell et al. 2014). Given that sea cucumbers have been commercially fished in the Pacific for almost 200 years (Ward 1972; Hair et al. 2016) reliable baseline data is difficult to find. In the Torres Strait, Skewes et al. (2000) reported *H. scabra* stocks declined by 53 – 81% between 1995 / 1996 and 2000, the *H. scabra* population in 1995 was already considered overexploited. In the Autonomous Region of Bougainville, Papua New Guinea the population of sea cucumbers were surveyed in 1992 and 2008. Over this 16 year period,

six of the eight species observed in both years had declined by 95 – 99% of their 1992 abundances (Hamilton and Lokani 2011), highlighting the rapid rate at which sea cucumbers are being removed from marine ecosystems.

Holothuria scabra are one of the highest value tropical species in the bêche-de-mer trade, fetching prices of up to US\$ 1668 kg⁻¹ (Purcell et al. 2014). *H. scabra* is also one of the few species that can be reliably cultured (Battaglene et al. 1999; Hair 2008; Purcell et al. 2012), *H. scabra* therefore has the potential to provide a sustainable source of income to Pacific island communities (PICs).

2.4 Sea cucumbers as ecosystem engineers

Sea cucumbers predominantly inhabit soft bottom sediments (Purcell et al. 2012), therefore interacting directly with and influencing the quality of bed sediment. Permeable sediments, particularly carbonate sands, are able to efficiently trap organic matter (OM) and operate as a biocatalytical filter as it provides a medium for the sediment community to efficiently degrade OM (Rusch et al. 2006). Thus permeable sediment plays crucial role in a biocatalytical recycling system in the marine environment (Wild et al. 2004).

Through ingestion and movement sea cucumbers bioturbate marine sediment (Uthicke 1999). Bioturbation of marine sediments alters geochemical gradients, modifies nutrient fluxes, and redistributes food resources (Meysman et al. 2006; MacTavish et al. 2012). Consequently bioturbators are able to enhance ecosystem function through inducing these complex biogeochemical interactions (Lohrer et al. 2004). Organisms that modify, maintain, and create habitats are ecosystem engineers (Jones et al. 1994), thusly sea cucumbers in the high densities they were previously found can be considered ecosystem engineers. *H. scabra* exhibits a natural burrowing cycle (Mercier et al. 1999), playing a key role in bioturbation.

Benthic-pelagic coupling implies that changes in overlying water quality are integrated and reflected in sediments, and correspondingly changes within sediments affect the

overlying water quality (Wild et al. 2004). Sedimentary metabolism plays a significant role in OM degradation and remineralisation, thus benthic-pelagic coupling is of particular significance after a pulse of increased OM (Wild et al. 2004; Wild et al. 2005; Rusch et al. 2006; Wild et al. 2008). The buffering capacity provided by functioning sediments towards OM pulses becomes increasingly important as coastal environments face threats of intensifying land-derived nutrient and OM input (Maragos 1972; Barnes 1973; Mosley and Aalbersberg 2003).

Sea cucumbers are able to bioturbate a considerable amount of sediment through ingestion alone (Uthicke 1999), in doing so they are able to alter the grain size composition of carbonate sediment (Hammond 1981). Despite these changes to the grain size composition being “geologically insignificant” (Hammond 1981) they could potentially alter pore water transport. Current state of the art suggests that grazing and bioturbation by sea cucumbers may play a functional role in maintaining the operation of permeable sediment as a biocatalytical filter. Grazing by sea cucumbers reduces microalga biomass and OM concentration in sediment (Uthicke and Karez 1999; Michio et al. 2003). In doing so, sea cucumbers excrete and remineralize nutrients; primarily Ammonium (NH_4^+) (Uthicke 2001a). An after-effect of remineralizing nutrients by sea cucumbers is enhanced primary production (Uthicke 2001b), which may subsequently contribute to reducing nutrient concentrations, as well as absorbing carbon dioxide (CO_2). Sea cucumbers are able to shift the microbial balance in organically enriched sediment, further enhancing mineralization and nutrient cycling (MacTavish et al. 2012).

2.5 Research gaps

Several studies have investigated the role of sea cucumbers in a benthic recycling system based on how sea cucumbers directly affect components of the benthic environment such as OM concentration and benthic primary production (Refer 2.4). However few studies approach the question from a sediment perspective i.e. how does the presence and absence of sea cucumbers affect sediment characteristics and function in a biocatalytical recycling system. Even fewer studies investigating the role of sea cucumbers on sediment

function are conducted *in situ* and those of which are, are often for relatively short time scales (less than a month). Considering the complex biogeochemical interactions within sediment (Herman et al. 1999) and the extensive overexploitation of sea cucumbers, there is a need for long-term *in situ* studies focusing on effects of sea cucumber removal on sediment function. In marine systems, removal of a single key species can have extensive biogeochemical implications to that system (Lohrer et al. 2004), implying that removal of sea cucumbers could ultimately interfere with critical ecosystem services that Pacific island communities rely on. Sediment turnover by sea cucumbers is a useful and tangible way of providing scale to the bioturbation potential of sea cucumbers (Uthicke 1999), however this data does not exist for *H. scabra* despite this species being one of the most abundant on reef flats and shallow lagoons (Preston 1993).

2.6 Research objectives

The study focused on the deposit-feeding sea cucumber *H. scabra*, which according to historical accounts was commonly found in high densities (2900 ind. ha⁻¹) on reef flats throughout the Pacific (Ward 1972; Preston 1993), is of commercial interest for both the export bêche-de-mer market and local Fijian markets, and has successfully been cultured. The current study aimed to investigate the role of *H. scabra* in maintaining sediment function as a biocatalytical filter system, and thereby to quantify their effect on grain size distribution, sediment porosity, O₂ penetration depth, and sedimentary O₂ consumption (SOC) *in situ*. Additionally, we quantified the amount of sediment bioturbated by *H. scabra* through ingestion in order to provide scale to the bioturbation effect provided by this species. We hypothesized firstly that high densities of sea cucumbers would cause (i) a shift in sediment composition towards larger grain size fractions, (ii) an increased sediment porosity, (iii) an increased O₂ penetration depth, and (iv) would increase and maintain high SOC rates. Densities of *H. scabra* were manipulated *in situ* to simulate high densities and removal; parameters were monitored for six months.



MATERIALS AND METHODS

3 MATERIALS AND METHODS

3.1 Site selection

Between August 2015 and February 2016 fieldwork was conducted on an extensive reef flat in front of Natuvu village, Wailevu East District, Vanua Levu, Fiji ($16^{\circ} 44.940'S$ $179^{\circ} 0.280'E$) (Refer Figs. 1 and 2). The site was selected because it was identified by Pakoa et al. (2013) and Wildlife Conservation Society as having a relatively high *Holothuria scabra* density for the region, which we assumed would be closer to baseline densities.

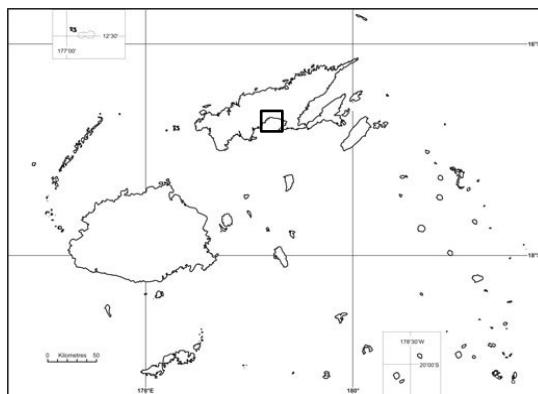


Figure 1: Map of Fiji, black rectangle indicates the site. Natuvu, Wailevu East District, Vanua Levu, Fiji. Map modified from naturefiji.org



Figure 3: Satellite image of the Natuvu reef flat. White rectangle shows the extent of the reef flat and Natuvu's *qoliqoli* (traditional fishing grounds). Google Earth

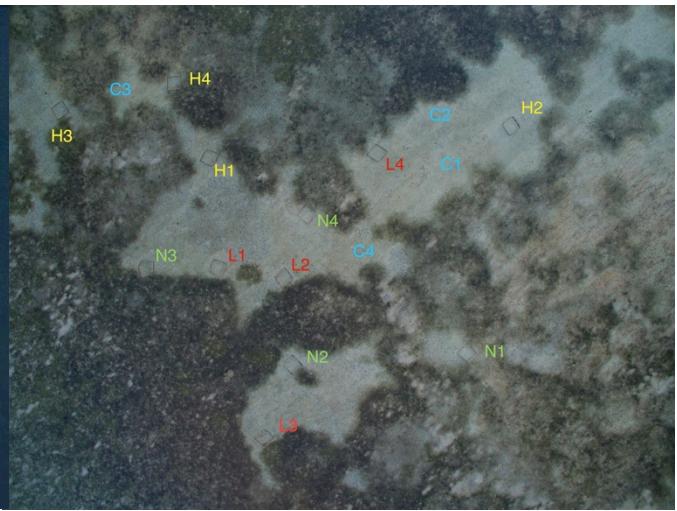


Figure 3: Aerial image showing positions of enclosures at our site. Each colour represents a treatment; each treatment had four enclosures (1 - 4). Yellow = High density, Red = Exclusion, Green = Natural control, Blue = Cage control

3.2 Enclosure design and construction

Sixteen square enclosures ($3\text{ m} \times 3\text{ m}$) were constructed on the reef flat (Refer Fig. 3): three treatments and one control ($n=4$ treatment $^{-1}$). High-density ca. 350 g m^{-2} , exclusion (0 g m^{-2}), natural control (ca. 60 g m^{-2}), and cage controls, which had no walls/mesh. Natural density (ca. 55 g m^{-2}) was determined in a pilot study at the enclosure site, this biomass equated to ca. 3000 individuals ha^{-1} . High density (350 g m^{-2}) was modeled against high and maximum stocking biomass for *H. scabra* used in previous studies for sea pens or at natural ranching sites; 650 g m^{-2} in Madagascar (Lavitra et al. 2010), 225 g m^{-2} in Solomon Islands (Battaglene et al. 1999), 300 g m^{-2} in Tanzania (Namukose et al. 2014). Enclosures were monitored six days a week for any signs of damage or disturbance and to maintain stocking densities, a length-weight relationship was established (Refer 9.1.4) to allow restocking enclosures without the need to weight individual *H. scabra*.

Enclosures were constructed out of eight 1.5 m pieces of 16 mm steel re-bar driven vertically into the sediment until ca. 70 cm remained exposed. High-density polyethylene (HDPE) diamond mesh with a 40 mm aperture formed the enclosure walls. The mesh was driven ca. 20 cm into the sediment, leaving ca. 80 cm exposed. Enclosures were designed according to recommendations by Miller and Gaylord (2007) in order to minimise cage effects. Enclosure construction was completed on the second week of September after initial measurements ($t=0$, September); the experiment ran from September 2015 to February 2016. Weather was monitored throughout the course of the study using national and regional weather reports, and reports from villagers in Natuvu.



Figure 4: Enclosures at low tide.

3.3 Sediment turnover

Sediment turnover is the quantity of sediment defecated by *H. scabra*; this was used as proxy for how much sediment is being reworked/bioturbated by *H. scabra*. Sediment turnover was studied over the course of two weeks in January 2016 for the three size classes (8 – 10, 11 – 13, 14 - 16 cm) of *H. scabra*, and covering day and night, flood and ebb tide, and three habitats: sand, *Halodule* spp. bed (*Halodule* spp.), and *Syringodium* spp. bed (*Syringodium* spp.). The method used to quantify sediment turnover was based off *in situ* methodology for a similar study by Uthicke (1999). The quantity of sediment defecated during 24 h was measured *in situ* using 28 individuals of *H. scabra* (mean length = 13 cm ± 0.22 SE; mean mass = 145 g ± 6.27 SE).

Trails of pellets/sediment sausages behind *H. scabra* was used to quantify sediment defecated. Markers made using Number 8 wire (4.0 mm gauge) were driven vertically into the sediment at a standardized distance (ca. 1 cm) behind the caudal end of the animal. If the animal appeared disturbed (e.g. contracted its body or stopped feeding) then another was chosen. Approximately every hour the animal was relocated, and an additional marker placed at its caudal end. After three hours the third and final marker was placed. The animal's length and weight were recorded (for method refer 9.1.4). Time was recorded each time a marker was placed; and the behavior (feeding or burrowing) was noted after placing the last marker. Surveys were conducted from mid to high, or mid to low tide, during day and night, in a stratified fashion in order to compare the influence of these factors on feeding intensity.

Six pellets per sea cucumber were collected in individual polymerase chain reaction (PCR) tubes. The number of pellets between each marker (wire marker driven into sediment behind *H. scabra*) was counted, and the distance between each marker was measured to the nearest centimeter. Sediment pellets were placed in a dry oven at 70°C for approximately 12 h before weighing on an analytical balance (± 0.02 g).



Figure 5: *Holothuria scabra* feeding and a trail of sediment pellets.

In order to model sediment turnover by *H. scabra* the quantity of ingested sediment was assumed equal to the quantity of defecated sediment (Hammond 1981; Uthicke 1999). Mean pellet mass ($0.36\text{g} \pm 0.0095$) was multiplied by the number of pellets defecated per hour (26 ± 1.78) providing the mean mass of sediment perturbed per sea cucumber per hour. It was observed that *H. scabra* actively feed for ca. 10 h day^{-1} ; they only fed during day light hours (12 h in Fiji), and remained buried for an hour before and after low tide. Additionally habitat preference, population structure and density, and diurnal and tidal abundance were quantified for *H. scabra* at the site (Refer 9.1).

3.3.1 Bulk density (Enclosure sediment)

The bulk density of sediment in enclosures was required in order to quantify the mass of sediment *in situ m⁻³*. Dividing the mass of sediment turnover animal⁻¹ and bulk density of sediment *in situ m⁻³* provides us with the area of sediment-reworked m⁻³.

Bulk density of enclosure sediment was calculated by dividing the volume of a sediment core (30 cm^3) by the mass of dry sediment contained within the core n=3 cores enclosure⁻¹. Sediment cores collected from each enclosure were kept together in airtight containers ($90\text{ cm}^3 \pm 0.56$). Sediment dry weight ($75.34\text{ g} \pm 0.56$) was determined by washing the sediment into an aluminum tray using distilled water then drying it in a dry oven at 70°C for 24 h. Bulk density ($0.83\text{ g cm}^{-3} \pm 0.01$) of sediment was calculated as:

$$\text{Mean dry mass of sediment (g) / Mean volume of sediment core (cm}^3\text{)}$$

3.4 Grain size distribution

Sea cucumbers can alter grain size distribution (Hammond 1981; Schneider et al. 2011). The composition of sediment's grain size plays a role in sediment oxygen dynamics, therefore changes to the grain size distribution caused by altered densities of sea cucumbers may account for changes in sediment function. Grain size analysis of the grain size distribution quantifies sorting, skewness, and kurtosis, the relationships of these parameters can help to explain how sediment is being changed, and thus how changes in sediment may be affecting sediment oxygen dynamics (Masch and Denny 1966; Urumović and Urumović 2014).

After dry sediment samples were weighed, they were transferred to a column of sieves (Refer Table 1). Sediment trays were opened up and any remaining sediment on them was brushed onto the sieve. The columns of sieves were shaken for seven minutes in a sieve shaker. Sediment in each sieve was collected by emptying the sieve's contents and brushing out any remaining contents onto an aluminum sheet and then transferring sediment onto a pre-weighed tray, which was then, weighed using an analytical balance (± 0.02 g).

Grain size analysis, textural classifications and distribution of sediments was done on R Studio using the `gran.stats` function of the 'rysgran' package (Eliandro et al. 2015) based on methods and verbal classifications by Folk and Ward (1957). Sorting describes the homogeneity of grain size within sediment and how widely scattered these are about the mean. Lower sorting values (expressed as Phi Φ) means the grain sizes within sediment are more homogenous, where as higher values indicate the spread is more heterogenous. Skewness describes the shape of the grainsize distribution; higher skewness values indicate sediment is skewed towards finer grain sizes, where as lower skewness values indicate sediment is skewed towards larger grain sizes. Kurtosis describes the flatness or peakness of the grain size distribution, lower values indicate a more platykurtic (flat) distribution and higher values indicate a more leptokurtic (peaked) distribution.

Table 1: **Sieve fraction and size of sediment retained.** x = size of sediment retained by sieve

Sieve fraction/size (μm)	x (size of sediment retained (μm))
≥ 2000	≥ 2000
1000	$2000 > x \leq 1000$
500	$1000 > x \leq 500$
250	$500 > x \leq 250$
125	$250 > x \leq 125$
<125	<125



Figure 6: **Sediment cores stored in an airtight container.**

3.5 Porosity

Porosity is the measurement of void (empty) spaces within the sediment; hydraulic conductivity is the ease with which a fluid can move through pore spaces and is generally considered being dependent upon porosity and sorting (Morin 2006; Urumović and Urumović 2014). As porosity increases the hydraulic conductivity of coarse-grained sediment monotonically increases (Nelson 1994) i.e. the ease of water to flow through the bed sediment increases in steps. Subsequently the ability of water to flow through sediment can influence oxygen penetration depth and oxygen consumption rates of sediment (Kristensen 2000).

The procedure used to determine porosity of the sediment samples was based on methods by Case and Hnatow (2007). Ca. 20 cm³ sediment cores were collected, n=3 enclosure⁻¹. Sediment cores were placed into airtight chambers. Sediment was washed out of the jars into aluminum foil trays using distilled water, and dried in a dry oven at 70°C for ca. 24 h. Sediment samples were removed from the oven and immediately weighed on an analytical balance (± 0.02 g).

Mass of dry sediment was determined as:

$$\text{Mass of tray with dry sediment} - \text{mass of tray}$$

Mass of wet sediment was determined as:

$$\text{Mass of wet sediment} - \text{mass of dry sediment}$$

Porosity was determined as:

$$\text{Mass of water in wet sediment} / \text{Mass of dry sediment}$$

3.6 Oxygen penetration depth

The penetration depth of oxygen into sediment determines the REDOX reactions occurring within that sediment (anoxic vs. oxic respiration), and thus the efficiency of OM remineralization. Consistently dark sediment and a “rotten egg” smell indicate sulphate reduction by sulphate reducing bacteria, producing Hydrogen Sulphide (H_2S), which is symptomatic of anoxia (Castro and Huber 2012).

Oxygen penetration depth was quantified based on methods adapted from Kemp et al. (2015). Ca. 30 cm^3 sediment cores were collected in 100 ml syringes (depth = ca. 30 mm), $n=3\text{ treatment}^{-1}$. Each core was photographed and the oxygen penetration depth was measured as the distance from the syringe’s plunger seal to the start of the anoxic layer (sediment is consistently darker – Refer Fig. 7).



Figure 7: Sediment core from an enclosure showing layer of consistently darker sediment (anoxic layer) – indicated by a white arrow.

3.7 Sedimentary oxygen consumption

Sedimentary oxygen consumption (SOC) measurements integrate the respiration of the entire sedimentary community contained within a core and the overlying water column. Increased SOC rates indicates higher OM degradation within sediments therefore SOC rates provide a proxy for the ability of permeable sediment to function as a biocatalytical recycling system (Wild et al. 2004; Ford et al. *in prep*).

In situ determination of SOC was based on methods used by Ford et al. (*in prep*). Sediment cores (ca. 10 cm³) were collected from enclosures and transferred immediately to glass incubation chambers (160 ml). Chambers were subsequently filled with undisturbed water from the same location (n=4 treatments enclosure⁻¹, n=3 controls enclosure⁻¹). Controls only contained undisturbed water from our site; this allowed us to account for activity in the overlying water column. Samples were placed into opaque bags and placed in an icebox filled with water from the site to maintain temperature consistency. Approximately 30 ml of water was removed from the chamber to prevent overflowing from chamber whilst measuring; oxygen (O₂) concentration was measured using a WTW O₂ sensor and salinity probe. O₂ saturation was consistently between 70 – 120% at initial measurements. Water removed (ca. 30 ml) was replaced, additional water (3-5 ml) collected from the site was used to top-up the incubation chamber to ensure the chamber was sealed airtight with no air bubbles. Chambers were incubated in the corresponding opaque bags, in an icebox filled with water from the site for ca. 1 h. Chambers were collected and O₂ concentration was remeasured. Salinity and temperature within the chambers were remeasured to ensure consistency throughout measurements. The exact duration (minutes) of each incubation was recorded.

3.8 Salinity and temperature

Salinity and temperature measurements allowed us to monitor natural variation of seawater at the study site during sampling, and monitor the consistency of conditions during sedimentary oxygen consumption incubations. Salinity and temperature of water contained within sedimentary O₂ chambers prior to incubation were measured using a WTW salinity sensor (7 samples enclosure⁻¹, 4 enclosures treatment⁻¹).

3.9 Summary of statistical analysis

All statistical analysis was done using:

RStudio Version 0.99.893 (Team 2014)

Excel for Mac 2011 Version 14.6.3

All results reported are mean \pm standard error (SE) of the mean, unless reported otherwise.

Table 2: **Description of statistical functions used in R Studio**, R code for each function and respective package

Description of function	R function	Package	Reference
Akaike information criterion	step	stats	(Team 2014)
Q-Q normal plot	qqnorm	stats	(Team 2014)
ANOVA	Anova	car	(Fox and Weisberg 2011)
Repeated measures ANOVA	aov	stats	(Team 2014)
Linear model	lm	stats	(Team 2014)
Mann-Whitney-Wilcox U test	wilcox.test	stats	(Team 2014)
Student's T Test	t.test	stats	(Team 2014)
Statistical analysis of grain size	gran.stats	rysgran	(Eliandro et al. 2015)

All models in R were reduced using an Akaike information criterion. The effect of habitat and time on number of pellets defecated per hour and mean mass of pellets defecated was analyzed using a type II analysis of variances (ANOVA). Data from enclosures was modeled using repeated-measures ANOVA as samples from within individual enclosures were not independent of each other and data was tested over time. Cage controls and Natural controls were tested against each other for significant differences at each sampling date, if no significant differences were found then treatments were compared. Changes to grain size distribution between treatments were analyzed using a Chi-squared analysis. For all models a Quantile-Quantile normal plot of the residuals verified whether or not the data approximated a normal distribution. If the data assumed a normal distribution a student's *t*-test was used to test for significant differences between levels of a factor, if not a Mann-Whitney-Wilcox U test was used. All multiple comparison post-hoc tests used a Bonferroni adjustment to correct for alpha error.



RESULTS

4 RESULTS

A total of 959 *H. scabra* were found in transects at Natuvu between August and December 2015, at a density of $0.23 \text{ individuals m}^{-2}$. These values were gathered by surveying the whole reef flat. Additional data was collected on *H. scabra* population structure, diurnal and tidal abundances, habitat associations, and a length-weight relationship were quantified (Refer 9.1).

4.1 Sediment turnover

The mass of sediment pellets defecated by *H. scabra* was not affected by the habitat in which the animal was feeding (ANOVA; $F(2,15)= 2.42, p=0.12$), however the mean number of pellets defecated per hour by *H. scabra* was (ANOVA; $F(2,17)=4.39, p=0.03$). *H. scabra* defecated significantly more frequently in sand ($23 \text{ pellets h}^{-1} \pm 2$) than *Syringodium* spp. (pairwise t-test; $p=0.01$), and insignificantly more in *Halodule* spp. ($18 \text{ pellets h}^{-1} \pm 3$) than *Syringodium* spp. (pairwise t-test; $p=0.055$). Generally *H. scabra* defecated least frequently when in *Syringodium* spp ($9 \text{ pellets h}^{-1} \pm 3$) (Refer Fig. 8).

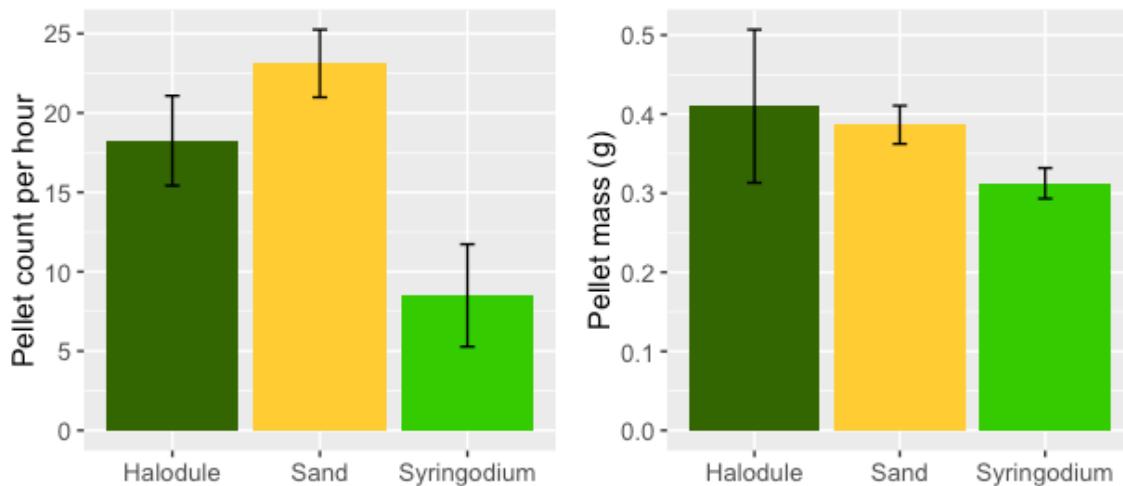


Figure 8: Number of pellets defecated by *Holothuria scabra*, and mass of individual pellets defecated, against the habitat in which the animal was grazing. Mean value with standard error of the mean.

Bulk density of sediment was $0.83 \text{ g} \pm 0.01$. The mean pellet mass and mean pellets defecated per hour for all habitats and *H. scabra* size classes were pooled to produce the following flow charts:

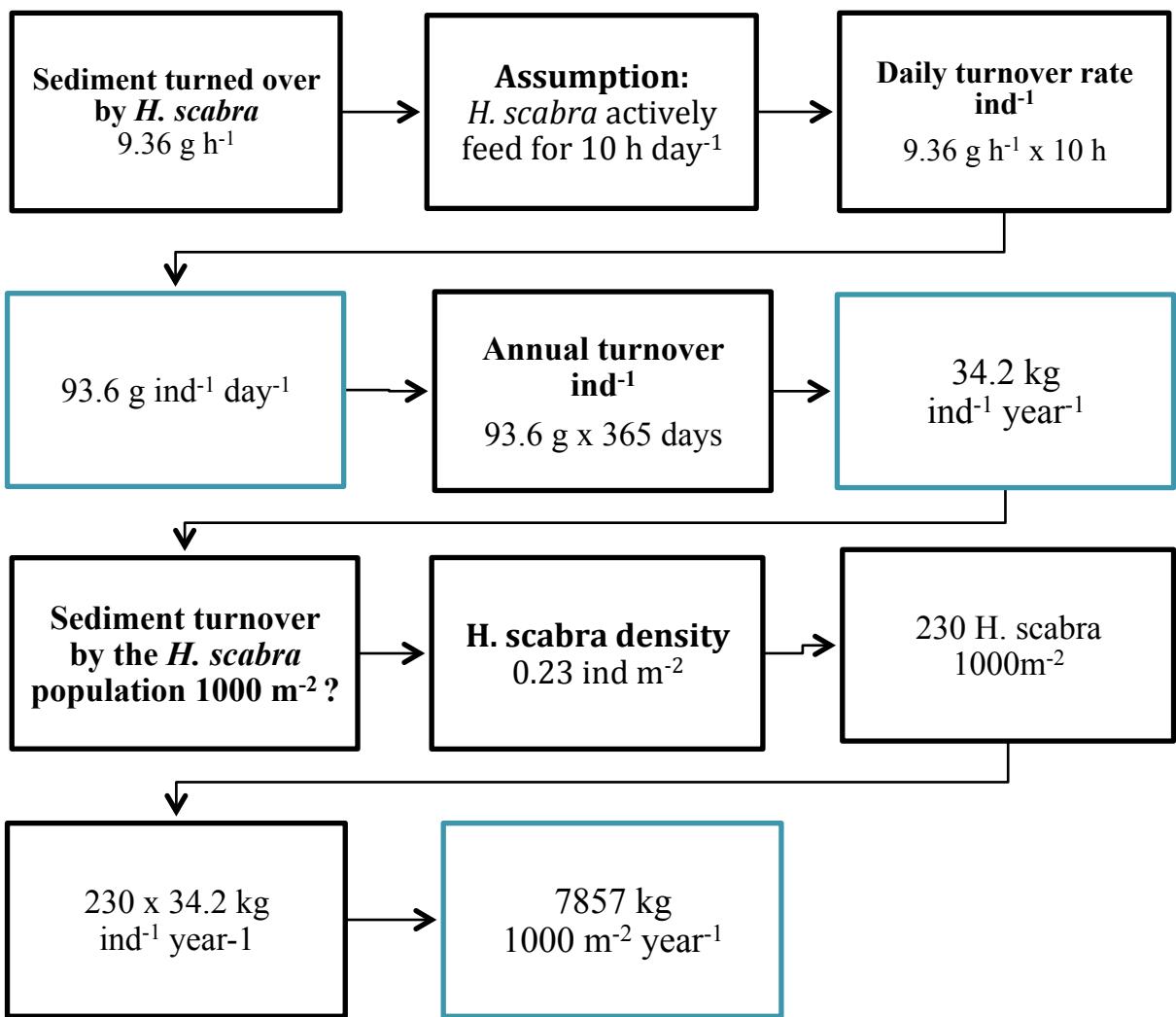


Figure 9: Mass of sediment turned over (defecated) by *Holothuria scabra*.

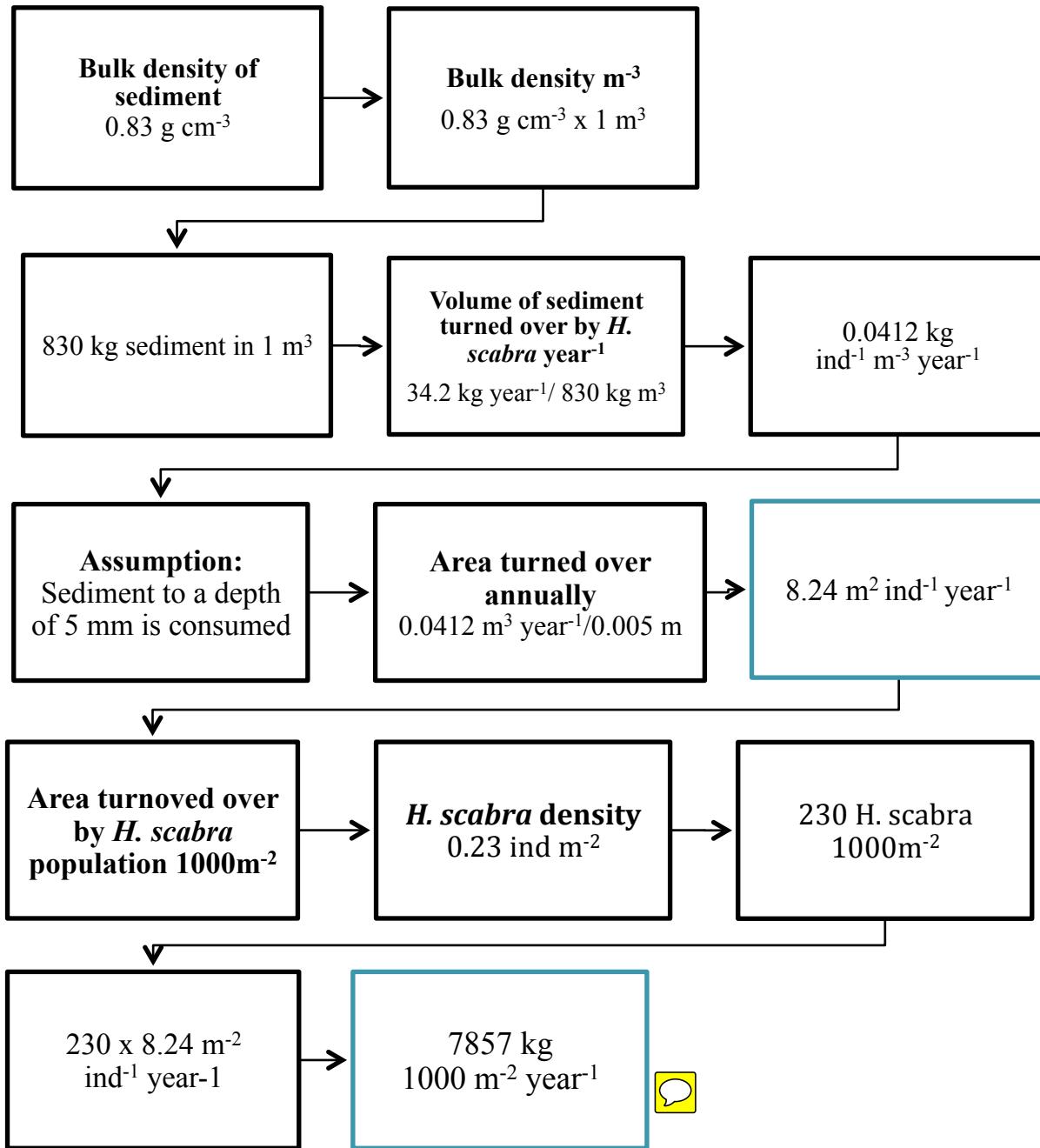


Figure 10: Volume of sediment turned over (defecated) by *Holothuria scabra*.

4.2 Grain size distribution

Through out the course of the study sediment remained poorly sorted in all treatments, had a near symmetrical skewness, and was for the most part Mesokurtic (Refer Table 3), implying grain size distribution within enclosures was not homogenous (ANOVA; $F(5,333)=878.19, p<0.01$). The grain size distribution of sediment at the study site approximated a normal distribution, and was comprised primarily of 500 – 250 μm grain. Grain size composition significantly changed with time from the onset of the experiment (ANOVA; $F(15,333)=4.71, p<0.01$)

The most noticeable change in percentage of each fraction occurred between December and January (Refer Fig. 11). A tropical depression between December 31st and January 2nd caused storm surges at the study site, which appeared to have shifted a considerable amount of sediment prior to sampling in January. Therefore results of the analysis from January and February are unlikely to be affected by treatment (i.e. caused by manipulated *H. scabra* densities in enclosures). However, it should be noted that there were no significant differences in grain size distribution between treatments in February.

Prior to any beginning the experiment (September) and three months following from the onset of the experiment (December) Cage control and Natural control grain size composition's showed no significant differences for all fractions. Between September and December, for all fractions, the grain size composition within the Exclusion treatment showed no significant changes (Chi-square test; Chisq(5)=0.23, $p=1$) (Refer 9.2 Table 10). However for the same time period there is a significant change in the grain size composition of High treatment enclosures (Chi-square test; Chisq(5)=11.25, $p=0.045$) (Refer 9.2 Table 12).

Despite no significant differences between High and Exclusion treatments in September, by December there were significant differences in the composition of two fractions; 125 μm and 1000 μm . The mean proportion of sediment within each fraction was compared; there was a significantly higher proportion of 125 μm sediment (U test; $p=0.03$) and a significantly lower proportion of 1000 μm sediment (U test; $p=0.03$) in the High treatment enclosures (Refer Fig. 11).

Table 3: **Sediment descriptive statistics**, per month, for each treatment. Results reported as mean \pm standard error of mean

	Treatment	Sorting (Φ)	Skewness	Kurtosis
September	High	1.31 ± 0.03	0.06 ± 0.04	0.87 ± 0.03
	Exclusion	1.26 ± 0.02	0.07 ± 0.03	0.90 ± 0.02
	Cage control	1.26 ± 0.04	0.04 ± 0.02	0.94 ± 0.02
	Natural	1.34 ± 0.04	0.02 ± 0.03	0.88 ± 0.01
December	High	1.33 ± 0.01	0.03 ± 0.03	0.91 ± 0.01
	Exclusion	1.24 ± 0.01	0.04 ± 0.03	0.90 ± 0.01
	Cage control	1.26 ± 0.05	0.03 ± 0.02	0.93 ± 0.03
	Natural	1.32 ± 0.06	0.05 ± 0.03	0.90 ± 0.02
January	High	1.33 ± 0.03	0.01 ± 0.04	0.92 ± 0.01
	Exclusion	1.31 ± 0.03	0.03 ± 0.03	0.92 ± 0.01
	Cage control	1.26 ± 0.04	0.03 ± 0.01	0.92 ± 0.02
	Natural	1.24 ± 0.02	0.01 ± 0.03	0.91 ± 0.01
February	High	1.34 ± 0.01	0.01 ± 0.03	0.92 ± 0.02
	Exclusion	1.30 ± 0.04	0.05 ± 0.02	0.93 ± 0.03
	Cage control	1.31 ± 0.02	0.04 ± 0.004	0.93 ± 0.01
	Natural	1.33 ± 0.03	0.05 ± 0.03	0.92 ± 0.01
Verbal description		Poorly sorted (1 - 2 Φ)	Near symmetrical (+0.10 – 0.10)	Platykurtic (0.67 – 0.90) Mesokurtic (0.90 – 1.11)

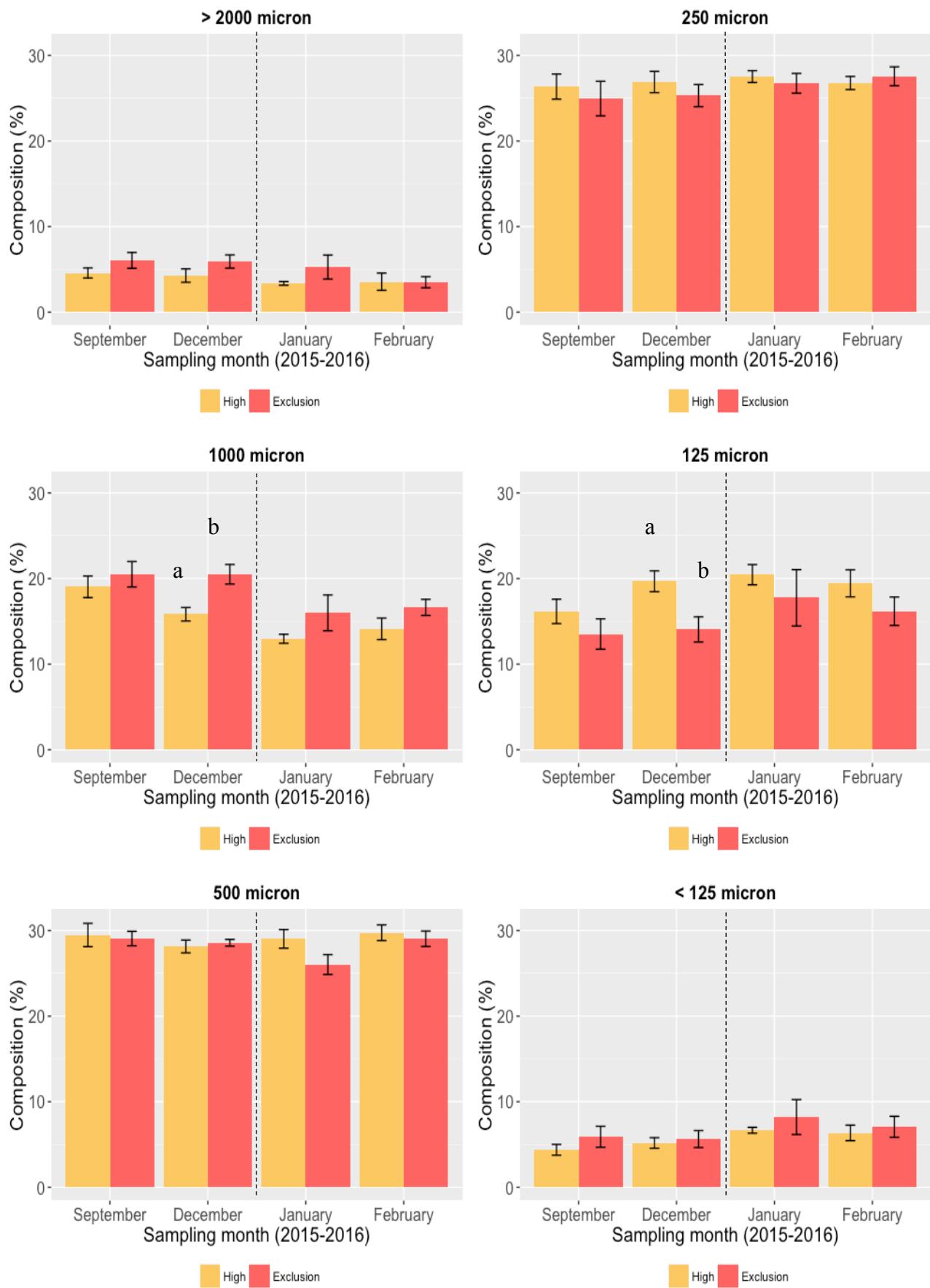


Figure 7: **Grain size distribution of sediment within high density and exclusion treatments.** Mean values with standard error. Vertical break indicates flooding and storm surge five days prior to January sampling. Letters above bars (a, b) indicate significant differences ($p < 0.05$)

4.3 Porosity

Porosity showed no significant change among treatments from December to January. Porosity changed significantly over time since the beginning of the experiment (ANOVA; $F(2,36)=3.20, p=0.05$), however there were no significant differences among treatments (ANOVA; $F(3,36)=1.05, p=0.38$).

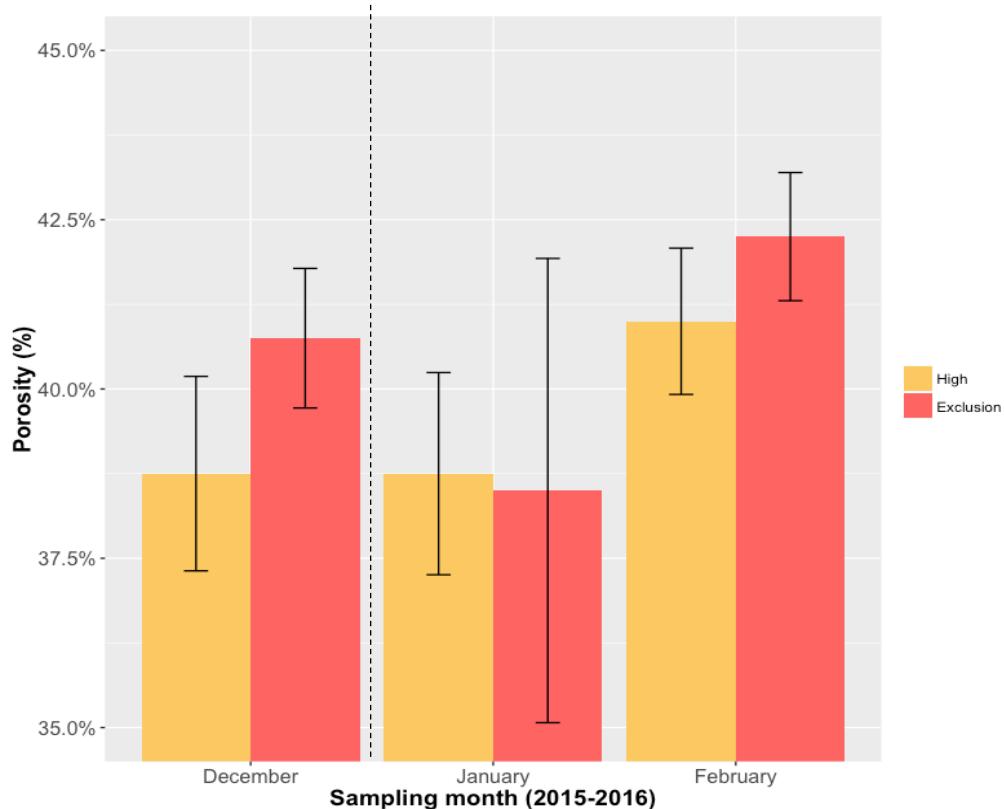


Figure 12: **Porosity (%) in enclosures of high density and exclusion treatments.** Vertical break indicates flooding and storm surge 5 days prior to January sampling. Mean values with standard error.

4.4 Temperature and salinity

Temperature increased steadily between August and December 2015 ($26.7^{\circ}\text{C} \pm 1.92$ (SD)), followed by a severe increase in January and February 2016. Salinity varied significantly ($34\text{ ppt} \pm 2.23$ (SD)) between each month. However, values should not be taken as the mean salinity per month as sampling was restricted to the same dates as SOC measurements i.e. first week of every month. Rather these values show the salinity for each treatment on a given sampling date.

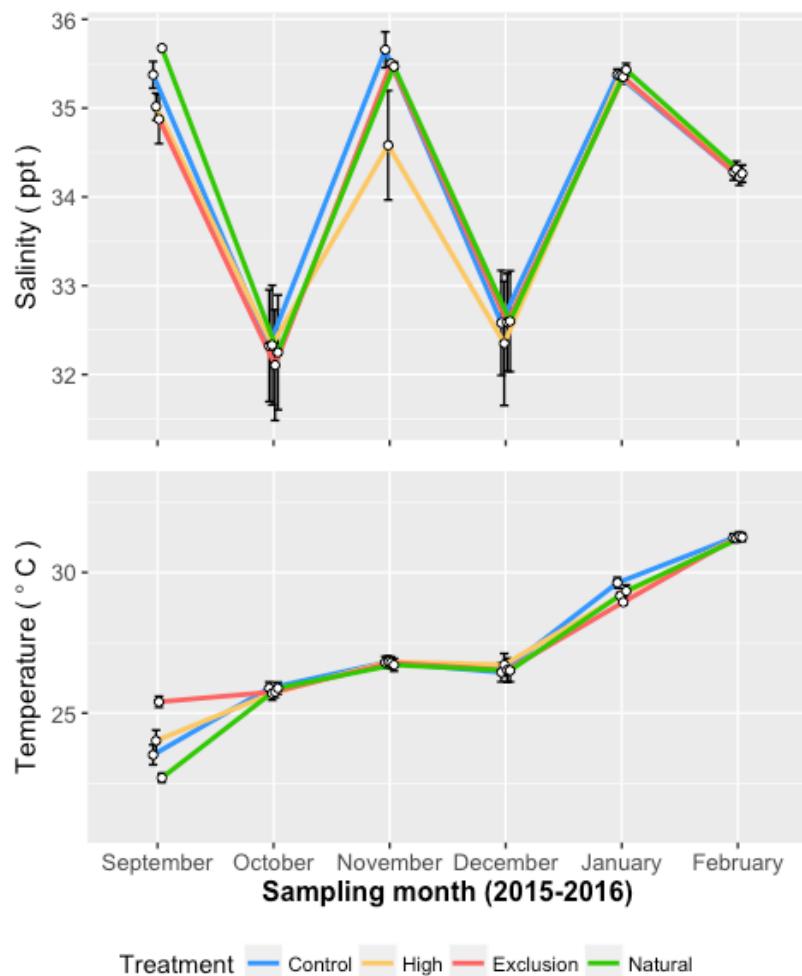


Figure 13: Top: Salinity (ppt), bottom: Temperature ($^{\circ}\text{C}$). Both parameters sampled on the first week of each month, during initial sedimentary oxygen consumption measurements

4.5 Oxygen penetration depth

Over time the O₂ penetration depth between treatments differed significantly (ANOVA; F(9,166)=3.16, $p<0.01$). O₂ penetration depths were similar between High and Exclusion treatments in November (U-test; $p=0.73$). However from November to December the O₂ penetration depth of High treatment increased significantly (U-test; $p=0.03$), for the same time period there was no change in Exclusion treatment (U-test; $p=0.37$). Despite the increased O₂ penetration depth for the High treatment, comparing the mean O₂ penetration depths showed no significant differences between High and Exclusion treatments in December (U-test; $p=0.32$).

Due to a storm prior to sampling in January, results from January are assumed to have been caused by the storm and not due to treatment (i.e. manipulated *H. scabra* densities); therefore they are excluded from post-hoc analysis. However, February shows a distinctly different pattern from prior months. Whilst the High treatment returns to November values (U-test, $p=1$), oxygen penetration depth in the Exclusion treatment decreased by 62.5% from 32mm ± 3.4 in December to 12mm ± 2.4 in February, resulting in a strongly significant difference between treatments in February (U-test, $p<0.01$).

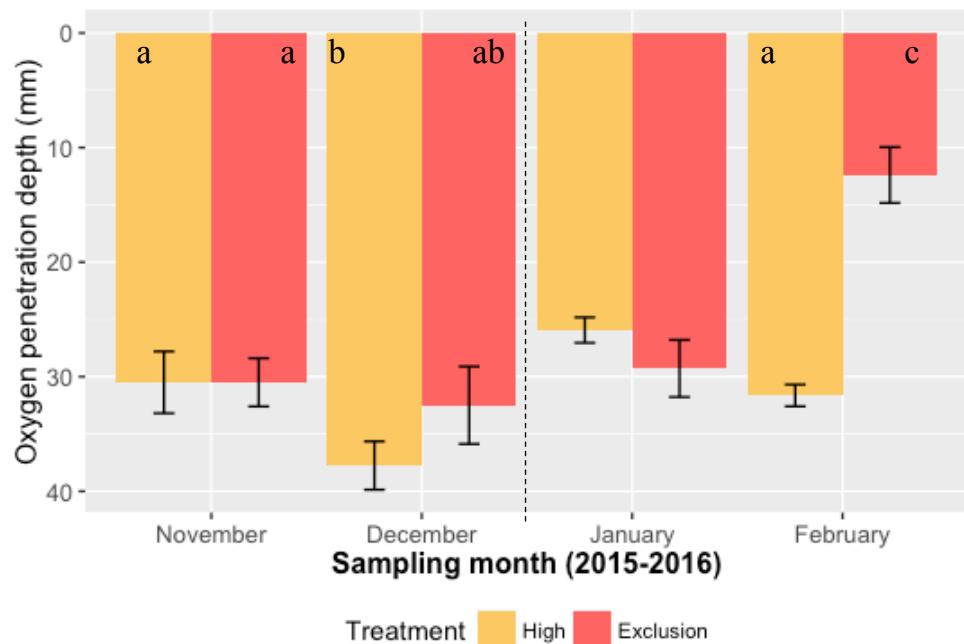


Figure 8: **Oxygen penetration depth (mm) of high density and exclusion treatments**, mean values with standard error. Vertical break indicated flooding and storm surge 5 days prior to January sampling. Different letters (a, b, c) indicate significant differences ($p <0.05$) same letters indicate no significant differences ($p >0.05$)

4.6 Sedimentary oxygen consumption

Over the course of the study sedimentary oxygen consumption (SOC) rates varied significantly between treatments (ANOVA; $F(15,360)=943$, $p<0.01$). There was a significant difference in SOC between Natural and Control for September (U-test; $p<0.01$), however there were no significant differences between Natural and Control for all other months (U-test, $p>0.05$).

Initially, both High and Exclusion treatments display similar SOC rates (U-test; September; $p=0.55$). Four weeks following manipulation, Exclusion treatment SOC rates increased significantly (U-test; September - October; $p<0.01$) from $43.03 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1} \pm 4.59$ to $75.96 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1} \pm 4.69$. No changes occur within the High treatment enclosures during the same time period (U-test, September – October; $p=0.74$).

Heavy flooding occurred less than ten days prior to sampling at the start of November (Refer Fig. 15). Following the flooding, SOC rates within the Exclusion treatment showed a decreasing, though insignificant (U-test; October – November; $p=0.38$) trend, while the High treatment increased significantly (U-test; October – November; $p=0.03$). Thus, in November there were no longer significant differences between treatments (U-test; November; $p=0.25$).

From November to December, SOC rates within the Exclusion treatment showed a slight but insignificant increase. Conversely, SOC rates within the High treatment showed a decreased trend to equivalent SOC levels as September (Refer Fig. 15). Following further heavy rains prior to sampling in January, both treatments showed similar patterns as before (i.e. SOC rates within the Exclusion treatment decreased and within the High treatment increased) to the extent that treatments were no longer significantly different (U-test, January; $p=0.99$). Consistent with the pulse pattern in previous months, both treatments separated again in February, though not to a significant level.

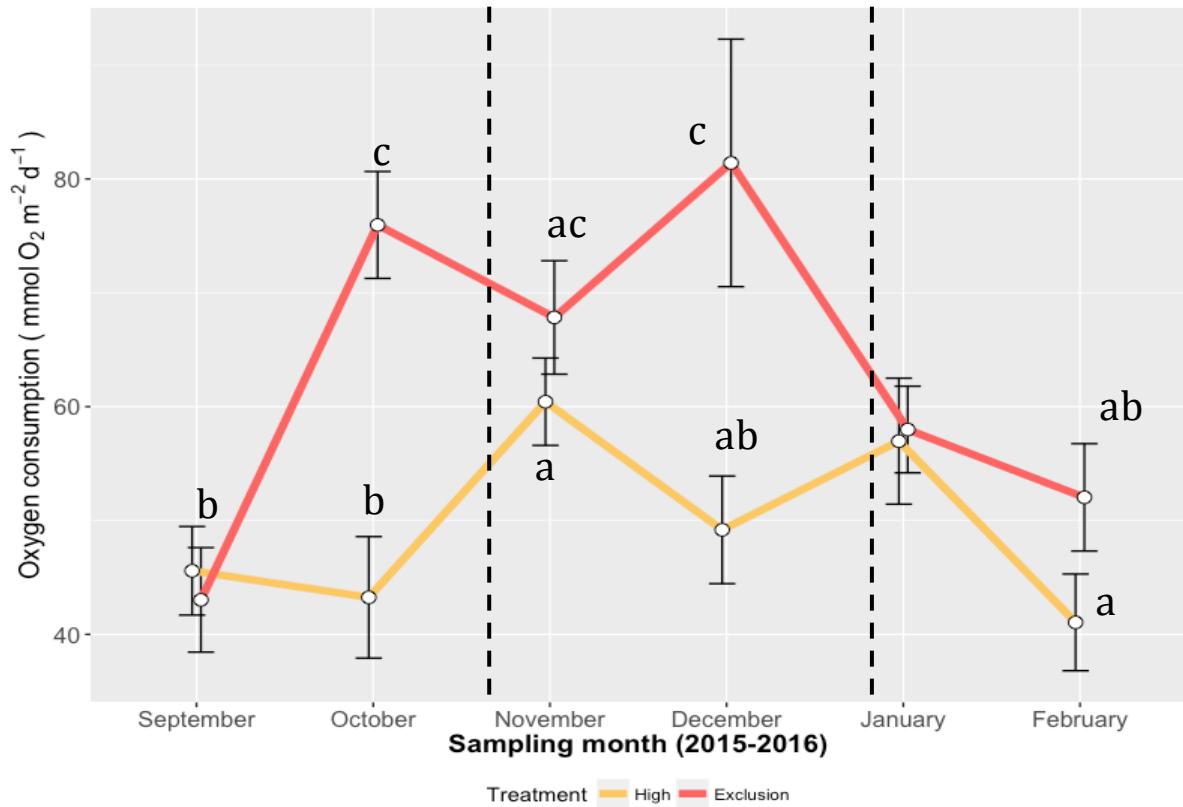


Figure 9: **Sedimentary oxygen consumption (mmol O₂ m⁻² day⁻¹) of high density and exclusion treatments**, mean values with standard error. Vertical break between October and November indicates flooding <10 days prior to November sampling, vertical break between December and January indicate flooding and storm surge 5 days prior to January sampling. Different letters (a, b, c) indicate significant differences ($p < 0.05$), same letters (a, a / b, b) indicate no significant differences ($p > 0.05$).

An aerial photograph of a small, densely forested island in a tropical lagoon. The island is covered in lush green trees and has a sandy beach along its coastline. In the harbor, numerous sailboats of various sizes are anchored. The surrounding water is a deep blue, and in the background, a range of mountains covered in green forests stretches across the horizon under a cloudy sky.

DISCUSSION

5 DISCUSSION

Throughout this discussion the terms high densities, high density, etc. and absence, removal, exclusion, void, etc. refer to High density treatment i.e. ca. 350g m^{-2} and Exclusion treatment i.e. 0g m^{-2} respectively.

H. scabra generally tended to burrow an hour before low tide and emerge an hour after. At night, the abundance of *H. scabra* was significantly lower than during day (Refer 9.1.2), and no active feeding or movement of individuals was observed at night. It is therefore assumed that *H. scabra* at the study site were active for approximately 10 h day^{-1} , similar to findings in Vonavona lagoon, Solomon Islands (Mercier et al. 2000). In order to investigate the effect of habitat on grazing intensity the mass of sediment pellets defecated, and number of sediment pellets defecated in the three most abundant habitats; *Halodule* spp., Sand, and *Syringodium* spp were quantified. Generally differences in grazing intensity were subtle among habitats. Consequently, the sediment turnover calculations presented in this thesis pool results from all three habitats and assume *H. scabra* actively fed for 10 h day^{-1} .

During the study only sediment pellets defecated by *H. scabra* were counted. However, we assume our calculations are a conservative estimate as some of the sediment defecated was released as loose sand and did not form into pellets, and results are for *H. scabra* only, despite several other species being present in the area. The sediment turnover study only took place during one season, which may affect results as several species do exhibit different feeding behaviors according to season (Dar and Ahmad 2006). Despite this, our findings show that *H. scabra* are capable of reworking a considerable amount of sediment through consumption and defecation alone – sediment turned over through burrowing was not considered (Refer Fig. 24). Deposit feeding sea cucumbers consume sediment to a depth of 5 mm (Klinger et al. 1994; Uthicke 1999). Given the density of *H. scabra* at our site ($0.23\text{ individuals m}^{-2}$) we calculated that the population of *H. scabra* in an area of 1000 m^2 is capable of turning over the upper 5 mm of sediment almost twice a year. The rate at which *H. scabra* reworks sediment is considerably more than *Stichopus*

chloronotus and *Holothuria atra* (Uthicke 1999), highlighting a heterogeneity in the bioturbation potential among sea cucumbers thusly the need for more species specific studies.

The lack of any significant changes to grain size distribution in the absence of *H. scabra* indicates that sediment was not significantly reworked when sea cucumbers are excluded from the ecosystem. Under high densities of *H. scabra* sediment composition shifted to finer fractions; reduced composition of 1000 µm and an increased composition of 125 µm in December (Refer Fig. 11). Sea cucumbers may thus play a critical role in the physical reworking and change of sediment structure. Due to storm surge affecting the study site five days prior to sampling in January 2016, results from January are not considered as caused by the experimental treatments.



Figure 10: *H. scabra* emerging from a burrow exposing anoxic sediment to the overlying water and breaking up the microbial mat growing over the sediment (February 2016)

Changes to the grain size distribution in the presence of high densities of *H. scabra* may be caused by dissolution of calcium carbonate of ingested sediment as several species of sea cucumbers have a slightly acidic gut (Hammond 1981; Schneider et al. 2011). The nonlinearity of specific surface area: size relationship of carbonate reef sands (Hammond 1981) and grain selection by sea cucumbers (Dar and Ahmad 2006; Mezali and Soualili 2013) may explain why changes to grain size distribution were specific to the 1000 µm and 125 µm fractions. Gravel (2000 – 1000 µm) and coarse sands (1000 – 500 µm) were the main sediment components in the guts of several holothurians in the Red Sea regardless of season, even during the spawning period (spawning at our site observed during October – Refer 9.1.6) when several holothuria tended to assimilate fine sediments (<125 µm) more (Dar and Ahmad 2006). In the present study, significant changes in grains were observed approximately within these ranges; therefore grain selection may have played a large role in the observed changes. Enclosures were stocked with *H. scabra* between lengths 14 – 16 cm, as this size class was the most abundant at the study site. It is possible that different size classes would select other fractions, inferring that if enclosures were stocked with a different size class, the fraction affected could have varied. It has also been postulated that bioturbation by sea cucumbers may also activate meio- and microfauna, which may also affect grain size (Michio et al. 2003).

As the grain size distribution showed significant changes in enclosures stocked with high densities of *H. scabra* a significant difference in sediment porosity between areas with high densities of *H. scabra* and those void of *H. scabra* was expected. However no significant differences in porosity were observed between high and exclusion treatments throughout the study. There was a considerable standard error for porosity (Refer Fig. 12), which may be due to low replication ($n=3$ enclosure $^{-1}$) and/or methods used as samples had to be stored in airtight containers for up to three weeks before drying, due to logistical reasons.

In December, where a shift towards finer grain fractions was observed in the presence of high densities of *H. scabra*, there was a lower porosity than in sediment void of *H.*

scabra. As sediment was poorly sorted, the shift towards finer fractions likely resulted in finer sediment moving into interstitial spaces thus reducing porosity. Variation in porosity may also be due to natural variation in sediment and weather conditions at the study site such as waves. The unexpected trend between porosity and grain size distribution may also be due to a confounding variable or factor such as the shape and nature of material on or in sediment e.g. OM, which may have degraded while samples were in transit.

Despite few significant changes to the physical characteristics of sediment between areas with high densities of *H. scabra* and those void of *H. scabra*, oxygen dynamics within sediment exhibited a strong variation over the course of the study. Generally in the presence of high densities of *H. scabra* sedimentary oxygen consumption (SOC) rates exhibited a buffered response, recovering to approximately background (September) levels following flooding at the study site (Refer Fig. 15). A similar ‘buffered’ response was observed in oxygen penetration depth; the absence of *H. scabra* caused erratic changes in O₂ penetration depth, however high densities of *H. scabra* seemed able to buffer and maintain the depth of O₂ penetration into sediment at background (November) levels. The absence of *H. scabra* caused SOC rates to double within the first month of the study and remain high. The SOC rate of sediment void of *H. scabra* only significantly decreased after storm surge affected the area in January. Water temperatures increased rapidly from December to February (ca. 26° C in December to ca. 31° C in February – Refer Fig. 13), during this time period the O₂ penetration depth under high *H. scabra* densities remained at approximately December levels however where *H. scabra* were excluded the O₂ penetration depth decreased significantly; by more than half that of December levels (Refer Fig. 14).

Increased SOC rates are primarily driven by respiration of benthic fauna, microbially mediated oxygenation of OM, and reduced inorganic metabolites (Kristensen, 2000; Nickell et al. 2003). Sea cucumbers actively feed on OM, thus reducing OM concentration in sediment (Uthicke and Karez 1999; Michio et al. 2003; Wolkenhauer et al. 2009). Therefore, it is likely that there was a reduced concentration of OM in sediment

where high densities of *H. scabra* were present compared to areas where *H. scabra* were excluded. Furthermore as high concentrations of OM facilitate increased microbial abundance and activity (MacTavish et al. 2012) the SOC rate in areas with high OM concentrations are likely to be enhanced, as observed in areas deprived of *H. scabra*. The buffered response of SOC and O₂ penetration depth in sediment of high-density enclosures is also likely to have been mediated by the considerable bioturbation impact *H. scabra* has on sediment. Bioturbation of sediment by *H. scabra* is further enhanced by their burrowing cycle (Hamel and Mercier 1996; Mercier et al. 1999). Bioturbation increases bed sediment complexity, which creates pressure imbalances that help to drive advective porewater flow (Thibodeaux and Boyle 1987). Increased porewater flow increases the supply of degradable material and electron acceptors (such as O₂) to the sediment (Rusch et al. 2006), promoting aerobic degradation. Aerobic degradation in marine sediments is faster and more efficient than anaerobic degradation (Kristensen et al. 1995; Hulthe et al. 1998). Consequently high densities of *H. scabra* mediate an increased bioturbation of bed sediment, promoting aerobic degradation, which enhances the rate of OM remineralization.

Heavy rain and flooding less than ten days prior to sampling in November, which likely resulted in an increased input of OM into the site (Briand et al. 2015). The decomposition of this OM would have increased O₂ demand (Wild et al. 2004). Thus, a faster ‘recovery’ to a low SOC rate would be expected in sediment where OM is being efficiently decomposed. Correspondingly, recovery to OM pulses was observed in sediment stocked with high densities of *H. scabra*, whilst no such recovery was observed in the absence of *H. scabra* (Refer Fig. 15).

Less than five days prior to sampling in January, there was storm surge and severe flooding at the study site. The flooding prior to sampling in January likely deposited OM onto sediment at the site (Briand et al. 2015), however the storm surge had visibly shifted and resuspended a considerable amount of sediment within the site. The disturbance of sediment would have functioned similarly to bioturbation - oxygenating sediment and redistributing OM, which likely caused the significant decrease in SOC from December

to January even in the absence of *H. scabra* (Refer Fig. 15). Local disturbances such as storms may thus be able to induce sufficient mixing of bed sediment capable of significantly reducing SOC.

As grain size distribution and porosity between high density and exclusion treatments did not show any significant differences in February, changes in oxygen penetration depth from January to February are assumed due to treatment. The abrupt and significant reduction in O₂ penetration depth of exclusion treatment from December to February was likely due to the local stressor of sea cucumber removal and the global stressor of increased water temperature (assumably due to the 2016 El Niño). Warmer water temperatures induce microbial growth and respiration (Nydale et al. 2013) and the relatively calm conditions from January to February meant limited mixing of bed sediment and the overlying water column. Both these factors favor a reduced O₂ penetration depth and enhance the development of hypoxic conditions (Kristensen 2000; Friedrich et al. 2014). However areas in which high densities of *H. scabra* were present, despite being subject to the same environmental conditions, exhibited no such reduction in O₂ penetration depth. Accordingly high densities of sea cucumbers are able to enhance the buffering capacity of sediment to such stressors and potentially ameliorate the development of hypoxic conditions.

Being an *in situ* study, there is likely to have been considerable ‘background noise’ from waves, wind, currents, other marine benthos, etc. that likely affected the results. A stronger signal among treatments may be more likely in *ex situ* experiments or in calmer conditions such as those within a sheltered bay e.g. Marovo Lagoon, Solomon Islands where sea cucumber have been extensively overfished in recent years (Buckius et al. 2010).

A photograph showing the silhouettes of two young boys from behind, sitting in a small boat on the ocean. They are looking towards a bright sunset or sunrise over a cloudy sky. The water is slightly choppy. The word "CONCLUSION" is overlaid in white text in the center of the image.

CONCLUSION

6 CONCLUSION

Trends seen in SOC and O₂ penetration depth indicate sediment function as a biocatalytical filter is compromised as a result of sea cucumber removal. The limited sediment function in the absence of sea cucumbers is likely due to reduced reworking of bed sediment, potentially 34 kg dry weight *H. scabra*⁻¹ year⁻¹.

The current study was conducted on a reef flat with a relatively high flushing rate, and low residual volume. Many sea cucumber fisheries take place in bays and lagoons (Friedman et al. 2009). Relative to reef flats, lagoons are generally characterized by lower flushing rates, higher sedimentation rates, and a higher residual volume, and are therefore more prone to trapping inorganic sediment and OM (Kjerfve and Magill 1989; Knoppers 1994; Kjerfve 1994). Under such lagoonal conditions, the observed negative effects of sea cucumber removal on sediment function would be expected to be amplified. Furthermore benthic-pelagic coupling (Wild et al. 2005; Wild et al. 2004; Wild et al. 2008) indicates that the changes we observed in sediment will affect the overlying water quality. Thus if sediment becomes anoxic, the oxygen conditions of the overlying water will be affected which could lead to fish kills, inhibit larval development, etc. (Helfrich 2009).

A recent study by Ford et al. (*in prep*) indicated that anthropogenically-derived OM from untreated sewage is driving a shift from coral towards cyanobacterial mats in a remote island in Papua New Guinea. Small patches of cyanobacterial mats (< 25cm²) were seen in areas void of *H. scabra* following increased water temperatures and calm conditions in January, these cyanobacterial mats continued to proliferate for the remaining duration of the study. If these cyanobacterial mats are able to establish themselves in sediment as a result of reduced sea cucumber densities, areas where sea cucumbers have been extensively overfished may be at risk of a shift towards cyanobacterial mats. These cyanobacterial shifts are likely to reduce the value and productivity of soft bottom habitats such as sandy lagoons to tourism, fisheries, and other vital sectors (Anderson et al. 2000), and may pose a potential health threat in the form of toxic algal blooms.

A large fish kill in Marovo Lagoon, Solomon Islands, in June 2011 illustrates a potential worst-case scenario. Multiple local stressors; increased nutrient input from logging, and decreased processing of nutrients and OM caused by the extensive commercial harvest of sea cucumbers. Coupled with global stressors (prolonged calm weather and warm water), these factors likely intensified what has historically been a small-scale (few hundred m²) red tide into a widespread (> 20 km²) and severe event (Albert et al. 2011). Concentrations of a toxic diatom and toxic dinoflagellate increased to millions of cells litre⁻¹ during the phytoplankton bloom, the senescent phytoplankton bloom subsequently caused a complete de-oxygenation of the water column. Following the phytoplankton bloom reef recovery was extremely slow and deep reefs (>6 m) and sediment shifted towards a cyanobacterial mat dominated state (Albert et al. 2012).

Sea cucumbers play a critical role in maintaining sediment integrity. Their removal from the environment reduces sediment capacity to function as a biocatalytical filter. The resistance and resilience of coastal ecosystems to local and global stressors are likely being compromised by the extensive reduction in sea cucumber stocks worldwide. Consequently, the ecosystem services that Pacific island communities rely heavily on for their livelihoods are being undermined by the removal of sea cucumbers, leaving coastal ecosystems and the communities that rely upon them increasingly vulnerable.

7 ACKNOWLEDGEMENTS

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Finally I would like to thank my parents for their support over the years and helping me get to where I am. This thesis is dedicated to my grandparents Nurn and Pa, grandma and grandpa; I hope I have done you proud.

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9 APPENDIX

9.1 Additional data: *H. scabra* ethology and ecology

Data were generally collected on foot, as the reef flat was easily accessible at all tides, with snorkeling gear used when necessary. Within belt transects (100 m x 2 m), all sea cucumbers were identified to species level. For each individual their length (ventral, mouth to anus), position along the transect line in meters (distance from start) and the habitat immediately (ca. 10 cm) around them was recorded. Seagrasses were identified to genus level and substrate type was recorded using the following categories; sand and rubble. The transect locations were chosen systematically by using a fixed distance of ca. 150 m between transects in order to get a uniform representation of the site and to cover all different microhabitat types. Two deeper pools of water (blue holes) were snorkeled; these areas were ca. 3 m deep over a sandy bed. All *H. scabra* within the blue holes were collected, and their length, weight and associated habitat were recorded using the same method outlined in section 9.1.4.

9.1.1 *H. scabra* population structure

The mean and median length of the entire *H. scabra* population was 11 and 10 cm respectively, this equates to a mean and median weight of 103 and 71 g respectively (Refer 9.1.4). The main size classes were 5 – 7 and 8 – 10 cm per transect. *H. scabra* of lengths of 2, 4, and 21 cm were underrepresented in transects.

H. scabra <5 cm in length are likely under-represented. Individuals <5 cm are difficult to locate visually and burrow into the substratum (Mercier et al. 1999). To obtain more accurate representation of the smaller individuals in a population a detailed survey of 1 m² quadrats using sieves to collect burrowed animals should be used following methods by Mercier et al. (2000b). Furthermore, a pulse harvest of *H. scabra* in December 2014 which targeted individuals of length ≥16 cm may account for the relatively low abundance of *H. scabra* ≥18 cm (contracted body length).

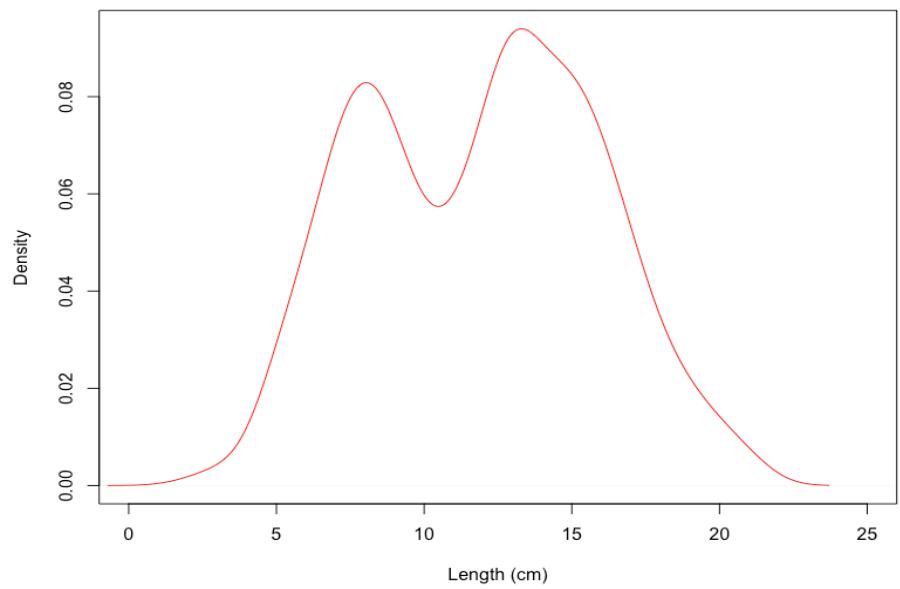


Figure 16: *Holothuria scabra* population length-frequency Kernel density plot.

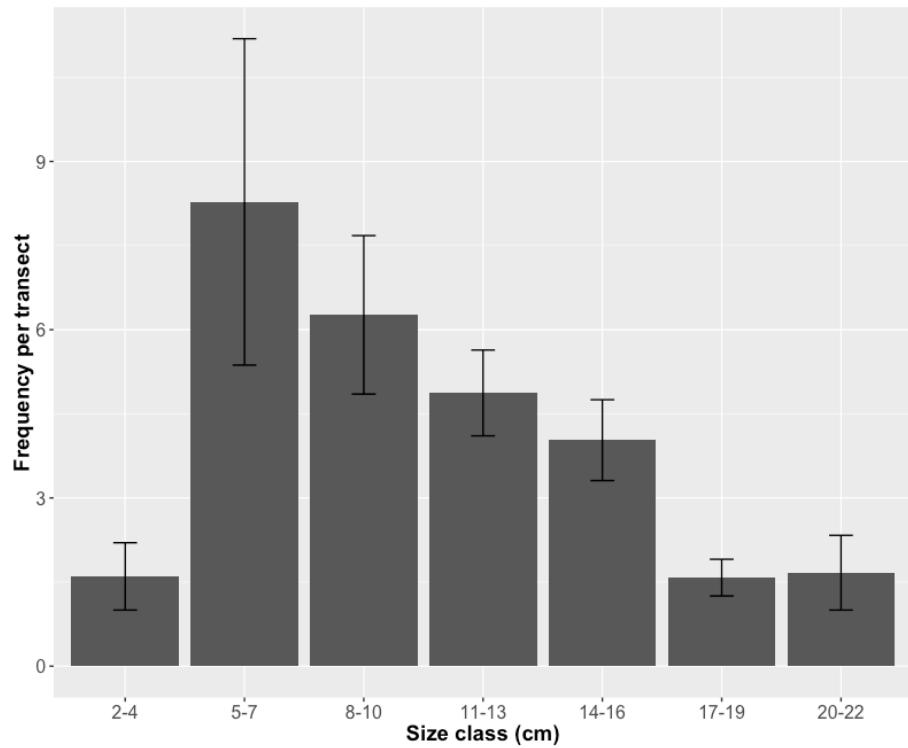


Figure 17: *Holothuria scabra* size class distribution per transect. Mean with standard error

9.1.2 Tidal and diurnal abundance

Each transect was categorized as being during one tide (Flood/Ebb) and time of day (Day/Night) e.g. Transect 1 during an ebb tide during daylight hours. The abundance of *H. scabra* was significantly higher during day than night (Welch Two Sample t-test ; $p < 0.001$), tide did not have a significant effect on *H. scabra* abundance.

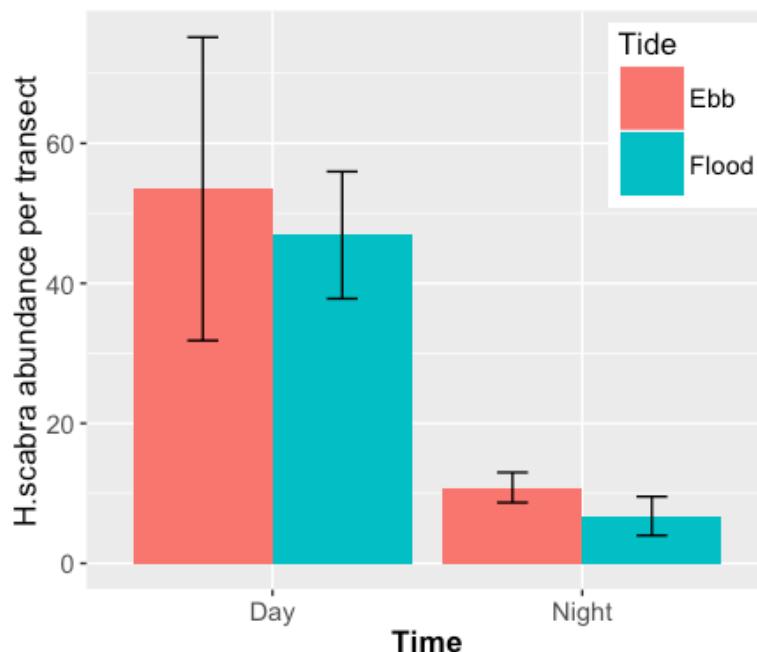


Figure 18: Abundance of *Holothuria scabra* per transect during day and night, broken down by tide. Mean with standard error

9.1.3 Habitat associations

In order to investigate habitat preference, a habitat electivity index adapted from Ivlev's electivity index (Lechowicz and Url 2011) was used. The relative availability of habitat types at our site (p) was compared to their relative utilization (r). A sea cucumber was classified as utilizing a habitat when it was found in that habitat. An electivity index was calculated per transect, the mean value of all transects combined is presented in the results.

Habitat electivity index:

$$\text{Electivity index} = (\text{relative habitat availability} - \text{habitat utilization}) / (\text{relative habitat availability} + \text{habitat utilization})$$

$$E_i = (r_i - p_i) / (r_i + p_i)$$

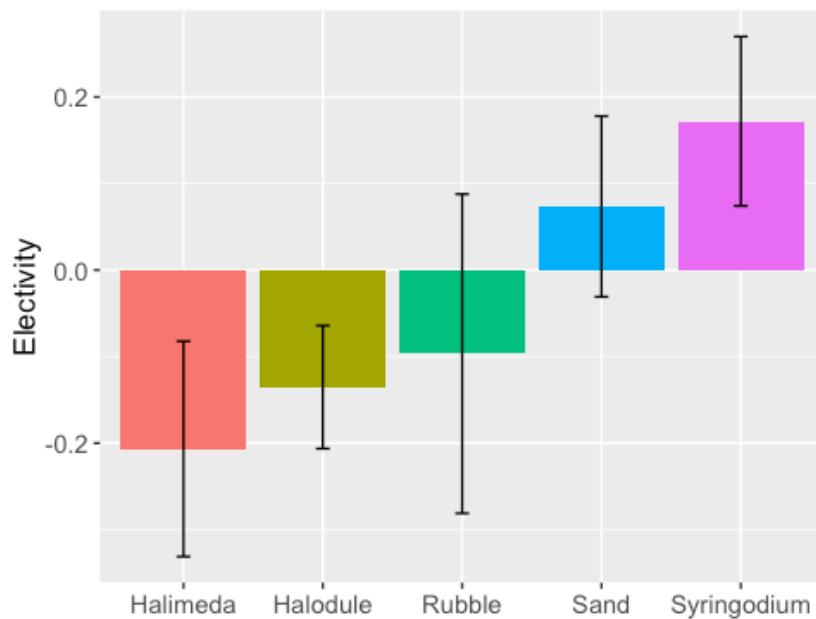


Figure 11: **Habitat electivity index for *Holothuria scabra*.** At zero (0) the animal randomly chooses its habitat, positive values indicate preference, negative avoidance

H. scabra displayed preference for both sand and *Syringodium* spp. habitats, despite *Halodule* spp. being the dominant habitats on the reef flat. *Halodule* spp. have strap-like leaves, whereas *Syringodium* spp. have cylindrical-like leaves (McKenzie and Yoshida 2010). Therefore our findings contradict those from previous studies, which indicated *H. scabra* were predominantly associated with seagrasses that had strap-like leaves such as *Cymodocea serrulata* and *Enhalus acoroides* (Mercier et al. 2000b; Wolkenhauer et al. 2009). There was considerable standard error in our habitat electivity index, which may be due to limited habitat coverage data for the entire site. However, the study by Mercier et al. (2000b) reported low densities of large *H. scabra* (>ca.10 cm) and that even when found in areas with high seagrass coverage, these larger individuals generally moved into denuded areas. Avoidance of dense seagrass beds was possibly due to the dense root systems interfering with their burrowing and feeding behaviour (Mercier et al. 2000b). The root systems of *Halimeda* spp. and *Halodule* spp. beds were denser than that of *Syringodium* spp. beds. As our site had relatively high densities of *H. scabra* >10 cm length, habitat electivity results may be indicative of the preference of larger individuals, whereas findings by Mercier et al. (2000b) may be more relevant to smaller (<ca.10 cm) individuals.

Habitat size-class associations show that all size classes surveyed were present in *Halodule* spp. and sand; only three size classes were found in *Syringodium* spp (Refer Fig. 20). Our findings may be biased due to limited habitat coverage data, or it may indicate that the ability to burrow into sediment plays a critical role in the habitat selection of *H. scabra*, regardless of size.

Only *H. scabra* \geq 11 cm (contracted body length) were found in the blue holes, similar to previous findings by Mercier et al. (2000b) (Refer Fig. 21). As the blue holes were deeper, water conditions above the bed sediment such as temperature and salinity may have been more stable, and the sandy substrate allowed *H. scabra* to easily burrow. There may be a trade-off between predation risk and stress in a habitat – deeper habitats may have less stressful conditions but an increased predation risk. The quality and or quantity

of OM available in different habitats may also appeal to varying size classes of *H. scabra* depending on their nutritional needs at that size (Hamel and Mercier 1996).

While dense seagrass beds are important for juvenile *H. scabra*, our results suggest that larger individuals (> ca.10 cm) prefer deeper areas with soft bed sediment and limited seagrass coverage. The ability to shelter (burrow or move into shade) may be key features that influence their choices, especially in high stress environments such as reef flats. Having all of these habitats available in a reef flat may improve the chances for restocking success, or indicate an area with the potential to support a high density *H. scabra*, as optimum conditions for all parts of the animal's life cycle are present in a relatively small area.

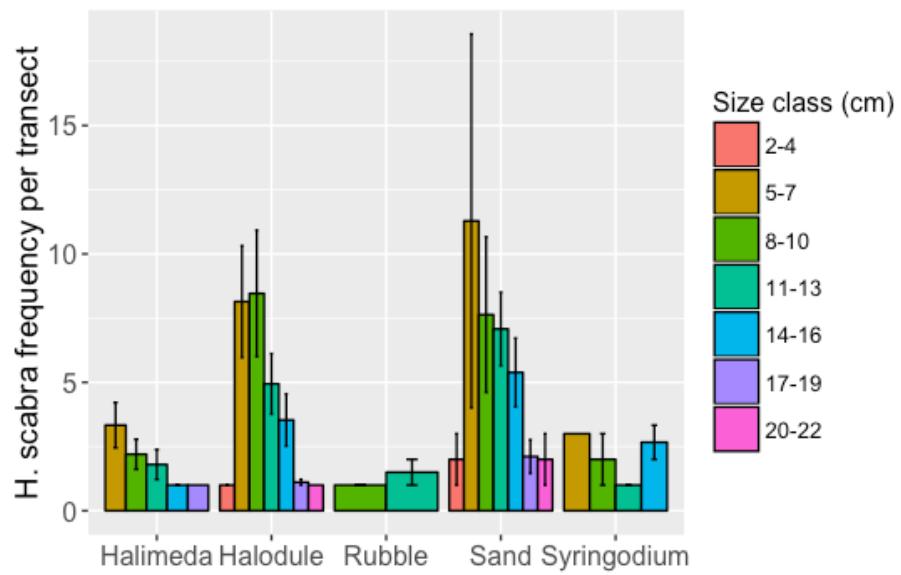


Figure 20: **Size class distribution of *Holothuria scabra* within each habitat.**
Mean frequency per transect with standard error

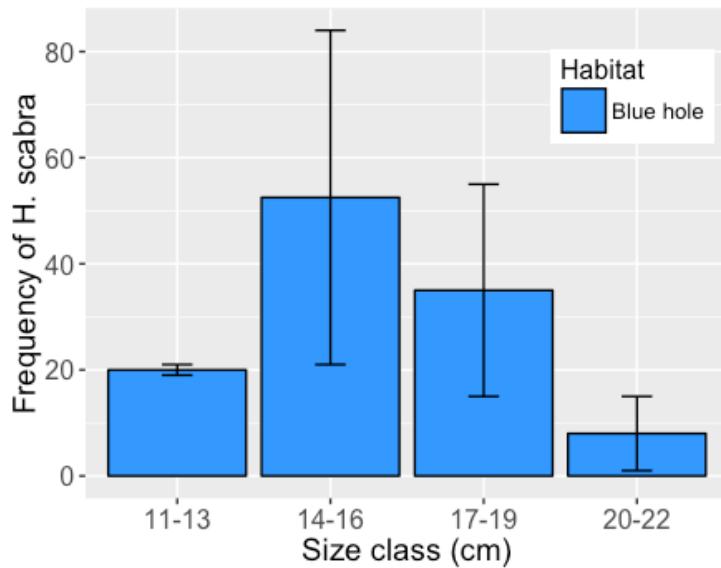


Figure 212: **Size class distribution of *Holothuria scabra* within the blue hole habitat.** Mean values with standard error

9.1.4 Length-weight relationship

Measuring the body length (mouth to anus) of sea cucumbers is relatively simple and quick; hence it is a more reliable and common measurement to do during population surveys (Poot-Salazar et al. 2014). Measuring weight however can be time consuming. By establishing a length-weight relationship based off a simple standardized method for measuring length, the total biomass of sea cucumber stocks can be determined, improving data for management. Method for measuring *H. scabra* length was based of methods used to assess length of *Holothuria atra* by Seeto (1994).

H. scabra individuals were removed from the water and were allowed to initially contract and expel water which happened almost immediately following handling (<3 seconds). The animal's length was measured from anus to mouth by placing a ruler along its ventral surface; all measurements were recorded to the nearest centimeter. The animal was then placed into a container filled with water from the site; water was continuously exchanged to reduce stress on the animals. All sea cucumbers within a size group (1 cm) were kept together. Following each transect the animals were taken ashore and weighed on a digital scale to the nearest gram. Time from initial capture to weighing was ca. one hour. Individuals were allowed to contract and expel water before being weighed. Following weighing, all sea cucumbers were released back to the area from where they were collected. The equation provided in this thesis is limited to *H. scabra* between lengths 5 - 21 cm, as there was a lack of data outside of this size range (Refer Fig. 22). Four collected *H. scabra* weighed more than 500g, however these were excluded from analysis as the scale used only measured up to 500g. A study of *H. scabra* in Oman using a similar method to that here produced a length-weight allometric equation that explained 80% of variation (Al-Rashdi et al. 2007). The dataset produced by Al-Rashdi et al. (2007) had considerably more data for lengths ≥ 20 cm, and weight ≥ 500 g but lacked data for lengths ≤ 10 cm. Based off both graphs, *H. scabra* exhibit exponential growth well past their size at maturity (ca. 15 cm for the present site).

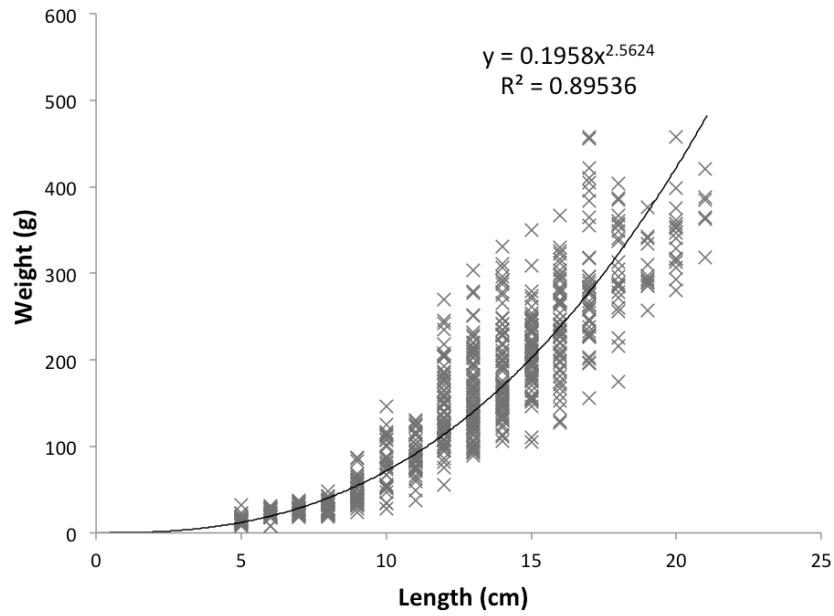


Figure 22: **Length weight relationship of *Holothuria scabra*.** Log-transformed regression (Power regression Microsoft Excel 2011), regression line is extrapolated towards (0,0). $R^2 = 0.8954$. $y = 0.1958x^{2.5624}$

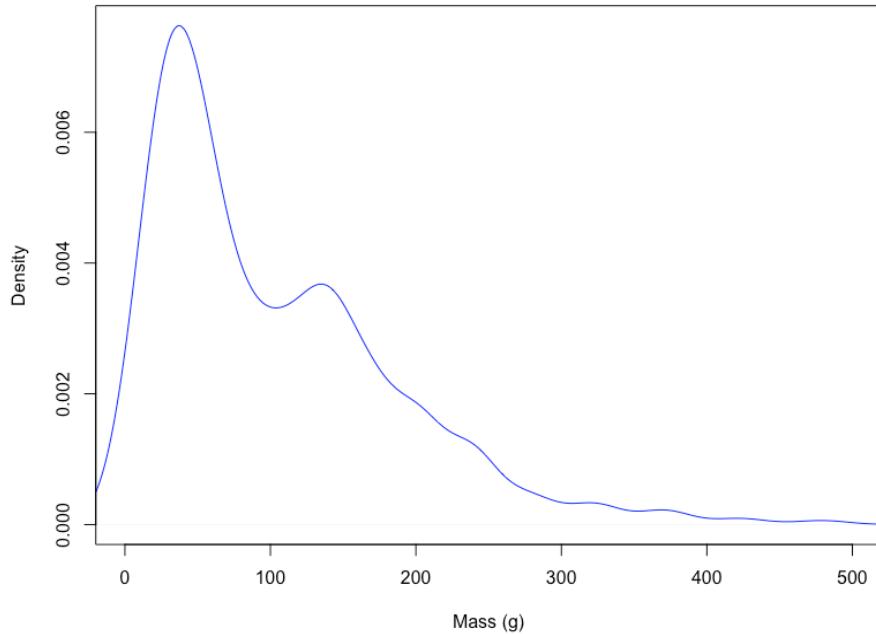


Figure 23: ***Holothuria scabra* population mass Kernel density plot.** Mass calculated using provided length weight allometric equation.

9.1.5 Distance travelled

Distance travelled by *H. scabra* was measured as straight distance; distance between each marker (Refer. 3.3) was used to quantify distance travelled by each *H. scabra*. Mean distance travelled by all surveyed *H. scabra* was used to produce the following table:

Table 4: Distance travelled *in situ* by *Holothuria scabra*

Distance travelled (per individual)	Distance (m)
(40 cm h ⁻¹ x 10 h)	4
(4 m day ⁻¹ x 30 days)	120
(120 m month ⁻¹ x 12 months)	1440

H. scabra are capable of moving up to 4 m day⁻¹, thus an individual could potentially travel ca. 120m month⁻¹ – however this assumes they move in a straight line, which is not the case. Traditional Fijian fishing grounds (*qoliqolis*) and their respective traditional marine protected areas (MPAs) (*tabu*) vary in size according to custom (Iwakiri 1983). Particularly in small *qoliqolis* loss of marine resources to neighbouring *qoliqolis* can be a cause for conflict. Distance crawled *in situ* was measured as straight distance, however observed movement patterns were similar to findings by Mercier et al. (2000), and ‘home range’ behaviour similar to findings by Purcell et al. (2016). Movement patterns may be linked to the distribution of food, as some species of sea cucumbers exhibit patch selectivity (Uthicke and Karez 1999). In the case of *H. scabra*, movement may be driven by the availability of soft-bottom sediment in which to shelter through burrowing (Mercier et al. 1999). Due to the restricted movement and home range behaviour, when *H. scabra* were not seen above sediment it was assumed they were burrowed. Given their limited movement, the main method by which *H. scabra* would spread into other areas may be during the larval stage (Mercier et al. 2000b). Therefore, *qoliqolis* and *tabu* areas may be able to contain stocks of *H. scabra* with little loss to neighboring *qoliqolis*.

9.1.6 *H. scabra* *in situ* spawning

Conditions during *H. scabra* spawning at our site coincided with the full moon and spring tide in October (dry season), and occurred during the afternoon/early evening. The peak spawning period in the Solomon Islands occurs during similar conditions; dry season (August – November), coincides with the full moon, and generally occurs during the afternoon/early evening (Battaglene et al. 2002). *H. scabra* spawning *ex situ* is induced through thermal stress followed by immersing brood stock in a bath with high concentrations of microalgae (Hair et al. 2011). Spring tides likely induce thermal stress and algal blooms are more likely to occur during the dry season (warmer waters) (Sibrizzi and Moore 2013). Therefore the *H. scabra* peak spawning period in Fiji is likely to be in the dry season and spawning events coincide with spring tides. Aggregate behavior prior to and during spawning only took place in and around enclosures stocked with high densities of *H. scabra*, indicating density-dependent spawning possibly due to chemical cues (Lovatelli et al. 2004). Reduced densities of *H. scabra* may inhibit the ability of *H. scabra* to spawn or reduce fertilization rates of gametes, further inhibiting chances of population recovery. As only large *H. scabra* (contracted body length >15 cm) were observed spawning in these aggregations, length at maturity for *H. scabra* in Fiji may be ca. 15 cm.

9.1.7 Management recommendations

Reef flat surveys for *H. scabra* should be done during daylight hours and should avoid the hour before and the hour after low tide in order to capture a more accurate representation of the *H. scabra* population. All harvest of *H. scabra* in Fiji should be prohibited during the peak spawning period of August – November to allow higher recruitment within a population. Densities may have to be artificially increased during the peak spawning period in order to improve chances of successful spawning and/or fertilization of gametes. Minimum legal harvest size for *H. scabra* in Fiji should be set higher than 15 cm (contracted body length) to allow for at least one spawning cycle.

9.2 Tables

Table 3: **Mass of pellets defecated per individual *Holothuria scabra*.** Sediment turnover model, ANOVA Type II results

Independent variable: Mass of pellets defecated per individual				
Dependent variables (factors)	Degrees of freedom	F value	p value	Significance
sizeclass	3	5.7499	0.0080	***
habitat	2	2.4197	0.1228	
Tide	1	0.4156	0.5288	
sizeclass:habitat	3	2.8785	0.0708	.
sizeclass:tide	1	5.0399	0.0402	*
habitat:tide	1	11.6240	0.0038	**
residuals	15			

Table 6: **Mean number of pellets defecated per hour per *Holothuria scabra*.** ANOVA Type II results table

Independent variable: Mean number of pellets defecated h ⁻¹ individual ⁻¹				
Dependent variables (factors)	Degrees of freedom	F value	p value	Significance
sizeclass	3	1.8469	0.1770	
habitat	2	4.3902	0.0290	*
tide	1	2.3732	0.1418	
sizeclass:habitat	3	3.3372	0.0442	*
sizeclass:tide	1	12.2551	0.0027	**
habitat:tide	1	1.5027	0.2370	
Residuals	17			

Table 7: **Mean pellet mass (g), per habitat.** Pairwise t-test with Bonferroni adjustment

	Halodule	Sand
Sand	$p = 0.82$	-
Syringodium	$p = 0.37$	$p = 0.032$

Table 8: **Mean number of pellets defecated per individual per hour, in each habitat.** Pairwise t-test with Bonferroni adjustment

	Halodule	Sand
Sand	$p = 0.19$	-
Syringodium	$p = 0.055$	$p = 0.0093$

Table 9: **Grain size composition, repeated measures ANOVA results table.** '·' indicates an interaction between

Independent variable: grain size composition (%)					
Dependent variables	Degrees of freedom	Sum of Squares	F value	P value	Significance
Fraction	5	29940	878.186	<2e-16	***
Treatment	3	0	0.009	0.999	
Date	3	0	0.000	1.000	
Fraction: Treatment	15	508	4.966	8.20e-09	***
Fraction: Date	15	482	4.710	3.04e-08	***
Treatment: Date	9	0	0.007	1.000	
Residuals	333	2271			

Table 10: **Grain size composition for Exclusion treatment**, September and December results only. Type II Wald chi-

Independent variable: grain size composition (%)				
Dependent variables	Chi-square	Degrees of freedom	P value	Significance
Fraction	587.4971	5	<2e-16	***
Date	0.0000	1	0.9982	
Fraction:Date	0.2281	5	0.9988	

Table 4: **Grain size composition for High treatment**, September and December results only. Type II Wald chi-

Independent variable: grain size composition (%)				
Dependent variables	Chi-square	Degrees of freedom	P value	Significance
Fraction	954.402	5	<2e-16	***
Date	0.0000	1	0.9989	
Fraction:Date	11.253	5	0.04659	*

Table 5: **Grain size distribution. Mann-Whitney-Wilcox U test with Bonferroni adjustment comparing treatments from September to December**

September - December	Sieve fraction (micron)	p value	Significance
High	≥ 2000	0.62	
	1000	0.12	
	500	0.25	
	250	0.25	
	125	0.12	
	≤ 125	0.62	
Exclusion	≥ 2000	1	
	1000	0.88	
	500	0.88	
	250	0.88	
	125	0.62	
	≤ 125	0.62	

Table 6: **Grain size distribution. Mann-Whitney-Wilcox U test with Bonferroni adjustment:** controls and treatments, per month.

Cage control vs. Natural	Sieve fraction (micron)	p value	Significance
September	≥ 2000	0.9	
	1000	0.89	
	500	1	
	250	0.49	
	125	0.38	
	≤ 125	0.4	
December	≥ 2000	0.56	
	1000	0.34	
	500	0.69	
	250	0.49	
	125	0.25	
	≤ 125	0.23	
High vs. Exclusion	Fraction (micron)	p value	
September	≥ 2000	0.49	
	1000	0.49	
	500	0.89	
	250	0.69	
	125	0.34	
	≤ 125	0.34	
December	≥ 2000	0.34	
	1000	0.029	**
	500	0.69	
	250	0.69	
	125	0.029	**
	≤ 125	0.89	
January	≥ 2000	0.3	
	1000	0.31	
	500	0.11	
	250	0.66	
	125	0.88	
	≤ 125	0.31	
February	≥ 2000	1	
	1000	0.19	
	500	0.49	
	250	0.49	
	125	0.2	
	≤ 125	0.89	

Table 7: **Porosity, all treatments, repeated measures ANOVA results table**

Independent variable: porosity (%)					
Dependent variables	Degrees of freedom	Sum of Squares	F value	P value	Significance
Treatment	3	0.0028	1.054	0.3805	
Date	2	0.0057	3.201	0.0526	*
Treatment: Date	6	0.0015	0.280	0.9427	
Residuals	36	0.0322			

Table 8: **Oxygen penetration depth, all treatments, repeated measures ANOVA results table**

Independent variable: oxygen penetration depth (mm)					
Dependent variables	Degrees of freedom	Sum of Squares	F value	P value	Significance
Treatment	3	961	3.557	0.0157	*
Date	3	6183	22.883	1.89e-12	***
Treatment: Date	9	2564	3.163	0.0015	**
Residuals	166	14951			

Table 16: **Oxygen penetration depth; Mann-Whitney-Wilcoxon U test with Bonferroni adjustment**

High vs. Exclusion	p value	Significance
November	0.73	
December	0.32	
January	0.53	
February	0.000039	***
<hr/>		
Cage control vs. Natural	p value	
November	0.95	
December	0.86	
January	0.22	
February	0.48	
<hr/>		
November – December	p value	
High	0.026	**
Exclusion	0.37	
<hr/>		
November – February	p value	
High	1	