Auswirkung des Abfischens - Eine Fallstudie zur ökologischen Rolle der wirtschaftlich genutzten Seegurke *Holothuria scabra* (Echinodermata: Holothuroidea) in Moreton Bay, Australien

Dissertation
zur
Erlangung des akademischen Grades
doctor rerum naturalium (Dr. rer. nat.)
der Mathematisch-Naturwissenschaftlichen Fakultät
der Universität Rostock

vorgelegt von:
Svea-Mara Wolkenhauer, geb. am 5. November 1973 in Ottersberg
aus Brisbane, Australien

Brisbane, 3. Juli 2008

urn:nbn:de:gbv:28-diss2008-0101-2

Betreuung und Gutachtenerstellung durch:

Prof. Dr. Ragnar Kinzelbach, Allgemeine und Spezielle Zoologie, Universität Rostock

Prof. Dr. Gerhard Graf, Meeresbiologie, Universität Rostock

Tag der Verteidigung: 04.11.2008

Impact of removal - A case study on the ecological role of the commercially important sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea) in Moreton Bay, Australia

To be submitted for the degree of Doctor of Philosophy to the
University of Rostock
July 2008

Svea Mara Wolkenhauer
(Institute of Biosciences)

Statement of Originality

The work presented in this thesis is to the best of my knowledge original. It presents my own work except as acknowledged in the text. The material has not been submitted, either in whole or in part, for any degree at this or any other institution.

July 2008

Statement of Contributions of Others

Intellectual support for the research conducted in this thesis was provided by my PhD advisors Tim Skewes, Dr. Sven Uthicke and Dr. Roland Pitcher. Editorial comments for all thesis chapters were provided by Tim Skewes, Dr. Sven Uthicke, Dr. Roland Pitcher and Prof. Ragnar Kinzelbach. Dr. Charis Burridge and Dr. Sven Uthicke provided intellectual and editorial support for statistical methods for Chapter 2 and Matthew Browne provided intellectual and editorial support for predictive models and graphs presented in Chapter 3 and 4. Doug Chetwynd provided essential support to data manipulation and extraction for the long-term study in Chapter 3.

Financial support for the research conducted in this thesis was provided by an Australia-Europe Scholarship through the Department of Education, Science and Training, Australia, the Daimler-Benz Foundation, Germany and by Dr. Roland Pitcher and Tim Skewes, CSIRO Marine & Atmospheric Research, Brisbane.

Laboratory and field assistance was provided by A. Anas, D. Chetwynd, S. Corrie, D. Dennis, M. Haywood, R. Kenyon, P. Kiehl, F. Manson, V. McClure, I. McLeod, T. Skewes and R. Soares.

____________________________	__________________
Candidate’s signature	Date
____________________________	__________________
Supervisor’s signature	Date

Acknowledgements

Independently pursuing a PhD abroad and creating the self-motivation to finalise this manuscript was a magnificent as well as challenging experience for me. Many people have been instrumental in shaping my work, my career and my personality during these years in Australia. Without the support of those precious people I would not have been able to thrive in my doctoral work and personal life. Here is a small tribute to some of these very special people.

First and foremost, I would like to express my deepest gratitude to my co-supervisor Tim Skewes whose dedicated and constant support has made him my primary advisor. He taught me how to write academic papers, had confidence in me when I doubted myself. More importantly, he taught me how to refocus on my work after my maternity leave. He was always there to meet and talk about my ideas, always believing in my ability to actually finish. Not only did his advice and ideas play a crucial role in the process of this manuscript, but his constant trust, reassurance and encouragements during all stages, made it possible for me to persevere and turn field work with vast data sets into this PhD. Above all, he has been a trusted friend to me during all those years, both in work and personal life, and I will truly miss him when I move further in my career.

Furthermore, I am deeply grateful to my co-supervisor Dr Sven Uthicke for his essential contribution to the experimental design and for his patient feedback during the early years of my PhD and again during the last year, when his constructive advice greatly improved this manuscript and my publications.

I wish to express my sincere thanks to my main supervisors Prof Ragnar Kinzelbach in Germany and Dr Roland Pitcher in Australia. They both provided excellent feedback and constructive criticism to experimental design and reviewing the final draft of this manuscript. Especially Dr Pitcher's "Popperian" ideals and concepts of a PhD had a remarkable influence on how I approached my hypotheses and structured my research.

Special thanks go to Charis Burridge and Matthew Browne, my two most valuable people in terms of mathematical and statistical advice to this manuscript. Without their guidance and original contribution, my results would have had no substance to defend.

During my work at the Cleveland Labs I have collaborated with many colleagues for whom I have great respect and I wish to extend my warmest thanks to all those who have helped me with my work and kept me sane due to countless games of 'Awesome Touch Footy'. In particular, thanks are due to A. Anas, M. Barclay, A. Butler, R. Butler, J. Carroll, D. Chetwynd, F. Coman, Q. Dell, D. Dennis, C. Dichmont, K. Forcey, G. Fry, M. Haywood, R. Kenyon, I. McLeod, F. Manson, D. Milton, C. Moeseneder, F. Pantus, N. Preston, S. Tabrett, T. Taranto and M. Tonks.

My warm thanks are due to the staff at the Moreton Bay Research Station on North Stradbroke Island and the Centre for Microscopy and Microanalysis at the University of Queensland for all their effort and help during my field studies and handling the endless supply of sediment.

Victoria McClure, Petra Kiehl and Shaun Corrie were my most dedicated volunteers in those long hours of field and laboratory work. I would like to express my warmest thanks for their commitment, their patience and valuable ideas to this work; especially to Victoria, whose cheerful nature kept me alive during the dark hours of sediment-sieving and poo-collecting.

I cannot leave out mentioning three of my closest and longest friends in science, Dr Caroline Gaus, Dr Conny Guenter and Dr Fiona Manson who have provided me with indispensable support during difficult times and celebrated with me during the height of success.

I am forever indebted to the Daimler-Benz-Foundation, Germany, as well as the Department of Education, Training and Youth Affairs of the Australian Government for their financial support. Logistical support was provided by CSIRO Marine & Atmospheric Research, Australia.

Last but not least I owe my loving thanks to Fabien, Yannick and Mamoune, who have believed in me ever since, helped me to structure and focus my life (yes, sometimes Frenchmen can help Germans in those regards) and brought incredible joy, respect and warmth to my life outside of science. I would also like to thank my own mother, who has raised me to be independent, self-sufficient and always aim high.

To conclude in the words of Wilhelm Bush: “Und die Moral von der Geschicht…” …philosophical thinking makes all the difference when trying to balance social life, working and writing a PhD. I wish you all happy reading!

Abstract

The ecology of holothurians, their ecological role in marine ecosystems, and the potential impacts of their removal (through over-fishing) was the subject of this study. This was investigated by focusing on important aspects of holothurian behavioural dynamics, such as burying and feeding, as well as assessing their impact on important habitat variables such as sediment mixing, and seagrass and algae biomass and productivity.

Certain species of holothurians have been harvested for the human food consumption for centuries. The dry products of the body wall (also called 'bêche-de-mer' or 'trepang') of these animals are considered as a delicacy to the Asian (particularly Chinese) food industry. Currently, there are more than 20 holothurian species that are commercially used around the world. *Holothuria scabra* is one of those most targeted species due to their thick body wall and large size. Since most commercial species inhabit shallow waters and are easily harvested by hand at low tide, high value species such as *H. scabra* are easily over-exploited. *H. scabra* are already over-exploited or extinct in many locations such as Solomon Islands, some places along the Indian coast and in many places along the South-East Asian coastline.

The main objectives of this study were to: (a) document the difference in the productivity and biomass of seagrass and benthic microalgae (BMA) with and without *H. scabra* by means of *in situ* exclusion cages, (b) quantify the rate and extent of vertical sediment transport associated with feeding and burying of *H. scabra* using luminophores as tracers in aquaria, and (c) investigate the relationship between burying and feeding behaviour and temperature (within a seasonal context) by means of continuous long-term monitoring of *H. scabra* behaviour in mesocosms.

Exclusion experiments were conducted in shallow seagrass habitat to investigate the impact of holothurians on seagrass biomass and productivity. In addition, sediment samples were taken to measure BMA biomass and organic matter (OM). Holothurians appear to be beneficial for seagrass, with significantly higher seagrass productivity (12%, ANOVA, $p = 0.008$) and slightly higher seagrass biomass (18%, ANOVA, $p = 0.348$) under natural holothurian densities compared to exclusion areas. Conversely, the presence of holothurians appeared to reduce BMA biomass and OM, as suggested by higher BMA biomass (ANOVA, $p = 0.089$) and OM content (ANOVA, $p = 0.110$) in exclusion cages. Combining all major response variables in a Principal Component Analysis (PCA) suggested that the exclusion of holothurians caused marginally significant differences in those variables compared to natural densities (MANOVA, $p = 0.074$).

The results of bioturbation experiments demonstrated that *H. scabra* caused mixing of the surface sediment layers during their feeding and burying activities. Instantaneous sediment mixing rates (IMR) of 0.016% d^{-1} were calculated for the top 2 cm for *H. scabra* at natural densities (0.48 ind. m^{-2}). Moreover, the holothurians did not influence sediment deeper than 6 cm and their pattern of bioturbation created a relatively smooth sediment surface with the formation of a shallow anoxic layer (3-6 cm). A new mode of bioturbation for these animals is suggested, categorising holothurians as "conveyor diffusors".

Overall findings of the behaviour study showed that differences in burying and feeding behaviour of adult *H. scabra* were strongly related to water temperature, thus resulting in drastic seasonal changes in behaviour. Austral winter (Jun-Aug) was a time of very low activity with most animals being buried for whole or part of the day, feeding only a few hours each day and displaying very little to no searching activity. Austral summer (Nov-Feb) was a time of high activity with short periods of being buried (early morning), frequent feeding and searching (morning, afternoon and late evening) and some spawning activity occurring (late afternoon). The behavioural pattern during shoulder seasons (Mar-May and Sep-Oct) was similar to that observed during summer. The exception was searching activity, which occurred more frequently during the shoulder seasons, due to higher food requirements (e.g. preparation for spawning). Hence, the ecosystem function of holothurians was altered dependent on seasons and needed to be taken into account when establishing an ecological role of those animals within their habitat.

Results of this study demonstrated a potential mechanism by which fisheries for holothurians may impact their surrounding habitats and result in indirect cascading ecological consequences for the animal's ecosystem function. Furthermore, given that seagrass habitat is a known nursery for other important fishery species (e.g. prawns), there is the potential for an impact in one fishery (holothurians) to be linked to another. Over-fishing of holothurians might alter the overall habitat structure in unpredictable ways and could thus have consequences for the ecology of tropical seagrass beds in the long term. Furthermore, findings in this study have implications for population surveys for *H. scabra* when relying mainly on visually counting animals along transects. Surveys should be conducted at consistent diel and seasonal timing if results are to be compared with previous data. Based on burying data presented in this study, it is suggested that the most suitable time to conduct population surveys on *H. scabra* would be during austral summer (Nov-Feb) from midday to late afternoon.

Most of the findings in this study were based on individual *H. scabra* and their ecological role as ecosystem engineers. However, one of the most important aspects of this research was the ability to use the acquired results and draw conclusions as to the wider population of *H. scabra* within Moreton Bay. By using models of activity dynamics in combination with sediment transport rates, this study estimated the overall impacts *H. scabra* population had on their associated habitat in Moreton Bay.

List of Figures

List of Tables

Table of contents

Glossary and list of abbreviations

AFDW	ash-free dry weight (AFDW = DW – AW)
ANOVA	Analysis of Variance (statistical term)
Aspidochirotide	Sub-class of Holothuroidea containing *Holothuria scabra*
Bêche-de-mer	French: 'spade of the sea', common name for the dried product of commercially harvested sea cucumbers (also called trepang)
bioturbation	activities of living organisms that mix and redistribute sediments and associated pore water laterally and vertically
BMA	benthic (bottom-dwelling) microalgae
chl-*a*	chlorophyll *a* (photosynthetic pigment of algae and plants)
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DF	degrees of freedom (statistical term)
diel cycle	24-hr cycle (incorporating day and night)
DW	dry weight
epibenthic	living on top of the sea floor
evisceration	voluntary expulsion of some or all interior organs (caused by predator attacks, rough handling of the animal or strong water temperature change)
GDA	Geocentric Datum of Australia (coordinate system that replaced the old Australian Geodedic Datum (AGD) in 1994 to be compatible with Global Positioning System (GPS))
GF/F	a type of glass fibre filter
GI	gonad index (expressed as ratio of gonad-weight divided by body weight multiplied by 100)
GLM	Generalised Linear Model (statistical term)
GPP	gross primary productivity (measured in μmol O_2 m^{-2} hr^{-1})
GPS	Global Positioning System
HCl	hydrochloride acid
IMR	instantaneous mixing rate
LM	general Linear Model (statistical term)

LUX	unit to measure intensity of light (illuminance)
macrofauna	animals that can be seen without a microscope (generally sized 1-200 mm)
MANOVA	Multivariate Analysis of Variance (statistical term)
megafauna	animals larger than 20 cm (in the marine sense)
microfauna	animals between 0.06 and 0.5 mm in size, mainly living within the sediment grains (interstitial)
MDD	minimal detectable difference (statistical term)
NNCHC	Nunukul Ngugi Cultural Heritage Corporation
OM	organic matter (detritus)
PCA	Principal Component Analysis (statistical term)
phaeophytin	inactive degradation product of chlorophyll *a* after the central magnesium atom has been replaced with two hydrogen atoms
protandrous	of or relating to an organism or species in which the male releases its reproductive cells before the female
PSU	partial salinity units (1 PSU = 1.005 part per thousand (PPT))
PVC	polyvinyl chloride, a thermoplastic polymer (hard plastic)
QDPI&F	Queensland Department of Primary Industries and Fisheries
QPWS	Queensland Parks and Wildlife Services
R	statistical software package (compare: SYSTAT, S-PLUS)
RPDL	redox potential discontinuity layer (also called anoxic layer)
SE	standard error, also STER (statistical term)
sonifier	instrument (metal rod with pointy tip) that disrupts cells using ultrasound (sonification)
S-PLUS	statistical software package (compare: SYSTAT and R)
STDEV	standard deviation (mathematical term)
SYSTAT	statistical software package (compare: S-PLUS and R)
TAC	total allowable catch
trophic	of or involving the feeding habits or food relationship of different organisms in a food chain, more general: of or relating to nutrition

1 General introduction

Holothurians, or ‘sea cucumbers’ (Echinodermata: Holothuroidea) are ubiquitous around the world’s ocean floors. Although adults of all species live on or near the seabed (benthic), they have developed ways of utilising two very distinct food sources: either extracting organic matter from the substrate or from the water column. This study will focus on the deposit-feeding holothurians of the order ‘Aspidochirotidea’ and, in particular, the species *Holothuria scabra* Jaeger, 1833 – also known as ‘sandfish’, a tropical species of the Indo-Pacific.

Certain species of holothurians have been harvested for the human food consumption for centuries. Currently, there are more than 20 holothurian species that are commercially harvested around the world, *H. scabra* being one of them (reviewed in Conand and Byrne 1993). Their thick body wall and large size make them attractive to the food industry. Particular Asian countries like China, Japan and Malaysia consider the dry product of these animals, also called ‘bêche-de-mer’ or ‘trepang’, as a delicacy.

Research on these animals so far has been focused mainly on reproduction and physiology, facilitating studies on fisheries and aquaculture feasibility (Battaglene *et al.* 1999; Morgan 2000b; Purcell *et al.* 2006b). However, research concerning the ecology of holothurians is necessary to fully understand their role within the marine ecosystem and the potential impacts of harvesting by humans. The following overview, mainly concerning *H. scabra* (based on Hamel *et al.* 2001), provides an outline of the current knowledge, particularly in relation to the importance of holothurians in regard to their ecological role in marine ecosystems.

1.1 General biology

1.1.1 Anatomy

The outer morphology of adult *H. scabra* (sandfish) is cylindrical, elongated and with a flattened ventral side (Figure 1-1). The colour of its dorsal side is variable ranging from black over sandy-brown to grey with irregular patterns and wrinkles (Conand 1999; Uthicke and Benzie 1998). The ventral side is mostly whitish and has small tube feet to facilitate the animal's peristaltic movement.

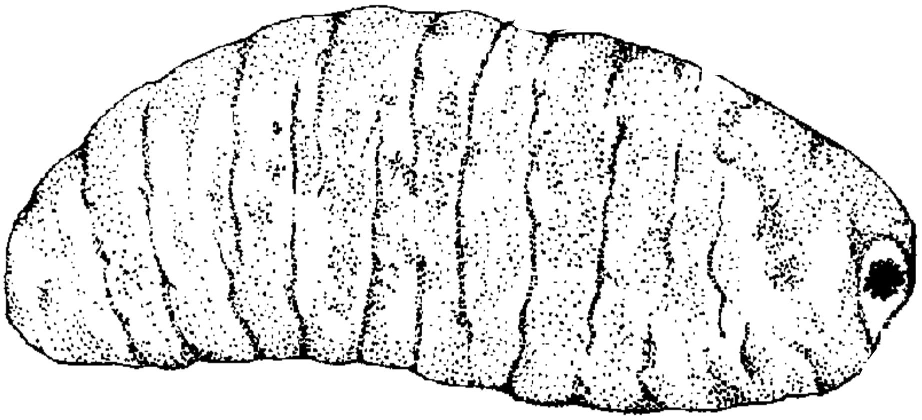

Figure 1-1: Exterior view of *Holothuria scabra* with anal region visible on the right side

Mature specimens usually measure between 150 and 400 mm in length and weigh between 500 and 2000 g, with both size and weight highly dependent on the animal's geographical range (Hamel *et al.* 2001). The body wall is about 5 mm thick, rough and accounts for about 56% of the total wet weight (Conand 1993).

The internal morphology (Figure 1-2) reveals the affiliation to the group of Echinodermata with its pentamerous partition and endoskeleton just under the outer skin.

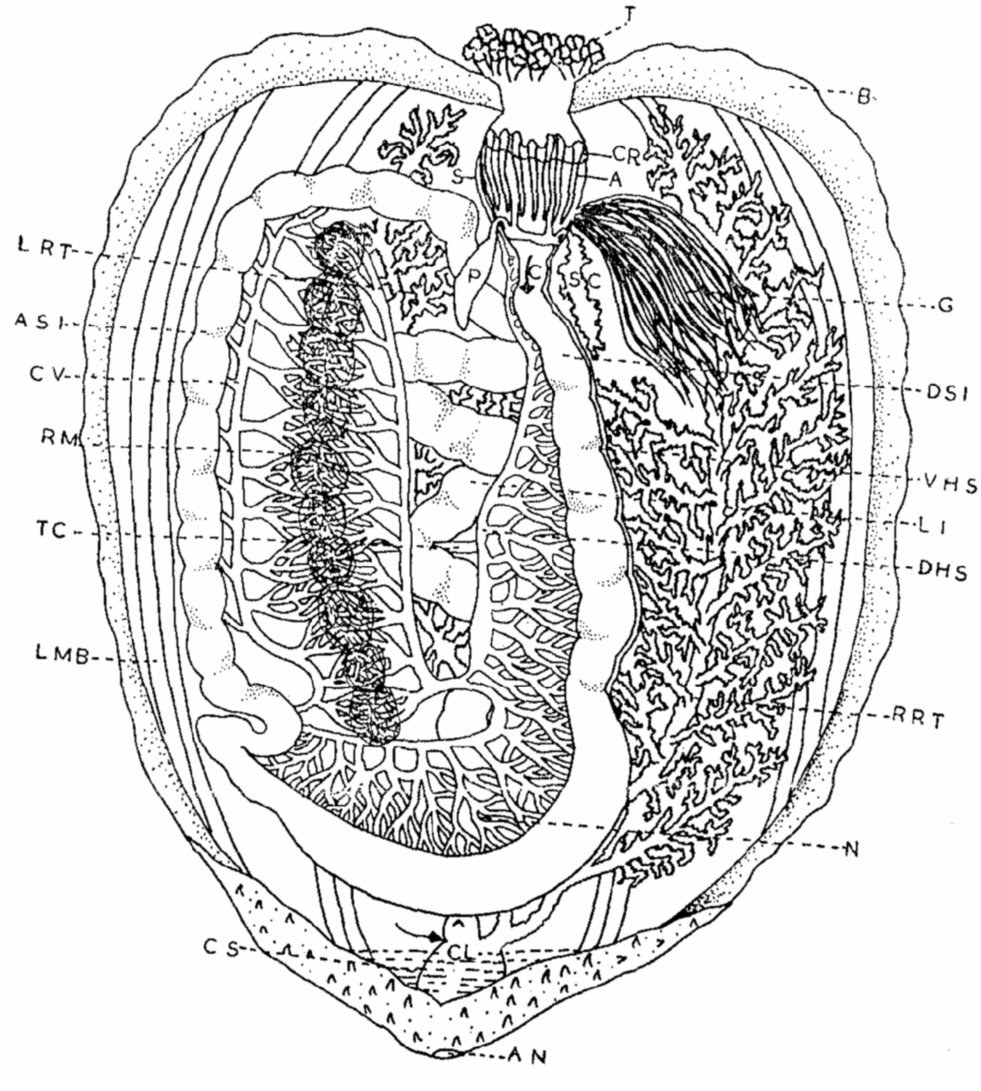

Figure 1-2: Interior view of *Holothuria scabra* (taken from Bai 1971). Abbreviation used: A = ampulla; AN = anus; ASI = ascending small intestine; B = body wall; C = constriction; CL = cloaca; CR = calcareous ring; CS = cloacal suspensor; CV = collecting vessel; DHS = dorsal haemal sinus; DSL = descending small intestine; G = gonad; LI = large intestine; LMB = longitudinal muscle band; LRT = left respiratory tree; N = network of dorsal haemal sinus; P = polian vesicle; RM = rete mirabile; RRT = right respiratory tree; S = stomach, SC = stone canal; T = tentacle; TC = transverse connective of ventral haemal vessel and VHS = ventral haemal sinus

The gonad is a tubular shaped reproductive organ, and males and females release sperms or eggs through a single opening at the front dorsal region (James 1996). Apart from spawning events, the only way to distinguish between male and female specimens is to dissect the gonad under a microscope (Baskar 1994).

The digestive system is composed of a descending and ascending small intestine and a large intestine, which is highly coiled and opens into a cloacal chamber at the posterior (Bai 1978). Also terminating in the cloaca is the respiratory tree, the main organ involved in respiration beside the outer integument and the tube feet (Bai 1980). The tree is divided into right and left tubes and smaller branches reach all through the coelomic cavity. In order to transport oxygen to the respiratory tree, the animal performs 'cloacal pumping', forcing water in and out of the anus (Hamel *et al.* 2001).

To facilitate the transport of nutrients as well as oxygen to all parts of the body and discarding waste products, sandfish use two types of body fluids: the coelomic fluid and the haemal system. The transparent coelomic fluid fills most of the body's cavity and contains free cells called coelomocytes. The haemal (colourless blood vascular) system is restricted to two main sinuses, centrally running along the top and the bottom of the small intestine and five radial sinuses branching of the haemal ring around the oesophagus and then running along the body wall to the anterior (Bai 1980).

The nerve system is not centralised (e.g. brain-like), however, is concentrated into numerous ganglia along radial nerve cords. The tube feet are highly sensitive towards vibration and direct contact, and signals can spread across the body in a matter of seconds. This is especially important in regard to the animal's defence mechanisms like building up a solid endoskeleton.

Another defence mechanism many holothurians have is called ‘Cuvierian tubules’ (commonly known as ‘threads of defence’), but sandfish do not possess these (Hamel *et al.* 2001). Instead, they commonly use ‘evisceration’ (expulsion of the interior organs). During field studies where the animals were tagged or transported this occurred in up to 14.6% of the stock (Conand 1989). However, auto-evisceration under natural conditions has never been reported (Bai 1971). After evisceration, the internal organs can be regenerated. In *H. scabra* this can take approximately 40 days; although feeding, or rather production of faecal pellets, can start as early as seven days after the event (Bai 1971).

1.2 Distribution

1.2.1 Geographical range

H. scabra are found mainly within 30 degree of latitude north and south throughout the Indo-Pacific. Their distribution ranges from the African coast, to Asia and Indo-, Micro-, Mega- and Polynesia (reviewed in Hamel *et al.* 2001). However, this species has never been reported along the eastern side of the Pacific or anywhere in the Atlantic (Massin 1999). Throughout their range *H. scabra* occur mainly in low energy environments behind fringing reefs or within protected bays and shores (Mercier *et al.* 2000a, b). The animal is known to tolerate salinity as low as 20 PSU but avoids direct contact with brackish water by burying and lowering their cloacal pumping frequency (Mercier *et al.* 1999). The general habitat preference of *H. scabra* ranges from intertidal flats to depths of around 10 m on muddy to sandy sediments in close proximity of seagrass beds.

1.2.2 Density

Density of *H. scabra* is variable throughout the animal's geographical range. Conand (1989) reported maximum densities of 0.6 individuals m^{-2} for New Caledonia. In the Solomon Islands density of up to 3.4 animals m^{-2} were found if juveniles were incorporated (Mercier *et al.* 2000a). The highest recorded adult densities were from India with figures from 0.4 to 2 individuals m^{-2} (James 1994a). Skewes *et al.* (2002) reported a maximum density of around 1.5 individuals m^{-2} in Moreton Bay, Australia. Hamel *et al.* (2001) pooled most of the existing data, and found that an average density of *H. scabra* throughout the Pacific and Indian Ocean ranged between 0.2 and 0.6 individuals m^{-2}.

1.2.3 Locomotion

H. scabra move by means of three rows of ventrally located tube feet (the "trivium") and peristaltic muscular movement of the body wall enabling the animal to climb over hard surfaces such as rocks (James 1989) as well as soft sediments (Hamel *et al.* 2001). Mercier *et al.* (1999; 2000a) recorded juvenile *H. scabra* moving 125 cm d^{-1} on average (specimens captured and in the field). Lokani *et al.* (1996) reported faster movement of adults with a speed of 12 cm min^{-1}; however, this was most likely related to short-term searching behaviour. Locomotion of sandfish is supposed to be mostly a random movement, changing direction every day (Mercier *et al.* 2000a). However, Lokani *et al.* (1996) suggested that movement was not always random but may be orientated towards specific areas (such as preferable grain size or food sources). Most publications concur that the movement of *H. scabra* is greater on non-optimal substrata such as coarse or silty sediment compared to favourable substrata like muddy sand with organic content around 4% (Mercier *et al.* 2000a).

Long-term (years) movement has not yet been studied on adult sandfish. Only data about individual movement of juveniles are available (Mercier *et al.* 2000a). They showed that *H. scabra* only moved up to 80 cm d^{-1} and thus remained relatively steady over a two-month study period. However, *H. whitmaei*, a closely related species, can move up to 1100 cm d^{-1} (Shiell, pers. com.). In general it can be said: the larger the sea cucumber, the greater distance it travels (Hamel *et al.* 2001). Therefore, movement of *H. scabra* along different coastal habitats as well as into deeper areas and recruitment from the deeper areas back to the shallow environment need a closer insight.

1.2.4 Habitat

The spatial distribution of sandfish is highly age dependent and related to preferences of different substrata (Dance *et al.* 2003; Mercier *et al.* 2000b). In general it is believed that animals of all ages occur in coastal areas, which include sandy habitats as well as seagrass beds as part of an estuarine, low energy system (Mercier *et al.* 2000a, b). Most individuals occur where sediments comprise about 5-10 % organic matter (Mercier *et al.* 2000a). In New Caledonia, sediment with terrigenous inputs is the preferred substratum of the animal (Conand 1990). Mercier *et al.* (2000b) described that late larval stages of *H. scabra* settle preferably on substrata other than sand, like rocks or seagrass. Only when they reach a certain length (around 6 mm) do they start moving down to sandy areas with a transition period of approximately a week where they move on and off the seagrass leaves. After transitioning from seagrass to sediment habitats, juvenile sandfish prefer muddy-sandy (grain size ~ 0.4 mm) habitats (Mercier *et al.* 1999). However, a specific choice of grain size might also have a close relationship to food richness of the substratum (Moriarty 1982). In turn, Mercier *et al.* (1999) pointed out that their study did not substantiate this hypothesis, but showed *H. scabra* differentiate between substrata irrespective of organic content. Therefore, *H. scabra* may prefer sediment compositions representing a compromise between burying energetics and feeding efficiency. Hence, a substratum with medium grain size and sufficient organic matter (2-5%) should be ideal for juveniles as well as adults.

1.3 Development

1.3.1 Reproduction

There are two main methods used to identify the reproductive stages of holothurian: gonad index (GI) and histological examination. The GI is expressed as a ratio between weight of the gonad divided by the drained body weight (body without the coelomic fluid) multiplied by 100. Histological examinations provide a more thorough insight into the tissue of the gonads by microscopic analyses, such as measuring the diameter and/or thickness of the different stages of maturing gametes.

There has been some confusion about spawning periods of sandfish. Most authors report that *H. scabra* spawn all year around (Battaglene 2000; Conand 1990, 1993; Krishnan 1968; Ong Che and Gomez 1985; Tuwo 1999). Some authors also report a semi-annual reproductive cycle, signifying two major spawning peaks around May and November throughout the Indo-Pacific, which are mainly related to the dry and wet season. However, Morgan (2000a) found only one single spawning peak in November within the sandfish population of Moreton Bay, Australia. Despite some controversy in the literature, temperature seems to be the major spawning cue. All authors report a concordance of spawning events with either a rise or a decrease in water temperature, although some also mention an associated salinity variation, mainly caused by monsoonal rainfall in the Indian Ocean regions (Krishnaswamy and Krishnan 1967; Moiyadeen 1994).

During a spawning event (Figure 1-3) the release of the gametes takes place over 2-3 hours around noon. Males normally spawn first and females release their eggs about 1-2 hr later.

Figure 1-3: Picture of *Holothuria scabra* during a spawning event in a mesocosm tank (Nov 2005)

Individuals lift two-thirds of their body up into the water column and rotate the anterior in a circular movement. At the same time gametes are discharged, sperm in a continuous stream and eggs as intermittent jets (Battaglene 2000; James and James 1993). Cues for synchronous spawning of males and females have rarely been investigated and are still poorly understood. Studies on the Solomon Islands showed aggregative behaviour prior to spawning (possibly through chemical cues), corresponding with the full moon (Hamel *et al.* 1999; Mercier *et al.* 2000a). James *et al.* (1994) suggested that the protandrous spawning occurrence might thereafter stimulate the release of gametes in females.

1.3.2 Larvae

Larval development of sandfish undergoes three main stages. After initial cell division (48 hr), the first plankto-trophic stage is the feeding auricularia larvae, which is fully developed after 5-6 days (James 1994b). After a further week larvae transform into the doliolaria larvae. The animal remains for about 5-8 days in this non-feeding stage before settling as pentactula. At this stage, aged about 10-14 days, sandfish larvae prepare to settle on preferred substrata, with seagrass leaves yielding the highest settlement rates (Mercier *et al.* 2000b). In the absence of a suitable substratum, larvae delay settlement for up to four days after which they die (>95%, Mercier *et al.* 2000b).

1.3.3 Juveniles

Early settled sandfish feed on epiphytes and detritus upon seagrass leaves for 4-5 weeks until the animal's transition to the sediment when approximately 6 mm long (Mercier *et* *al.* 2000b). At this stage sandfish have high mortality rates (up to 70%) due to predators such as polychaetes, gastropods, crustaceans and fish (Mercier *et al.* 2000a). Hereafter, juveniles distribute on muddy sand substratum near seagrass beds. Juveniles have been found exposed on fine sand on an inner reef flat (Long and Skewes 1997); however, most animals stay in the shallow zone of coastal areas under constant water cover.

Growth rates have been assessed mainly under laboratory conditions and vary between 0.07-1.5 cm mo^{-1} with a corresponding weight gain of 6-27 g mo^{-1} (Battaglene 1999b; Battaglene *et al.* 1999; James *et al.* 1994; Mercier *et al.* 2000a; Shelley 1985a, b). However, some authors noted that when in captivity, some individuals could also lose weight (Battaglene 1999b; Conand 1983). Studies of growth rates in the field are limited. Mercier *et al.* (2000a) measured growth rates of 10-15 cm mo^{-1} on hatchery-reared juveniles released in the wild, which is 10-30 times the growth rate found under laboratory conditions. However, more studies are needed to establish growth rates in the field and to relate size to age. James *et al.* (1994) stated that *H. scabra* could live for up to to about 10 years.

1.4 Behaviour

1.4.1 Burying

Sandfish are often cryptic, by partly or totally burying into the sediment. Yamanouchi (1939; 1956) ascribed the burying behaviour of *H. scabra* in Palau to light intensity. The observed animals remained buried during the day, emerging in late afternoon to feed on the surface during the night. He suggested that this behaviour could minimise predation during the day and hypothesised it to be under neural control or a circadian rhythm triggered by the amount of light reaching the anal region of the holothurian. This, besides water exchange for breathing purposes, could also explain why most adult sandfish ensure their anal region is in constant contact with the water while buried.

Animals do not seem to show any detectable movement while buried (Mercier *et al.* 1999) and their metabolism and respiratory rate are low when not feeding (Uthicke 2001b; Yamanouchi 1956). Highest metabolic, locomotary and feeding rates were measured within 30 min. of animals emerging from the sediments (Mercier *et al.* 1999).

Mercier *et al.* (1999) and Battaglene *et al.* (1999) reported some new detailed aspects of the burying behaviour of juvenile *H. scabra*. Small animals (<80 mm) buried at sunrise and emerged at sunset. Larger animals (>80 mm) began to emerge earlier in the afternoon and spent longer periods exposed. Hence, burying cycles had different triggers depending on the size and age of the animal. The smallest juveniles (10-40 mm) were only affected by sunlight. Continuous darkness inhibited their burying behaviour. Intermediate juveniles (40-140 mm) began to surface earlier (mid afternoon), however, responded more to changes in temperature. Under constant high temperatures these juveniles remained exposed. Mercier *et al.* (1999; 2000b) also revealed a dependency of burying behaviour to salinity regimes. Decreased salinity (as occurs on reef flats at low tide during the rainy season) led to immediate burying and animals remained cryptic all day. This may facilitate osmoregulation. Tides can also be detected by sandfish and influence their burying behaviour; however, different studies show conflicting results. Constant low water significantly reduced emergence during laboratory studies (Mercier *et al.* 1999; 2000a). James (1994a), however, reported that juveniles, normally living buried under the sand, emerged during low tide. Similarly, Skewes *et al.* (2000) observed that only a third of the studied adult sandfish were visible during high tide. *H. scabra*'s ability to tolerate low salinity as well as low water levels may reflect their adaptation to shallow, tidal habitats (Hamel *et al.* 2001). Nevertheless, more thorough insights, especially long-term studies, are needed concerning the burying behaviour of adult sandfish.

1.4.2 Feeding

There are two fundamentally different feeding strategies amongst holothurians: deposit- or suspension-feeders. Deposit-feeders scavenge the organic detritus on or within the sediment, whereas suspension feeders usually have more developed tentacles to filter organic matter out of the water column. The family of Holothuriidae, of which *H. scabra* are a member, are exclusively deposit-feeders, and diet descriptions are relatively uniform in the literature. The animals gain their nutrition by ingesting sediment and digesting bacteria and microalgae, as well as detritus (dead organic matter of plant and animal origin) thereof (Moriarty 1982; Yingst 1976). Holothurians function as omnivores and the term 'detritivores' is no longer suitable to describe their trophic level.

James (1989; 1996) suggested that *H. scabra* have no food preference. However, Mercier *et al.* (1999) demonstrated that sandfish are able to choose between different types of substrata and therefore being able to identify sediments containing suitable organic content. Some authors believe that *H. scabra* are able to discriminate between sediments just on the basis of grain size (Baskar 1994; Wiedemeyer 1992). The conclusion that *H. scabra* feeds selectively can be debated, as the findings reflect a substratum preference but not necessarily a patch selectivity within a given substratum (*sensu* Hamel *et al.* 2001; Uthicke and Karez 1999). It is believed that *H. scabra* stop eating when burying down into the sediment in the early hours of the morning, and start feeding again in late afternoon when emerging (Mercier *et al.* 1999). However, findings are contradictory and need further confirmation. There is a particular need in differentiating between the physiology of sediment ingestion and actual food assimilation.

Various authors have estimated feeding rates and digestive transits. Mercier *et al.* (1999) determined high intestinal transit of about 20 cm hr^{-1}, whereas Wiedemeyer (1992) estimated considerable lower rates of 2.6 cm hr^{-1}. Complete passage of ingested sediment through the gut is estimated to take between 30 and 60 minutes (Mercier *et al.* 1999) or up to two hours (Yamanouchi 1939). Maximum feeding rates in juvenile *H. scabra*, 4.8 g dry weight (DW) sediment hr^{-1}, were measured by Mercier *et al.* (1999), while Yamanouchi (1939) reported *in situ* values of 8.2 g DW hr^{-1} on adult sandfish. Studies on closely related species ascertained 2.8 g DW hr^{-1} for *H. atra* and 2.6 g DW hr^{-1} for *S. chloronotus* (Uthicke 1999).

Recent studies have shown that benthic microalgae not only get stimulated by constant grazing but also receive high levels of ammonium through excretion (ejecting water) by holothurians (Uthicke 2001a, b). Thus, nutrient regeneration by holothurians could have an important ecological role when considering a reef or seagrass community. These findings, like nutrient uptake, ingestion rates and patch and/or habitat preferences, yet need to be substantiated for *H. scabra*.

1.4.3 Bioturbation

Activities of living organisms that mix and redistribute sediments and associated pore water horizontally and vertically are called ‘bioturbation’ (Mahaut and Graf 1987; Schink and Guinasso 1977; Welsh 2003). The activities of holothurians, such as feeding and burying, cause bioturbation and contribute to oxygen supply, sediment turnover rates and vertical transport of organic matter, thus may have a substantial impact on their habitat (Rhoads and Young 1971). Irrigation and aeration of deeper sediment layers through bioturbation, as well as increased nutrient input through interstitial waters, affects sediment chemistry and thus has ecological significance (Rosenberg *et al.* 2001; Rosenberg *et al.* 2000).

Uthicke (2001a) noted that high levels of bioturbation decrease total pigment (chlorophyll *a* and phaeophytin) content in the sediment. Hence, high densities of holothurians can reduce algal biomass. However, when taking the natural *in situ* holothurian densities of a coral reef into consideration, they were all in a range low enough to be non-destructive and were thus likely to be beneficial for the algal community. A review by Yingst and Rhoads (1980) concludes that metabolic activities and population growth rates of both bacteria and micro-organisms can be enhanced through bioturbation.

Uthicke (1999) estimated that a mixed population of *H. atra* and *S. chloronotus* with a density of about 0.1 individual m^{-2} on a coral reef flat in Australia had the potential to rework about 4600 kg of dry weight (DW) sediment $year^{-1}$ on a 1000 m^2 surface. This was approximately the weight of the upper 5 mm of sediment (the layer bioturbated by these holothurians) in this area. Mercier *et al.* (1999) estimated an annual sediment intake of a juvenile *H. scabra* at a maximum of 41 kg DW yr^{-1}, while Yamanouchi's (1939) results for an adult sandfish can be extrapolated to 71 kg DW yr^{-1}. These findings yet need to be confirmed in accordance with daily and seasonal variation in activity levels, for variation in abundance and density and in relation to habitat types (organic content and nutrient input).

1.5 Global bêche-de-mer fishery and history

Holothurians are used as food source for humans in two different ways: either the dried skin is traded as bêche-de-mer (also called trepang) or the gonads are consumed raw. The dominant consumer markets for bêche-de-mer are in China, with Hong Kong and Singapore being the main centres of trade and redistribution (Sachithananthan 1994a, b). The main producers (in order of quantities produced) are surrounding Asian countries such as Indonesia, Philippines, Japan, Korea, India, but also Africa and many Pacific islands (van Eys 1986). The Chinese have been trading bêche-de-mer with Southern India and Sri Lanka for more than 1000 years (Hornell 1917). The use of sea cucumbers as a tonic and in traditional medicine dates back to the Ming Dynasty (1368-1644 BC) (Chen 2003). Bêche-de-mer is attractive for Chinese cuisine due to its high protein content (up to 83%) and a strong anti-cancerous as well as anti-viral property (Chen 2003).

Bêche-de-mer fisheries have always followed a 'boom and bust' cycle and *H. scabra* were not always first grade product. In 1972 the market price was only US$ 0.3 kg^{-1} (Sachithananthan 1972) and in 1986 it was still only US$ 0.7 kg^{-1} (James 1989). In the early 1990s sandfish was upgraded to medium value bêche-de-mer at a price of about US$ 14-20 kg^{-1} (Holland 1994; Sommerville 1993). For comparison, the estimated value of world trade in bêche-de-mer was around US$ 60 million in 1994 with a global production of all valuable holothurians at 12 000 metric tonnes (mt) (Conand 1997). In 1996 the annual landing of processed product was estimated at around 23 000 mt. However, on a global scale it is difficult to differentiate between holothurian species in multi-species fisheries either having no species-based regulations or making it hard to enforce a single-species ban (Bruckner *et al.* 2003; Roberts and Hawkins 1999; Uthicke *et al.* 2004). In recent years, sandfish has become regarded as a high value species and prices were about US$ 50-100 kg^{-1} (Conand 2001; Jun 2002; Mercier and Hamel 1997). Latest estimates price high value bêche-de-mer as much as US$ 400 kg^{-1} for the dried product (Chen 2003; Rimmer 2006). Consequently, *H. scabra* is now among the most intensively exploited holothurians in the Indo-Pacific (Bruce 1983; McElroy 1990; Preston 1990; Taylor-Moore 1994; van Eys 1986).

The increasing value of holothurians has encouraged artisanal fishermen – in particular in Indonesia, Papua New Guinea, India, Madagascar, Solomon Islands, Philippines and many other Indo-Pacific countries – to harvest sandfish intensely. Hence, from the 1990s onwards a severe over-fishing around the Indo-Pacific depleted the natural stock and brought *H. scabra*, among other species, close to local extinction in many regions including Australia (Conand 1998; Conand and Byrne 1993; Morgan and Archer 1999; Preston 1990; Skewes *et al.* 2000).

Most fisheries are managed with respect to the effect of fishing on the target species, and the desire to control the fishery so that the removal through fishing is done in a sustainable fashion. However, there is increasing realisation that fished populations perform ecosystem functions and that the management of these fisheries needs to take these factors into account when assessing sustainability.

1.6 Local bêche-de-mer fishery

A developmental fishery for sandfish in Moreton Bay commenced in April 2003 and has since been closely surveyed and monitored by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Queensland Department of Primary Industries and Fisheries (QDPI&F) and Queensland Parks and Wildlife Services (QPWS). The Nunukul Ngugi Cultural Heritage Corporation (NNCHC) on North Stradbroke Island is the sole permit holder with two small vessels and the only method of collection is by hand while snorkelling. There is an annual total allowable catch (TAC) of 86 t wet weight (45 t gutted weight) from an estimated virgin stock of around 950 t wet weight, or 3.6 million individuals (Skewes *et al.* 2002) in three restricted zones.

1.7 Thesis objectives

The overall objective of this study is to investigate the ecological role of holothurians in the ecosystem and the effects of their removal (e.g. by fishing), using sandfish as the example. This study will aim at identifying the connection between *H. scabra* and its surrounding environment (e.g. seagrass and algae) by describing and assessing the ecological role of: burying, feeding, excretion and bioturbation.

In order to assess the ecological role of *H. scabra* this study has two major objectives:

(**A**) to determine the biological influence of *H. scabra* on associated flora and

(**B**) to determine the physical influence of *H. scabra* on the substrate.

Objective A will consider following questions:

A1) What is the influence of *H. scabra* on the benthic microalgae (BMA) community (biomass)?

A2) What is the influence of *H. scabra* on the seagrass community (biomass and/or productivity)?

A3) What is the influence of *H. scabra* on total organic matter (OM) in the sediment?

Objective B will consider following questions:

B1) What is the influence of *H. scabra* on chemical processes in the sediment (anoxic layer)?

B2) What is the influence of burying and feeding of *H. scabra* on sediment reworking, and is there a diel and/or seasonal cycle (mixing rate and behaviour)?

After addressing the above questions it is intended to extrapolate findings to a population of *H. scabra* under subtropical conditions (Moreton Bay) as well as applying them to tropical populations in general (Pacific). In conclusion, it is intended to comment on the relevance of these findings to the design of population surveys and management strategy plans for this species, applying the findings to a wider conceptual model of the importance of deposit-feeding holothurians situated within a complete ecosystem framework.

2 Biological role of sandfish for seagrass and benthic microalgae

2.1 Introduction

One of the main habitats for aspidochirotide (deposit-feeding) holothurians such as *H. scabra* in tropical and subtropical regions are seagrass beds, since larvae and juveniles rely heavily on seagrass for their settling cues and early life stages (Mercier *et al.* 2000b). The interaction between seagrass beds and associated organisms can be either beneficial, detrimental or have no effect on its host. Marine benthic communities have generally proven to be beneficial to primary producers like seagrass and algae by releasing and recycling nutrients (Reise 2002). Possible positive effects of holothurians on seagrass and algae could be through direct release or recycling of nutrients as they feed on bacteria, microalgae and organic detritus attached to sediment grains (Moriarty *et al.* 1985; Wiedemeyer 1992); thus, increasing nutrient levels in the water column in close proximity (Grall and Chauvaud 2002; Uthicke 2001b). Holothurians have been found to increase productivity of benthic microalgae through nutrient (especially ammonium) enhancement for carbonate sands in oligotrophic coral reef ecosystems (Uthicke 2001a).

In general, under conditions of nitrogen limitation, ammonium enrichment is likely to be beneficial to seagrass growth (Lee and Dunton 2000). Seagrass growth in subtropical Australia is known to be nitrogen rather than phosphorus limited (Udy and Dennison 1997). Furthermore, offshore seagrass beds are more susceptible to changes in nutrient regimes than their coastal counterparts (Ferdie and Fourqurean 2004). Hence, direct excretion of holothurians could have a positive effect on algae and seagrasses as shown in lower latitude (Uthicke 2001a).

The burying behaviour of holothurians can also beneficially affect benthic primary producers (Mercier *et al.* 1999; Wiedemeyer 1992); the movement potentially irrigating and aerating deeper sediment layers. This behaviour may release nutrients trapped in the interstitial waters, keep sediments oxygenated and/or displace benthic microalgae vertically as well as horizontally. However, there has been indication that burying holothurians might have also a negative impact on seagrass by accumulating sediment which may interfere in seagrass growth and dispersion (Mosher 1980).

Looking at complex subtropical seagrass habitats, the removal of *H. scabra* may affect productivity and biomass of seagrass and benthic microalgae. It is hypothesised that exclusion of holothurians from experimental treatments, thus reducing excretion and bioturbation, would have a negative effect on those benthic primary producers.

2.2 Methods

2.2.1 Pilot study

Pilot study methods

A pilot study was conducted for 16 days in March/April 2003 to trial the feasibility of field equipment, sampling and lab techniques. Only two cages and one control were set up at the study site (see Section 2.2.2 and 2.2.3). In addition, specially marked stakes were deployed inside and outside the fences (directly adjacent to the fence) to measure possible sediment accumulation near the fences. Samples were taken in 10 cm cores and subsequently sub-sampled with 60 ml cut-off syringes (as described below). The pilot study focused mainly on methods such as acetone extraction (volume and time), and sonification time, but this data set was also used for a power analysis. The following parameters were analysed during the pilot study: BMA biomass (chlorophyll *a*), OM (% g DW^{-1}) and the depth of the anoxic layer (mm). Statistical analyses were performed in R 2.5.1 (R 2005) and sample means were compared using Welch Two Sample t-test.

Acetone volume

The amount of acetone used to extract chlorophyll *a* (chl-*a*) of a known volume of sediment (here 2 cm^3) was trialled. Chl-*a* in samples was extracted with 10 ml of 90% acetone, a volume commonly used in the literature for similar chlorophyll concentrations (Jeffrey and Welshmeyer 1997). Results of this extraction were set to a 100% and sub-samples were then extracted with increasing volumes of acetone.

Sonification

The sonification method, based on ultrasound, destroys most micro-organisms and cells and releases the chlorophyll molecules into the solution. The extent of sonification necessary to extract all chl-*a* from a given sample was trialled with both, the standard 10 ml and 15 ml of 90% acetone. The sonification time most commonly found in the literature is 60 seconds (Jeffrey and Welshmeyer 1997; Parsons *et al.* 1984). Samples sonified for 60 sec were set to a 100% and sub-samples were processed with either shorter or longer durations of sonification.

Acetone extraction

Literature recommends an extraction period of 1 hour in the dark (Parsons *et al.* 1984). Although recently chl-*a* extraction procedures recommend samples to be filtered directly after sonification (Jeffrey and Welshmeyer 1997), it was decided nevertheless to test the extraction efficiency under these particular sediment conditions. It was investigated how different times of acetone extraction altered the efficiency of extracting chl-*a* from the sediment. Samples were filtered after 0.25, 0.5, 1 and 2 hours and the 1h-samples were set to a 100%.

Anoxic layer

Sediments in the study area are oxygen-rich only in the upper layers (5-20 mm). Below that, sulphur and hydrogen sulphide are the dominant chemical compounds, sediments are reduced (no oxygen, black in colour) and most organisms cannot survive under these conditions. The transition zone between oxidised and reduced sediments is called 'redox potential discontinuity layer', also known as anoxic layer. As an indicator of bioturbation through sea cucumbers, the depth of the overlaying oxic sediment was measured to calculate an average depth of the anoxic layer. The 60 ml cut-off syringes were marked with three vertical lines prior to sub-sampling to ensure random measure of each core. The oxic layer was measured to the nearest 5 mm along these markers. An Analysis of Variance (ANOVA) was used to analyse the log-transformed data set using R 2.5.1 (R 2005).

Power analysis

Chl-*a* and OM data were used to calculate a power and cost-benefit analysis prior to the main experiments. The results were used to determine how much effort was needed for yielding adequate precision and power. Number of cages, number of cores in each cage and number of sub-samples in each core were compared. Minimum detectable differences are given with 5% error and 80% or 90% power. S-PLUS was used for those statistical analyses (S-PLUS 2005).

Pilot study results

There was no accumulation of sediment along the fences, thus 4 m^2 cages set 5 m apart were thought to be adequate to ensure that hydrodynamic interactions would not affect the sediment. Sampling with 10 cm cores was considered to be too much of an intrusion. Subsequently, the cages were sampled with small 60 ml cut-off syringes, and the big cores were used only to sample seagrass biomass at the start and the end of each experiment.

Acetone volume

When comparing samples amongst different volumes of acetone extraction (5 ml increments), variation was high which was mainly due to the low number of replicates (Figure 2-1).

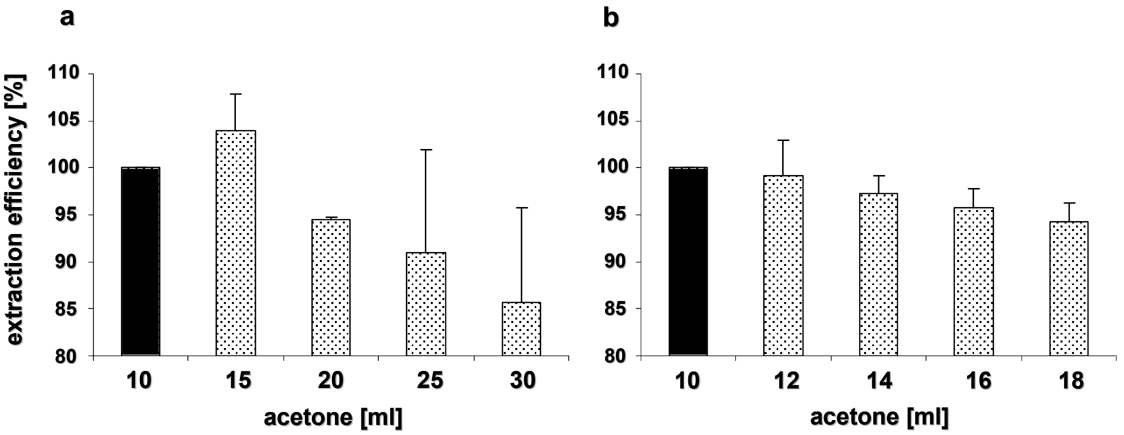

Figure 2-1: Chlorophyll *a* extraction efficiency (%) with different amounts of acetone in (a) large (5 ml) and (b) small (2 ml) increments. Common extraction volume (10 ml) was set to a 100% (solid bar); n = 2 (for a) and 10 (for b), error bars = 1 SE

Nevertheless, it was apparent that with increasing volume of acetone the chl-*a* content of the sample was diluted, thus decreasing the accuracy of its measurement. After increasing the resolution of volumes of acetone (2 ml increments), it was concluded that the maximum efficiency of extraction in acetone lied round 14-16 ml for approximately 4 g of sediment (Figure 2-1 b). Thus, it was decided that 15 ml, which was about 3.5 times the volume of the sediment sample used in the trials, was an appropriate volume of acetone for the type of sediment used in this study.

Sonification

Sub-samples in 10 ml acetone that were sonified for 30 seconds had significantly lower (t = 2.99, p = 0.03) chl-*a* content than sub-samples that had the standard 60 sec treatment (Figure 2-2 a). When samples were sonified in 15 ml acetone means did not differ significantly, however, sonification for 30 sec yielded in general less chl-*a* than for 60 sec (Figure 2-2 b). When comparing sub-samples of 60 and 90 sec sonification time with sub-samples being treated the usual 60 sec (Figure 2-2 c-f), no clear trend was visible and means were not statistically significant.

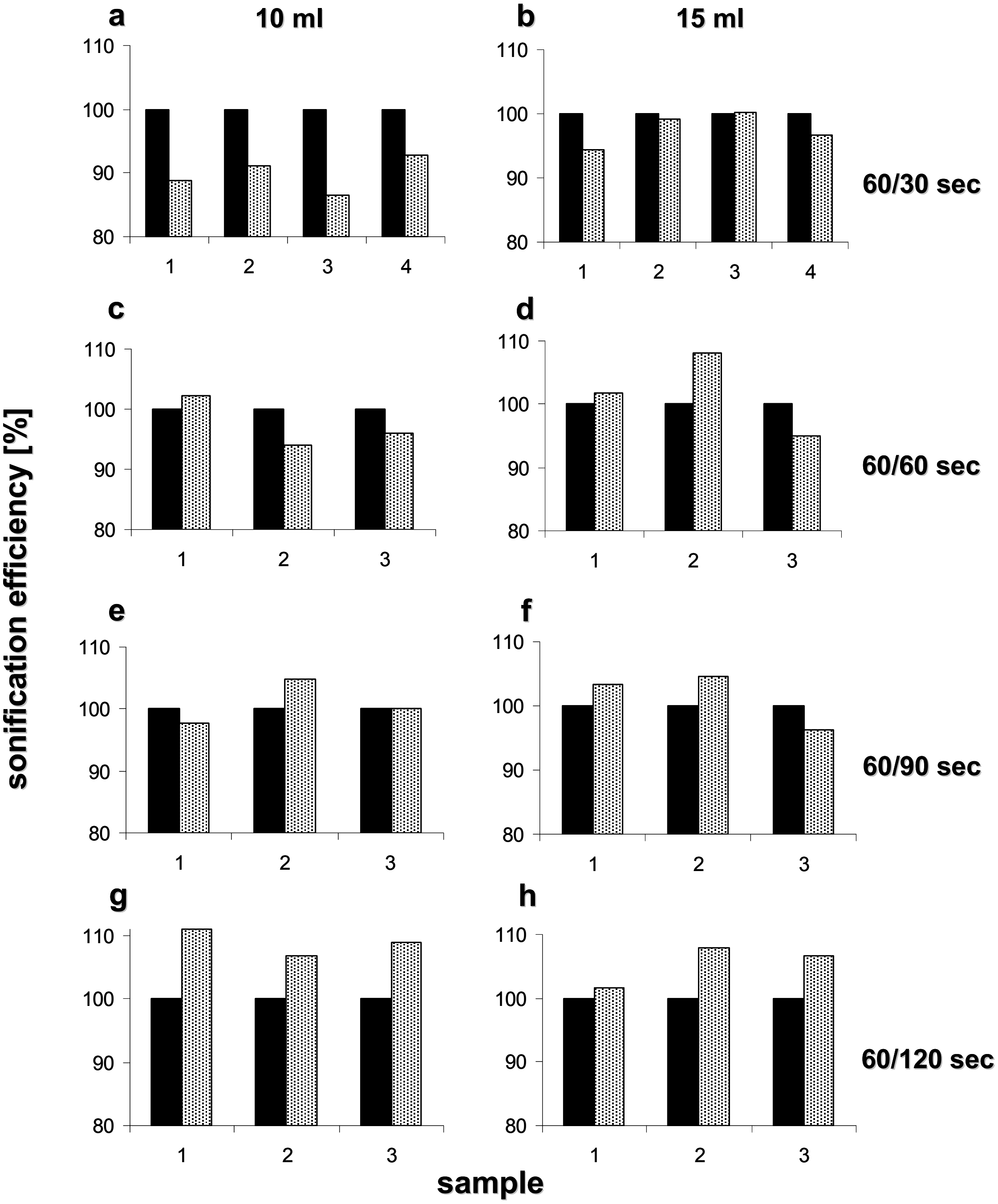

Figure 2-2: Sonification duration and its efficiency in extracting chlorophyll *a* with 10 ml (left column) and 15 ml (right column) of acetone; figures on right represent sonification times; solid bars indicate samples treated with the standard sonification (60 sec, set to 100%), dotted bars represent the second sub-sample. Note: each bar represents a single sample; bar-pairs represent two sub-samples from the same core

However, after 120 sec of sonification sub-samples had significantly higher ($t = 5.69$, $p < 0.001$) amount of chl-*a* than after only 60 sec (Figure 2-2 g-h). Hence, the sonifier was used for at least 120 sec when extracting chlorophyll from these sediments. A trade-off is apparent when sonification is applied for any longer duration, since the sample tends to heat up even though it is kept on ice during the process. This trial did not test for any longer period than 180 sec, and saw neither a decrease in extraction efficiency amongst those longer durations nor a significant increase between 120 and 180 sec. Based on these results it was recommended not to overheat both sonifier and samples and to keep sonification time to a maximum of 120 sec.

Acetone extraction

When comparing different duration of acetone extraction (0.5, 1 and 2 hr), variation was high and only trial 5 showed significantly higher extraction after 2 hr (Figure 2-3 a). When analysing the data from the higher resolution trial (0.25, 0.5 and 1 hr), sample means were not significantly different between to the usual 1 hr extraction time (Figure 2-3 b).

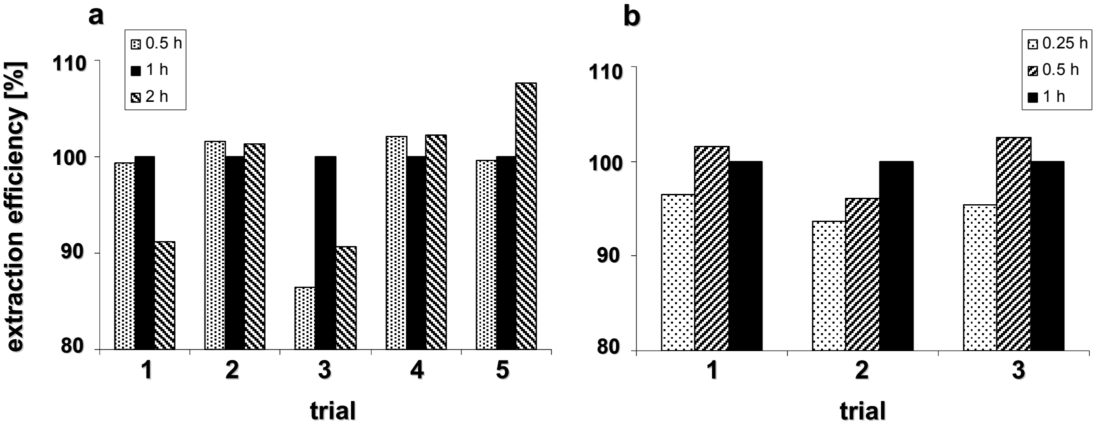

Figure 2-3: Chlorophyll *a* extraction efficiency (%) in acetone after different durations in (a) large (0.5 hr) and (b) small (0.25 hr) time increments. Note: scales are different; standard extraction time (1 hr) was set to a 100% (solid bar); n = 2 (a) and 10 (b)

Based on these data it was concluded that 0.5 to 1 hr extraction in the dark would be sufficient to release all chl-*a* trapped in these sediments into the acetone.

Sonification should release all trapped chlorophyll into solution (here acetone). However, an extraction period of 2 hours in the dark is recommended. This trial (Figure 2-2) determined that shorter extraction periods come to similar result than the standard 2 hr (set to a 100 %).

Anoxic layer

The depth of the overlying oxic layer was highly variable in all treatments (Figure 2-4 a). Even though oxic sediment in one cage (F 2) was significantly thinner (F = 6.50, p = 0.017) than in the control (C), in general, it was only marginally significantly different (F = 3.05, p = 0.058) between cages and the control.

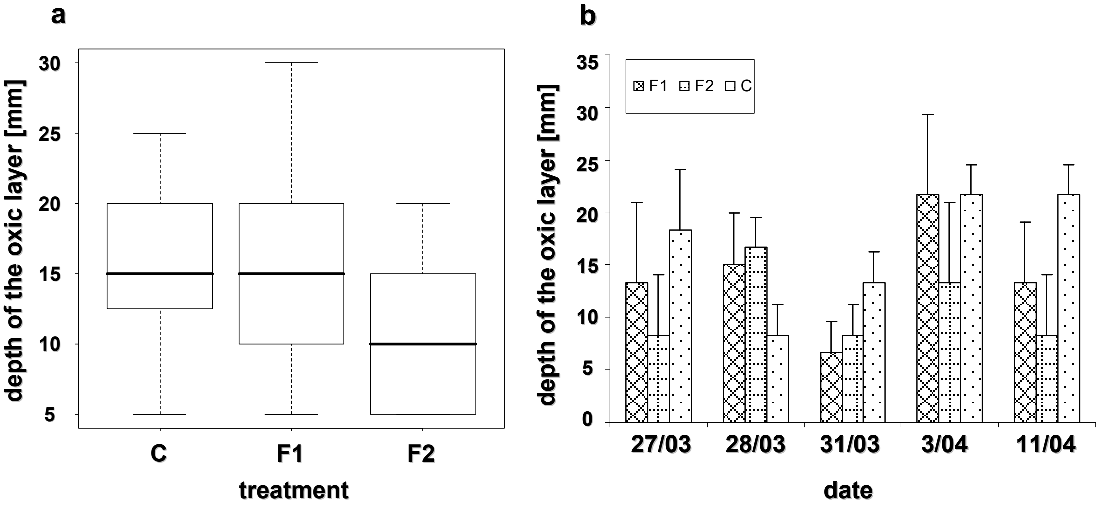

Figure 2-4: Depth of the oxic sediment layer as (a) overall treatment average and (b) treatments over time. F1 = fenced area 1; F2 = fenced area 2; C = unfenced control area; n = 3; error bars = 1 STDEV (a) and 1 SE (b)

When data were analysed as repeated measures over time, no significant difference could be found (Figure 2-4 b). Nevertheless, this method was used to measure the anoxic layer during the first main experiment (Nov. 2003), thus collecting a larger data set to adequately judge the variability in the system.

Organic content (OM)

Samples did not significantly vary amongst treatments (cage and control) over time (Figure 2-5). This data set was the baseline for an initial power analysis (see below).

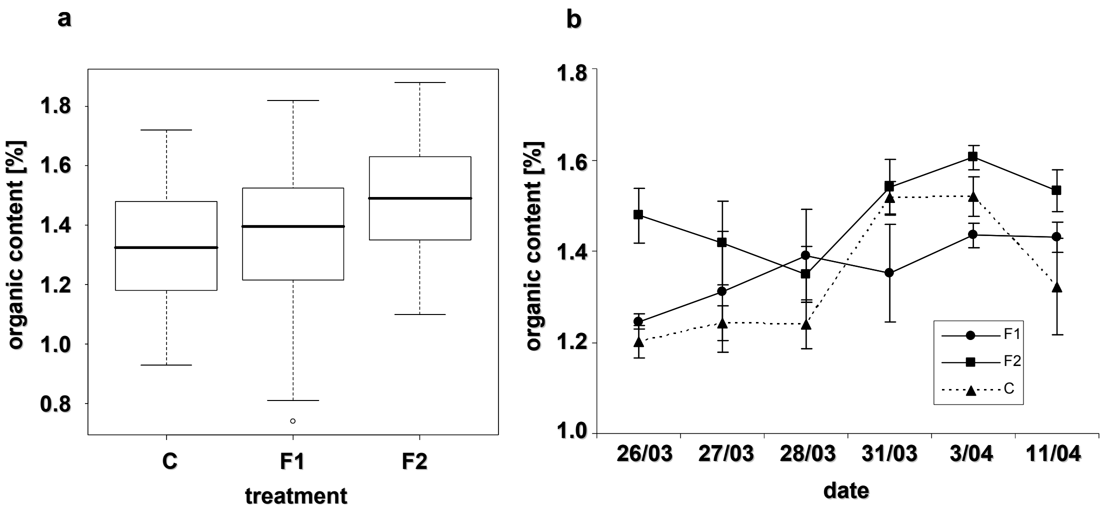

Figure 2-5: Organic content as (a) overall treatment average and (b) treatments over time. F1 = fenced area 1; F2 = fenced area 2; C = unfenced control area; n = 9; error bars = 1 STDEV (a) and 1 SE (b)

Benthic microalgae (BMA) biomass

BMA biomass samples (based on chl-*a* content in the sediment) did not significantly vary amongst treatments over time (Figure 2-6). Data were used for the initial power analysis (see below).

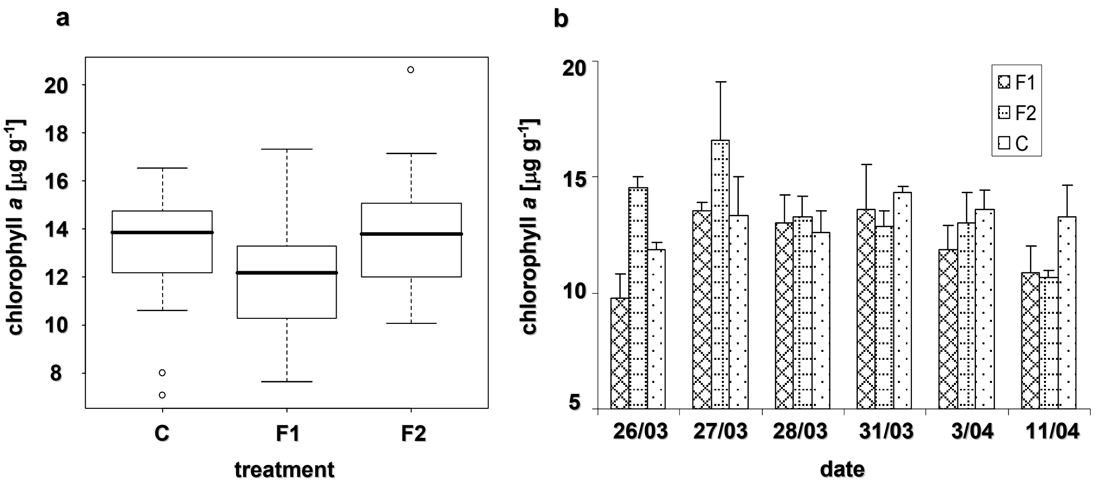

Figure 2-6: Chlorophyll *a* as (a) overall treatment average and (b) treatments over time. F1 = fenced area 1; F2 = fenced area 2; C = unfenced control area; n = 9; error bars = 1 STDEV (a) and 1 SE (b)

Power analysis

Data showed that the highest variance was amongst cores and the highest cost (effort) involved was correlated with increasing number of cages. Based on these results and an 80% power (Figure 2-7), a minimum of 12 cages (4 replicates for each treatment) was used, and 12 cores were sampled out of each cage on any given sampling occasion. Sub-sampling was reduced to two per core and considered to be sufficient for the variability of this design and habitat.

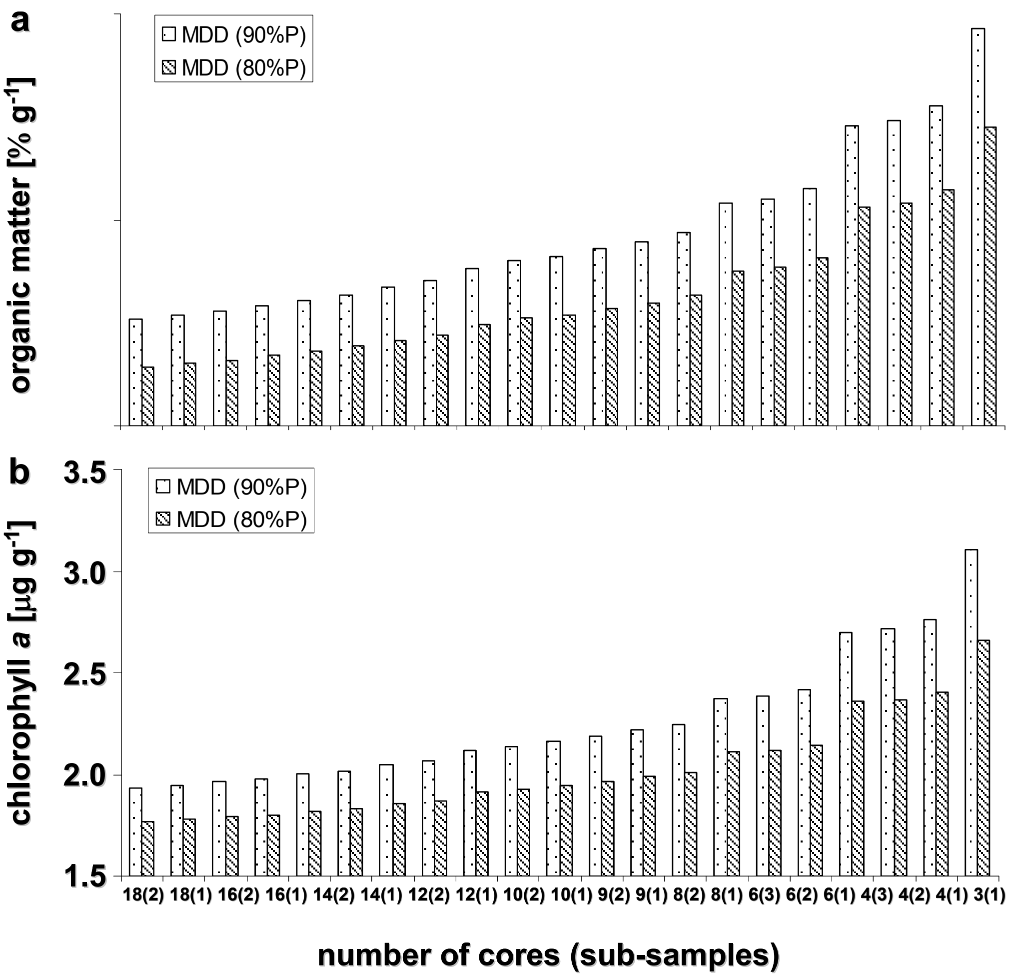

Figure 2-7: Power analysis for (a) organic matter and (b) benthic microalgae with 80% and 90% power. MDD = minimal detectable difference; n = 3

2.2.2 Study site

Moreton Bay is a semi-enclosed embayment in south-east Queensland, Australia with water exchange occurring through its northern and eastern margins. It is adjacent to a major city (Brisbane, population = 1.8 million) and bounded to the east by two main islands, Moreton Island and Stradbroke Island. Of an approximate total area of 1,500 km^2, it contains around 203 km^2 of seagrass beds (Dennison and Abal 1999). Six different seagrass species occur either in mixed or in monospecific beds on both eastern and western sides of Moreton Bay.

Field experiments were conducted at Myora Gutter (27 º 27.876’ S; 153 º 25.146‘ E (Datum: GDA), a small bay adjacent to the western side of North Stradbroke Island (see arrow in Figure 2-8). This area is mostly sheltered but occasionally subjected to strong tidal currents. Tidal sea levels in the bay vary by 1-2 m. The study site was located in about 3-4 m depth below Mean Low water level, approximately 300 m from the island’s shore line.

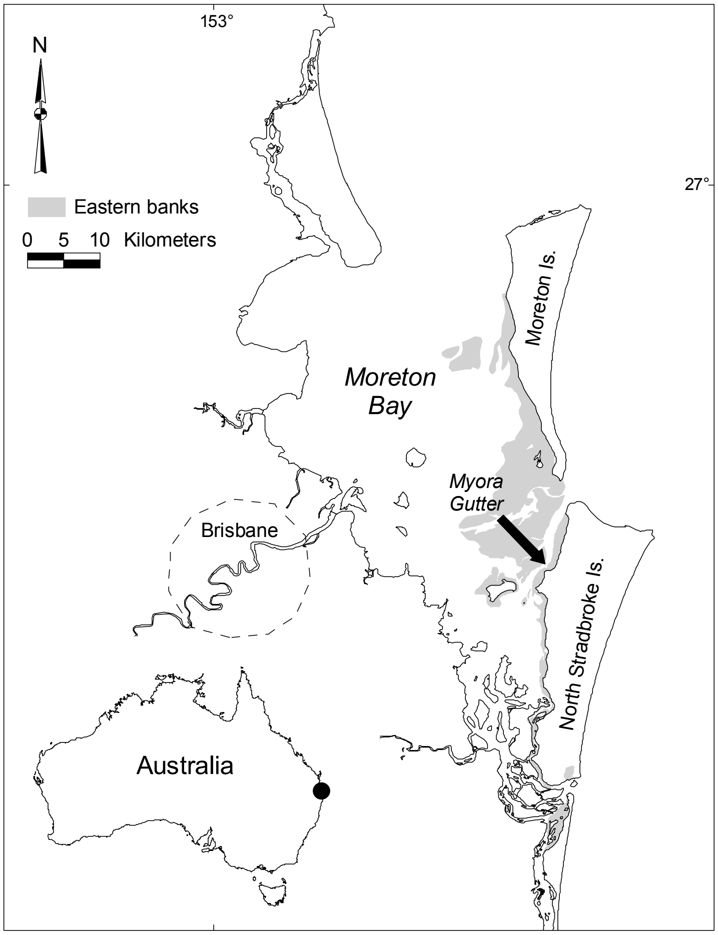

Figure 2-8: Map of Moreton Bay (insert indicates location within Australia). Arrow shows study site at Myora Gutter

Most of Myora Gutter has 80-90% seagrass coverage, which is dominated by two seagrass species; *Cymodocea serrulata* (R. Brown) Ascherson and Magnus 1871 and *Zostera muelleri* Irmisch ex Ascherson 1867. The former is known to occur predominantly in monospecific beds within Moreton Bay and has its highest coverage at Myora Gutter (Dennison and Abal 1999). The cage experiments were conducted in a monospecific *C. serrulata* seagrass bed and all seagrass results presented are based on measurements of this species.

2.2.3 Experimental design

The experiment was based on 2 by 2 m square exclusion cages that were placed in shallow seagrass beds in 3-4 m below Mean Low water level. Each cage was constructed from chicken wire (13 mm mesh) 30 cm high, fixed to rigid mesh (25 mm mesh) that extended 10 cm down into the sediment. The cages were held together by four wooden poles at each corner.

Three different treatments were used in the experiment: exclusion cages (EX) where *H. scabra* were excluded from the caged area; cage controls (CC) with chicken wire only (no solid mesh) allowing *H. scabra* to crawl underneath the fencing and therefore occupy the cage at approximately natural densities; and natural controls (NC) with no fencing (four posts only) and therefore with *H. scabra* at natural densities (Figure 2-9).

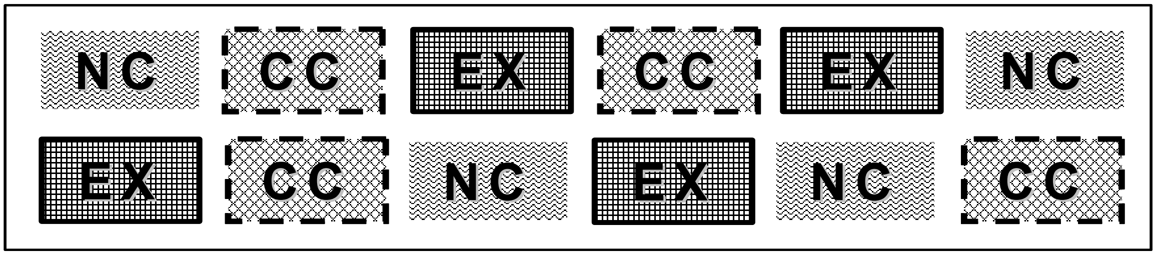

Figure 2-9: Schematic design of the cage experiment; NC = natural control (no fencing), CC = cage control (wired mesh only), EX = exclusion (wired and solid mesh)

Twelve cages were erected parallel to the shore line in two rows of six. Each row had two blocks of three cages, with the location of each treatment randomly located within the block. Based on preliminary experiments, each cage was placed 5 m apart to minimise hydrodynamic disturbances. A random number decided where each treatment was placed within each block.

Two field experiments were carried out; the first in austral spring (September to November 2003), thereafter referred to as 2003, and the second in austral autumn (February to April 2004), referred to as 2004. The cage area, incl. a 100-m buffer zone around them, was not disturbed by fishermen for the entire duration of both experiments. Samples were collected by means of SCUBA diving and several response variables were measured to examine the effect *H. scabra* may have had on the seagrass community: seagrass productivity and biomass, benthic microalgae (BMA) biomass and general organic matter (OM) in the sediment.

2.2.4 Holothurian density

Animals inside the cages were counted every week to ensure that sandfish densities in CC were close to natural densities (NC) and EX had close to no *H. scabra*. Observer interference took place only in EX where animals that had intruded into cages were manually removed and placed in the vicinity of the site, no further than 20 m away from where they were found. Based on observations conducted during the pilot study, adult *H. scabra* bury in the sediment during late hours of the night until the morning depending on water temperature. Monitoring and counting of the cages coincided with the highest probability of every animal being on the surface feeding (10:00 – 16:00) to ensure no buried animal was overlooked.

2.2.5 Seagrass productivity

Seagrass productivity was measured by determining the growth in leaves following the "hole-punch method" (Zieman 1974 with modifications described in Dennison 1990) during the last week of each experiment. In this approach a small area (20 × 20 cm) was selected at random and about 40 shoots were marked in each cage. They were punctured twice with a hypodermic needle about 2 cm above the sheath (Figure 2-10). Six days later all the shoots within the marked area were harvested by clipping whole shoots directly off the rhizome. Shoots were carefully placed into linen bags to prevent leaves separating from the sheath and kept refrigerated until analysed within the next three weeks.

In the laboratory each shoot was cut off at the needle mark of the oldest leaf (see solid line in Figure 2-10), since the oldest leaf does not grow anymore (Kenyon *et al.* 1997) and can therefore be used as a stationary reference point. Leaves were then separated into 'standing crop' and 'new growth'. All parts of leaves which were above the needle mark were considered standing crop (O) and below the marks was new growth (N). Totally new small leaves that had no marks (and had no signs of grazing at the tip) were considered new growth as well. Length and width measurements were taken for both leaf sections. Shoots were collected in aluminium trays and dried at 60°C for 24 hours. Dry weight of shoots was pooled for each sample (cage) and leaf sections (new growth and standing crop).

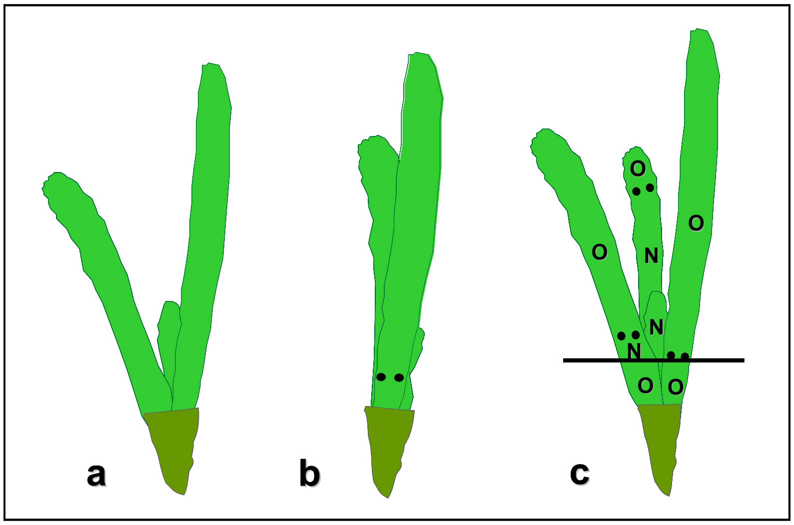

Figure 2-10: Schematic picture of a seagrass shoot (a) before marking, (b) held together while marking and (c) when harvested after 6 days with subsequent cut-off mark (solid line). N = new growth below needle markers; O = old standing crop above needle markers and sheath

2.2.6 Seagrass biomass

Seagrass biomass in cages was sampled at the start and conclusion of both experiments. On each occasion one seagrass biomass sample (randomly placed) was taken from each cage with a 10 cm diameter corer. Samples were sieved through a mesh bag to extract the sediment and plant material was kept refrigerated in a moist linen bag for less than two weeks before being processed. Shoots were counted and all green (living) leaves were measured (length and width) to the nearest mm. Since *C. serrulata* has relatively thick leaves, short term storage (less than a month) did not affect their colour. Dry weight for each sample was determined for leaves and shoots (above-ground biomass) and roots and rhizomes (below-ground biomass) after removing any epiphytic growth by means of scraping and then drying the seagrass material at 60°C for 24 hours.

2.2.7 BMA biomass and general OM

Sediment samples were collected using 60 ml cut-off syringes (corers) with rubber stoppers. On each sampling occasion 12 cores were taken from each cage to about 60 mm depth, six for BMA and six for OM analyses. The top 30 mm of sediment from each core was transferred to a plastic bag for analysis. All samples were kept on ice during the field trip until transferred to the freezer and stored at -30° until processed.

Sediment samples for BMA analysis were thawed in a water bath (20°C) in the dark. Any large particles such as seagrass leaves, roots or stones were removed and sediment thoroughly mixed. Two sub-samples (2 cm^3, approx. 3.7 g wet weight) were removed from the homogenised sample and transferred into 15 ml of 100% acetone. A third sub-sample was placed in a pre-weighed aluminium tin and dried at 60 ºC for 24 hours for moisture content analysis.

Extraction of chl-*a* from BMA cells was achieved by means of sonification. Based on results from the pilot study (see Section 2.2.1), samples were sonified for two minutes directly after acetone was added, and then left to extract for one hour in the dark on ice. After extraction, liquid samples were transferred into a 60 ml syringe and manually filtered through glass micro-fibre paper (GF/75 or grade 453). The extract was then used for spectrophotometric analyses (Parsons *et al.* 1984) at four wavelengths (630, 647, 665 and 750 nm). To determine the content of degradation products (phaeopigements) of the sample, 30 µl of 0.2 M hydrochloric acid (HCl) was added to the sample and a second reading recorded after 10 sec. Chl-*a* and phaeopigements were then calculated as in Parsons *et al.* (1984).

Sediment samples for OM analysis were thawed and homogenised as described above. Two sub-samples (approx. 5 cm^3) were transferred into pre-weighed crucibles. Samples were dried at 60ºC for 24 hours and then weighed to record their dry weight (DW). Subsequently, crucibles were transferred to a muffle furnace for two hours at 550ºC to determine the ash weight (AW). Finally, ash free dry weight (AFDW = DW - AW) was used to calculate OM content.

2.2.8 Statistical analyses

For each measurement variable, a General Linear Model (LM) was fitted (SYSTAT 1999) to two explanatory factors: cage treatment (NC, CC and EX) and block (a four-level factor handled as a fixed effect). For those variables measured at both the start and end of the experiment, the change in measured response for each cage was analysed. This simplifies the model while retaining information on important interactions between time and the other experimental factors. Analysis of Variance (ANOVA) was used to test the null hypothesis that the response variable is unaffected by presence or absence of *H. scabra*. The response variable was always continuous and either expressed as weight or length. Initial data exploration showed data were more or less normally distributed and the variances were homogeneous.

During 2003, one cage (EX II) was destroyed by a vessel impact, thus resulting in an unbalanced design and lower precision for this treatment compared to the controls (NC and CC).

Variation amongst treatments was partitioned into two orthogonal contrasts: (1) comparing the average of the controls (NC and CC) with that of the exclusion treatment

(EX) and (2) comparing the two controls. A large F-ratio for contrast 1 and a small F-ratio for contrast 2 would be strong evidence that excluding *H. scabra* has an impact.

Holothurian density

Holothurian counts were modelled using a Generalised Linear Model (GLM) with Poisson distribution and log link (R 2005) to demonstrate that exclusion of animals in EX was effective. An analysis of deviance using a chi-square test was used to assess the explanatory importance of terms in the model.

Seagrass biomass and productivity, BMA biomass and OM

Values from sub-samples in each cage were averaged on each occasion before being differenced over time, allowing a General Linear Model (LM) with a single error term to be fitted.

Multivariate analyses

A Principal Component Analysis (PCA) was carried out to further investigate if holothurians have a significant effect on multiple aspects of seagrass systems. Six response variables were included in this analysis: above and below-ground seagrass biomass change, seagrass productivity (based on leaf length and weight), change in BMA and OM. PCA was conducted on z-transformed data using S-PLUS (S-PLUS 2005). In addition, Multivariate Analysis of Variance (MANOVA) was used to assess the impact of the exclusion treatment on the combined suite of response variables.

Post hoc power analysis

A post hoc power analysis was carried out for all response variables (including seagrass data), by substituting the fitted values from the linear model as if these were the true mean response for the given combination of time, treatment and block. The observed residual mean squared error was used and the actual sample size (including number of replicates) was preserved. Under this set of conditions, the probability of correctly rejecting the null hypothesis of no change for each parameter between the three treatments was evaluated, given a false positive rate of 5%.

2.3 Results

2.3.1 Holothurian density

Animals in NC and CC had an average density of 0.45 ± 0.07 SE m^{-2} (Figure 2-11). Only on one occasion (Figure 2-11 a: 31.10.03) were densities below 0.25 m^{-2}. Densities as high as 1.25 m^{-2} were recorded in CC on several occasions. For both experiments the average density inside exclusion cages (EX, 2003: 0.12 m^{-2} ± 0.04 SE; 2004: 0.09 m^{-2} ± 0.03 SE) was significantly (GLM, $p < 0.00001$) lower than in the cage controls (CC, 2003: 0.44 m^{-2} ± 0.08 SE; 2004: 0.47 m^{-2} ± 0.06 SE) and in the natural controls (NC, 2003: 0.48 m^{-2} ± 0.06 SE, 2004: 0.40 m^{-2} ± 0.06 SE). Mild over-dispersion occurred during 2003 (65.1 on 55 df), probably due to low numbers for CC on 31.10.03 as mentioned above. During 2004, however, the model showed a good fit (49.4 on 51 df). Holothurian densities in the controls (NC and CC) were similar to naturally occurring densities in this area of Moreton Bay (Skewes *et al.*, 2002). As densities in the exclusion cages (EX) were, on average, less than a quarter of the NC and CC, it provided an opportunity to assess the impact of deposit-feeding holothurians on primary producers such as seagrass and microalgae.

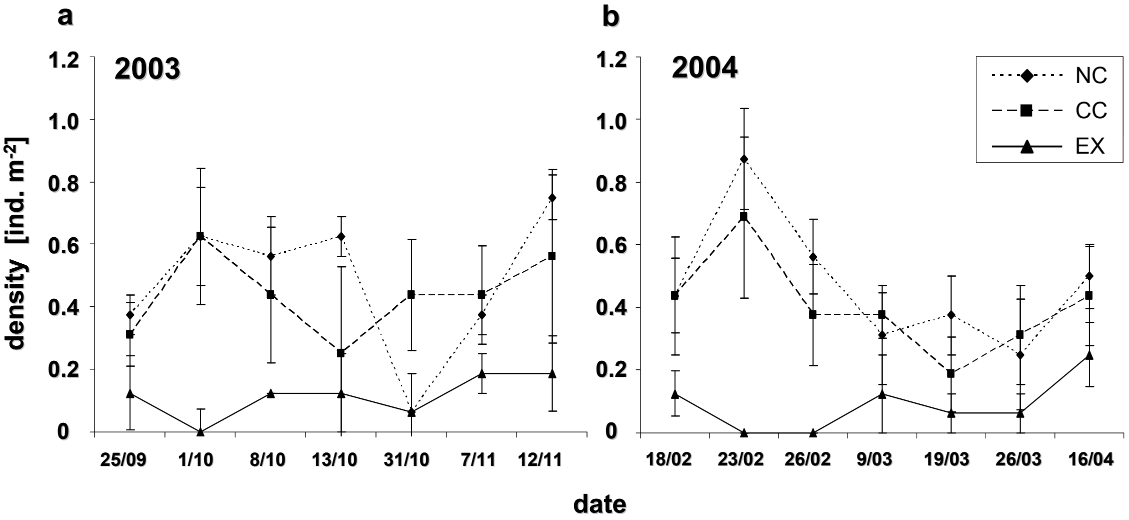

Figure 2-11: Holothurian density in experimental cages over the duration of (a) the 2003 and (b) the 2004 experiment. NC = natural control; CC = cage control, EX = exclusion; error bars = 1 SE; n = 4

2.3.2 Seagrass productivity

Seagrass productivity expressed as growth of new leaf weight in mg DW shoot^{-1} day^{-1} (W) was almost identical among treatments during 2003 (Figure 2-12 b), reinforced by non-significant p-values for treatment and linear contrasts (Table 2-1). However, during 2004, there were marked differences among treatments (Figure 2-12). NC and CC had significantly (p = 0.008) higher productivity than EX, which was in general 12% lower than the combined controls, supporting a holothurian exclusion effect (orthogonal contrasts, Table 2-1). The post hoc statistical power of detecting treatment differences in shoot productivity was poor in 2003 (8%) but excellent in 2004 (89%).

Seagrass productivity, expressed as growth of new leaf length in mm shoot^{-1} day^{-1} (L), was again similar amongst the three treatments in 2003 (9.34 ± 1.10 SE) (7.49 ± 0.78 SE) (Figure 2-12 a), reinforced by non-significant p-values for treatments (Table 2-1). However, in 2004 EX had the highest average growth of new leaf length, and the difference between exclusion treatment and combined controls (NC and CC) was larger (p = 0.099) than between the two controls (p = 0.251) (Table 2-1). The post hoc statistical power of detecting treatment differences was low in 2003 (17%) and fairly good in 2004 (57%).

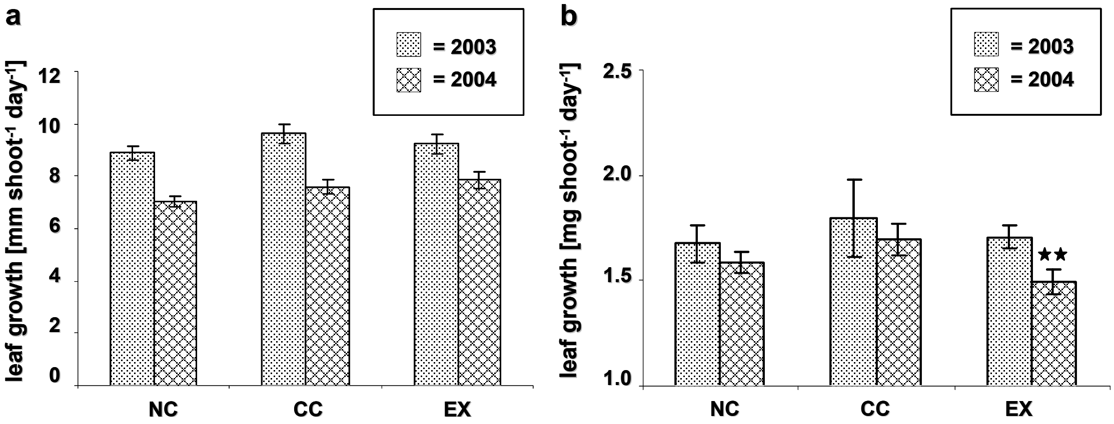

Figure 2-12: Average seagrass productivity for the first (2003) and second (2004) experiment (a) in terms of leaf length and (b) in terms of leaf weight. Note: different scales on y-axis. NC = natural control; CC = cage control, EX = exclusion; error bars are 1 SE; n = 4; ** = $p < 0.01$

Table 2-1: Linear contrast for seagrass productivity and biomass for the first (2003) and the second (2004) experiment. df = degrees of freedom; MS = mean squares; NC = natural control; CC = cage control, EX = exclusion; DW = dry weight; L = leaf length in mm shoot^{-1} day^{-1}; W = leaf weight in mg DW shoot^{-1} day^{-1}, biomass = g DW m^{-2}; A = above-ground; B = below-ground

Variable:		Productivity (L)		Productivity (W)		Biomass (A)		Biomass (B)	
	df	MS	P	MS	P	MS	P	MS	P
2003									
block	3	1.847	0.245	0.080	0.338	27506	0.010*	14489	0.431
treatment	3	1.149	0.377	0.015	0.771	1509	0.605	2544	0.834
residual	5/6	0.962		0.056		2756		13596	
orthogonal contrasts:									
(NC+CCvEX)	1	<0.000	9.890	0.003	0.830	2884	0.346	5086	0.563
(NCvCC)	1	2.299	0.183	0.028	0.513	134	0.833	<1	0.994
2004									
block	3	2.344	0.027*	0.041	0.007*	2307	0.439	14593	0.205
treatment	2	0.996	0.145	0.040	0.010*	1437	0.555	1447	0.820
residual	6	0.368		0.004		2212		7034	
orthogonal contrasts:									
(NC+CCvEX)	1	1.396	0.099	0.056	0.008*	2294	0.348	1296	0.683
(NCvCC)	1	0.595	0.251	0.025	0.040*	580	0.627	1598	0.650

2.3.3 Seagrass biomass

Above-ground seagrass biomass was initially similar amongst the three treatments during both experiments and averaged 165 g DW m^{-2} ± 27 SE in 2003 and 104 g DW m^{-2} ± 10 SE in 2004 (Figure 2-13). However, in both experiments average biomass decreased in all three treatments over the 2-month study period — by 32% in 2003 and 19% in 2004; although only 2003 was statistically significant (p = 0.013).

Below-ground seagrass showed a similar pattern with an initial average biomass of 240 g DW m^{-2} ± 28 SE in 2003 and 273 g DW m^{-2} ± 27 SE in 2004 (Figure 2-13). It also decreased during both experiments in all three treatments over the 2-month study period — by 20% in 2003 and 17% in 2004, however, neither change was statistically significant.

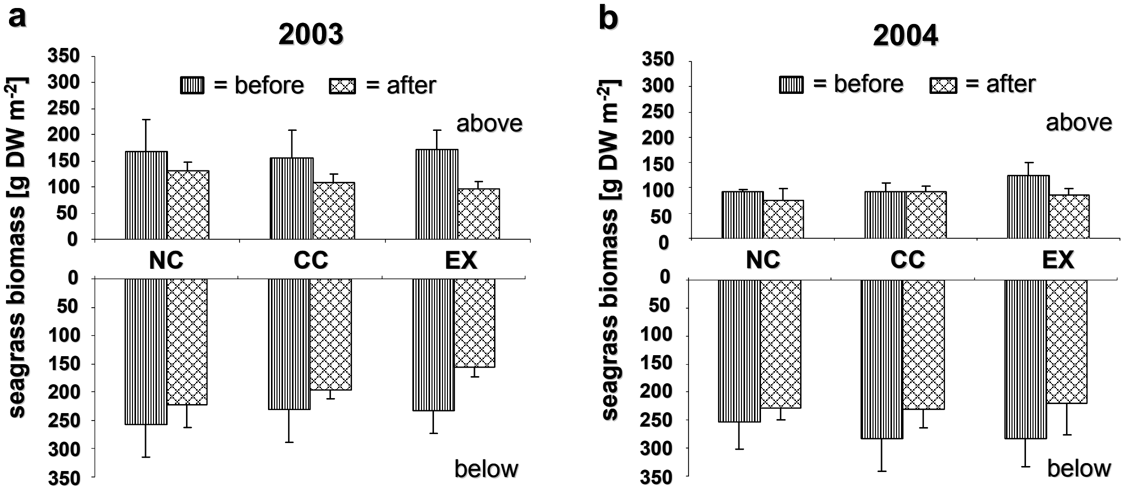

Figure 2-13: Average seagrass biomass: (a) during the first (2003) and (b) during the second (2004) experiment. Bars pointing upwards represent above-ground biomass (leaves and shoots), bars pointing downwards represent below-ground biomass (rhizomes and roots). NC = natural control, CC = cage control, EX = exclusion; error bars are 1 SE; n = 4

EX had a consistently greater decrease in seagrass biomass compared to NC and CC within both experiments (Figure 2-14), suggesting that holothurian exclusion had an impact. In 2003, above-ground biomass decreased by 18% more in EX than controls (Figure 2-14 a). Below-ground biomass showed a similar trend where reduction was 19% more in EX compared to the controls (Figure 2-14 b). In 2004, above-ground biomass decreased by 21% more in EX, and below-ground biomass showed still a 7% greater reduction in the EX than in the controls.

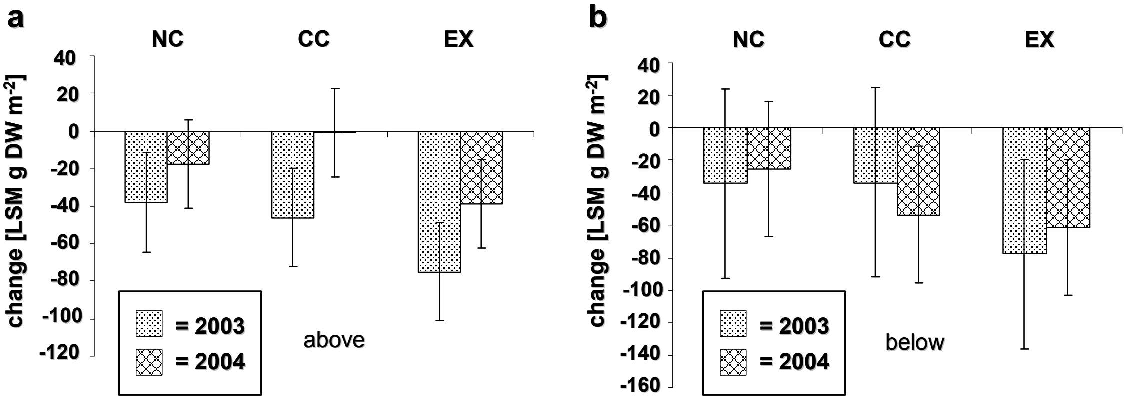

Figure 2-14: Average change in seagrass biomass during both experiments for (a) above and (b) below-ground biomass. NC = natural control, CC = cage control, EX = exclusion; LSM = Least Squares Means; DW = dry weight; error bars are 1 SE; n = 4

While none of the above-mentioned differences were statistically significant (contrasts NC+CC v EX, Table 2-1), the relative size of mean squares (MS) for contrasts shows that this difference was appreciably larger than that between controls.

The significant block effect, which occurred in seagrass biomass data in 2003 and seagrass productivity data in 2004 (Table 2-1), was followed up with simple correlation analysis. A possible relationship with depth (distance to water surface), seagrass cover and holothurian density was investigated, but none of these factors could be correlated to the fact that during both experiments one block behaved differently to the other three.

There was high variability between samples within treatments, and the low number of samples meant that the experiment had a low power; the post hoc statistical power of detecting the actual treatment differences was only 7-10% in 2003 and 9-12% in 2004.

2.3.4 Benthic microalgae (BMA) and organic matter (OM)

During the 2003 experiment, BMA biomass (average = 15.31 ± 0.42 SE µg chl-*a* g sediment^{-1}) increased in CC and EX, whereas NC showed an overall decrease in biomass (Figure 2-15 a, Table 2-2). During 2004, BMA biomass (average = 12.67 ± 0.43 SE µg chl-*a* g sediment^{-1}) increased slightly in NC and CC (non-significant), whereas EX showed a large increase in BMA biomass that was marginally statistically significant ($p = 0.089$) (Figure 2-15 a, Table 2-2).

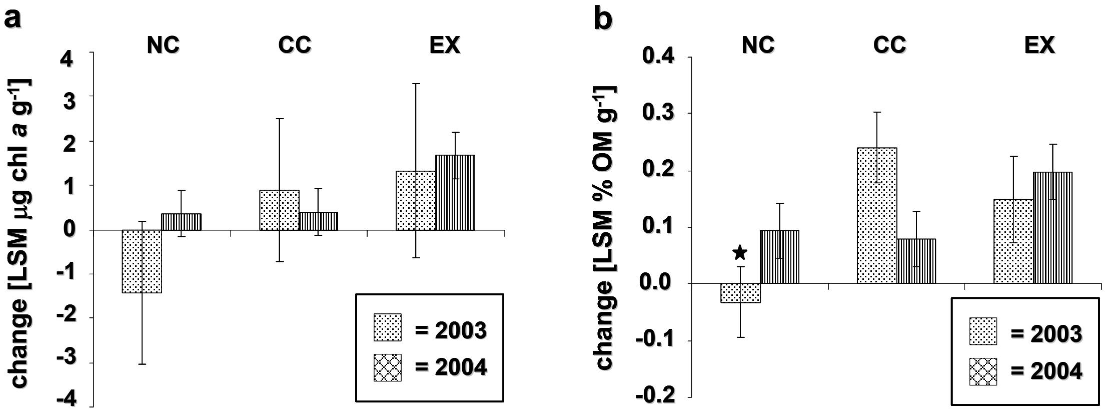

Figure 2-15: Average change in (a) benthic microalgae biomass (expressed as µg chlorophyll *a* g DW^{-1}) and (b) organic matter (expressed as % of sediment) during both experiments. NC = natural control, CC = cage control, EX = exclusion; LSM = Least Squares Means; DW = dry weight; error bars are 1 SE; n = 4; * = $p < 0.05$

Table 2-2: Linear contrast for organic matter (OM, % g sediment^{-1}) and benthic microalgae biomass change (BMA, in μg chl-*a* g sediment^{-1}) for the first (2003) and the second experiment (2004). df = degrees of freedom; MS = mean squares; NC = natural control; CC = cage control, EX = exclusion

Variable:		BMA		OM	
	df	MS	P	MS	P
2003					
block	3	1.751	0.912	3.515	0.193
treatment	2	7.911	0.511	7.625	0.064
residuals	5	10.288		1.521	
orthogonal contrasts:					
(NC+CCvEX)	1	5.038	0.515	0.404	0.628
(NCvCC)	1	10.784	0.353	14.846	0.026*
2004					
block	3	0.886	0.535	0.080	0.965
treatment	2	2.263	0.209	1.657	0.248
residuals	6	1.101			0.934
orthogonal contrasts:					
(NC+CCvEX)	1	4.526	0.089	3.273	0.110
(NCvCC)	1	0.001	0.983	0.040	0.842

Average OM in the sediment, which was 1.25% ± 0.05 SE in 2003 and 1.37% ± 0.03 SE in 2004, showed a similar pattern to changes in BMA. The 2003 experiment revealed a slight decrease of OM for NC which was significantly different ($p = 0.034$) to the increase in the two cages (CC, EX) (Figure 2-15 b, Table 2-2). During 2004, organic matter increased in all treatments, but EX showed a higher, however, not statistically significant, increase in OM than controls (Figure 2-15 b, Table 2-2).

Both response variables indicate that there may have been a cage effect during 2003 and a marginal significant holothurian exclusion effect during 2004 (see contrasts in Table 2-2).

2.3.5 Multivariate analysis

The Principal Component Analysis (PCA) for 2003 did not show any apparent differences between EX and the controls (Figure 2-16 a). However, during 2004 EX were clearly separated from the controls (Figure 2-16 b).

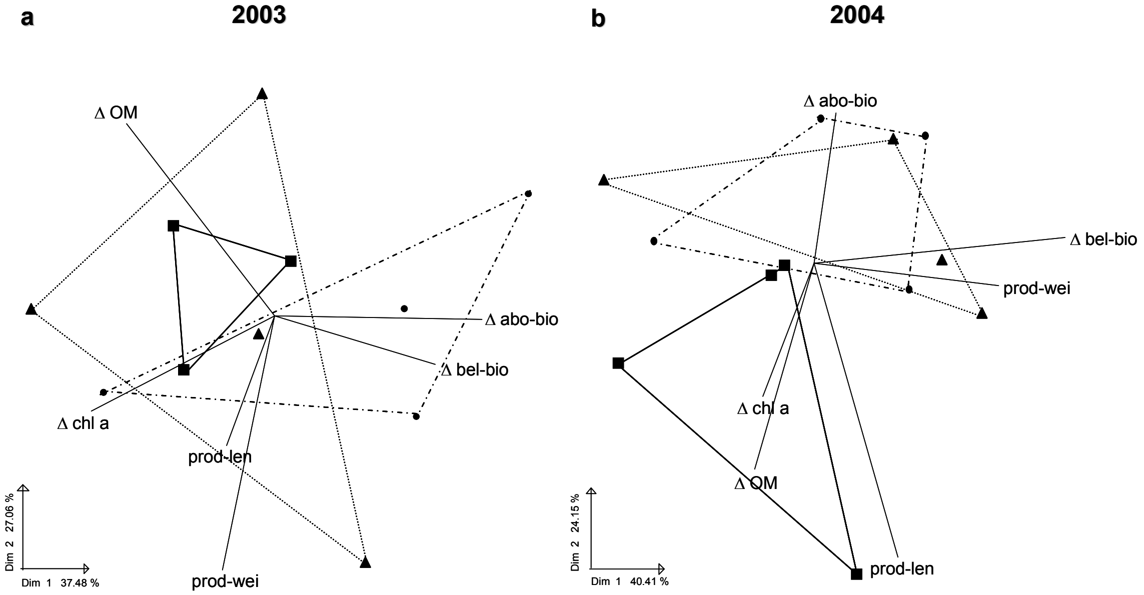

Figure 2-16: Principal component analysis (PCA) biplot illustrating differences between exclusion cages (EX) and controls (NC and CC) during (a) 2003 and (b) 2004. Data were z-transformed before analysis and individual cages from the same treatment were surrounded by polygons; Δabo-bio and Δbel-bio = above and below-ground biomass change; prod-wt and prod-lg = seagrass productivity based on leaf weight and length; Δchl-*a* = benthic microalgae biomass change, ΔOM = change in organic matter; NC = broken line; CC = dotted line; EX = solid line

The main contributors to this separation were the difference in BMA biomass and OM (which increased more in EX). No statistically significant difference ($p = 0.397$) was found between EX and the controls in 2004 when multivariate tests (MANOVA) were applied to all six variables, but when the four variables with greater individual statistical power (BMA biomass, OM, and shoot productivity — length and weight) were selected, the difference bordered on statistically significant ($p = 0.074$; Wilks' lambda test).

2.4 Discussion

Holothurians appear to benefit seagrass, as evidenced by the higher productivity (weight) and lower decrease in biomass in NC compared with EX during both years. Conversely, the foraging habits of holothurians appears to reduce BMA biomass and OM, as evidenced by the lower BMA biomass and OM content in NC compared to EX during both years. This particular combination of results from five key variables, occurring over two years, supported the hypothesis that the removal of holothurians may have impacted on their surrounding habitat.

2.4.1 Seagrass productivity

Our range of productivity values corresponds closely to growth maxima for *C. serrulata* in other studies: 1.38 g DW m^{-2} in northern Queensland (Pollard and Greenway 1993), 11.4 mm $shoot^{-1}$ day^{-1} and 1.48 g DW m^{-2} in Malaysia (Kamal *et al.* 1999) and 7.2 mm $shoot^{-1}$ day^{-1} and 2.4 g DW m^{-2} in Mozambique (de Boer 2000). However, in the current study, growth in shoot length and shoot weight was affected in opposite ways during the 2004 experiment (austral summer). Despite new growth in EX being longer than in controls, it weighed less. This suggested that low densities of *H. scabra* may have caused some form of stress reaction within the seagrass by possibly producing thinner leaves with bigger internal gas spaces (Enriquez 2005). The stress may have been caused by limited nutrient availability or increased shading through increased organic litter. Thus, either nutrient or light limitation or a combination of both could have caused possible physiological alterations within the leaves.

2.4.2 Seagrass biomass

The greater decrease in seagrass biomass in EX also suggested a positive impact on seagrass when holothurians were present. Here again, the data range (min to max) corresponded well with other studies: 115-235 g DW m^{-2} (Boon 1986) and 63-79 g DW m^{-2} (Udy and Dennison 1997) in Moreton Bay, 88.8-186.7 g DW m^{-2} (Birch and Birch 1984) and 80-100 g DW m^{-2} (Lanyon and Marsh 1995) in Northern Queensland. Previous studies have found a decrease in biomass of tropical seagrass species (incl. *C. serrulata*) directly related to decreased abundance of other benthic macrofauna (e.g. polychaetes, amphipods, decapods) (Klumpp and Kwak 2005 and citations therein). Variation amongst cages in the present study was high and statistical power low; therefore no assumption can be made about holothurian effects on standing seagrass biomass until further data can be collected. This also justifies the exclusion of seagrass biomass (above and below-ground) as variables from the MANOVA, which resulted in a marginally significant difference ($p = 0.074$) for the remaining four variables. Seagrass productivity, BMA biomass and OM, thus, seemed to have been key indicators of healthy habitats in which *H. scabra* were foraging and reproducing in this subtropical study.

2.4.3 Seagrass habitat and nutrients

Even though tropical seagrass beds are in general mainly influenced by wet and dry seasons rather than strong light and temperature variations (Lanyon and Marsh 1995), Moreton Bay’s seagrasses take a special position at a latitude considered to be a boundary between subtropical and temperate environment (Carruthers *et al.* 2002). This study was situated at the eastern side of Moreton Bay with low turbidity and low levels of nutrients. Hence, seagrass beds such as *C. serrulata* were more susceptible to changes in nutrients (Dennison and Abal 1999) and may have been able to pick up elevated nutrient levels and convert them into physiological changes of above-ground biomass quicker than seagrass beds in nearshore nutrient-saturated waters (Ferdie and Fourqurean 2004). A recent meta-analysis found that sediment nutrients are the key limiting factors to seagrass growth and biomass rather than light (Hughes *et al.* 2004).

Nutrients excreted by holothurians consist mainly of ammonium (Uthicke 2001b), and individual *H. scabra* can excrete up to 3.46 mg d^{-1} (Mukai *et al.* 1989). Using an excretion rate of $E_{(\mu mol\ N\ hr^{-1})} = 1.68(W)^{0.44}$ of Mukai *et al.*, an average excretion rate of 3.08 mg N m^{-2} d^{-1} for *H. scabra* was calculated in this study (animal’s median wet weight = 321 g, density = 0.43 m^{-2}). This was comparable to another study of the coral reef holothurian *H. atra*, with estimated excretion rates of 5.35 mg N m^{-2} d^{-1} (Uthicke 2001b). Comparing these values to average ammonium concentration in the water column (<0.002 mg L^{-1}, EHMP data from 2005/06, station E00531, 27º 43.864’ S;

153° 41.340' E) and in the interstitial waters (38 μmol L^{-1} in winter and 60 □μmol L^{-1} in summer) (Boon 1986), suggest that holothurians may have considerable input into the nutrient budget of this seagrass system. However, in seagrass beds in subtropical regions such as Moreton Bay, the exclusion of holothurians might not show immediate consequences, and possible cascading effects could be obvious only after months or even years. Further studies need to focus on nutrient excretion of *H. scabra* as well as determining ammonium uptake of *C. serrulata* and sampling sediment surface water directly amongst the seagrass to accurately calculate what percentage *H. scabra* may contribute.

Another possible reason why *H. scabra* may have a beneficial impact on seagrass could be through indirect physical effects on sediments. Bottom-dwelling holothurians bioturbate their habitat by ingesting large amount of sand (Purcell 2004; Uthicke 1999) and by burying in the substrate (Mercier *et al.* 2000a). This can cause organic matter and detritus to become resuspended (de Jonge and van den Bergs 1987) and may release additional nutrients that can be used by seagrasses and benthic microalgae.

2.4.4 Benthic microalgae and nutrients

Results of this study showed that BMA biomass and OM consistently increased inside EX during both years, which may have been caused by the lack of holothurians consuming algae, bacteria and detritus. However, during 2003 the CC increased as well, indicating that an accumulation of BMA and OM also may have been caused by the cage structure. To avoid cage effects in any future field exclusion experiments, cages probably need to be larger (>16 m^2) and situated further apart (>10 m).

An increase in BMA in the absence of holothurians has been shown in other studies. For example, exclusion of *H. atra* in aquaria has lead to development of substantial algal mats (Uthicke 1999). Similar results were shown in temperate waters where BMA biomass increased significantly from 6 to 60 µg chl-*a* g sediment^{-1} when deposit feeding holothurians (*Stichopus japonicus*) were absent (Kitano *et al.* 2003). Furthermore, algal and bacterial production was reduced under holothurian grazing, and animals (here *H. atra*) are believed to consume 10-40% of bacterial carbon produced in summer (Moriarty *et al.* 1985).

In Moreton Bay, BMA productivity is strongly limited by temperature during the colder months (Grinham 2006) and this may result in an additional stress to the community. Furthermore, a comparison between productivity of BMA communities in subtropical Moreton Bay and an equatorial lagoon indicated higher rates in comparable areas in the warmer equatorial waters (Grinham 2006). This might mean that BMA productivity in Moreton Bay can remain stable biomass values when grazed upon, whereas, if those grazers are removed, biomass would be able to increase.

In contrast, a direct positive link has been suggested between nutrients (ammonium) excreted by holothurians and BMA biomass and productivity from the results of laboratory and field experiments using exclusion, density manipulation and direct addition experiments (Uthicke 2001a; Uthicke and Klumpp 1998). However, these studies were based on carbonated coral reef sediments under highly oligotrophic tropical conditions, which make it difficult to compare to a muddy-sandy habitat where BMA share the available nutrients with seagrasses.

2.4.5 Holothurian-plant interaction

Holothurians have been overlooked previously in most conceptual models of animal-plant interactions in seagrass habitats. Complex multispecies interactions, such as present in seagrass beds, result in ecosystem resilience (*sensu* Lundberg and Moberg 2003). They can be seen as a buffer to most external influences (e.g. fishing) and may help stabilising systems subject to moderate habitat changes (Nakaoka 2005). Those potential broader ecological consequences when over-fishing marine species are of increasing importance to management under policy that requires sustainable fishing practises (Coleman and Williams 2002).

The present study has illustrated a potential mechanism by which fisheries for deposit feeding holothurians could have indirect cascading ecological consequences. Furthermore, given that seagrass habitat is a known nursery for other important fishery species (e.g. prawns) (Haywood *et al.* 1995), there is the potential for an impact in one fishery to be linked to another, and the effects of over-fishing can be extrapolated on an ecosystem as a whole (Tegner and Dayton 1999). Removal of holothurians might alter the habitat structure and could thus have consequences for the ecology of tropical seagrass beds in the long term.

3 Physical role of sandfish behaviour on the sediment by burying and feeding

3.1 Introduction

H. scabra feed on bacteria and detritus by means of ingesting sediment and extracting organic matter from it (Baskar 1994; Conand 1999; Moriarty 1982; Yingst 1976). They predominantly forage in the vicinity of seagrass beds, and shallow inshore waters are their preferred habitat and nursery areas (Mercier *et al.* 2000a, b).

H. scabra show various cyclical patterns of burying depending on their age (Battaglene 1999a; Mercier *et al.* 1999, 2000a; Yamanouchi 1939, 1956). Juveniles, probably due to their higher risk of predation, are synchronised by day/night regimes, burying at sunrise and re-emerging at sunset. When the juveniles reach about 40 mm in length, they respond more to diel changes in water temperature, by burying earlier at night (~03:30 hr) and emerging sooner during the day (~12:00 hr) (Mercier *et al.* 1999). However, many triggers of burying behaviour change across geographical regions and are not well understood.

Studies on the burying behaviour of adult sandfish are scarce and fragmentary (Purcell and Kirby 2005; Skewes *et al.* 2000; Yamanouchi 1939, 1956). Seasonal variations in burying behaviour, based on long-term studies, have not yet been addressed. Field experiments in Moreton Bay showed that *H. scabra* were much less abundant during winter (Wolkenhauer, unp. data) and local fishers reported that *H. scabra* ‘disappeared’ during winter (D. Burns, pers. com.).

Yamanouchi (1939, 1956) studied *H. scabra* along with several deposit-feeding species. However, his most detailed results about burying and feeding behaviour are in relation to 'brown sandfish' (*Bohadschia marmorata*, then named *H. vitiensis*) and he did not mention the effect of temperature on the animals. Skewes *et al.* (2000) studied *H. scabra in situ* on Warrior Reef (Torres Strait) during daylight hours and found that the animal's burying was cyclical and related to tides. However, sampling was done on only five occasions and results were highly variable depending on location and seagrass cover, and any relationship with water temperature was not investigated. Purcell *et al.* (2005) were focused more on *in situ* locomotion than burying of *H. scabra* and mentioned adults burying during daylight only in relation to the coldest period of the year and did not specify at what temperature changes occurred.

H. scabra's feeding behaviour can be somewhat independent of their burying cycle, in that exposed animals are not necessarily feeding and burying animals may still ingest sediment (Mercier *et al.* 1999; Wiedemeyer 1992; Yamanouchi 1939, 1956). While there are some conflicting reports in regards to the periodicity of feeding when the animals are exposed, authors agree that juvenile *H. scabra* generally have two distinct feeding cycles per day. Feeding behaviour for adults is described as pulsed and variable (Hamel *et al.* 2001; Purcell 2004), with some studies indicating temperature as cues (Roberts *et al.* 2000). However, no study has investigated the relationship between temperature and feeding rates for adult *H. scabra*.

Seasonal variation in burying and feeding behaviour may affect ecosystem function and bioturbation rates attributed to holothurians within their habitat. Additionally, when addressing conservation and fishery management of *H. scabra*, an understanding of burying behaviour is critical to avoid errors in population and distribution surveys.

The aim of this study is to investigate a possible relationship between burying/feeding behaviour and temperature, while excluding other possible factors that may influence the animal's burying and feeding pattern such as tides, current and light. The first study, hereafter referred to as the 'short-term study', investigated burying and feeding behaviour in a controlled environment under a constant summer light regimes. Temperature was reduced in increments over the duration of the study down to a minimum that the animals might experience in the wild during autumn/winter. The second study, hereafter referred to as the 'long-term study', investigated close-to-wild changes in burying and feeding behaviour in mesocosms exposed to natural light and temperature with constant sea water flow-through. This mesocosm study focused on time-lapse video monitoring over a 2-year period to estimate the burying and feeding behaviour based on seasonal changes.

3.2 Methods

3.2.1 Substrate and animal collection

Adult *H. scabra* and sediment (muddy sand) used in both studies were collected from a shallow (~2 m) holothurian habitat in Moreton Bay, south-east Queensland, Australia (Myora Gutter, 27 º 27.876' S; 153 º 25.146' E (Datum: GDA)). Sediment in aquaria and tanks was left to settle for at least 48 hr before any animals were placed inside. Transporting the animals took approximately one hour and was done in plastic tanks with ambient site water.

3.2.2 Short-term study

Experimental set up

In order to facilitate the understanding of terms used throughout this chapter, specific definitions are presented in Table 3-1.

Table 3-1: Definitions of terms in relation to sandfish behaviour

term	**definition**
behaviour	combination of burial state and animal's activity
activity cycle	diel (24-hr) cycle of animal's behaviour pattern
behaviour of interest	2 feeding activities: not feeding and feeding 3 burial states: buried, half-buried and exposed
resting	animal is inactive, meaning it has not moved for the last 5 minutes; this can occur whilst fully exposed, partially or fully buried
feeding	animal is actively feeding either on substrate or on walls; tentacles are exposed and head performs sweeping movements
burying/emerging	animal is actively burying into or emerging out of the substrate
buried	animal is partially or fully buried into the substrate and inactive (see also 'resting')
burying cycle	diel (24-hr) cycle of animal's burying pattern
excretion rate	rate at which animal excretes sediment (measured through dry weight of excreted sediment per 24-hr)

The experiment was carried out in a temperature controlled room at the Moreton Bay Research Station on North Stradbroke Island, Queensland, Australia. Six aquaria each with 10 cm of substrate were set up. Based on results from previous studies and personal observations (Wiedemeyer 1992`; Wolkenhauer unpubl. data) this is an adequate sediment depth to allow for normal burying behaviour of adult *H. scabra,* since their anus is usually in constant contact with the water column to facilitate respiration. Aquaria were filled with seawater and aerated.

Three artificial lamps (DegenPai 36W ATT BR-HG (UV bulb) and DegenPai 36W D-HG FL6500KT8" (daylight bulb)) were placed over the aquaria, each covering two aquaria to simulate natural summer light regimes (14h light and 10h darkness). At 10 cm below (water surface) the light reading was 1200 LUX, at 30 cm below (sediment-water interface) it was 450 LUX. Temperature was set to 24°C. Each of the six aquaria was stocked with one adult sandfish (~17 cm length; ~300 g wet weight) collected from the above field location. The animals were left to acclimatise in the aquaria for two days before the experiment. Subsequently, temperature was decreased one degree every day for a week until reaching 17°C at the end of the experiment.

In addition, another 12 aquaria were set up in a wet laboratory as control animals for faeces collection in the same way as described above. The only difference to the aquaria set-up in the controlled room was ambient light (through ceiling flood lights and windows) and flow-through sea water at a constant ambient temperature of 24°C. The sampling design and data collection for ingestion rates of these animals was the same as the ones in the temperature controlled room and is described below.

Sampling design and data collection

Animals' behaviours in aquaria were monitored every two hours for seven days and on each occasion classified as various combinations of burial state and behaviour (Table 3-2). Furthermore, faeces produced by each individual were collected two-hourly and weighed for each 24-hr period.

Table 3-2: Activity score of *Holothuria scabra* in aquaria classed as combinations of burial state and behaviour

Burial State Behaviour	Fully buried	Half buried	Fully exposed
Resting	**1**	**2**	**3**
Burying		**4**	
Emerging		**5**	
Feeding levelled (on substrate)			**6**
Feeding upright (on walls)			**7**
Searching			**8**
Spawning			**9**
Rolling			**10**

Two common 'behaviours of interest' were established for the analysis of burying cycles: (a) buried (score 1, 2, 4 and 5) and (b) exposed (score 3, 6, 7, 8, 9 and 10). Two 'behaviours of interest' were also established for the analysis of feeding behaviour: (a) not feeding (score 1, 2, 3, 4, 5, 8, 9 and 10) and (b) feeding (score 6 and 7). During this study *H. scabra* did not ingest any sediment while stationary or searching, since oral tentacles were retracted. Those behaviours were considered resting/searching periods (score 3 or 8/10). Thus, only hours spent by the animals moving along the substrate/walls with oral tentacles extended were considered as feeding periods (score 6 and 7).

Two main approaches were used to characterise both burial state and feeding behaviour: (i) the time of day of each animal's state within the diel cycle; and (ii) the average amount of time per day the animals spent in each state.

Statistical analysis

Statistical analyses were done using R 2.5.1 (R 2005). Scores of different 'behaviours of interest' were converted to a binomial form (true/false) and the responses were analysed using a Generalised Linear Model (GLM) with binomial error structure. Each state, e.g. feeding/not feeding or buried/not buried, was therefore treated as a binary response and the probability of this behaviour occurring was estimated as a probability between 0 and 1. Furthermore, harmonic transformation of the time-of-day using sine and cosine functions, representing the daily feeding and burying cycles, were used as supplementary explanatory variables. A linear regression was used to analyse the relation between temperature and excretion rates.

3.2.3 Long-term study

Experimental set up

Mesocosm tanks were situated at the outdoor facilities at CSIRO Marine & Atmospheric Research, Cleveland. Three 1000 l tanks (height = 90 cm, diameter = 120 cm) were filled with approximately 113 l of sediment (= a 10 cm deep layer) and connected to water flow-through at a rate of 1.5 l minute^{-1}. The tanks were continuously aerated using air stones. Each tank was stocked with three holothurians of similar weight (250-350 g) and length (17-20 cm), which simulated a maximum density of around 2.65 animals m^{-2} (about 5-times the natural density, see Section 2.3.1). In each tank animals were of different colour morphs to facilitate a clear distinction and recognition for repeated measures. After one month of acclimation, animal behaviour in each tank was monitored with time-lapse video for 24 hours once a month around the full moon. Previous studies have shown that lunar cycles may influence holothurian behaviour, especially reproductive activity (Guzman *et al.* 2003; Morgan 2000b; Uthicke 1997). A video camera of the type Panasonic WV-CP654 (Panasonic WV-LA210C3 Aspherical TV lens, 2.1 mm, 1:1.0) in a water-proof housing was used, and red spectrum lights were installed above each tank to ensure night viewing. It was assumed that, beside the natural microalgal and bacterial food found in the sediments, we assumed that the microflora growing on tank walls was sufficient for the animals' food requirements. Animals were removed every 3-5 months for measuring and weighing to verify that animals were healthy and growing.

Data collection and statistical analysis

Video tapes were viewed and the date and time was recorded each time there was a change in behaviour (e.g. from feeding to searching) for individual sandfish. The duration spent on each behaviour was then summed to produce the total duration of each 'behaviour of interest' (see Section 3.2.2; Table 3-2) in a 24-hr period with an accuracy of ± 1 min. Scores were converted into true or false categories for both burying and feeding behaviour, and analysed with a Generalised Linear Model (see Section 3.2.1 for details).

In addition, rare behaviours like spawning or rolling were also recorded. The times spent feeding on algae and/or detritus on artificial substratum like walls (vertical), as opposed to feeding naturally on the sand surface (horizontal), was analysed separately. Temperature was recorded with an automated data logger situated in the middle tank.

Prior to analysis, an interpretive dendrogram (Figure 3-1) with all ten behaviours (scores) was set up to facilitate the modelling process. The schema was not based on a traditional cluster analysis; rather, it was used in a hypothetical sense to show similarities in relation to different behavioural patterns. In brief, the higher the different activities split (from top to bottom), the greater the dissimilarity between them in terms of subjective behaviour type and their potential energy usage in relation to each behaviour.

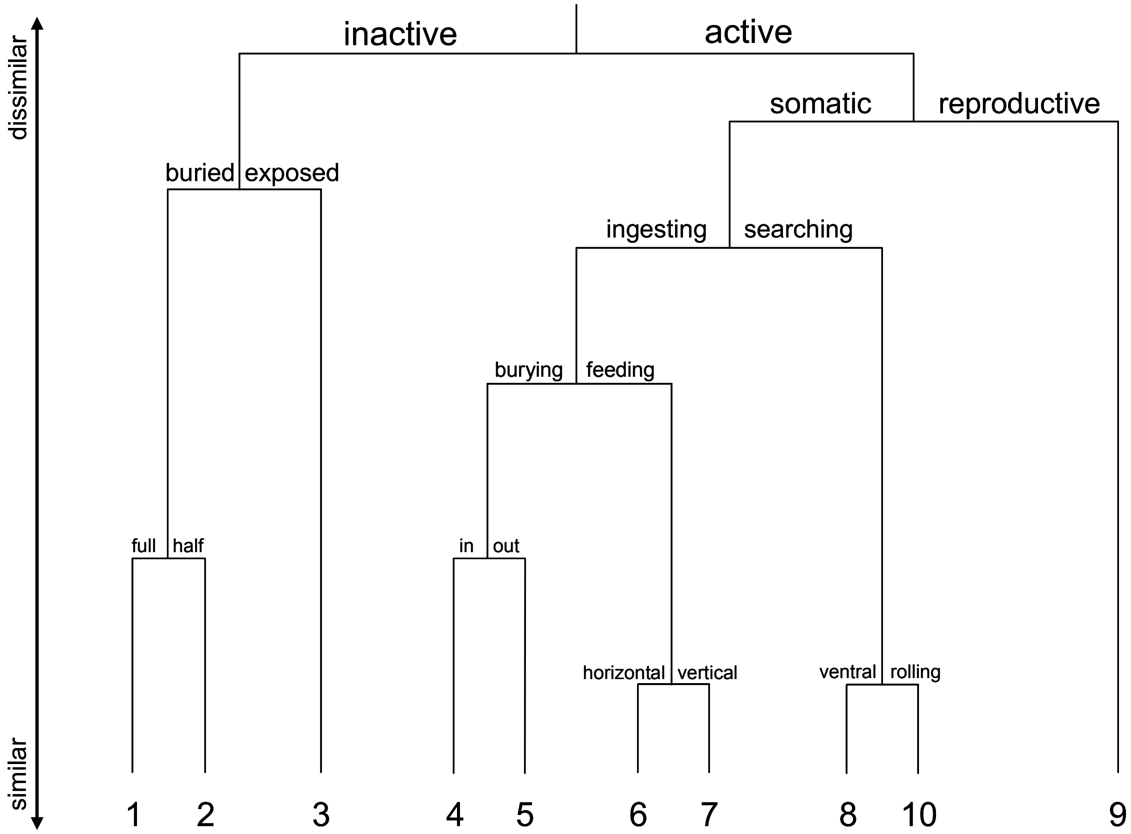

Figure 3-1: Interpretative dendrogramm of *Holothuria scabra*'s behaviour dynamics based on the scoring system used in this study. The length of the vertical lines represents a hypothesised estimate of dissimilarity of behaviours. See text for detailed explanation

In addition, the interpretative dendrogram provided an organising structure for the categorical response as well as the means to analyse the full behavioural range along the pre-categorised 'behaviours of interest'. A series of nested conditional binomial models were used to achieve this analysis. Initial analyses treated each binomial split as conditional based on the model generated on the parent, using standard binomial regression given a truncated data-set. However, in the interest of transparency, the analyses presented were the results of unconditional models. For example, 'buried' behaviour was treated by simply combining categories 1, 2, 4 and 5 into a single binomial response.

The data for the long-term study was categorised into five minute intervals, yielding a total of 38,473 non-independent observations for the total period. Each "composite binomial model" of the probability of observing some class of behaviour had the following form:

$$\mathrm{logit}\left(E\left[y_i\right]\right) = \beta_0 + \boldsymbol{\beta}_1\mathbf{h}'_{1,i} + \boldsymbol{\beta}_2\mathbf{h}'_{2,i} + \sum \boldsymbol{\beta}_{12} \cdot \left(\mathbf{h}'_{1,i}\mathbf{h}_{2,i}\right)$$

assuming a binomial error distribution

$$\begin{aligned} &y_i = \pi_i + \varepsilon_i, \\ &\varepsilon_i \sim \mathrm{binomial}\left(0, \pi_i\left(1-\pi_i\right)\right) \end{aligned}$$

and where

$$\begin{aligned} &\mathrm{logit}\left(\pi\right) = \log\left(\frac{\pi}{1-\pi}\right), \\ &\mathbf{h}_{1,i} = \left\{\sin(2\pi t_{1,i} \cdot \mathbf{n}), \cos(2\pi t_{1,i} \cdot \mathbf{n})\right\}, \qquad \mathbf{n} = \left\{1, \ldots, n\right\}, \\ &\mathbf{h}_{2,i} = \left\{\sin(2\pi t_{2,i} \cdot \mathbf{m}), \cos(2\pi t_{2,i} \cdot \mathbf{m})\right\}, \qquad \mathbf{m} = \left\{1, \ldots, m\right\} \end{aligned}$$

Also, $t_{2,i}$ and $t_{2,i}$ represented temporal variables "time of day" and "day of year", normalised so as to vary between 0 and 1, on the daily and yearly intervals, respectively. The behavioural models were therefore time-domain models, with cyclic behaviour assumed to occur on two different scales. Inspection of the model above showed that the complexity of the modelled cycles was determined by the meta-parameters *n* and *m*, which determined highest harmonics that the model could capture, and also the number of free parameters in the models:

$$N = 2n + 2m + nm\,.$$

The models served the purposes of estimating the baseline changes in the probability of observing behaviours at particular times of day and year. These models assumed independence of observations y_i, and an assumption was clearly violated, since behavioural dynamics are time-dependent. Therefore, the utility of the time-domain harmonic predictors was determined by models that incorporated the previous state as a predictor, thus taking into account order 1 auto-dependence. After determining the optimal model complexity (in terms of setting *n* and *m*), the data were modelled using the time-domain effects only, consciously ignoring the violation of model assumptions. This was necessary to generate estimates that were not dependent on the previous state (i.e. recursive). Thus, presented were only the mean estimates and no explicit reference to variable significance, *F*-statistics or other diagnostics were made. However, it should be emphasised that the model estimates presented were determined by optimising the Akaike Information Criterion (AIC) statistic for the models given proper accommodation of auto-regressive effects. Tank number was also incorporated as another control variable, although it was found to have relatively little explanatory ability.

3.3 Results

3.3.1 Short-term study

Temperature effects on burial state

H. scabra showed a distinct diel burying cycle (Figure 3-2), with most of the animals exposed and active between the hours of 13:00 and 22:00 and most buried and inactive between the hours of 01:00 and 09:00. As experimental temperatures decreased, fewer animals spent time exposed and active, while more remained buried or half buried (Figure 3-2). However, the trend of burying during the day did not change as such, but rather the burial duration lengthened.

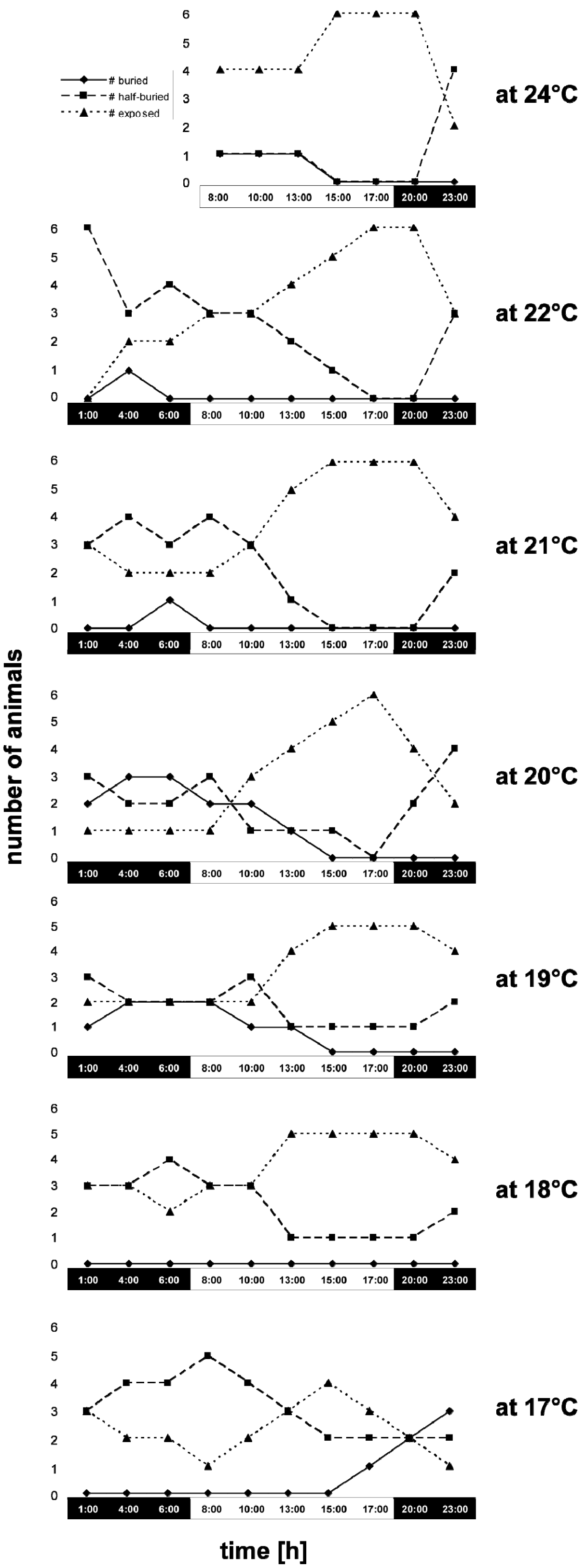

Figure 3-2: Diel burying cycle of *Holothuria scabra* with decreasing temperature. Open and solid bars on x-axis represent light and darkness

The number of animals being buried (combining partially and fully buried) showed a significant (p = 0.002) negative relationship with temperature (Figure 3-3 a, Table 3-3). While there was at least some period when no animals were buried above 20°C, a minimum of two out of six animals were buried at 17°C (Figure 3-2). This effect was particularly obvious in the morning (08:00 to 10:00), with only one animal out of six being buried at 24°C, compared to four out of six animals being buried when temperature reached 17°C (Figure 3-2).

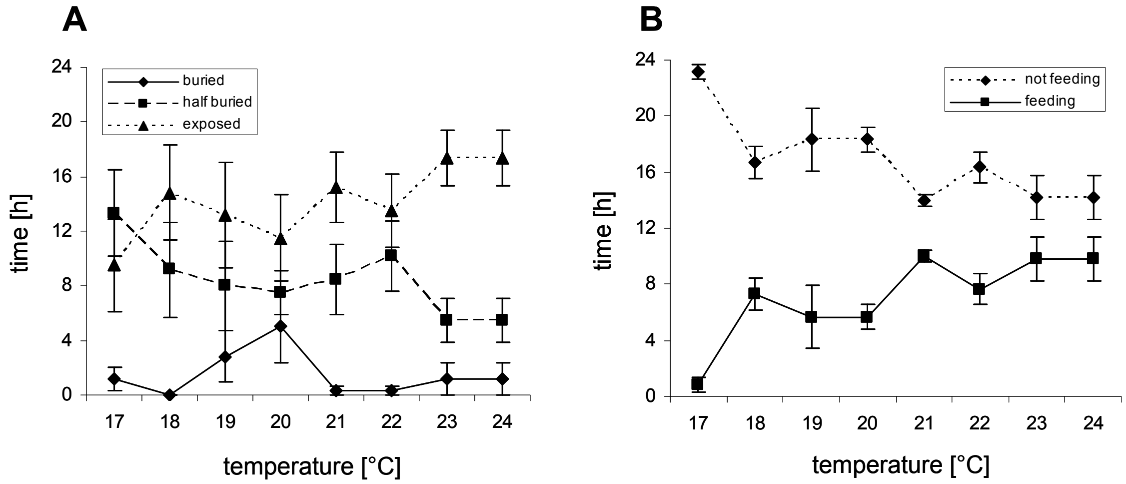

Figure 3-3: Burying and feeding behaviour of *Holothuria scabra* depending on temperature. (a) buried/half-buried versus exposed periods expressed as average hours spent in a day; (b) non-feeding versus feeding periods expressed as average hours spent in a day; error bars = 1 SE with n = 6

H. scabra's average duration of being buried increased with decreasing temperature (Figure 3-3 a). When comparing combined values of being buried (fully and partially) against being exposed, periods being buried increased from 6.7 hr at 24°C to 14.5 hr at 17°C within 24 hours.

Table 3-3: Results of GLM analyses of burial state (exposed/not exposed) in relation to temperature

Effect	**Estimate**	**Standard Error**	**z Value**	**Pr (>|z|)**
intercept	-3.270	1.172	-2.791	0.005
sin (t)	-1.266	0.162	-7.802	<0.000
cos (t)	-0.462	0.153	-3.027	0.003
temperature	0.175	0.057	3.075	0.002

Temperature effects on feeding behaviour

There was a significant ($p < 0.001$) relationship of feeding behaviour with temperature (Figure 3-4, Table 3-4). Daily periods of feeding decreased by 9 hr from 9.8 hr at 24°C to about 0.8 hr at 17°C. Especially during the last two days when temperature fell from 18°C to 17°C, a strong decrease in feeding behaviour was noticeable which might indicate a threshold temperature (Figure 3-3 b).

Table 3-4: Results of GLM analyses of feeding behaviour (feeding/not feeding) in relation to temperature

Effect	**Estimate**	**Standard Error**	**z Value**	**Pr (>|z|)**
intercept	-8.402	1.597	-5.263	<0.000
sin (t)	-1.977	0.230	-8.602	<0.000
cos (t)	-1.139	0.207	-5.502	<0.000
temperature	0.335	0.075	4.452	<0.000

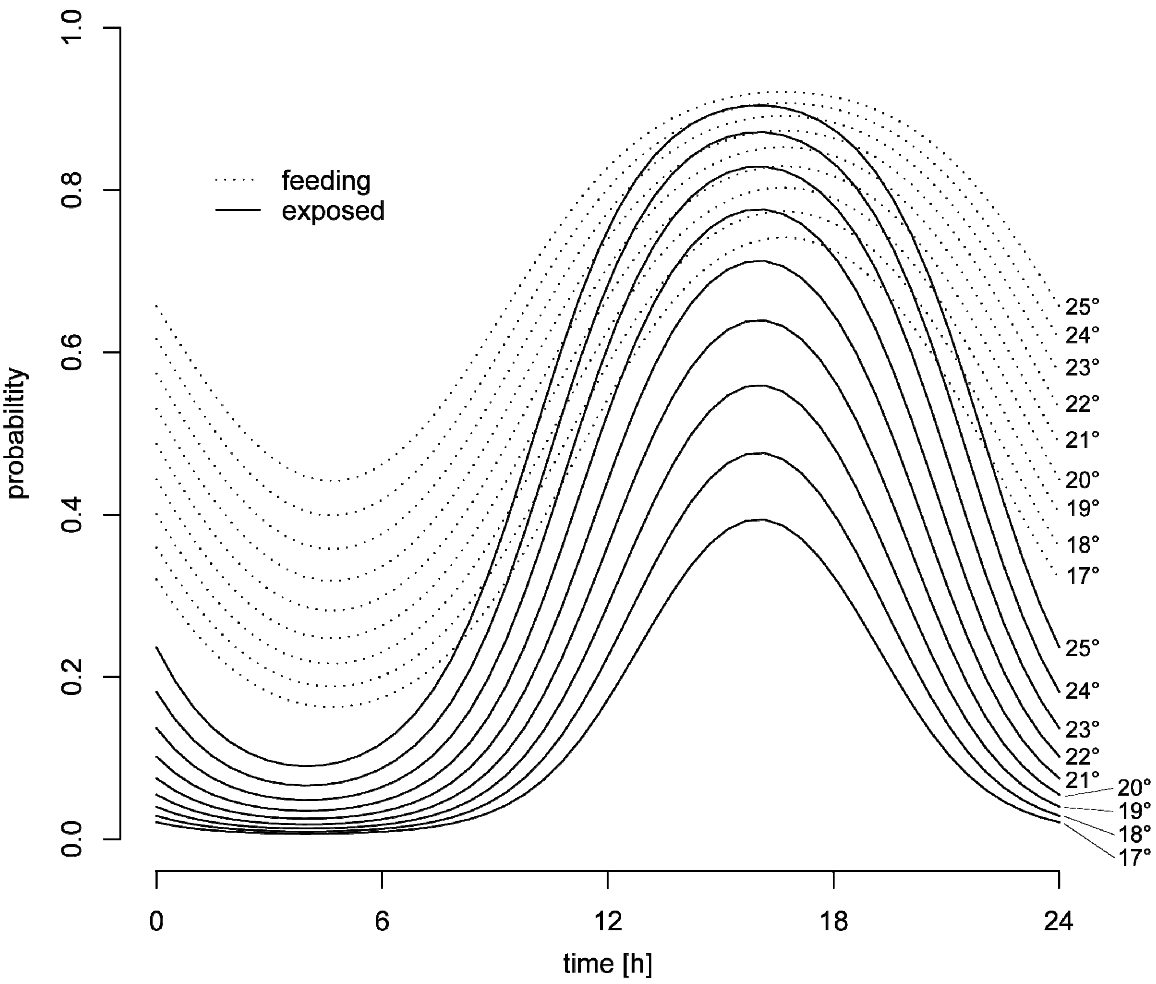

Figure 3-4: Generalised Linear Model prediction of probability of feeding and burying behaviour (cos-sin function). Dotted line = probability of animals feeding, solid line = probability of animals being exposed. Numbers on the right represent the temperature in degrees Celsius for each line.

Temperature effects on faeces production

Average daily faeces production decreased with decreasing temperature (Figure 3-5), which formed a significant linear relationship ($r^2 = 0.82$, $p < 0.001$, Table 3-5). The regression analysis indicated that there would be 5.7g (DW) increase of faeces production with every degree of temperature up to 22°C.

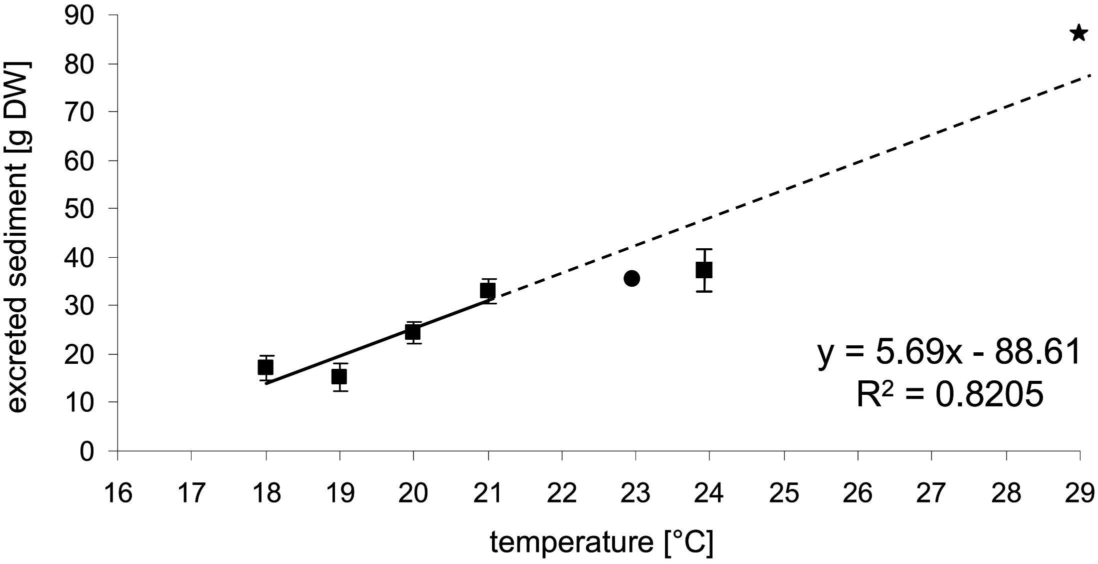

Figure 3-5: Regression of faeces production of adult *Holothuria scabra* versus temperature expressed in gram dry weight (DW) per 24-hr period. Error bars = 1 SE with *n* = 6, df = 22. Solid line shows the actual regression, dotted line is an extrapolation. Three data points are added from other studies (but not included in the analysis). Square represents 39.2 g faeces production at 24°C (control animals in this study), dot represents 38.4 g faeces production at 23°C (Purcell, 2004) and star represents 88.8 g faeces production at 29°C (Mercier *et al.*, 1999)

Table 3-5: Results of regression analyses of faeces production in relation to temperature

Effect	Coefficient	Standard Error	t	P
intercept (constant)	-88.611	23.973	-3.696	0.001
temperature	5.693	1.227	4.638	<0.000

The results were close to the predicted regression line (Figure 3-5) when the average faeces production from the twelve animals kept at a constant 24°C in flow-through aquaria (39.2 g) as well as data from two other studies were added to the graph.

3.3.2 Long-term study

Animal health and growth

During the first 6-7 months of the study, animals gained weight (3.6%) from an average of 317 ± 11.7 g to 326 g ± 11.5 g (Figure 3-6). Nine months later all animals had lost substantial weight (25.1%) and their average weight of 236 ± 8.9 g was significantly different to the initial weight. A final weighing, conducted a few months before the end of the study, showed an average weight increase of 37.8 ± 6.2 g (16.7%) from the previous weighing, however, this was still 13.6% short of the initial weight. Animals showed no signs of illness, bacterial infection or any other indication of unhealthy conditions for the entire duration of the study.

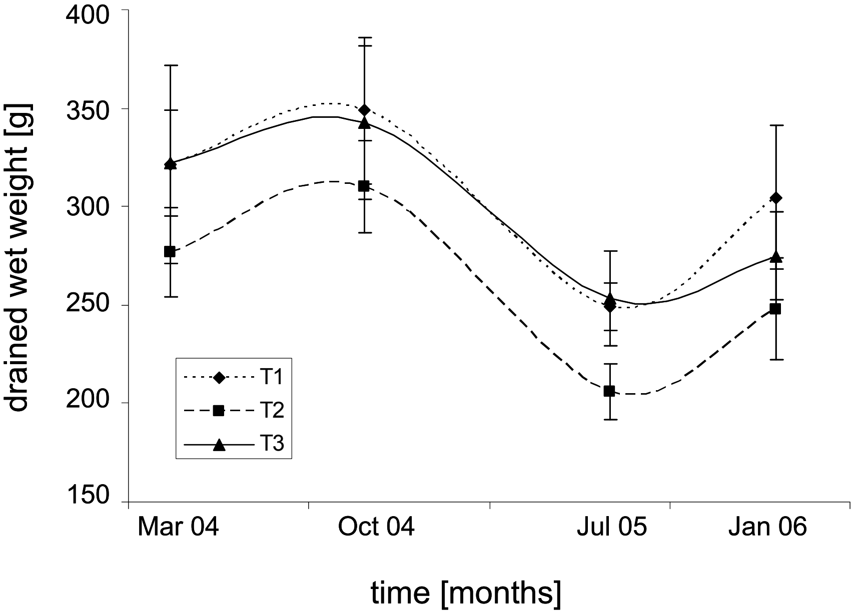

Figure 3-6: Average weight change of *Holothuria scabra* over the 2-year study period. T 1-3 = tank numbers. Error bars = 1 SE; n = 3

Seasonal dynamics based on raw data

H. scabra showed distinct seasonal activity behaviour with high levels of activity during summer (September to February) and little to no activity during winter months (June and July). Burial state (fully and partially, Figure 3-7 a-b) was highly significantly related to season and increased from 3 hr d^{-1} in summer to an average of 16 hr d^{-1} in winter.

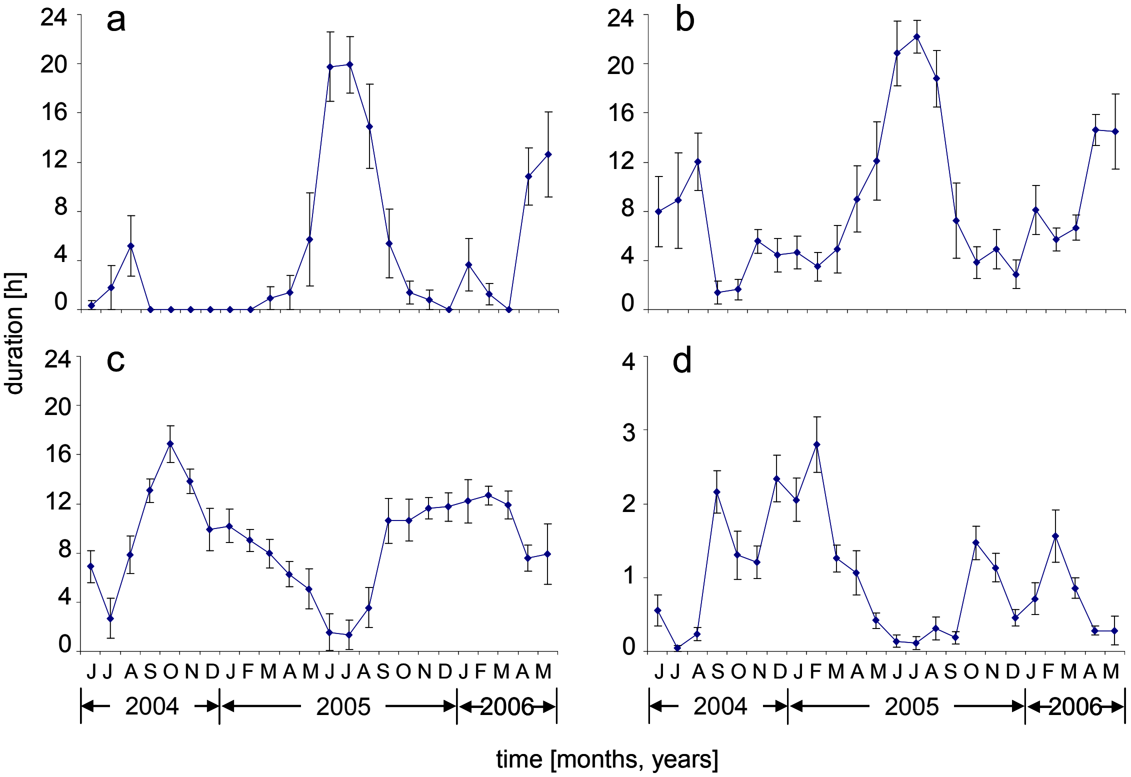

Figure 3-7: Seasonal dynamics of *Holothuria scabra*'s core behaviours over the 2-year study period. (a) fully buried, (b) buried (fully or partially), (c) feeding and (d) searching. Note: (d) y-axis has different scale; 2004, 2005 and 2006 = years; error bars = 1 SE; n = 9

During the winter of 2004, animals spent an average of 12 hr d^{-1} buried (Figure 3-7 b). This was followed by a summer-period of six months (Sep-Feb), where animals spent on average 3 hr d^{-1} partially buried, but not a single animal was fully buried at any time during this period (Figure 3-7 a-b). Subsequently, the period buried or partially buried increased to 23 hr d^{-1} during the second winter (2005) and decreased to an average of 5 hr d^{-1} during the following summer, however, with only two months (Dec, Mar) where not a single animal was fully buried (Figure 3-7 a-b). During the third winter (2006), observations ceased at the end of May, nevertheless, burying rates increased to an average of 14 hr d^{-1}.

Average time spent feeding (Figure 3-7 c) increased rapidly during spring 2004/05 (Jul-Oct), and peaked at 18 hr d^{-1} (Oct); then decreased and plateaued at 10 hr d^{-1} (Dec) until further decreasing and reaching a winter minimum at less than 2 hr d^{-1} (Jul). In 2005/06 feeding behaviour again showed a rapid increase in spring (Jul-Sep). However, unlike in the previous spring, plateaued at 11 hr d^{-1} (Oct) and slowly increased further until reaching a maximum monthly average of 13 hr d^{-1} (Feb) before decreasing again in autumn 2006 (Figure 3-7 c).

Searching behaviour (Figure 3-7 d) exhibited a winter minima (around 20 min d^{-1}) during all three winters sampled, similar to the pattern of other activities described above. However, the maxima (approx. 2 hr d^{-1}) did not occur during summer, but rather during both shoulder seasons (spring and autumn) forming a bimodal pattern and halving their searching activity (to approx. 1 hr d^{-1}) during the height of summer (Nov-Jan).

Seasonal dynamics based on model estimates

The composite binomial models (i.e. those estimating behaviours defined as particular combinations of 'behaviours of interest') estimated the activity dynamics of *H. scabra* in two ways. In the following figures, contour plots (left side) show the probability of a certain behaviour occurring dependent on the time of day and year. The accompanying plot (right side) shows a daily average cross section for winter (July), summer (January) and spring/autumn of the corresponding behaviour.

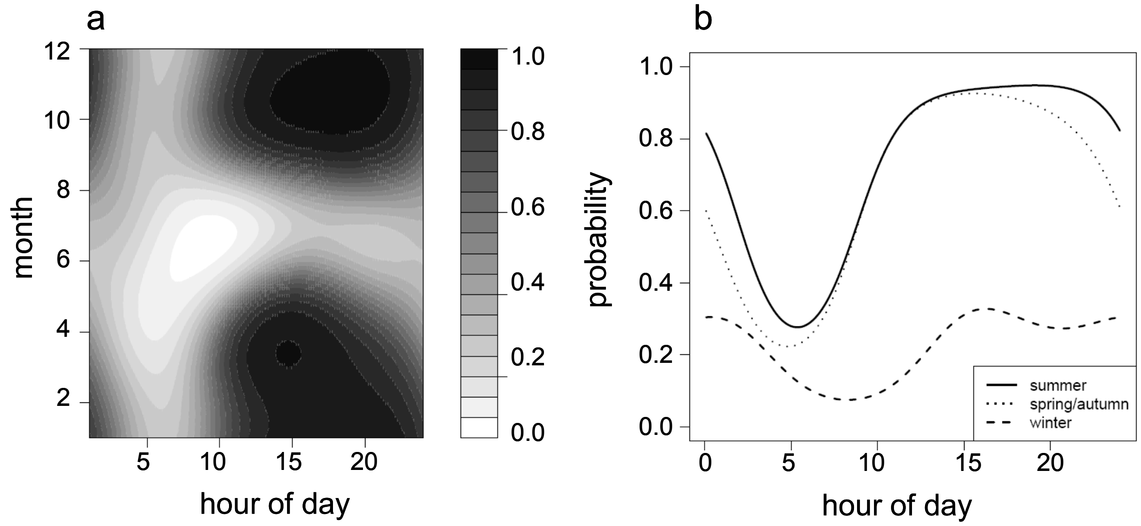

Figure 3-8: Probability of *Holothuria scabra* being active (a) over the year and (b) over the 24-hr day for each season

First, the probability of *H. scabra* having any form of activity was investigated (Figure 3-8). Both plots show a very distinct period of inactivity in the early hours of the morning (03:00-07:00) irrespective of the season. During winter (months 6-8) animals were almost completely inactive. This pattern changed to a period of high activity (10:00-02:00) during spring (months 9-10) and autumn (months 3-4). During summer, times of high activity were only interrupted during late morning (11:00-12:00) and late evening (22:00-00:00) when the animals were resting (dark bottleneck in Figure 3-8), and a few hours (03:00-05:00) when they were buried.

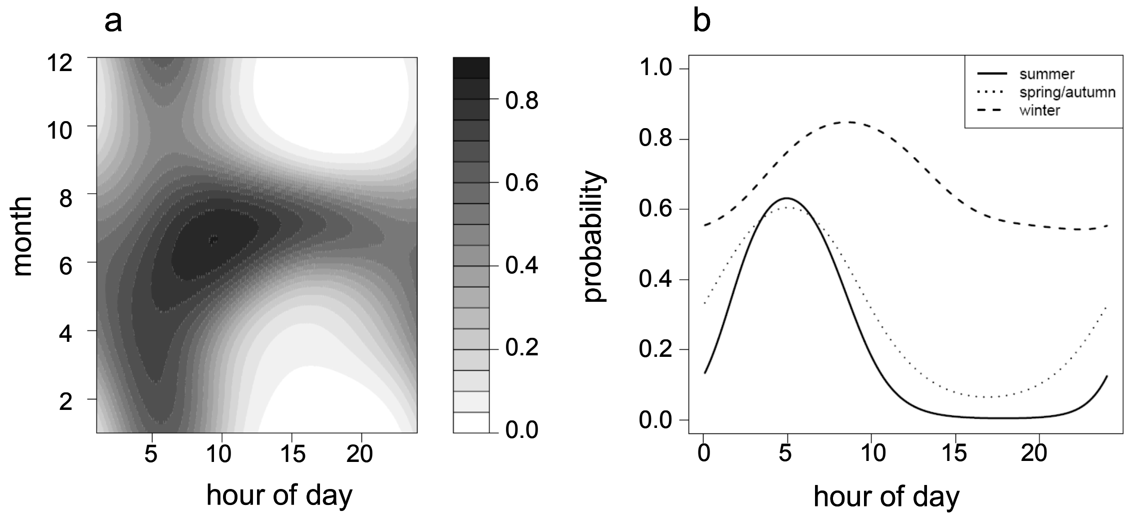

Figure 3-9: Probability of *Holothuria scabra* being buried (a) over the year and (b) over the 24-hr day for each season

Second, the probability of *H. scabra*'s overall burial state was modelled (Figure 3-9). The plots show a significantly higher probability that the animals were buried, partially buried, burying or emerging (score 1-2 and 4-5) during winter months compared to summer or shoulder seasons (spring and autumn). The probability of being buried in summer had a rather narrow maximum in the early morning (04:00-06:00) with the probability being considerably lower at all other times.

Modelling the probabilities for animals being fully buried only (Figure 3-10) showed that in winter the pattern displayed was different to those in summer and shoulder seasons. However, at any given time of day the probability of being fully buried was always at least two-fold higher in winter.

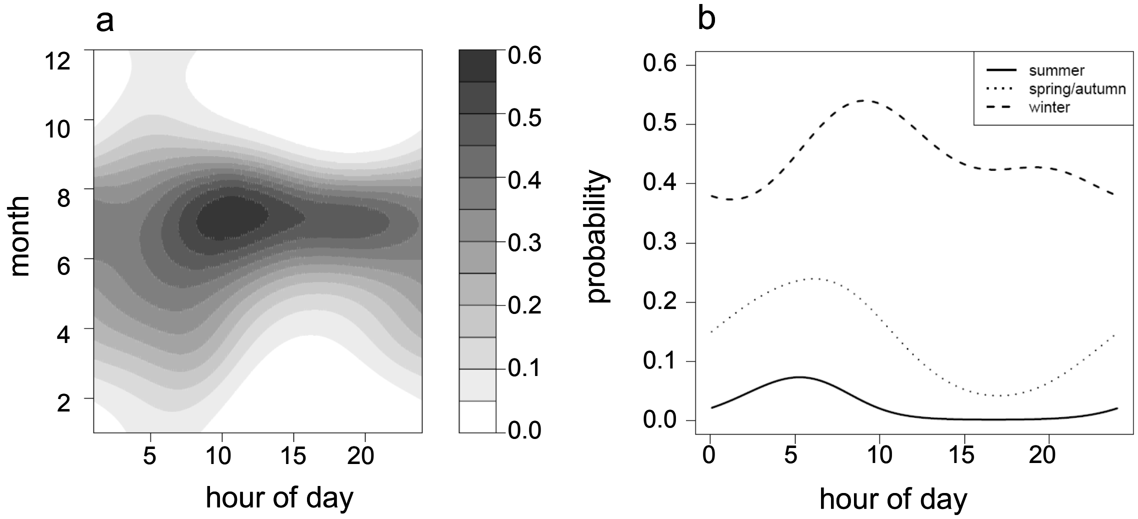

Figure 3-10: Probability of *Holothuria scabra* being fully buried (a) over the year and (b) over the 24-hr day for each season

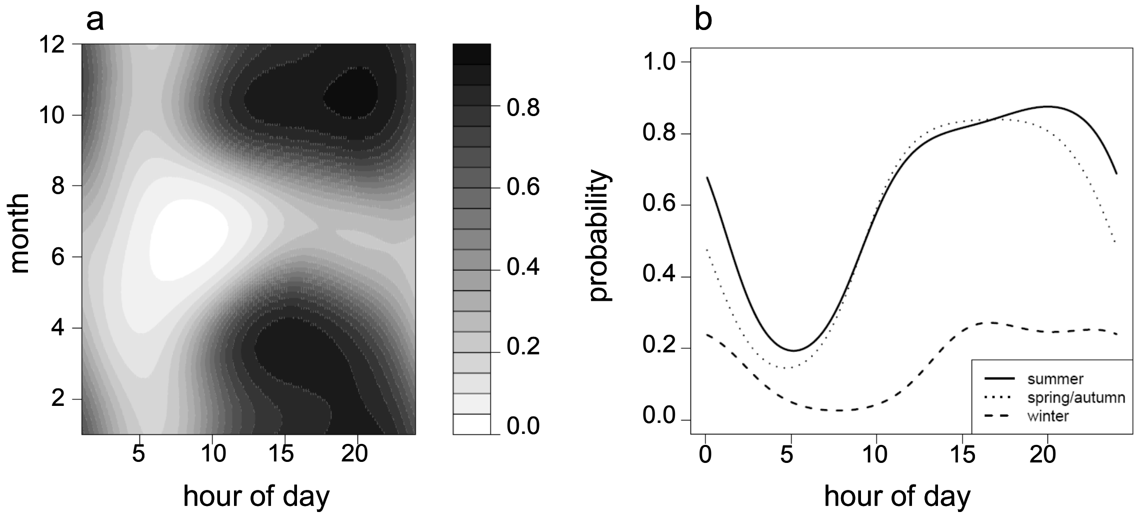

Figure 3-11: Probability of *Holothuria scabra* feeding (a) over the year and (b) over the 24-hr day for each season

When the feeding behaviour of *H. scabra* was compared with non-feeding behaviour (Figure 3-11), the composite binomial models showed significant differences between summer and winter and with the hours of the day. While there was a high probability of feeding occurring during the afternoon and evening (12:00–21:00), there was only a very slight chance of feeding predicted during the early morning (04:00–07:00). This pattern was evident during both seasons, but was amplified during summer months.

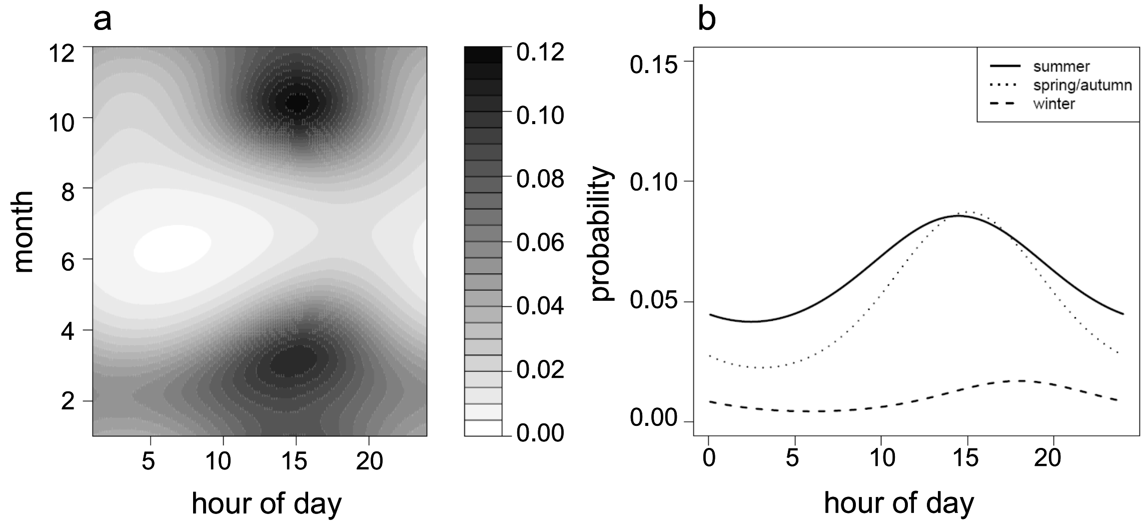

Figure 3-12: Probability of *Holothuria scabra* searching (a) over the year and (b) over the 24-hr day for each season

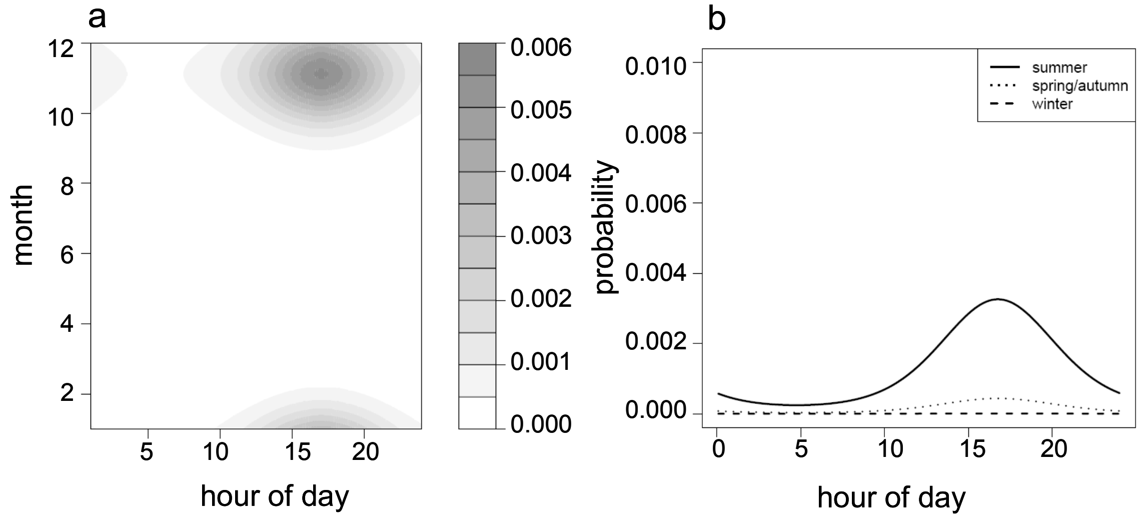

Figure 3-13: Probability of *Holothuria scabra* spawning (a) over the year and (b) over the 24-hr day for each season

Probability of searching behaviour of *H. scabra* was analysed (Figure 3-12). This activity occurred for a maximum of 2-4 hours a day during spring and autumn (see also Figure 3-7 d) and was recorded only very rarely during winter.

Spawning behaviour was observed only on two animals on one occasion (Oct 2004) during the 2-year period. Model predictions of spawning probability (Figure 3-13) thus have to be interpreted cautiously.

To summarize the dynamics of *H. scabra*'s behaviour, a conceptual model was established to visualise the auto-correlation and dependence of their behaviour. Total time spent on each behaviour (over the 2-year period) and the frequency of each behavioural transition was transformed into percentage and visualised in the size of circles and thickness of arrows (Figure 3-14).

Nearly half of the total recorded time (46.6%) was spent feeding, either on the sediment or on the walls. More than a third of the total time (37.4%) was spend buried, either fully buried, partially buried, burying into or emerging out of the sediment. The remaining time (16%) was spent either resting or searching. Rare behaviour, like rolling or spawning, made up less than 0.0001% of the total recorded time (40 and 205 minutes, respectively).

Transitions between feeding (horizontally and vertically) and searching were the dominant switches (>1000) (**Table 3-6**). Transitions between the two feeding stages (score 6 and 7) and resting (score 3) were the next most frequent switches (600-1000). Transitions between feeding (score 6) and resting (score 3) occurred less frequently (100-150). Transitions between all other behaviours were considerably less frequent (<50).

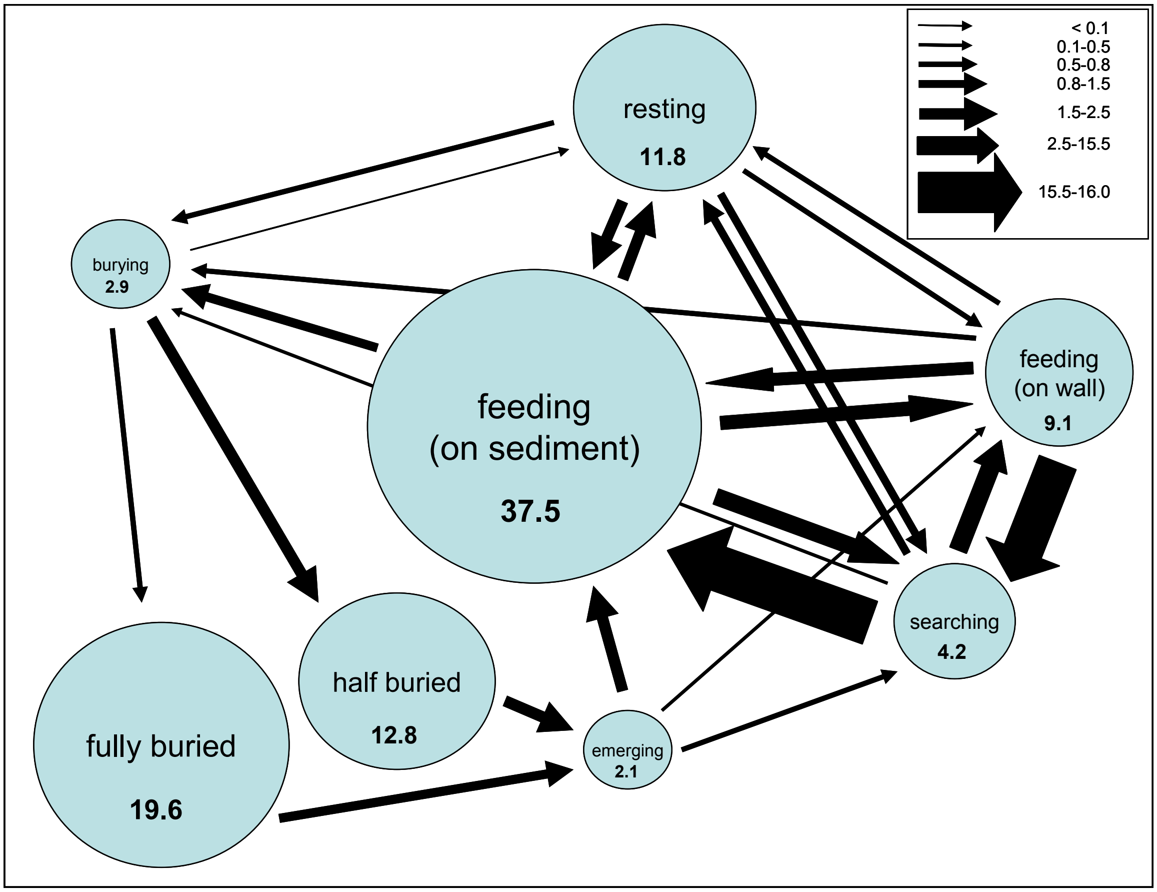

Figure 3-14: Conceptual model of *Holothuria scabra*'s behaviour. Numbers in circles represent percent time spent over two years. Legend in top right corner shows frequency of transition (between behaviours) in percentage.

Table 3-6: Total transitions between behaviours over the 2-year recording period (6471): 1 = full buried; 2 = half buried; 3 = resting; 4 = burying; 5 = emerging; 6 = feeding (horizontal); 7 = feeding (vertical); 8 = searching; 9 = spawning; 10 = rolling

to from	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	0	0	0	0	56	0	0	0	0	0
2	0	0	0	1	112	0	0	0	0	0
3	0	0	0	24	0	142	17	36	2	0
4	50	107	2	0	0	0	0	0	0	0
5	0	7	1	0	0	108	14	35	1	1
6	0	0	152	108	0	0	814	909	10	1
7	0	0	29	22	0	661	0	1009	1	2
8	0	0	30	10	0	1034	939	0	3	0
9	0	0	0	0	0	7	5	5	0	0
10	0	0	1	0	0	1	2	0	0	0

3.4 Discussion

3.4.1 Burying cycles

Both studies (short-term and long-term) showed that adult *H. scabra* have a diel burying cycle as described for juveniles (Battaglene *et al.* 1999; Mercier *et al.* 1999). Furthermore, the length of time spent buried showed a significant relationship to temperature and season. Purcell and Kirby (2005) also found more adult sandfish buried for longer periods during the day as water temperature decreased. However, they did not specify any temperature range and did not investigate the duration of the animals being buried over a 24-hr cycle, since their observations were made during daylight hours only. Mercier *et al.* (2000a) found most adult *H. scabra* on the surface did not follow their usual burying cycle when water temperature increased to more than 30°C.

There are other known factors that cause sandfish to bury for prolonged periods of time such as stress (Purcell *et al.* 2006a), spring tides and strong current (Skewes *et al.* 2000), predation (Dance *et al.* 2003) and desiccation or changes in salinity (Mercier *et al.* 2000a). These factors might interact and prolong the effect temperature has on their burying cycle in the wild. However, the short-term study aimed specifically to exclude those variable factors to find a potential underlying pattern in response to temperature alone. The long-term study also excluded tides and strong currents, but could not distinguish between light or temperature effects.

Further study is needed to determine how light and temperature interact and whether adult *H. scabra* have the potential to reverse their burying cycle in accordance with reversed light regimes, overruling the temperature effect, as has been shown for smaller juveniles (Mercier *et al.* 1999).

3.4.2 Feeding cycles

Decreasing temperature has a significant effect on *H. scabra*'s time spent exposed and feeding. The significant decrease in feeding behaviour between 18°C and 17°C supports a potential threshold temperature at 18°C for *H. scabra*, since this is the usual winter minimum in Moreton Bay and sandfish do not occur any further south than this estuary (see distribution in Hamel *et al.* 2001), suggesting a temperature limit to the species' southern distribution range.

Studies on feeding behaviour of other echinoderms show similar effects with temperature (Hollertz and Duchêne 2001; Schinner 1993; Thompson and Riddle 2005). For example, Thompson and Riddle (2005) showed that the sea urchin *Abatus ingens* increased its displacement activity with increasing temperature.

The increased searching activity displayed by *H. scabra* during shoulder seasons was directly linked to food availability and nutritional requirements prior to the reproductive season. Benthic microalgae and bacteria were visibly scarce during these months and animals preferably fed on walls or searched on substrate and walls. Other studies have found searching behaviour in holothurians (Hudson *et al.* 2004) and other echinoderms (Himmelman *et al.* 2005; Kidawa 2005) mainly to be linked to food requirements. However, they did not record data over the course of a whole year describing possible seasonal differences.

A rare behaviour displayed by the animals, which is closely connected to searching, was a type of 'rolling', where the animal rolled considerable distances (metres) in short periods of time (minutes). Reasons for this behaviour may not only be related to food requirement but also to the absence of seagrass in experimental mesocosm or the need to aggregate during spawning events. 'Rolling' has been documented in *H. scabra* and has been mainly related to spawning, migration or strategies to avoid desiccation during low tides (Aquila 1996; Battaglene *et al.* 2002).

The fact that *H. scabra* displayed a marked reduction in feeding behaviour during winter, very likely accompanied with decreased metabolism as well, hints at a form of 'hibernation', similar to that found for temperate deposit-feeding sea cucumbers (*Stichopus japonicus*) during high water temperatures ('estivation' described in Kitano *et al.* 2003). These behaviour related impacts will have inevitable implications (negative and positive) for the flora grazed by *H. scabra*, both in regards to its co-community as well as its nutriment.

3.4.3 Excretion rates

In the short-term study, the amount of faeces excreted by *H. scabra* had a significant positive relationship to temperature, and the average excretion rate measured (33g per 24-hr period at 22°C) corresponded well with other studies (Mercier *et al.* 1999; Purcell 2004). Studies on ingestion rates of other echinoderms showed similar responses to decreasing temperature. For example, the heart urchin *Brissopsis lyrifera* decreased its ingestion rates from 1.92 g to 0.48 g dry sediment d^{-1} when kept at 13°C and 7°C, respectively (Hollertz and Duchêne 2001).

To estimate a potential peak efficiency of ingesting food in relation to temperature, further investigation is needed at higher temperatures. *H. scabra* is a tropical species with its best performance of growth rate and reproduction expressed at water temperature ranging from 25 to 30°C (Hamel *et al.* 2001).

3.5 Conclusion

The data presented here indicate that observed differences in burying and feeding behaviour of adult *H. scabra* are strongly related to changes in water temperature. These findings have implications for population surveys of this species and related holothurians, which relying on visually counting animals for distribution and abundance surveys. Surveys should be conducted at consistent diel and seasonal timing if results are to be compared with previous data. Based on burying data presented here, it is suggested that the most suitable time to conduct population surveys on *H. scabra* would be during summer (Nov-Jan) from midday to late afternoon. A reduction in ingestion rates at lower temperatures also indicates that bioturbation does not occur at equal rates during the year. Hence, the ecosystem function of holothurians is altered dependent on seasons and needs to be taken into account when establishing an ecological role of those animals within their habitat.

4 Quantifying sediment displacement by means of fluorescent tracers

4.1 Introduction

Bioturbation, the reworking of soils and sediments by living organisms, was first recognised as an important ecological function by Charles Darwin (Darwin 1881) when studying the behaviour of terrestrial earth worms (Meysman *et al.* 2006). Since then many studies have described bioturbation in terrestrial (e.g. Jones *et al.* 1994), freshwater (Matisoff and Wang 2000) and marine (Gerino 1990; Thayer 1979; Welsh 2003) habitats, predominantly caused by the burrowing and feeding activities of macrofauna (*sensu stricto*).

There are a number of modes of bioturbation and the complex interactions between these modes have led to the classification of bioturbators depending on their reworking ability and direction of sediment displacement (Duport *et al.* 2006; François *et al.* 1997). Organisms can be classified into five types of functional bioturbators, depending on their burrowing and feeding characteristics. There are: biodiffusors, upward conveyors, downward conveyors, regenerators and gallery diffusors (François *et al.* 1997). Biodiffusion is usually considered spatially constant, whereas the other modes are grouped into 'non-local' mixing, occurring at irregular intervals in time and space. Biodiffusors move sediment a random distance and direction to an adjacent location, usually through the displacement of sediment by friction of their bodies. This results in a spatially constant diffusive transport of sediment. Examples of biodiffusors are small bivalves or amphipods. Upward conveyors are animals living head-down in the sediment, ingesting sediment at depth and excreting it on the surface. However, unlike biodiffusors, they do not excrete sediment in transit. This behaviour is typically seen in oligochaetes and some polychaetes. Conversely, downward conveyors are animals living head-up in the sediment, ingesting sediment at the top sediment layers and excreting it at the bottom of their burrow, again leaving the intermediate layers free of deposit. Sipunculida (peanut worms) exhibit this kind of bioturbation behaviour. Regenerators move sediments in two directions, actively transferring sediment from depth to the surface during burrowing and passively causing surface sediments to fall into the burrow after leaving it. Fiddler crabs are an example for this group of bioturbators. Gallery diffusors exhibit a behavioural combination of biodiffusors and downward conveyors, constructing a complex system of burrows that allows for diffusion as well as active downward transport. Mobile, foraging polychaetes can be classified into this group of bioturbators.

Epibenthic megafauna, such as deposit-feeding holothurians, have not yet been classified into any of the above listed functional modes of bioturbators. However, it has been demonstrated that by feeding and burying, these animals turn over large amounts of sediments and shift it horizontally (through delayed excretion) as well as vertically (through feeding and burying) (Mercier *et al.* 1999; Purcell 2004; Wiedemeyer 1992).

Bioturbation rates of holothurians have been estimated in several studies using a range of methods. Traditionally, Hauksson (1979) used dry weight (DW) of faeces and assimilation efficiency of organic material to calculate daily sediment reworking rates (20 g m^{-2} yr^{-1}) of *Stichopus tremulus* (a deep-sea deposit-feeding holothurian found here at 350-500 m depth). Uthicke (1999) used DW of faeces to calculate individual sediment reworking rates of *H. atra* (67 g d^{-1}) and *S. chloronotus* (59 g d^{-1}). Additionally, he modelled a spatial estimate of reworked sediment for this mixed population (4.6 kg (DW) yr^{-1} m^{-2}) which was equivalent to the upper 5 mm of sediment of that same area. Purcell (2004) differentiated between sediment reworking through feeding and burying of *H. scabra*. He estimated the displacement of sediment after each burying event by measuring the actual depression left by the emerging animal (1087 cm^3) and measured their faeces production (38.4 g d^{-1}).

None of the studies directly measured the vertical mixing of sediment. In order to calculate this, it is necessary to mark the sediment with a tracer. Sediment grains coated with a fluorescent paint (luminophores) were found to be the most effective and non-intrusive technique to quantify sediment reworking for bivalves and polychaetes (Mahaut and Graf 1987). Numerous studies have since used this technique to quantify bioturbation of macrofauna by estimating a diffusion coefficient (D_b) and modelling sediment reworking rates (g m^{-2} yr^{-1}) (Duport *et al.* 2006; Gerino 1990; Gerino *et al.* 1998; Katrak and Bird 2003; Maire *et al.* 2007; Mermillod-Blondin *et al.* 2005; Mugnai *et al.* 2003). However, in most of these studies sediment bioturbation rates were calculated using single-coloured luminophores spread on the sediment surface, thus, authors were able to describe and quantify only a downward mixing of surface particles (Duport *et al.* 2006; Gerino 1990; Maire *et al.* 2007; Mermillod-Blondin *et al.* 2005). Multi-coloured tracers have been used in previous studies; however, they were utilised only to differentiate between grain size dependent effects (Gerino *et al.* 1998; Mahaut and Graf 1987), not bidirectional mixing.

Studies using tracers to estimate reworked sediment rates for echinoderms are scarce. Hollertz and Duchêne (2001) calculated that a spatangoid urchin (*Bissopsis lyrifera*) can rework their own body volume (~38 cm^3) in an hour solely due to sediment displacement by movement. This was estimated to be 60-150 times greater than bioturbation through ingestion rates (~0.08 g hr^{-1}). Thompson and Riddle (2005) showed that another spatangoid urchin *Abatus ingens* did not bioturbate deeper than 4 cm and calculated comparable ingestion rates (~0.05 g hr^{-1}) to Hollertz and Duchêne (2001). Lohrer *et al.* (2005) modelled the bioturbation of another group of spatangoid urchins (*Echinocardium* sp.) and estimated the volume of sediment displaced by that population (average of 21 ind. $m^{-2)}$ to be 20,000 cm^{-3} m^{-2} d^{-1}. They also calculated that the upper 5 cm of surface sediment was reworked every three days. Vertical sediment displacement rates of holothurians have not yet been calculated. Furthermore, it has not been reliably established how deep *H. scabra* bury and by what functional mode they bioturbate.

This study aims to quantify the vertical sediment transport rate, also called the instantaneous mixing rate, associated with feeding and burying of *H. scabra*, using luminophores to measure vertical sediment movements. Three different-coloured tracers were used, making it possible to track any vertical movement of sediment (up- or downward). In addition, this study aims to define a maximum reworking depth of *H. scabra* and assign one of the existing five bioturbation modes to its behaviour or alternatively describe a new functional mode of bioturbation for these animals.

4.2 Methods

4.2.1 Experimental set-up

Aquaria were placed in an indoor wet lab at the Moreton Bay Marine Research Station on North Stradbroke Island, south-east Queensland. Twenty-four aquaria, each measuring

L 60 × W 30 × H 34 cm (61 l), were set up on three racks. Racks were connected to a sea water flow-through system with an inlet into each aquarium. Animals were collected as described in Section 3.2.1. One adult *H. scabra* was placed in each of 20 aquaria, with the remaining four aquaria used as controls with sediment but no *H. scabra*.

Sediment was collected at low tide from Myora Gutter (27º 27.876’ S; 153º 25.146‘ E), a location where there were relatively high densities of *H. scabra* (0.8 ind. m^{-2}; Wolkenhauer, unp. data). The sediment was sieved through 5 mm mesh to exclude any macrofauna such as polychaetes, bivalves or gastropods that could interfere with the bioturbation of holothurians. Movement and sediment displacement caused by meiofauna was assumed to be negligible, not interfering with the set-up of this experiment. Approximately 520 l of sediment was needed to fill all 24 aquaria to a height of 12 cm (21.6 l for each aquarium). Based on observations in existing mesocosm experiments and a pilot study in aquaria, it appeared that adult *H. scabra* did not bury much deeper than their body depth (approx. 6-8 cm), since their anus is in constant contact with the water column to facilitate sufficient oxygen supply. As the animals may bury on a slight angle to the sediment surface, the sediment depth had to be deep enough to allow for this possibility.

4.2.2 Tracer

The tracers used in this study were EcoTrace® Fluorescent luminophores and were purchased from Environmental Tracing Systems Ltd (Helensburgh, Scotland). The tracers were thermoplastic polystyrene shaped like sediment grains and coated with a fluorescent paint to make them visible under a UV light source. Their density was slightly less than sediment grains of similar size; however, the surface of the tracer particles has the same irregular shape as that of sand grains, giving tracers the highest possible resemblance to natural sediments.

Three fluorescent colours were used in the experiment to model the movement of three different sediment layers within the aquaria (Table 4-1). Since *H. scabra* prefer grain sizes of 125-250 μ (Baskar 1994), each tracer colour was sieved to the appropriate size by the supplier to accommodate this. The tracers were soaked in sea water 24-hr prior to use, to break its surface tension.

Table 4-1: Details of the tracers used in each aquarium

Tracer Colour	**Location**	**Layer (cm)**	**Mixing Ratio**	**Sediment (l)**	**Tracer (g)**
green	top layer	0-2	1:150	3.6	24.96
orange	middle layer	2-6	1:150	7.2	49.92
violet	bottom layer	6-12	1:150	10.8	74.88

Each tracer was then manually mixed with the sediment in 10 l buckets to make up an even sediment-tracer mixture. Pre-experiment trials were conducted to determined how much tracer needed to be mixed with a litre of sediment to deliver a sufficient number of countable tracer particles (>50 but <300) in 0.25 g of dried sediment. A mixing ratio of 1:150 (one part of tracer to 150 parts of sediment) was found to be suitable for all three colours. The sediment-tracer mix was transferred into each of the aquaria in the following order: bottom layer = 6 cm of violet tracer, middle layer = 4 cm of orange tracer and top layer = 2 cm of green tracer. Each of the aquaria was then carefully filled with salt water, using a cloth so not to disturb the sediment layers. Water flow was set to a constant rate of about half a litre per minute.

4.2.3 Sampling design

It was likely that holothurians held in captivity would spend a considerably greater time along the walls of a tank (Hudson *et al.* 2005, Wolkenhauer, pers. obs.). Thus, it was hypothesised that holothurians may cause more bioturbation at the periphery of the tank. Accordingly, a stratified sampling regime was designed that allocated two sets of 5 × 5 cm grid cells to the area of each aquarium (Figure 4-1); a central set consisting of 12 cells and a peripheral set consisting of 60 cells.

1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	I	II	III	IV	V	VI	28	29	30
31	32	33	VII	VIII	IX	X	XI	XII	34	35	36
37	38	39	40	41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56	57	58	59	60

Figure 4-1: Stratified sampling grid. 12 central and 60 peripheral sampling points; quadrat size = 5 × 5 cm

The sediment in the aquaria was sampled with corers (PVC pipe, diameter = 2 cm, length = 13 cm, surface area ~ 3 cm^2) and was taken from the middle of each selected square (area = 25 cm^2). The corers were carefully pushed into the sediment all the way to the bottom of the aquarium and a stopper was used to seal the top of the corer before extraction. On each sampling occasion, five cores were taken out of each aquarium; four from the peripheral area (Arabian numbers in Figure 4-1) and one from the inner rectangle (Roman numbers in Figure 4-1). Each sampling point along the grid was randomised with non-repetitive numbers prior to the start of the experiment to prevent re-sampling of the same square or any bias towards bioturbated areas. When squares were obstructed by pipes or by the animal itself at time of sampling the next random number on the list was chosen. Once a sample was extracted from the aquarium the overlying water at the top end of the core was siphoned off carefully. The cores were then sealed with Parafilm® at both ends and frozen at -80°, while remaining in an upright position.

The trial ran for eight weeks and sampling was conducted on day 1, day 10 and a final sample taken after eight weeks (day 56). Visual observations of holothurian behaviour were made regularly for the first 10 days. Observers recorded the animals' position and behavioural status (using the coding scheme detailed in Chapter 3) every hour during the day (09:00h to 17:00h), and at night their behaviour was monitored every three hours for about 15 minutes. In addition, an anoxic layer that established itself in some aquaria was recorded by measuring an average thickness of the overlying oxic sediment from the outside of the aquarium.

4.2.4 Laboratory analyses

The sediment samples frozen in PVC corers were run under hot water for a short time to thaw only the outer surface of the sediment plug. Each sediment core was extracted from the PVC corer by pushing with a wooden plunger through the pipe. Possible ‘smearing’ and cross-contamination of tracers along the core surface during sampling and later extraction from corers was thought to be negligible based on pilot trials (data not shown). Sediment cores were divided into 2 cm long segments and each slice was dried for 24 hours at 60°C.

Dried samples were homogenised and a sub-sample of 0.25 g was taken to analyse under a UV light microscope (Olympus BX51 Fluorescence light microscope). A blue filter was used to visualise all three fluorescent colours. The sub-sample was evenly distributed in a phytoplankton counting chamber and then every fluorescent particle within the chamber was counted. The chamber was cleaned with high-pressure air after each count to ensure no particles remained in the chamber, thus avoiding cross-contamination. Pilot trials were conducted prior to the actual sample counting to ensure that variation was low in multiple sub-sampling indicating that only one count per sample was needed. The standard error of the differences between sub-samples was 3.95 (data not shown), which was very small in comparison to actual counts (50 to 300). In addition, fifty sub-samples were recounted to ensure accuracy of full-chamber counts. All samples were counted by the same person (SMW), reducing the risk of personal bias and keeping the possible counting error constant throughout the analysis.

4.2.5 Polychaetes

At the termination of the experiment (day 56) 19 of the 24 aquaria contained large polychaetes that had established in the aquaria since previous sampling occasion (day 10). Sediment was therefore sieved to count and identify polychaetes and to determine their biomass in each aquarium. Most polychaetes were of the genera *Arenicola* and

Abarenicola (lugworms), some of which grew to a length of 200 mm and 0.5 mm in diameter. Since these animals are head-down conveyor-belt deposit-feeders, they may have had a significant influence on the topography of the sediment surface (mounds and pits) and the redistribution of tracers in the aquaria. Therefore, the 24 aquaria were divided into four treatments for the analysis of the samples from day 56 to distinguish between bioturbation effects of holothurians and polychaetes (weight based on their biomass) and their mixing rates separately (Table 4-2).

Table 4-2: Classification of all 24 aquaria for the final sample (day 56) in regard to the presence of the two animal types

Animals in Aquaria	**Designation in Analysis**	**Number of Aquaria**
holothurians present polychaetes present	+ holos + polychaetes (HP)	16
holothurians present polychaetes absent	+ holos – polychaetes (H)	4
holothurians absent polychaetes present	– holos + polychaetes (P)	3
holothurians absent polychaetes absent	– holos – polychaetes (C)	1

4.2.6 Statistical analyses

Instantaneous mixing rates (IMR)

Assuming that sediment is mixed from one layer to another at a constant rate, and that movement occurs in both directions (down and up), then the observed concentration in a given layer will follow a log-function with an asymptote equivalent to the long term average concentration over all the mixing layers.

In this case, the average actual sediment mixing rate (instantaneous mixing rate) of the surface layer was calculated by modelling the decreasing concentration of the green tracer in the surface layer using the following function:

$$p(t) = p_\infty + (p_0 - p_\infty)\mathrm{e}^{-\beta t};\ \mathrm{t} \geq 0 \tag{1}$$

where $p(t)$ is the overall ratio of total green tracer in the surface layer with respect to green tracer in the other two layers. At the beginning of the experiment, the percentage of green tracer in the surface layer is $p_0 \approx 100$. As the sediment becomes totally mixed, the percentage of green tracer in the surface layer tends towards the fully mixed concentration p_∞. The mixing rate is represented by β.

In the statistical model, p_0 and p_∞ are regarded as fixed parameters, while the parameter β is estimated from the data. After finding β, the 'instantaneous mixing rate' $\partial R/\partial t$, is estimated by calculating the derivative of the slope of the line R per time step Δt and at p_0.

$$R = p_\infty + (p_0 - p_\infty)\mathrm{e}^{-\beta 0} - (p_\infty + (p_0 - p_\infty)\ \mathrm{e}^{-\beta \Delta t}) \tag{2}$$

$$R = p_0 - p_\infty - (p_0 - p_\infty)\ \mathrm{e}^{-\beta \Delta t} \tag{3}$$

$$\partial R/\partial t = -\beta(p_0 - p_\infty). \tag{4}$$

To model the increasing concentration of the middle and bottom layers into the surface layer (orange and violet tracer mixing into the surface layer), the following alteration of equation (1) was used:

$$p(t) = p_\infty - (p_\infty - p_0)\mathrm{e}^{-\beta t};\ \mathrm{t} \geq 0 \tag{5}$$

where $p(t)$ is the overall ratio of orange or violet tracer in the surface layer with respect to orange and violet tracer in the combined middle (2-6 cm) and bottom (6-12 cm) layers. At the beginning of the experiment, the percentage of tracer (orange or violet) in the surface layer is $p_0 \approx 0$. As the sediment becomes totally mixed, the percentage of either tracer in the surface layer tends towards the fully mixed concentration p_∞. The mixing rate again is represented by β.

The 'instantaneous mixing rate' ($\partial R/\partial t$) is estimated by calculating the derivative of the slope of the line R per time step Δt and at p_0.

$$\partial R/\partial t = -\beta(p_\infty - p_0). \tag{6}$$

Instantaneous mixing rates represent the actual uni-directional movement of sediment in a two-way mixing scenario. It was calculated for all treatments separately to distinguish between holothurian (H), polychaete (P) and their combined (HP) influence on the sediment displacement (see Table 4-2). As the polychaetes established in the aquaria over the duration of the experiment (by growing from <0.5 mm to ~200 mm long in 6 weeks), their impact on sediment mixing increased over time. This made it difficult to determine their full effect on the mixing rates and could have led to an underestimate of their capacity to redistribute sediment. Under these circumstances, mixing rates calculated for polychaetes alone need to be interpreted with caution.

Pair-wise comparison

Tracer concentrations in the top layer (0-2 cm) at day 56 were used to compare mixing rates between treatments (Table 4-2). This comparison potentially underestimates the actual difference in IMR, because as tracer concentrations track to an asymptote, the differences between treatments become smaller. However, because the tracer concentration at the day 56 mark was still near the start of the mixing curve, the tracer concentration was a suitable proxy for the actual difference in the IMR between treatments. The Bonferoni pair-wise comparisons were done in SYSTAT 9.0 (SYSTAT 1999).

4.3 Results

4.3.1 Observations

Between day 1 and day 10 most animals commenced burying in the early hours of the morning (02:00h – 04:00h). On day 1 about 60% of the animals (n = 20) excreted faeces containing orange tracer from the second sediment layer (2-6 cm) approximately 3-4 hours after they began burying. The excretion of only distinctly orange tracers was observed also the following day (5 out of 20 animals, 25%). However, since some of the surface layer had been already mixed during the burying events of day 1, it was hard to differentiate between the coloured tracers with the naked eye. Nevertheless, it supported the hypothesis that *H. scabra* were ingesting sediment while burying (Wiedemeyer 1992).

The second observation was made throughout the whole experiment whenever holothurians were excreting faeces. During previous observations (Chapter 3), *H. scabra* was observed to excrete its faeces as a “string-of-pearls” together with a strong contraction of the posterior end of its body accompanied by a strong jet of water. This was usually followed by a few quicker contractions, much weaker in strength, until the entire string (including the organic sheath that surrounds the faeces) was disconnected from the anus. However, during observations of the animals in this experiment, it was noticed that many animals “buried” their faeces. They lifted the posterior end of their body and pointed it straight down into the sediment to dig a small indentation behind them before beginning the contractions. This left some of their faeces buried under the sediment surface.

The most noticeable observation made during the experiment was due to the activities of polychaetes in several tanks. When only holothurians were present, sediment topography was relatively even and smooth with an anoxic layer forming at a depth of about 3-6 cm (Figure 4-2 a-b). However, in aquaria that held polychaetes only, the topography of the sediment surface was changed substantially (Figure 4-2 c-d). The conveyor-belt activities of the polychaetes had created large mounds (up to 20 cm high) and deep pits (down to 6 cm), substantially altering the initial sediment set-up (12 cm deep). The anoxic layer was broken up and pushed to deeper layers, increasing the depth of the overlying oxic sediment. In contrast, the topography of the surface sediment in aquaria with both holothurians and polychaetes present (Figure 4-2 e-f) was more even and similar to the initial set-up. The anoxic layer was also broken up in these tanks, allowing at least 4 cm of overlying oxic sediment to develop.

Figure 4-2: Change in sediment surface topography and anoxic layer depth for aquaria during sediment reworking experiment (day 56) with (a-b) only holothurians, (c-d) only polychaetes and (e-f) holothurian and polychaetes present

4.3.2 Vertical distribution of tracers

Top layer (green tracer)

At the start of the experiment (day 1), the green tracer was located predominantly in the top layer (0-2 cm) in all tanks, with only a small quantity (~5%) in the second layer (2-4 cm) due to sediment compacting (Figure 4-3 a-b). After 10 days this distribution was essentially unchanged even when holothurians were present (Figure 4-3 c). The final sampling (day 56) showed that the green tracer had been diluted considerably in the surface layer: by 14.7% in aquaria with holothurians only (H) and by 23.6% with polychaetes (P) only or when both animals were present (HP). By the end of the experiment (day 56), a considerable amount of tracer had been transported to the middle layer (2-6 cm). The concentration of green tracer in the middle layer increased from 5.8% on day 1 to 20.5% (H), 27.5% (P), and 29.4% (HP) of green tracer on day 56 (Figure 4-3 e-g). Very little tracer (0.0%, 3.6% and 0.2%, respectively) was transported to the bottom layer (6-10 cm) in the three treatments (Figure 4-3 e-g).

The absolute sediment transport from the surface layer (0-2 cm) into the underlying layer (2-6 cm) at day 56 was about a third less for holothurians than for polychaetes and holothurians and polychaetes together (Figure 4-4). Also, polychaetes were transporting some surface sediment (3.6%) from the surface layer to the deepest sediment layer (>6 cm), whereas holothurians were not transporting any surface sediment deeper than 6 cm (Figure 4-4).

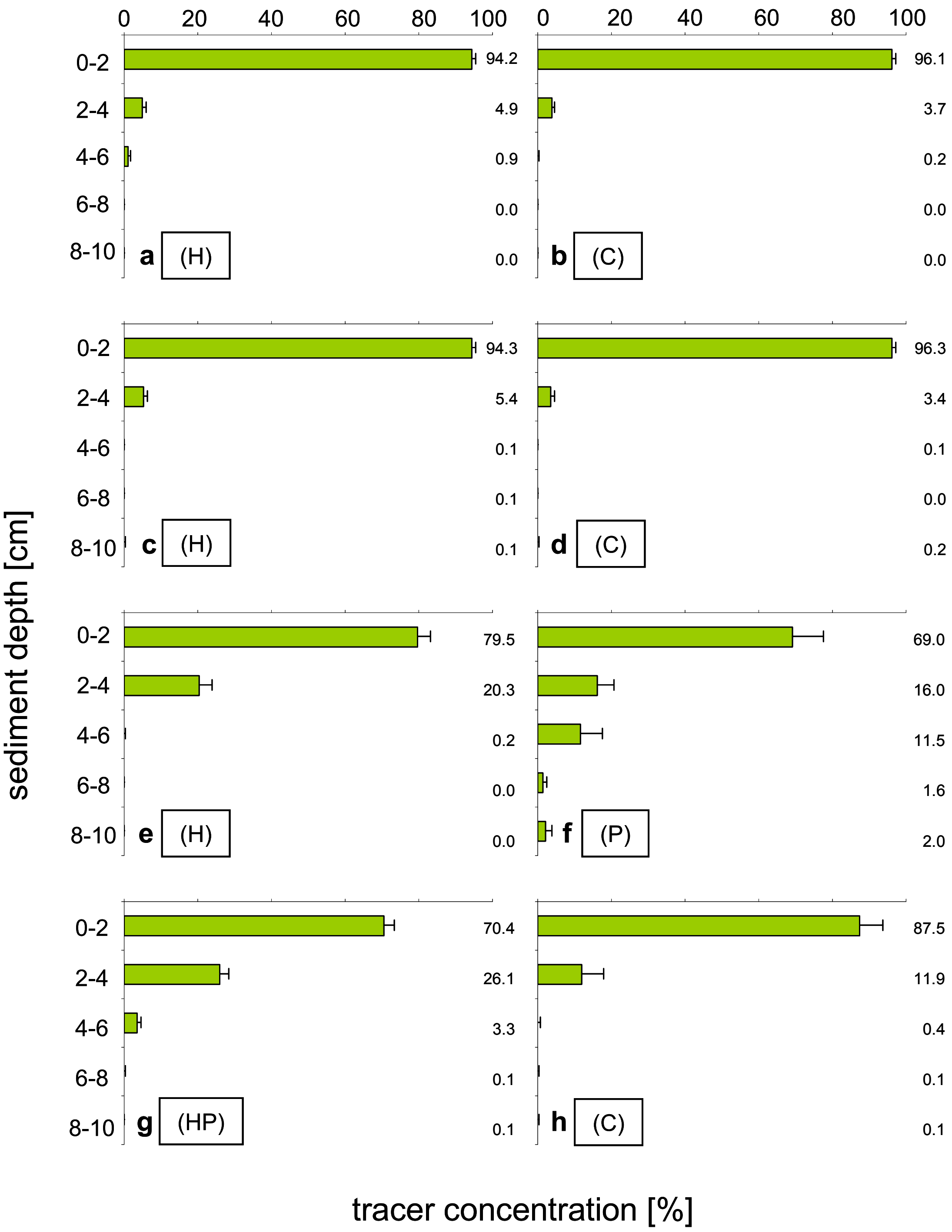

Figure 4-3: Depth distribution of green tracers at day 1 (a-b), at day 10 (c-d) and at day 56 (e-h). C = control; H = holothurian present; P = polychaetes present; HP = holothurians and polychaetes present; n (number of cores) = 100 (a, c), 80 (g), 20 (b, d, e), 15 (f) and 5 (h). Figures on the right hand side indicate individual values in percentage. Error bars = 1 SE

Sediment displacement, when both animal types were present, showed that transport of surface sediment to deep layers by polychaetes appeared to be inhibited by the presence of holothurians. Conversely, this was not observed in the middle layer (2-6 cm) where the same amount (23.6%) of surface sediment was accumulated when both animal types were present (Figure 4-3 g). The increase of surface sediment in the control (~8%) in the middle layer (2-6 cm) may have been due to the settling of the sediment or due to physical diffusion processes when air in the interstitial spaces was slowly replaced by water.

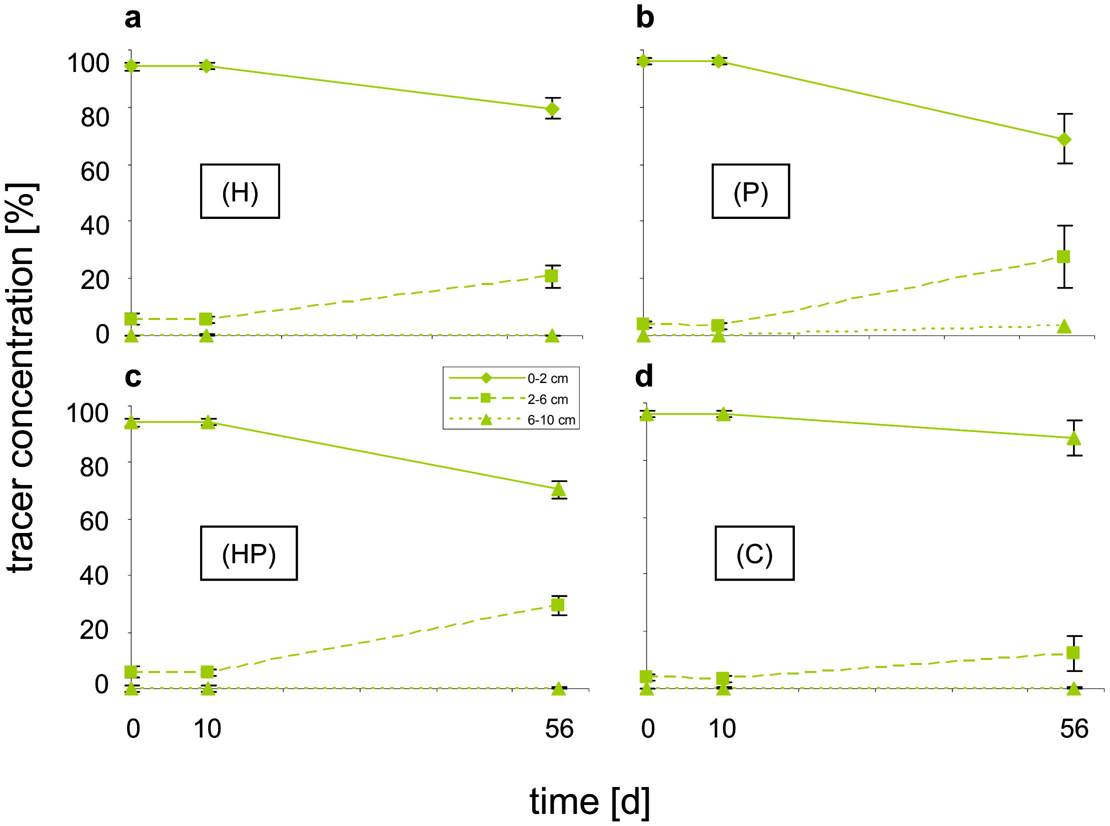

Figure 4-4: Mean relative concentration of green tracers (%) in aquaria over time with holothurians (H), polychaetes (P), both (HP) and control (C). Solid line represents surface layer (0-2 cm), dashed line the middle layer (2-6 cm) and dotted line the bottom layer (>6 cm). Error bars = 1 SE

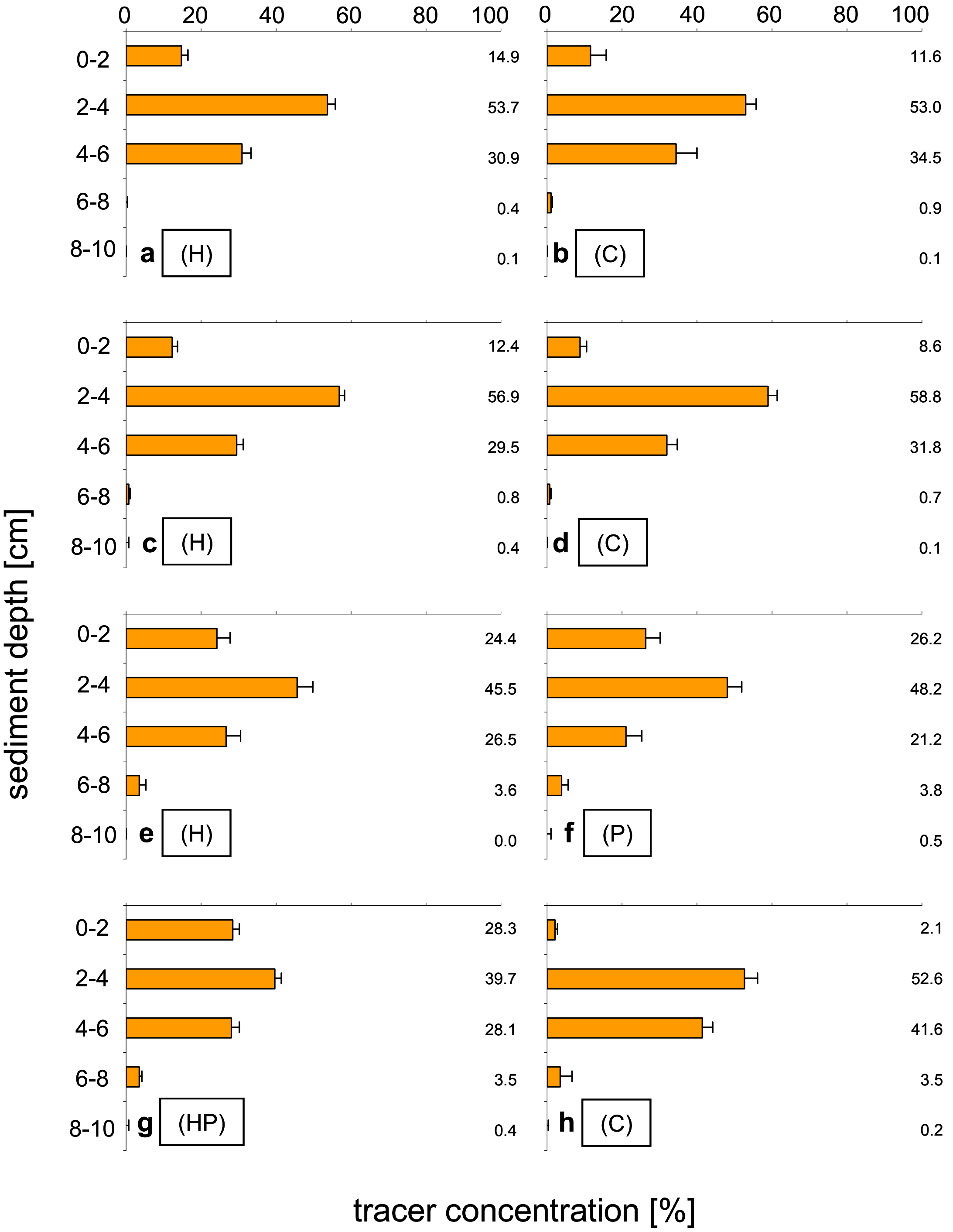

Figure 4-5: Depth distribution of the orange tracers at day 1 (a-b), at day 10 (c-d) and at day 56 (e-h). C = control; H = holothurian present; P = polychaetes present; HP = holothurians and polychaetes present; n (number of cores) = 100 (a, c), 80 (g), 20 (b, d, e), 15 (f) and 5 (h). Figures on the right hand side indicate individual values in percentage. Error bars = 1 SE

Middle layer (orange tracer)

Originally deployed in 2-6 cm depth, there was some orange tracer in the surface layer (~13%) and ~0.5% in the bottom layer at day 1 (Figure 4-5 a-b). This was due to sediment compaction after water had forced out air trapped during the experimental set-up. This sediment distribution was relatively stable up to day 10, with ~10% in the top and ~1% in the bottom layer (Figure 4-5 c-d). The final core sampling (day 56) showed that the orange tracer concentration had decreased in the middle layer: by 12.6% in aquaria with holothurians only (H), 18.1% with polychaetes only (P) and 16.8% when both animal types were present (HP).

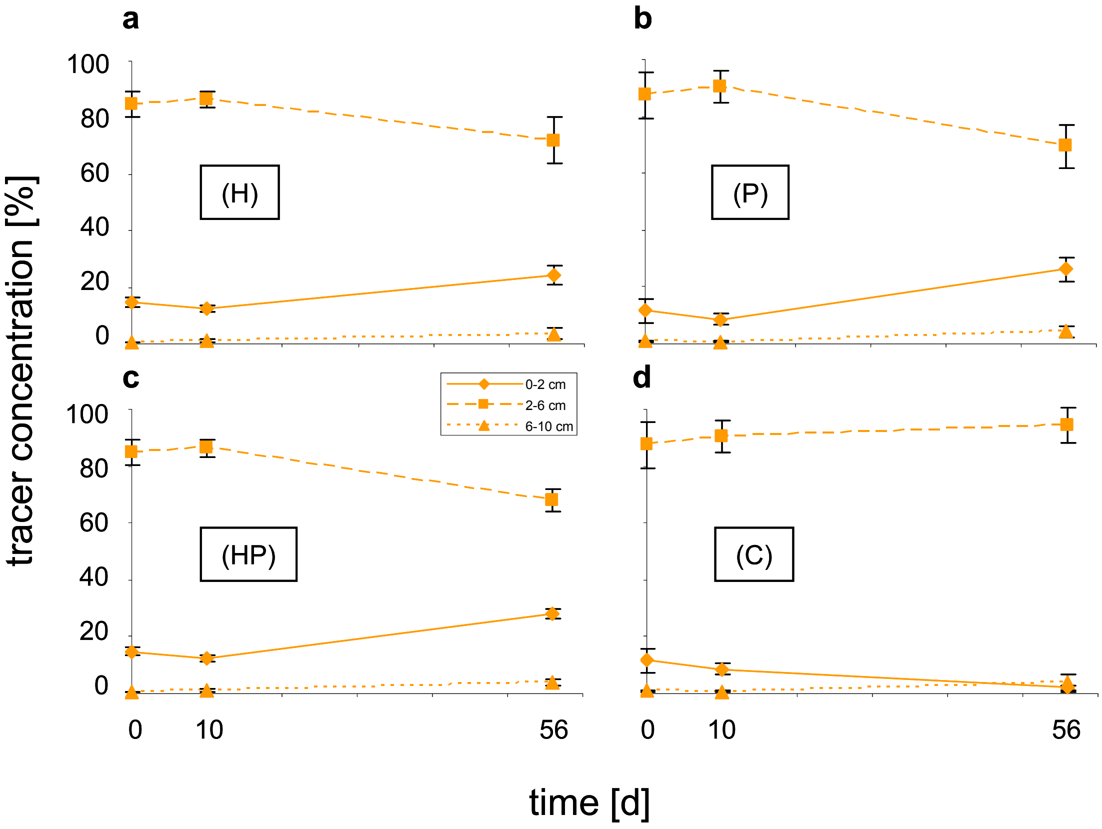

Figure 4-6: Mean relative concentration of orange tracers (%) in aquaria over time with holothurians (H), polychaetes (P), both (HP) and control (C). Solid line represents surface layer (0-2 cm), dashed line the middle layer (2-6 cm) and dotted line the bottom layer (>6 cm). Error bars = 1 SE

Most of this middle layer had been transported to the surface layer (0-2 cm), with concentrations increasing from around 13% to 24.4% (H), 26.2% (P), and 28.3% (HP) (Figure 4-5 e-g). A small amount of orange tracer had been transported to the bottom layer (3.1%, 3.3% and 3.4%, respectively) (Figure 4-5 e-g). Sediment transports from the middle layer (2-6 cm) to the upper or lower layers were similar for holothurians and polychaetes (Figure 4-6).

Bottom layer (violet tracer)

On day 1, the violet tracer was located predominantly in the bottom sediment layer (6-10 cm), with ~16% located in the adjacent layer directly above (4-6 cm) (Figure 4-7 a-b). After day 10, only slight mixing of the bottom sediment layer occurred: ~2% more sediment was mixed from the bottom into the adjacent middle layer but not higher (Figure 4-7 c-d). The final sampling (day 56) showed that the violet tracer had not been mixed at all into the upper sediment layer of aquaria with holothurians only (H). However, in the aquaria with polychaetes only (P), 11.7% of sediment had been transported from the bottom layer: ~7% to the middle layer and ~4% to the surface layer. In the aquaria with both animal types present (HP), the violet tracer concentrations in the bottom layer were reduced by ~5% over the 56-days experiment coinciding with a similar increase in the surface layer (Figure 4-7 e-g). Sediment transport from the bottom layer (6-10 cm) to the upper layer was significantly different for holothurians and polychaetes (Figure 4-8). Polychaetes shifted sediment directly from the bottom to the surface layer (Figure 4-8 b-c), whereas holothurians did not mix the bottom layer at all (Figure 4-8 a).

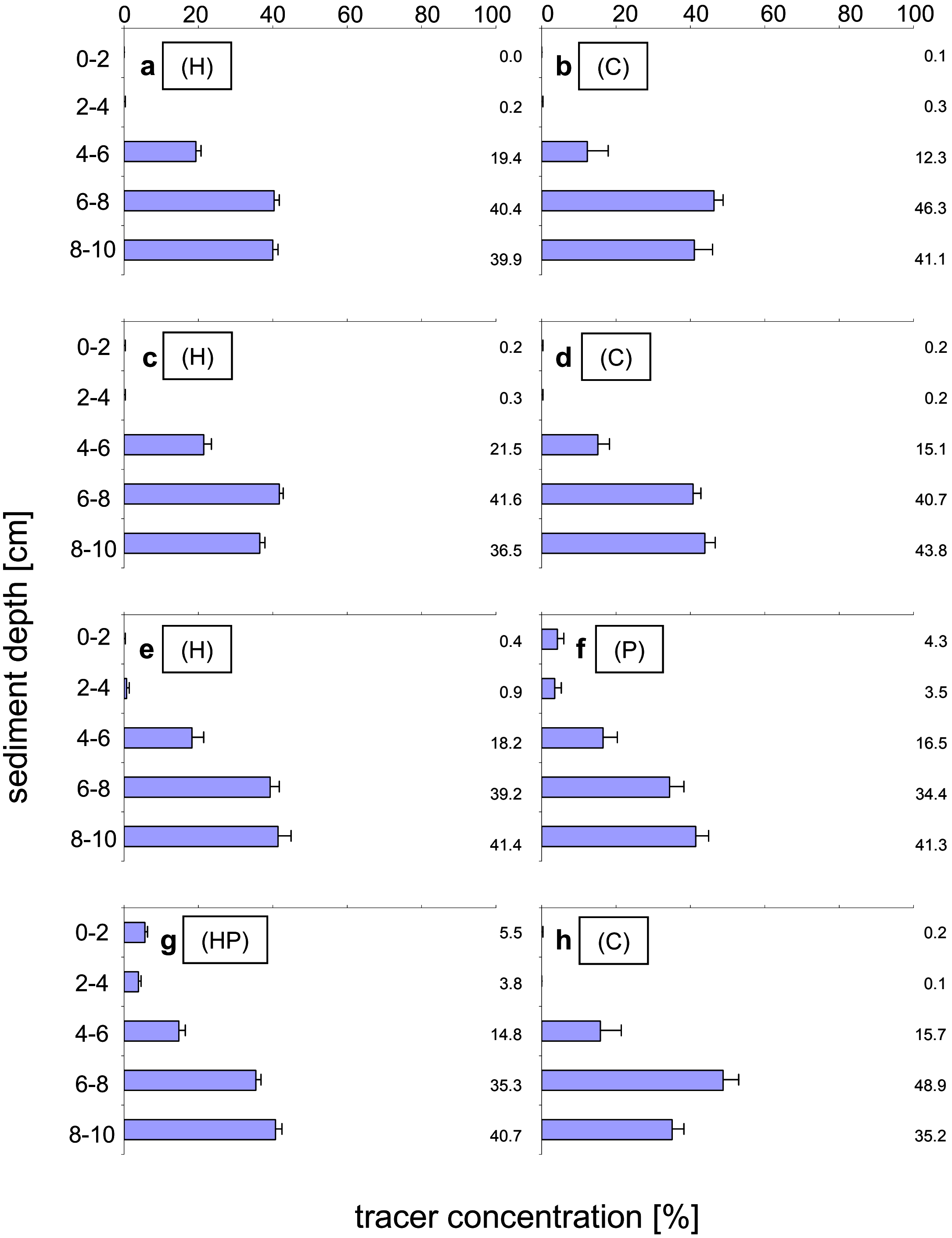

Figure 4-7: Depth distribution of the violet tracers at day 1 (a-b), at day 10 (c-d) and at day 56 (e-h). C = control; H = holothurian present; P = polychaetes present; HP = holothurians and polychaetes present; n (number of cores) = 100 (a, c), 80 (g), 20 (b, d, e), 15 (f) and 5 (h). Figures on the right hand side indicate individual values in percentage. Error bars = 1 SE

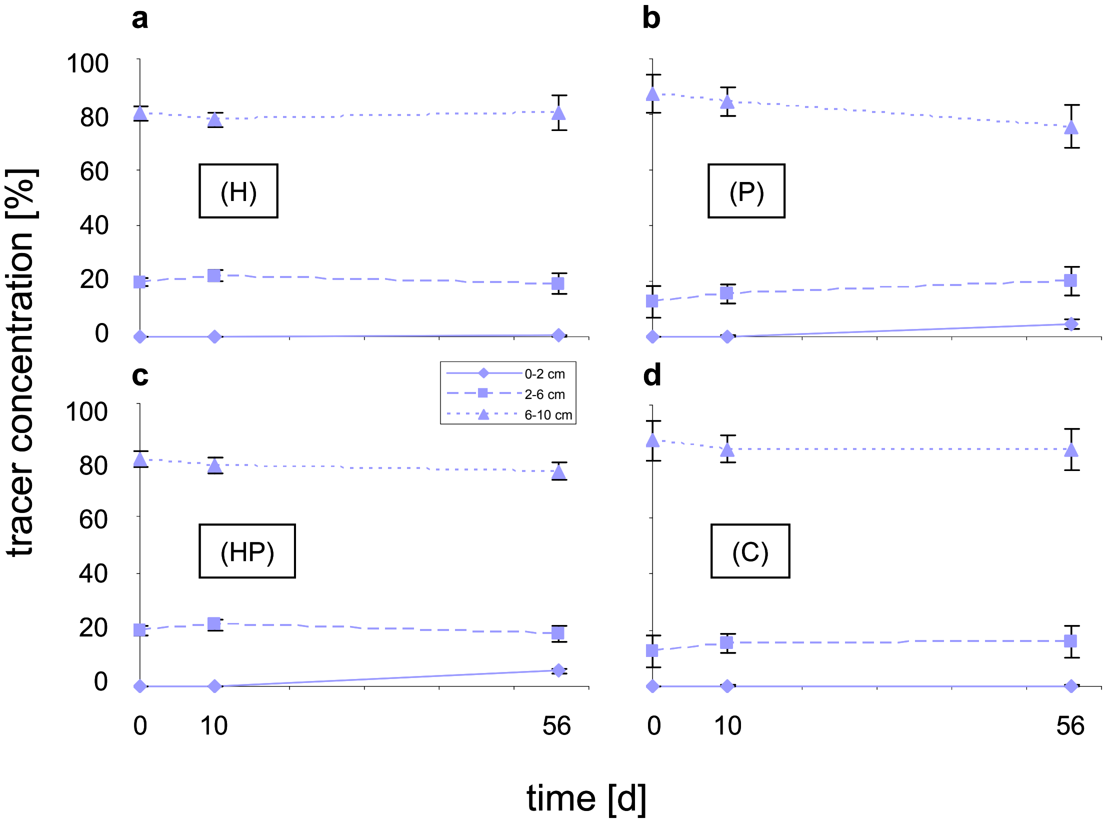

Figure 4-8: Mean relative concentration of violet tracers (%) in aquaria over time with holothurians (H), polychaetes (P), both (HP) and control (C). Solid line represents surface layer (0-2 cm), dashed line the middle layer (2-6 cm) and dotted line the bottom layer (>6 cm). Error bars = 1 SE

4.3.3 Instantaneous mixing rates (IMR)

Based on the model presented in Section 4.2.6, instantaneous mixing rates (IMR) were calculated for the surface layer (Table 4-3). The IMR of the surface layer (0-2 cm) was twice as high for holothurians and polychaetes combined (HP, 0.50% d^{-1}) compared with holothurians only (H, 0.25% d^{-1}), while the mixing rate was highest with polychaetes only (P, 0.57% d^{-1}).

Table 4-3: Instantaneous mixing rates of selected sediment layers out of (for green tracer) and into (for other two tracers) the surface layer. Green = transport of surface layer (0-2 cm) into the adjacent layer (2-6); orange = transport of middle layer (2-6 cm) into surface layer (0-2 cm); violet = transport of bottom layer (6-10 cm) into the surface layer (0-2 cm); H = holothurians present; P = polychaetes present; HP = holothurians and polychaetes present; C = control (no animals present); P_∞ = proportion of tracer in surface layer when totally mixed; P_0 = proportion of tracer at time zero in surface layer; ß = calculated mixing rate; IMR = instantaneous mixing rate, sig = level of significance based on Bonferoni pair-wise comparison of day 56 tracer concentration in treatments (same letter means P > 0.05)

Depth (cm)	**Colour**	**Treatment**	**n**	P_∞	P_0	β	**IMR (% d^{-1})**	**STER (IMR)**	**Sig**
0-2	green	H	20	33.3	94.2	0.004063	-0.24746	0.04871	ac
0-2	green	HP	80	33.3	94.2	0.008222	-0.50069	0.05623	ab
0-2	green	P	15	33.3	96.1	0.009112	-0.57224	0.14231	abc
0-2	green	C	5	33.3	96.1	0.002214	-0.13905	0.05221	c
2-6	orange	H	20	33.3	14.9	0.006647	-0.12231	0.06447	a
2-6	orange	HP	80	33.3	14.9	0.016748	-0.30816	0.06493	a
2-6	orange	P	15	33.3	11.6	0.014278	-0.30983	0.12237	a
2-6	orange	C	5	33.3	11.6	-0.00692	+0.15011	0.04922	b
6-10	violet	H	20	20.0	0.0	0.000223	-0.00445	0.0012	a
6-10	violet	HP	80	20.0	0.0	0.003077	-0.06154	0.0060	b
6-10	violet	P	15	20.0	0.1	0.002341	-0.04659	0.0119	b
6-10	violet	C	5	20.0	0.1	0.000111	-0.00220	0.0017	a

The IMR of the middle layer (2-6 cm) into the surface layer (0-2 cm) was nearly three times as high when polychaetes and holothurians were both present (HP, 0.31% d^{-1}) compared when only holothurians were present (H, 0.12% d^{-1}). When polychaetes were the only bioturbators, mixing rates were similar to HP conditions (P, 0.31% d^{-1}).

The IMR of the bottom layer (6-10 cm) into the surface layer (0-2 cm) in aquaria with both animal types was more than ten times higher (HP, 0.062% d^{-1}) compared with when only holothurians were present (H, 0.005% d^{-1}). When polychaetes were the only bioturbators, mixing rates were slightly less than those with both animal types present (P, 0.047% d^{-1}).

Based on these mixing rates the top 2 cm are displaced every 200 days when both polychaetes and holothurians are present. However, holothurians can only account for about 50% of this sediment transport; displacing the top 2 cm about every 400 days. The density of holothurians in these aquaria (at 5.6 m^{-2}) were about 10-times higher than found in nature (0.48 m^{-2}), thus, their real mixing capability was overestimated in this experiment. Furthermore, the experiment was done under summer conditions, and seasons were shown to have a strong influence on the feeding and burying activities of holothurian (see Chapter 3).

The IMR of the treatments was not corrected using the control value due to considerable uncertainty in the control value. Firstly, the control value was based only on one tank. Secondly, there was some evidence of bioturbation in the controls by small animals, which were not detected by the 0.5 mm sieve at the end of the experiment.

4.4 Discussion

This study demonstrated that holothurians contributed to mixing of the surface sediment layers during their feeding and burying activities. Nevertheless, sediment mixing rates calculated for *H. scabra* in this study were much lower than for other echinoderms like urchins, that were reported to turnover the top 5 cm in three days (Lohrer *et al.* 2005). However, sampling for that study occurred in shallow coastal waters off the North Island of New Zealand during austral summer (Nov-Dec) under relatively high densities (average 21 ind. m^{-2}, max 64 ind. m^{-2}). The present study showed that holothurians did not influence sediment deeper than 6 cm and that their pattern of bioturbation created a relatively smooth sediment surface with the formation of a shallow anoxic layer (3-6 cm).

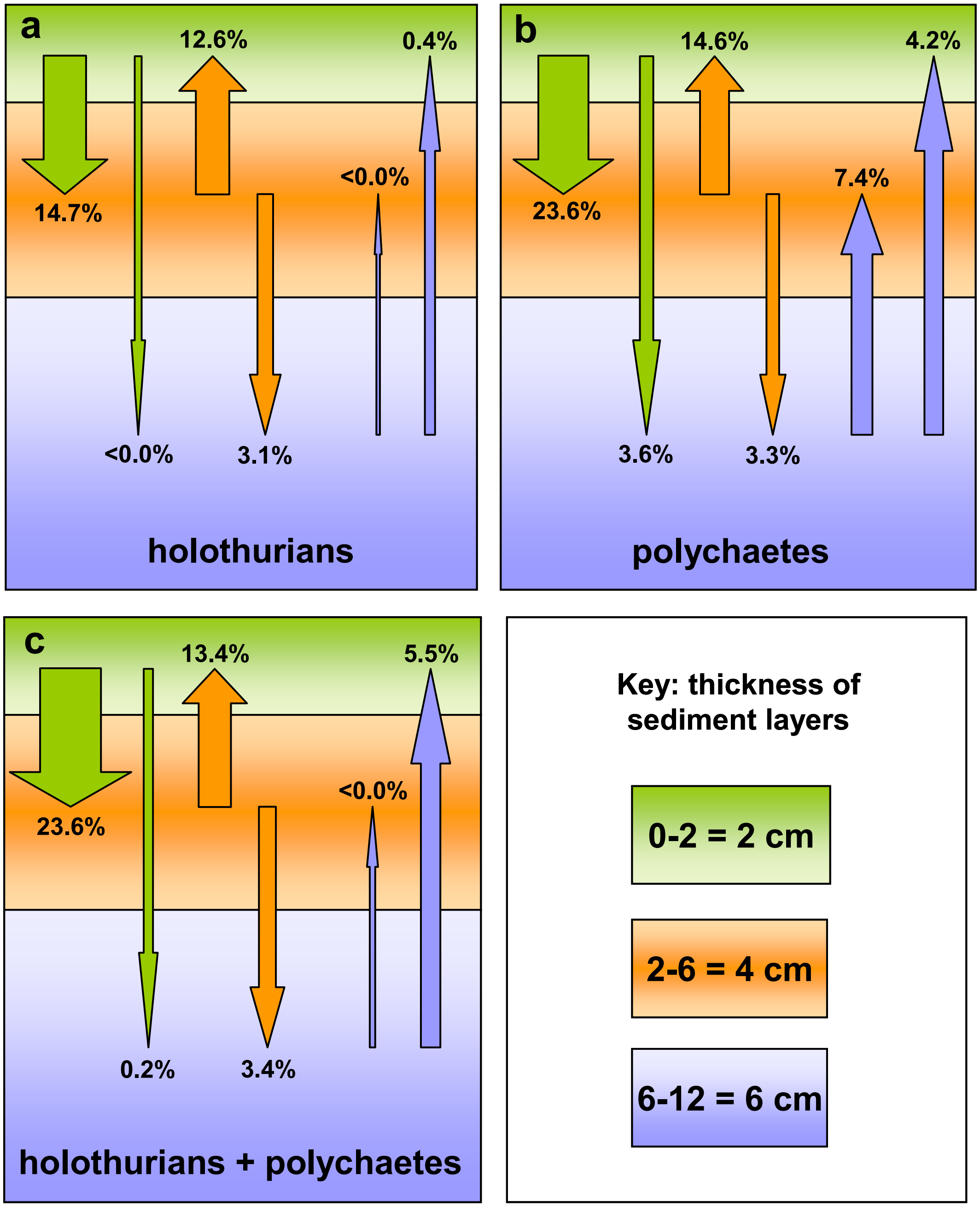

Figure 4-9: Tracer displacement as a percentage at day 56 for (a) holothurians only, (b) polychaetes only and (c) with both animal types. Direction of arrows represents the movement from one layer to another; arrow thickness represents the amount of tracer transported in relation to the others.

While sandfish did not impact on sediment in the bottom layer, polychaetes displaced considerable amounts of this bottom sediment to the middle and surface layers (Figure 4-9). Under the presence of both animal types, polychaetes transported more violet tracer into the top 2 cm than into the middle layer (2-4 cm). This is a typical result of sediment being displaced via the upward-conveyer-belt method (Duport *et al.* 2006; François *et al.* 2002). Such sediment transport from the bottom (>6 cm) was not visible when only holothurians were present. This suggested that holothurians and polychaetes used different functional modes to move the sediment.

Holothurians turn over sediment by a combination of two mechanisms, diffusion (smearing) and upward conveyor-belt movement. The first occurs only during burying, the latter during burying and feeding. To accurately categorise holothurians into a functional group of bioturbators, a new mode of bioturbation is suggested: ‘conveyor diffusors’. As holothurians spend much of their time foraging for food, they mix sediments mainly through their movement, but also some during diel burying (see Chapter 3). Similar results have been found for heart urchins, where sediment mixing due to burying movement (diffusion) can be 60 – 150 times higher than the volume moved due to ingestion (conveyor) (Hollertz and Duchêne 2001).

In addition to bioturbation caused by holothurians (conveyor diffusion), this study showed polychaetes can have a significant effect on vertical sediment displacement. They mix sediment mainly through upward conveyor-belt movements and, when left undisturbed, can drastically transform the sediment surface and also oxygenate deeper layers (anoxic layer at 10-12 cm). Polychaetes, at the densities observed, also exhibit significantly higher mixing rates of surface sediments than holothurians (P, 0.57% d^{-1}).

When both animal types coexist, the polychaetes' upward conveyor-belt mixing from deeper layers to the surface and the extensive oxygenation of the sediment may be suppressed (anoxic layer at 6-8 cm), as is the overall sediment mixing rate (HP, 0.50% d^{-1}) and the influence on the topography of surface sediments. Hughes and Gage (2004) have described similar results for deep-sea sediments where the interplay of macro- and megafaunal sediment transport resulted in a lack of tracer peaks in the subsurface.

Under natural conditions with both animal types present, holothurians may be the main ecosystem engineers (*sensu* Jones *et al.* 1994) in regards to micro-topography of the sediment surface; though polychaetes have a much greater influence on the condition of subsurface sediments. Their foraging behaviour appeared to have had a levelling effect on the sediment surface, destroying mounts and pits and counteracting against the higher sediment mixing out of deeper layers caused by polychaetes. Conveyor diffusors seem to inhibit the 'upward conveyors' in this study, but the latter are still considered being the most efficient and thus true ecosystem engineers, in terms of sediment mixing, in this habitat.

When multi-species coexist in a habitat, the rate of sediment reworking is assumed to be directly linked to the dominant macrobenthic species, both in size (Sandnes *et al.* 2000) and in density (Duport *et al.* 2006). In this experiment *H. scabra* was the dominant species in size and biomass relative to the polychaetes. Nevertheless, the holothurians had far less influence on the sediment reworking than originally assumed. This emphasises the importance of a particular life mode (e.g. feeding and burying behaviour) of functional groups, rather than its size and abundance (Mermillod-Blondin *et al.* 2004). For example, Grant (1983) found that 33% of sediment reworking was due to the bioturbation of one single polychaete species (*Paraonis fulgens*), greatly exceeding the effect of larger bioturbators as well as sediment movement caused by tidal currents. He regarded the bioturbation effect of all other groups (mainly sting rays, acorn worms and amphipods) as 'background noise'. The type of behaviour is therefore an important factor in determining a bioturbator's position in the natural community compared to size or abundance of a particular dominant species.

One of the most important factors for successful coexistence of species in the same habitat is the effective partitioning of food sources (e.g. McPeek 1996). Hylleberg (1975) found that the reworking of sediment and production of faecal pellets by the polychaete *Abarenicola pacifica* stimulated the metabolic activities and growth rates of microorganisms, thus adding to the bioturbator's own food requirements. Anoxic sediment layers were found to have increased microbial productivity (especially of bacteria), often higher than at the sediment-water interface (Yingst and Rhoads 1980). A review by Aller (1994) suggested that even brief redox oscillation (periodic re-exposure to O_2) can result in more complete decomposition of organic matter by bacteria. Thus, if sediment reworking leads to increased bacterial metabolism, it could benefit all deposit-feeders. Gerino *et al*. (1998) also found that sediment transport by macrofaunal communities moved organic material out of the oxic zone, thus promoting anaerobic metabolism in deeper strata. Hence, holothurians and polychaetes could benefit from bacteria both in the oxic as well as anoxic zone with their sediment reworking constantly aiding each others food requirements.

5 Extrapolation of findings to holothurian populations in topical environments

Most of the conclusions on the ecological role of *H. scabra* have focused on the effects of individual sandfish, however, one of the most important aspects of this research is the possibility to use the acquired results to draw conclusions about the wider population of *H. scabra* within Moreton Bay. By using activity dynamics in combination with sediment turn-over rates, the overall impact of the *H. scabra* population on its habitat in Moreton Bay can be estimated, and thus the possible ecological impact of fishery related depletion of the population.

5.1 Sandfish density and seagrass cover in Moreton Bay

Using data on seagrass cover and sandfish abundance from surveys conducted in 2000 (Skewes *et al.* 2002), a map with *H. scabra* distribution overlain with seagrass cover was created (Figure 5-1). This illustrated that *H. scabra* were mainly associated with seagrass meadows. Only a very small percentage of sites with sandfish were on bare sand, however, they were still in the vicinity of adjacent seagrass meadows. Also, *H. scabra* seemed to be less associated with areas that had more than 70% seagrass cover. This became more apparent when looking at the relationship between seagrass cover and *H. scabra* density (Figure 5-2). Meadows with >70% seagrass cover were potentially too dense, making it hard for the animal to access the sediment for food and shelter.

Similarly, abundance of *H. scabra* has been positively correlated (Gaussian) with seagrass meadows on Warrior Reef (Skewes *et al.* 2000).

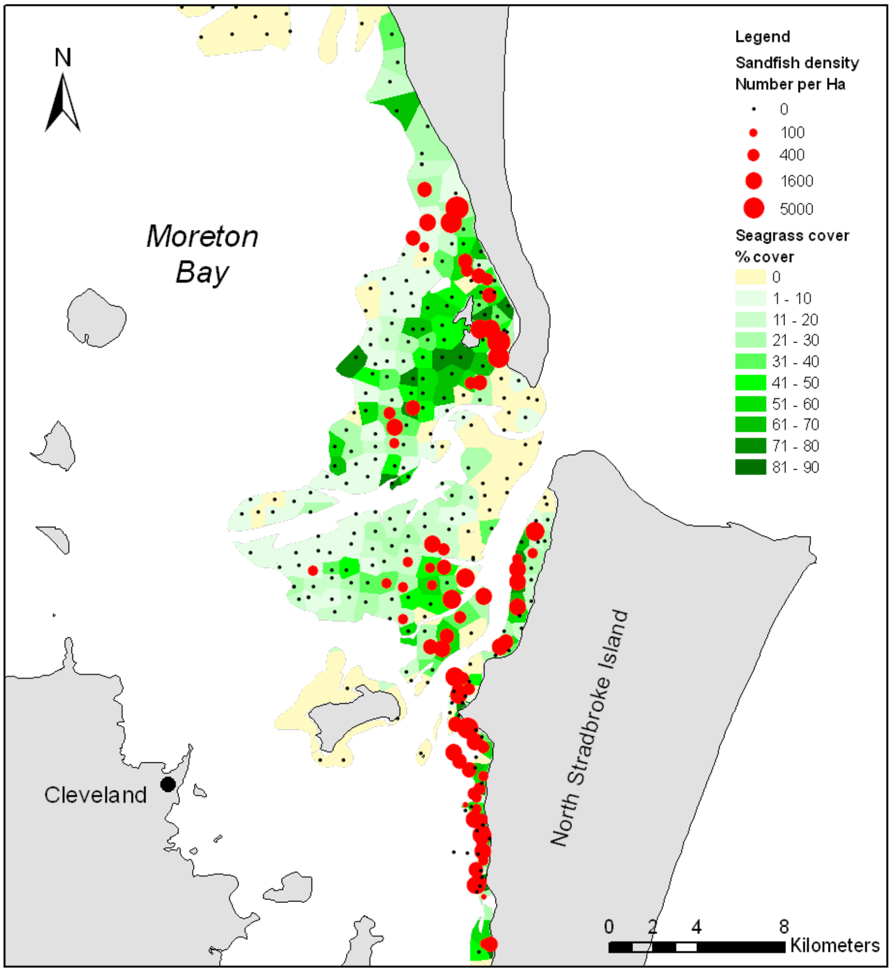

Figure 5-1: Seagrass cover (green shades) and abundance of *Holothuria scabra* (red circles) in eastern Moreton Bay

The sandfish surveys in Moreton Bay did not extend to the western side of the bay. However, given the predominance of muddy sediments (< 65 µm) deposited in the western Bay from several rivers (92,000 t yr^{-1} being exported from Brisbane River alone (Eyre *et al.* 1998)), and given the coarser sediment preferences (muddy sand) of *H. scabra* (Mercier *et al.* 2000a), it is very unlikely that *H. scabra* will inhabit these areas. Furthermore, the same areas had substantial seagrass losses between 1987 and 1996 due to increasing turbidity in the water and have not recovered since (Abal and Dennison 1996).

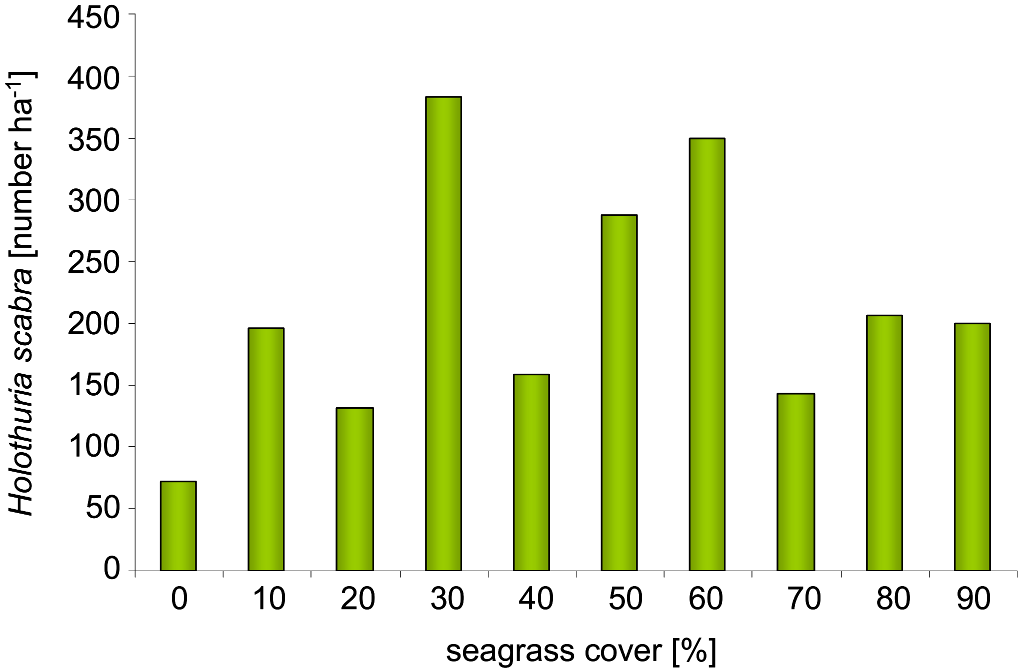

Figure 5-2: Histogram of holothurian density (*Holothuria scabra*) and seagrass cover in Moreton Bay (data provided by Skewes *et al.*, 2002)

5.2 Sediment transport by sandfish in Moreton Bay

Adult *H. scabra* are able to transport the top 2 cm at rate of 0.25% d^{-1} at a density of 5.6 ind. m^{-2} established in aquaria experiments presented in Chapter 4. To put these estimations into perspective, transport estimates need to be converted to natural densities. Therefore, the following calculation will use the weight of sediment transported by an average sized adult *H. scabra* (~17cm length and ~300 g wet weight) in summer (density independent).

The estimated volume of bioturbated sediment was calculated based on the instantaneous mixing rate of 0.25% d^{-1} (see Section 4.3.3) of a single animal and the area of sediment (top 2 cm) in an aquarium (60 × 28 × 2 cm), thus:

$$0.0025\ d^{-1}\ animal^{-1} \times 60cm \times 28cm \times 2cm = 8.4\ cm^3\ d^{-1}\ animal^{-1} \qquad (1)$$

To convert volume into weight, the calculation was based on field measurements of moist sediment from the same site (see Section 4.2.1), such that one tonne of sediment wet weight (WW) was equivalent to 510 litres.

$$510{,}000\ cm^3 = 1{,}000{,}000\ g \qquad (2)$$

Therefore the weight of 1 cm^3 is:

$$1{,}000{,}000\ g/510{,}000\ cm^3 = \quad 1.96\ g\ (WW)\ cm^{-3} \qquad (3)$$

Now this sediment density was applied to equation (1) and the weight of moist sediment that a single animal transports in a day was calculated:

$$1.96\ g\ (WW)\ cm^{-3} \times 8.4\ cm^3\ d^{-1}\ animal^{-1} = 16.46\ g\ (WW)\ d^{-1} animal^{-1} \qquad (4)$$

To convert this estimate of sediment weight to dry weight (DW), an average sediment moisture content of 24.94% was used, which was obtained during chlorophyll analysis (see Section 2.2.7):

$$1\ g\ (DW) = 0.7506\ g\ (wet\ weight) \qquad (5)$$

Therefore the weight of sediment that a single animal transports in a day is:

$$16.46\ g\ (wet\ weight)\ d^{-1} animal^{-1} \times 0.7506 = 12.36\ g\ (DW)\ d^{-1} animal^{-1} \qquad (6)$$

This sediment transport estimate is based on sandfish activity during shoulder seasons, where animals bury approximately once each day (see Section 3.3.1). However, at the height of summer this is reduced to once every 48 hr, due to the animals switching to several exposed resting modes instead of one burial for each 24-hr-period (see Chapter 3 for details). Similarly, regular burying patterns are broken during the coldest months in winter where the animals stay buried for up to 48 hr. Taking these variations in burying activity into consideration, the following factor is calculated to adjust daily bioturbation rates over the year:

$$(3\text{ m} / 12\text{ m} \times 0.5) + (3\text{ m} / 12\text{ m} \times 0.5) + (6\text{ m} / 12\text{ m} \times 1) = 0.75 \qquad (7)$$

Thus, using the result from equation (6), the average daily transport rate for a single animal on an annual basis would be

$$12.36\text{ g (DW) d}^{-1}\text{ animal}^{-1} \times 0.75 = 9.27\text{ g (DW) d}^{-1}\text{ animal}^{-1} \qquad (8)$$

To calculate the transport rate at natural densities, the ratio of aquarium density to natural density and the seasonal correction factor were used:

$$0.25\%\text{ d}^{-1}\text{ animal}^{-1} \times (0.48\text{ animal}^{-1}\text{ m}^{-2} / \ 5.6\text{ animal}^{-1}\text{ m}^{-2}) \text{ x } 0.75 =$$
$$0.016\%\text{ animal}^{-1}\text{ d}^{-1} \qquad (9)$$

For the sandfish population of 3.6 million animals in Moreton Bay (Skewes *et al.* 2002), the average daily sediment transport is:

$$9.27\text{ g (DW) d}^{-1}\text{ animal}^{-1} \times 3.6\text{ M animals} = 33.4\text{ t (DW) d}^{-1} \qquad (10)$$

and a yearly estimate of

$$33.4\text{ t (DW) d}^{-1} \times 365\text{ d yr}^{-1} = 12{,}180.8\text{ t (DW) yr}^{-1} \qquad (11)$$

5.3 Sediment turnover by sandfish excretion in Moreton Bay

The annual average sediment turnover cause by excretion of the sandfish population in Moreton Bay was estimated using the daily excretion rate of 39.2 g (DW) d^{-1} animal^{-1} under summer conditions (24°C, see Section 3.3.1). To convert this estimate to an annual excretion rate, average daily feeding hours for the different seasons were incorporated (see Section 3.3.2).

Summer (feeding for 15.5 hr):

$$39.2 \text{ g (DW) d}^{-1} \text{ animal}^{-1} \tag{12}$$

Spring/Autumn (feeding for 10.5 hr):

$$39.2 \text{ g (DW) d}^{-1} \text{ animal}^{-1} \times (10.5 \text{ hr}/15.5 \text{ hr}) = 26.6 \text{ g (DW) d}^{-1} \text{ animal}^{-1} \tag{13}$$

Winter (feeding for 2 hr):

$$39.2 \text{ g (DW) d}^{-1} \text{ animal}^{-1} \times (2 \text{ hr}/15.5 \text{ hr}) = 5.1 \text{ g d}^{-1} \text{ animal}^{-1} \tag{14}$$

Thus, the annual daily average sediment excreted by a single animal is:

$$(3/12 \times 39.2 \text{ g}) + (6/12 \times 26.6 \text{ g}) + (3/12 \times 5.1 \text{ g}) = 24.38 \text{ g (DW) d}^{-1} \text{ animal}^{-1} \tag{15}$$

For the sandfish population in Moreton Bay this equates to:

$$24.38 \text{ g (DW) d}^{-1} \text{ animal}^{-1} \times 3.6 \text{ M animals} = 87.8 \text{ t (DW) d}^{-1} \tag{16}$$

Lastly, the turnover per square meter was calculated in order to compare results with published data of turnover rates of other holothurian populations, thus:

$$0.46 \text{ ind. m}^{-2} \times 24.38 \text{ g (DW) d}^{-1} \text{ animal}^{-1} = 11.22 \text{ g d}^{-1} \text{ m}^{-2} \tag{17}$$

$$11.22 \text{ g d}^{-1} \text{ m}^{-2} \times 365 \text{ d yr}^{-1} = 4.09 \text{ kg yr-1 m}^{-2} \tag{18}$$

Comparing these figures to model calculations presented in the literature, the *H. scabra* population in Moreton Bay is bioturbating about the same quantity of sediment as estimated for a population of two closely related deposit-feeding holothurians with similar densities on a coral reef habitat (4.6 kg yr^{-1} m^{-2}, Uthicke 1999). Other studies for single individuals of *H. scabra* calculated maximum annual excretion rates of 41 kg DW yr^{-1} (Mercier *et al.* 1999) and 71 kg DW yr^{-1} (Yamanouchi 1939). However, these values are potentially an overestimation due to the fact that average yearly values were based on experiments conducted during summer and not adjusted to seasonal differences in feeding behaviour (with respect to tropical environments).

The figures presented in this study provide a powerful tool to estimate long-term physical effects on sediments in Moreton Bay caused by holothurians and offer a reliable measure to assess their ecological role within this shallow coastal habitat.

5.4 Extrapolation to tropical sandfish populations

Population of *H. scabra* are found in tropical and subtropical environments only (see Section 1.2.1). The productivity (growth and reproduction) of this species is optimal in tropical conditions with water temperatures above 27°C (Hamel *et al.* 2001). Thus, investigating a subtropical population at the edge of the species range, like the one in Moreton Bay, has some limitations when extrapolating findings to other environments.

Mercier *et al.* (1999; 2000a) found that the optimal temperature range for young adult *H. scabra* is 27-29°C and that animals might change their usual burying behaviour when water temperature exceeds 29°C. They also reported higher excretion rates for *H. scabra* in tropical environments such as Solomon Islands. Moreton Bay's water temperature ranges from 16°C in winter to 27°C in summer (Gabric *et al.* 1998), thus never reaching *H. scabra*'s optimal range.

Hence, it is important to note that figures presented in the present study probably represent the lower range of bioturbation and sediment excretion for *H. scabra*. Animals found in Moreton Bay usually are smaller and have slower growth rates than their counterparts in tropical habitats (O'Brien 1994).

These findings emphasise the importance of maintaining different populations of *H. scabra* especially in the tropics, not only to preserve their genetic diversity, but also because subtropical environments are not necessarily a suitable refuge for this species.

6 General discussion

6.1 Review of thesis objectives

The two major objectives of this study were to determine (A) the biological and (B) the physical influence of *Holothuria scabra* on their habitat to assess and quantify their ecological role. The following questions were posed:

A1) What is the influence of *H. scabra* on the benthic microalgae (BMA) community (biomass)?

A2) What is the influence of *H. scabra* on the seagrass community (biomass and/or productivity)?

A3) What is the influence of *H. scabra* on total organic matter (OM) in the sediment?

B1) What is the influence of *H. scabra* on chemical processes in the sediment (anoxic layer)?

B2) What is the influence of burying and feeding of *H. scabra* on sediment reworking and is there a diel and/or seasonal cycle (mixing rate and behaviour)?

Assessing the biological role of *H. scabra* (questions A1-A3) was achieved by means of *in situ* holothurian exclusion experiments and measurements of seagrass biomass and productivity, BMA biomass and OM content in the sediment (Chapter 2). Assessing the physical role of *H. scabra* in terms of behavioural influences (question B1-B2) was achieved by means of short-term controlled aquarium experiments (10 days) as well as longer-term mesocosms and *in situ* observations (25 months) (Chapter 3). This information was used to calculate bioturbation rates for *H. scabra* (Chapter 4) and these results were the basis for the extrapolations to broader field populations of this species (Chapter 5).

The following sections will restate the conclusions of this study in the context of the original objectives and place them into the larger question of how and why holothurians like *H. scabra* are important to the health of their habitat. Statements about their biological role are based on the change of associate flora like seagrass and benthic microalgae when holothurian densities are reduced. Their physical role is put into perspective by commenting on their bioturbation caused by burying and feeding. Finally, conclusions are drawn onto an ‘interaction web’ to consider what may happen to shallow marine ecosystems if holothurian density is reduced.

6.2 Ecological roles of *Holothuria scabra*

6.2.1 Effects on associated flora

An important ecological role of *H. scabra* is recycling nutrients. They directly convert organic N and P into inorganic nutrients (excretion), but also indirectly influence the nutrient availability through resuspension of organic matter in the sediment (Kitano *et al.* 2003; Uthicke 2001b; Uthicke and Klumpp 1998). This study showed that the presence of holothurians not only benefited seagrass productivity, but also that BMA biomass was influenced by holothurian density.

BMA and bacteria are the main food source for deposit-feeding holothurians and are preferred over filamentous brown, red and blue-green algae (Yingst 1976). Based on bacterial productivity on a coral reef and holothurians (*H. atra*) being the main grazer

(1.5 ind. m^{-2}), holothurians are estimated to consume 10-40% of daily bacterial carbon production (Moriarty *et al.* 1985), which converts to about 20-50 mg bacterial carbon d^{-1} for each individual (Moriarty 1982). Similar results have been described for temperate holothurians in Japan, where *S. japonicus* inhibited benthic algal growth and anaerobic processes in aquaria sediments and decreased OM deposits (Kitano *et al.* 2003).

As only BMA biomass was measured, this study was unable to establish the relationship between holothurian density and BMA productivity. Without having measured the BMA productivity under grazed and ungrazed conditions, it is difficult to quantify the overall gross effects that these holothurians might have on the microorganisms.

6.2.2 Sediment bioturbation (through burying and feeding)

By conducting *in situ* cage exclusion experiments and long-term behavioural observations in mesocosms as well as producing bioturbation estimates and instantaneous mixing rates, this study has substantially increased knowledge of the physical role of holothurians in a shallow marine ecosystem. A major ecological function of these animals is to mix and change the properties of sediments and their associated microflora and fauna by exposing sediments to various hydrodynamic and chemical conditions, either by transporting it to the sediment-water interface or by depositing it into deeper, anoxic layers. Thayer (1979) has introduced the term ‘biological bulldozing’ (overturning or burial of other organisms), and considers that holothurians have played a major role in this sort of bioturbation since the Devonian (410-355 MYBP).

This study showed that sediment mixing due to holothurians was only to a depth of ~ 6 cm, and that the mixing rate was relatively slow compared to polychaetes (see Chapter 3). In this case, ‘big’ is not ‘best’, and smaller, more efficient bioturbators (e.g. polychaetes) can have a substantially greater effect on the overall sediment transport.

The existence of cyclical patterns in the feeding and burying behaviour of adult *H. scabra* was another major finding of this study. The timing and duration of burying was highly temperature related, thus giving the behaviour a distinct seasonal pattern. Seasonal variations in burying and feeding activities may affect the ecosystem function of holothurians within their habitat. Observations of the seasonal behaviour dynamics of these animals make it possible to infer seasonal movement and influences of holothurians on other organisms. For example, more intense searching periods observed during shoulder seasons may mean a greater range of movement, utilising a greater area for increased food requirements in relation to reproduction, however, on the other hand less influence on the actual sediment bioturbation. Furthermore, almost all animals remained buried for long periods during the coldest months of the year, which can mean drastic reductions in sediment transport, therefore potentially affecting sediment chemistry and indirectly seagrass and microalgae.

The relatively low sediment transport rates associated with holothurian bioturbation, and the negative interaction effect on other bioturbators such as polychaetes, indicate that holothurians might not be absolutely critical for this habitat function, and other animals with similar functional modes might be able to compensate for a certain reduction in holothurian abundance, at least for the short term. Nevertheless, this study did not investigate the long-term effects (years) of excluding holothurians. The results presented here carefully suggest that a delayed rather than immediate ‘impact of removal’ may occur when holothurians are completely removed from a certain habitat. This in turn might push an otherwise balanced multi-species ecosystem to an environment with lower biodiversity, vulnerable to any further disruption by anthropogenic as well as natural forces.

It has been suggested that biodiversity can act within functional groups as an ‘insurance policy’ or ‘buffer’ against major ecosystem changes (Doherty *et al.* 2000) and that ‘species do not need to be extinct before there is a loss of function’ (Meysman *et al.* 2006; Thrush and Dayton 2002).

6.2.3 Implication for surveys and management

Stock surveys and population monitoring are common tools used to assess and manage holothurian fisheries. Burying causes stock surveys to underestimate actual density and stock size, and variable burying rates make comparison of different surveys difficult and prone to error. As described in Chapter 3, burying and feeding behaviour of adult *H. scabra* exhibit seasonal variability strongly related to changes in water temperature. Bioturbation caused by this behaviour thus does not occur at equal rates during the year (Chapter 4). Information on the timing and extent of holothurian burying behaviour is critical for the design of surveys and interpretation of results. This is also true for the interpretation of fishery dependant data (catch per unit effort) due to changes in catchability caused by varying burying rates. Based on the findings of this study, the most suitable time to conduct population surveys on *H. scabra* would be during summer (Nov-Jan) from midday to late afternoon.

This illustrates the importance of understanding the dynamics of holothurian activity when formulating conservation or fishery management plans for this commercially important group. Not only can population and distribution surveys be based on the exact daily cycle of behavioural patterns presented in this study, but also seasonal changes of those patterns can be accounted for and incorporated into the strategic planning of tools and management for the fishery.

6.2.4 Ecosystem engineers

The term ‘ecosystem engineer’ (*sensu* Jones *et al.* 1994) can be applied to the role of holothurians in shallow marine habitat: these animals can be considered as ‘earthworms of the sea’, true ‘allogenic engineers’ in the sense of changing the environment by transforming living and non-living materials from one physical state to another via mechanical means (*sensu* Lawton 1994). Nevertheless, these non-trophic interactions are closely linked to the trophic role that holothurians also have in their habitat. Lawton (1994) calls this phenomenon the ‘interaction web’ (as oppose to trophic web), which should reflect any given interaction in communities and ecosystems.

Integrating holothurians into such an ‘interaction web’ (**Figure 6-1**) suggests that over-exploitation of these animals may have cascading effects to many other organisms either directly or indirectly linked to the holothurian habitat. Hence, bioturbation as well as nutrient recycling and chemical transformation are fundamental attributes that give holothurians their main ecological role in the habitat they shape and live in.

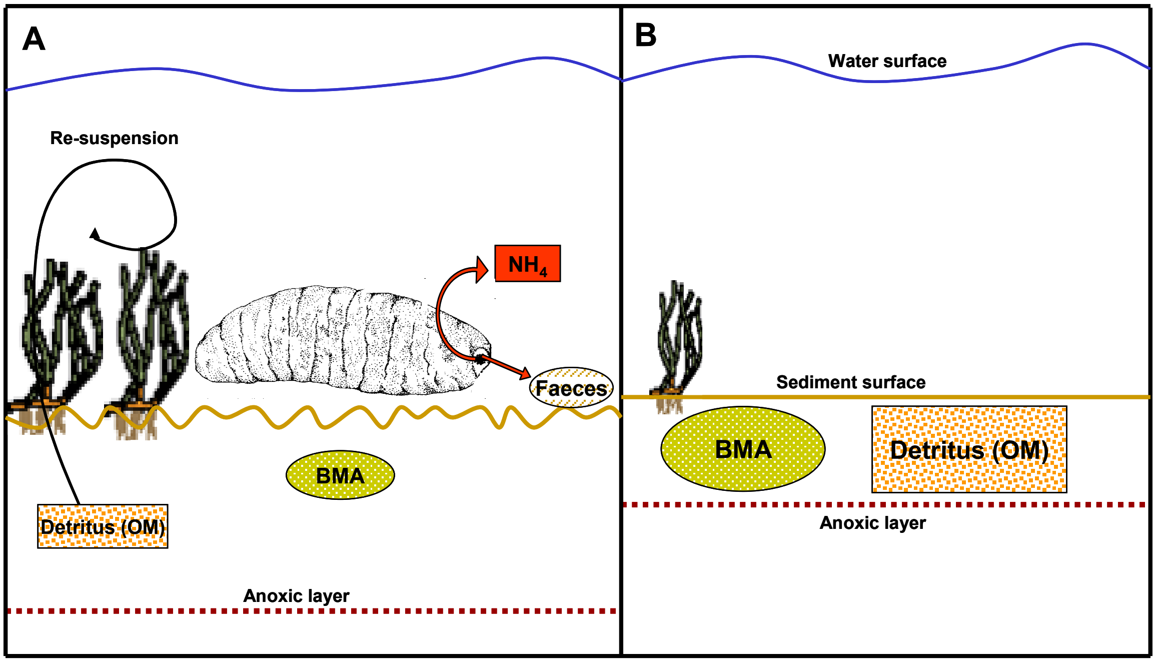

Figure 6-1: Conceptual model of an ‘interaction web’ (A) with and (B) after removing holothurians from their environment, and the possible effect this can have on seagrass, benthic microalgae (BMA), organic matter (OM) and the anoxic layer

References

Abal E.G. and Dennison W.C. 1996. Seagrass depth range and water quality in southern Moreton Bay, Queensland, Australia. Marine Freshwater Research 47:763-771.

Aller R.C. 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. Chemical Geology 114(3-4):331-345.

Aquila S.P. 1996. Migration of *Holothuria scabra*. South Pacific Commission Bêche-de-mer Information Bulletin 8:45.

Bai M.M. 1971. Regeneration in the holothurian, *Holothuria scabra* Jaeger. Indian Journal of Experimental Biology 9:467-471.

Bai M.M. 1978. The anatomy and histology of the digestive system of *Holothuria scabra* Jaeger. Journal of Marine Biological Association India 20(1-2):22-31.

Bai M.M. 1980. Monograph on *Holothuria* (*Metriatyla*) *scabra* Jaeger. Memoirs of the Zoological Survey of India 16:1-75.

Baskar B.K. (1994) Some observations on the biology of the holothurian *Holothuria* (*Metriatyla*) *scabra* (Jaeger). In 'Proceedings of the National Workshop on Bêche-de-mer'. Indian Council of Agricultural Research, Cochin, India. (Eds K Rengarajan and DB James) pp. 39-43. (Bulletin of the Central Marine Fisheries Research Institute)

Battaglene S.C. 1999a. Culture of tropical sea cucumbers for stock restoration and enhancement. NAGA, The ICLARM Quarterly 22(4):4-11.

Battaglene S.C. (1999b) Progress in the production of tropical sea cucumbers *Holothuria scabra* and *Holothuria fuscogilva* for stock enhancement. In 'World Aquaculture Society Conference'. Sydney, Australia. (Ed. WA Society) p. p 57. (World Aquaculture Society)

Battaglene S.C. (2000) Culture of tropical sea cucumbers for stock restoration and enhancement. In 'NAGA, The ICLARM Quarterly'. pp. 4-11.

Battaglene S.C., Seymour J.E. and Ramofafia C. 1999. Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra*. Aquaculture 178(3/4):293-322.

Battaglene S.C., Seymour J.E., Ramofafia C. and Lane I. 2002. Spawning induction of three tropical sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*. Aquaculture 207(1):29-47.

Birch W.R. and Birch M. 1984. Succession and pattern of tropical intertidal seagrasses in Cockle Bay, Queensland, Australia: a decade of observations. Aquatic Botany 19:343-367.

Boon P.I. 1986. Nitrogen pools in seagrass beds of *Cymodocea serrulata* and *Zostera capricorni* of Moreton Bay. Aquatic Botany 25(1):1-19.

Bruce C. (1983) Sea cucumber - extraordinary but edible all the same. In 'Infofish Marketing Digest'. pp. 19-21.

Bruckner A.W., Johnson K.A. and Field J.D. 2003. Conservation strategies for sea cucumbers: Can a CITES Appendix II listing promote sustainable international trade? South Pacific Commission Bêche-de-mer Information Bulletin 18:24-33.

Carruthers T.J.B., Dennison W.C., Longstaff B.J., Waycott M., Abal E.G., McKenzie L.J. and Lee Long W.J. 2002. Seagrass habitats of North-East Australia: Models of key processes and controls. Bulletin of Marine Science 71(3):1153-1169.

Chen J. 2003. Overview of sea cucumber farming and sea ranching practices in China. South Pacific Commission Bêche-de-mer Information Bulletin 18:18-23.

Coleman F.C. and Williams S.L. 2002. Overexploiting marine ecosystem engineers: potential consequences for biodiversity. Trends in Ecology & Evolution 17(1):40-44.

Conand C. 1983. Methods of studying growth in holothurians (bêche-de-mer), and preliminary results from a bêche-de-mer tagging experiment in New Caledonia. South Pacific Commission Fisheries Newsletter 26:31-38.

Conand C. (1989) Les holothuries aspidochirotes du lagon de Nouvelle-Calédonie: biologie, écologie et exploitation. Etudes et Thèses thesis, O.S.T.R.O.M.

Conand C. (1990) 'The fishery resources of Pacific Island countries. Part 2: Holothurians.' Food and Agriculture Organization of the United Nations, No. 272.2, Rome, Italy.

Conand C. 1993. Reproductive biology of the holothurians from the major communities of the New Caledonian lagoon. Marine Biology 116(3):439-450.

Conand C. (1997) Are holothurian fisheries for export sustainable? In 'Proceedings of the 8th International Coral Reef Symposium'. Malboa, Republic of Panama. (Eds HA Lessios and IG Macintyre) pp. 2021-2026. (Smithsonian Tropical Research Institute)

Conand C. (1998) Overexploitation in the present world sea cucumber fisheries and perspectives in mariculture. In '9th International Echinoderm Conference'. San Francisco, USA. (Eds R Mooi and M Telford) pp. 449-454. (Balkema, A. A., Rotterdam, The Netherlands)

Conand C. (1999) World sea cucumber exploitation and the market for trepang: an overview. In 'The conservation of sea cucumbers in Malaysia, their taxonomy, ecology and trade - Proceedings of an International Conference'. Kuala Lumpur, Malaysia. (Ed. M Baine) pp. 1-10. (Herriot-Watt University and the Fisheries Research Institute)

Conand C. 2001. Sea cucumber retail market in Singapore. South Pacific Commission Bêche-de-mer Information Bulletin 14:12-14.

Conand C. and Byrne M. 1993. A review of recent developments in the world sea cucumber fisheries. Marine Fisheries Review 55(4):1-13.

Dance S.K., Lane I. and Bell J.D. 2003. Variation in short-term survival of cultured sandfish (*Holothuria scabra*) released in mangrove-seagrass and coral reef flat habitats in Solomon Islands. Aquaculture 220(1-4):495-505.

Darwin C.R. (1881) 'The formation of vegetable mould, through the action of worms, with observations on their habits.' (John Murray: London)

de Boer W.F. 2000. Biomass dynamics of seagrasses and the role of mangrove and seagrass vegetation as different nutrient sources for an intertidal ecosystem. Aquatic Botany 66:225-239.

de Jonge V.N. and van den Bergs J. 1987. Experiments on the resuspension of estuarine sediments containing benthic diatoms. Estuarine, Coastal and Shelf Science 24:725-740.

Dennison W.C. and Abal E.G. (1999) 'Moreton Bay Study: a scientific basis for the healthy waterways campaign.' (South East Queensland regional water quality management strategy team: Brisbane)

Doherty M., Kearns A., Barnett G., Sarre A., Hochuli D., Gibb H. and Dickman C. (2000) The interaction between habitat conditions, ecosystem processes and terrestrial biodiversity – a review. In 'Australia: State of the Environment, Second Technical Paper Series (Biodiversity)' pp. 114. (Department of the Environment and Heritage: Canberra)

Duport E., Stora G., Tremblay P. and Gilbert F. 2006. Effects of population density on the sediment mixing induced by the gallery-diffusor *Hediste* (*Nereis*) *diversicolor* OF Müller, 1776. Journal of Experimental Marine Biology and Ecology 336(1):33-41.

Enriquez S. 2005. Light absorption efficiency and the package effect in the leaves of the seagrass *Thalassia testudinum*. Marine Ecology Progress Series 289:141-150.

Eyre B., Hossain S. and McKee L. 1998. A suspended sediment budget for the modified subtropical Brisbane River estuary, Australia. Estuarine, Coastal and Shelf Science 47(4):513-522.

Ferdie M. and Fourqurean J.W. 2004. Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. Limnology and Oceanography 49(6):2082-2094.

François F., Gerino M., Stora G., Durbec J.P. and Poggiale J.C. 2002. Functional approach to sediment reworking by gallery-forming macrobenthic organisms: modeling and application with the polychaete *Nereis diversicolor*. Marine Ecology Progress Series 229:127-136.

François F., Poggiale J.C., Durbec J.P. and Stora G. 1997. A new approach for the modelling of sediment reworking induced by a macrobenthic community. Acta Biotheoretica 45(3):295-319.

Gabric A.J., McEwan J. and Bell P.R.F. 1998. Water quality and phytoplankton dynamics in Moreton Bay, south-eastern Queensland. I. Field survey and satellite data. Marine & Freshwater Research 49(3):215-225.

Gerino M. 1990. The effects of bioturbation on particle redistribution in Mediterranean coastal sediment. Preliminary results. Hydrobiologia 207:251-258.

Gerino M., Aller R.C., Lee C., Cochran J.K., Aller J.Y., Green M.A. and Hirschberg D. 1998. Comparison of different tracers and methods used to quantify bioturbation during a spring bloom: 234-Thorium, luminophores and Chlorophyll-a. Estuarine, Coastal and Shelf Science 46(4):531-547.

Grall J. and Chauvaud L. 2002. Marine eutrophication and benthos: the need for new approaches and concepts. Global Change Biology 8:813-830.

Grant J. 1983. The relative magnitude of biological and physical sediment reworking in an intertidal community. Journal of Marine Research 41:673-689.

Grinham A.R. (2006) Downstream effects on land use in shallow-water benthic microalgae communities in Moreton Bay, Australia and Marove Lagoon, Solomon Islands. PhD thesis, University of Queensland.

Guzman H.M., Guevara C.A. and Hernandez I.C. 2003. Reproduction cycle of two commercial species of sea cucumber (Echinodermata: Holothuroidea) from Caribbean Panama. Marine Biology 142(2):271-279.

Hamel J.-F., Conand C., Paeson D.L. and Mercier A. 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and exploitation as bêche-de-mer. Advances in Marine Biology 41:129-223.

Hamel J.-F., Ng P.K.L. and Mercier A. 1999. Life cycle of the pea crab *Pinnotheres halingi* sp. nov., an obligate symbiont of the sea cucumber *Holothuria scabra* Jaeger. Ophelia 50(3):149-175.

Hauksson E. 1979. Feeding biology of *Stichopus tremulus*, a deposit-feeding holothurian. Sarsia 64(3):155-160.

Haywood M.D.E., Vance D.J. and Loneragan N.R. 1995. Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semisulcatus* and *P. esculentus*) in a tropical Australian estuary. Marine Biology 122(2):213-223.

Himmelman J.H., Dutil C. and Gaymer C.F. 2005. Foraging behavior and activity budgets of sea stars on a subtidal sediment bottom community. Journal of Experimental Marine Biology and Ecology 322(2):153-165.

Holland A. 1994. The bêche-de-mer industry in the Solomon Islands: recent trends and suggestions for management. South Pacific Commission Bêche-de-mer Information Bulletin 6:2–9.

Hollertz K. and Duchêne J.-C. 2001. Burrowing behaviour and sediment reworking in the heart urchin *Brissopsis lyrifera* Forbes (Spatangoida). Marine Biology 139(5):951-957.

Hornell J. 1917. The Indian bêche-de-mer industry, its history and recent revival. Madras Fisheries Bulletin 11(4):119-150.

Hudson I.R., Wigham B.D., Solan M. and Rosenberg R. 2005. Feeding behaviour of deep-sea dwelling holothurians: Inferences from a laboratory investigation of shallow fjordic species. Journal of Marine Systems 57(3-4):201-218.

Hudson I.R., Wigham B.D. and Tyler P.A. 2004. The feeding behaviour of a deep-sea holothurian, *Stichopus tremulus* (Gunnerus) based on in situ observations and experiments using a Remotely Operated Vehicle. Journal of Experimental Marine Biology and Ecology 301:75-91.

Hughes A.R., Bando K.J., Rodriguez L.F. and Williams S.L. 2004. Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. Marine Ecology Progress Series 282:87-99.

Hughes D.J. and Gage J.D. 2004. Benthic metazoan biomass, community structure and bioturbation at three contrasting deep-water sites on the northwest European continental margin. Progress in Oceanography 63(1-2):29-55.

Hylleberg J. 1975. Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lugworms. Ophelia 14:113-137.

James D.B. 1989. Bêche-de-mer - Its resources, fishery and industry. Marine Fisheries Information Service 92:1-30.

James D.B. (1994a) Ecology of commercially important holothurians of India. In 'Proceedings of the National Workshop on Bêche-de-mer'. Cochin, India. (Eds K Rengarajan and DB James) pp. 37-38

James D.B. 1994b. Seed production in sea cucumbers. Aquacult. Int. 1:15-26.

James D.B. (1996) Culture of sea cucumber. In 'Artificial Reef and Seafarming Technology'. (Ed. K Rengarajan) pp. 120-126. (Bulletin of the Central Marine Fisheries Research Institute: Cochin, India)

James D.B., Gandhi A.D., Palanisamy N. and Rodrigo J.X. (1994) 'Hatchery techniques and culture of sea cucumber *Holothuria scabra*.' (Central Marine Fisheries Research Institute: Cochin, India)

James P.S.B.R. and James D.B. 1993. Ecology, breeding, seed production and prospects for farming of sea cucumbers from the sea around India. Fishing Chimes 13:28-34.

Jeffrey S.W. and Welshmeyer N.A. (1997) Spectrophotometric and fluorometric equations in common use in oceanography. In 'Phytoplankton Pigments in Oceanography. Monographs on Oceanographic Methodology No. 10'. (Eds SW Jeffrey, RFC Mantoura and SW Wright) pp. 597-615. (UNESCO Publishing: Paris)

Jones C.G., Lawton J.H. and Shachak M. 1994. Organisms as Ecosystem Engineers. Oikos 69(3):373-386.

Jun A. 2002. Trepang exploitation in the Philippines: Updated information. South Pacific Commission Bêche-de-mer Information Bulletin 17:17-21.

Kamal A., Misri K., Japar S., Hishamuddin O. and Hidir H. (1999) Leaf growth, production and ecological aspects of toothed seagrass *Cymodocea serrulata* (R. Br.) Aschers. et Magnus at Port Dickson, Negeri Sembilan, Malaysia. In 'International Conference on the International Oceanographic Data and Information Exchange in the Western Pacific

(IODE-WESTPAC) - ICIWP '99'. Langkawi (Malaysia), 1-4 Nov 1999 pp. 173-184. (IOC Paris, France)

Katrak G. and Bird F.L. 2003. Comparative effects of the large bioturbators, *Trypaea australiensis* and *Heloecius cordiformis*, on intertidal sediments of Western Port, Victoria, Australia. Marine and Freshwater Research 54:701-708.

Kenyon R.A., Conacher C.A. and Poiner I.R. 1997. Seasonal growth and reproduction of *Enhalus acoroides* (L.f.) Royle in a shallow bay in the western Gulf of Carpentaria, Australia. Marine and Freshwater Research 48:335-342.

Kidawa A. 2005. Behavioural and metabolic responses of the Antarctic sea star *Odontaster validus* to food stimuli of different concentration. Polar Biology 28(6):449-455.

Kitano M., Kurata K., Kozuki Y., Murakami H., Yamasaki T., Yoshida H. and Sasayama H. 2003. Effects of deposit feeder *Stichopus japonicus* on algal bloom and organic matter contents of bottom sediments of the enclosed sea. Marine Pollution Bulletin 47:118-125.

Klumpp D.W. and Kwak S.N. 2005. Composition and abundance of benthic macrofauna of a tropical sea-grass bed in North Queensland, Australia. Pacific Science 59(4):541-560.

Krishnan S. 1968. Histochemical studies on reproductive and nutritional cycles of the holothurian, *Holothuria scabra*. Marine Biology 2:54-65.

Krishnaswamy S. and Krishnan S. 1967. A report on the reproductive cycle of holothurian *Holothuria scabra* Jaeger. Current Science 36:155-156.

Lanyon J.M. and Marsh H. 1995. Temporal changes in the abundance of some tropical intertidal seagrasses in North Queensland. Aquatic Botany 49(4):217-237.

Lawton J.H. 1994. What do species do in ecosystems? Oikos 71(3):367-374.

Lee K.-S. and Dunton K.H. 2000. Effects of nitrogen enrichment on biomass allocation, growth, and leaf morphology of seagrass *Thalassia testudinum*. Marine Ecology Progress Series 196:39-48.

Lohrer A.M., Thrush S.F., Hunt L., Hancock N. and Lundquist C. 2005. Rapid reworking of subtidal sediments by burrowing spatangoid urchins. Journal of Experimental Marine Biology and Ecology 321(2):155-169.

Lokani P., Polon P. and Lari R. 1996. Management of bêche-de-mer fisheries in the Western Province of Papua New Guinea. South Pacific Commission Bêche-de-mer Information Bulletin 8:7-11.

Long B. and Skewes T. 1997. Distribution and abundance of bêche-de-mer on Torres Strait reefs. South Pacific Commission Bêche-de-mer Information Bulletin 9:17-22.

Lundberg J. and Moberg F. 2003. Mobile Link Organisms and Ecosystem Functioning: Implications for Ecosystem Resilience and Management. Ecosystems 6(1):87-98.

Mahaut M.-L. and Graf G. 1987. A luminophore tracer technique for bioturbation studies. Oceanologica Acta 10(3):323-328.

Maire O., Duchêne J.C., Grémare A., Malyuga V.S. and Meysman F.J.R. 2007. A comparison of sediment reworking rates by the surface deposit-feeding bivalve *Abra ovata* during summertime and wintertime, with a comparison between two models of sediment reworking. Journal of Experimental Marine Biology and Ecology 343(1):21-36.

Massin C. (1999) 'Reef-dwelling Holothuroidea (Echinodermata) of the Spermonde archipelago (South-West Sulawesi, Indonesia).' Leiden, The Netherlands.

Matisoff G. and Wang X. 2000. Particle mixing by freshwater infaunal bioirrigators: midges (Chironomidae: Diptera) and mayflies (Ephemeridae: Ephemeroptera). Journal of Great Lakes Research 26(2):174-182.

McElroy S. 1990. Bêche-de-mer species of commercial value - an update. South Pacific Commission Bêche-de-mer Information Bulletin 2:2-7.

McPeek M.A. 1996. Trade-Offs, food web structure, and the coexistence of habitat specialists and generalists. American Naturalist 148(S1):124.

Mercier A., Battaglene S.C. and Hamel J.-F. 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology 239(1):125-156.

Mercier A., Battaglene S.C. and Hamel J.-F. 2000a. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. Hydrobiologia 440(1-3):81-100.

Mercier A., Battaglene S.C. and Hamel J.-F. 2000b. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. Journal of Experimental Marine Biology and Ecology 249(1):89-110.

Mercier A. and Hamel J.-F. (1997) Finding solutions for a bêche-de-mer industry. In 'Island Business Magazine'. pp. 44-47.

Mermillod-Blondin F., François-Carcaillet F. and Rosenberg R. 2005. Biodiversity of benthic invertebrates and organic matter processing in shallow marine sediments: an experimental study. Journal of Experimental Marine Biology and Ecology 315(2):187-209.

Mermillod-Blondin F., Gaudet J.P., Gerino M., Desrosiers G., Jose J. and Chatelliers M.C. 2004. Relative influence of bioturbation and predation on organic matter processing in river sediments: a microcosm experiment. Freshwater Biology 49(7):895-912.

Meysman F.J.R., Middelburg J.J. and Heip C.H.R. 2006. Bioturbation: a fresh look at Darwin's last idea. Trends in Ecology & Evolution 21(12):688-695.

Moiyadeen N.M. (1994) The biannual reproductive activity in *Holothuria* (*Metriatyla*) *scabra* (Jaeger, 1833), the most abundant commercial holothuriod of the north-western coastal waters. In 'Proceedings of the First Annual Scientific Session NARA'. Colombo, Sri Lanka pp. 123-129

Morgan A.D. 2000a. Aspects of the reproductive cycle of the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea). Bulletin of Marine Science 66(1):47-57.

Morgan A.D. 2000b. Induction of spawning in the sea cucumber *Holothuira scabra* (Echinodermata: Holothuroidea). Journal of the World Aquaculture Society 31(2):186-194.

Morgan A.D. and Archer J. 1999. Overview: aspects of sea cucumber industry research and development in the South Pacific. South Pacific Commission Bêche-de-mer Information Bulletin 12:15-17.

Moriarty D.J.W. 1982. Feeding of *Holothuria atra* and *Stichopus chloronotus* on bacteria, organic carbon, and organic nitrogen in sediments of the Great Barrier Reef. Australian Journal of Marine and Freshwater Research 33(2):255-263.

Moriarty D.J.W., Pollard P.C., Hunt W.G., Moriarty C.M. and Wassenberg T.J. 1985. Productivity of bacteria and microalgae and the effect of grazing by holothurians in sediments on a coral reef flat. Marine Biology 85(3):293-300.

Mosher C. 1980. Distribution of *Holothuria arenicola* Semper in the Bahamas with observations on habitat, behaviour and feeding activity (Echinodermata: Holothuroidea). Bulletin of Marine Science 30(1):1-12.

Mugnai C., Gerino M., Frignani M., Sauvage S. and Bellucci L.G. 2003. Bioturbation experiments in the Venice Lagoon. Hydrobiologia 494(1):245-250.

Mukai H., Koike I., Nishihira M. and Nojima S. 1989. Oxygen consumption and ammonium excretion of mega-sized benthic invertebrates in a tropical seagrass bed. Journal of Experimental Marine Biology and Ecology 134(2):101-115.

Nakaoka M. 2005. Plant-animal interaction in seagrass beds: ongoing and future challenges for understanding population and community dynamics. Population Ecology 47:167-177.

O'Brien C.J. 1994. Population dynamics of juvenile tiger prawns Penaeus esculentus in south Queensland, Australia. Marine Ecology Progress Series 104:247-256.

Ong Che R.G. and Gomez E.D. 1985. Reproductive periodicity of *Holothuria scabra* Jaeger at Calatagan, Batangas, Philippines. Asian Marine Biology 2:21-29.

Parsons T.R., Maita Y. and Lalli C.M. (1984) 'A manual of chemical and biological methods for seawater analysis.' (Pergamon Press: Oxford)

Pollard P.C. and Greenway M. 1993. Photosynthetic characteristics of seagrasses (*Cymodocea serrulata*, *Thalassia hemprichii* and *Zostera capricorni*) in a low-light environment, with a comparison of leaf-marking and lacunal-gas measurements of productivity. Australian Journal of Marine and Freshwater Research 44:127-139.

Preston G.L. 1990. Mass bêche-de-mer production in Fiji. South Pacific Commission Bêche-de-mer Information Bulletin 1:4-5.

Purcell S. (2004) Rapid growth and bioturbation activity of the sea cucumber *Holothuria scabra* in earthen ponds. In 'Proceedings of Australasian Aquaculture'. Sydney p. 244

Purcell S., Blockmans B. and Agudo N. 2006a. Transportation methods for restocking of juvenile sea cucumber, *Holothuria scabra*. Aquaculture 251:238-244.

Purcell S., Patrois J. and Fraisse N. 2006b. Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jaeger), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson). Aquaculture Research 37:515-522.

Purcell S.W. and Kirby D.S. 2005. Restocking the sea cucumber *Holothuria scabra*: Sizing no-take zones through individual-based movement modelling. Fisheries Research 80:53-61.

R (2005) R: A language and environment for statistical computing. In 'R Foundation for Statistical Computing'. (Ed. Development Core Team). (Vienna, Austria)

Reise K. 2002. Sediment mediated species interactions in coastal waters. Journal of Sea Research 48:127-141.

Rhoads D.C. and Young D.K. 1971. Animal-sediment relations in Cape Cod Bay, Massachusetts II. Reworking by *Molpadia oolitica* (Holothuroidea). Marine Biology 11(3):255-261.

Rimmer M.A. (2006) Regional Review on Existing Major Mariculture Species and Farming Technologies. In 'FAO/NACA Regional Mariculture Workshop'. Guangdong Hotel, Guangzhou City, Guangdong Province, China pp. 7-11

Roberts C.M. and Hawkins J.P. 1999. Extinction risk in the sea. Trends in Ecology & Evolution 14(6):241-246.

Roberts D., Gebruk A., Levin V. and Manship B.A.D. 2000. Feeding and digestive strategies in deposit-feeding holothurians. Oceanography and Marine Biology: an Annual Review 38:257-310.

Rosenberg R., Nilsson H.C. and Diaz R.J. 2001. Response of benthic fauna and changing sediment redox profiles over a hypoxic gradient. Estuarine, Coastal and Shelf Science 53(3):343-350.

Rosenberg R., Nilsson H.C., Hellman B. and Agrenius S. 2000. Depth correlated benthic faunal quantity and infaunal burrow structures on the slopes of a marine depression. Estuarine, Coastal and Shelf Science 50:843-853.

S-PLUS (2005) Programmer's Manual. In. (Insightful Corporation: Seattle, WA, USA)

Sachithananthan K. (1972) 'South Pacific Islands, bêche-de-mer fishery.' South Pacific Islands Fisheries Development Agency, Food and Agriculture Organization of the United Nations, Rome, Italy.

Sachithananthan K. (1994a) Bêche-de-mer: global perspectives. In 'Proceedings of the National Workshop on Bêche-de-mer'. Cochin, India. (Eds K Rengarajan and DB James) pp. 106-

109. (Central Marine Fisheries Research Institute, Indian Council of Agricultural Research)

Sachithananthan K. 1994b. A small-scale unit to process sand-fish *Holothuria* (*Metriatyla*) *scabra*. Bulletin of the Central Marine Fisheries Research Institute 46:79-80.

Sandnes J., Forbes T., Hansen R., Sandnes B. and Rygg B. 2000. Bioturbation and irrigation in natural sediments, described by animal-community parameters. Marine Ecology Progress Series 197:169-179.

Schink D.R. and Guinasso N.L. 1977. Effects of bioturbation on sediment-seawater interaction. Marine Geology 23:133-154.

Schinner G.O. 1993. Burrowing behaviour, substratum preference and distribution of *Schizaster canaliferus* (Echinoidea: Spatangoida) in Northern Adriatic Sea. Marine Ecology 14:129-145.

Shelley C.C. (1985a) Growth of *Actinopyga echinites* and *Holothuria scabra* (Holothuroidea: Echinodermata) and their fisheries potential (as bêche-de-mer) in Papua New Guinea. In 'Proceedings of the 5th International Coral Reef Congress'. Tahiti pp. 297-302

Shelley C.C. (1985b) Potential for re-introduction of a bêche-de-mer fishery in the Torres Strait. In 'Torres Strait Fisheries Seminar'. Port Moresby, PNG. (Eds AK Haines, GC Williams and D Coates). (Australian Government Publishing Service Canberra)

Skewes T., Dennis D. and Burridge C.M. (2000) 'Survey of *Holothuria scabra* (sandfish) on Warrior Reef, Torres Strait, in January 2000.' CSIRO Division of Marine Research, Brisbane.

Skewes T., Dennis D., Wassenberg T.J., Austin M., Moeseneder C., Koutsoukos A., Haywood M., Pendrey R. and Bustamante R. (2002) 'Surveying the distribution and abundance of *Holothuria scabra* (sandfish) in Moreton Bay.' CSIRO Division of Marine Research Final Report, Brisbane.

Sommerville W.S. 1993. Marketing of bêche-de-mer. South Pacific Commission Bêche-de-mer Information Bulletin 5:2-4.

SYSTAT (1999) Programmer's Manual. In. (Systat Software, Inc: San Jose, CA, USA)

Taylor-Moore N. (1994) 'Bêche-de-mer fishery management arrangements.' Department of Primary Industries and Queensland Fish Management Authority, Brisbane, Queensland, Australia.

Tegner M.J. and Dayton P.K. 1999. Ecosystem effects of fishing. Trends in Ecology & Evolution 14(7):261-262.

Thayer C.W. 1979. Biological bulldozers and the evolution of marine benthic communities. Science 203:458-461.

Thompson B.A.W. and Riddle M.J. 2005. Bioturbation behaviour of the spatangoid urchin *Abatus ingens* in Antarctic marine sediments. Marine Ecology Progress Series 290:135-143.

Thrush S.F. and Dayton P.K. 2002. Disturbance to marine benthic habitats by trawling and dredging: implications for marine biodiversity. Annual Review of Ecology and Systematics 33(1):449-473.

Tuwo A. 1999. Reproductive cycle of the holothurian *Holothuria scabra* in Saugi Island, Spermonde Archipelago, Southwest Sulawesi, Indonesia. South Pacific Commission Bêche-de-mer Information Bulletin 11:9-12.

Udy J.W. and Dennison W.C. 1997. Growth and physiological response of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. Journal of Experimental Marine Biology and Ecology 217(2):253-277.

Uthicke S. 1997. Seasonality of asexual reproduction in *Holothuria* (*Halodeima*) *atra*, *H.* (*H.*) *edulis* and *Stichopus chloronotus* (Holothuroidea: Aspidochirotida) on the Great Barrier Reef. Marine Biology 129(3):435-441.

Uthicke S. 1999. Sediment bioturbation and impact of feeding activity of *Holothuria* (*Halodeima*) *atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. Bulletin of Marine Science 64(1):129-141.

Uthicke S. 2001a. Interactions between sediment-feeders and microalgae on coral reefs: grazing losses *versus* production enhancement. Marine Ecology Progress Series 210:125-138.

Uthicke S. 2001b. Nutrient regeneration by abundant coral reef holothurians. Journal of Experimental Marine Biology and Ecology 265:153-170.

Uthicke S. and Benzie J. (1998) 'Improving the conservation management of the commercial sea cucumber *Holothuria scabra* (sandfish).' Australian Institute of Marine Science, project no. WRCP-019, Townsville.

Uthicke S. and Karez R. 1999. Sediment patch selectivity in tropical sea cucumbers (Holothuroidea: Aspidochirotida) analysed with multiple choice experiments. Journal of Experimental Marine Biology and Ecology 236(1):69-87.

Uthicke S. and Klumpp D.W. 1998. Microphytobenthos community production at a near-shore coral reef: seasonal variation and response to ammonium recycled by holothurians. Marine Ecology Progress Series 169:1-11.

Uthicke S., Welch D. and Benzie J.A.H. 2004. Slow growth and lack of recovery in overfished holothurians on the Great Barrier Reef: Evidence from DNA fingerprints and repeated large-scale surveys. Conservation Biology 18(5):1395-1404.

van Eys S. 1986. The international market for sea cucumber. Infofish Marketing Digest 5:41-44.

Welsh D.T. 2003. It's a dirty job but someone has to do it: The role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column. Chemistry and Ecology 19(5):321-342.

Wiedemeyer W.L. (1992) Feeding behaviour of two tropical holothurians, *Holothuria* (*Metriatyla*) *scabra* (Jaeger 1833) and *H.* (*Halodeima*) *atra* (Jaeger 1833), from

Okinawa, Japan. In 'Proceedings of the 7th International Coral Reef Symposium'. Mangilao, Guam. (Ed. RH Richmond) pp. 853-860. (University of Guam Press)

Wolkenhauer S.-M. 2008. Burying and feeding activity of adult *Holothuria scabra* (Echinodermata: Holothuroidea) in a controlled environment. South Pacific Commission Bêche-de-mer Information Bulletin 27:25-28.

Yamanouchi T. 1939. Ecological and physiological studies on the holothurians in the coral reef of Palao Islands. Palao Tropical Biological Station Studies 1(4):603-634.

Yamanouchi T. 1956. The daily activity rhythms of the holothurians in the coral reef of Palao Island. Publications of the Seto Marine Biological Laboratory 5(3):45-60.

Yingst J.Y. 1976. The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. Journal of Experimental Marine Biology and Ecology 23:55-69.

Yingst J.Y. and Rhoads D.C. (1980) The role of bioturbation in the enhancement of microbial turnover rates in marine sediments. In 'Marine Benthic Dynamics'. (Eds KR Tenore and BC Coull) pp. 407–421. (University of South Carolina Press: Columbia)

Zieman J.C. 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* König. Aquaculture 4:139-143.