Element cycling on tropical coral reefs: The cryptic carbon shunt revealed

Het onderzoek gepresenteerd in dit proefschrift werd uitgevoerd met subsidie van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO).

Het drukwerk van dit proefschrift werd mede mogelijk gemaakt dankzij een financiële bijdrage van het Nederlands Instituut voor Onderzoek der Zee (NIOZ).

© Jasper M. de Goeij, Amsterdam, 2009

ISBN 978-90-367-3664-0
Ontwerp: Geurts & De Goeij Eijsden
Foto omslag (door P. Frade): Binnenwand van kunstmatige grot met grotorganismen
Druk: PrintPartners Ipskamp Enschede
Dit proefschrift werd gedrukt op papier met FSC keurmerk

RIJKSUNIVERSITEIT GRONINGEN

Element cycling on tropical coral reefs: The cryptic carbon shunt revealed

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde- en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 30 januari 2009
om 13.15 uur

door

Jasper Merijn de Goeij
geboren op 12 augustus 1977
te Leiderdorp

Promotor: Prof. dr. G.J. Herndl
Copromotor: Dr. F.C. van Duyl

Beoordelingscomissie:
Prof. dr. R.P.M. Bak
Prof. dr. P.M.J. Herman
PD Dr. C. Wild

Diving into an unknown world...

Contents

ABBREVIATIONS 9

CHAPTER 1 11
General introduction

CHAPTER 2 21
Objectives and outline

CHAPTER 3 29
Coral cavities are sinks of dissolved organic carbon

CHAPTER 4 55
Major bulk dissolved organic carbon removal by encrusting coral reef cavity sponges

CHAPTER 5 81
Tracing ^{13}C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM-feeding

CHAPTER 6 105
Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding

CHAPTER 7 127
Element cycling on tropical coral reefs: The cryptic carbon shunt revealed

CHAPTER 8 147
The cryptic carbon shunt, the sponge loop, and beyond…

REFERENCES 155

SUMMARY 171

SAMENVATTING 179

ACKNOWLEDGEMENTS 187

ABBREVIATIONS

AFDW	ash free dry weight
ASW	artificial seawater
BA	bacterial abundance
BC	bacterioplankton carbon
CW	cavity water
DIC	dissolved inorganic carbon
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphorus
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
DOP	dissolved organic phosphorus
DOM	dissolved organic matter
DTN	dissolved total nitrogen
DTP	dissolved total phosporus
FA	fatty acid
O_2	oxygen
POC	particulate organic carbon
PON	particulate organic nitrogen
POP	particulate organic phosphorus
RW	reef water
TOC	total organic carbon
TON	total organic nitrogen
TOP	total organic phosphorus
TSA	total cavity surface area

CHAPTER 1

General introduction

Coral reef ecosystems

"(…) every one must be struck with astonishment, when he first beholds one of these vast rings of coral-rock, often many leagues in diameter, here and there surmounted by a low verdant island with dazzling white shores, bathed on the outside by the foaming breakers of the ocean, and on the inside surrounding a calm expanse of water, which, from reflection, is of a bright but pale green colour. The naturalist will feel this astonishment more deeply after having examined the soft and almost gelatinous bodies of these apparently insignificant creatures, and when he knows that the solid reef increases only on the outer edge, which day and night is lashed by the breakers of an ocean never at rest."

Darwin, Charles R. (1842)

When viewing upon the underwater structures built of limestone and inhabited by numerous colourful creatures for the first time, one can understand, naturalist or not, the emotion Charles Darwin expressed after his first encounter with the coral reef ecosystem on his voyage with the Beagle. But the coral reef is not only astonishing to observe. From an ecological point of view, it is also one of the most productive ecosystems on earth. The impressive limestone constructions possess only a thin layer of living organic material. Hatcher (1997a) compared this bioactive layer (in terms of biomass) to "a large jar of peanut butter (…) spread over each meter of reef". Nonetheless, gross primary production (P) ranges from 2-40 g C m^{-2} d^{-1} (Kinsey 1983, 1985; Hatcher 1988), with a 'standard' reef flat P-value of 7 ± 1 g C m^{-2} d^{-1} (Kinsey 1983). These values are in the upper range of highest sustainable yields possible from (land) plants (Larkum 1983). Paradoxically, the tropical reef is surrounded by waters containing very low concentrations of nutrients (Crossland 1983), like an "oasis in the desert" (Odum 1971). For decades a major question to understand coral reef ecosystems has been: How can such a relatively highly productive ecosystem occur in these oligotrophic conditions? In order to answer this question it is important to be aware that the metabolic rate of the reef is determined by the balance between gross primary production (P) and community respiration (R). The biogeochemistry of a reef system is dominated by processes like photosyn-

thesis, calcification and community respiration, which are determined by the dynamics of nutrient fluxes (Kinsey 1985). For coral reef ecosystems a remarkable consistency exists between their estimated net (or excess) community productions (E, i.e. E = P - R), which is approximately zero. The net gain (or loss) of matter within the systems is, thus, very low (Hatcher 1990; Crossland et al. 1991). In fact, the net production by reefs and their surrounding waters indicates that the excess production by coral reefs is similar to that found in oligotrophic plankton communities in the oceans (Crossland et al. 1991). A highly productive, oligotrophic ecosystem has a high demand of nutrients, which is met by highly efficient systems of nutrient trapping, uptake and recycling. In addition, there is plenty of nutrient supply from the surrounding ocean. Although the oceanic waters hold very few nutrients per volume, an enormous volume of flowing water constantly replenishes the reef (Atkinson 1988).

The metabolic rate of a coral reef ecosystem is reflected in the balanced values of flows of consumption and excretion of elements or compounds by different communities within the reef. The main elements contributing to reef primary production, or the autotrophic part of the reef, are the zooxanthellae of symbiotic animals like corals, the benthic algae, the seagrasses, and the phytoplankton. Organic matter produced by this part of the reef is then incorporated in the heterotrophic food web, together with external sources of organic matter. The latter include trapping of plankton from the ocean by "a wall of mouths", represented by coral reef fish and suspension feeders (Hamner et al. 1988), or land-based sources like terrestrial run-off (Furnas et al. 2005).

The major part of the organic matter introduced into the food web - produced by benthic plants in coastal marine communities, and on reefs also by symbiotic animals (coral mucus) - is dead matter, or detritus (Alongi 1988). More than 80% of organic matter would pass via the "detritus chain", in the form of dissolved organic matter, organic aggregates (marine snow) and detritus (Sorokin 1993).

Dissolved organic matter in the coral reef waters

The total pool of organic matter consists of two main sub-pools: The particulate and the dissolved organic matter fraction. In the oligotrophic tropical waters, the particulate organic matter (POM) fraction consists mainly of phytoplankton and bacterioplankton (Ayukai 1995; Richter et al. 2001). The POM fraction represents less than 3% of the total organic matter pool (Benner 2002). The other 97% consists of dissolved organic matter, which is, in turn, the largest standing stock of carbon in the oceans (Martin and Fitzwater 1992). Technically, dissolved organic matter (DOM) does not have to be truly 'dissolved'. It is a fraction operationally defined as the organic carbon passing through a fine filter, typically GF/F, with a pore size of approximately 0.8 µm (Benner 2002; Carlson 2002). Carbon is the most common currency for analysis of matter, because it is the major component of all organic material.

In coral reef and lagoon waters the concentration of DOC is usually elevated as compared with DOC levels in surrounding ocean surface waters (Johannes 1967; Ducklow 1990; Torréton et al. 1997). This indicates that the production of DOC on the reef exceeds the losses (Van Duyl and Gast 2001). Sources of DOC are corals in the form of mucus (Richman 1975; Wild et al. 2004), or free amino acids (Schlichter and Liebezeit 1991). Algae also release DOC as products of photosynthesis (Mague et al. 1980; Zlotnik and Dubinsky 1989). The primary consumers of DOM are bacteria (Fenchel 1988), mediating the organic matter through the microbial loop (Azam et al. 1983).

DOC measurements in coral reef waters are scarce due to methodological constrains. Moreover, the informative value of data is limited since the largest part of DOC (70-80%) is an inert or refractory fraction, and only the smaller labile fraction is readily available for bacteria (Carlson 2002) and possibly other 'DOC-feeders' as well.

Benthic-pelagic coupling: Grazing on coral reefs

The importance of detritus as a stock of organic matter in reef trophodynamics was already stressed by the first investigators of this problem (Odum and Odum 1955). They pointed out the abundance of filter feeders in reef benthic communities and the dominance of the detrital mass in their guts. It is therefore remarkable that the role of the detritus pathways is still largely unknown. What we do know is that the microbial community plays an important role in the decomposition and recycling of dissolved organic matter in the oceans and on coral reefs through the so-called 'microbial loop' (Azam et al. 1983).

Communities of microorganisms form virtually isolated food webs in the water column over coral reefs (Ducklow 1990). Bacteria recycle organic matter efficiently, and as a result little of this bacterial production moves up to larger members of the reef food web. However, coral reefs are inhabited by an abundant fauna of filtering organisms. About half of the reef benthic animals are filter feeders, or suspension feeders. Initially, it was hypothesised that grazing on plankton by filter or suspension feeders was indeed a way to receive "new" nutrients on the reef, but mainly in the form of larger zooplankton (Glynn 1973). However, in addition to zooplankton and phytoplankton, these organisms can effectively filter bacterioplankton from the water column (DiSalvo 1971; Buss and Jackson 1981). Moreover, smaller-sized plankton (<10 μm) are the main component of plankton communities in coral reef waters (Ferrier-Pagès and Gattuso 1998). Organic matter can thus enter the reef food web via the microbial link, making bacterioplankton a key component in reef trophodynamics (Sorokin 1993).

Only recently the significance of capturing plankton, termed 'grazing', by the Great Barrier Reef of Australia has been quantified (Ayukai 1995). In a later study, Yahel and co-workers (1998), found a significant depletion of phytoplankton over the Red Sea reefs in the Gulf of Aqaba. The dominant grazers in marine benthic environments are bivalves, polychaetes, ascidians, and sponges (Riisgård and Larsen 1995). Due to their abundance and ability to filter huge volumes of water in time, they are considered to play a key role in the benthic-pelagic coupling in their ecosystems (Gili and Coma 1998). Plankton is a major source of nitrogen for the coral reef benthos (Ribes et al. 2003) and its removal is controlled by ascidians and sponges (Ribes et al. 2005).

A significant part of the particulate organic matter pool consists of bacterioplankton, although for a long time, not much was known on the distribution and function of these very small-sized (<2 µm) plankton particles. On the Caribbean reefs of Curaçao, the concentration of bacteria in the water column overlying the reef declines near the bottom (Gast et al. 1998). Moreover, water collected from coral reef framework crevices, formed by the three-dimensional structure of the reef showed an even further depletion in bacterial numbers (Gast et al. 1998). This work, together with earlier studies on the coral reef framework and its biota revealed a hitherto overlooked part of the coral reef ecosystem. This is very remarkable, since this part represents the largest habitat of the whole coral reef ecosystem and consequently could have a major impact on the element cycling and overall functioning of the whole ecosystem.

The reef framework

> "The variety of life on the surface of a coral reef holds one's attention so completely that it takes considerable experience to realize that most of the bulk volume of reefs is empty space. However, the cavities of coral reefs are by no means just empty space. They are an integral, perhaps even essential, element of the reef ecosystem."
>
> *Ginsburg, Robert, N. (1983)*

Even two decades later, one of the few papers written on the coral framework started with the following sentence: "Framework cavities are the largest, but least explored coral reef habitat" (Richter et al. 2001), referring to the work of Ginsburg cited at the opening of this chapter. This is not without reason, since the reef framework consists of highly complex and highly variable and irregular three-dimensional structures of cracks, holes, overhangs, cavities, and caves, which makes it indisputably difficult to study. However, it does not imply that information on the cryptic habitat is absent. Garrett and co-workers (1971) described the complex and irregular cavity systems and how these resulted from the growth forms of massive corals. Cavities can also be formed by roofing of spaces between coral colonies and by curtain-like growth of individual colonies (Jackson et al. 1971). Large caves and tunnels (up to five meters high

and ten meters long) are formed by combination of vertical and horizontal growth - in mushroom shapes - of corals (Zankl and Schroeder 1972). Generally, the cryptic habitat encompasses all the interstitial spaces of the reef, ranging from size classes of just a few microns (the pores of the sediment), to centimeters (tubular intra-skeletal cavities made by worms and boring sponges), to meters (framework cavities and (sub) marine caves). The volume of the framework has been estimated to be more than three-quarters (75-90%) of the total volume of the reef (Ginsburg 1983). In terms of surface area, the cavity walls show a large internal surface, estimated to be up to eight times the surface of the projected planar reef (Richter et al. 2001; Scheffers et al. 2004).

Cavity organisms or 'coelobites'

The cavity walls are inhabited by a thin veneer of shade-loving organisms like sponges, coralline algae, bivalves, and foraminifera, only a few millimeters in thickness (Garett et al. 1971). These organisms are referred to as 'cryptofauna' (Peyrot-Clausade 1974) or 'coelobites' (Ginsburg and Schroeder 1973), and the numerous (and still incomplete list of) species have been subject to many studies (e.g. Vasseur 1977; Buss and Jackson 1979; Jackson and Winston 1982; Logan et al. 1984; Meesters et al. 1991; Wunsch et al. 2000).

In addition, the cavity floor is covered with coral sand containing sediment dwellers like crustaceans, worms and, mostly, bacteria (DiSalvo 1971; Ginsburg 1983). The biomass of the cryptofauna might even exceed that of the reef surface (Hutchings 1974). The abundance of coelobites is extremely high, covering on average 93-99% of the cavity walls (Jackson et al. 1971; Richter and Wunsch 1999; Scheffers et al. 2004). Due to this abundance there is a high competition for space and resources within the cavity system (Buss 1979). Only a small fraction of the cryptic habitat receives sufficient light for photosynthesis. Therefore, most coelobites are heterotrophic filter or suspension feeders, depending on organic matter from outside the framework (Ginsburg 1983).

BIOGEOCHEMICAL CYCLING IN THE REEF FRAMEWORK

The large area of the cryptic habitat, together with the abundance of cryptic organisms provides a potentially important interface in the exchange of materials between the framework of the reef and the overlying water column. Several studies showed a depletion of particulate organic matter in the form of bacterioplankton (Gast et al. 1998; Scheffers et al 2004), and phytoplankton (Richter et al. 2001), in the reef framework as compared with the overlying water column. Moreover, the cryptic habitat shows signs of respiration, like oxygen depletion (DiSalvo 1971) and dissolved inorganic carbon (DIC) release (Tribble et al. 1988). Also, the framework is a source of inorganic nutrients (DiSalvo 1974; Andrews and Müller 1983; Tribble 1988; Gast et al. 1998; Rasheed et al. 2002). It is assumed that the depletion in organic carbon and the elevated inorganic nutrient concentrations are due to extensive decomposition and mineralisation of organic matter by the cryptic organisms and the reef sediment (Richter et al. 2001; Rasheed et al. 2002; Scheffers et al. 2004).

CHAPTER 2

Objectives and outline

Thesis objectives

Coral cavities are among the largest and least explored habitats in coral reef environments. Hardly anything is known about the ecological role of this cryptic habitat in the carbon cycling on the reef. The large area of the cryptic habitat and the high degree of cover by encrusting organisms provide a potentially important interface in the exchange of material between the cavities and the overlying water column. At the level of the ecosystem, coral cavities are significant sinks of phytoplankton (Richter et al. 2001) and bacterioplankton (Gast et al. 1998), making up the major part of the particulate organic carbon (POC) in tropical reef waters. However, by far the largest component (>97%) of organic matter is dissolved organic matter (DOM) (Benner 2002), which, in turn, is the largest carbon standing stock in the oceans (Martin and Fitzwater 1992). DOC may also be a potential carbon source for the cavity and its marine benthic invertebrates but this has never been addressed in this respect. The cavity biota consists predominantly of sponges.

The goals of the PhD project which forms the basis of this thesis were to determine the role of DOC as a carbon source for coral cavities, and to link the fluxes of carbon and nutrients to the activity of the surface-dominating cavity communities, in particular the cavity sponges. An additional aim was to study the hypothesised crucial role and function for the cryptic habitat in the carbon and nutrient cycling within the coral reef ecosystem. To achieve these goals, several steps were taken to unravel this puzzle.

Thesis outline

Chapter 3 - Coral cavities are sinks of dissolved organic carbon

The potential importance of DOC in the carbon budgets of coral cavities and for the cryptofauna has been discussed (Richter et al. 2001; Yahel et al. 2003; Van Duyl et al. 2006), but has yet not been determined.

In this study our main questions are: Are coral cavities sinks of DOC? And if so, is DOC a quantitative important source of carbon for coral cavities? We studied the removal of DOC by coral cavities of 50-250 dm^3 at a depth range of 5-17 m along the coral reefs of Curaçao, Netherlands Antilles,

and the Berau area, East Kalimantan, Indonesia. We compared the concentration of DOC and bacterial abundance between cavity water and overlying reef water in 19 cavities on the fringing reefs of Curaçao and 21 cavities on different types of reefs in the Berau area, East Kalimantan, Indonesia. To determine DOC uptake rates, we closed cavities in both areas and followed the removal of DOC over time and compared that with the removal of bacterioplankton carbon (BC). We estimated fluxes of DOC and BC for the cryptic habitat. The biodegradability of DOC was determined in a series of bioassays with ambient reef water bacterioplankton as key DOC consumers.

Chapter 4 - Major bulk dissolved organic carbon removal by encrusting coral reef cavity sponges

Sponges, which represent the dominating cryptic community, are very efficient suspension feeders, feeding on a wide variety of types and sizes of plankton. They prefer particles smaller than 2 μm (Pile et al. 1996, Ribes et al. 1999, Kötter and Pernthaler 2002), but the qualitative and quantitative role of dissolved organic matter (DOM) as food source for sponges is still debated. Generally, only sponges containing associated bacteria are assumed to be capable of utilising DOC (Frost 1987, Ribes et al. 1999). Yahel et al. (2003) were the first to show that DOC intake by the sponge *Theonella swinhoei* accounted for more than 90% of the total organic carbon intake.

In this study we investigated the role of three encrusting coral cavity sponge species, *Halisarca caerulea, Mycale microsigmatosa* and *Merlia normani*, in the removal of DOC and bacterioplankton carbon. The concentrations of DOC and BC were monitored *in situ*, using incubation chambers. For *H. caerulea*, the fate of organic carbon was further studied by measuring dissolved oxygen consumption and dissolved inorganic carbon release.

Chapter 5 - Tracing ^{13}C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM-feeding

In incubation experiments *in situ*, ~40% of the removed DOC by the sponge *Halisarca caerulea* was found to be respired, whereas ~60% of the carbon is assimilated (De Goeij et al. 2008a). However, virtually no data are avail-

able on the actual utilisation, partitioning, and possible translocation of natural diets, like DOM or particulate food sources, between sponge cells and sponge-associated bacteria. Fatty acid biomarkers have been repeatedly used as source-specific (i.e. prokaryotic or eukaryotic specific fatty acids) indicators of dissolved and particulate organic matter both in environmental and food web studies (e.g. Canuel et al. 1995; Hall et al. 2006).

In this study, the uptake and processing, or carbon flow pathways, of DOC as compared with particulate sources of nutrition in sponges were aimed to be unraveled. We examined the assimilation and respiration of stable carbon isotope-enriched substrates by the sponge *Halisarca caerulea*. The assimilation and respiration of the ^{13}C-enriched substrates glucose, algal derived dissolved and particulate organic matter (diatom-DOM and -POM), and bacteria was followed in 1 and 6 h incubations. In addition, we followed ^{13}C-enrichment in fatty acid biomarkers to elucidate sponge-associated-bacteria-mediated versus direct sponge uptake of DOM, glucose, POM, and bacterioplankton for *Halisarca caerulea.*

Chapter 6 - Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding

For the cavity sponge *Halisarca caerulea* a major discrepancy was found between the high amount of organic carbon assimilated by the sponge (35-40% body C d^{-1}) and the low net increase of biomass by the sponge (close to zero). This organic carbon may be used for reproduction activities or the production of secondary metabolites, but may also point to a high turnover of matter. Moreover, sponges have a high plasticity, or regeneration capacity, with up to 2,900 times the normal growth rate after tissue damage (Ayling 1983) and a high telomerase activity (Koziol et al. 1998). Both features imply the potential for a relatively rapid cell proliferation. Since sponges have an integrated multicellular organisation they should have control mechanisms for rates of cell proliferation and cell death to maintain homeostasis in the various cell populations. Sponges are considered to be the oldest, still existing metazoan phylum and to function as an important link between a unicellular and multicellular way of living (Müller 1998). Sponges ought to have an appropriate functional organisation for the processing of enormous amounts of nutrients from the water column. However, the required struc-

tural and functional characteristics of the sponge, such as the gain and loss of different cell types and compartmentalization in the sponge anatomy, are largely unknown.

The major discrepancy of organic carbon uptake in relation to growth found in *H. caerulea* and, subsequently, the lack of knowledge on *in vivo* growth characteristics of sponges, prompted us to investigate the process of cell turnover in this sponge. To investigate the cell kinetics in *H. caerulea* we labelled the sponge tissue in vivo with 5-bromo-2'-deoxyuridine (BrdU) and analysed the cell kinetic parameters using immunohistochemical staining of BrdU in tissue sections. In order to study cell loss we determined *in vivo* apoptosis by immunohistochemical staining of sponge tissue using an antibody against active Caspase-3. Furthermore, general and immunohistochemical staining methods were used to gain more insight in the structural organisation and cell turnover in sponge tissue.

Chapter 7 - Element cycling on tropical coral reefs: The cryptic carbon shunt revealed.

The Chapters 3-6 will evidently lead to a concluding chapter for the coral reef framework ecosystem. And the crucial question: "who 's done it?" has been posed many times, but has yet to be answered. The conventional and well-established view on the functioning of coral cavities is that these are the key regenerative spaces and hot spots of mineralization on coral reefs, processing particulate organic matter and releasing inorganic nutrients (Tribble et al. 1988; Richter et al. 2001; Van Duyl et al. 2006). The removal of organic matter and the release of inorganic nutrients by the coral reef framework have always eagerly been ascribed to decomposition and remineralization processes of the cryptofauna (Richter and Wunsch 1999; Richter et al. 2001; Van Duyl et al. 2001; Scheffers et al. 2004, 2005; Van Duyl et al. 2006). However, at the community levels (i.e. the dominant coelobites groups) information on carbon and nutrient fluxes is scarce. In fact, to date, no data is available on the turnover rates of cryptofaunal communities in this regenerative space. Because of the fierce competition within the cryptic habitat, it is suggested to be rapid (Hutchings 1983). Cavity sponges have been shown to remove substantial amounts of organic carbon from the overlying water column (De Goeij et al. 2008a). However, as previously described, an average coral cavity is made up of four surface dominating

communtities, accounting together for almost 90% of the total surface area (TSA) (Scheffers 2005; Van Duyl et al. 2006). According to cover percentage of the TSA these communities include: (1) The coral sandy bottom or 'sediment', (2) the sponges, (3) the coralline algae, and (4) the uncovered coral rock or 'bare substrate'.

In this study, we examined the role of the framework cavities in the carbon and nutrient cycling on the coral reef ecosystem by quantifying net fluxes of organic and inorganic matter. Furthermore, the relative importance of the four dominant cavity communities in framework nutrient cycling was assessed. We constructed mass balances for C, N, and P for coral cavities and the sponge and sediment communities to unravel the functioning of cavities and the cryptic communities.

Chapter 8 - The cryptic carbon shunt, the sponge loop, and beyond...

This chapter continues with a broader discusson of some of the major results from the previous chapters and by giving some considerations, implications, and ideas for future work.

To conclude, I hope to have encouraged you to read on, and to have interested you for the beautiful, complex and ingenious coral reef ecosystem. Especially the largely overlooked and underestimated coral reef framework cavities deserve some attention by the reader. I have tried to answer or explain some of the many questions raised. What I hope to have achieved even more is to have raised many more questions.

CHAPTER 3

Coral cavities are sinks of dissolved organic carbon

Abstract

We studied the removal of dissolved organic carbon (DOC) by coral cavities of 50-250 dm^3 at a depth range of 5-17 m along the coral reefs of Curaçao, Netherlands Antilles, and the Berau area, East Kalimantan, Indonesia. We found significantly lower DOC concentrations in cavity water compared with ambient reef water. On average, DOC concentrations in cavity water were 15.1 ± 6.0 µmol L^{-1} (Curaçao) and 4.0 ± 2.4 µmol L^{-1} (Berau) lower than in reef water. When the cavities were closed, DOC concentrations in the cavities declined by 22 ± 8% and 11 ± 4% in Curaçao and Berau, respectively, within 30 min. This corresponded to average DOC removal rates per cavity surface area of 342 ± 82 mmol C m^{-2} d^{-1} in Curaçao and 90 ± 45 mmol C m^{-2} d^{-1} in Berau. Bioassays showed that bacterioplankton are not responsible for this DOC removal by coral cavities. DOC fluxes exceeded bacterioplankton carbon (BC) fluxes into cavities by two orders of magnitude. On average, BC fluxes per cavity surface area were 3.6 ± 1.3 mmol C m^{-2} d^{-1} (Curaçao) and 1.9 ± 1.3 mmol C m^{-2} d^{-1} (Berau area). The net DOC removal per square meter of cryptic surface likely exceeded the gross primary production per square meter of planar reef area. We conclude that coral cavities and their biota are net sinks of DOC and play an important role in the energy budget of coral reefs.

Introduction

Coral cavities are among the largest and least-known habitats in coral reef environments. Their total volume comprises up to two-thirds of the reef volume (Garret et al. 1971; Ginsburg 1983) and their inner surface represents 60-75% of the total available surface of the reef (e.g. Jackson et al. 1971; Logan et al. 1984; Scheffers 2005). Yet, hardly anything is known about the ecological role of this cryptic habitat in the carbon cycling on the reef.

The relatively sheltered cryptic habitat is inhabited by a high abundance of different organisms, called cavity dwellers or coelobites (Ginsburg and Schroeder 1973). The biomass of this cryptofaunal community might exceed that of the reef surface (Hutchings 1974; Brock and Brock 1977; Meesters et al. 1991) and the encrusting biota can cover more than 93% of the available hard substrate (Richter and Wunsch 1999; Richter et al. 2001; Scheffers 2005). As a consequence, the competition for space is high in coral cavities (Jackson et al. 1971; Buss 1979; Buss and Jackson 1979). Heterotrophic organisms generally dominate the coelobite community due to low light conditions in the cavities. Two-thirds of the cavity walls are inhabited by suspension feeders (sponges, tunicates, bryozoans, bivalves, and polychaetes), with sponges usually dominating this group. Approximately one-third of the cavity walls consists of calcareous algae (e.g. Vasseur 1974; Gili and Coma 1998; Wunsch et al. 2002).

The large area of the cryptic habitat and the high cover of encrusting organisms provide a potentially important interface in the exchange of material between the cavities and the overlying water column. Several studies showed a depletion of phyto-, nano-, pico-, and bacterioplankton in waters overlying coral reefs (e.g. Ayukai 1995; Yahel et al. 1998; Van Duyl et al. 2002). Gast et al. (1998) were the first to describe bacterioplankton depletion and accumulation of inorganic nutrients in coral crevices of Curaçao compared with overlying reef water. On the reefs along Curaçao, bacterial abundance was usually lower inside cavities than outside and the inorganic nutrient concentrations often differed from that in overlying water. Scheffers et al. (2004) and Van Duyl et al. (2006) reported bacterial removal rates by coral cavities of Curaçao of, on average, 3 mmol C m^{-2} d^{-1}. A net influx of chlorophyll *a* was found in framework cavities in the Red Sea at an estimated phytoplankton removal rate in cavities of 75 mmol C m^{-2} d^{-1} (Richter et al. 2001).

Phytoplankton and bacterioplankton make up the major part of the particulate organic matter (POM; *see* List of Abbreviations) in the oligotrophic reef waters. However, by far the largest component (>97%) of organic matter is dissolved organic matter (DOM) (Benner 2002), which, in turn, is the largest carbon standing stock in the oceans (Martin and Fitzwater 1992). This fraction is operationally defined as the organic carbon passing through a fine filter, typically GF/F (Benner 2002; Carlson 2002). Dissolved organic carbon (DOC) is composed of a small labile and a much larger refractory fraction, which is not readily available to bacteria (Carlson 2002). DOC levels are usually enhanced over coral reefs and in lagoons compared to ocean surface waters (e.g. Johannes 1967; Ducklow 1990; Torréton et al. 1997), indicating that in reef waters, the production of DOC exceeds its loss (Van Duyl and Gast 2001). Sources of DOC in coral reefs are, for example, the release of DOC by benthic algae as a function of photosynthesis (Mague et al. 1980; Zlotnik and Dubinsky 1989), and release of DOC by corals through mucus production (Johannes 1967; Richman et al. 1975) or as free amino acids (Schlichter and Liebezeit 1991). The main consumers of DOC are heterotrophic bacteria (Fenchel 1988) mediating the flux of DOC through the microbial loop (Azam et al. 1983).

However, DOC may also be a potential food source for marine benthic invertebrates, as has been suggested already since the end of the nineteenth century (reviewed by Jørgensen 1976). Reiswig (1981) found a discrepancy between the supply and demand of carbon in benthic suspension feeders. However, although DOC has been suggested as the missing carbon (Reiswig 1981; Gili and Coma 1998), not much is known on the uptake of DOC by the benthic community. Yahel et al. (2003) were the first to show extensive feeding on bulk DOC by the sponge *Theonella swinhoei.* Although the potential importance of DOC in the carbon budgets of coral cavities and for the cryptofauna has been discussed (Richter et al. 2001; Yahel et al. 2003; Van Duyl et al. 2006), DOC removal by the crytofauna has not been determined.

Our main question in this study is: Are coral reef cavities net sinks of DOC? If so, is DOC quantitatively an important food source for cryptic coral reef habitats? To answer these questions, we carried out a series of measurements and experiments. We compared the concentration of DOC and bacterial abundance between cavity water and overlying reef water in 19 cavities on the fringing reefs of Curaçao and 21 cavities on different types of reefs in the Berau area, East Kalimantan, Indonesia. To determine DOC uptake rates, we closed cavities in both

areas and followed the removal of DOC over time and compared that with the removal of bacterioplankton carbon (BC). We estimated fluxes of DOC and BC for the cryptic habitat. The biodegradability of DOC was determined in a series of bioassays with ambient reef water bacterioplankton as key DOC consumers.

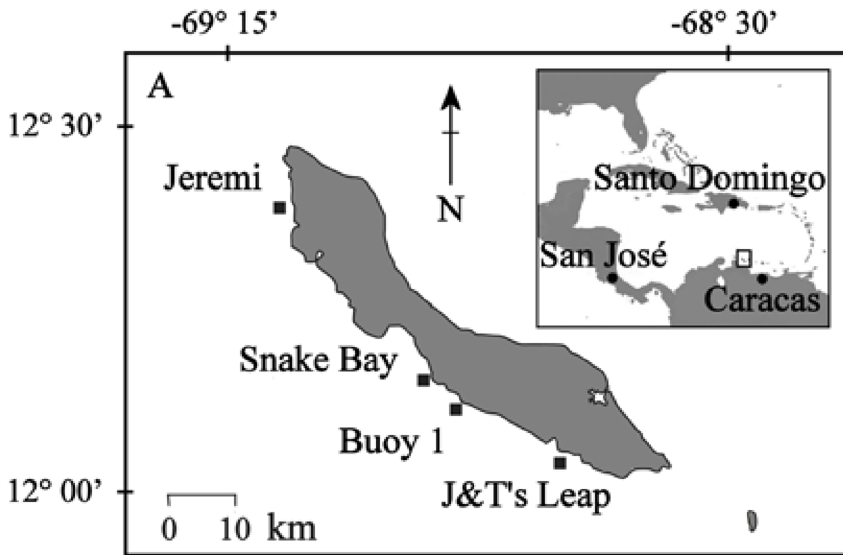
Materials and methods

Study area and sites

This study was conducted on the Caribean island Curaçao, Netherlands Antilles (12°12'N, 68°56'W) in the Caribbean (*Fig. 3.1A*) and in the Berau area along the east coast of Kalimantan, Indonesia (2°15'N, 118°15'E) (*Fig. 3.1B*). Most of the experimental work was performed at the fringing reefs along the leeward side of Curaçao. For station selection, the long-term mean current was taken into account running from SE to NW along the island. Cavities (two per site) were selected at four sites: J&T's Leap, Buoy 1, Snake Bay, and Jeremi, along the SW coast of Curaçao (*Fig. 3.1A; Table 3.1*). Cavity Buoy 1.2 is an artificial cavity constructed with a steel skeleton in August 2004. It was covered with concrete plates and a coral cement top layer. The cavity was placed in the Buoy 1 area on 08 September 2004. Station J&T's Leap is upcurrent from the area around the city of Willemstad. Buoy 1, Snake Bay, and Jeremi are all downcurrent from Willemstad. Cavities were located in the fore-reef slope between 10 and 15 m water depth, close to the drop-off. In Berau, 21 cavities, in a water depth range of 5-17 m, were selected. Cavity volumes ranged from 50-250 dm^3, where a sandy bottom made up approximately one-third of the total cavity surface area (TSA) and the remainder consisted of cavity wall. The framework of the Berau reefs was more porous than the reefs of Curaçao, and cavities showed more openings to the outside reef than cavities at Curaçao. Samples were collected by SCUBA diving.

Sample collection

DOC concentration and BA in cavity water and reef water: To compare the concentrations of dissolved organic carbon (DOC) and bacterial abundance (BA) between cavity water and overlying reef water we defined two types of water. Cavity water (CW) was sampled from the middle of the cavity and reef water (RW) ~1 m from the opening of the cavity. For each cavity, a RW sample was



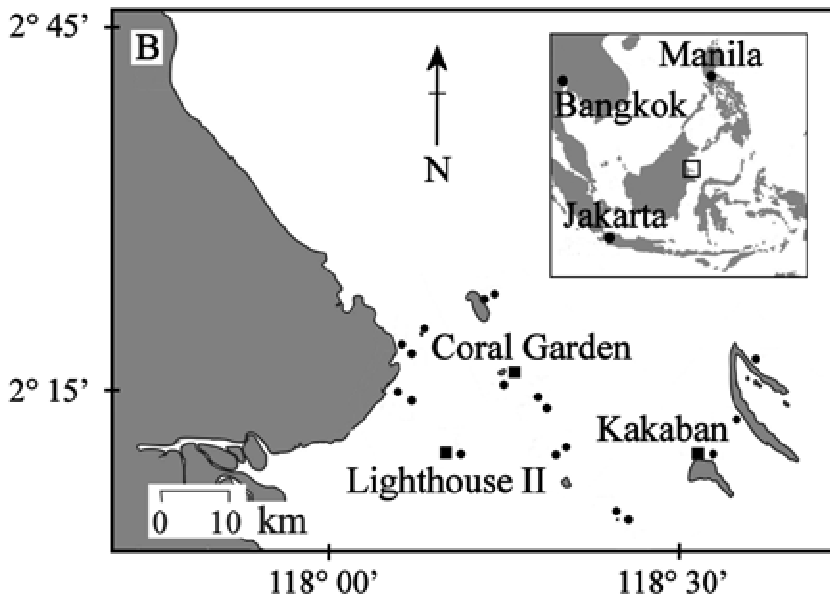


Fig. 3.1A - Map of Curaçao, locating four sites along the SW coast, with an inset of the location of Curaçao in the Caribbean Sea.

Fig. 3.1B - Map of Berau, East Kalimantan, Indonesia, with an inset of the location of Berau in Indonesia. The sampled area consists of 21 sites, of which three sites (Lighthouse II, Coral Garden and Kakaban) were used in cavity closure experiments.

taken first, against the current to avoid contamination of samples, and then a CW sample was taken. On Curaçao, 20 cavities were sampled along the house reef of the CARMABI Foundation (Buoy 1) in August 2003 (*Fig. 3.1A*). Samples for DOC and BA were taken between 09:00 h and 10:00 h local time with an acid-washed 750 mL polycarbonate syringe. The water was collected in acid-washed glass bottles, stored in the dark at 4°C prior to further processing in the lab within 4 h. In Berau, 21 cavities in the Berau area were sampled in October 2003. 100 mL polypropylene syringes were used for sampling in the morning and afternoon around slack tide to avoid strong currents. Morning samples were processed on the boat. Afternoon samples were kept in the 100-mL syringes in the dark at 4°C prior to further processing within 4 h.

DOC and BC removal rates: To study *in situ* net fluxes of DOC and bacterioplankton carbon (BC) in coral cavities, water exchange was restricted by closing the cavity with a tightly woven cloth (Scheffers et al. 2004). Water samples were taken at 0, 5, 10, 15, 30, and 60 min after closing the cavities by an acid-washed silicon tube and 100-mL polypropylene syringes. Closure experiments were performed over the course of four fieldwork periods, in July-August 2003, March-August 2004, March-May 2005, and January-April 2006. Berau cavity close-off experiments were carried out at three stations (Lighthouse II, Coral Garden and Kakaban; *Fig. 3.1B; Table 3.1*) in October 2003. Volume and TSA (i.e. the sum of the surface of the inner walls and surface of the sandy bottom combined; *Table 3.1*) of the cavities along Curaçao were determined with the Cave-Profiler, a tool to measure the three-dimensional structure of cavities (Scheffers et al. 2003). In Berau, cavity volumes were estimated on basis of depth, width, and height measurements of cavities. The TSA was derived from the empirical linear relation between cavity volume (CV, in a size range of 50-250 dm^3) and TSA, given by the following equation:

$$TSA = 0.0108 \times CV + 0.460 \qquad (1)$$

($R^2 = 0.943$; $F = 82.676$; df = 5; $p < 0.001$). In Curaçao, samples for DOC and BA were taken between 10:00 and 12:00 h. Water was collected in acid-washed glass bottles, stored in the dark at 4°C prior to further processing in the lab within 4 h. In Berau, samples for total organic carbon (TOC) and BA were taken both in the morning and the afternoon. Samples of the morning session were directly processed on the boat. Water collected in the afternoon was kept

in the 100 mL syringes in the dark at 4ºC, prior to further processing in the lab within 4 h.

Bio-assays: Bioavailability of DOC for ambient reef water bacterioplankton was determined by a series of bioassays. Water used in the bioassay was collected in August 2003 at the station Buoy 1 at 15 m water depth in front of coral cavities. Aliquots of seawater (1.5 L) were gently filtered through 0.8 μm polycarbonate filters (Millipore, 47 mm) to remove grazers and incubated in acid-washed glass bottles in the dark at *in situ* temperature of 26°C (n = 6). Duplicate samples for bacterial abundance and DOC were taken at t = 0, 5, 10, 20, and 30 d. To keep the system C-limited, inorganic N and P (10 μmol L^{-1} N as $NaNO_3$ and 1 μmol L^{-1} P as K_2HPO_4) were added at day 1.

Table 3.1 - Volume, surface area, and in situ *depth of seven coral cavities in Curaçao, Netherlands Antilles, and three coral cavities in Berau, East-Kalimantan, Indonesia.*

Cavity	Depth (m)	Volume (dm^3)	Wall surface (m^2)	Floor surface (m^2)	Total surface (m^2)
Curaçao					
Buoy 1.1	15.2	111	1.0	0.7	1.7
Buovy 1.2†	15.0	250	1.7	0.8	2.4
Jeremi 1	16.8	175	1.3	1.0	2.3
Jeremi 2	16.3	86	0.5	0.8	1.3
Snake Bay 1	15.8	248	1.8	1.3	3.1
J&T's Leap 1	12.7	194	1.1	1.8	2.9
J&T's Leap 2	16.1	159	0.9	1.4	2.3
Berau					
Coral Garden	12.7	50			1.00
Lighthouse II	9.6	150			2.08
Kakaban	15.8	200			2.62

†Buoy 1.2 is an artificial cave.

Sample treatment and analysis

Water samples were processed in the laboratory, prior to transportation to the Netherlands for analyses. Samples for bacterial abundance (10 mL) were stained with acridine orange and filtered (max. 20 kPa suction pressure) onto 0.2 μm black polycarbonate membrane filters (Millipore, 25 mm), mounted on slides and stored at -20°C. Bacterial numbers were counted using an epifluorescence microscope (×1,250). Per slide, 10 fields were counted or up to a minimum of 200 bacteria. In Berau, bacterial abundance was determined with a flow cytometer (FCM, Beckton Dickinson FACScalibur equipped with a 15 mW, 488 nm air cooled argon-ion laser) as described by Marie et al. (1999) with the modification of using 0.2 μm filtered glutaraldehyde as a fixative (20 μL 25% glutaraldehyde per sample, EM grade, Merck). In the field, duplicate 1 mL samples were collected in cryovials (Greiner), fixed and kept at 4°C before shock-freezing and storage in liquid nitrogen. To convert bacterial abundance to carbon biomass a conversion factor of 30 fg per bacterial cell was used (Fukuda et al. 1998). Bacterioplankton carbon removal rates in closed coral cavities were calculated assuming exponential clearance of prey in a closed system with homogenous mixed water (Scheffers et al. 2004).

Samples for DOC analysis were gently (max. 20 kPa Hg suction pressure) filtered through a 0.2 μm polycarbonate filter (Millipore, 47 mm). Prior to filtration, filters, glassware and pipette tips were washed with acid (3×10 mL 0.4 M HCl), 0.2 μm filtered double distilled water (3×10 mL) and sample water (3×10 mL). Duplicate 8 mL DOC samples were collected in precombusted (4 h 450°C) glass ampoules. Ampoules were sealed immediately after acidification with 1-2 drops of concentrated H_3PO_4 (80%) and stored at 4°C until analysis. In Berau, TOC instead of DOC concentration was measured. Measurements of DOC and TOC were performed by the high-temperature combustion method, using a TOC analyser (Model TOC-5000A, Shimadzu). The TOC analyser was calibrated with potassium phthalate in Milli-Q water. As an internal control of the DOC measurements, consensus reference material provided by Hansell and Chen of the University of Miami, USA (Batch 4, 2004) was used.

TOC is composed of DOC and particulate organic carbon (POC). POC in tropical reef water consists mainly of phytoplankton and bacterioplankton. The phytoplankton carbon retention rates by reefs are comparable with (Ayukai 1995), or, in coral cavities, even lower than (Kötter and Pernthaler 2002) bacterioplankton carbon (BC) retention rates. Therefore, we assumed,

for conservancy, that bacterioplankton carbon and phytoplankton carbon retention rates were equal. DOC removal rates (RR) for the Berau area was calculated as:

$$DOC_{RR} = TOC_{RR} - 2 \times BC_{RR} \quad (2)$$

DOC concentrations for RW and CW in the Berau area were calculated as:

$$DOC_{RW} = TOC_{RW} - 2 \times BC_{RW}, \text{ and} \quad (3)$$

$$DOC_{CW} = TOC_{CW} - 2 \times BC_{CW} \quad (4)$$

Estimation of DOC removal rates

The removal rates of DOC in closed cavities were determined with a 2G-model. DOC represents a very heterogenic group of organic compounds, both in size fractions, chemical composition as in bioavailability or biodegradability. The degradation of carbon is often described as a first-order process, in which velocity of degradation is given as the product of a reaction constant and the actual carbon concentration, or:

$$\frac{dC}{dt} = -kC \quad (5)$$

This equation assumes that only one carbon fraction has a constant biodegradation. Application of this model to experimental data often gives a poor description or trend, while in fact there are multiple fractions with different degradation constants and with different concentrations within the total DOC pool. A simplified model to describe the course of carbon in time assumes that the DOC pool is composed of two major fractions, a fast removable fraction, $C_{f,}$ and a slow removable fraction, C_s. These two fractions will be consumed according to characteristic removal constants, k_f and k_s. The total DOC removal will then be described as the sum of all individual removal rates, or:

$$\frac{dDOC}{dt} = -(k_f C_f + k_s C_s) \quad (6)$$

By integration of Eq. 2 in reference to time, *t*, we arrive at the equation describing the concentration of DOC as a function of time:

$$DOC(t) = C_{f,0} e^{-k_f t} + C_{s,0} e^{-k_s t} \quad (7)$$

Using a minimalisation routine, the experimental data can be described with the model by estimating the model variables $C_{f,0}$, k_f, $C_{s,0}$, and k_s. The initial uptake rate of DOC (the flux on $t = 0$) was calculated from the estimated values of these variables and is given by:

$$Flux_{DOC} = -(k_f C_{f,0} + k_s C_{s,0}) \tag{8}$$

The computed fluxes are the expected DOC fluxes in the field. For our closure experiments, we assume a well-mixed closed system without exchange with overlying reef water.

Results

The concentrations of DOC in the cavity waters (70.1 ± 5.4 µmol L^{-1}) at the Curaçao sampling sites were significantly lower than in the overlying reef water (84.9 ± 9.1 µmol L^{-1}; paired *t*-test: t = -4.863, df = 18; $p < 0.0005$; *Table 3.2*). In 15 out of 19 different cavities on Curaçao, the DOC concentrations were lower inside the cavity than outside. Out of the remaining four measurements, three showed no difference in DOC concentration (<3 µmol L^{-1}) between CW and RW. In one cavity, the concentration of DOC was higher than in RW. The average difference in DOC concentration between RW and CW was 14.8 ± 6.0 µmol L^{-1}. This corresponded to a 15.2 ± 6.8% lower DOC concentration in the coral cavities on Curaçao.

The cavities in Berau were characterised by a significantly lower concentration of DOC in CW (67.0 ± 7.1 µmol L^{-1}) than in RW (71.0 ± 7.2 µmol L^{-1}; paired *t*-test: t = -3.845, df = 20; $p < 0.005$; *Table 3.2*). In Berau, 16 out of 21 cavities showed a lower concentration of DOC in CW as compared to RW. There was no difference in four cavities (<3 µmol L^{-1}) and in one cavity the concentration DOC in CW was higher than RW. On average, difference in DOC concentrations in CW were 4.0 ± 2.4 µmol L^{-1} or 5.7 ± 3.7% lower than in RW.

The bacterial abundance was always significantly lower in CW than in RW, in cavities on Curaçao (paired *t*-test: t = -4.364, df = 19; $p < 0.0005$) and Berau (paired *t*-test: t = -3.3845, df = 20; $p < 0.005$; *Table 3.2*). The average bacterial abundance in RW in Curaçao was 10.5 ± 3.4×10^5 cm^{-3}, compared to an average CW bacterial abundance of 7.2 ± 1.6×10^5 cm^{-3}. Bacterial numbers were on average 27.9 ± 16% (Curaçao) and 38.0 ± 16.8% (Berau) lower in CW compared with RW. The average bacterioplankton carbon concentration in RW of Berau was 2.2 ± 0.5 µmol L^{-1}, or 3% of the TOC pool. TOC consisted for 94% of DOC.

Table 3.2 - Overview of average concentrations of dissolved organic carbon (DOC) and bacterial abundances in reef water (RW) and cavity water (CW), for coral cavities in Curaçao, Netherlands Antilles, and coral cavities in Berau, East-Kalimantan, Indonesia (average ± SD).

DOC	Average RW (μmol L^{-1})	Average CW (μmol L^{-1})	Average difference (μmol)	Relative difference (%)
Curaçao (n = 19)	84.9 ± 9.1	70.1 ± 5.4	14.8 ± 6.0	15.2 ± 6.8
Berau area* (n = 21)	71.0 ± 7.2	67.0 ± 7.1	4.0 ± 2.4	5.7 ± 3.7
Bacterial abundance	**Average RW (10^5 cm^{-3})**	**Average CW (10^5 cm^{-3})**	**Average difference (10^5 cm^{-3})**	**Relative difference (%)**
Curaçao (n = 20)	10.5 ± 3.4	7.2 ± 1.6	3.3 ± 2.8	27.9 ± 16.7
Berau (n = 21)	8.6 ± 1.9	5.5 ± 2.1	3.2 ± 1.3	38.0 ± 16.8

*calculated from TOC

The DOC (Curaçao; paired *t*-test: $t = -9.852$, df = 22; $p < 0.001$), and TOC (Berau area; paired *t*-test: $t = -5.908$, df = 6; $p < 0.0025$) concentrations significantly decreased in time in cavities after closure. In all 30 time series in 10 different cavities in both regions, the concentration of DOC or TOC declined after 30 min of cavity closure (*Table 3.3*). In seven time series in different cavities on Curaçao, we measured changes in DOC concentration up to 60 min, but in all cases, the changes in DOC concentration stabilised after 30-45 min. Figure 3.2 illustrates the pattern of DOC concentration change in time at six different cavities at four different stations on Curaçao. Cavity Buoy 1.1 was studied in more detail. The average concentration drop of DOC after 30 min in cavity Buoy 1.1 was 20.3 ± 4.9 μmol L^{-1}, the average calculated flux per cavity surface area was 353 ± 57 mmol C m^{-2} d^{-1} ($n = 12$; *Table 3.3*).

Table 3.3 - Complete list of cavity closure experiments conducted from 2003 to 2006 in seven different coral cavities at four sites in Curaçao and three different coral cavities at three sites in Berau. DOC concentration at the start of each experiment, the DOC removal rate based on both the linear (DOC_{lin}) and 2G-model (DOC_{2G}) fit, the bacterial abundance (BA) at the start of each experiment, and the bacterial carbon removal rate (BC) are given. Based on the individual time series, the averages for coral cavities in both Curaçao and Berau are presented.

Cavity	Date	DOC t=0 (µmol L^{-1})	DOC_{lin} removal rate (mmol C m^{-2} d^{-1})		DOC_{2G} removal rate (mmol C m^{-2} d^{-1})		BA t=0 (10^5 cm^{-3})	BC removal rate (mmol C m^{-2} d^{-1})
Curaçao								
Buoy 1.1	21 Aug 03	63	53		336		8.2	2.5
Buoy 1.1	22 Aug 03	101	63		386		8.3	6.1
Buoy 1.1	23 Aug 03	97	57		345		7.6	3.2
Buoy 1.1	24 Aug 03	97	75		462		6.8	2.3
Buoy 1.1	25 Aug 03	94	61		370		6.4	3.0
Buoy 1.1	19 Sep 04	98	118		340		7.8	4.9
Buoy 1.1	20 Sep 04	106	52		285		9.3	3.9
Buoy 1.1	21 Sep 04	119	101		363		7.7	5.0
Buoy 1.1	18 May 05	140	73		380		5.8	2.5
Buoy 1.1	06 Jun 05	121	42		263		6.4	2.7
Buoy 1.1	07 Jun 05	112	63		286		8.6	4.5
Buoy 1.1	11 Apr 06	112	49		419		8.9	4.7
Buoy 1.2	19 May 05	109	79		324		7.6	3.8
Jeremi 1	26 Jun 05	140	163		410		6.5	4.1
Jeremi 1	27 Jun 05	140	180		432		7.6	4.7
Jeremi 1	19 Apr 06	146	212		493		6.5	3.1
Jeremi 2	19 Apr 06	101	64		314		8.2	4.6
Snake Bay 1	25 Jun 06	122	151		444		6.5	5.4
Snake Bay 1	12 Apr 06	140	124		273		8.0	2.2
J&T's Leap 1	20 Apr 06	129	106		220		7.0	1.9
J&T's Leap 1	21 Apr 06	94	58		269		5.8	1.8
J&T's Leap 2	20 Apr 06	97	30		151		7.3	2.9
J&T's Leap 2	21 Apr 06	114	70		300		4.5	1.9
Average		113	86		342		7.3	3.6
Berau		*	*	**	*	**		
Coral Garden	18 Oct 03	84	36	34	135	133	4.0	1.0
Coral Garden	19 Oct 03	74	17	9	33	25	5.0	3.9
Lighthouse II	21 Oct 03	80	36	29	167	160	5.5	3.3
Lighthouse II	21 Oct 03	72	28	25	90	87	3.2	1.5
Kakaban	23 Oct 03	77	27	23	77	73	6.5	1.9
Kakaban	23 Oct 03	74	18	16	89	87	2.8	1.1
Kakaban	25 Oct 03	63	16	15	63	62	9.0	0.6
Average		75	28	24	93	89	5.1	1.9

*TOC **DOC

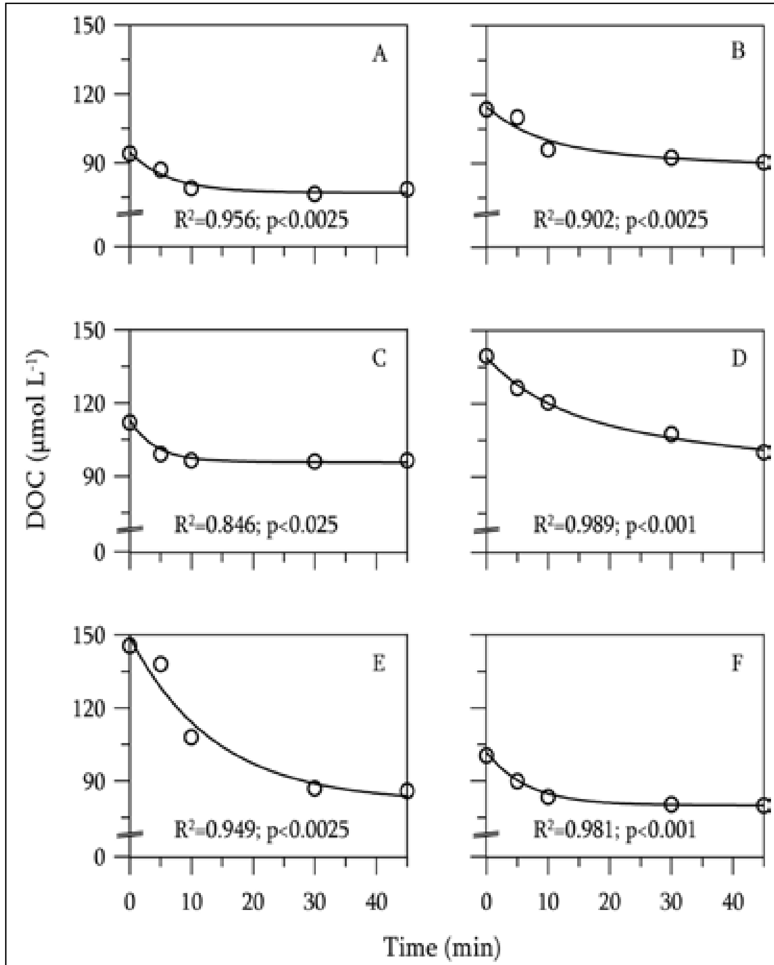


Fig. 3.2 - The decrease of DOC concentration in time in six different coral cavities after cavity closure at four different stations: (A,B) J&T's Leap, (C) Buoy 1, (D) Snake Bay, and (E,F) Jeremi, along the SW coast of Curaçao. Trend lines are given by a 2G-model fit.

Artificial cavity Buoy 1.2 was sampled in May 2005, eight months after its placement underwater. Occasional observation and underwater photography indicated that within a time frame of eight months, the artificial cavity walls had a thin veneer of encrusting cryptic organisms. The estimated DOC flux into cavity Buoy 1.2 was 324 mmol C m^{-2} d^{-1}, which was in the same order of magnitude as DOC fluxes estimated for natural coral reef cavities (*Table 3.3*). For all cavities the average concentration drop of DOC (Curaçao) or TOC (Berau) after 30 min was respectively 25.6 ± 12.3 µmol L^{-1} and 8.1 ± 3.6 µmol

L^{-1}, estimating fluxes with the 2G-model of respectively 342 ± 82 mmol C m^{-2} d^{-1} for the cavities on Curaçao (DOC; *n* = 23) and 93 ± 44 mmol C m^{-2} d^{-1} for the cavities in Berau (TOC; *n* = 7) (*Fig. 3.3*). The DOC removal rates for cavities in Berau were on average 90 ± 45 mmol C m^{-2} d^{-1}, or 96% of the TOC removal rates.

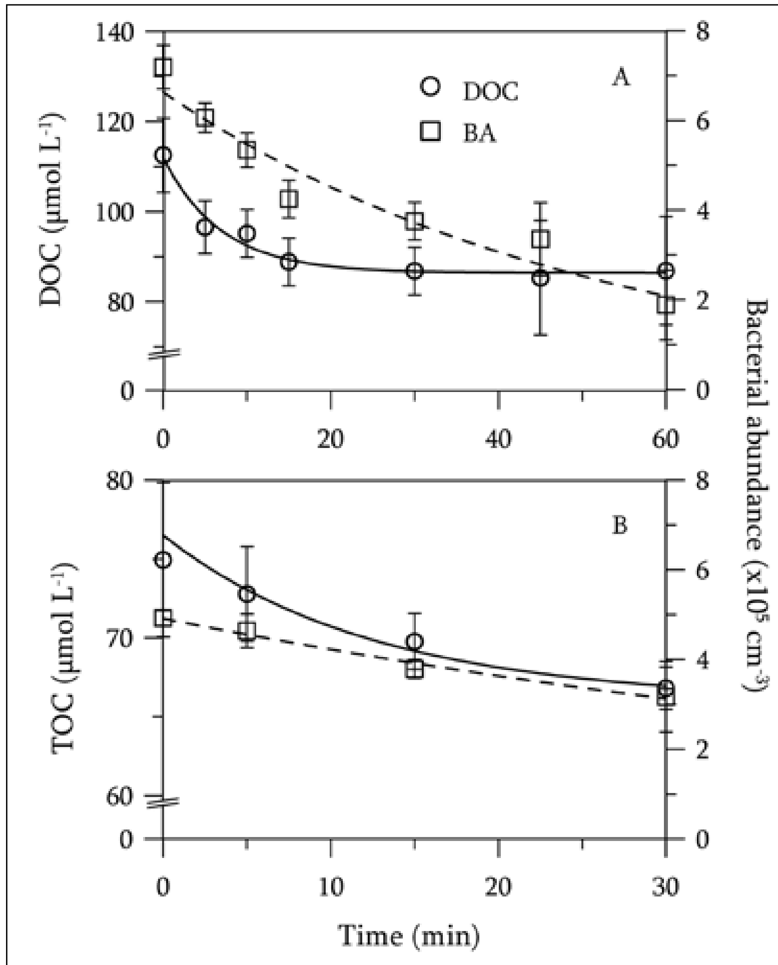


Fig. 3.3 - (A) Curaçao: The average decrease of DOC concentrations (n = 23; trend line is given by a 2G-model fit) and bacterial abundance (n = 23; trend line is given by an exponential fit) in time in coral cavities after closure. (B) Berau: The average decrease in TOC concentrations (n = 7; trend line is given by a 2G-model fit) and bacterial abundance (n = 7; trend line is given by an exponential fit) in time in coral cavities after cavityclosure; mean ± SD.

We determined the following effects on the variance in organic carbon removal rates: (1) The concentration of DOC or TOC at $t = 0$, (2) the cavity volume, and (3) the station (area effect). There was no significant correlation between the concentration of DOC (Curaçao) at $t = 0$ and the removal rate of DOC in cavities (Pearson; $r = 0.256$, df = 21, not significant (n.s.)), nor between the concentration of TOC (Berau) at $t = 0$ and the TOC removal rates in cavities (Pearson; $r = 0.644$, df = 5, n.s.). Cavity volume did not have a significant effect on carbon removal rates in Curaçao (General Linear Models (GLM);

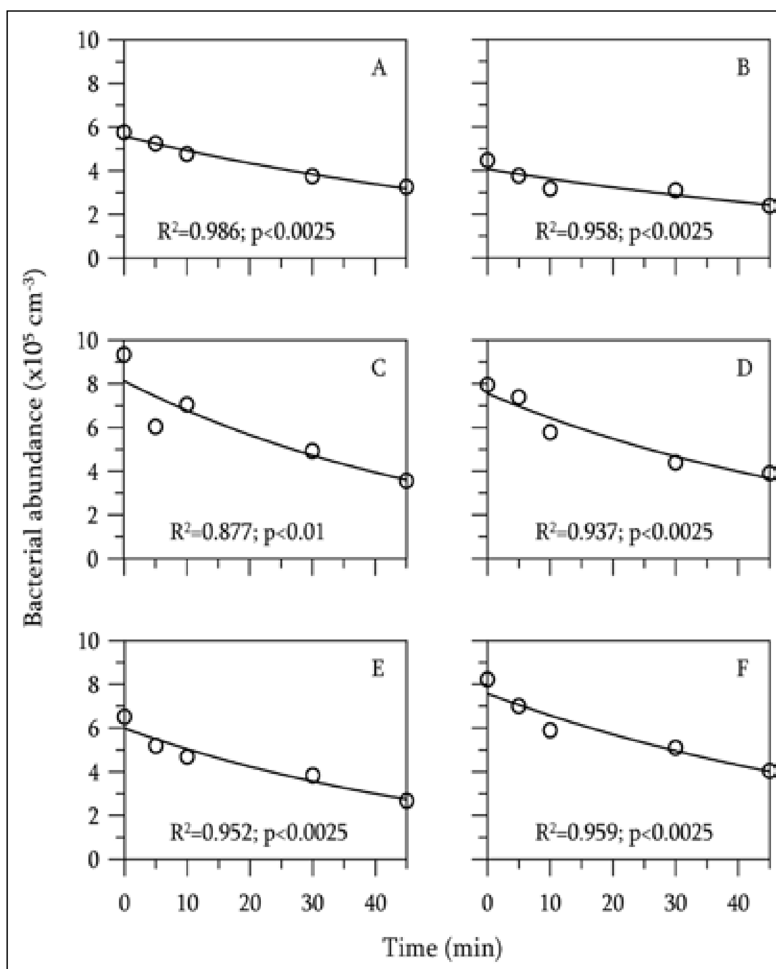


Fig. 3.4 - The decrease of bacterial abundance in time in six different coral cavities after cavity closure at four different stations: (A,B) J&T's Leap, (C) Buoy 1, (D) Snake Bay, and (E,F) Jeremi, along the SW coast of Curaçao. Trend lines are given by an exponential fit.

F = 0.058, df = 21, n.s.), and in Berau (GLM; F = 0.007, df = 5, n.s.). The location of the stations did not have a significant effect on DOC removal rates (GLM; F = 3.236, df = 6, n.s.), or TOC removal rates (GLM; F = 0.826, df = 2, n.s.). The bacterial numbers decreased exponentially in coral cavities in Curaçao and Berau in time (*Figs. 3.3* and *3.4; Table 3.3*). This decrease corresponded on average to a removal of 49 ± 11% (Curaçao) and 40 ± 20% (Berau) of bacterioplankton by coral cavities. Bacterioplankton carbon removal by coral cavities of Curaçao was on average 3.6 ± 1.3 mmol C m^{-2} d^{-1} (n = 15). In the Berau area the average bacterioplankton carbon removal was 1.9 ± 1.3 mmol C m^{-2} d^{-1} (n = 6).

The bioassays showed an average DOC removal of 19.6 ± 8.4 µmol L^{-1} after 30 days (*Fig. 3.5*). During the bioassay, bacterial numbers increased from 6.3 ± 0.25×10^5 bacteria cm^{-3} at t = 0 to 24.3 ± 0.77×10^5 bacteria cm^{-3} at t = 4 d, and slowly decreased again to 6.5 ± 2.1×10^5 bacteria cm^{-3} at t = 30 d (*Fig. 3.5*). Inorganic N and P levels remained high after enrichment at approximately 10 µmol L^{-1} and 1 µmol L^{-1}, respectively.

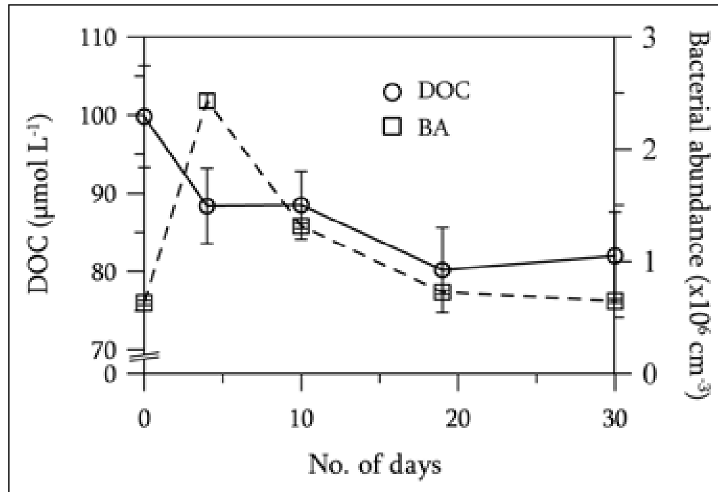


Fig. 3.5 Bioassays (n = 6) of grazer free sea water cultures showing the uptake of DOC by bacterioplankton in time and the bacterial abundance in time; mean ± SD.

Discussion

This is the first study on the flux of dissolved organic carbon (DOC) in coral cavities, and two independent methods indicated that they are sinks of DOC. DOC removal rates in coral cavities were on average two orders of magnitude higher than bacterioplankton removal rates. All our results strongly support a net influx of DOC and bacterioplankton into cavities. Whereas Richter et al. (2001) suggested phytoplankton and, indirectly, bacterioplankton as the most important organic matter sources for biota in cryptic habitats on coral reefs, we, however, find that the removal of DOC in cavities is more likely to be one to two orders of magnitude larger than these particulate sources.

The concentration of DOC in cavity water compared to reef water is 15% and 6% lower than the bulk DOC concentration in ambient reef water in Curaçao and in Berau, respectively (*Table 3.2*). Depletion of bacterioplankton (29% at Curaçao and 38% at Berau) is higher than of DOC. Bacterioplankton is apparently more efficiently removed from cavity water than bulk DOC in coral cavities. These findings may not be surprising because the cryptic biota is dominated by suspension feeders, particularly by sponges (e.g. Vasseur 1977; Richter and Wunsch 1999; Wunsch et al. 2002). Sponges are very efficient filter feeders, especially in feeding on particles smaller than 2 μm, like bacterioplankton (e.g. Reiswig 1974a; Pile et al. 1996; Kötter and Pernthaler 2002). Smaller differences between cavity and reef water DOC concentrations in Berau as compared to those in Curaçao could be explained by shorter residence times of water in the cavities of the Berau area. This argument is supported by the observation that the coral cavities in Berau had more openings to the ambient reef water compared with those in Curaçao, allowing enhanced water exchange. It is also possible that DOC is less efficiently removed from the water in the cavities in Berau as compared to the cavities in Curaçao. The ratio DOC:bacterioplankton carbon in reef water is comparable in both regions, i.e. 33.3 and 34.5 for Curaçao and Berau, respectively, but not in cavity water, i.e. 38.9 for Curaçao and 50.7 for Berau. There is no clear discrimination in DOC and bacterioplankton carbon uptake in cavities of Curaçao. Cavities in Berau, however, remove relatively more bacterial carbon than DOC. The composition of DOC in Berau could be less favourable in terms of utilisation by cryptic organisms, and this could explain the positive discrimination for bacterioplankton in cavities of the Berau area.

DOC fluxes in cavities found in this study are high and unequaled in the literature, with estimated removal rates per cryptic surface area of 342 ± 82 mmol C m^{-2} d^{-1} (range: 151-493) in Curaçao and 90 ± 45 mmol C m^{-2} d^{-1} (range 33-167) in Berau. After closure of cavities organic carbon is clearly removed in two major fractions, a fast removable fraction C_f of 28 ± 12 µmol L^{-1} (Curaçao) and 12 ± 4 µmol L^{-1} (Berau), and a slow removal fraction C_s of 84 µmol ± 14 µmol L^{-1} (Curaçao) and 65 ± 4 µmol (Berau) (*Figs. 3.2 and 3.4*). Therefore, fluxes based on a linear model are grave underestimations and are presented here only as the most conservative values of DOC fluxes into coral cavities. Another approach to determine fluxes of matter into coral cavities is given by Van Duyl et al. (2006). They calculated fluxes in an open system, based on the relation between differences in concentration of matter in cavity water and overlying reef water and the water exchange coefficient of a series of cavities at different current velocities along the reef bottom. They found an average water exchange coefficient of 0.0041 s^{-1}, which is equivalent to an average residence time of water in coral cavities in Curaçao of 4.07 minutes. This approach rules out any possible closure effects. The average difference between cavity and reef water DOC concentration in this study is 14.8 µmol L^{-1}. Using the average water exchange coefficient of 0.0041 s^{-1}, the weighed average volume (148 dm^3) and total cavity surface area (TSA; 2.11 m^2), the DOC flux into cavities is 367 mmol C m^{-2} d^{-1}, which is surprisingly close to the average DOC flux calculated with a 2G-model, namely 342 mmol C m^{-2} d^{-1}. This implies that DOC fluxes based on the 2G-model are reliable.

Bacterioplankton abundance declines exponentially in closed cavities. This supports our assumption that the suspension or filter feeding activity by the cryptofauna is not arrested nor inhibited by closure of the cavities. Bacterial carbon uptake rates by coral reef cavities (*Table 3.3*) closely resemble those reported in literature. Scheffers et al. (2004) found significant bacterioplankton depletion within cavities on Curaçao of on average 2.5 mmol C m^{-2} d^{-1}, whereas Van Duyl et al. (2006) found a bacterial carbon flux into cavities in the same area of 3.8 mmol C m^{-2} d^{-1}. Ayukai (1995) found bacterioplankton carbon retention rates on the Great Barrier Reef of on average 2.2 mmol C m^{-2} d^{-1}. The average bacterioplankton carbon removal in cavities in this study is 3.6 ± 1.3 (range: 1.8-6.1) and 1.9 ± 1.3 (range: 0.6-3.9) mmol C m^{-2} d^{-1} for cavities on Curaçao and Berau, respectively. This represents only 1-2% of the DOC removal. It is evident that DOC is quantitatively a far more important organic

carbon source for coral cavities than bacterioplankton in Curaçao as well as in Berau.

In our bioassays in the reef water of Curaçao we recorded an uptake of 20 µmol L^{-1}, or 19%, of DOC by bacterioplankton in 20 days. We consider this fraction to be the average readily available part of the DOC. Because this percentage is close to the average DOC concentration reduction in closed cavities, it is tempting to suggest that the depletion in DOC concentration in CW as compared to RW was due to removal of labile DOC. The most likely candidate to remove labile DOC from cavity water is bacterioplankton. In the bioassays, it takes bacterioplankton 20 days to take up 19% of the total DOC, while cavities (with a 10-fold lower abundance of bacterioplankton as compared to the bacterial abundance in the bioassays) remove the same amount of DOC within 30 min. In addition, the residence time of water in our coral cavities is more in the range of minutes than days (Van Duyl et al. 2006). It is, therefore, unlikely that bacterioplankton is responsible for the DOC depletion in coral cavities.

Uptake of DOC by coral cavities appears to have been an overlooked general function in coral reef ecology. Considering the sheer size of the cryptic habitat and the significance compared to other sources of organic matter, DOC may be a key factor in the carbon and energy budget on coral reefs. Net influx of DOC into cavities is shown in a wide variety of cavities in two distinct coral reef regions, i.e. an Atlantic and Indo-Pacific region. Bulk DOC uptake rates by coral cavities vary in time and between cavities sampled in different areas. Neither the concentration of bulk DOC at the start of an experiment, nor cavity geometry accounts significantly for the variation in DOC fluxes. The difference in DOC flux size between the sampled areas can be explained by differences in composition and quality of DOC. The composition of the dissolved organic matter (DOM) pool is very diverse with a size range of low-molecular-weight organic molecules like amino acids, to high-molecular-weight molecules (e.g. mucus, polysaccharides), to minute particles like viruses and colloids. At least 10% of oceanic DOM is colloidal material (>95% consists of non-living particles) in the size range 0.4-1.0 µm that easily passes the pores of the GF/F filters commonly employed in the separation of DOM and particulate organic matter (POM) and a significant part still passes through the pores of the 0.2 µm filters that we used (Koike et al. 1990). It could

well be that the cryptofauna mainly takes up colloidal material in this size range. Sponges can take up minute particles like viruses (Hadas et al. 2006) and 0.1 µm-sized beads from ambient water (Leys and Eerkes-Medrano 2006). Pile et al. (1996, 1997) showed that the sponges used in their studies did not show selective feeding on any component of the plankton community. They suggested that the composition of the plankton community and the variability in the water column can affect sponge nutrition. The opportunistic feeding of sponges is further strengthened by Ribes et al. (1999) who argued that the composition of the ingested carbon by *Dysidea avara* mainly varied according to the availability of the different prey types in the water column. In this respect, DOM should also be taken into account as a food source available for the cryptic biota, which is dominated by suspension feeders. There is evidence of extensive DOM feeding by suspension feeders. DOC intake can explain up to 50% of the carbon demand of zebra mussels (Roditi et al. 2000; Baines et al. 2005). Yahel et al. (2003) showed evidence of extensive *in situ* DOC feeding, representing more than 90% of the total carbon intake, by the marine sponge *Theonella swinhoei*.

The variation in DOC removal rates between cavities may be attributed to differences in cryptofaunal composition. Interestingly, we know from previous data that each cavity investigated on Curaçao has its unique cryptofaunal composition (Scheffers 2005). Yet, there is no significant difference in carbon fluxes between cavities on Curaçao, and the proportion of the main functional groups that might influence variation in DOC removal rates, like sponges, (calcareous) algae, ascidians, bryozoans, and polychaetes, is relatively constant in coral cavities on Curaçao.

Depending on reef zone, the cryptic surface may range from less than 1× to 8× the planar reef area (Richter et al. 2001; Scheffers 2005). Hatcher (1997b) reviewed the importance of regenerative spaces in reefs for the carbon budget of coral reefs. His gross primary production rates of entire reefs, where back reefs were the most productive reef zones (Hatcher 1990), may, however, be insufficient (200-500 mmol C m^2 planar reef d^{-1}) to meet the organic carbon demands of cryptic biota. We measured removal rates of 1,000 mmol C m^{-2} planar reef d^{-1}, assuming an average cryptic surface of 2.8 m^2 m^{-2} planar reef for the entire Curaçaoan reef (Scheffers 2005), omitting the particulate organic carbon removal by cryptic habitats and omitting the DOC consumption by benthos of the open reef. So, removal of DOC by cryptic habitats alone is

already two times more than the gross primary production. Where is all this carbon coming from? Because bulk DOC concentrations are usually higher in reef overlying waters than in adjacent ocean (Ducklow 1990; Torréton et al. 1997; Van Duyl and Gast 2001), the carbon budget is unlikely to be matched by net import of bulk DOC from the ocean to the reef, unless organic carbon is actively taken up against a concentration gradient. This implies that DOC production by reefs and reef overlying waters, and possibly DOC supply from land-based sources is probably larger than currently anticipated. A net input of external particulate organic matter (POM) to the reef, for instance by trapping of oceanic plankton and other particles by the reef (Hamner and Wolanski 1988; Richter et al. 2001), may possibly result in extra DOC supply via the benthic food web. This, however, may not be sufficient to cover the gap between gross primary production and consumption. Coral mucus, a part of colloidal DOC, has been suggested to be an important carrier of energy to the benthic food chains of the reef (Wild et al. 2004). Therefore, we hypothesise that the bulk DOC production by noncryptic reef communities is significantly higher than presently assumed. It is evident that the high DOC removal rates we measured in cryptic habitats of coral reefs in Curaçao and Berau influence our present understanding of energy budgets of coral reefs.

CHAPTER 4

Major bulk dissolved organic carbon removal by encrusting coral reef cavity sponges

Abstract

We studied the removal of dissolved organic carbon (DOC) and bacterioplankton by the encrusting sponges *Halisarca caerulea*, *Mycale microsigmatosa* and *Merlia normani* in coral reefs along Curaçao, Netherlands Antilles. Sponge specimens were collected from coral reef cavities and incubations were done on the fore reef slope at 12 m water depth. The concentrations of DOC and bacterioplankton carbon were monitored *in situ*, using incubation chambers with sponges and without sponges (incubations with coral rock or ambient reef water only). Average (± SD) DOC removal rates (in µmol C cm^{-3} sponge h^{-1}) amounted to 13.1 ± 2.5, 15.2 ± 0.9, and 13.6 ± 2.4 for *H. caerulea, M. microsigmatosa*, and *M. normani*, respectively. The DOC removal rates by the three sponges were on average two orders of magnitude higher than bacterioplankton carbon removal rates and accounted for more than 90% of the total organic carbon removal. Total carbon removal rates presented here were the highest ever reported for sponges. In an additional experiment with *H. caerulea*, the fate of organic carbon was reconstructed by measuring dissolved oxygen (O_2) removal and dissolved inorganic carbon (DIC) release in a laminar flow chamber. *H. caerulea* respired 39-45% of the organic carbon removed. The remaining 55-61% of carbon is expected to be assimilated. We argue that *H. caerulea* may have a rapid turnover of matter. All three sponge species contained associated bacteria, but it is unclear to what extent the associated bacteria are involved in the nutrition of the sponge. We conclude that the three sponge-microbe associations are (related to the availability of dissolved and particulate carbon sources in the ambient water) 'dissolved organic matter (DOM)-feeders' and encrusting sponges are of quantitative importance in the removal of dissolved organic carbon in coral reef cavities.

Introduction

Sponges are considered very efficient suspension feeders and their filtering capacity in combination with their abundance may have a profound impact on their environment (Gili and Coma 1998). Sponges can feed on a wide variety of types and sizes of plankton up to the capture of zooplankton (Vacelet and Boury-Esnault 1995). However, the preferred utilisation of nano- and picoplankton (particles smaller than 2 μm) by sponges has been shown both in laboratory studies (Van de Vyver et al. 1990; Riisgård et al. 1993; Turon et al. 1997) and field studies (e.g. Reiswig 1974a; Pile et al. 1996; Ribes et al. 1999; Kötter and Pernthaler 2002).

There is still debate on the qualitative and quantitative role of dissolved organic matter (DOM) in the nutrition of marine benthic animals (Wright and Manahan 1989; Thomas 1997). As early as 1872, it was suggested that DOM could be a potential food source for marine invertebrates and ever since it has been a topic for discussion (reviewed by Jørgensen 1976). Reiswig pioneered studies on the retention of organic carbon by sponges (Reiswig 1971) and the vast volumes of water these sponges can process over time (Reiswig 1974b). He found a discrepancy between the supply and demand of carbon in benthic suspension feeders, and DOM was supposed to be the missing link (Reiswig 1981). It is generally assumed that only sponges with sponge-associated bacteria, sometimes comprising up to half of the total biomass of the sponge, are capable of utilising DOM (Frost 1987; Ribes et al. 1999). Sponges have been demonstrated to take up the amino acid glycine (Stephens and Schinske 1961), 0.1 μm beads (Leys and Eerkes-Medrano 2006), as well as virus particles (Hadas et al. 2006) from ambient water. Both viral particles and 0.1 μm beads easily pass a 0.2 μm filter and are therefore operationally defined as 'dissolved'. Yahel et al. (2003) were the first to show extensive removal of bulk dissolved organic carbon (DOC) by the sponge *Theonella swinhoei*. The DOC intake by this sponge accounted for more than 90% of the total organic carbon (TOC) intake.

Coral cavities, ranging in volume between 50-250 dm^3, have been identified as major sinks of organic carbon (De Goeij and Van Duyl 2007). Framework cavities in reefs of the Berau area, East-Kalimantan, Indonesia and in the reefs of Curaçao, Netherlands Antilles have shown extensive removal of organic carbon. The surface of the framework exceeds that of the open reef

(Richter et al. 2001; Scheffers et al. 2004) and the cavity walls are densely covered with cryptic organisms, dominated by (mostly encrusting) sponges. The natural sponge coverage in relation to the total cavity surface (including the sandy bottom) in coral cavities ranges from 10-27% (Wunsch et al. 2000; Richter et al. 2001; Van Duyl et al. 2006). Particle uptake from the water column by the benthic reef community is mainly accounted for by suspension feeders, such as sponges and ascidians (Ribes et al. 2005). Encrusting cryptic sponges feed effectively on ultraplankton (more than 90% accounted for by bacteria) resulting in high carbon fluxes, up to 373 mg C g ash free dry weight $(AFDW)^{-1}$ day^{-1} (Kötter and Pernthaler 2002). However, although particulate organic carbon (POC) removal rates by the cryptic habitat are considered to be high (Richter et al. 2001; Scheffers et al. 2004), DOC removal rates by coral cavities were two orders of magnitude higher in comparison (De Goeij and Van Duyl 2007). No efforts have been made to investigate the contribution of DOC to total carbon uptake by encrusting sponges in coral cavities.

In this study we investigate the role of three encrusting coral cavity sponge species, *Halisarca caerulea*, *Mycale microsigmatosa* and *Merlia normani*, in the removal of DOC and bacterioplankton carbon. For *H. caerulea*, the fate of organic carbon was further studied by measuring dissolved oxygen (O_2) consumption and dissolved inorganic carbon (DIC) release.

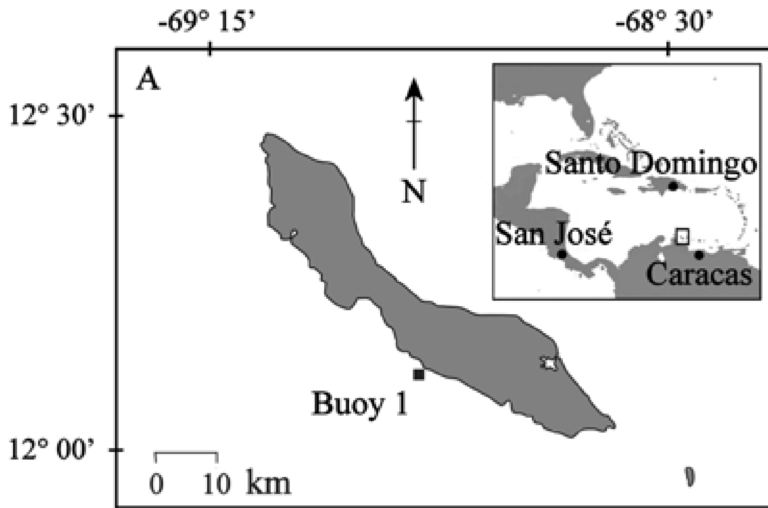


Fig. 4.1 - Map of Curaçao, locating Buoy 1 along the SW coast. Inset: location of Curaçao in the Caribbean Sea. The long-term mean current runs from SE to NW along the island.

Materials and methods

Study area and sponge collection

The study was conducted on the Caribbean island of Curaçao, Netherlands Antilles (12°12'N, 68°56'W). Sponge experiments were performed at station Buoy 1 using incubation chambers on the reef flat of the fringing reefs along the leeward side of Curaçao at a water depth of approximately 12 m (*Fig. 4.1*). Sponges were collected by chiselling them from overhangs and cavity walls between 15-25 m depth. Attached pieces of coral rock were cleared of epibionts and shaped to a maximum total volume of approximately 70 cm^3 and a sponge surface of approximately 24 cm^2, with an average sponge thickness of 2.5 mm. Sponges were stored upside down in wire cages (20×20×15 cm; maximum of four pieces per cage) to protect them from sediment accumulation and predation. Cages were stored inside coral reef cavities at 15 m water depth. Sponges were acclimatised for at least one week prior to experiments. Pieces of sponge were kept to a maximum of six months before use in an incubation experiment. They were regularly visually checked and when necessary the substratum was cleaned and debris removed. Sponges were resized by cutting them to their original surface area when overgrowth of the edges of the coral

rock substratum by the sponge occurred. After each experiment two pieces of sponge tissue (0.5 cm^2) were cut loose from the substratum and fixed for both taxonomy and the determination of abundance of sponge-associated bacteria. Sponge collection and all data were collected by SCUBA diving.

Incubation chambers

Two types of incubation chambers were used *in situ* to determine DOC and bacterioplankton carbon removal over time for the selected sponges. During the first fieldwork period (2004) we used 1.05 L plexiglass cylinders with a magnetic stirring device, powered by a 9 V battery (*Fig. 4.2*). Sample water withdrawn from the chamber during incubation was replaced by air, supplied by an air-filled bottle attached to the cylinder. Air was supplied and flow was controlled by a three-way valve and clamps, which were opened during sampling. Pieces of sponge (7.7-31.2 cm^2) were placed on the bottom of the cylinder next to the magnetic stirrer and sampling point. The cylinder was sealed by two 10 cm diameter lids containing an O-ring. This incubation chamber will be referred to as Chamber 1.

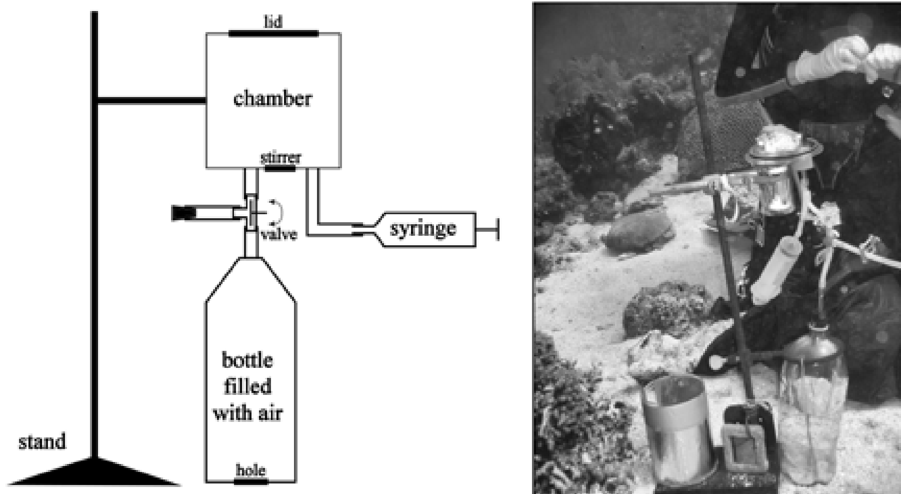


Fig.4.2 - Incubation Chamber 1. Pieces of sponge are placed in the chamber and water is mixed through a magnetic stirrer. During sampling process, sample water is replaced by air from the bottle attached underneath the benthic chamber. The chamber is sealed from outside water by a lid with an O-ring. Air release is regulated by a 3-way valve and clamps.

To measure O_2 and DIC fluxes in the sponge *Halisarca caerulea* we designed a portable plexiglass flow chamber. The design of the flow chamber is given in *Figure 4.3*. The flow chamber (35×8×12 cm) has a volume of 1.7 L and an average flow velocity of approximately 1.5 cm s^{-1}, generated by a magnetic stirrer, powered by 8 AA batteries (total 12 V). Downstream the stirrer, water passes a mesh screen with 2 mm pores, to reduce vortices and to create a quasi-laminar flow. Water was sampled downstream the sponge, using a glass Pasteur pipette and a silicon tube. The sample water was replaced by ambient reef water through a PVC tube (2 mm in diameter and 0.5 m in length to reduce diffusive exchange) placed after the magnetic stirring device. The surface area of the sponge compartment (6.5×4.5×3 cm) relative to the volume of the chamber was scaled to the proportion of sponge surface on total cavity surface area (TSA) and coral cavity volume (CV). The empirical linear relation of CV (dm^3) and TSA (in m^2) in coral cavities on Curaçao (for cavities volumes ranging from 86-248 dm^3) is described as:

$$TSA = 0.0134 \times CV \qquad (1)$$

($R^2 = 0.993$; $F = 908.533$; df = 5; $p < 0.001$). If CV is assumed to be 1.7 L, a sponge surface area of 24.5 cm^2 used in the flow chamber corresponds to a sponge cover of approximately 11% of the TSA. Pieces of coral rock with sponge were shaped to the size of the sponge compartment and sponges were cut to a surface area of 24.5 cm^2 (6.1 cm^3). The incubation chamber was sealed with a 10-cm diameter lid containing an O-ring. This laminar flow chamber will be referred to as Chamber 2. Both Chambers 1 and 2 were covered with duct tape and with a black bag to conduct the experiments in the dark.

Sample collection

In a first series of experiments, changes in DOC concentration and bacterial abundance were measured over time in incubation Chamber 1 to determine DOC and bacterioplankton carbon removal by the encrusting cavity-dwelling sponges *Halisarca caerulea* ($n = 7$), *Mycale microsigmatosa* ($n = 6$), and *Merlia normani* ($n = 3$) (*Fig. 4.4*). Incubations with only ambient reef water were performed as blank measurement (control). In addition, incubation experiments were performed with a piece of coral rock (cleared of epibionts) to measure possible coral rock metabolism. Differences in DOC and bacterioplankton carbon removal rates between the three sponges were determined. Experiments

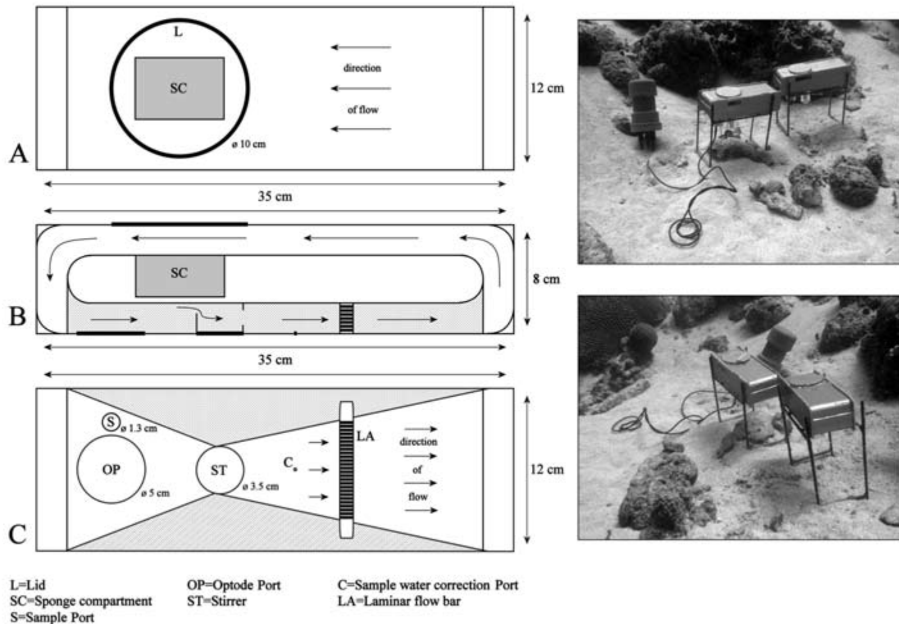


Fig. 4.3 - Incubation Chamber 2. (A) top, (B) side, and (C) bottom views. (D, E) Flow chamber on the reef slope at approximately 12 m water depth.

were done between March and September 2004 between 10.00 and 12.00 h. Prior to the experiment, pumping activity of the sponges was checked visually and only specimens with open oscula (outflow openings) were selected for the experiments. Sponges were carefully positioned, and after closing the chamber, water samples were taken at t = 0, 2, 4, 10, 30 min and in some series up to 45 min. Samples for DOC and bacterial abundance were taken with acid-washed 100 mL polypropylene syringes. Syringes were stored in the dark underwater during the experiment. Sponges were monitored for pumping activity during the experiment. After the incubation experiment, syringes were kept in the dark at 4°C prior to further processing within 4 h.

In a second series of experiments, between March and June 2005 and between January and April 2006, O_2, DOC and bacterioplankton carbon uptake, and DIC release by *H. caerulea* were monitored in Chamber 2. Two control experiments (with coral rock and with ambient reef water only; n = 6) were performed. Before the incubation, trapped air was carefully removed from the chambers, tubings and syringes. The flow chambers were placed on a specially designed stand and were allowed to acclimatise prior to the positioning of the

sponge (*Fig. 4.3*). Before closing the chamber, $t = 0$ samples were taken for DOC and bacterial abundance (80 mL) and O_2 (220 mL). After closing Chamber 2, samples for DOC and bacterial abundance were taken at $t = 5$, 10, 30, 60 min, and in some series up to 90 min. Samples for O_2 and DIC were taken at the end of each experiment. Due to the large volumes of water needed per sample, O_2 and DIC concentration were determined only at $t = 0$ and $t =$ end. All syringes were kept underwater in the dark during the whole experiment. To determine the influence of keeping the syringes underwater during the experiments on oxygen concentration, we measured a series of $t = 0$ oxygen samples ($n = 8$) that were both kept underwater during the whole experiment as well as directly processed (brought to the surface by a second diver after sampling $t = 0$). After the incubation experiment, samples for oxygen concentration were directly processed. Samples for DOC, bacterial abundance and DIC were kept in the dark at 4°C prior to further processing within 4 h.

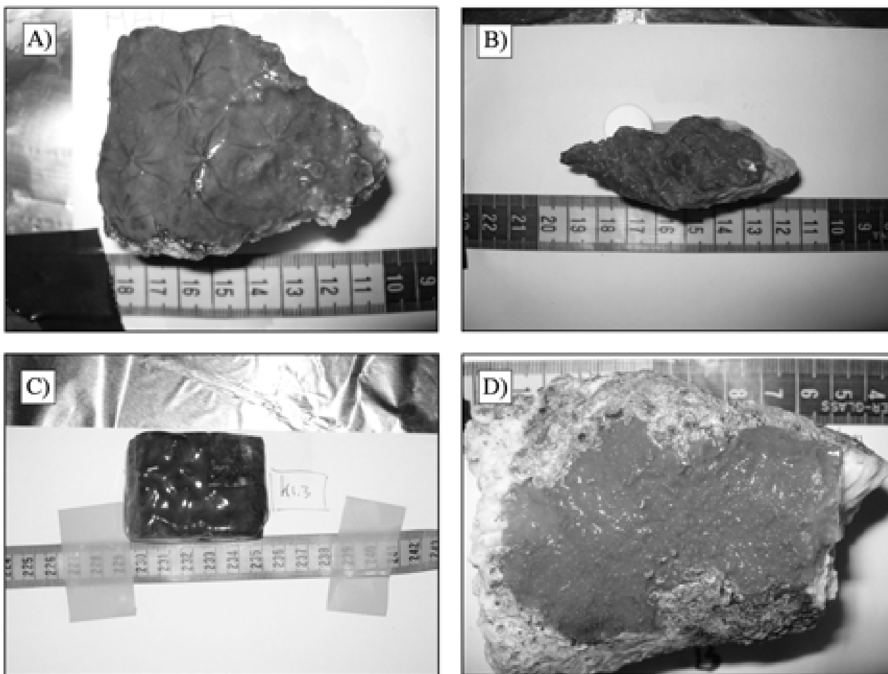


Fig. 4.4 - (A) Halisarca caerulea*, (B)* Mycale microsigmatosa*, (C)* Halisarca caerulea *shaped to fit sponge compartment of Chamber 2, and (D)* Merlia normani.

Sample treatment and analysis

Water samples, collected for the determination of DOC, DIC, and bacterial abundance, and sponge tissues for the determination of sponge-associated bacteria, were processed in the laboratory, prior to transportation to the Netherlands for analysis. Samples for DOC and DIC analysis were gently (max. 20 kPa suction pressure) filtered through a 0.2 μm polycarbonate filter (Millipore, 47 mm). Prior to filtration, filters, glassware and pipette tips were washed with acid (3×10 mL 0.4 M HCl), 0.2 μm filtered double distilled water (3×10 mL) and sample water (3×10 mL).

Duplicate 8 mL DOC samples were collected in pre-combusted (4 h 450°C) glass ampoules. Ampoules were sealed immediately after acidification with 1-2 drops of concentrated H_3PO_4 (80%) and stored at 4°C until analysis. Measurements of DOC were performed by the high temperature combustion method, using a TOC Analyser, Model TOC-5000A (Shimadzu). The TOC analyser was calibrated with potassium phthalate in Milli-Q water. As an internal control of the DOC measurements, consensus reference material provided by Hansell and Chen of the University of Miami, USA (Batch 4, 2004; 45 μmol L^{-1}, every 10-20 samples) was used. DOC concentrations measured for the batch were 45 ± 2 μmol L^{-1}. The average analytical precision of the instrument was <3% (every sample was measured in fivefold).

DIC samples (5 mL) were transferred to glass vials (Alltech, clear screw cap 4 mL capped with a TFE liner) amended with 10 μL of a saturated $HgCl_2$ solution (8 g 100 mL^{-1}). Analysis of DIC was done spectrophotometrically by a continuous flow set-up run on a Technicon Traacs 80 autoanalyser using the method of Stoll et al. (2001). The instrument was calibrated for the typical range for natural seawater (1900-2500 μmol L^{-1}) with Certified Reference Materials (CRMs) (Godec et al. 1992).

O_2 was measured according to the method of Winkler (1888), adjusted by Carpenter (1965) and Culberson (1991). Winkler bottles of approximately 60 mL (volume of each bottle was precisely measured up to 10 μL) were rinsed 3× in seawater. Sample water of two syringes was carefully divided over three Winkler bottles (overflowing approximately 40 mL). Whole bottle end point titration was determined by adding a 1% starch solution using a microburette. The coefficient of variation between the triplicate measurements was <0.5%.

Samples for bacterial abundance in seawater (10 mL) were fixed in 4% paraformaldehyde (PFA), stained with acridine orange, and gently (max. 20 kPa

suction pressure) filtered onto 0.2 μm black polycarbonate membrane filters (Millipore, 25 mm), which were mounted on slides and stored at -20°C.

Sponge tissues for taxonomy were fixed in 5 mL 80% ethanol. For determination of sponge-associated bacteria, sponge tissue was fixed in 3 mL 4% PFA for 30 min at 4°C. Subsequently, tissues were washed twice with 1×PBS and stored in 5 mL 1×PBS:80% ethanol (1:1) at -20°C. Sponge tissue was crushed in a reaction vial, containing 200 μL of Lysis T (Sigma). The dissociated cells were resuspended in 200 μL of artificial sea water (ASW) and centrifuged at 5000 rpm for 30 s. Supernatant was collected into a 15 mL tube. The pellet was resuspended in 10 mL ASW, and filtered through a 0.8 μm membrane filter (Millipore, 25 mm), to separate sponge cells from bacterial cells. Filters were placed on a microscopic slide with a drop of DAPI-mix to stain possible retained bacteria, and stored in the dark at -20°C. The filtrate was added to the supernatant and diluted to 10 mL with ASW, of which 2.5 mL was filtered over a 0.2 μm polycarbonate filter (Millipore, 25 mm), supported by a 0.45 μm HA filter (Millipore, 25 mm). The filters were air dried, mounted on a microscopic slide in a DAPI-mix and stored at -20°C. Bacterial numbers were counted using an epifluorescence microscope (×1250). Per slide, 10 fields were counted or up to a minimum of 200 bacteria. The DAPI counts were recalculated per cubic cm sponge. Data presented in this study is to only show the presence of associated bacteria qualitatively.

C-uptake rates and budgets

The initial DOC removal rates by sponges were estimated by applying a 2G-model (see also De Goeij and Van Duyl 2007). DOC represents a very heterogenic group of organic compounds, both in size fractions, chemical composition as in bioavailability or biodegradability. A simplified model to describe the course of carbon in time assumes that the DOC pool is composed of two major fractions. In a well mixed system, the fast (C_f) and a slow (C_s) removable fractions will be consumed according to their specific removal rate constant k_f and k_s, respectively. The total DOC removal will then be described as the sum of all individual removal rates, or:

$$\frac{dDOC}{dt} = -(k_f C_f + k_s C_s) \tag{2}$$

By integration of this equation in reference to time, t, we arrive at the equation describing the concentration of DOC as function of time:

$$DOC(t) = C_{f,0} \times e^{-k_f t} + C_{s,0} \times e^{-k_s t} \quad (3)$$

The experimental data can be described with the model by estimating the model variables $C_{f,0}$, k_f, $C_{s,0}$, and k_s using a minimalisation routine. The initial uptake rate of DOC (the flux on $t = 0$) was calculated from the estimated values of these variables and is given by:

$$Flux_{DOC} = -(k_f C_{f,0} + k_s C_{s,0}) \quad (4)$$

Bacterioplankton removal rates in closed coral cavities were calculated assuming exponential clearance of bacterial cells in a closed system with homogenous mixed water (Scheffers et al. 2004). To convert bacterial abundance to carbon biomass a conversion factor of 30 fg per bacterial cell was used (Fukuda et al. 1998). Clearance rates (CR) were calculated according to Riisgård et al. (1993):

$$CR = (V_w / t) \times \ln(C_0 / C_t) \quad (5)$$

where V_w is water volume in the incubation chamber, C_0 and C_t are the bacterioplankton concentrations at times 0 and t, calculated from the regression equation (Riisgård et al. 1993). CRs were calculated only to compare clearance rates with those found in literature.

To establish a mass balance, and to reconstruct the carbon flow for *H. caerulea*, exchange rates for DOC, O_2 and DIC were calculated from the concentration difference between $t = 0$ and t = end. The total organic carbon (TOC) pool comprises of DOC and particulate organic carbon (POC). POC in tropical reef water consists mainly of phytoplankton and bacterioplankton. Phytoplankton carbon removal rates were not directly measured. The contribution of phytoplankton carbon to the total C pool in tropical waters is low and in the same order of magnitude as bacterioplankton carbon (BC) (Ayukai 1995; Yahel et al. 1998; Van Duyl et al. 2002), or lower (Richter et al. 2001; Kötter 2003). For conservancy, TOC and POC removal rates used in the mass balance are calculated as follows:

$$TOC = DOC + POC, \text{ where } POC = 2 \times BC \quad (6)$$

DOC removal kinetics

The DOC concentration decreased exponentially with time in the presence of either three sponges, regardless of the chamber used. The 2G-model provided a fairly accurate description of the decrease in DOC concentration in the presence of the three sponges (*Fig. 4.5*) and was therefore applied to estimate the

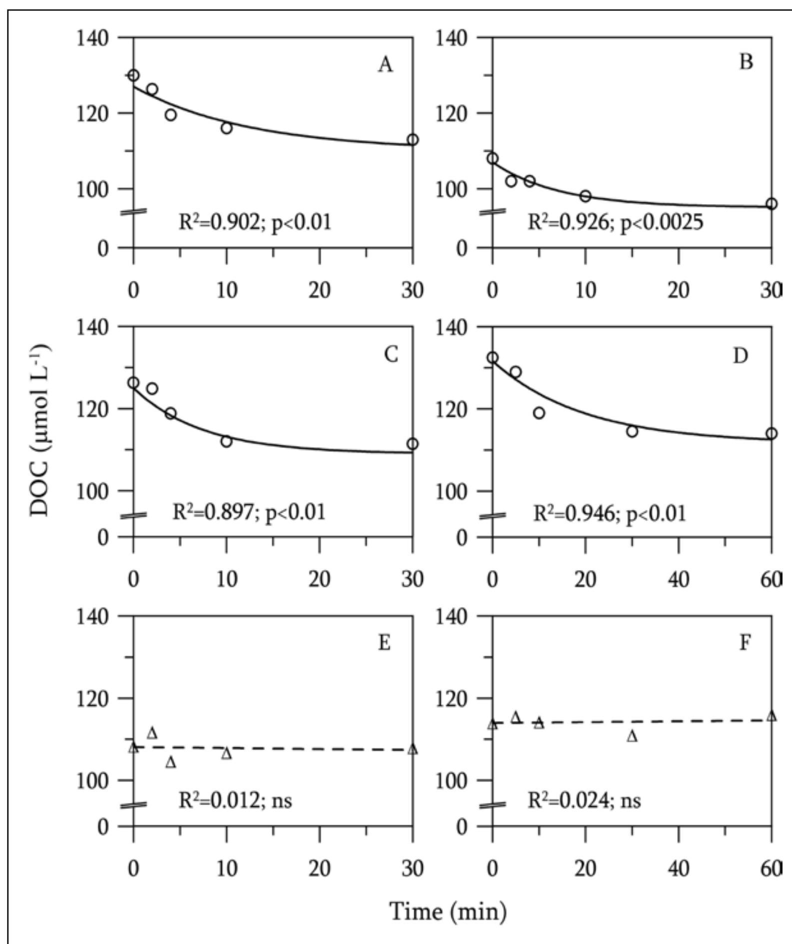


Fig. 4.5 - Exponential decrease of DOC concentration with time for incubations with three encrusting sponges. Chamber 1: (A) Halisarca caerulea *Hal 2, (B)* Mycale microsigmatosa *Myc 2, and (C)* Merlia normani *Mer 3.Chamber 2: (D)* Halisarca caerulea *Hal 16. (E) and (F) are blank incubations in Chamber 1 and 2, respectively. Sponge designations correspond to those used in Table 4.1. Note that Chamber 1 incubations are up to 30 min and Chamber 2 incubations up to 60 min. Trend lines are given by a 2G-model fit. ns: not significant.*

Table 4.1 - Halisarca caerulea, Mycale microsigmatosa*, and* Merlia normani. *Incubation experiments and average dissolved organic carbon (DOC) and bacterioplankton carbon (BC) removal rates, measured in two different incubation chambers. Values are average ± SD. Hal:* H. caerulea*, Myc:* M. microsigmatosa*, Mer:* M. normani*; t_0: time 0; DOC_{2G}: DOC 2G-model fit; BA: bacterial abundance; BC_{exp}: BC exponential fit; nd: no data*

Sponge	Volume sponge (cm^3)	DOC t_0 ($\mu mol\ L^{-1}$)	DOC_{2G} removal rate ($\mu mol\ cm^{-3}\ h^{-1}$)	BA t_0 ($10^5\ cm^{-3}$)	BC_{exp} removal rate ($\mu mol\ cm^{-3}\ h^{-1}$)	Clearance rate ($cm^3\ cm^{-3}\ min^{-1}$)
Chamber 1						
Hal 1	4.2	117	13.4	nd	nd	nd
Hal 2	7.0	131	16.4	9.2	0.3	2.3
Hal 3	6.1	94	10.6	9.1	0.6	4.3
Hal 4	5.6	nd	nd	9.5	0.7	4.8
Hal 5	6.2	nd	nd	9.9	0.6	4.0
Hal 6	6.8	105	10.7	8.1	0.5	4.2
Hal 7	5.7	130	14.3	7.9	0.6	5.2
Average			13.1 ± 2.5		0.6 ± 0.1	
Myc 1	7.2	108	14.0	8.8	0.5	3.9
Myc 2	5.0	109	14.4	11.0	0.9	5.5
Myc 3	7.4	144	15.1	7.4	0.4	3.9
Myc 4	1.9	154	15.3	nd	nd	nd
Myc 5	3.5	106	15.5	9.3	0.6	4.0
Myc 6	5.0	105	16.5	9.1	0.4	3.0
Average			15.2 ± 0.9		0.6 ± 0.1	
Mer 1	7.6	136	11.9	7.3	0.3	2.9
Mer 2	3.8	142	11.8	8.0	0.3	2.8
Mer 3	5.8	126	17.1	8.3	0.4	3.5
Average			13.6 ± 3.1		0.4 ± 0.1	
Chamber 2						
Hal 8	6.1	112	18.7	8.3	1.5	11.7
Hal 9	6.1	130	13.4	10.7	0.4	2.2
Hal 10	6.1	135	14.0	9.4	0.6	4.1
Hal 11	6.1	120	21.0	9.4	0.3	2.1
Hal 12	6.1	160	18.9	8.6	0.7	5.1
Hal 13	6.1	133	16.7	8.9	0.4	2.8
Hal 14	6.1	139	16.7	12.1	0.4	2.3
Hal 15	6.1	110	16.3	12.6	0.8	4.2
Hal 16	6.1	111	18.2	9.0	1.1	8.4
Hal 17	6.1	nd	nd	8.9	1.0	7.3
Average			17.1 ± 2.4		0.7 ± 0.4	

initial DOC removal rates for all experiments. In contrast, the concentration of DOC in the blank incubations (with ambient reef water only) did not significantly change (*Fig. 4.5*). In incubation experiments with coral rock, there was no significant removal or release of DOC (paired *t*-test; $t = 0.358$, df = 5; not significant (n.s.)).

Table 4.1 gives the volumes of the three incubated encrusting sponges, based on an average thickness of 0.25 cm, the DOC concentration at the start of each experiment and the initial removal rate. No significant differences in initial removal rates could be detected between the three species (General Linear Models (GLM); $F = 1.433$, df = 2; n.s.). The average initial removal rates for the three sponges are given in *Table 1*. The average (± SD) initial DOC removal rate of all sponges in Chamber 1 was 14.1 ± 2.1 µmol C cm^{-3} sponge h^{-1}. For *Halisarca caerulea*, the average initial removal rates measured in Chamber 2 (17.1 ± 2.4 µmol C cm^{-3} sponge h^{-1}) were higher than those observed in Chamber 1 (13.1 ± 2.5 µmol C cm^{-3} sponge h^{-1}) (two-sample *t*-test; $t = -3.076$, df = 12; $p < 0.025$). This may have resulted from a (seasonal) difference in DOC composition and bio-availability, the physiological status of the sponges, and from a difference in transport efficiency (different hydrodynamical conditions due to different design) between the two chambers. Since the design of Chamber 2 combines the surface-to-volume ratio of natural cavities with optimal mixing conditions, these initial DOC removal rates were considered as maximum values.

Bacterial clearance

The bacterial abundance significantly decreased exponentially with time for all sponges, whereas bacterial abundance did not significantly change in the control experiments without sponges (with ambient reef water only; *Fig. 4.6*). In incubation experiments with coral rock, there was no significant removal or release of DOC (paired *t*-test; $t = 1.342$, df = 5; n.s.) Initial abundance, bacterioplankton carbon removal rate, and the clearance rates are presented in Table 4.1. There was no significant difference in bacterioplankton carbon fluxes measured for the three different sponges used in Chamber 1 (GLM: $F= 2.324$, df = 2; n.s.). Moreover, *H. caerulea* BC fluxes did not significantly change between Chamber 1 and 2 (two-sample *t*-test, $t = -0.769$, df = 14; n.s.). The average (± SD) initial bacterioplankton carbon removal rate of all sponges in Chamber 1 was 0.52 ± 0.16 µmol C cm^{-3} sponge h^{-1}, and for *H. caerulea* in Chamber 2 was 0.70 ± 0.38 µmol C cm^{-3} sponge h^{-1}. The initial bacterioplankton carbon removal

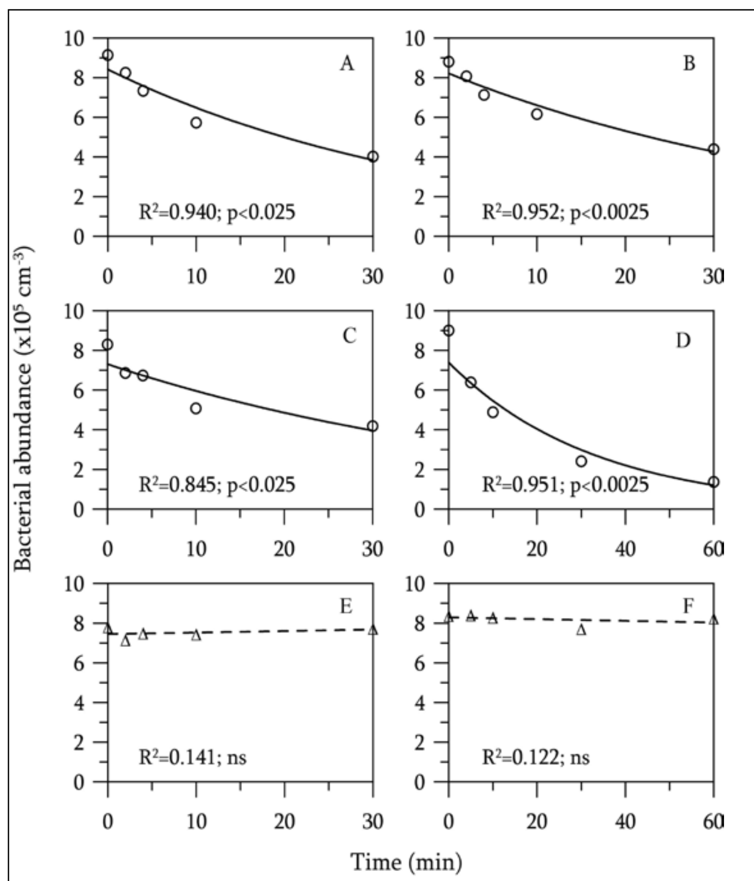


Fig. 4.6 Exponential decrease in bacterial abundance with time for incubations with three encrusting sponges. Chamber 1: (A) Halisarca caerulea *Hal 2, (B)* Mycale microsigmatosa *Myc 2, (C)* Merlia normani *Mer 3, (E) blank incubation. Chamber 2: (D)* Halisarca caerulea *Hal 16, (F) blank incubation. Sponge designations correspond to those used in Table 4.1. Note that Chamber 1 incubations are up to 30 min and Chamber 2 incubations up to 60 min. Trend lines are given by an exponential fit.*

rates were two orders of magnitude lower than the initial DOC removal rates (*Table 4.1*). There was no correlation between size (volume) of the three encrusting sponges in Chamber 1 and the clearance rates (Pearson two-tailed; $R^2 = 0.0196$, $n = 14$; n.s.).

Sponge-associated bacteria

All three encrusting sponges contain sponge-associated bacteria. On average 2.1×10^9 cm^{-3} sponge (*Halisarca caerulea*), 2.1×10^9 cm^{-3} sponge (*Mycale microsigmatosa*), and 1.5×10^9 cm^{-3} sponge (*Merlia normani*) were counted

with DAPI. Preliminary results of the bacterial communities of the three sponges determined by a catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) procedure on the filters (according to Pernthaler et al. 2002, adjusted by Teira et al. 2004) show that DAPI-counts represented 76% (*H. caerulea*), 66% (*M. microsigmatosa*), and 60% (*M. normani*) of the CARD-FISH counts, using a probe against eubacteria (F.C. van Duyl, unpubl.). This data is only presented here to qualitatively confirm the presence of sponge-associated bacteria.

Halisarca caerulea oxygen respiration

The concentration of O_2 significantly decreased with time in the presence of *H. caerulea* (paired *t*-test, $t = 8.646$, df = 21; $p < 0.001$). In the control experiment (incubation experiment with ambient reef water only), the O_2 concentration significantly increased with time, at an average rate of 1.4 µmol L^{-1} h^{-1} (paired *t*-test, $t = -5.000$, df = 3; $p < 0.01$). In addition, the O_2 concentration of sample water directly processed on board during an experiment was significantly higher than the O_2 concentration of sample water kept underwater in the dark and processed at the end of an experiment (paired *t*-test, $t = 8.148$, df = 6; $p < 0.001$), yielding an average water column respiration rate of 2.7 µmol L^{-1} h^{-1}. The average (± SD) respiration rate for *H. caerulea*, corrected for control and water column respiration, was 2.7 ± 0.8 µmol O_2 cm^{-3} sponge h^{-1}, or 6.7 ± 1.9 mmol m^{-2} h^{-1} (*Table 4.2*). There was no respiration rate measured for coral rock (paired *t*-test, $t = -1.464$, df = 5; n.s.). During the sponge incubation experiments, the average drop in oxygen levels was 4.3 ± 1.2% at an average initial seawater O_2 concentration of 208 ± 7 µmol L^{-1} ($n = 22$; range 196-220 µmol L^{-1}).

Halisarca caerulea DIC release

The DIC concentration significantly increased with time in the presence of *H. caerulea* (paired *t*-test, $t = -12.304$, df = 12; $p < 0.001$). In the control incubations with ambient reef water only, the DIC concentration did not significantly change with time. However, DIC concentration significantly increased during incubations with a piece of coral rock without sponge (paired *t*-test, $t = -3.124$, df = 4; $p < 0.05$). This increase probably results from passive, chemical dissolution of $CaCO_3$, and was on average 23 µmol C L^{-1} h^{-1}. In the presence of *H. caerulea*, the increase of DIC was on average 47 µmol C L^{-1} h^{-1}. After correction for passive chemical dissolution of $CaCO_3$ the release rate of DIC

in the presence of *H. caerulea* was on average 6.4 ± 3.3 µmol C cm^{-3} sponge h^{-1} (*Table 4.2*). The increase in DIC levels during the incubations was on average 0.3 ± 0.2% at an average seawater DIC concentration of 2073 ± 23 µmol L^{-1} (n = 13; range: 2048-2103 µmol L^{-1}).

Halisarca caerulea mass balance

More than 90% of the total organic carbon (TOC) removal by the three encrusting coral cavity sponges was accounted for by DOC. Although the sponges removed bacteria very efficiently on an absolute scale, the relative BC removal was only 2.5-4.1% of the TOC removal by sponges (*Table 4.1*). The contribution of POC to TOC removal was only 5.0-8.2%, leaving 91.8-95.0% accounted for by DOC. *Table 4.2* shows the ΔO_2/ΔTOC and ΔDIC/ΔTOC for a selection of time series. On average (± SD), per mol organic C removed by *H. caerulea*, 0.39 ± 0.12 mol of O_2 was consumed and 0.90 ± 0.43 of DIC was released (*Table 4.2*).

Table 4.2 - Halisarca caerulea *carbon mass balance. The concentration of DOC, POC, O_2 and DIC were measured simultaneously per incubation, and the ΔO_2/ΔTOC and ΔDIC/ΔTOC calculated. Values are average ± SD. Note that fluxes of DOC, POC, TOC, and O_2 are removal rates and DIC is a release rate.*

Date	DOC	POC	TOC (µmol cm^{-3} h^{-1})	O_2	DIC	ΔO_2/ ΔTOC	ΔDIC/ ΔTOC
06 Jun 05	8.3	3.0	11.3	3.2	8.9	0.29	0.79
23 Jun 05	3.3	0.8	4.1	2.0	2.7	0.49	0.66
24 Jun 05	5.3	1.2	6.5	4.0	2.8	0.62	0.43
06 Apr 06	6.9	0.6	7.5	2.2	10.8	0.29	1.43
07 Apr 06	6.9	1.4	8.3	3.4	7.4	0.41	0.89
07 Apr 06	5.1	0.8	5.9	2.5	6.6	0.43	1.12
10 Apr 06	5.3	0.8	6.1	1.9	9.5	0.31	1.55
10 Apr 06	6.1	1.6	7.7	3.0	1.8	0.38	0.24
13 Apr 06	5.3	2.2	7.5	1.9	7.4	0.26	0.99
Average (µmol cm^{-3} h^{-1})	5.9 ± 1.4	1.4 ± 0.8	7.2 ± 2.0	2.7 ± 0.8	6.4 ± 3.6	0.39 ± 0.12	0.90 ±0.43
Average (mmol m^{-2} h^{-1})	14.6 ± 3.6	3.4 ± 2.0	18.0 ± 4.9	6.7 ± 1.9	16.1 ± 8.1		

Discussion

DOM-feeding

Sponges are opportunistic feeders and tend to select their food on the basis of availability (Pile et al. 1996, 1997; Ribes et al. 1999). Similar to oceanic waters, dissolved organic carbon (DOC) in the oligotrophic tropical waters represents the largest fraction of total organic carbon (TOC), with only a minor contribution of particulate organic carbon (POC). TOC in tropical reef water of Curaçao consists mainly (average ± SD) of DOC (118.4 ± 20.5 μmol L^{-1}; range 63-160, n = 46), bacterioplankton carbon (2.1 ± 0.4 μmol L^{-1}; range 1.1-3.1, n = 47), and phytoplankton carbon (measured as chlorophyll *a*) (0.9 ± 0.2 μmol L^{-1}; range 0.6-1.1, n = 41) (Van Duyl et al. 2002; De Goeij and Van Duyl 2007; this study). In our incubation experiments the DOC is clearly removed in (at least) two major fractions, where a large part (the slow removable fraction) of DOC is not available to the sponge in the time frame of the incubation. The residence time of water in the coral cavities (the natural environment of the sponges) is in the order of minutes (Van Duyl et al. 2006), suggesting that the slow removable fraction in our model is, on average, not readily available as a source of carbon for the cavity sponges. For the three encrusting coral cavity sponges studied here, the amount of DOC uptake in relation to total organic carbon intake is comparable with values found for the sponge *Theonella swinhoei* (Yahel et al. 2003). In fact, in both studies, more than 90% of the TOC removed by the sponges is accounted for by DOC, suggesting that these species, in spite of being classified as particle feeders, are (in quantitative terms related to the availability of organic carbon sources) actually 'DOM-feeders'. This supports the suggestion by Reiswig (1974b, 1981) that DOC uptake may explain the >70% discrepancy between the particulate gain and respiratory demand of several tropical sponges.

Little is known on the uptake mechanism for DOM in sponges. It has been suggested that only sponges with large amounts of sponge-associated bacteria can utilise DOM. The three encrusting sponge species used in this report indeed contain sponge-associated bacteria. Tritium labelled proline was more rapidly incorporated into symbiotic bacteria of the marine sponge *Chondrosia reniformis*, than in sponge cells (Wilkinson and Garrone 1979). However, in *Theonella swinhoei* (Magnino et al. 1999), *Verongia fistularis* (Reiswig 1981), and in the three sponge species presented in this report, most

sponge-associated bacteria reside in the mesohyl and are not in direct contact with the passing water, and the removed DOM is likely to pass sponge cells first. Sponges feed by using flagellated cells (choanocytes) lining the choanocyte chambers, which constitute the basic pumping and filtering elements. The sponge choanocytes are functionally comparable with choanoflagellates, which are closely related with sponges (Leys and Eerkes-Medrano 2006, and references therein). Flagellates can ingest a variety of macromolecules, including carbohydrates and proteins, components of the colloidal fraction of DOM (Tranvik et al. 1993). Choanoflagellates can feed on high molecular weight molecules (Sherr 1988; Christoffersen et al. 1996) and have been demonstrated to prefer smaller sized (viral-sized) particles (50 nm latex beads) over larger bacterial-sized beads (500 nm latex beads) (Marchant 1990; Gonzalez & Suttle 1993). Sponges have been reported to take up virus particles (Hadas et al. 2006), and to remove 0.1 μm beads from ambient water (Leys & Eerkes-Medrano 2006). At least 10% of oceanic DOM is in the form of amorphous detrital particles in the size range 0.4 to 1.0 μm that easily pass the pores of the 0.2 μm filters employed in the separation of DOM and POM (Koike et al. 1990). It is possible that sponge choanocytes take up particles, to molecular weight size range, residing in the dissolved fraction mainly in colloidal form and transport part of the DOM to the sponge-associated bacteria in the mesohyl. First evidence from experiments with ^{13}C enriched DOC substrate show that both sponge cells and associated bacteria can assimilate DOM (De Goeij et al. 2008b). It is not yet clear to what extent (quantitatively and qualitatively) the sponge cells or the associated bacteria cells are involved in the utilisation and metabolism of DOM. Therefore, 'DOM-feeding by sponges' should be more appropriately described as: 'DOM-feeding by the sponge-microbe association'.

Carbon removal rates

The total carbon removal rates by the three cryptic encrusting sponges presented in this study are the highest ever reported. Ingestion rates reported in literature range from 0.08-1.97 g C m^{-2} sponge d^{-1} (Gili and Coma 1998, and references therein) and 0.04-1.80 μmol cm^{-3} sponge h^{-1} (Yahel et al. 2003, and references therein). This study, assuming an average daily pumping activity of 12 h (Pile et al. 1997), yields a carbon flux of 5.15-6.66 g C m^{-2} sponge d^{-1}. Fluxes per volume of sponge in this report range 14.3-18.5 μmol C cm^{-3} sponge h^{-1}. To our knowledge there is only one study on extensive DOC removal by

sponges, reporting the highest total carbon intake rates at that time (Yahel et al. 2003). Since other studies do not report DOM fluxes, (Reiswig 1971; Pile et al. 1996, 1997; Kötter and Pernthaler 2002), or did not find DOM retention in sponge species lacking bacterial symbionts (Ribes et al. 1999; Yahel et al. 2007), it is difficult to compare total carbon fluxes. Nonetheless, the fluxes presented here are very high compared to published values. The estimated clearance rates (in cm^3 water cm^{-3} sponge min^{-1}) of *Halisarca caerulea* (2.1-11.7), *Mycale microsigmatosa* (3.0-5.5) and *Merlia normani* (2.8-3.5), however, are in the range of values reported in literature, of 2.5 ± 1.7 for the sponge *Haliclona ureolus* (range 1.1-6.0) (Riisgård et al. 1993). Kötter and Pernthaler (2002) reported average clearance rates for *H. caerulea* (6.1 ± 4.6) and *M. normani* (2.5 ± 1.1), so there is no reason to suspect abnormal clearance capacity of the three sponges used in this study. Rates of bacterioplankton carbon removal (in µmol C cm^{-3} h^{-1}) for tropical encrusting sponges is on average 0.75 (Kötter and Pernthaler 2002), close to our average bacterioplankton carbon removal rates by encrusting sponges of 0.59 (*Tables 4.1 and 4.2*). Kötter and Pernthaler (2002) found removal rates of bacterioplankton carbon for *H. caerulea* ranging 0.33-1.17 and for *M. normani* ranging 0.59-0.88. We found bacterioplankton carbon fluxes of 0.60-2.92 and 0.62-0.88 for *H. caerulea* and *M. normani,* respectively. Again, the feeding behaviour of the three encrusting sponges does not seem to be out of range.

Size and body morphology of sponges can have an effect on clearance rates (Reiswig 1974b; Riisgård et al. 1993; Ribes et al. 1999), but also on the supply of certain food fractions and, thus, the capability of feeding on different sized particles (Alberson et al. 1993), like DOM (Yahel et al. 2003). Clearance rates have been observed to decrease with increasing sponge size (Reiswig 1974b; Riisgård et al. 1993; Ribes et al. 1999). We did not find any correlation between sponge size (in the measured size range 1.9-7.6 cm^3) and clearance rates, but cannot exclude that larger sized sponges in the field (ranging <1 cm^3 >100 cm^3) (J.M. de Goeij, pers. observ.) have lower clearance rates and might effect community organic carbon removal. The high surface:volume ratio of encrusting sponges as compared with massive sponges is suggested to increase their retention efficiency (Kötter 2003), and the ability of invertebrates to ingest DOC (Siebers 1982). The sheet-like body form can have a competitive edge over more massive growth forms in the particle depleted coral cavities.

Mass balance and fate of carbon

The O_2 respiration rates for *H. caerulea* (in μmol O_2 cm^{-3} sponge h^{-1}) are within the range of reported values for other sponges (1.82-3.98 and 0.21-24.6, respectively) reviewed by Osinga (1999), and comparable with reported rates for *H. caerulea* (1.56-2.67) measured by Kötter and Pernthaler (2002). *H. caerulea* has a $\Delta O_2/\Delta TOC$ of 0.39, or 39% of the ingested carbon is respired. Assuming a respiratory quotient of 1, this would yield a ΔDIC/ΔTOC value of 0.39, whereas a value of 0.90 (corrected for passive chemical dissolution) has been observed. We argue that the excess DIC release is not due to possible coral rock metabolism by epi- or infauna, since the coral rock (cleared of epibionts) did not remove or release any DOC, bacterioplankton carbon, or O_2. Excess DIC release is attributed to *H. caerulea* respiration driven dissolution of the attached coral rock. Dissolution of $CaCO_3$ increases DIC by 1 mol for each mol of calcium carbonate dissolved (Gattuso et al. 1995), leaving a ΔDIC/ΔTOC for *H. caerulea* respiration of 0.45. The similarity between O_2-based respiration estimation (39%) and CO_2-based respiration estimation (45%) illustrates the accuracy for the *H. caerulea* carbon mass balance.

To determine the fate of carbon it is assumed that 1 mol of organic C removed is respired by 1 mol of O_2. The discrepancy between total organic carbon uptake and oxygen respiration can be explained by microbial processes like sulfate reduction (Hoffmann et al. 2005), or fermentation (Santavy et al. 1990). Fermentation is a common feature in benthic invertebrates (Grieshaber et al. 1994). Alternatively, or in addition, the fate of the removed organic carbon is determined by assimilation. If it is assumed that *H. caerulea* respires approximately 39-45% of the removed organic carbon, 55-61% of the removed organic carbon can be used for growth, reproduction or the production of metabolites. The net increase of cryptic sponge biomass is not likely to be high. Competition for space is high in coral cavities (Jackson et al. 1971) and especially for the thin encrusting species, which are highly surface-dependent, and growth and mortality rates are influenced by strong space competition with neighbours (Turon et al. 1998). If more than half of the carbon uptake is assimilated by *H. caerulea*, but net growth is close to 0, then a rapid turnover of biomass is suggested. Encrusting sponges are known to have a high plasticity, or regeneration capacity, with growth rates of 2,900 times the normal growth rate after tissue damage (Ayling 1983), showing the potential for rapid cell proliferation is present. The resulting cell remnants could have been missed

from our incubation measurements, because it is likely to be exported as detrital particulate carbon, which we did not measure. Both Reiswig (1971) and Yahel and co-workers (2003) found significant excretion of detrital material by the examined sponges (possible sponge cell material or faeces). Decomposition by the deep-sea sponge community of particles >2 μm was argued to have a major contribution to the total sedimentation rate of the Greenland-Iceland-Norwegian (GIN) seas (Witte et al. 1997).

Sponges and coral cavities

The cryptic coral reef framework is a significant sink of carbon, where most (>90%) of the removed carbon is accounted for by the dissolved fraction. The flux of carbon even exceeds the estimated gross production of the reef (De Goeij and Van Duyl 2007). But which organisms are responsible for this important carbon retention in cryptic habitats? The walls of coral cavities walls are covered by highly abundant groups of coelobites, like coralline algae, ascidians, bryozoans, and polychaetes. To directly link the organic carbon removal of sponges with coral cavities, qualitatively and quantitatively, the activity of other compartments of the cavity (e.g. benthic communities on cavity walls and in the sediment) has to be included in the carbon budget. The cover is, however, dominated by encrusting sponges (Wunsch et al. 2000; Richter et al. 2001; Van Duyl et al. 2006). In the present study the encrusting cryptic sponges *Halisarca caerulea, Mycale microsigmatosa*, and *Merlia normani* remove carbon of which the largest part (>90%) is DOC, comparable to organic carbon removal by coral cavities. It is likely that the removal of DOC by the reef framework is influenced by the removal of DOC by cavity sponges. Thus, a thin veneer of encrusting sponges, only a few millimetres thick, may play a key role in organic carbon removal by coral cavities and thus in the overall carbon cycling of coral reefs.

CHAPTER 5

Tracing ^{13}C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM-feeding

Abstract

Here we report on the trophodynamics of the bacteria-containing coral reef sponge *Halisarca caerulea*. The assimilation and respiration of the ^{13}C-enriched substrates glucose, algal-derived dissolved and particulate organic matter (diatom-DOM and -POM), and bacteria was followed in 1 h and 6 h incubations. Except for glucose, all substrates were readily processed by the sponge, with assimilation being the major fate. ^{13}C-enrichment patterns in fatty acid biomarkers revealed that sponge dissolved organic ^{13}C assimilation was both direct and bacteria mediated as tracer carbon was recovered both in bacteria-specific and nonbacterial fatty acid. This is the first direct evidence of DOM incorporation by sponges. The present study demonstrates that the encrusting sponge *H. caerulea* feeds on both DOM and POM and given their dominant coverage of the largest coral reef habitat (coral cavities) it is proposed that organic matter assimilation by cryptic reef sponges may represent an important, largely overlooked ecological function. Quantitatively significant DOM processing may not be the exclusive function of the microbial world on coral reefs; sponges transform DOM to biomass, and thus retain and store organic matter in the reef system.

Introduction

Sponges are common inhabitants on coral reefs and are widely acknowledged as efficient suspension feeders, preferably filtering small particles (<10 μm) like bacteria and phytoplankton from the passing water (Pile et al. 1996; Ribes et al. 1999). On the open reef, sponges co-occur with benthic organisms like corals and algae, whereas in the cryptic reef framework, which forms the largest habitat on well-developed coral reefs (Ginsburg 1983), sponges dominate the cover (Vasseur 1974; Wunsch et al. 2000; Van Duyl et al. 2006). Coral cavities are sinks of bacterio- and phytoplankton (Richter et al. 2001; Scheffers et al. 2004). Recently it has been demonstrated that the cryptic reef framework is a major sink of dissolved organic carbon (DOC), with DOC removal rates exceeding bacterio- and phytoplankton removal rates by two orders of magnitude (De Goeij and Van Duyl 2007). Heterotrophic bacterioplankton are the dominant consumers of reactive DOC in the ocean (Harvey 2006). However, although the magnitude of eukaryotic uptake of DOC is likely to be small relative to prokaryotic uptake, there is growing evidence that some eukaryotes may be directly fuelled by DOC, such as flagellates (Tranvik et al. 1993), and invertebrates like bivalves (Roditi et al. 2000). Moreover, evidence is accumulating that tropical sponges are important removers of bulk DOC from the passing water (Yahel et al. 2003; De Goeij et al. 2008a). Substantial bulk DOC removal was found by three common encrusting coral cavity sponges, including the sponge *Halisarca caerulea*, with DOC removal accounting for more than 90% of the total organic carbon removal (De Goeij et al. 2008a). The uptake and processing, or carbon flow pathways, of DOC compared with particulate sources of nutrition in sponges need to be unraveled.

Sponge-associated bacteria have been assumed to be capable of utilising dissolved organic matter (DOM) from ambient water (Frost 1987; Ribes et al. 1999). Proline uptake by sponge-associated bacteria in *Chondrosia reniformis* corroborates these assumptions, but direct uptake of amino acids by sponge cells may also occur, considering the presence of required uptake systems (Wilkinson and Garrone 1980). On the actual utilisation, partitioning, and possible translocation of natural diets, like DOM, or particulate food sources between sponge cells and sponge-associated bacteria, virtually no data are available.

Fatty acid biomarkers have been repeatedly used as source-specific indicators of DOM and POM both in environmental and food web studies (e.g. Canuel et al. 1995; Hall et al. 2006). In this study, fatty acid source designation was achieved using the different data now available on distinctive fatty acids for bacteria, algae, and sponges (Carballeira et al. 1987; Volkman et al. 1998; Boschker and Middelburg 2002).

We examined the assimilation and respiration of stable carbon isotope-enriched substrates by the sponge *Halisarca caerulea* (Porifera: Demospongiae). *H. caerulea* is an encrusting coral cavity sponge with sponge-associated bacteria and is common on the fringing reefs along Curaçao, Netherlands Antilles (Vacelet and Donadey 1987; De Goeij et al. 2008a). In incubation experiments *in situ* with natural seawater only ~40% of the removed DOC by *H. caerulea* was found to be respired, where ~60% of the carbon was assimilated (De Goeij et al. 2008a). In this study, tracer assimilation and respiration by the sponge is tracked as excess ^{13}C in tissue (bulk and compound specific) and dissolved inorganic carbon (ΣCO_2) (Middelburg et al. 2000; Moodley et al. 2000). In addition to commercially available ^{13}C-enriched glucose, we utilised organic matter extracted from diatoms enriched in ^{13}C, representing biogenic dissolved and particulate organic matter (POM), and ^{13}C-enriched bacteria.

We used fatty acid analysis for *H. caerulea* to establish assimilation and allocation of DOM and POM in sponge cells and sponge-associated bacteria. We followed ^{13}C-enrichment in fatty acid biomarkers to elucidate sponge-associated bacteria mediated versus direct sponge uptake of DOM, glucose, POM, and bacterioplankton for *H. caerulea*. We investigated the possible translocation of matter from the sponge-associated bacteria and the sponge cells by taking samples at different time intervals (1 h and 6 h, respectively).

MATERIALS AND METHODS

Location and sponge collection

This study was conducted on the Caribbean island of Curaçao, Netherlands Antilles (12°12'N, 68°56'W). Incubation experiments were performed at the aquarium building of the Caribbean Marine Biology & Ecology (CARMABI) foundation. Sponges were collected by SCUBA diving at station Buoy 1 (De Goeij and Van Duyl 2007). We used the common, thin (0.8-2.5 mm) cavity-dwelling encrusting sponge *Halisarca caerulea* for our experiments (*Fig. 5.1*).

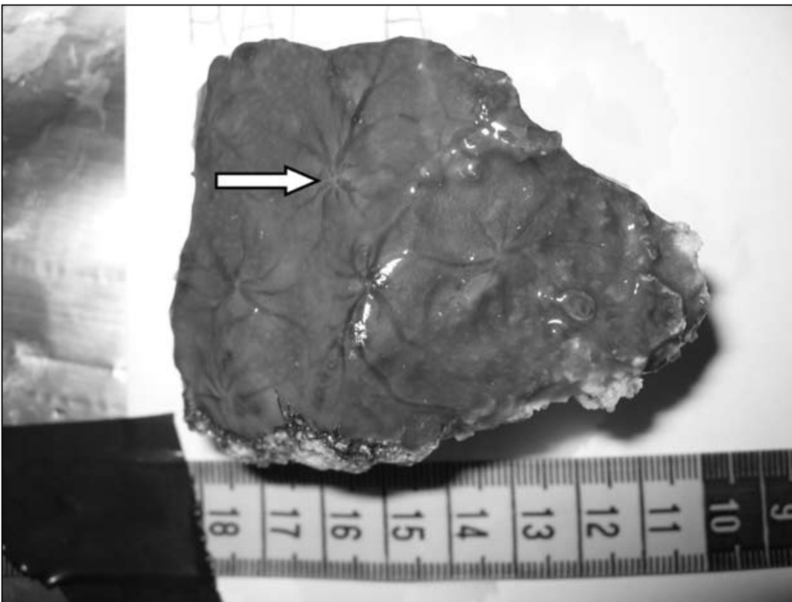


Fig. 5.1 - Halisarca caerulea *(Porifera: Demospongiae). Encrusting, coral reef cavity sponge (~2.5 mm thick; color purple), showing six oscula (1 osculum indicated with arrow).*

Pieces of sponge were chiselled from overhangs and coral cavity walls between 15 and 25 m of water depth. Attached pieces of coral rock were cleared from epibionts and sponges were transferred to aquaria of ~100 L. Before the incubation experiments, sponges were acclimatised for at least one week. They were regularly visually checked and when necessary, the substratum was cleaned and debris removed. The aquaria were kept at around 26°C in the dark and in reef water, pumped from 10 m of depth from the reef slope near the institute, which was refreshed at 3 L min^{-1}.

^{13}C-labelled substrates

We administered four ^{13}C-labelled substrates to *H. caerulea*: glucose, DOM extracted from the axenic diatom *Skeletonema costatum* (diatom-DOM), particulate residue of *S. costatum* after extraction of DOM (diatom-POM), and prelabelled bacteria. The substrates were prepared to aim at using an addition of 100 µmol L^{-1} organic carbon in the incubation experiments.

Tracer glucose containing 20% ^{13}C was made by mixing unlabelled glucose with isotope-enriched glucose (Cambridge Isotope Laboratories, 98-99% ^{13}C). This mix was dissolved in 0.2 µm filtered seawater and aliquots (3 mL) were stored at -20°C.

The two algal-derived substrates, diatom-DOM and diatom-POM, were extracted from an axenic diatom culture, labelled and concentrated as described earlier (Moodley et al. 2002). Axenic diatom cells were thoroughly rinsed to remove residue ^{13}C-enriched bicarbonate, concentrated by centrifugation and then freeze-dried. First, DOM was isolated: a known weight of freeze-dried diatom cells (we estimated the amount required by assuming 20% C for this diatom) was mixed with MilliQ, vortexed, centrifuged, and the supernatant collected. After three extractions, collective supernatant was passed through a 0.2 µm polycarbonate filter (Millipore) to isolate DOM caught in glass tubes that were subsequently freeze-dried and stored frozen until used in the experiment. Before the experiment 0.2 µm filtered seawater was added to dissolve and mix the DOM and aliquots (6 mL) were stored at -20°C.

The residue diatom cells were again rinsed and centrifuged and the pellet freeze-dried and used as particulate diatom organic matter substrate. Similarly, a known amount of dried substrate (8 mg of C) was kept in centrifuge tubes and just before to the experiments, 0.2 µm filtered seawater was added and well-mixed aliquots (6 mL) were stored at -20°C. For both algal derived substrates, a sub-sample was stored and used for later accurate measurement of carbon content and isotope labelling (atomic % ^{13}C).

Bacteria were prelabelled with ^{13}C. Natural seawater (1 L) was collected, prefiltered (0.7 µm; GF/F), and concentrated (approximately 10^7 bacteria mL^{-1}) by ultrafiltration (0.2 µm; Vivaflow). The inoculum was added to culture medium M63 (Miller 1972) amended with thiamine (0.0001%) and $MgSO_4$ (67 µmol L^{-1}) and glucose as carbon source (20% ^{13}C, *see* above). The culture was grown for 48 h in the dark at 25°C and ^{13}C-labelled bacteria were isolated by centrifuge (7 min, 500 rpm), rinsed in 0.2 µm filtered seawater, centrifuged,

and the pellet resuspended in 0.2 μm filtered seawater (30 mL), divided in six aliquots (5 mL) and stored at 4°C.

The exact amount of C added and the amount of ^{13}C-labelling was determined simultaneously on the gas chromatograph-isotope ratio mass spectrometer (GC-IRMS) (Moodley et al. 2002), except for the prelabelled bacteria, where the amount of C used in the incubations was estimated from bacterial abundances at t = 0, and the subsequent estimate of the amount of bacterial C. Samples for bacterial abundance in seawater (10 mL) were fixed in 4% paraformaldehyde (PFA) and stained with acridine orange and gently (max. 20 kPa suction pressure) filtered onto 0.2 μm black polycarbonate membrane filters (Millipore, 25 mm), mounted on slides, and stored at -20°C. Bacterial volume was established using epifluorescence microscopy (Zeiss, × 1,000) and digital imaging software (Axiovision, Zeiss), according to Heldal et al. (1985). A bacterial C-to-volume ratio of 100 fg μm^{-3} was used for cultured bacteria (Fagerbakke et al. 1996). The percent labelling was equal to the percentage ^{13}C-glucose used as carbon source (20%).

To facilitate interpretation of substrate assimilation on the fatty acid level, substrates were also analysed for fatty acid composition (*see* below for methods); fatty acids were extracted from a known amount of freeze-dried substrate.

Incubation experiments

Pieces of sponge of similar size (three replicate incubations per treatment) were placed at the bottom of 2 L incubation chambers in 0.2 μm filtered seawater and closed with a lid equipped with a magnetic stirrer. The chambers were closed without trapping air in the system and experiments were performed in the dark at 26°C. To quantify respiration of the added substrate, water samples (5 mL) for ΣCO_2 were taken with a 5 mL polycarbonate syringe first, before adding ^{13}C-labelled substrate, and second, after addition of ^{13}C-labelled substrate (at t = 0 and 60 min and t = 0 and 360 min for 1 h and 6 h incubation experiments, respectively). The substrate was injected with a sterile syringe. Sampling water was replaced by 0.2 μm filtered seawater. After the incubation experiment sponges were thoroughly washed in 0.2 μm filtered seawater and surface area was measured. Sponges were dipped in 0.2 μm filtered double distilled water and tissue was collected in precombusted (4 h, 450°C) glass vials and subsequently dried at 50°C for 24 h and stored at -20°C until further processing. The incuba-

tion chambers were chemically sterilised in hydrochloric acid (overnight, 0.4 mol L^{-1}) before the experiments. Control incubations were incubations without sponge to quantify possible bacterial respiration in the incubation water (0.2 µm filtered seawater). Controls were done only with the glucose substrate, but, being a substrate generally rapidly respired by bacteria, this correction was considered maximum and also applied to the diatom-DOM substrate.

Sample treatment and analysis

Duplicate water samples (2 mL) for ΣCO_2 were transferred through a precombusted GF/F filter into preweighed 5 mL N_2-filled headspace vials, immediately acidified (1 µL 80% H_3PO_4 per mL sample), and stored refrigerated upside down until analysis. Dried sponge material was carefully homogenised and after determining total dry weight, a subsample of known weight was taken for bulk tissue carbon isotope analysis. The organic carbon content (biomass) of each individual sponge was determined directly from the area counts given in the standard output of the IRMS (Moodley et al. 2000, 2002). Details of the measurement of δ^{13}-ΣCO_2 and $\delta^{13}C$ of tissue are given in Moodley et al. (2000). Headspace ΣCO_2 and sponge tissue (acidified in silver capsules) carbon content and isotopic composition was measured using a Carlo Erba 1500 elemental analyser coupled online with a Finnigan Delta S IRMS. Carbon content of the different pools was determined directly from area counts extracted from the isotope measurements (Moodley et al. 2000, 2002).

Extraction and preparation of fatty acid methyl esters (FAME) were carried out according to Masood et al. (2005) with reagent volumes adapted for 2.5 mL GC vials using fatty acid 19:0 as internal standard to calculate concentration of fatty acids. Total fatty acids were extracted from 10 mg dry weight sponge material. Concentration and carbon isotopic composition of individual FAME was determined with a GC-combustion interface-IRMS consisting of a HP G1530 GC (Hewlett Packard) connected to Delta-plus IRMS via a type III combustion interface from Thermo Finnigan. Identification of FAMEs is based on comparison of retention times with authentic reference materials on a HP 5MS analytical column, confirmed by GC-MS analysis. Stable carbon isotope ratios ($\delta^{13}C$) of individual fatty acids were calculated from FAME data by correcting for the one carbon atom in the methyl group that was added during derivatisation (Boschker and Middelburg 2002).

Carbon isotopes ratios ($\delta^{13}C$) were calculated using conventional methods relative to Vienna Pee Dee Belemnite and tracer carbon incorporation was quantified through determining excess (above background) ^{13}C content in the different pools (bulk sponge tissue, sponge fatty acid, and ΣCO_2). The product of fraction excess ^{13}C and carbon content of the pool examined equals tracer ^{13}C incorporation, which divided by fraction labelling of the substrate (0.20 for glucose and bacteria and 0.12 for organic matter extracted from diatoms, *Table 5.1*) provides the amount of total tracer carbon ^{12}C + ^{13}C incorporated (Middelburg et al. 2000; Moodley et al. 2000). For the background isotope signature of ΣCO_2 (as measure of substrate respiration) we did not use ambient $\delta^{13}CO_2$, but that of water samples taken from incubation chambers at t = 0. In the case of extracted fatty acids from sponge bulk tissue we used $\delta^{13}C$ of these components from sponges not exposed to isotope-enriched substrates (Moodley et al. 2002).

Table 5.1 - Characteristics of ^{13}C-enriched substrates. Amount of tracer organic C added per incubation (2 liters), the percentage of ^{13}C labelling, and the percentage of fatty acid C.

Substrate	C added (μmol incubation^{-1})	^{13}C labelling (% of added C)	Fatty acid C (% of added C)
Glucose	196	20	0
Diatom-DOM	156	12	12
Diatom-POM	168	12	49
Bacteria	254	20	5

RESULTS

Bulk organic carbon respiration and assimilation

The substrates were adequately labelled with ^{13}C as a tracer (12-20% ^{13}C compared to ~1% under natural conditions) and ultimately 156-254 µmol C was added to the incubations (each incubation with a volume of 2 L; *Table 5.1*). The biomass of sponges used for the incubations averaged 3999 ± 816 µmol C (± SE; n = 24) with a corresponding surface area of 26 ± 8 cm^2 (± SE; n =24). Because of the variation in sponge biomass, the tracer processing is expressed per unit biomass sponge (mmol C_{sponge}). In addition, to avoid confusion on terminology, by *H. caerulea* or sponge-microbe association we mean the sponge with associated bacteria. The compartmentalisation between sponge cells and associated bacteria is specifically referred to in the text. Control incubations without sponge revealed limited respiration (after 6 h 0.4-4.4% of that measured with sponges with glucose or diatom-DOM additions) and measurements were corrected accordingly.

Uptake and respiration of the different substrates was rapid but there was clear differential processing of the different substrates by *Halisarca caerulea* (*Table 5.2; Fig. 5.2*). On the basis of the trends after 1 h incubations, fastest and comparable uptake was recorded in diatom-DOM and bacteria additions (*Table 5.2; Fig. 5.2*) in spite of strong difference in the amount of substrate offered (*Table 5.1*). For diatom-DOM >90% of the uptake was recorded already within 1 h (*Fig. 5.2*). Clearly more time was required to metabolise diatom-POM, and glucose was slowest and least processed. Assimilation by the sponge was on average the major fate of metabolised glucose (64 ± 7%), diatom-DOM (65 ± 25%), diatom-POM (97 ± 5%), and bacteria (91 ± 2%) (± SD; n = 3) after 1 h of incubation. Respiration accounted of a small fraction after 1 h and 6 h (*Table 5.2 and Fig. 5.2*).

Fatty acid composition of substrates and *Halisarca caerulea*

No fatty acids were detected in the glucose substrate, as expected. Fatty acids typical of diatoms were encountered in both diatom-DOM and diatom-POM (*Fig. 5.3A,B*). Verified axenic conditions (Moodley et al. 2000) validate algal carbon origin. There were slight differences between these two substrates: diatom-POM(*Fig. 5.3B*) was dominated by four fatty acids (14:0, 16:1ω7c, 16:0, and 20:5ω3), together accounting for ~90% of total fatty acids. In diatom-DOM, fatty

Table 5.2 Halisarca caerulea *bulk assimilation and respiration of the four ^{13}C-enriched substrates after 1 h and 6 h of incubation (% tracer C mmol C_{sponge}^{-1}; ± SD; n = 3).*

Substrate	1-h Incubation		6-h Incubation	
	Respiration	Assimilation	Respiration	Assimilation
	Tracer C processed (% tracer C mmol C_{sponge}^{-1})			
Glucose	0.03 ± 0.01	0.06 ± 0.03	0.35 ± 0.05	0.17 ± 0.03
Diatom-DOM	1.33 ± 1.65	1.99 ± 1.08	0.91 ± 0.29	2.54 ± 0.19
Diatom-POM	0.01 ± 0.02	0.25 ± 0.07	0.55 ± 0.43	1.43 ± 0.83
Bacteria	0.41 ± 0.11	3.93 ± 0.04	1.09 ± 0.51	12.47 ± 3.89

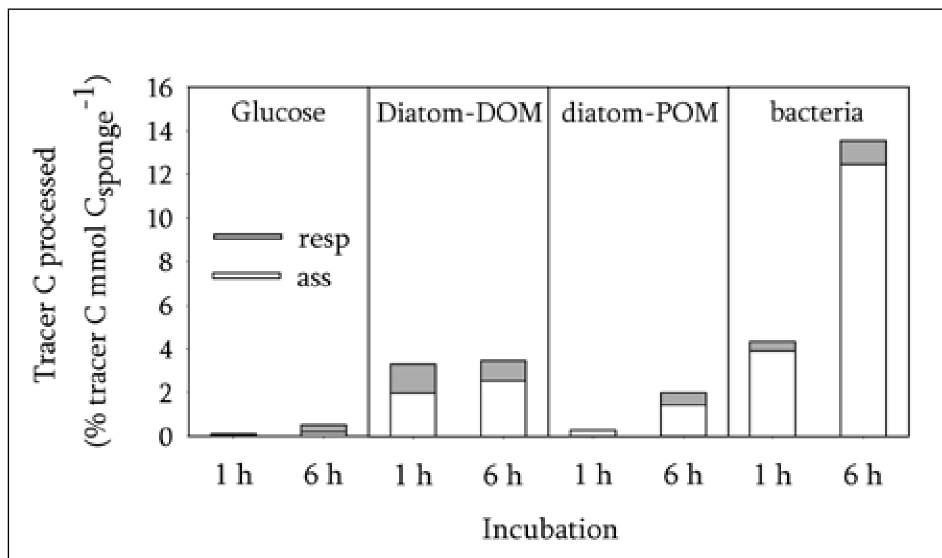


Fig. 5.2 - The amount of tracer C recovered in sponge tissue (% tracer C mmol C_{sponge}^{-1}) after 1 h and 6 h incubation with the different ^{13}C-labelled substrates. Values are average of three replicates (see Table 5.1) of respiration (gray bars) and assimilation (open bars) measurements.

acids were more evenly distributed (*Fig. 5.3A*). Furthermore, fatty acid C accounted for 12% of the total C in diatom-DOM and 48% in diatom-POM (*Table 5.1*).

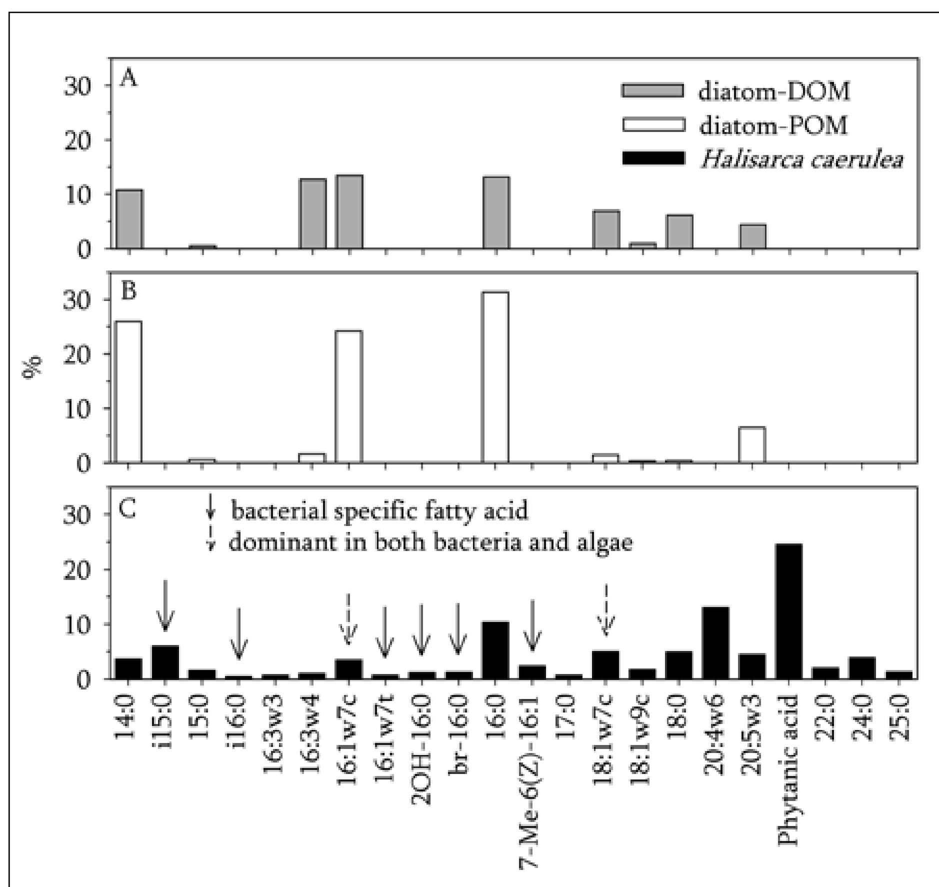


Fig 5.3 - Fatty acid composition of diatom-derived substrates (A) diatom-DOM, (B) diatom-POM, and (C) the fatty acid composition of the encrusting coral cavity sponge Halisarca caerulea. Depicted are fatty acids having an occurrence of ≥0.5% of the total fatty acid C of H. caerulea; mean (n = 3).

For *H. caerulea*, total fatty acid C was on average 1.61 ± 0.08% (average ± SE; n = 25) of the sponge-microbe carbon biomass and its composition is depicted in *Fig. 5.3C*. Fatty acids shown in *Figs. 5.3 and 5.4* are only those with ≥0.5% share of total fatty acids for *H. caerulea* (41 fatty acids have been identified, of which 22 have an occurrence of ≥0.5%). Phytanic acid, common in some sponges (Carballeira et al. 1989) and assigned as nonbacteria fatty acid, was the most dominant, accounting for 24.6 ± 0.6% (average ± SE; n = 25) of fatty acid

C. Bacteria-specific fatty acids in the sponge (listed in *Table 5.3*) accounted for a significant fraction of total fatty acids. Together they accounted on average for 13.8 ± 0.3 or 22.2 ± 0.5% (± SE; *n* = 25) without or with fatty acids 18:1ω7c and 16:1ω7c; although dominant in bacteria, 18:1ω7c can also contribute to algal C (*see* also *Fig. 5.3A,B*). Similarly, fattyacid 16:1ω7c can be a strong component of both algae and bacteria (Volkman 2006, and references therein), also evident in substrates used in this study (*Fig. 5.4*). However, these two fatty acids can still be reliably assigned to a source depending on whether they are complemented with a large bacteria-specific or algal-specific contribution (Dalsgaard et al. 2003).

Table 5.3 - Amount (μmol tracer C mmol C_{sponge}^{-1}; ± SD; n = 3) and distribution of tracer C in fatty acids of Halisarca caerulea*, as percentage of total assimilation and proportion of tracer in bacteria-specific fatty acids after 1 h and 6 h incubations.*

	Glucose		Diatom-DOM		Diatom-POM		Bacteria	
	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h
Tracer C in fatty acids	-	0.01 ± 0.00	0.48 ± 0.20	0.53 ± 0.08	0.08 ± 0.02	0.95 ± 0.43	0.69 ± 0.12	1.53 ± 0.43
% Total assimilation	-	3	16	13	19	40	7	5
% Bacteria specific[a]	-	37	20	20	7	7	47	45

[a] i15:0 ai15:0 i16:0 16:1ω7t 2OH-16:0 br-16:0 i17:0 7-Me-6(Z)-16:1 18 :1ω7c (see also Fig. 5.3C)

Compound specific tracing of substrate assimilation by *Halisarca caerulea*

As in *H. caerulea* bulk tissue analysis, substrate assimilation was also clearly evident in fatty acids extracted from sponge-microbe associations postincubation (*Fig. 5.4*), and represented different fractions (3-40%) of total tracer C assimilation (*Table 5.3*). Similar to trends observed in bulk tissue analysis, minimum tracer recovery in fatty acids was found in incubations with glucose (*Table 5.3*). However, given that fatty acids were absent in the glucose substrate itself, this demonstrates that fatty acids were synthesised *de novo* from glucose after 6 h (*Fig. 5.4A*) and confirm active assimilation of substrates. Of the total tracer carbon recovered in fatty acids after incubation with glucose, a large fraction (37%, including 18:1ω7c) was bacteria-specific (*Table 5.3*). The strong dominance of 18:1ω7c complemented with bacteria-

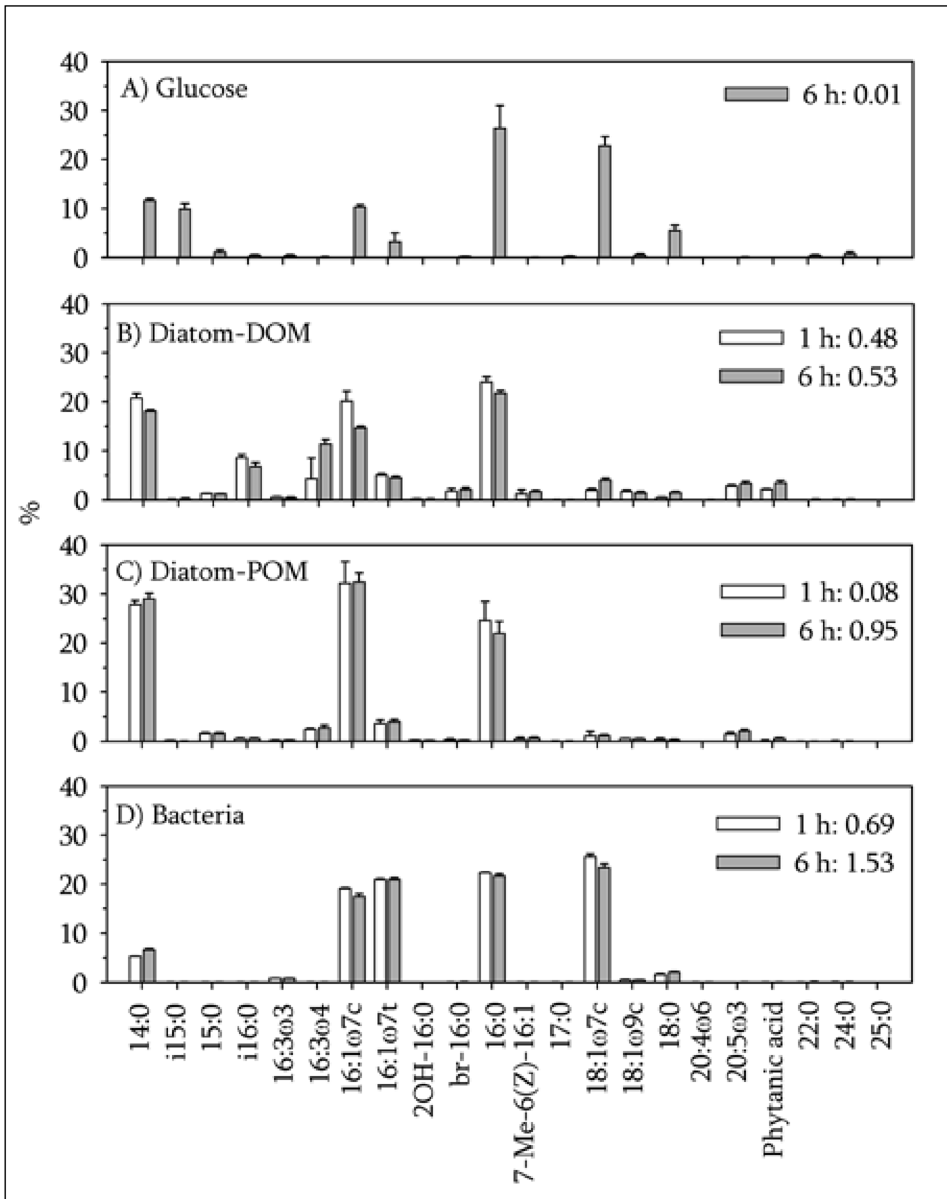


Fig. 5.4 - Distribution of tracer C among the sponge fatty acids (% of total) after 1 h and 6 h incubation (absolute tracer C in fatty acids is presented in box insert in upper right of each panel; see also Table 5.3). Depicted are fatty acids having an occurrence of ≥0.5% of the total fatty acid C of H. caerulea *(for complete list see Table 5.4). Open and gray bars represent patterns respectively after 1 h and 6 h incubation for (A) glucose, (B) diatom-DOM, (C) diatom-POM, and (D) prelabelled bacteria; mean ± SD (n = 3).*

specific 16:1ω7t and i15:0, together with the absence of eukaryotic phytoplankton fatty acids (e.g. 16:3ω4 and 20:5ω3) support uptake of glucose by sponge-associated bacteria. This indicates that glucose assimilation in fatty acids was primarily bacteria-mediated, but does not exclude nonbacterial uptake and remains unresolved because of fatty acids being common in both prokaryotes and eukaryotes.

A totally different trend was found for the fatty acid assimilation in *H. caerulea* after diatom-DOM and diatom-POM substrate incubations (*Fig. 5.4B,C*). Tracer carbon was recovered both in bacteria-specific fatty acids and in nonbacterial fatty acids. First, the observed patterns of tracer C allocation among fatty acids extracted from incubated *H. caerulea* reflect strongly that of the diatom derived substrate. For diatom-DOM, the dominant fatty acids 14:0, 16:3ω4, 16:1ω7c, 16:0, and 20:5ω3 together accounted for ~60% both in the substrate and tracer allocation from sponge extracted fatty acids (compare *Figs. 5.3A* and *5.4B*). For diatom-POM, >80% was accounted for by fatty acids both present in the substrate and tracer found in *H. caerulea* extracted fatty acids (14:0, 16:1ω7c, 16:0, and 20:5ω3; compare *Figs. 5.3B* and *5.4C*). This indicates that the major fatty acids were directly ingested by *H. caerulea* in the diatom-DOM as well as in the diatom-POM treatment and not bacteria-mediated.

The direct use of substrate-specific fatty acids by the sponge-microbe association also explained the larger total tracer assimilation in fatty acids for diatom-POM after incubation (40% after 6 h, *Table 5.3*) as compared to diatom-DOM (13% after 6 h, *Table 5.3*), in spite of the overall higher bulk removal of diatom-DOM (*Table 5.1*). The POM substrate contained a much higher absolute fatty acid carbon content (49%, *Table 5.1*), than the DOM substrate (12%, *Table 5.1*).

In both the diatom-DOM and diatom-POM additions, tracer carbon was also recovered in fatty acids that were not present in the substrates (i15:0, i16:0, 16:1ω7t, 2OH-16:0, br-16:0, 7-Me-6(Z)-16:1, and phytanic acid; absent in *Fig. 5.3A,B* and present in *Fig. 5.4B,C*). These fatty acids were primarily bacteria specific (i15:0, i16:0, 16:1ω7t, 2OH-16:0, br-16:0, and 7-Me-6(Z)-16:1) and one non-bacteria specific (phytanic acid). Phytanic acid was only found after incubations with diatom-derived substrates, primarily after diatom-DOM additions and not after administration of glucose or bacteria, indicating algal carbon origin. Clearly, tracer uptake was both bacteria and direct sponge mediated and

consequently fatty acids common to both organisms containing high fraction of tracer carbon such as 14:0 and 16:0 reflect both consumers. POM is assumed to be primarily incorporated by sponge cells.

The bacteria substrate was not profiled for fatty acid composition but the assimilation pattern in *H. caerulea* possessed a fatty acid distribution (depicted in *Fig. 5.4D*) almost identical (>95%) to estuarine bacteria cultured and labelled identical as in this study (L. Moodley, unpubl.). Thus, the fatty acid pattern reflected directly the trophic resource and indicates limited modification or *de novo* fatty acid synthesis in the sponge-microbe association. Indeed, fatty acid profiles of bacteria change when exposed to single substrates away form natural conditions and depend upon the carbon source that can produce identical patterns in bacteria from different locations and even lack otherwise bacteria-specific branched fatty acids such as iso and anteiso fatty acids (Harvey 2006; L. Moodley, pers. obs.). Consequently, this fatty acid composition pattern provides bacteria biomarkers for this setting (strong dominance of 16:1ω7t and 18:1ω7c) and an indication of fatty acid carbon contribution to total bacteria carbon content (~5%), similar to values estimated for natural populations (Middelburg et al. 2000).

For the substrates glucose, diatom-POM, and bacteria, there was a consistent increase of tracer C in *H. caerulea* during the 6 h incubation (*Tables 5.3* and *5.4*). In contrast, diatom-DOM was almost depleted after 1 h, with more than 90% of the tracer recovered. Therefore, shifts in label distribution in different fatty acids between 1 h and 6 h can be ascribed to possible reallocation of tracer C such as in a pulse-chase experiment. However, there was no significant difference in the fatty acid signature, and distribution of tracer in *H. caerulea* after 1 h and 6 h of incubation with diatom-DOM (ANOVA; F_{40} = 1.226, p = 0.189; *Fig. 5.4B*).

Table 5.4 - Complete list of ($\times 10^{-3}$ µmol tracer C mmol C_{sponge}^{-1}) tracer carbon distribution in fatty acids of Halisarca caerulea after 1 h and 6 h incubations; average ± SD (n = 3).

Fatty acid	Glucose		Diatom-DOM		Diatom-POM		Bacteria	
	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h
10:0	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
12:0	-	< 1	< 1	< 1	< 1	< 1	15 ± 1	29 ± 6
i14:0	-	< 1	< 1	< 1	< 1	< 1	1 ± 0	2 ± 1
14:0	-	1 ± 0	102 ± 41	95 ± 5	21 ± 2	286 ± 131	37 ± 4	102 ± 17
ai15:0	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
i15:0	-	1 ± 0	< 1	1 ± 1	< 1	< 1	< 1	< 1
15:0	-	< 1	6 ± 3	7 ± 0	1 ± 0	16 ± 7	< 1	1 ± 0
i16:0	-	< 1	43 ± 18	36 ± 5	< 1	5 ± 2	< 1	< 1
16:3ω3	-	< 1	3 ± 1	2 ± 1	< 1	3 ± 1	6 ± 1	14 ± 2
16:3ω4	-	< 1	25 ± 25	60 ± 5	2 ± 0	26 ± 14	< 1	< 1
16:1ω7c	-	1 ± 0	91 ± 33	77 ± 4	24 ± 2	305 ± 140	132 ± 13	269 ± 49
16:1ω7t	-	< 1	25 ± 10	24 ± 1	3 ± 0	38 ± 19	145 ± 14	323 ± 56
2OH-16:0	-	< 1	1 ± 0	1 ± 1	< 1	1 ± 0	< 1	< 1
br-16:0	-	< 1	7 ± 2	11 ± 2	< 1	3 ± 1	< 1	1 ± 0
16:0	-	3 ± 0	119 ± 48	114 ± 7	19 ± 4	207 ± 83	154 ± 15	332 ± 51
ai17:0	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
i17:0	-	< 1	2 ± 1	2 ± 0	< 1	1 ± 0	< 1	< 1
7-Me-6(E)-16:1	-	< 1	1 ± 0	1 ± 0	< 1	1 ± 0	< 1	< 1
7-Me-6(Z)-16:1	-	< 1	3 ± 0	9 ± 1	< 1	7 ± 3	< 1	< 1
cy-17:0	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
17:0	-	< 1	1 ± 0	1 ± 0	< 1	1 ± 0	< 1	1 ± 0
18:3ω6	-	< 1	1 ± 0	1 ± 0	< 1	2 ± 1	< 1	< 1
18:1ω7c	-	2 ± 0	10 ± 5	21 ± 3	1 ± 1	12 ± 6	178 ± 21	356 ± 49
18:1ω9c	-	< 1	9 ± 4	8 ± 2	< 1	6 ± 3	4 ± 0	7 ± 1
18:0	-	1 ± 0	2 ± 1	8 ± 1	< 1	4 ± 2	12 ± 2	33 ± 5
19:1	-	< 1	1 ± 0	< 1	< 1	< 1	< 1	< 1
20:4ω6	-	< 1	< 1	< 1	< 1	< 1	< 1	1 ± 0
20:5ω3	-	< 1	13 ± 5	17 ± 3	1 ± 0	20 ± 11	< 1	< 1
20:3ω6	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Phytanic	-	< 1	10 ± 4	18 ± 3	< 1	7 ± 3	< 1	1 ± 0
20:1ω9c	-	< 1	1 ± 0	2 ± 0	< 1	1 ± 1	4 ± 1	27 ± 6
20:2ω9	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
20:0	-	< 1	< 1	< 1	< 1	< 1	< 1	2 ± 1
21:0	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
22:6ω3	-	< 1	2 ± 1	2 ± 0	< 1	1 ± 0	< 1	< 1
22:1ω9	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1
22:1	-	< 1	1 ± 0	2 ± 0	< 1	< 1	1 ± 0	14 ± 2
22:0	-	< 1	< 1	1 ± 0	< 1	< 1	< 1	3 ± 0
24:1ω9	-	< 1	1 ± 0	3 ± 0	< 1	1 ± 0	1 ± 0	11 ± 1
24:0	-	< 1	< 1	1 ± 0	< 1	< 1	< 1	1 ± 0
25:0	-	< 1	< 1	< 1	< 1	< 1	< 1	< 1

Discussion

DOM assimilation

Most studies on sponge feeding (Reiswig 1971; Pile et al. 1996; Ribes et al. 1999), including recent studies on DOC removal from ambient water by sponges (Yahel et al. 2003; De Goeij et al. 2008a), report indirect observations of net fluxes of organic matter through the sponge. Actual proof of assimilation of organic matter - and DOM in particular - by sponges was lacking. We traced ^{13}C excess in the bulk tissue of the tropical encrusting sponge *Halisarca caerulea* and in its fatty acids after supply of dissolved and particulate ^{13}C-labelled organic substrates. The observed bulk ^{13}C-enrichment in sponge tissue and the excess $^{13}CO_2$ release reveals that *H. caerulea* actually processes both DOM and POM. To the best of our knowledge, this is the first direct evidence of bulk DOC processing by a sponge. The ^{13}C-enrichment in the sponge is evidently not an artefact such as accumulation of the food source in or on the sponge, because the assimilation of substrate is also evidently compound-specific (in sponge-microbe fatty acids). This is especially convincing for ^{13}C-enrichment in fatty acids that are not present in the substrate, i.e. conversion of nonfatty acid compounds into fatty acids (i.e. all the fatty acids recorded in the case of the 6 h glucose additions, and both the bacteria-specific fatty acids and phytanic acid in the cases of diatom-DOM and diatom-POM treatments (*Figs. 5.3 and 5.4*)).

We could not assess a clear quantitative preference for particulate over dissolved food except for glucose, which seemed to be an unpreferred substrate. This suggests discrimination by the sponge between algal-derived DOM (containing carbohydrates, amino acids, some lipids, and fatty acids) and glucose. A comparable pattern has been reported for the zebra mussel: limited glucose uptake and rapid assimilation of fatty acids (Baines et al. 2005). Glucose may be an unpalatable food source for invertebrates.

The other substrates seem to be processed by the sponge proportionally to the availability of the source. The concentration of bacterioplankton in the experiments was 50 times higher than under natural conditions, where the DOM concentration was only two times higher than in the field (Van Duyl et al. 2002; De Goeij and Van Duyl 2007). Moreover, the utilisable part of diatom-DOM was virtually depleted after 1 h incubation, when 12% of the added C was processed. Diatom–POM and bacteria were not depleted during

the experiment. Because POM sources in the coral reef framework cavities are one to two orders of magnitude lower in concentration than DOM, the absolute DOM assimilation on total nutrition seems to exceed plankton and detritus assimilation by this sponge under *in situ* conditions as suggested in De Goeij and co-workers (2008a).

The finding that the sponge-microbe association is an important sink of DOM draws attention to how they might exceed bacterioplankton DOM cycling within their ecosystem. It is well established that free-living heterotrophic bacterioplankton (or prokaryotes) are the dominant consumers of reactive DOC in the ocean (Harvey 2006); they represent the largest living surface area in the sea and have the ability to directly transport low-molecular-weight compounds (500-1,000 Da) through their cell membranes (Carlson 2002). However, a significant fraction of operationally defined DOC in the dissolved phase consists of colloidal material; submicron colloidal organic carbon (1 kDa – 0.22 μm) is abundant in natural waters (Guo and Santschi 1997). The importance of the colloidal fraction of DOC as intermediate between "truly dissolved" and particulate organic carbon has been recognized (Koike et al. 1990). Colloids and aggregates formed in the DOC fraction constitute a reactive carbon pool for active filter-feeding shellfish (Roditi et al. 2000; Baines et al. 2005). This may also hold for sponges that filter large volumes of water through choanocyte chambers. Chambers are lined with choanocyte cells, that are potentially capable of assimilating both "truly dissolved" and DOC aggregates besides plankton, potentially providing a competitive edge over small particles like bacterioplankton. Whereas the associated bacteria utilise true DOM, sponge cells are capable of utilising the colloidal fraction of DOM. Together; the sponge-microbe association is able to utilise a unique palette of nutrients over single-cellular organisms or organisms lacking associated prokaryotes.

Fatty acids

Except for glucose, all substrates contain fatty acids, of which a proportional fraction is recovered being assimilated in the sponge. After 6 h of incubation with diatom-DOM, diatom-POM, and bacteria, the percentageof total tracer assimilation in fatty acids in *H. caerulea* (13%, 40%, and 5%, respectively), is comparable with the percentage of fatty acid C present in the substrates (12%, 49%, and 5%). Conform the application of fatty acid bio-

markers - "you are what you eat" - *H. caerulea* fatty acid labelling patterns reflect primarily the fatty acid pattern of the substrate.

The ^{13}C-labelled fatty acids present in the substrates as well as in *H. caerulea* after incubation are ingested by the sponge-microbe association. We cannot be conclusive on the fate of the ingested matter. Is it processed by *H. caerulea* or only an accumulation of food? However, respiration found is evidence for actual C processing. The processing of glucose into fatty acids and the presence of fatty acids in *H. caerulea* not present in the substrates is the ultimate evidence of C processing by the sponge-microbe association. It is therefore highly unlikely that all of the ingested fatty acids are only accumulated in the sponge-microbe association. In addition, we want to point out that there were also several fatty acids found in the diatom-DOM and diatom-POM substrates (e.g. 16:2ω7, 16:2ω4, 16:4ω1, 18:2ω6c, 18:4ω3, 22:6ω3; 16 fatty acids in total, showing only those with a presence of >0.5% of the total fatty acids) that were in fact not found postincubation in *H. caerulea*, suggesting that the ingested food has been altered by the sponge-microbe association.

Analysis of fatty acid composition is a very useful tool to unravel the source of carbon in bulk POM, DOM, or eukaryotic cells and tissue (Graeve et al. 1994; Meziane and Tsuchiya 2000; Hall et al. 2006). Phytanic acid was the most dominant fatty acid in *H. caerulea* and has its origin in phytol, a decomposition product of chlorophyll (Rontani and Volkman 2003), which is ubiquitous in the marine environment (Boon et al. 1975) occurring both in POM and DOM (Loh et al. 2006). An important pathway of phytol degradation producing phytanic acid is marine invertebrate feeding (Rontani and Volkman 2003). A link between chlorophyll and phytanic acid as a product of sponge feeding is supported by observations in this study. There was no phytanic acid present in the diatom-derived substrates but clear evidence of tracer carbon in phytanic acid extracted from the sponges post incubation, most clearly in the diatom-DOM treatment. Consistently, ^{13}C-enrichment in phytanic acid was absent in the glucose and bacteria additions. Therefore DOM may not only be the most abundant food source, it may also provide a necessary part of the sponges' diet.

Assuming that indeed the ultimate source of the most dominant fatty acid (phytanic acid) is algal chlorophyll derived, this and the other algal carbon fatty acid biomarkers found in sponge fatty acids (16:3ω3, 16:3ω4, 20:4ω6, and 20:5ω3) together account for ~45% of the total fatty acids of *H. caerulea.*

This suggests that algal-derived C (dissolved and particulate) constitutes the major source of carbon for this sponge. The second most abundant fatty acid was 20:4ω6 and although some marine invertebrates can synthesise 20:4ω6 from 18:2ω6 through chain elongation (Hall et al. 2006) and this precursor (18:2ω6) was present in both algal derived substrates used in this study (not shown, this fatty acid occurs in less than 0.5% of total fatty acids in *H. caerulea*), no tracer C was recovered in 20:4ω6 in the sponge after feeding on diatom-derived organic matter. This indicates an exogenous source of 20:4ω6 for the sponge under natural conditions. On coral reefs, the main sources of this fatty acid entering the particulate or dissolved pool include corals (Latyshev et al. 1991) from which mucus release represents a potential important source of organic matter, both particulate and dissolved, within the reef (Wild et al. 2004). Additionally, coralline algae including *Rhodophyta* species, have been observed to account for a major coverage in coral cavities (Wunsch et al. 2000; Van Duyl et al. 2006), and *Rhodophyta* species are a major source of 20:4ω6 (Viso and Marty 1993). This may imply that an important algal carbon source for the sponge originates nearby, on the reef and in coral cavities.

Sponge-microbe association

On the partitioning of organic matter between the sponge cells and the sponge-associated bacteria, knowledge is still limited. It is often assumed that if DOM consumption by sponges occurs, this is mediated by sponge-associated bacteria (Ribes et al. 1999; Yahel et al. 2003). Sponge direct uptake of substrate fatty acids (eukaryotic fatty acids), sponge *de novo* synthesis of phytanic acid, and tracer carbon recovery in bacteria-specific fatty acids (particularly after feeding of the sponge on diatom-DOM) provides evidence that sponge cells and sponge-associated bacteria both mediate in organic matter assimilation. This implies that sponges without associated bacteria may also assimilate (colloidal) DOM. Phytanic acid is the only fatty acid that can specifically be ascribed to sponge fatty acid but it is important to note that the direct uptake of eukaryotic fatty acids present in the substrates was also clearly evident (*Fig. 5.4*). Sponge cells can utilise small chain bacterial fatty acids, such as i-15:0 and ai-15:0 as well as 16:0, and elongate these fatty acids to very long-chain fatty acids (Carballeira et al. 1986) and may be traced in longer incubations to gain further insight into possible translocation of carbon between different compartments of the sponge-microbe association. In the present study, such

very long-chain fatty acids were not found in *H. caerulea*. The contribution of bacteria-specific fatty acids was more apparent after the dissolved substrates diatom-DOM (15%) and glucose (40%) than after incubation with diatom-POM (7%). It is possible that "truly" dissolved substrates are relatively more readily assimilated by bacteria than by sponge cells in the sponge than particulate sources, but it may depend on the compound to be assimilated. Therefore, we cannot be conclusive whether dissolved organic substrates are preferentially processed by the sponge-associated bacteria or by the sponge cells. Carbon transfer between bacteria and sponge was not evident after 1 h of incubation within the following 5 h but this observation does not exclude exchange or reallocation after a longer period.

Sponges and carbon flow modulation on the reef

The present study demonstrates that the common encrusting coral cavity sponge *H. caerulea* consumes and incorporates DOM as well as POM. The reported massive removal of ambient DOC in the presence of sponges (Yahel et al. 2003; De Goeij et al. 2008a) can definitely be ascribed to assimilation and respiration of DOM by the sponge-microbe association. It is highly likely that more sponges on coral reefs have the capacity to process DOM. Exploitation of DOM by sponges may be a common and profitable strategy on reefs, where the supply of utilisable DOC by far exceeds the supply of particulate carbon to the reef benthos (De Goeij and Van Duyl 2007; De Goeij et al. 2008a). Moreover, sponges occupy a considerable surface on coral reefs, particularly in the cryptic habitat, which is equally extensive, and often even larger than the "open" reef surface. Therefore, we conclude that DOM assimilation by sponges is an efficient strategy to retain locally produced DOM in the reef system, otherwise lost in the microbial loop or to the adjacent ocean. This eminent and quite unique capacity of sponges combined with their high cover may play a central role in modulating organic matter fluxes in the coral reef ecosystem.

CHAPTER 6

Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding

Abstract

This study reveals the peculiar *in vivo* cell kinetics and cell turnover of the marine sponge *Halisarca caerulea* under steady state conditions. The tropical coral reef sponge shows an extremely high proliferation activity, a short duration of the cell cycle, and massive cell shedding. Cell turnover is predominantly confined to a single cell population, i.e. the choanocytes, and in this process apoptosis only plays a minor role. To our knowledge, such fast cell kinetics and high turnover have not been observed previously in any other multi-cellular organism. The duration of the cell cycle *in vivo* resembles that of some uni-cellular organisms in culture. Morphological and histochemical studies demonstrate compartmentalisation of choanocytes in the sponge tissue. The functional morphology of the sponge *H. caerulea* corresponds well with its remarkable cellular kinetics. Coral reef cavity sponges, like *Halisarca caerulea*, inhabit very low nutrient tropical waters, forcing these organisms to filter large volumes of water and to capture the few nutrients very efficiently. Under these oligotrophic conditions, a high cell turnover can be considered as a very useful strategy of the sponge, preventing permanent damage by environmental stress. These data can be helpful to improve the culturing of sponges, which are recognised as important potential sources for new drugs, antibiotics and biomaterials. It is concluded that studies on cell kinetics and functional morphology may be important new steps in acquiring essential information on the regulation of sponge growth *in vivo* as well as *in vitro* and the role of choanocytes in tissue homeostasis.

Introduction

Sponges and other benthic suspension and filter feeders play a profound role in the food web of marine ecosystems (Gili and Coma 1998). These organisms filter vast volumes of water, efficiently remove organic matter from the water column and subsequently release inorganic nutrients. A tropical coral reef ecosystem is a highly productive system (Kinsey 1983; Hatcher 1988) surrounded by an oligotrophic tropical sea (Crossland 1983). The gross primary production, amongst the highest of any natural ecosystem, is almost completely balanced by the community respiration (Hatcher 1990; Croslland et al. 1991). The low net production suggests a tight coupling between the producing, autotrophic, part and the consuming, heterotrophic, part of the reef, to prevent loss of valuable nutrients from the system. In order to maintain the high gross production, the high demand of nutrients is met by efficient trapping, uptake, and recycling of nutrients, which can be accomplished by rapid decomposition and turnover. The major site for decomposition and regeneration of nutrients is the coral reef framework, the largest habitat of the reef (Richter et al. 2001; De Goeij and Van Duyl 2007). It has therefore been suggested that the turnover rates of the cryptic biota are presumably high (Hutchings 1983), although no data have yet confirmed this hypothesis. The walls of the reef framework cavities are dominated by, mostly encrusting, sponges.

Sponges and their associated bacteria play a key role in the uptake of organic matter within the cryptic habitat of the reef (De Goeij et al. 2008a). Here, the measured fluxes of organic carbon for sponges are the highest observed to date (De Goeij et al. 2008a). The sponge-microbe association is important for the ability to feed on a wide range of food sources and nutrient sizes (De Goeij et al. 2008b), from dissolved organic matter (Yahel et al. 2003; De Goeij et al. 2008a,b) to the capturing of zooplankton (Vacelet and Boury-Esnault 1995). For the cavity sponge, *Halisarca caerulea* (Porifera: Demospongiae) a major discrepancy was found between the high amount of organic carbon assimilated by the sponge (35-40% body C d^{-1}) and the low net increase of biomass by the sponge (close to zero). This organic carbon may be used for reproduction or the production of secondary metabolites, but may also point to a high turnover of matter. This is corroborated by observations of massive deposition of material by sponges (Witte et al. 1997). Moreover, sponges have a high plasticity, or regeneration capacity, with up to 2,900 times the normal growth rate after

tissue damage (Ayling 1983) and a high telomerase activity (Koziol et al. 1998). Both features imply the potential for a relatively rapid cell proliferation.

Since sponges have an integrated multicellular organisation they should have control mechanisms for growth, which is the net result of increase in cell number (cell proliferation) and cell loss. This way, organisms are able to maintain homeostasis in the various cell populations (Koziol et al. 1998). The rate of proliferation is determined by the proportion of cells actively proliferating (the growth fraction) and the duration of the cell cycle. Cell loss can occur by different mechanisms, including necrosis, programmed cell death (apoptosis), and shedding of cells. Knowledge on the cell kinetics is important to understand the growth characteristics of a cell or cell populations in tissues or organisms (Wilson 2007). Sponges possess most of the core elements of apoptosis, including members of the caspase family (Wiens and Müller 2006), which are pivotal enzymes in apoptosis (Kumar 2007). Sponge cell proliferation has been studied by the incorporation of the thymidine analogue 5-bromo-2'-deoxyuridine (BrdU) into cellular DNA (Custodio et al. 1998; Sun et al. 2007). So far, data on cell kinetic parameters are few, and analyses were only performed *in vitro*, not under *in vivo* conditions (Shore 1971).

Sponges possess various types of cells, of which the kinetic behaviour is largely unknown. These various cell types are structurally organised in the body of the sponge. The diploblastic sponge body is built around a system of chambers and canals, the aquiferous system, through which water is channelled for nutrition and respiration. The spherical chambers (choanocyte chambers) are lined by choanocytes, which are flagellated cells containing the basic pumping and filtering elements to take up material from the surrounding water. The aquiferous system is surrounded by the mesohyl, containing the granulo-fibrillar matrix and collagen fibrils, and various other cell types, such as archaeocytes and spherulous cells. Sponges lack internal organ. However, sponges may show compartmentalisation, allowing or preventing the exchange of cells from one compartment to the other. The relation of this functional organisation of the sponge to the kinetic behaviour of cells, proliferation and loss, is largely unknown.

The major discrepancy of organic carbon uptake in relation to growth found in *H. caerulea* and, subsequently, the lack of knowledge on *in vivo* growth characteristics of sponges, prompted us to investigate the process of cell turnover in this sponge.

To investigate the cell kinetics in *H. caerulea* we labelled the sponge tissue *in vivo* with BrdU and analysed the cell kinetic parameters using immunohistochemical staining of BrdU in tissue sections (Nowakowski et al. 1989). In order to study cell loss we determined *in vivo* apoptosis by immunohistochemical staining of sponge tissue using an antibody against active caspase-3. Furthermore, general and immunohistochemical staining methods were used to gain more insight in the structural organisation and cell turnover in sponge tissue.

Materials and Methods

Sponge collection

The thin (0.8-2.5 mm) cavity-dwelling encrusting sponge *Halisarca caerulea* was collected by SCUBA diving on the Caribbean island of Curaçao, Netherlands Antilles (12°12'N, 68°56'W). Pieces of sponge were chiselled from overhangs and coral cavity walls ranging from 15 to 25 m water depth. Attached pieces of coral rock were cleared from epibionts. In a first series of experiments the sponges were stored upside down in wire cages (20×20×15 cm; maximum of four pieces per cage) to protect them from sediment accumulation and predation. Cages were stored inside coral reef cavities at 15 m water depth. In a second series of experiments sponges were kept in aquaria (100 L) at 26°C in the dark. Water flow, reef water pumped up from 10 m of water depth from the reef slope, was regulated at 3 L min^{-1}. Sponges were acclimatised for at least one week, prior to incubation experiments.

BrdU-incorporation experiments

To determine the proliferation rate of cells in *H. caerulea*, sponges were placed in 1.7 L incubations chambers with a magnetic stirring device at 12 m water depth (first series) or in 2 L incubation chambers in aquaria (second series). Sponges were continuously labelled with 5-bromo-2'-deoxyuridine (BrdU, Sigma) in a final concentration of 50 µmol L^{-1} and experiments were performed in the dark at 26°C. Sponges (n = 3) were sacrificed at t = 0, 0.5, 1, 3, 6, and 10 h after BrdU exposure. Sponge tissue was fixed in 4% buffered paraformaldehyde (PFA; 4 h at 4°C), subsequently washed in phosphate buffered saline (PBS) and stored in PBS/ethanol (1:1) at -20°C. Fixed sponge tissue was dehydrated in a graded series of ethanol and embedded in butyl-methyl-methacrylate (BMM).

Sections (3 μm) were cut on a LKB2 ultra microtome and collected on microscopic glass slides (10 wells; Erie scientific company).

BMM was removed using acetone. The sections were washed in PBS and incubated in citric acid (0.2% pH 6.0, 30 min at 90°C), washed and DNA was denatured in HCl (2 N, 30 min at 37°C). Tissue sections were neutralised in a sodium borate buffer (pH 8.5) and washed with PBS. Blocking was done with 1% BSA (Sigma) in PBS (30 min this step and all subsequent steps at RT). After washing in PBS, sections were incubated with mouse-anti-BrdU monoclonal antibody (MuBio Products BV, clone IIB5, 1:50 in PBS with 1% BSA, 30 min) and washed. Primary antibody was detected with the avidin-biotin enzyme complex (Vectastain Elite ABC Kit, Vector labs). Sections were first incubated with biotinylated horse anti-mouse antibody (in PBS with 1% BSA, 30 min), washed and incubated in avidin-biotin-peroxidase complex in PBS (30 min). Peroxidase activity was visualised with DAB (0.05% with 0.015% H_2O_2, 5-10 min). The slides were washed in tap water and mounted in glycergel (DAKO). The slides were examined under the light microscope (Axiovert 200, Zeiss) and photographs were taken with a Zeiss AxioCam MRc5 digital camera.

To estimate the proportion of BrdU-positive choanocytes, three tissue (1-2 cm^2) samples were taken from each sponge (edge, middle with, and without osculum). From each tissue sample four sections (>1 mm apart, containing >250 choanocyte cells per section) were counted. Per sponge >3,000 (4×250×3) choanocytes were counted, and three sponges were used for each time point (six time points in total). Estimates of labelling index (LI), growth fraction (*GF*), duration of cell cycle (T_C) and duration of the S-phase (T_S) were calculated from the continuous labelling curve, according to an iterated least squares fit to the data, using the following specified initial conditions:

$$f(t) = GF \times (t + T_S) / T_C \text{, for} \quad (1)$$

$$t \leq T_C - T_S \text{, and} \quad (2)$$

$$f(t) = GF \text{, for} \quad (3)$$

$$t \geq T_C - T_S \quad (4)$$

These equations are based on a 'one population model' (Nowakowski et al. 1989).

In order to estimate the carbon demand of *H. caerulea* choanocyte proliferation in time (μmol C cm^{-3} h^{-1}), the number of BrdU-positive choanocytes per cm^3 sponge in time was determined. We assumed that the carbon content of one choanocyte is close to the carbon content of a choanoflagellate (CC) - 100 fg C μm^{-3}, with a volume of:

$$W^2 \times L \times P / 6 \qquad (5)$$

(*W*=cell width, *L*=cell length (μm) (Børsheim and Bratbak 1987). Choanocytes have no obvious homologue among metazoans, but are functionally comparable with choanoflagellates, which are closely related with sponges (Leys and Eerkes-Medrano 2006), and share the general cell structure of choanoflagellates (Maldonado 2004). The thickness of a living and pumping sponge (filled with water) is larger (2.5 mm) than of a fixed sponge (0.8 mm), and values were corrected (*F*) accordingly to compare carbon removal rates. The number of choanocytes was counted in four randomly chosen areas (A; 0.0214×0.0160 cm) from eight sponges. The flux was calculated according to:

$$carbon\ demand = Ch \times GF / V / Tc, \text{ with} \qquad (6)$$

$$Ch = CC \times 10^{-9} / 12, \text{ and} \qquad (7)$$

$$V = A \times th \times F \qquad (8)$$

Ch is the amount of choanocytes (μmol); *V* the volume (cm^3); *th* is the thickness of a section (0.3 x 10^{-3} cm); The percentage of choanocytes in total tissue was established by using image analysing software (Axiovision, Zeiss). The 95% confidence limits are given, unless stated otherwise.

Caspase-3

After removing BMM, slides were washed in PBS, incubated in citric acid and washed. The tissue sections were incubated with purified rabbit anti-active caspase-3 (BD Biosciences, 0.25 μg mL^{-1} PBS with 1% BSA, 1 h at RT). The slides were washed in PBS and incubated with goat anti-rabbit-biotin (IgG Jackson Immunoresearch laboratories, in PBS with 1% BSA, 30 min). After washing, the sections were incubated with avidin-biotin-peroxidase complex and peroxidase activity was visualised with DAB. The sections were rinsed in tap water and mounted with Glycergel.

Extracellular matrix

For all staining, BMM was removed with acetone as described above. A subset of sections washed in distilled water and incubated for 5 min in 0.2% phosphomolybdic acid in water and then for 90 minutes in 0.1% Sirius red in saturated picric acid. The sections were quickly rinsed in 0.01N HCl, dehydrated and mounted using Entellan. Other sections were stained with Alcian Blue solution (pH 2.5) for 30 min, quickly rinsed with tap water and incubated with 1% periodic acid (10 min). After rinsing with distilled water the sections were incubated with Schiff's reagent (20 min), rinsed with tap water and counterstained with Hematoxylin (5 min).

For immunohistochemical staining of components showing compartmentalisation, endogenous peroxidase was blocked by incubation in methanol containing 0.3% H_2O_2 for 20 min, followed by rinsing with Tris buffered saline, pH 7.5 (TBS). Sections were incubated in citrate buffer (pH 6.0; 30 min, 80°C) and pretreated by digesting the tissue with 0.1% pepsin in 0.01 N HCl (30 min, RT). Antibodies used were: Polyclonal rabbit anti type-IV collagen antibody (Eurodiagnostica) (Havenith et al. 1987; Cleutjens et al. 1990), and polyclonal rabbit anti-laminin antibody (Eurodiagnostica). Sections were incubated with the primary antibodies for 1 h. After washing with TBS, biotinylated sheep anti-mouse IgG (Amersham, dilution 1:1000) or biotinylated swine anti-rabbit IgG (Amersham, dilution 1:1000) were applied to the sections (1 h). Subsequently, sections were washed and incubated with Horse radish peroxidase coupled ABC reagent (DAKO, Glostrup, Denmark; 30 min). The sections were washed with TBS and incubated with diaminobenzidine containing H_2O_2 (3-5 min). After washing with water the sections were counterstained with hematoxylin, dehydrated and mounted with Entellan.

Electron microscopy

The sponges were fixed in 2.5% glutaraldehyde in sea water for 20 to 24 h. The specimens were then rinsed in sea water, postfixed in osmium tetroxide (2% in sea water) for 2 h, and imbedded in araldite. Semithin sections were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a Hitachi HU600 electron microscope.

Cell kinetics

Figure 6.1 demonstrates the percentage of BrdU-positive choanocytes generated in time after continuous *in vivo* labelling. A linear increase of BrdU-labelled proliferating cells was observed until a maximum was reached when all proliferating cells have been labelled and re-enter the S-phase of the cell cycle. This maximum represents the growth fraction (GF), or the proportion of the total population of proliferating cells, estimated at 46.6 ± 2.6%. The y-intercept, or labelling index (LI), i.e. the proportion of cells in the S-phase, was 3.9 ± 1.2%, and the x-intercept represents the duration of the S-phase (T_S): 0.5 ± 0.3 h. The

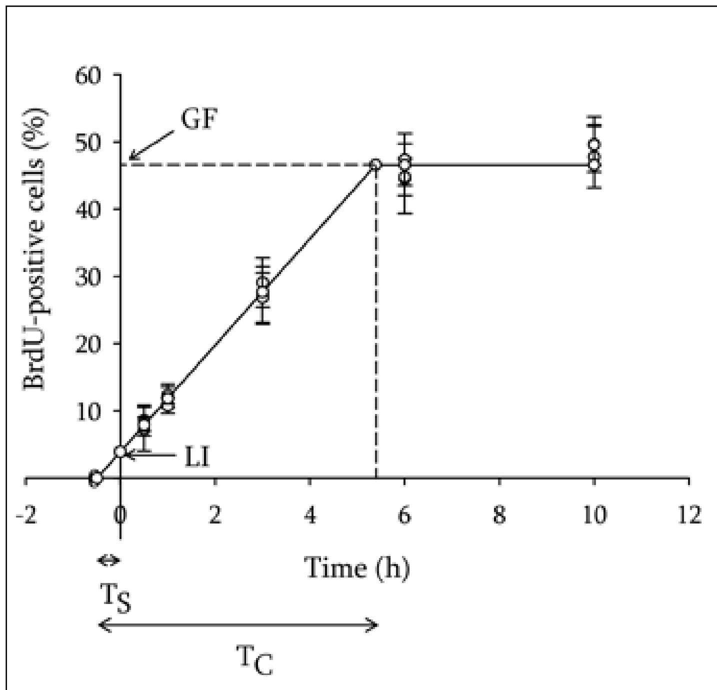
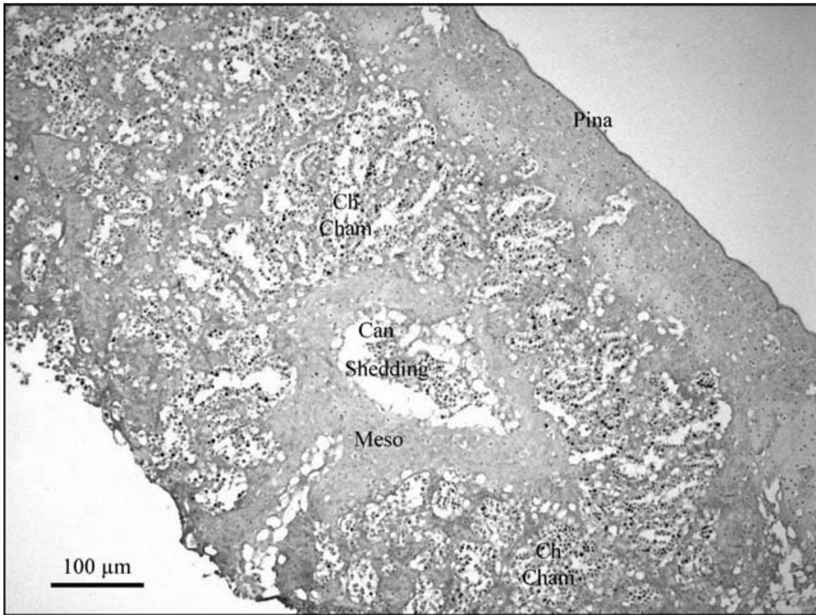
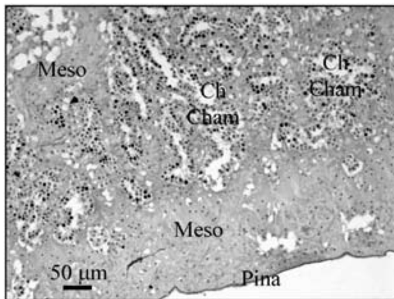


Fig. 6.1 - Cell kinetics of Halisarca caerulea *choanocyte cell population. BrdU positive choanocytes (%) in time (h) after labelling, showing a linear increase of BrdU-labelled proliferating cells until a maximum was reached. This maximum represents the growth fraction (GF), or the proportion of the total population of proliferating choanocytes. The duration of the linear increase represents the duration of the cell cycle (T_C). The y-intercept represents the labeling index (LI; the proportion of cells in the S-phase) and the x-intercept calculated the duration of the S-phase (T_S). The line is the least squares fit obtained to the conditions for a 'one population model' as described in Nowakowski et al. (1989): mean ± SD.*





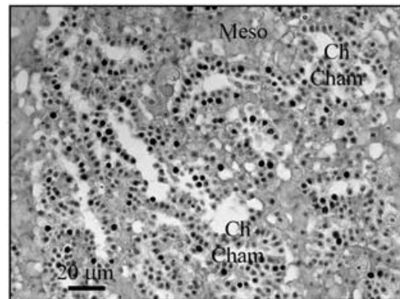


Fig. 6.2 - Cell proliferation in Halisarca caerulea*. Incorporation of BrdU in cells (brown stained nuclei) of the sponge* Halisarca caerulea *after* in vivo *(10 h) exposure to BrdU. The labelled cell population consists primarily of choanocytes lining the well developed choanocyte chambers (**Ch Cham**).Very few BrdU positive cells are found in the mesohyl (**Meso**) or pinacoderm (**Pina**). The cross-section (top picture) shows shedding of BrdU-positive cells (**Shedding**) in the lumen of a canal (**Can**).*

duration of the cell cycle (T_C) was 5.4 ± 0.4 h. These cell kinetic parameters were determined on tissue samples obtained from various locations in the sponge, including central and peripheral areas, and did not show significant differences according to the spot of sampling.

The results of the cell kinetic analysis as well as the incorporation of BrdU in *Halisarca caerulea* choanocytes in time correlate significantly with a 'one population model' (Nowakowski et al. 1989), strongly suggesting choanocytes are the sole proliferating cell population.

Cell turnover and carbon assimilation

Choanocytes comprise 18 ± 1% of the total tissue of *Halisarca caerulea*. The carbon content of one choanocyte (with an average volume of 28 ± 2 μm^3) is 2.8 pg. On average, 218 ± 44 choanocytes were counted per 0.34×10^{-3} cm^2 (i.e. one image field 400×). The carbon needed to produce new choanocytes, based on the cell proliferation kinetics for *H. caerulea*, was found to be 13.8 ± 0.9 µmol C cm^{-3} sponge h^{-1}.

Site of proliferation

Incorporation of BrdU in cells of *H. caerulea* was demonstrated after *in vivo* exposure of sponges to BrdU and immunohistochemical analysis of sponge sections sampled from different regions of the sponge. The labelled cell population consisted primarily of choanocytes, small cells of approximately 3-6 µm in diameter lining the well-developed choanocyte chambers (*Fig. 6.2*). Only a few BrdU-positive cells were observed scattered in the mesohyl and in the pinacoderm. This pattern of BrdU-labeled cells did not change with increasing BrdU exposure time. Per sponge, no differences were observed between the percentages of positive choanocytes per section. Similarly, analysis at various locations taken from the sponge (the edge or middle, with or without osculum) showed no significant differences with respect to the percentage of positive choanocytes, which is indicative for steady state conditions, instead of a response to injury. In addition, there were no significant differences between the measurements done *in situ* (at 12 m of water depth) and the experiments performed in aquaria.

Cell loss by apoptosis and shedding

The number of active caspase-3 positive cells, as a measure for apoptosis, was detected in *H. caerulea* tissue. Only a few positive cells were found, i.e. 0-4 cells per 0.34×10^{-3} cm^2 sponge. Apoptotic cells were not distributed evenly throughout the sponge tissue. Positive cells were located both in the mesohyl adjacent to the choanocyte chamber, and near or inside canals leading to the outflow opening (*Fig. 6.3A,B*). The caspase-3-positive cells do not have the characteristics of choanocytes, but strongly resemble spherulous cells and archaeocytes. TEM images confirm spherulous cells being extruded from the mesohyl into the exhalant canals (*Fig. 6.3C*). Some cells in the mesohyl showed active caspase-3 positive inclusions. A few small active caspase-3 positive

cellular fragments were found close to choanocyte chambers and - within the eight sponges examined - only two caspase-3-positive choanocytes were detected.

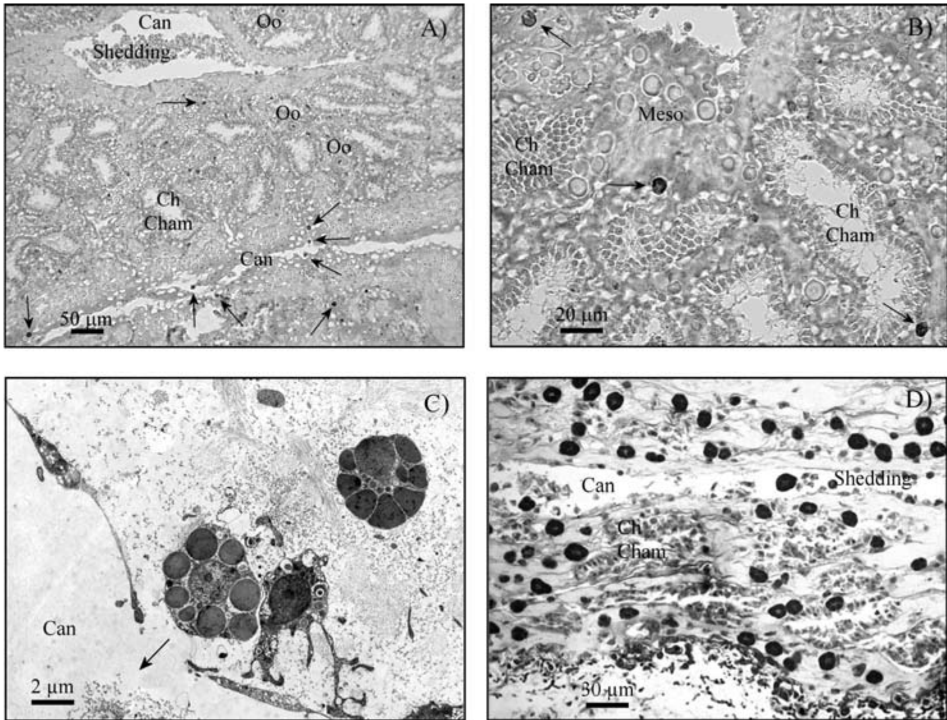


Fig. 6.3 - Apoptosis and cell shedding in Halisarca caerulea *tissue. Brown stained cells are caspase-3 positive cells (**Arrows**) representing apoptosis. (A) Overview of a distinct area of choanocyte chambers (**Ch Cham**) and two canals (**Can**) leading to the outflow opening. The upper canal shows massive shedding of cells (**Shedding**). The lower canal shows several caspase-3 positive cells located near or inside canals leading to the outflow opening. Three oocytes (**Oo**) are shown. (B) Three caspase-3 positive cells located in the mesohyl (**Meso**). (C) TEM of spherulous cell being extruded in canal (derived from Vacelet and Donadey (1987)). (D) Canal showing shedded cells (choanocytes and spherulous cells).*

Choanocytes were found in the lumen of the choanocyte chambers and large amounts of cellular debris were found in the lumen in canals leading to the oscula of *H. caerulea* (*Fig. 6.3A,D*). This debris contains predominantly choanocytes and few spherulous cells. In sponges exposed up to a few hours to BrdU, the shedded material, including the shedded cells, was clearly BrdU-negative. In contrast, sponges that were labelled with BrdU for more than six

hours showed extensively positive staining in the shedded cells and debris *Fig. 6.2*). These observations demonstrate massive shedding of BrdU-labelled, and hence newly formed cells, and point toward their choanocyte origin.

Structural organisation of *Halisarca caerulea*

Picrosirius red staining was observed in the extracellular matrix of the mesohyl and under the cuticle layer and the pinacoderm, below the surface cells of the sponge. The typical wavy patterns of interstitial collagen fibers could be observed using bright field and polarised light illumination (*Fig. 6.4A,B*).

Alcian blue stains proteoglycan-like extracellular material. The Alcian blue staining was observed mainly in the mesohyl and not in the pinacoderm. Alcian blue-positive material could be observed at the borders of individual cells in the mesohyl and at the transition of the basal part of the choanocytes towards the mesohyl. Generally, a sharp demarcation was visualised, strongly indicating compartmentalisation (*Fig. 6.4C*).

The immunohistochemical detection of type IV collagen and laminin indicate the presence of a compartmentalising layer at the transition of the basal side of the choanocyte, which separates the choanocytes from the surrounding mesohyl (*Fig. 6.4D,E*).

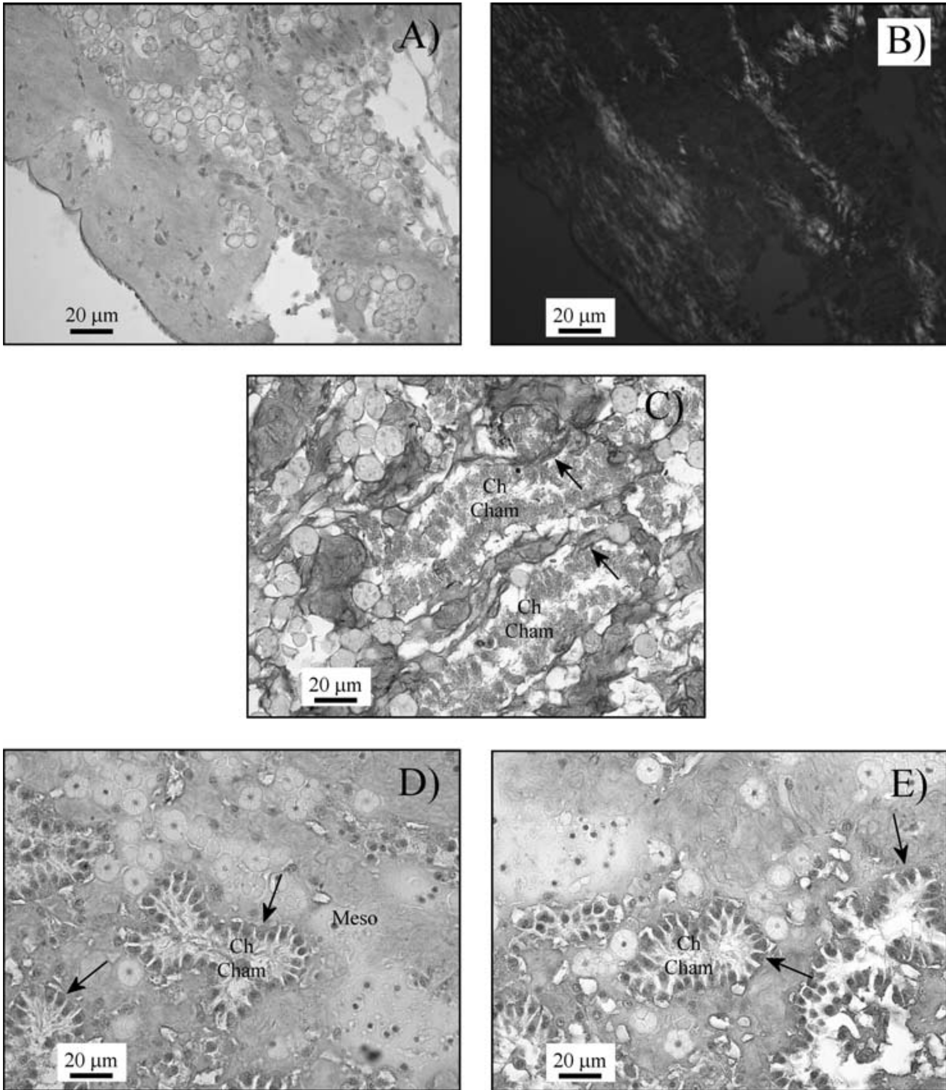


Fig. 6.4 - Structural organisation of Halisarca caerulea. *Typical wavy patterns of collagen is shown after Picrosirius red staining in the extracellular matrix of the mesohyl and in the cuticle layer below the surface cells of the sponge using bright field (A) and polarised light (B). Sharp demarcation of proteoglycan-like extracellular material (**Arrows**) after Alcian Blue staining at the borders of individual cells in the mesohyl and at the transition of the basal site of the choanocytes towards the mesohyl, indicating compartmentalisation (C). Compartmentalising layer at the transition of the basal side of the choanocytes (**Arrows**), separating the choanocytes from the surrounding mesohyl, following combined immunohistochemical detection of laminin (D) and type IV collagen (E).*

Discussion

We have examined *in vivo* cell kinetics of the marine sponge *Halisarca caerulea* by measuring proliferation characteristics, using continuous BrdU labelling and quantitative analysis of BrdU incorporation. In addition, apoptosis was determined using caspase-3 immunohistochemistry. The tropical coral reef sponge shows remarkable proliferation kinetics, i.e. a very high proliferative activity and a short duration of the cell cycle. The analysis of cell kinetic parameters indicates the proliferation of a single population of cells in the sponge, represented by the choanocytes. Remarkably, under steady state conditions, high levels of cell loss were found, not by apoptosis, but through shedding of predominantly choanocytes. The high cell turnover, indicated by the fast proliferation and concomitant shedding of choanocytes, corresponds well with the observed discrepancy between the high assimilation of organic carbon and the absence of a corresponding net increase in the sponge biomass. Additionally, the estimated costs of proliferation for this sponge (13.8 µmol C cm^{-3} h^{-1}), are in the same order of magnitude as the organic carbon removal by *H. caerulea* (18.5 ± 2.8 µmol C cm^{-3} sponge h^{-1}) previously shown (De Goeij et al. 2008a). In line with the cell kinetics, the histochemical studies have demonstrated two other features of the sponge that correspond well with a crucial role of choanocytes in this organism. The morphological studies identify the choanocytes as the predominantly proliferating and shedding cells and strongly suggest compartmentalisation of choanocytes in the sponge tissue. The functional morphology of the sponge *H. caerulea* was found to correspond well with its remarkable cellular kinetics.

Cell kinetics

The extremely high proliferation rates *in vivo*, indicated by the high growth fraction (46.6%), the short duration of the cell cycle (T_c = 5.4 h) as well as the short S-phase (T_s = 0.5 h) is remarkable and have - to our knowledge - not been observed to date in sponges and other multi-cellular organisms.

The *in vivo* cell kinetics as determined in our study do not corroborate well with cell proliferation rates as measured for *in vitro* sponge systems, like cell cultures or primmorphs. In these studies choanocytes were not specifically measured. In contrast, the focus with respect to proliferating cells was on archaeocytes. It is thought that archaeocytes represent the predominant, if not

the only, proliferative cell type in sponges (Sun et al. 2007). In cell aggregates only 6.5% of the cells (archaeocytes and spherulous cells) were BrdU-labelled after 24 h; in a later stage (primmorphs, lacking choanocytes), only 33% after three days (Custodio et al. 1998). In one study the cell cycle of choanocytes of the marine sponge *Hymeniacidon sinapium* was studied, showing a cell cycle of 20-40 h (Shore 1971). In another study, choanocytes of sponge larvae showed a cell cycle duration of 13-15 h (Efremova and Efremov 1979). Both studies, however, are hard to compare with our study. The first study was performed *ex situ*, using artificial seawater, and it is well known that ideal conditions for keeping sponges are still not established. Moreover, very small nubbins of sponge were used for the experiments, resembling more *in vitro* than *in vivo* conditions. The second study has been done on larvae of a freshwater sponge, which may be considered as quite different from adult marine sponges. For *Hydra* - a well studied dipoblastic, lower metazoan related to the Porifera and well known for its regenerative power - the fastest cell cycles measured are 16-27 h for interstitial (stem) cells, with a S-phase length (T_S) of 11-12 h (Campbell and David 1974). Gill cells of a suspension feeder like the mussel have a T_C of 24-30 h (Martínez-Expósito et al. 1994). A fast proliferating tissue such as the intestinal epithelium of the mouse shows a T_c between 12-15 h and a T_S of approximately 7-8 h (Rowinski et al. 1977). The proliferation rate of the sponge choanocytes resembles that of uni-cellular organisms in culture, like, for example, choanoflagellates. The doubling time of the choanoflagellate *Monosiga* in culture during log phase was approximately 6 h (King et al. 2003), almost identical to the T_c of *H. caerulea* choanocytes. It should be noted that under natural conditions marine bacteria have doubling times ranging from 8 h to a few days (Fuhrman and Azam 1982).

Cellular homeostasis under steady state conditions: Cell loss by shedding of choanocytes

The proliferation characteristics of the choanocyte cell population correspond with a steady state situation and not with a regenerative process, as was concluded from a series of observations. No differences in proliferative patterns were found between the sponges kept in their natural environment and the sponges kept in aquaria. Trauma, evoking regeneration of tissue, was most likely to occur at the sponge edges (where sponges were cut), but clearly no differences in the growth characteristics were observed between sections

taken from different locations in the sponge, including the edges. Additionally, the regenerative power of sponges in response to trauma has been shown to depend on the migration and proliferation of archaeocytes, which are considered to be the toti/multi-potent “stem cells” (Simpson 1984). Since the mass and overall structure of sponge tissue do not change significantly in time, the observed cell kinetics strongly suggest a rapid cell turnover in the choanocyte cell compartment. In order to maintain cellular homeostasis within the sponge, an increase in cell number has to be balanced by loss of cells from the same compartment, either by apoptosis, by autophagy (Xie and Klionsky 2007) or by shedding.

Specific loss of choanocytes through apoptosis is very unlikely, since immunohistochemical staining with anti-active caspase-3 antibody shows only very few positive cells and these active caspase-3-positive cells were predominantly cells residing in the mesohyl, and not in the proliferation compartment. The caspase-3-positive cells show the morphological characteristics of spherulous cells and archaeocytes. It is remarkable that in many of these cells caspase-3-positive staining is observed in distinct entities in the cytoplasm. In all these cells an intact nucleus is present. This corroborates with findings of spherulous cells passing through the pinacocyte layer of canals in *Halisarca caerulea* (Vacelet and Donadey 1987).

Therefore, most choanocytes are lost by shedding of cells from the choanocyte chambers into the central canals leading to the osculum, or outflow opening. Evidence for such cell shedding is strongly supported by the presence of large amounts of cellular debris and choanocytes in these compartments. The presence of BrdU-labelled cells and debris after relatively long (6 and 10 h) exposure of the sponge to BrdU (*Fig. 6.2*), and the absence of BrdU staining in the shedded material after relatively short (0.5-3 h) labelling indicates that this cellular material originates from the rapidly proliferating choanocytes. Excretion of large amounts of detritus by sponges has been reported (Witte et al. 1997). Shedding of tissue also occurs in *Hydra*, in order to maintain a constant size and cellular composition of the organism under steady state continuous growth (David and Campbell 1972). A similar equilibrium between continuous cell proliferation and cell shedding can be observed in the lumen of mammalian colonic tissue *in vivo* (Van de Wetering et al. 2002).

Compartmentalisation in sponge tissue

The *in vivo* cell kinetic analysis of *H. caerulea* demonstrates that the massive proliferation is not a random phenomenon, but is restricted to a single population of cells. Our (immuno)histochemical studies indicate shedding as the predominant mechanism of cell loss and identify the choanocyte as the cell type responsible for the high cell turnover. These processes require a structural and functional basis, and, in other words, the question is whether these are reflected in the morphological characteristics of the sponge.

The kinetic and morphological data strongly suggest functional compartmentalisation of choanocytes in the sponge. In the mesohyl only few scattered proliferating cells were observed, suggesting that no exchange, migration, or differentation of BrdU-labelled cells occurs between the choanocyte compartment and the mesohyl. *H. caerulea* has a distinct distribution of extracellular matrix components in differently organised compartments, such as bands with fibrillar mature collagen in the mesohyl and cuticle. This was demonstrated with Picrosirius red staining, bright field and polarised light illumination. The combined results of these techniques prove the presence of mature collagen (Puchtler et al. 1973; Junqueira et al. 1979). These collagenous structures, as well as the cells in the choanocyte chambers, are delineated with other extra-cellular matrix components that stain with Alcian blue, like proteoglycan-like or hyaluronic acid containing structures (Zierer and Mourao 2000).

Furthermore, the choanocytes are separated from the mesohylic collagen and proteoglycans by a structure which contains type IV collagen as well as laminin components. The combination of the structural organisation, together with the cell kinetics of a distinct cell population, indicates a functional compartmentalisation in the sponge, which might function in the cellular homeostasis, physiology, differentiation and polarity of these sponges, in particular of the choanocytes. Further studies should be performed to investigate the presence of basement membranes-like structures.

Implications

Halisarca caerulea is an encrusting coral reef sponge, inhabiting coral overhangs and framework cavities, playing a key role in the recycling of nutrients within the reef framework (De Goeij et al. 2008a). Its habitat provides shelter and protection, and therefore the competitive struggle for space and food for

these benthic organisms is high (Buss and Jackson 1979). The tropical waters surrounding coral reefs are very low in nutrients and considered the marine equivalent of a desert. Marine benthic organisms have adapted to their environment by becoming very efficient and opportunistic suspension feeders. Sponges are known for pumping vast volumes of water in time (Reiswig 1974b) and removing enormous amounts of material from their environment (Gili and Coma 1998). As a consequence, the sponge, as a benthic filter feeder, is continuously in contact with large volumes of water containing nutrients, but also potential mutagenic, toxic, viral, bacterial and physical stress factors. A relevant survival strategy to cope with this function and the hostile environment may be a high turnover of cells. Before possible structural damage of the adsorptive system, the cells are shedded and replenished by new cells. Not surprisingly, sponges have a high telomerase activity (Müller and Müller 2003), preventing highly proliferative cells from senescence, but cells that loose their contact with other cells, for example by shedding, subsequently loose their telomerase activity (Müller and Müller 2003). We are aware of the fact that this study is performed on only one species, but we found discrepancies in carbon removal rates and net growth in two other types of cavity sponges, similar to *H. caerulea* (De Goeij et al. 2008a).

Sponges may be important in the search for new drugs and antibiotics. They produce secondary metabolites that may have a great potential for the development of new therapeutics and cosmetics (Sipkema et al. 2005). Currently, a major drawback to implement sponges as a scientific or biotechnological model is the difficulty in culturing sponges. This cell kinetic study provides unique and essential information on the regulation of growth of sponges. The present *in vitro* systems do not take into account the pivotal role of choanocytes in tissue homeostasis of sponges. Cell kinetic parameters of sponges will be inseparably linked with future growth studies on sponges.

CHAPTER 7

Element cycling on tropical coral reefs: The cryptic carbon shunt revealed

Abstract

The exchange of C, N, and P between reef water and coral cavities, and between reef water and distinct cavity inhabiting communities has been quantified to assess the role of coral cavities in overall coral reef element cycling. Coral cavities appear major sinks of dissolved organic matter (DOM) as well as sources of particulate organic matter (POM). The release of inorganic nutrients represented only a minor pathway as compared to the assimilation of organic matter into biomass in the cryptic habitat. Covering only one-quarter of the total surface area (TSA) of cavities, sponges determined three-quarters of the net cavity fluxes and were quantitatively important in transforming dissolved to particulate organic matter. Extrapolation of the results suggests that coral reef framework cavities are the major habitat in respiration processes in the coral reef ecosystem, accounting for 27-68% of the gross community respiration of an entire reef ecosystem. We hypothesise that coral cavities support a 'cryptic carbon shunt', capturing locally produced DOM and converting it to particulate matter, which may facilitate the retention of nutrients within the ecosystem.

INTRODUCTION

Gross primary production rates of tropical coral reefs rank amongst the highest on earth (Odum 1971). However, the net community production of the coral reef ecosystem is relatively low (Odum and Odum 1955; Smith and Marsh 1973). The ratio of gross community production (*P*) and community respiration (*R*), i.e. the *P/R* ratio, is approximately 1, and the excess production is close to zero (Kinsey 1983). Surrounded by oligotrophic waters (Crossland 1983), the oceanic supply of nutrients to the reef is limited, whereas the advective loss of nutrients may be substantial. To sustain high rates of gross primary production in these settings an efficient recycling and conservation of organic and inorganic nutrients within the reef ecosystem is required (Erez 1990; Atkinson and Falter 2003).

The cryptic habitat, one of the largest subsystems of the reef, is net heterotrophic and a significant sink of organic matter. The three-dimensional structure of a coral reef is composed of a complex and irregular framework of caves, cavities, crevices, holes, and cracks. Most of the available hard substratum of the reef can be found here (Jackson et al. 1971), covered by a dense community of cavity organisms, or coelobites (Ginsburg and Schroeder 1973). Coral cavities remove phytoplankton (Richter et al. 2001), bacterioplankton (Scheffers et al. 2004), and dissolved organic carbon (De Goeij and Van Duyl 2007) from the passing ambient water. Depending on the dissolved organic carbon (DOC) and particulate organic carbon (POC) concentrations however, DOC removal rates may exceed the POC uptake rates by one to two orders of magnitude (De Goeij and Van Duyl 2007). The importance of DOC for cryptic habitats as established for two distinct coral reef ecosystems, i.e. an Atlantic reef (Curaçao) and an Indo-Pacific reef (Indonesia), suggests the dominance of DOC over POC removal rates to be a more general characteristic of reef carbon cycling. The total organic carbon (DOC + POC) removal rates are in the range of the average gross primary production by entire coral reef ecosystems (De Goeij and Van Duyl 2007). Within the reef framework, the organic matter is decomposed and major nutrients are regenerated and released as inorganic nitrogen (N) and phosphorus (P) (Andrews and Müller 1983; Tribble et al. 1988; Rasheed et al. 2002). Studies on C, N, and P exchange between coral cavities and surrounding waters are scarce (Tribble et al. 1988, 1990; Richter et al. 2001) and have neglected the potential contribution of dissolved organic C, N, and P.

Moreover, the role of the cavity biota in the C, N, and P cycling in coral cavities is as yet largely unknown.

In this study, we examine the role of the framework cavities and its separate inhabiting communities in the C, N and P cycling of coral reef ecosystems by quantifying net fluxes of organic and inorganic matter and by the construction of mass balances.

Materials and methods

Study area and sites

The fieldwork for this study was conducted on the fringing reefs of the Caribbean island of Curaçao, Netherlands Antilles (12°12'N, 68°56'W). Coral cavities were chosen at four stations (two cavities per station) along the leeward (SW) side of the island. The long-term mean current runs from SE to SW along the island. Hydrographic descriptions of the stations, the cavity volumes, and total surface areas (TSA) have been presented in De Goeij and Van Duyl (2007). A cavity TSA was divided in four surface dominating cavity communities: Sponge, sediment, calcareous algae, and bare substratum, which all together cover ~90% of TSA.

All incubation experiments were performed at station Buoy 1. Sponges, calcareous algae and bare substratum were chiselled from overhangs and coral cavity walls between 15 and 25 m water depth. Pieces of coral rock were cleared from epibionts. Pieces were filed to fit the incubation chambers (6.5×4.5×3 cm), and were stored upside down in wire cages (20×20×15 cm; maximum of four pieces per cage) to protect them from sediment accumulation and predation. Cages were stored inside coral reef cavities at 15 m water depth. Sponges were acclimatised for at least one week prior to experiments. The omnipresent, thin (0.8-2.5 mm), encrusting cavity-dwelling sponge *Halisarca caerulea* (Porifera: Demospongiae) was selected as a model species for detailed element budget studies on encrusting sponges. Sediments were incubated in a cavity at station Buoy 1 and at the drop-off at approximately 12 m water depth.

All data were collected within four fieldwork periods from July-August 2003, March-August 2004, March-May 2005, and January-April 2006, while SCUBA diving.

Cavity sampling

Open cavity: Cavity water (CW) and overlying reef water (RW) were sampled for 20 different cavities at station Buoy 1. With an acid-washed 750 mL polycarbonate syringe, samples were taken for dissolved organic carbon (DOC), bacterioplankton abundance, total dissolved nitrogen (TDN), dissolved inorganic nitrogen (DIN), total dissolved phosphorus (TDP), and dissolved inorganic phosphorus (DIP). CW was sampled from the center of the cavity and RW approximately 1 m from the opening of the cavity. For each site, a RW sample was taken first, against the current to avoid contamination of samples, and then a CW sample was taken. The water was collected in acid-washed glass bottles, and stored in the dark at 4°C prior to further processing in the lab within 4 h.

Closed cavity: The mass exchange for carbon, nitrogen, and phosphorus, as well as cavity oxygen consumption rates were determined by halting the water flow through the cavity. For details *see* De Goeij and Van Duyl (2007). Samples for DOC, bacterial abundance, dissolved oxygen (O_2), TDN, DIN, TDP, and DIP were collected in time series of up to 60 min with an acid-washed polypropylene syringe. In total 23 experiments were performed on eight different cavities, with at least two experiments per cavity.

Community sampling

Pieces of sponge, calcareous algae, and bare substratum were carefully placed in 1.7 L incubation chambers (De Goeij et al. 2008a) and DOC, bacterial abundance, O_2, TDN, DIN, TDP, and DIP concentration changes were recorded in time series up to 60 min. For sediment incubations, a plexiglas ring was designed (ø 10 cm, 6 cm in length) to fit the lid of the incubation chamber. The chamber was then turned upside down and the ring placed in the sediment. Incubation experiments with ambient reef water, without sponge, sediment, algae, or bare substratum, served as blank experiments.

Sample treatment and analysis

Sample treatment: Water, collected for DOC, TDN, TDP, and inorganic nutrients determination, was gently filtered (max. 20 kPa suction pressure) through a 0.2 μm polycarbonate filter (Millipore, 47 mm). Prior to filtration, filters, glassware, and pipette tips were washed with acid (3×10 mL 0.4 M HCl), 0.2 μm filtered double distilled water (3×10 mL) and sample water (3×10 mL).

Duplicate 8 mL DOC samples were collected in pre-combusted (4 h, 450°C) glass ampoules. Ampoules were sealed immediately after acidification with 1-2 drops of concentrated H_3PO_4 (80%) and stored at 4°C until analysis. Samples for bacterial abundance in seawater (10 mL) were fixed in 4% paraformaldehyde (PFA), stained with acridine orange, and gently (max. 20 kPa suction pressure) filtered onto 0.2 μm black polycarbonate membrane filters (Millipore, 25 mm). Filters were mounted on slides and stored at -20°C prior to count using epifluorescence microscopy. Duplicate samples for N and P (5 mL) were transferred to 6 mL polyethylene vials (Packard BioScience) and stored at -20 °C until further analysis. O_2 samples were carefully divided over three Winkler bottles (volume ~60 mL, accurate to 10 μL) and treated following the method of Winkler (1888).

Sample analysis: DOC was measured by high temperature combustion (Shimadzu, TOC Analyser, Model TOC-5000A), using potassium phthalate in Milli-Q water as a standard. As an internal control on the DOC measurements, consensus reference material provided by Hansell and Chen of the University of Miami, USA (Batch 4, 2004; 45 μmol L^{-1}, every 10 - 20 samples) was used. DOC concentrations determined for the batch were 45 ± 2 μmol L^{-1}. The average analytical precision of the instrument was <3%, and each sample was measured in fivefold. The concentrations of inorganic nutrients were measured in a TRAACS 800 autoanalyser system (Technicon). Ammonium was detected using the indo-phenolblue method (pH 10.5) at 630 nm (Helder and De Vries 1979). Nitrite was detected after diazotation with sulfanilamide and N-(1- naphtyl)-ethylene diammonium-dichloride as the reddish-purple dye complex at 540nm (Parsons et al. 1984). Nitrate was reduced in a copper cadmium coil to nitrite (using imidazole as a buffer) and measured as nitrite. DIN is defined as the sum of ammonium, nitrate, and nitrite. Soluble reactive phosphorus was determined via the formation of a blue-reduced molybdophosphate-complex at pH 0.9-1.1 with potassiumantimonyltartrate as catalyst and ascorbic acid as a reductant and measured at 880 nm (Murphy and Riley 1962). TDN and TDP were determined according to Valderrama (1981).

For TDN, an online chemical destruction of the sample by UV radiation and persulfate oxidation, using a pH range of 10.0-3.0, was followed by buffering the sample with borate at pH 9.0, and subsequent destruction by heat (95°C). The resulting nitrate is dialysed out of the destruction solution using an ammoniumchloride buffer. Nitrate was reduced to nitrite using a copperised

cadmium coil, which was measured colorimetrically on a Technicon AAIII system. TDP was determined by an online chemical destruction of the sample under UV radiation with persulfate, followed by a strong acid hydrolysis at a temperature of 95°C. The formed ortho-phosphate is colourimetrically measured on a Technicon AAIII system (Murphy and Riley 1962). Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were calculated as:

$$DON = TDN - DIN \text{, and} \tag{1}$$

$$DOP = TDP - DIP \tag{2}$$

O_2 was determined with whole bottle end point titration by adding a 1% starch solution using a microburette. The coefficient of variation between the triplicate measurements was <0.5%.

Flux calculations

Open cavity: Fluxes for the open system are calculated according to:

$$Flux = (C_{RW} - C_{CW}) / t_c \times (V_{cavity} / TSA) \tag{3}$$

C_{RW} and C_{CW} are the nutrient concentrations of reef water and cavity water, respectively. $1/t_c$ is the water exchange coefficient (0.0041 ± 0.0024 s^{-1}; range 0.0034-0.0053 s^{-1} (Van Duyl et al. 2006)), V_{cavity} the cavity volume (148 ± 51 dm^3; range 86-250 dm^3), and *TSA* the total surface area (2.11 ± 0.54 m^2; range 1.30-3.16 m^2).

Closed cavity and communities: The initial DOC, DON, and DOP removal rates for closed cavities and communities were estimated by applying a 2G-model, describing the solute concentration as a function of time (De Goeij and Van Duyl 2007; De Goeij et al. 2008a):

$$C(t) = C_{f,0} \times e^{-k_f t} + C_{s,0} \times e^{-k_s t} \tag{4}$$

The experimental data are described by estimating the variables $C_{f,0}$, k_f, $C_{s,0}$, and k_s using a minimisation routine. The initial uptake rate of C (the flux on $t = 0$) was calculated from the estimated values of these variables and is given by:

$$Flux = -(k_f C_{f,0} + k_s C_{s,0}) \tag{5}$$

Bacterial abundance removal rates were calculated assuming homogenous dis-

tribution and exponential clearance of bacterial cells. Bacterial abundance was converted to bacterial C, N, and P using 30 fg C per bacterial cell and assuming an elemental composition of 45C:9N:1P for bacteria. DIN and DIP release was calculated from the linear increase in concentration with time. The fraction of C uptake being respired (RQ) was calculated from:

$$RQ = (O_{2t=0} - O_{2t=end}) / (TOC_{t=0} - TOC_{t=end}) \quad (6)$$

Mass balances and assumptions

Mass balances were constructed for the entire cavity and for the sponge and sediment communities separately. Mass balances were constructed based on the following assumptions:

(1) Cavity water is well mixed and the system is in steady state with respect to solute concentrations and biomass.

(2) Chemical composition and assimilation of cavity biota occurs according to the Redfield ratio (106:16:1).

(3) The contribution of phytoplankton carbon (not measured) to the total C pool in tropical waters is low and in the same order of magnitude as bacterioplankton carbon (BC) (Ayukai 1995), or lower (Richter et al. 2001). For conservancy, total organic carbon (TOC) and particulate organic carbon (POC) removal rates used in the mass balance are calculated as follows:

$$TOC = DOC + POC \text{, where} \quad (7)$$

$$POC = 2 \times BC \quad (8)$$

For phytoplankton nitrogen and phosphorus content (half of particulate organic nitrogen and phosphorus) a stoichiometric conversion according to the Redfield ratio (106:16:1) was applied. Thus, the CNP ratio of the POM removed is the average of bacteria (45:9:1) and phytoplankton element ratios, or 106:19:1.7.

(4) The assimilation efficiency of sediment biota is assumed to be 20% (Alongi et al. 2008).

Results

Cavity fluxes

Open cavity: Despite the short residence times for cavity water (t_c = ~4 min), significant concentration differences were measured for cavity and reef water. Cavity waters consistently showed lower concentrations of DOC, bacterioplankton carbon, DON, and DOP and higher concentrations of inorganic N and P, relative to reef water (*Table 7.1*). These results suggest that cavities remove dissolved and particulate organic carbon and organic nutrients, returning inorganic nutrients. Using the concentration differences between cavity and reef water, equation 1 yielded a net influx of 367 mmol C m^{-2} d^{-1} as DOC and 20 mmol C m^{-2} d^{-1} as POC. Applying a C:N:P ratio of 106 :19 :1.7 for particulate organic matter (assumption 3), would result in a net particulate organic nitrogen influx of $20/106 \times 19 = 3.3$ mmol N m^{-2} d^{-1} and a net particulate organic P influx of $20/106 \times 1.7 = 0.3$ mmol P m^{-2} d^{-1} (*Table 7.1*). For the dissolved organic matter, influxes would amount to 9.2 mmol N m^{-2} d^{-1} and 1.2 mmol P m^{-2} d^{-1} (*Table 7.1*). Evidently, the organic carbon and nutrient supply to cavities is dominated by the dissolved fraction, not the particulate fraction. The fluxes of total organic carbon, nitrogen, and phosphorus (TOC, TON, and TOP) are estimated at 387, 12.5, and 1.5 mmol m^{-2} d^{-1}, respectively. The return fluxes of inorganic N and P amounted to 9.6 mmol N m^{-2} d^{-1} and 0.6 mmol P m^{-2} d^{-1}. These results indicate a net organic to inorganic conversion of 77% and 40%, or retention of 23% and 60% for N and P, respectively.

Closed cavity: Upon closing the cavity, the concentrations of DOC, DON, DOP, and bacterioplankton carbon all showed exponential decrease with time, whereas inorganic N and P increased linearly (*Fig. 7.1*). With respect to the magnitude and the direction of initial fluxes, the closed cavities showed a remarkable correspondence to the open cavities (*Table 7.1*). Fitting the time courses of DOC, DON, and DOP revealed a C:N:P stoichiometry of 560:35:1 (C:N = 16; C:P= 560) for removal (Fig 7.1). Since the corresponding ratio for reef water was 472:32:1 (C:N = 15; C:P = 472), this again suggests that the cavity removed DOP more efficiently than DOC and DON, whereas DOC and DON were removed according to their relative concentrations in reef water. As for the open cavity, the net removal of total organic N and P was not balanced by the release of inorganic N and P. Overall, a net conversion of 39% and 27% from organic into inorganic N and P was observed (*Table 7.1*).

Table 7.1 - Concentrations and fluxes of C, N, P (organic and inorganic; dissolved, particulate, and total fractions), and O_2, for open and closed cavities. Note that positive fluxes are cavity removal rates, whereas negative fluxes are release rates; mean (95% CI).

	Open cavity			Closed cavity	
Variable measured	RW (μmol L^{-1})	CW (μmol L^{-1})	Flux (mmol m^{-2} d^{-1})	t=0 (μmol L^{-1})	Flux (mmol m^{-2} d^{-1})
DOC	85 ± 9	70 ± 5	367 ± 148	103 ± 6	342 ± 33
BC	2.6 ± 0.4	2.2 ± 0.2	9.9 ± 4.0	3.6 ± 0.2	3.6 ± 0.5
DON	5.8 ± 0.9	5.4 ± 1.0	9.2 ± 8.6	6.3 ± 0.4	7.0 ± 0.7
DIN	0.9 ± 0.2	1.3 ± 0.1	-9.6 ± 3.7	1.0 ± 0.2	-3.2 ± 0.7
DOP	0.18 ± 0.03	0.12 ± 0.01	1.2 ± 0.5	0.20 ± 0.02	1.0 ± 0.2
DIP	0.05 ± 0.01	0.08 ± 0.01	-0.6 ± 0.3	0.09 ± 0.06	-0.3 ± 0.1
O2	nd	nd	nd	212 ± 6	-136 ± 38
Variable derived					
POC	5.3 ± 0.7	4.4 ± 0.4	19.8 ± 8.0	7.2 ± 0.5	7.2 ± 1.0
PON	0.9 ± 0.1	0.8 ± 0.1	3.3 ± 0.7	1.3 ± 0.1	1.3 ± 0.2
POP	0.08 ± 0.01	0.07 ± 0.01	0.3 ± 0.1	0.11 ± 0.01	0.1 ± 0.0
TOC	90 ± 10	74 ± 5	387 ± 156	110 ± 6.5	349 ± 34
TON	6.7 ± 1.0	6.2 ± 1.1	12.5 ± 9.3	7.6 ± 0.5	8.3 ± 0.9
TOP	0.26 ± 0.04	0.19 ± 0.02	1.5 ± 0.6	0.31 ± 0.03	1.1 ± 0.2

For every mole of organic carbon removed by the cavity, on average 0.39 mole of oxygen was removed (*Table 7.1*). Assuming unity for carbon-to-oxygen stoichiometry in respiration, cavities on average respired 39% of the organic carbon removed, leaving 61% for tissue assimilation (regeneration). Assuming that all TON and TOP is used for assimilatory purposes and correcting for carbon respiration would yield a C:N:P ratio of 106:4:0.5 (C:N = 26.5; C:P = 212) for assimilation.

Community fluxes

The relative contribution of different communities to the cavity surface was derived from the cryptofaunal composition of 11 cavities determined in two previous studies in the same study area (Scheffers 2005; Van Duyl et al. 2006). The four surface dominating cavity communities, covering on average

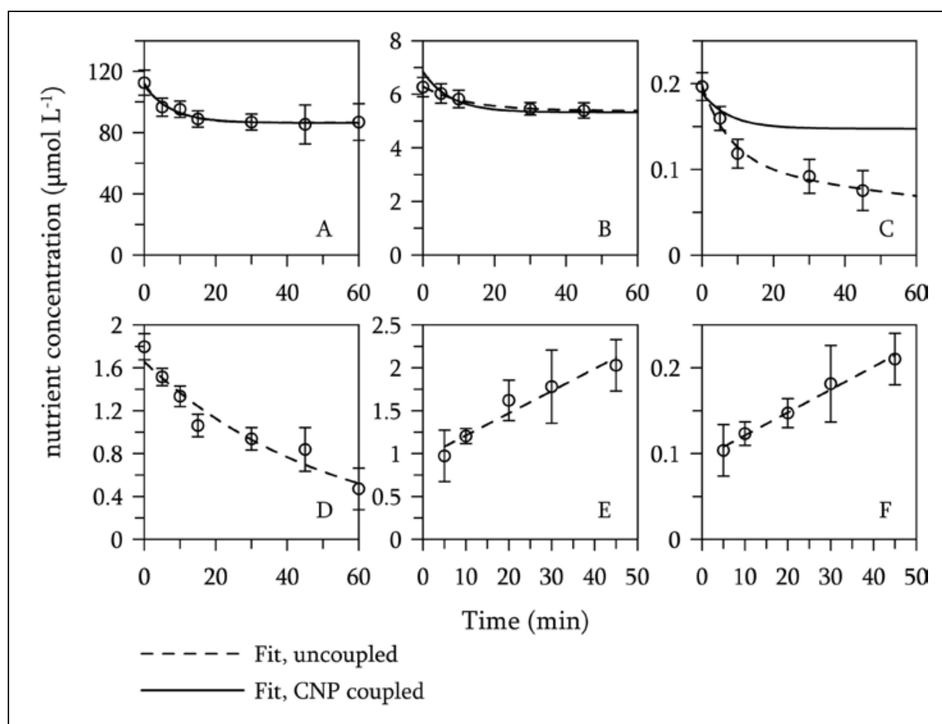


Fig. 7.1 - The average change in concentration of (A) dissolved organic carbon (DOC; n = 23), (B) dissolved organic nitrogen (DON; n = 11), (C) dissolved organic phosphorus (DOP; n = 12), (D) bacterioplankton (C; n=23), (E) dissolved inorganic nitrogen (DIN; n=13), and (F)dissolved inorganic phosphorus (DIP; n=15) with time in coral cavities after closure. Given are the trend lines (dashed) by a 2G-model fit (DOC, DON, and DOP), an exponential fit (bacterioplankton), and a linear fit (DIN and DIP). Additionally, a trend line (solid) is given for the coupled DOC, DON, and DOP removal rates by a 2G-model fit. DOC and DON showed a well coupled removal according to their initial C:N ratio, where more DOP is removed than according to its initial atomic ratio with C and N.; mean ± SD.

88% of the total surface area (TSA), were selected for flux incubation and detailed element budgets: Sponge (26 ± 5% TSA), sediment (39 ± 4% TSA), calcareous algae (19 ± 1% TSA), and bare substratum (4 ± 2% TSA; mean ± SD). The remaining 12% cover is ascribed to ascidians, bryozoans, polychaetes, corals, and bivalves.

Incubation experiments showed that the sponge and sediment community removed substantial amounts of organic matter from the ambient water (sponge almost 1 mol C m^{-2} d^{-1}), whereas the communities calcareous algae and bare substratum showed neither significant removal nor release of organic matter (*Table 7.2*). Particulate organic carbon was only removed by sponge, whereas

other communities did not significantly remove or release POC (*Table 7.2*). 93% of the organic C, 83% of the organic N, and 87% of the organic P removal by the sponge community originated from the dissolved fraction. Respiration accounted for 40-45% of the total organic matter consumption. Sponges released 16% of the consumed total organic N as inorganic N, and 23% of the consumed total organic P as inorganic P. The sediment released approximately as much P as it consumed (78%) as DIP, but released only 12% of the consumed TON as DIN. The remaining fraction may have served benthic assimilation or fuelled coupled nitrification-denitrification in the sediment (*Table 7.2*).

Table 7.2 - Community fluxes of C, N, P (organic and inorganic; dissolved, particulate, and total fractions), and O_2, for sponge and sediment. Note that positive fluxes are cavity removal rates, whereas negative fluxes are release rates; mean (95% CI).

Variable measured	Community flux Sponge (mmol $m^{-2} d^{-1}$)	Community flux Sediment (mmol $m^{-2} d^{-1}$)	Variable derived	Community flux Sponge (mmol $m^{-2} d^{-1}$)	Community flux Sediment (mmol $m^{-2} d^{-1}$)
DOC	915 ± 65	168 ± 25	POC	71 ± 14	ns
BC	36 ± 7	ns	PON	13 ± 2	ns
DON	63 ± 15	18 ± 9	POP	1.1 ± 0.2	ns
DIN	-12 ± 5	-2 ± 1	TOC	986 ± 79	168 ± 25
DOP	7.1 ± 1.4	0.9 ± 0.3	TON	76 ± 17	18 ± 9
DIP	-1.9 ± 0.7	-0.7 ± 0.3	TOP	8.2 ± 1.6	0.9 ± 0.3
O2	419 ± 79	134 ± 20			

The sponge community played a major role in the overall C, N, and P balance of coral cavities (*Table 7.3*). Covering 26% of the TSA, sponges accounted for 73% of the TOC removal by cavities. For every mole of organic carbon removed by the sponges, on average 0.425 mole of oxygen was removed (*Table 7.2*). Sponges respired on average 42.5% of the organic carbon removed, leaving 57.5% for tissue assimilation (regeneration). Assuming again that all TON and TOP is used for assimilatory purposes and correcting for carbon respiration would yield a C:N:P ratio of 106:14:1.5 (C:N = 7.5; C:P = 69) for assimilation.

Table 7.3 - The relative contribution of the surface dominating cavity communities to the total cavity surface (TSA); The community total organic carbon (TOC) flux, corrected for cover; Percentage of community TOC flux in relation to the total cavity TOC flux.

	Sponge	Sediment	Calcareous algae	Bare substrate	Unknown*
Cover (% of TSA)	26	39	19	4	12
TOC flux corrected for cover	256	66	ns	ns	27‡
% of total cavity TOC flux	73	19	ns	ns	8‡

* unknown cavity biota consists of (e.g.): ascidians, bryozoans, polychaetes, corals
ns: not significant
‡ calculated from total cavity removal rate minus compartment removal rates

Mass balances

The mass balances for the closed cavity and separate communities (*Fig. 7.2*) are all based on incubation experiments, using the same mathematical approach for C, N, and P fluxes (measurements obtained from *Tables 7.1* and *7.2*). Therefore, the budget for the closed cavity was used to evaluate the relative contribution of communities in overall cavity nutrient cycling, not the open cavity budget. The C, N, P mass balances for the open cavity (not shown), however, were strikingly similar to the balances for the closed cavity. Sponge (*Fig. 7.2B*) and sediment (*Fig. 7.2C*) community fluxes were normalised to overall cavity fluxes (*Fig. 7.2A*) using their respective cover percentage of TSA.

Cavity (*Fig. 7.2A*): The total uptake of organic carbon amounts to 349 mmol C m^{-2} d^{-1}, of which 136 mmol C m^{-2} d^{-1} is being respired to inorganic carbon and 213 mmol C m^{-2} d^{-1} is assimilated. Assuming Redfield ratio for the cavity biomass, the assimilation of 213 mmol C m^{-2} d^{-1} would require 32 mmol N m^{-2} d^{-1}, whereas only 8 mmol N m^{-2} d^{-1} is supplied by the uptake of DON and PON and 3 mmol N m^{-2} d^{-1} is excreted as DIN. Therefore, (-32+8-3) 27 mmol N m^{-2} d^{-1} is to be regenerated and resorbed during turnover of cavity biomass. Assuming steady state conditions for cavity biomass, this would leave 5 mmol N m^{-2} d^{-1} to be excreted in the particulate fraction. Likewise, the assimilation according to Redfield stoichiometry would require 2 mmol P m^{-2} d^{-1}, while in total 1.1 mmol P m^{-2} d^{-1} is supplied by uptake and 0.3 mmol P m^{-2} d^{-1} is excreted as inorganic P. Thus, (-2+1.1-0.3) 1.2 mmol P m^{-2} d^{-1} should be resorbed from

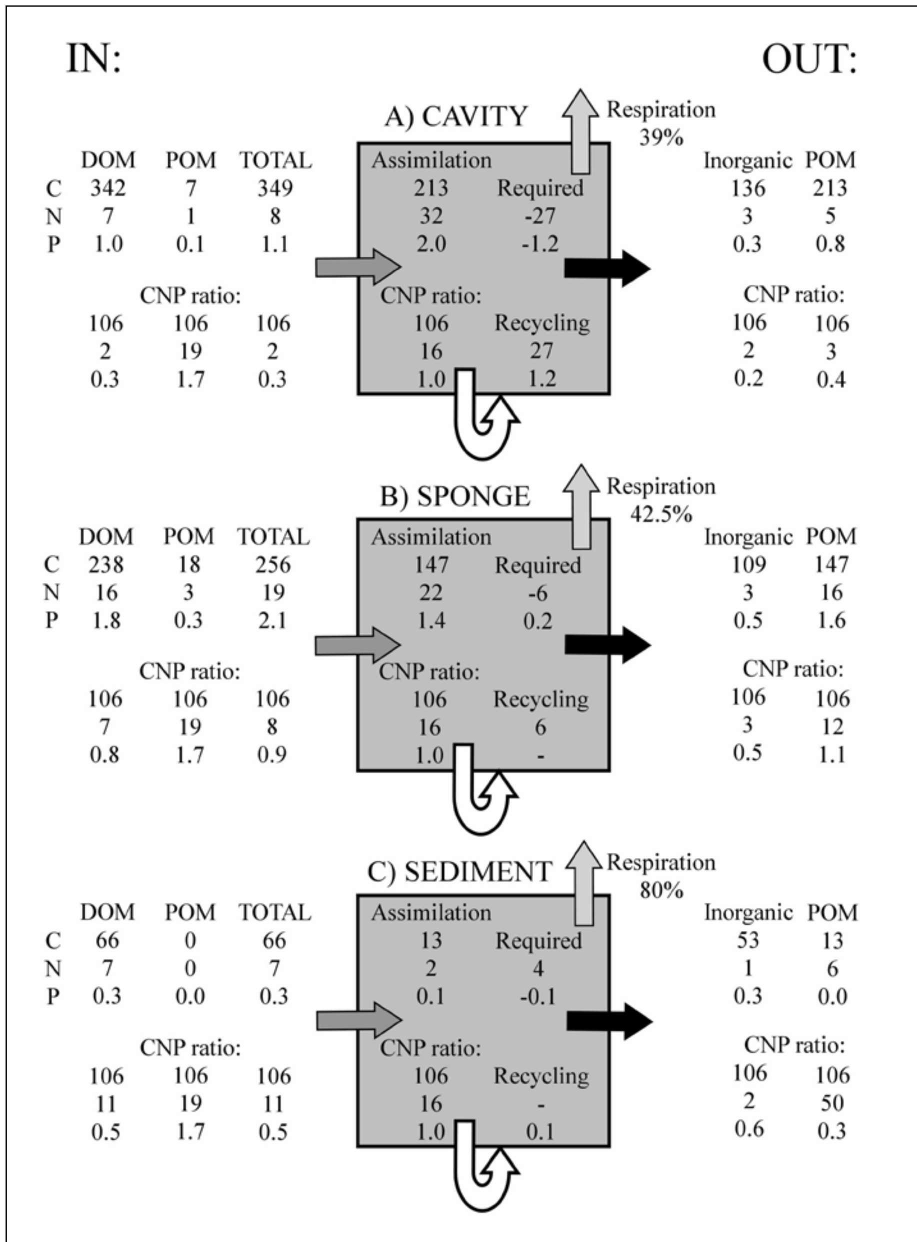


Fig. 7.2 - Mass balances of C, N, and P for: (A) closed cavity, and for the dominant cavity communities (B) sponge, and (C) sediment. In- and effluxes of organic and inorganic nutrients through the different communities are given. The communities are represented by grey boxes in which the gains and losses of C, N, and P for each community are shown (negative means required, whereas positive means a surplus). Consequently, requirement of N or P is met by internal recycling. The CNP ratios represent the stoichiometry of uptake or release of different nutrient pools and the stoichiometry of the cavity biota (in the grey box).

the turnover of cavity biomass, leaving 0.8 mmol P m^{-2} d^{-1} to be excreted in the particulate fraction. Based on these estimates, the predicted C:N:P elemental stoichiometry of the particulate cavity waste would be 106:3:0.4, which is very similar to the stoichiometry of total C, N, and P uptake by the cavity.

Communities (*Fig. 7.2B,C*): Only mass balances for the sponge and sediment communities were presented, as these were found to dominate the total fluxes of cavities. The total organic carbon removal by sponges amounted to 256 C m^{-2} TSA d^{-1}, of which 109 C m^{-2} TSA d^{-1} was respired (40-45%). Sponge carbon assimilation (147 mmol C m^{-2} TSA d^{-1}) would require 22 mmol N m^{-2} TSA d^{-1}, 19 mmol N m^{-2} TSA d^{-1} of which is supplied as dissolved and partciulate organic N. Since 3 mmoles N m^{-2} TSA d^{-1} are released as inorganic N, in total 6 mmol N m^{-2} TSA d^{-1} are to be resorbed from the tissue replaced by new assimilation. Similar calculations for P revealed no regeneration of sponge tissue P is required to cover the assimilatory P demand. Only a minor part of N and P was released as inorganic N and P. The major part of matter released by the sponge community was in the form of particulate organic matter with a predicted C:N:P stoichiometry of 106:12:1.1, relatively close to Redfield, and similar to the stoichiometry of total C, N, and P assimilation by sponges (106:14:1.5). Overall, sponges appear to convert dissolved organic C, N, and P into higher quality particulate organic matter. This conversion was predicted to be 84% and 76% for N and P, respectively, leaving 16% (N) and 24% (P) to be regenerated into inorganic nutrients.

The sediment released inorganic N and P and produces a relatively small amount of POM. This POM is enriched in N and depleted in P as compared to Redfield (106:50:0.3 *versus* 106:16:1). Recycling of neither N nor P was required in this mass balance.

Discussion

The conventional view on the functioning of coral cavities is that these are hot spots of mineralisation on coral reefs, processing particulate organic matter and releasing inorganic nutrients (Tribble et al. 1988; Richter et al. 2001; Van Duyl et al. 2006). This view emerged from studies in which particulate organic matter (POM) was considered the main source of organic carbon and nutrients for cavities. Based on the influxes of POM and the return fluxes of inorganic nutrients, regeneration efficiencies of 128-252% (N) and 182-249% (P) can be estimated from earlier studies (Richter et al. 2001; Van Duyl et al. 2006). These >100% efficiencies suggest non-steady-state conditions with respect to cavity biomass and element cycling or an additional source of carbon and organic nutrients. The estimated POM influxes and the return fluxes of inorganic nutrients for the present study are well in line with these earlier studies and would have resulted in mineralisation efficiencies of 246% (N) and 255% (P). However, by including DOM as a source of organic carbon and nutrients these regeneration efficiencies reduce to 39% for N and 27% for P. Apparently, coral cavities do represent important sinks for dissolved organic C, N, and P, but only play a modest role in the regeneration of inorganic nutrients. This predominance of DOM over POM uptake (1-2 orders of magnitude), has been observed for different reef types from various geographical domains (De Goeij et al. 2007) and urges us to drastically revise the concept of coral cavity functioning in terms of carbon and nutrient cycling.

By comparing cavity with community fluxes, we were able to asses the quantitative contribution of the communities to overall nutrient cycling within coral cavities. We identified the sponge community as the most important sink for organic matter. Similar to cavities, over 90% of the total uptake of organic carbon by sponges is accounted for by DOC. A recent study on the sponge *Halisarca caerulea* (De Goeij et al. 2008a) indicated that only ~40% of the TOC uptake was respired, leaving ~60% for assimilation. Tracer ^{13}C studies with different substrates provided evidence that $DO^{13}C$ is assimilated by the sponge, again showing a 40:60 respiration:assimilation ratio (De Goeij et al. 2008b). This would be equivalent to a biomass increase of ~38% body C d^{-1}, on a net growth rate of ~0 (1 m^2 sponge = 1540 mmol C sponge (De Goeij et al. 2008b)). Assimilated carbon may be used for reproduction or the production

of secondary metabolites, but can also point to a high turnover of sponge biomass. Evidence for the latter is provided by histological results, demonstrating high cell proliferation rates and the subsequent release of cellular debris by *H. caerulea*. These results corroborate well with reports on significant excretion of debris found for other tropical sponges (Reiswig 1971; Yahel et al. 2003).

Based on our mass balance for sponge communities, the predicted major pathway of element cycling in sponges is the net conversion of DOM into POM. In transforming dissolved organic matter into biomass and the subsequent production of particulate cellular debris, the sponge community provides the blueprint for carbon and nutrient cycling in the coral cavity (compare *Fig. 7.2A* and *B*). In contrast to the conventional view, coral cavities seem particularly important in the conversion of dissolved organic matter into particulate organic matter through assimilation and cellular debris formation. Although this pathway still requires experimental validation, its importance was illustrated by the strings of mucus and detritus detaching from the cavity walls (J.M. de Goeij, pers. obs.). The predicted elemental composition of this debris suggests it to be a good food source for particle feeding communities on the reef.

In order to qualify the role of cavities in overall reef nutrient cycling, fluxes need to be extrapolated. A squared meter of projected reef conceals on average 4.7 m^2 of TSA (Richter et al. 2001). However, per m^2 of reef, on average only 0.3 m^2 is covered by reef framework (Ginsburg 1983; Richter et al. 2001). Thus, one m^2 of reef equals approximately $0.3 \times 4.7 = 1.6$ m^2 TSA, which is a very rough estimate, considering the wide variety of community structures in different reef zones. For conservancy, we approximate the cavity surface-to-projected reef surface ratio to be 1:1. The range of gross primary production rates of entire reefs is estimated 200-500 mmol C m^{-2} reef d^{-1} (Hatcher 1990), which is roughly balanced by gross community respiration. The framework respiration rate of 136 mmol C m^{-2} d^{-1} from our study represents 27-68% of the gross community respiration for an entire reef ecosystem, whereas total organic carbon uptake by coral cavities is in the same order of magnitude as the gross primary production of the reef (De Goeij and Van Duyl 2007). It is hypothesised that the DOM, feeding the cavities is relatively young and reactive and therefore locally produced. The main sources of DOM identified on reefs are coral mucus (Wild et al. 2004) and the leakage from vascular plants, macro- and planktonic algae

(Benner et al. 1986). Corals can excrete 40-50% of their photosynthetically fixed carbon as mucus (Davies 1984; Crossland 1987), of which up to 80% is immediately dissolved in the seawater (Wild et al. 2004). Macroalgae (Newell et al. 1980) and vascular plants (Benner et al. 1986) may excrete 30-50% of their gross production as dissolved organic carbon. Although evidence is still lacking, these sources may significantly contribute to DOM feeding by coral cavity biota. Our results show that the reef framework or the 'lower reef' is the heterotrophic counterpart of the autotrophic 'upper reef'.

To conserve essential elements, mechanisms develop that promote recycling within the system, ultimately resulting in the stabilisation of the ecosystem (Pomeroy 1970; Odum 1971). The regeneration of nutrients by the reef framework has long been considered as an important source of inorganic nutrients for the highly productive reef surrounded by the highly oligotrophic oceans (Andrews and Müller 1983; Tribble et al. 1988). We hypothesise that the transformation of locally produced DOM into particulate matter enhances the retention of nutrients within the coral reef ecosystem. Due to their particulate nature, POM may go through successive cycles of resuspension and subsequent settling from the water column before being washed out to the adjacent ocean. Meanwhile, the particle is subject to bacterial degradation, slowly regenerating inorganic nutrients, and to the interception by particle feeding communities. In contrast, DOM would be lost more easily from the reef ecosystem. The conversion of DOM into particles as a mechanism for energy transfer in marine systems has been described in earlier studies as the so-called 'microbial-loop' (Azam et al. 1983). In coral cavities, not the bacterioplankton, but the sponge community functions as a 'sponge loop'. Since the carbon is not looped within the organism, but transferred to various trophic levels it would be more appropriate to define the functioning of coral cavities as a 'cryptic carbon shunt'. The validation of this hypothesis requires the resolution of two main issues: Identification of the sources of DOM fuelling the cavities and unraveling the composition of the excreted POM, both quantitatively and qualitatively.

CHAPTER 8

The cryptic carbon shunt, the sponge loop, and beyond…

“Living organisms and their nonliving (abiotic) environment are inseparably interrelated and interact upon each other. Any unit that includes all of the organisms (i.e., the “community”) in a given area interacting with the physical environment so that a flow of energy leads to clearly defined trophic structure, biotic diversity, and material cycles (i.e., exchange of materials between living and nonliving parts) within the system is an ecological system or *ecosystem*.”

Eugene P. Odum (1971)

Are sponges ‘DOM’-feeders?

It is an important and still unresolved debate whether only sponges containing associated bacteria can utilise DOM. We have found that both sponge cells and associated bacteria can assimilate DOM. However, focusing on the cycling of DOM within the coral reef ecosystem it is important to emphasize that in fact the invertebrates are processing the water and deliver the nutrients to the sponge-microbe association, by actively pumping and filtering. In this respect invertebrates, as opposed to bacterioplankton, play a key role in the uptake and remineralisation of DOM within the coral reef.

The discussion as to whether only sponges containing associated micro-organisms can use DOM is also a debate on the definition of the term ‘dissolved’, which is operationally defined as everything passing a GF/F (~0.7 µm!) filter. This definition may have a substantial impact on what is and what is not available for organisms other than microorganisms to feed on DOM. The DOM fraction definitely contains particles, as small as 0.1 µm that filter feeders, at least sponges, are keen to filter. Moreover, colloids with a size of a few µm can contain low and high molecular weight molecules, but may be filtered as a particle. In this respect the current definition should be considered as fuelling confusion and also as rather inappropriate for benthic aquatic systems with grazing activity by suspension feeders, capable of processing significant amounts of ‘DOM’. In these ecosystems, a more strict division of dissolved organic matter in confined size fractions would be very helpful to better distinguish between ‘truly’ (<0.1 µm) and ‘colloidal’ (>0.1 and <GF/F) DOM.

The 'sponge loop' and the 'cryptic carbon shunt'

Sponges are net converters of DOM into particulate organic matter. In fact, the conversion of predominantly DOM into particles as a manner of energy transfer in marine systems is described in earlier studies as the so-called 'microbial-loop' (Azam et al. 1983). In the microbial loop bacteria utilise DOM, which is mainly produced by phytoplankton. Bacteria are then consumed by flagellates, which in turn are eaten by ciliates. This way, dissolved organic matter is moving up the trophic levels in particulate form as an energy carrier. Subsequently, in analogy with processes in the coral cavities, inorganic nutrients are regenerated and carbon is lost through respiration. Another analogy with the microbial loop is the large surface to volume ratio of both bacteria and encrusting sponges. This increases their ability to take up nutrients at very low concentrations, like in oligotrophic tropical waters, and, additionally, the increased ability to take up DOM. Thus, the cavity sponges function as a 'sponge loop', surpassing the classical 'microbial loop' in coral cavities in terms of nutrient fluxes. Considered strictly, both 'loops' are actually no loops but shunts of energy, and the referred energy is not being looped within the organisms, but is carried to higher trophic levels. Therefore, we argue that a coral cavity is more appropriately defined as a 'cryptic carbon shunt'.

Damage control by coral cavity sponges: Living and surviving in oligotrophic conditions

Halisarca caerulea is an encrusting coral reef sponge, inhabiting coral overhangs and framework cavities. Its habitat provides shelter and protection, and therefore the struggle for space and food for these benthic organisms is high. The tropical waters surrounding coral reefs are very low in nutrients and considered as the marine equivalent of a desert. Marine benthic organisms have adapted to their environment by being very efficient and opportunistic feeders. Therefore, in order to gain enough energy for their metabolic processes, sponges pump enormous volumes of water in time. As a consequence, the sponge as a benthic filter feeder is permanently in contact with and threatened by potential mutagenic, toxic, viral, bacterial and physical stress. We hypothesise that sponges cope with this hostile environment by a high turnover of ("old") cells, to prevent potential damage. Since there is almost no space within the cavities to grow large, sponges may change their strategy to grow old and reproduce for many years. They are able to reach this goal by constantly renewing their

system of food uptake and gain of energy as most essential body part, which is also very vulnerable to exogenous stress. On the other hand, when space is not limited, sponges may show growth rates of almost 3,000 times their normal growth rate if presented a bare piece of substrate. It can be concluded that sponges are very well adapted to changes in their environment.

Sponge choanocyte chambers *versus* human gastrointestinal tract

Sponges are considered to be the oldest still existing metazoan phylum and an important link between a unicellular and multicellular way of living. One would expect that the first 'organs' to be constructed in a multicellular environment are those that provide energy for metabolic processes. In this view, it is quite remarkable how the cell kinetics and the functional organisation of the choanocytes in the sponge show a striking resemblance with epithelial cells lining the mammalian or human gastro-intestinal tract. Both type of cells, supported by extracellular matrix elements, form the functional cellular stuctures that are responsible for the primary and selective uptake of food, essential for energy and growth. In human colonic tissue the proliferating cells are found in the epithelial cell compartment in the lower two-thirds of the crypt, migrating upwards replacing the cells in the upper part of the crypt. At the top of the crypts the excess of cells loose their mutual binding, are detached from the basement membrane and are shed into the lumen (Van de Wetering et al. 2002). In the process of cell loss apoptosis seems to play only a minor role, since low numbers of apoptotic cells are found in the epithelial compartment (Anti et al. 2001). These processes closely resemble the fate of the choanocytes in the sponge, where large numbers of cells and cellular debris are shed into the lumen and low numbers of apoptotic cells are found in the mesohyl compartment. More similarities can be found in the functional organisation of *H. caerulea* and the human gastrointestinal tract (*Fig. 8.1*). The cuticle of the sponge resembles the basic morphology of the ectodermal or endodermal layers in vertebrates, i.e. the exopinacodermal cells. Furthermore, the endopinacodermal cells resemble the monolayer of absorptive cells in the gastrointestinal tract, whereas the wavy collagen matrix containing archaeocytes and other cells resemble the vertebrate dermis or the lamina propria and submucosa of the digestive system. In other words, the compartimentalisation of the sponge, which has a crucial role in the specific uptake of nutrients by the

organism, closely resemble the compartimentalisation in the human gastrointestinal tract.

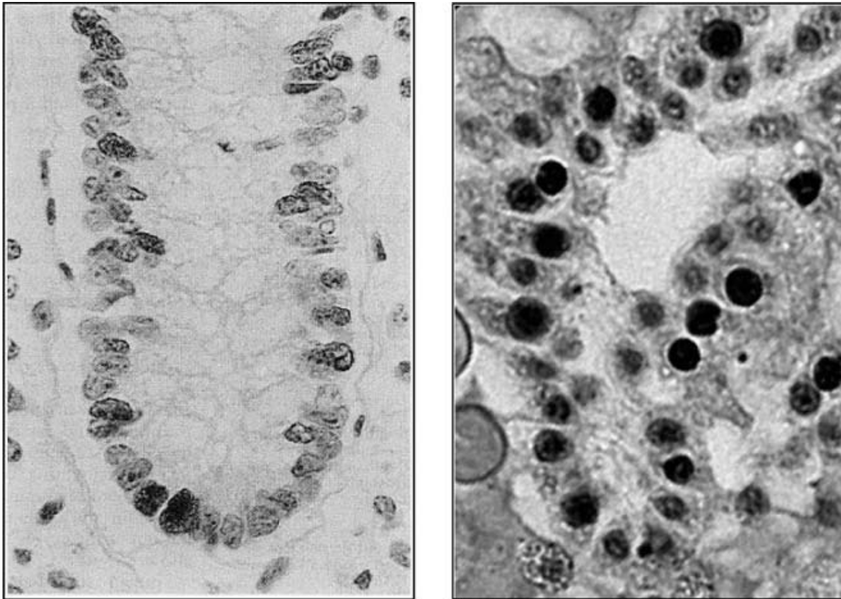


Fig. 8.1 Histological section of (left) human colonic crypt (derived from Potten et al. 1992) and (right) Halisarca caerulea *choanocyte chamber. Tissue was labelled with a monoclonal antibody against BrdU (brown stain), representing proliferative cells. Haematoxylin and Eosin staining.*

Another aspect of resemblance between sponges and the mammalian gastrointestinal tract is the presence of large numbers of symbiotic bacteria. An alliance between eukaryotic and prokaryotic cells is important in polytrophic nutrition. Recent ^{13}C-tracer studies on *H. caerulea* revealed an involvement of both sponge cells and associated bacteria in the assimilation of food within the sponge-microbe association (De Goeij et al. 2008b). The commensal interactions between intestinal microorganisms and animal hosts have been difficult to study in the past because of the diversity of microorganisms involved and the lack of culturability (Hentschel et al. 2003). Using this knowledge on cell kinetics and uptake of food components will allow a better culturing of sponges, and hence may provide new opportunities to study these bacteria-sponge interactions.

Sponges as animal models

Sponges like *Halisarca caerulea* have a great potential as an animal model for various scientific purposes. Their rapid turnover makes these sponges useful in toxicological or pharmaceutical studies. The ability to clone sponges by simply cutting in pieces provides a potential unlimited supply of genetically identical tissue. Genetic and physiological studies on sponges could provide important information on the mechanism of cell shedding. To emphasize the importance of shedding, it should be noted that disruption of shedding of differentiated, epithelial cells in the gastro-intestinal tract may consequently lead to polyp formation, which may develop into cancers of the digestive tract, stomach, and esophagus (Horii et al. 1992; Van de Wetering et al. 2002). In other words, cell shedding may be a very effective way to avoid aberrant growth or carcinogenesis in these filter feeders, which have to pump huge amounts of water, containing not only nutrients, but harmful components as well.

The simple body plan of sponges, hypothesised as the 'bauplan' of metazoan life forms, has recently been recognised in several evolutionary studies (Müller 1998; Maldonado 2004). Sponges are also known as a potential plentiful resource of new products for society, such as food additives, fine chemicals (biomaterials, for instance collagen and biosilica), cosmetics and natural compounds that may become leads for pharmaceutical drug development. A major drawback to implement sponges as a scientific or biotechnological model is the notorious difficulty to culture sponges. The studies in this thesis demonstrate that cell kinetic studies provide unique and essential information on the regulation of growth of these organisms.

REFERENCES

References

A

Abelson A, T Miloh, and Y Loya (1993) Flow patterns induced by substrata and body morphologies of benthic organsisms, and their availability of food particles. Limnol Oceanogr **38**: 1116-1124

Alongi DM (1988) Detritus in coral reef ecosystems: Fluxes and fates. *In* JH Choat et al [eds], Proc 6th Int Coral Reef Symp, p 29-36

Alongi DM, LA Trott, and J Pfitzner (2008) Biogeochemistry of inter-reef sediments on the northern and central Great Barrier Reef. Coral Reefs **27**: 407-420

Andrews JC and H Müller (1983) Space-time variability of nutrients in a lagoonal patch reef. Limnol Oceanogr **28**: 215-227

Anti M, A Armuzzi, S Morini, E Iascone, G Pignataro, C Coco, R Lorenzetti, M Paolucci, M Covino, A Gasbarrini, FM Vecchio, and G Gasbarrini (2001) Severe imbalance of cell proliferation and apoptosis in the left colon and in the rectosigmoid tract in subjects with a history of large adenomas. Gut **48**: 238-246

Atkinson MJ (1988) Are coral reefs nutrient-limited? *In* JH Choat et al [eds], Proc 6th Int Coral Reef Symp, p 157-166

Atkinson MJ, and JL Falter (2003) Coral reefs. *In* KD Black and GB Shimmield [eds], Biochemistry of marine systems, p 40-64. CRC Press

Ayling AL (1983) Growth and regeneration rates in thinly encrusting demospongiae from temperate waters. Biol Bull **165**: 343-352

Ayukai T (1995) Retention of phytoplankton and planktonic microbes on coral reefs within the Great Barrier Reef, Australia. Coral Reefs **14**: 141-147

Azam F, T Fenchel, JG Field, JS Gray, LA Meyer-Reil, and F Thingstad (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser **10**: 257-263

B

Baines SB, NS Fisher, and JJ Cole (2005) Uptake of dissolved organic matter (DOM) and its importance to metabolic requirements of the zebra mussel, *Dreissena polymorpha*. Limnol Oceanogr **50**: 36-47

Benner R, ER Peele, and RE Hodson (1986) Microbial utilization of dissolved organic matter from leaves of the red mangrove, *Rhizophora mangle*, in the Fresh Creek Estuary, Bahamas. Estuar Coast Shelf Sci **23**: 607-619

Benner R (2002) Chemical composition and reactivity. *In* DA Hansell and CA Carlson [eds], Biochemistry of marine dissolved organic matter, p. 59-90. Academic Press

Boon JJ, WIC Rijpstra, JW de Leeuw, and PA Schenck (1975) Phytenic acid in sediments. Nature **258**: 414-416

Børsheim KY and G Bratbak (1987) Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar Ecol Prog Ser **36**: 171-175

Boschker HTS and JJ Middelburg (2002) Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol Ecol **40**: 85-95

Boute N, J-Y Exposito, N Boury-Esnault, J Vacelet, N Noro, K Miyazaki, K Yoshizato, and R Garrone (1996) Type IV collagen in sponges, the missing link in basement membrane ubiquity. Biol Cell **88**: 37-44

Brock RE and JH Brock (1977) A method for quantitatively assessing the infaunal community in coral rock. Limnol Oceanogr **22**: 948-951

Buss LW (1979) Bryozoan overgrowth interactions—the interdependence of competition for space and food. Nature **281**: 475-477

Buss LW and JBC Jackson (1979) Competitive networks: Nontransitive competitive relationships in cryptic coral reef environments. Am Nat **113**: 223-234

Buss LW (1981) Planktonic food availability and suspension-feeder abundance: Evidence of in situ depletion. J Exp Mar Biol Ecol **49**: 151-161

C

Canuel EA, JE Cloern, DB Ringelberg, JB Guckert, and GH Rau (1995) Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnol Oceanogr **40**: 67-81

Campbell RD and CN David (1974) Cell cycle kinetics and development of *Hydra attenuata*. II. Interstitial cells. J Cell Sci **16**: 349-358

Carballeira N, JE Thompson, E Ayanoglu, and C Djerassi (1986) Biosynthetic studies of marine lipids. 5. The biosynthesis of long-chain branched fatty acids in marine sponges. J Org Chem **51**: 2751-2756

Carballeira NM, L Maldonado, and B Porras (1987) Isoprenoid fatty acids from marine sponges. Are sponges selective? Lipids **22**: 767-769

Carballeira NM, F Shalabi, and V Negron (1989) 2-Hydroxy fatty acids from marine sponges 2. The phospholipid fatty acids of the caribbean sponges *Verongula gigantea* and *Aplysina archeri*. Lipids **24**: 229-232

Carlson CA (2002) Production and removal processes. *In* DA Hansell and CA Carlson [eds], Biochemistry of marine dissolved organic matter, p. 91-152. Academic Press

Carpenter JH (1965) The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnol Oceanogr **10**: 141-143

Christoffersen K, C Bernard, and J Ekebom (1996) A comparison of the ability of different heterotrophic nanoflagellates to incorporate dissolved macromolecules. Arch Hydrobiol **48**: 73–84

Cleutjens JP, MG Havenith, C Beek, M Vallinga, J Ten Kate, and FT Bosman (1990) Origin of basement membrane type IV collagen in xenografted human epithelial tumor cell lines. Am J Pathol **136**: 1165-1172

Crossland CJ (1983) Dissolved nutrients in coral reef waters. *In* DJ Barnes [ed], Perspectives on coral reefs, p 56-68. Australian Institute of Marine Science

Crossland CJ (1987) In situ release of mucus and DOC-lipid from the corals *Acropora palmata variabilis* and *Stylophora pistillata* in different light regimes. Coral Reefs **35**: 35-42

Crossland CJ, BG Hatcher, and SV Smith (1991) Role of coral reefs in global ocean production. Coral Reefs **10**: 55-64

Culberson CH (1991) Dissolved oxygen. WOCE Operations and Methods **3**: 1-14

Custodio MR, I Prokic, R Steffen, C Koziol, R Borojevic, F Brümmer, M Nickel, and WEG Müller (1998) Primmorphs generated from dissociated cells of the sponge Suberites domuncula: A model system for studies of cell proliferation and cell death. Mech Ageing Dev **105**: 45–59

D

Dalsgaard J, M St John, G Kattner, D Müller-Navarra, and W Hagen (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol **46**: 225-340

Darwin CR (1842) The structure and distribution of coral reefs. Smith, Elder and Co

David CN and RD Campbell (1972) Cell cycle kinetics and development of *Hydra attenuata*. I. Epithelial cells. J Cell Sci **11**: 557-568

Davies PJ (1984) The role of Zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs **2**: 181-186
De Goeij JM and FC van Duyl (2007) Coral cavities are sinks of dissolved organic carbon (DOC). Limnol Oceanogr **52**: 2608-2617
De Goeij JM, H van den Berg, MM van Oostveen, EHG Epping, and FC van Duyl (2008a) Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. Mar Ecol Prog Ser **357**: 139-151.
De Goeij JM, L Moodley, M Houtekamer, NM Carballeira, and FC van Duyl (2008b) Tracing ^{13}C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM-feeding. Limnol Oceanogr **53**: 1376-1386
DiSalvo LH (1971) Regenerative functions and microbial ecology of coral reefs: Labelled bacteria in a coral reef microcosm. J Exp Mar Biol Ecol **7**: 123-136
DiSalvo LH (1974) Soluble phosphorus and amino-nitrogen release to the sea water during recoveries of coral reefs regenerative sediments. *In* AM Cameron et al [eds], Proc 2nd Coral Reef Symp, p 11-19
Dolbeare F (1995) Bromodeoxyuridine: A diagnostic tool in biology and medicine, Part I: Historical perspectives, histochemical methods and cell kinetics. Histochem J **27**: 339-369
Ducklow HW (1990) The biomass, production and fate of bacteria in coral reefs. *In* Z Dubinsky [ed], Ecosystems of the world: Coral reefs, p 265-289. Elsevier
E
Efremova SM and VI Efremov (1979) Prolifération cellulaire chez la larve nageante de l'éponge d'eau douce: *Baikalospongiae bacillifera* (Dybowski). *In* C Lévi C and N Boury-Esnault [eds], Biologie des Spongiaires, p 59-66. Editions du C.N.R.S.
Erez J 1990 On the importance of food sources in coral reef ecosystems. *In* Z Dubinsky [ed], Ecosystems of the world: Coral reefs, p 411-418. Elsevier
F
Fagerbakke KM, M Heldal, and S Norland (1996) Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. Aquat Microb Ecol **10**: 15-27
Fenchel T (1988) Marine Plankton Food Chains. Annu Rev Ecol Syst **19**: 19-38
Ferrier-Pagès C and JP Gattuso (1998) Biomass, production and grazing rates of pico- and nanoplankton in coral reef waters (Miyako Island, Japan). Micr Ecol **35**: 46-57
Frost BW (1987) Porifera. *In* TJ Pandian and FJ Vernberg [eds], Animal energetics, p 27-53. Academic Press
Fuhrman JA and Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. Mar Biol **66**: 109-120
Fukuda R, H Ogawa, T Nagata, and I Koike (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Appl Environ Microb **64**: 3352-3358
Furnas M, A Mitchell, M Skuza, and J Brodie (2005) In the other 90%: Phytoplankton responses to enhanced nutrient availability in the Great Barrier Reef Lagoon. Mar Pollut Bull **51**: 263-265.

G
Garrett P, DL Smith, AO Wilson, and D Patriquin (1971) Physiography, ecology, and sediments of two Bermuda patch reefs. J Geol **79**: 647-668
Gast GJ, S Wiegman, E Wieringa, FC van Duyl, and RPM Bak (1998) Bacteria in coral reef water types: Removal of cells, stimulation of growth and mineralization. Mar Ecol Prog Ser **167**: 37-45
Gili JM and R Coma (1998) Benthic suspension feeders: Their paramount role in littoral marine food webs. Trends Ecol Evol **13**: 316-321
Ginsburg RN and JH Schroeder (1973) Growth and submarine fossilization of algal cup reefs, Bermuda. Sedimentology **20**: 575–614
Ginsburg RN (1983) Geological and biological roles of cavities in coral reefs. *In* D Barnes [ed], Perspectives on coral reefs, p 148-153. Australian Institute of Marine Science
Godec R, K O'Neill, and R Hutte (1984) New technology for TOC analysis in water. Ultrapure Water **9**: 17-22
Gonzalez JM and CA Suttle (1993) Grazing by marine nanoflagellates on viruses and virus-sized particles: Ingestion and digestion. Mar Ecol Prog Ser **94**: 1-10
Graeve M, G Kattner, and W Hagen (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. J Exp Mar Biol Ecol **182**: 97-110
Grieshaber MK, I Hardewig, U Kreutzer, and HO Pörtner (1994) Physiological and metabolic responses to hypoxia in invertebrates. Rev Physiol Bioch P **125**: 43-147
Guo L and PH Santschi (1997) Composition and cycling of colloids in marine environments. Rev Geophys **35**: 17–40
H
Hadas E, D Marie, M Shpigel, and M Ilan (2006) Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnol Oceanogr **51**: 1548-1550
Hall D, SY Lee, and T Meziane (2006) Fatty acids as trophic tracers in an experimental estuarine food chain: Tracer transfer. J Exp Mar Biol Ecol **336**: 42-53
Hamner WM and E Wolanski (1988) Hydrodynamic forcing functions and biological processes on coral reefs: a status review. *In* JH Choat et al [eds], Proc 6th Int Coral Reef Symp, p 103-113
Harvey HR (2006) Sources and cycling of organic matter in the marine water column. *In* JK Volkman [ed], The Handbook of Environmental Chemistry, p 1-25. Springer-Verlag
Hatcher BG (1988) Coral reef primary productivity: A beggar's banquet. Trends Ecol Evol **3**: 106-111
Hatcher BG (1990) Coral reef primary productivity: A hierarchy of pattern and process. Trends Ecol Evol **5**: 149-155
Hatcher BG (1997a) Organic production and decomposition. *In* C Birkeland [ed], Life and death of coral reefs, p 140-174. Chapman & Hall
Hatcher BG (1997b) Coral reef ecosystems: How much greater is the whole than the sum of the parts? Coral Reefs **16**: 77-91
Havenith MG, JP Cleutjens, C Beek, E van der Linden, AF de Goeij, and FT Bosman (1987) Human specific anti-type IV collagen monoclonal antibodies, characterization and immunohistochemical application. Histochem Cell Biol **87**: 123-128
Heldal M, S Norland, and O Tumyr (1985) X-ray microanalytic method for measurement of dry matter and elemental content of individual bacteria. Appl Environ Microbiol **50**: 1251-1257

Helder W and RTP de Vries (1979) An automatic phenol-hypochlorite method for the determination of ammonia in sea- and brackish waters. Neth J Sea Res **13**: 154-160

Hentschel U, U Dobrindt, and M Steinert (2003) Commensal bacteria make a difference. Trends Microbiol **11**: 148-150

Hoffmann F, O Larsen, V Thiel, HT Rapp, T Pape, W Michaelis, and J Reitner (2005) An anaerobic world in sponges. Geomicrobiol J **22**: 1-10

Horii A, S Nakatsuru, Y Miyoshi, S Ichii, H Nagase, Y Kato, A Yanagisawa, and Y Nakamura (1992) The *APC* gene, responsible for familial adenomatous polyposis, is mutated in human gastric cancer. Cancer Res **52**: 3231-3233

Hutchings PA (1974) A preliminary report on the density and distribution of invertebrates living on coral reefs. *In* AM Cameron et al [eds], Proc 2nd Int Coral Reef Symp, p 285-296

Hutchings PA (1983) Cryptofaunal communities of coral reefs. *In* DJ Barnes [ed], Perspectives on coral reefs, p 200-208. The Australian Institute of Marine Science

J

Jackson JBC, TF Goreau, and WD Hartman (1971) Recent Brachiopod-Coralline Sponge Communities and Their Paleoecological Significance. Science **173**: 623-625

Jackson JBC and JE Winston (1982) Ecology of cryptic coral reef communities. I. Distribution and abundance of major groups of encrusting organisms. J Exp Mar Biol Ecol **57**: 135–147

Johannes RE (1967) Ecology of Organic Aggregates in the Vicinity of a Coral Reef. Limnol Oceanogr **12**: 189-195

Jørgensen CB (1976) August Pütter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. Biol Rev **51**: 291-328

Junqueira LCU, G Bignolas, and RR Brentani (1979) Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J **11**: 447-455

K

Kamada S, U Kikkawa, Y Tsujimoto, and T Hunter (2005) Nuclear translocation of caspase-3 is dependent on its proteloytic activation and recognition of a substrate-like protein(s). J Biol Chem **280**: 857-860

King N, CT Hittinger, and SB Carroll (2003) Evolution of key cell signaling and adhesion protein families predates animal origins. Science **301**: 361-363

Kinsey DW (1983) Standards of performance in coral reef primary production and carbon turnover. *In* DJ Barnes [ed], Perspectives on coral reefs, p 209-220. Australian Institute of Marine Science

Kinsey DW (1985) Metabolism, calcification and carbon production: 1 systems level studies. *In* C Gabrie and B Salvat [eds], Proc 5th Int Coral Reef Symp, p 505-526

Koike I, H Shigemitsu, T Kazuki, and K Kazuhiro (1990) Role of sub-micrometre particles in the ocean. Nature **345**: 242-244

Kötter I and J Pernthaler (2002) In situ feeding rates of obligate and facultative coelobite (cavity-dwelling) sponges in a Caribbean coral reef. *In* MK Moosa et al [eds], Proc 9th Int Coral Reef Symp, p 347-352

Kötter I (2003) Feeding ecology of coral reef sponges. PhD-thesis, Universität Bremen

Koziol C, R Borojevic, R Steffen, and WEG Müller (1998) Sponges (Porifera) model systems to study the shift from immortal to senescent somatic cells: The telomerase activity in somatic cells. Mech Ageing Dev **100**: 107-120

Kumar S (2007) Caspase function in programmed cell death. Cell Death Diff **14**: 32-43

L
Larkum AWD (1983) The primary productivity of plant communities on coral reefs. *In* DJ Barnes [ed], Perspectives on coral reefs, p 221-230. Australian Institute of Marine Science
Latyshev NA, NV Naumenko, VI Svetashev, and YY Latypov (1991) Fatty acids of reef-building corals. Mar Ecol Prog Ser **76**: 295-301
Leys SP and DI Eerkes-Medrano (2006) Feeding in a calcareous sponge: Particle uptake by pseudopodia. Biol Bull **211**: 157-171
Logan A, SM Mathers, and MLH Thomas (1984) Sessile invertebrate coelobite communities from reefs of Bermuda: Species composition and distribution. Coral Reefs **2**: 205-213
Loh AN, JE Bauer, and EA Canuel (2006) Dissolved and particulate organic matter source-age characterization in the upper and lower Chesapeake Bay: A combined isotope and biochemical approach. Limnol Oceanogr **51**: 1421-1431
M
Magnino G, A Sarà, T Lancioni, and E Gaino (1999) Endobionts of the coral reef sponge Theonella swinhoei (Porifera, Demospongiae). Invertebr Biol **118**: 213-220
Mague TH, E Friberg, DJ Hughes, and I Morris (1980) Extracellular release of carbon by marine phytoplankton; a physiological approach. Limnol Oceanogr **25**: 262-279
Maldonado M (2004) Choanoflagellates, choanocytes, and animal multicellularity. Invertebr Biol **123**: 1-22
Marchant HJ (1990) Grazing rate and particle size selection by the choanoflagellate *Diaphanoeca grandis* from the sea-ice of lagoon Saroma Ko, Hokkaido. Proc NIPR Symp Polar Biol **3**: 1-7
Marie D, F Partensky, D Vaulot, and C Brussaard (1999) Enumeration of phytoplankton, bacteria, and viruses in marine samples. Curr Protocols Cytom **11**: 1-15
Martin JH and SE Fitzwater (1992) Dissolved organic carbon in the Atlantic, Southern and Pacific oceans. Nature **356**: 699-700
Martínez-Expósito MJ, JJ Pasantes, and J Méndez (1994) Proliferation kinetics of mussel (*Mytilus galloprovincialis*) gill cells. Mar Biol **120**: 41-45
Masood A, KD Stark, and N Salem Jr (2005) A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. J Lipid Res **46**: 2299-2305
Meesters E, R Knijn, P Willemsen, R Pennartz, G Roebers, and RWM Soest (1991) Sub-rubble communities of Curaçao and Bonaire coral reefs. Coral Reefs 10: 189-197
Meziane T and M Tsuchiya (2000) Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar Ecol Prog Ser **200**: 49-57
Middelburg JJ, C Barranguet, HTS Boschker, PMJ Herman, T Moens, and CHR Heip (2000) The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol Oceanogr **45**: 1224-1234
Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Moodley L, HTS Boschker, JJ Middelburg, R Pel, PMJ Herman, E de Deckere, and CHR Heip (2000) Ecological significance of benthic foraminifera: 13C labelling experiments. Mar Ecol Prog Ser **202**: 289-295

Moodley L, JJ Middelburg, HTS Boschker, GCA Duineveld, R Pel, PMJ Herman, and CHR Heip (2002) Bacteria and Foraminifera: key players in a short term deep-sea benthic response to phytodetritus. Mar Ecol Prog Ser **236**: 23-29

Müller WEG (1998) Origin of Metazoa: Sponges as Living Fossils. Naturwissenschaften **85**: 11-25

Müller WEG and IM Müller (2003) The hypothetical ancestral animal the Urmetazoa: Telomerase activity in sponges [Porifera]. J Serb Chem Soc **68**: 257-268

Murphy J and JP Riley (1962) A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta **27**: 31-36

Newell RC, MI Lucas, B Velimirov, and LJ Seiderer (1980) Quantitative significance of dissolved organic losses following fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). Mar Ecol Prog Ser **2**: 45-59

N

Nowakowski RS, SB Lewin, and MW Miller (1989) Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol **18**: 311-318

O

Odum HT and EP Odum (1955) Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol Monogr **25**: 291-320

Odum EP (1971) Fundamentals of ecology. W.B. Saunders Company

Osinga R (1999) Cultivation of marine sponges. Mar Biotechnol **1**: 509-532

P

Parsons TR, Y Maita, and CM Lalli (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press

Pernthaler A, J Pernthaler, and R Amann (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl Environ Microbiol **68**: 3094-3101

Peyrot-Clausade M (1974) Ecological study of coral reef cryptobiotic communities: An analysis of the polychaete cryptofauna. *In* AM Cameron et al [eds], Proc 2nd Coral Reef Symp, p 269-284

Pile AJ, MR Patterson, and JD Witman (1996) In situ grazing on plankton <10 µm by the boreal sponge *Mycale lingua*. Mar Ecol Prog Ser **141**: 95-102

Pile AJ, MR Patterson, M Savarese, VI Chernykh, and VA Fialkov (1997) Trophic effects of sponge feeding within Lake Baikal's littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnol Oceanogr **42**: 178-184

Pomeroy LR (1970) The strategy of mineral cycling. Ann Rev Ecol Sys **1**: 171-190

Potten CS, M Kellet, SA Roberts, DA Rew, and GD Wilson (1992) Measurement of in vivo proliferation in human colorectal mucosa using bromodeoxyuridine. Gut **33**: 71-78

Puchtler H, FS Waldrop, and LS Valentine (1973) Polarization microscopic studies of connective tissue stained with picro-sirius red FBA. Beitr Pathol **150**: 174-187

R

Rasheed M, MI Badran, C Richter, and M Huettel (2002) Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. Mar Ecol Prog Ser **239**: 277–285

Reiswig HM (1971) Particle feeding in natural populations of three marine demosponges. Biol Bull **141**: 568-591

Reiswig HM (1974a) Bacteria as food for temperate-water marine sponges. Can J Zool **53**: 582-589

Reiswig HM (1974b) Water transport, respiration and energetics of three tropical marine sponges. J Exp Mar Biol Ecol 14: 231-249

Reiswig HM (1981) Partial carbon and energy budgets of the bacteriosponge *Verongia fistularis* (Porifera: Demospongiae) in Barabados. Mar Ecol **2**: 273-293

Ribes M, R Coma, and JM Gili (1999) Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Mar Ecol Prog Ser **176**: 179-190

Ribes M, R Coma, MJ Atkinson, and RA Kinzie III (2003) Particle removal by coral reef communities: Picoplankton is a major source of nitrogen. Mar Ecol Prog Ser **257**: 13-23

Ribes M, R Coma, MJ Atkinson, and RA Kinzie III (2005) Sponges and ascidians control removal of particulate organic nitrogen from coral reef water. Limnol Oceanogr **50**: 1480-1489

Richman S, Y Loya, and LB Slobodkin (1975) The rate of mucus production by corals and its assimilation by the coral reef copepod *Acartia negligens*. Limnol Oceanogr **20**: 918-923

Richter C and M Wunsch (1999) Cavity-dwelling suspension feeders in coral reefs - a new link in reef trophodynamics. Mar Ecol Prog Ser **188**: 105-116

Richter C, M Wunsch, M Rasheed, I Kötter, and MI Badran (2001) Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature **413**: 726-730

Riisgård HU, S Thomassen, H Jakobsen, JM Weeks, and PS Larsen (1993) Suspension feeding in marine sponges *Halichondria panicea* and *Haliclona urceolus*: Effects of temperature on filtration rate and energy cost of pumping. Mar Ecol Prog Ser **96**: 177-188

Riisgård HU and PS Larsen (1995) Filter-feeding in marine macro-invertebrates: pump characteristics, modelling and energy cost. Biol Rev **70**: 67-106

Roditi HA, NS Fisher, and SA Sañudo-Wilhelmy (2000) Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature **407**: 78-80

Rontani JF and JK Volkman (2003) Phytol degradation products as biogeochemical tracers in aquatic environments. Org Geochem **34**: 1-35

Rowinski J, K Kucharczyk, and T Boniecka (1977) The cell cycle in the small intestine transplanted beneath the kidney capsule in syngenic mice. Cell Tissue Res **177**: 141-144

S

Santavy DL, P Willenz, and RR Colwell (1990) Phenotypic study of bacteria associated with the caribbean sclerosponge, *Ceratoporella nicholsoni*. Appl Environ Microb **56**: 1750-1762

Scheffers SR, J de Goeij, FC van Duyl, and RPM Bak (2003) The cave-profiler: A simple tool to describe the 3-D structure of inaccessible coral reef cavities. Coral Reefs **22**: 49-53

Scheffers SR, G Nieuwland, RPM Bak, and FC van Duyl (2004) Removal of bacteria and nutrient dynamics within the coral reef framework of Curaçao (Netherlands Antilles). Coral Reefs **23**: 413-422

Scheffers SR (2005) Benthic-Pelagic Coupling in Coral Reefs: Interaction between framework cavities and reef water. Ph.D. thesis, University of Amsterdam

Schlichter D and G Liebezeit (1991) The natural release of amino acids from the symbiotic coral *Heteroxenia fuscescens* (Ehrb.) as a function of photosynthesis. J Exp Mar Biol Ecol **150**: 83-90

Sherr EB (1988) Direct use of high molecular weight polysaccharide by heterotrophic flagellates. Nature **335**: 348-351

Shore RE (1971) Growth and renewal studies of the choanocyte population in *Hymeniacidon sinapium* (Porifera: Demospongiae) using colcemid and 3-H thymidine. J Exp Zool **177**: 359-364

Siebers D (1982) Bacterial-invertebrate interactions in uptake of dissolved organic matter. Am Zool **22**: 723-733

Simpson TL (1984) The cell biology of sponges. Springer-Verlag

Sipkema D, R Osinga, W Schatton, W Mendola, J Tramper, and RH Wijffels (2005) Large-scale production of pharmaceuticals by marine sponges: Sea, cell, or synthesis? Biotechnol Bioeng **90**: 201-222

Smith SV and JA Marsh Jr (1973) Organic carbon production on the windward reef flat of Eniwetok Atoll. Limnol Oceanogr **18**: 953-961

Sorokin YI (1993) Coral reef ecology. Springer

Stephens GC and RA Schinske (1961) Uptake of amino acids by marine invertebrates. Limnol Oceanogr **6**: 175-181

Stoll MHC, K Bakker, GH Nobbe, and RR Haese (2001) Continuous-flow analysis of dissolved inorganic carbon content in seawater. Anal Chem **73**: 4111-4116

Sun LM, YF Song, Y Qu, X Yu, and W Zhang (2007) Purification and in vitro cultivation of archaeocytes (stem cells) of the marine sponge *Hymeniacidon perleve* (Demospongiae). Cell Tissue Res **328**: 223-237

T

Teira E, T Reinthaler, A Pernthaler, J Pernthaler, and GJ Herndl (2004) Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean. Appl Environ Microbiol **70**: 4411-4414

Thomas JD (1997) The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. Freshwater Biol **38**: 1-36

Torréton JP, J Pagès, P Dufour, and G Cauwet (1997) Bacterioplankton carbon growth yield and DOC turnover in some coral reef lagoons. *In* HA Lessions and IG Macintyre [eds], Proc 8th Int Coral Reef Symp, p 947-952

Tranvik LJ, EB Sherr, and BF Sherr (1993) Uptake and utilization of 'colloidal DOM' by heterotrophic flagellates in seawater. Mar Ecol Prog Ser **92**: 301-309

Tribble GW, FJ Sansone, Y-H Li, SV Smith, and RW Buddemeier (1988) Material fluxes from a reef framework. *In* JH Choat et al [eds], Proc 6th Int Coral Reef Symp, p 577-582

Tribble GW, FJ Sansone, and SV Smith (1990) Stoichiometric modeling of carbon diagenesis within a coral reef framework. Geochim Cosmochim Acta **54**: 2439-2449

Turon X, J Galera, and MJ Uriz (1997) Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. J Exp Zool **278**: 22-36

Turon X, I Tarjuelo, and MJ Uriz (1998) Growth dynamics and mortality of the encrusting sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation with population structure and investment in defense. Funct Ecol **12**: 631-639

V
Vacelet J and C Donadey (1987) A new species of *Halisarca* (Porifera, Demospongiae) from the Caribbean, with remarks on the cytology and affinities of the genus. *In* WC Jones [ed], European contribution to the taxonomy of sponges, p 5–12. Sherkin Island Marine Station Publications
Vacelet J and N Boury-Esnault (1995) Carnivorous sponges. Nature **373**: 333-335
Valderrama JC (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar Chem **10**: 109-122
Van de Vyver G, B Vray, S Belauane, and D Toussaint (1990) Efficiency and selectivity of microorganism retention by *Ephydatia fluviatilis*. *In* K. Rützer [ed.], New Perspectives in Sponge Biology, p 511-515. Smiths Inst Press
Van de Wetering M, E Sancho, C Verweij, K de Lau, I Oving, A Hurlstone, K van der Horn, E Batlle, D Coudreuse, AP Haramis, M Tjon-Pon-Fong, P Moerer, M van den Born, G Soete, S Pals, M Eilers, R Medema, and H Clevers (2002) The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell **111**: 241-250
Van Duyl FC and GJ Gast (2001) Linkage of small-scale spatial variations in DOC, inorganic nutrients and bacterioplankton growth with different coral reef water types. Aquat Microb Ecol **24**: 17-26
Van Duyl FC, GJ Gast, W Steinhoff, S Kloff, MJW Veldhuis, and RPM Bak (2002) Factors influencing the short-term variation in phytoplankton composition and biomass in coral reef waters. Coral Reefs **21**: 293-306
Van Duyl FC, SR Scheffers, FIM Thomas, and M Driscoll (2006) The effect of water exchange on bacterioplankton depletion and inorganic nutrient dynamics in coral reef cavities. Coral Reefs **25**: 23-36
Vasseur P (1974) The overhangs, tunnels and dark reef galleries of Tulear (Madagascar) and their sessile invertebrate communities. *In* AM Cameron et al [eds], Proc 2nd Int Coral Reef Symp, p 143-160
Vasseur P (1977) Cryptic sessile communities in various coral formations on reef flats in the icinity of Tulear (Madagascar). *In* DL Taylor [ed], Proc 3rd Int Coral Reef Symp, p 95-100
Viso AC and JC Marty (1993) Fatty acids from 28 marine microalgae. Phytochemistry **34**: 1521-1533
Volkman JK, SM Barrett, SI Blackburn, MP Mansour, EL Sikes, and F Gelin (1998) Microalgal biomarkers: A review of recent research developments. Organic Geochemistry **29**: 1163-1179
Volkman JK (2006) Lipid biomarkers for marine organic matter. *In* JK Volkman [ed], The Handbook of Environmental Chemistry, p 27-70. Springer-Verlag
W
Wiens M and WEG Müller (2006) Cell death in Porifera: Molecular players in the game of apoptotic cell death in living fossils. Can J Zool **84**: 307-321
Wild C, M Huettel, A Klueter, SG Kremb, MYM Rasheed, and BB Jørgensen (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature **428**: 66-70
Wilkinson C and R Garrone (1980) Nutrition of marine sponges. Involvement of symbiotic bacteria in the uptake of dissolved carbon. *In* DC Smith and Y Tiffon [eds], Nutrition in the lower metazoa, p 157-161. Pergamon press

Wilson GD (2007) Cell kinetics. Clin Oncol **19**: 370-384
Winkler LW (1888) Die bestimmung des im wasser gelösten sauerstoffes. Ber Deut Chem Ges **21**: 2843-2855
Witte U, T Brattegard, G Graf, and B Springer (1997) Particle capture and deposition by deep-sea sponges from the Norwegian-Greenland Sea. Mar Ecol Prog Ser **154**: 241-252
Wright SH and DT Manahan (1989) Integumental nutrient uptake by aquatic organisms. Annu Rev Physiol **51**: 585-600
Wunsch M, SM Al-Moghrabi, and I Kötter (2000) Communities of coral reef cavities in Jordan, Gulf of Aqaba (Red Sea). *In* MK Moosa et al [eds], Proc 9th Int Coral Reef Symp, p 595-600
X
Xie Z and DJ Klionsky (2007) Autophagosome formation: core machinery and adaptations. Nature Cell Biol **9**: 1102-1108
Y
Yahel G, AF Post, K Fabricius, D Marie, D Vaulot, and A Genin (1998) Phytoplankton distribution and grazing near coral reefs. Limnol Oceanogr **43**: 551-563
Yahel G, JH Sharp, D Marie, C Haese, and A Genin (2003) In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnol Oceanogr **48**: 141-149
Z
Zankl H and JH Schroeder (1972) Interaction of genetic processes in Holocene reefs off North Eleuthera Island, Bahamas. Int J Earth Sci **61**: 520-541
Zierer MS and PAS Mourao (2000) A wide diversity of sulfated polysaccharides are synthesized by different species of marine sponges. Carbohydr Res **328**: 209-216
Zlotnik I and Z Dubinsky (1989) The effect of light and temperature on DOC excretion by phytoplankton. Limnol Oceanogr **34**: 831-839

Publications

Scheffers SR, JM de Goeij, FC van Duyl, and RPM Bak (2003) The cave-profiler: A simple tool to describe the 3-D structure of inaccessible coral reef cavities. Coral reefs **22**: 49-53

De Goeij JM and FC van Duyl (2007) Coral cavities are sinks of dissolved organic carbon (DOC). Limnol Oceanogr **52**: 2608-2617

De Goeij JM, H van den Berg, MM van Oostveen, EHG Epping, and FC van Duyl (2008a) Major bulk dissolved organic carbon (DOC) removal by encrusting coral cavity sponges. Mar Ecol Prog Ser **357**: 139-151

De Goeij JM, L Moodley, M Houtekamer, NM Carballeira, and FC van Duyl (2008b) Tracing ^{13}C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM-feeding. Limnol Oceanogr **53**: 1376-1386

De Goeij JM, A de Kluijver, FC van Duyl, J Vacelet, RH Wijffels, AFPM de Goeij, JPM Cleutjens, and B Schutte. Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding. J Exp Biol (in review)

De Goeij JM, EHG Epping, JW van Dam, H Eijkelenboom, and FC van Duyl. Element cycling on coral reefs: The cryptic carbon shunt revealed. Ecology (in review)

SUMMARY

Summary

Coral cavities are the largest habitat in the coral reef ecosystem. Determining the role of this habitat and its inhabitants in the overall element cycling on reefs was the major goal of this PhD study. Coral cavities are major sinks of dissolved organic carbon (DOC). In two geographically distinct areas (the Caribbean island of Curaçao, Netherlands Antilles, and the Berau area, East-Kalimantan, Indonesia), in different reef types such as fringing, lagoon, barrier, and atoll reef, the fluxes of DOC were measured using two different methods. Fluxes were determined in an 'open' system by measuring concentration differences between cavity water and overlying reef water and by using a water exchange coefficient, i.e. the residence time of the cavity water. Subsequently, fluxes were determined by closing the cavities (and halting the water exchange with the overlying reef water) and monitoring the change in concentration of DOC in time. Both methods demonstrated a significant removal of DOC by coral cavities, wheras no significant differences in cavity fluxes using the two methods were observed. Moreover, in both geographically distinct areas, all cavities examined showed a DOC flux into coral cavities that appeared one to two orders of magnitude higher than particulate organic carbon (POC) removal rates. Bioassays showed that bacterioplankton is not responsible for this DOC removal by coral cavities.

For more than 95% the cavity walls are covered by a dense population of organisms. Cryptic organisms are mostly filter or suspension feeders, dominated by encrusting sponges, forming a layer with a thickness of only a few millimetres. In accordance with the extensive removal of DOC by coral cavities, we found that cavity sponges are also major sinks of DOC. This is contradictory to the conventional view that sponges can only "filter" particles, and are not able to use dissolved material for nutrition. We studied the removal of dissolved organic carbon and bacterioplankton by the encrusting sponges *Halisarca caerulea, Mycale microsigmatosa* and *Merlia normani* in coral reefs along Curaçao, Netherlands Antilles. We monitored the change in concentration of DOC in time, in experiments at 12 m water depth using flow chambers in the presence or absence of sponges. The DOC removal rates of all three sponges were on average two orders of magnitude higher than bacterial carbon removal rates and accounted for more than 90% of the total organic carbon removal. The

total carbon removal rates presented here were, to date, the highest reported for sponges. The three sponges are 'DOM-feeders', related to the availability of dissolved and particulate carbon sources in the ambient water. Sponges are very opportunistic feeders, feeding on anything available in their environment, and there is 10 to 100 fold more DOC that POC in the cavity waters. Furthermore, encrusting sponges could be very important in the removal of dissolved organic carbon in coral reef cavities.

Although cavity sponges remove large amounts of carbon from the water column, it is not known how the removed carbon is processed by sponges. Therefore, a mass balance was constructed to determine the fate of carbon for the sponge *Halisarca caerulea* that is used as a model species for cavity sponges. A major discrepancy was found between the total organic carbon removal and the net growth. Only ~40% of the carbon taken up by the sponge *Halisarca caerulea* is respired. The remaining ~60% of the removed organic carbon is assimilated. This is equivalent to a predicted increase in biomass of ~38% body C d^{-1}, which means a doubling in biomass every two to three days (!), with a net growth rate of approximately zero. Thus, an important question is: Where does all the removed carbon go? The answer cannot be found in the previous data, which are based on indirect observations of net fluxes of carbon through the sponge. To investigate actual evidence of assimilation of organic matter, and DOM in particular, by *H. caerulea,* we traced ^{13}C excess in the bulk tissue of the sponge and in its fatty acids after supply of dissolved and particulate ^{13}C-labelled organic substrates. The sponge actually processes both DOM and particulate organic matter (POM), showing a similar 40:60 respiration:assimilation ratio as was observed previously. To the best of our knowledge, we provided the first direct evidence of bulk DOC processing by a sponge. A clear quantitative preference of sponges for particulate over dissolved food could not be assessed, except for glucose, which seemed to be an unpreferred substrate. The other substrates seem to be processed by the sponge proportionally to the availability of the source.

Sponges, such as the three studied cavity sponges, contain numerous associated bacteria, which may be potentially very important in the processing of food (especially DOM) of the sponge-microbe association. Patterns of ^{13}C-enrichment in fatty acid biomarkers from sponges revealed that dissolved organic

13C assimilation was both direct and bacteria-mediated, as tracer carbon was recovered both in bacteria-specific and non-bacterial fatty acid. Carbon transfer between bacteria and sponge was not evident after 1 hour of incubation within the following 5 hours, but exchange or reallocation after a longer period of time could not be excluded.

Assimilated carbon may be used for reproduction or for the production of secondary metabolites, but may also be involved in a high turnover of matter. Indeed, a rapid turnover of matter within the cryptofaunal communities is likely, due to the combination of high rates of nutrient cycling and the high competition for space in coral cavities. By examining *in vivo* cell kinetics of the marine sponge *Halisarca caerulea* under steady state conditions, we found that the sponge shows an extremely high proliferation activity, a short duration of the cell cycle, and massive cell shedding. This corresponds well with the observed discrepancy between the high assimilation of organic carbon and the absence of a corresponding net increase in the sponge biomass. Additionally, the estimated costs of proliferation for this sponge are in the same order of magnitude as the organic carbon removal by *H. caerule*a. Cell turnover is predominantly confined to a single population of cells, i.e. the choanocytes, which are filtering cells primarily responsible for the uptake of food. Growth or cell proliferation must be counterbalanced by mechanisms of cell loss. Apoptosis, or programmed cell death, only plays a minor role. In contrast, similar to the shedding of absorptive cells lining the crypts of the human colon, the "old" cells of the sponge are being shed into the lumen of the choanocyte chambers and transported outside. To our knowledge, such fast cell kinetics in combination with high cell turnover by shedding in the sponges have not been observed previously in any other multi-cellular organism. The duration of the cell cycle *in vivo* resembles that of some uni-cellular organisms in culture. Morphological studies strongly suggest compartmentalisation of choanocytes in the sponge tissue. The functional morphology of the sponge *H. caerulea* corresponds well with its remarkable cellular kinetic behaviour. Both the kinetics and the functional organisation of the sponge show a striking resemblance with colonic tissue of the mammalian or human gastro-intestinal tract.

Thus, evidently sponges are involved in the extensive processing of DOC on coral reefs. Still, several relevant questions remain unanswered. How are other

elements, such as nitrogen (N) and phosphorus (P), processed within coral cavities? What is the function of other cavity communities? And what is the role and function of coral cavities within the energy budget of the coral reef ecosystem? Approximately 90% of an average coral cavity is covered by four cavity communities: sediment (sandy bottom), sponge, calcareous algae, and bare substrate. Mass balances were constructed for these four communities and for the total cavity in an attempt to unravel the puzzle.

The conventional view on the functioning of coral cavities is that these are the key regenerative spaces and hot spots of mineralisation on coral reefs: processing particulate organic matter, both living (phytoplankton and bacterioplankton) and nonliving (detritus), and releasing inorganic nutrients to the reef. Including DOM as a source for coral cavities, we found low mineralisation efficiencies for N and P. Coral cavities represent important sinks for predominantly dissolved organic C, N, and P, but only play a marginal role in the regeneration of inorganic nutrients. The sponge community is the most important sink for organic matter on the reef. In our mass balances, we predicted that the major pathway for the sponge community is a net conversion of DOM into POM. In this respect, the sponge community acts as a blueprint for the coral cavity, largely controlling carbon and nutrient fluxes within cavities. Covering on average only approximately one quarter of the cavity surface, sponges account for approximately three-quarters of the cavity fluxes. This is also reflected in the similar respiration (~40%) to assimilation (~60%) ratio for both the sponge community and coral cavities, as well as in the predicted net conversion of dissolved into particulate organic matter.

The coral reef framework cavities are the major sites for respiration within a coral reef ecosystem, representing 27-68% of the gross community respiration for an entire reef ecosystem. DOC removal rates were in the same order of magnitude as, or even exceed, gross primary production rates for entire coral reef ecosystems. DOM is locally produced by, among others, algae and corals on the reef. Coral cavities capture DOM, which would otherwise be washed away and lost from the ecosystem to the adjacent ocean. Cryptic organisms convert DOM as an energy carrier to higher trophic levels in particulate form (POM). These particles can, in turn, be used by the inhabitants of the 'upper' reef. This is a highly efficient recycling of elements, repeated many times. In the open

ocean this is in fact the function of the classical 'microbial loop', described in the eighties of the last century. In this thesis it is concluded that within the coral reef ecosystem, this function is performed by a 'sponge loop' and coral cavities are to be determined as a 'cryptic carbon shunt'. A thin veneer of encrusting sponges is highly efficient, by optimising nutrient input, and minimising output. A high turnover of organic matter is a useful strategy for the reef to survive in an environment which may be typed as a marine desert.

SAMENVATTING

Samenvatting

Grotten in het koraalrif vertgenwoordigen de grootste habitat van het rif ecosysteem. De functie van deze koraalgrotten en haar bewoners in het energiebudget van het rif was het belangrijkste onderwerp van studie in dit promotieonderzoek. Koraalgrotten blijken enorme hoeveelheden opgelost organisch materiaal, gemeten in de vorm van koolstof, op te nemen. De fluxen van opgelost organisch koolstof zijn gemeten in twee geografisch verschillende gebieden (het Caribische eiland Curaçao, Nederlandse Antillen en het gebied rond Berau aan de oostzijde van Kalimantan, Indonesië), en aan verschillende riftypen, zoals franje-, barrière-, lagune-, en atollriffen. Daarbij zijn twee verschillende methoden gebruikt. Bij de eerste methode zijn fluxen berekend in een 'open' systeem aan de hand van het concentratieverschil tussen het grotwater en het omgevende rifwater, daarbij gebruik makend van een wateruitwisselingsconstante. Bij de tweede methode zijn de grotten afgesloten met een kaasdoek, waardoor er geen wateruitwisseling meer plaatsvindt. De concentraties opgelost organisch koolstof worden vervolgens in de tijd gevolgd en de fluxen berekend aan de hand van een rekenkundig model. Beide methoden laten zien dat grotten een grote hoeveelheid koolstof wegnemen uit het water, waarbij er in de uitkomsten geen verschil bestaat tussen de methoden. Alle onderzochte grotten in beide, zeer verschillende, geografische gebieden, laten een influx van opgelost organisch koolstof zien die 10 tot 100 maal groter is dan de influx van niet-opgelost organisch koolstof (partikels of particulair organisch koolstof). Door middel van bioassays is aangetoond dat vrijlevende bacteriën niet verantwoordelijk zijn voor de opname van dit opgeloste koolstof door koraalgrotten.

De wanden van de grotten zijn voor meer dan 95% bedekt door organismen. Deze grotorganismen bestaan hoofdzakelijk uit vastzittende organismen die hun voedsel uit het water filteren en worden gedomineerd door voornamelijk korstvormende sponzen, die vaak een laag van maar enkele milimeters dik vormen. Net als voor de grotten geldt ook dat grotsponzen enorme hoeveelheden opgelost organisch koolstof kunnen wegnemen uit het water. Dat is in tegenspraak met de geldende gedachte dat filterende organismen alleen maar partikels (niet-opgeloste deeltjes) kunnen "filteren" en gebruiken als voedsel. De opname van opgelost koolstof en van bacteriën (als 'vertegenwoordigers'

van partikels) uit het water is vervolgens gemeten in drie verschillende soorten grotsponzen (*Halisarca caerulea*, *Mycale microsigmatosa* en *Merlia normani*) op het rif van Curaçao. In experimenten op het rif (op ongeveer 15 m diepte) met afgesloten bakken met en zonder sponzen is de verandering van de concentratie organisch koolstof in de tijd bepaald. De opname van opgelost organisch koolstof bleek 10 tot 100 maal hoger dan de opname van bacterieel koolstof en omvatte meer dan 90% van de totale opname van organisch koolstof. Opnamesnelheden van organisch koolstof door sponzen in deze studie zijn de hoogste gemeten tot dusver en deze bevindingen behoren, voor zover bekend, tot de eerste op het gebied van opgelost organisch koolstof. Op basis van de grootte (en dus beschikbaarheid) van verschillende voedselbronnen kan geconcludeerd worden dat deze sponzen, met name opgelost voedsel eten, in tegenstelling tot partikels. Tevens kunnen grotsponzen van belang zijn in de opname van opgelost organisch koolstof door koraalgrotten.

Sponzen in grotten blijken dus enorm veel opgeloste organische stoffen uit het water op te nemen, maar de vraag is of de sponzen dat materiaal slechts uit het water halen, of ook daadwerkelijk verwerken, met andere woorden respireren of verademen, c.q. assimileren of opslaan in biomassa. De lotsbestemming van het koolstof is vastgesteld door middel van het maken van een massabalans voor de spons *Halisarca caerulea*, als model voor een grotspons. Uit de balans bleek een grote discrepantie tussen de opname van organisch koolstof en de netto groei van de spons. In principe geldt voor een balans: wat er in gaat, moet er ook weer uitkomen. Voor *H. caerulea* werd gevonden dat 40% van de opgenomen koolstof wordt gerespireerd en dus 60% wordt geassimileerd. Dit komt overeen met een verwachte toename van sponsbiomassa van ongeveer 38% per dag; ofwel een verdubbeling van biomassa elke twee tot drie dagen! De waargenomen netto groei van de spons is echter ongeveer nul. Dus de vraag is: Waar blijft al het opgenomen koolstof? De data van dit experiment zijn gebaseerd op indirecte metingen van de afname van koolstof in water waarin zich een spons bevindt. Om het feitelijke kwalitatieve bewijs te leveren van de assimilatie van, met name opgelost, organisch voedsel door *H. caerulea*, zijn er verschillende voedselbronnen getraceerd in het weefsel van de spons. Na toediening van vier verschillende ^{13}C-gelabelde opgeloste en particulaire voedselbronnen is het label teruggevonden en gemeten in het totale weefsel en, specifieker, in vetzuren. De spons verwerkt zowel opgelost als particulair

voedsel in dezelfde respiratie:assimilatie verhouding van 40:60 als eerder werd gevonden. Hiermee is het allereerste bewijs voor het eten van opgelost voedsel door sponzen geleverd. Het is echter onduidelijk of de spons een voorkeur heeft voor opgelost danwel particulair voedsel. Alleen glucose vindt de spons niet 'lekker'. De andere voedselbronnen worden door de spons gegeten in de verhouding die ook aanwezig is in het water. Opgelost organisch koolstof is veruit de grootste bron van koolstof in het water.

Sponzen bestaan voor een groot deel uit bacteriën, soms voor meer dan de helft van de biomassa. Deze bacteriën kunnen een zeer belangrijke rol spelen in de verwerking van voedsel, met name opgelost voedsel. De ^{13}C-label experimenten laten zien dat zowel bacteriën als sponscellen opgelost koolstof assimileren, omdat label werd teruggevonden in bacterie-specifieke vetzuren en in niet-bacteriële vetzuren. Het is niet gebleken dat er translocatie van koolstof tussen bacteriën en sponscellen plaatsvindt na één uur incubatie en de daaropvolgende vijf uur. Dit sluit echter een uitwisseling van koolstof na langere tijd niet uit.

Het geassimileerde koolstof kan gebruikt worden voor reproductie of de productie van secondaire metabolieten, maar kan ook betrokken zijn bij een hoge omzetting van biomassa. Dat is aannemelijk voor de grotorganismen, aangezien de organismen maar zeer beperkt kunnen groeien door een felle competitie voor ruimte in koraalgrotten. Elk organisme verdedigt fel zijn of haar verworven plekje in de beschutte omgeving van de grot. Celkinetische studies van *Halisarca caerulea* laten een extreem hoge proliferatie (celdeling) activiteit zien, een korte celcyclus en een massale uitstoot van (oude) cellen. Dit verklaart de waargenomen discrepantie tussen de hoge mate van voedselopname en de zeer beperkte groei. De geschatte "kosten" van de celdeling komen dan ook overeen met de gemeten opname van voedsel door de spons. De omzetting van cellen is beperkt tot één celpopulatie, de choanocyten. Dit zijn de cellen die direct in contact staan met het omliggende water en verantwoordelijk zijn voor de opname van voedsel. Groei door celdeling wordt onder normale omstandigheden in evenwicht gehouden door het verlies van cellen. Apoptosis, of geprogrammeerde celdood, speelt hierin echter maar een kleine rol. Daarentegen worden 'oude' cellen, net als de absorptieve cellen die de crypten in onze dikke darm bedekken, door de spons uitgestoten in het lumen, zodat de cellen kunnen worden uitgescheiden. De celkinetiek van de

spons is qua snelheid en omzetting niet eerder waargenomen in enig ander meercellig organisme. De duur van de celcyclus van de spons lijkt meer op die van een bacterie of eencellige in kweek. De sponsmorfologie duidt op een sterke mate van compartimentalizatie in het weefsel, en correspondeert met de opmerkelijke celkinetiek. De functionele organisatie van de spons en de bijhorende celkinetische eigenschappen vertonen opvallend veel gelijkenis met die van het humane dikke darm weefsel.

Sponzen zijn dus betrokken bij het opnemen van opgelost koolstof (C) op het rif. Maar er is nog steeds een aantal vragen onbeantwoord. Hoe zit het met andere elementen als stikstof (N) of fosfor (P)? Wat is de functie van andere grotbewoners? En wat is dan de functie van de koraalgrotten binnen het energiebudget van het gehele koraalrif? Ongeveer 90% van een gemiddelde grot wordt bedekt door vier groepen: het sediment (zandbodem), kalkalgen, sponzen en onbedekt substraat. Voor die groepen en voor de totale grot zijn massabalansen ontwikkeld om de puzzel met haar stukjes op te lossen.

De conventionele kijk op koraalgrotten is dat zij belangrijke regeneratieve ruimtes zijn en “hot spots” van mineralisatie voor het rif. Met andere woorden: Grotten nemen levende partikels op, zoals vrijlevende algen en (cyano)bacteriën, danwel dode deeltjes, zoals detritus, en zetten dat om in anorganische verbindingen (fosfaat, nitraat, etc.) dat vrijkomt uit de grot. Opgelost organisch materiaal is tot nog toe niet meegenomen in de berekening van de budgetten. Als dat wel wordt meegenomen als potentiële voedselbron voor grotten, dan blijken grotten in verhouding slechts een kleine hoeveelheid anorganische stoffen te produceren. Koraalgrotten zijn dus belangrijk in het opnemen van, met name opgelost, organisch C, N en P, maar spelen slechts een marginale rol in de regeneratie van anorganische nutriënten. Van alle grotbewoners zijn sponzen de belangrijkste groep in de recycling van elementen. Daarbij zetten sponzen opgelost organisch materiaal om in organische partikels. Sponzen zijn wat dat betreft een blauwdruk van de grot en controleren de fluxes van elementen binnen de grot. Alhoewel sponzen slechts een kwart van het grotoppervlak bedekken zijn ze verantwoordelijk voor driekwart van de totale grotfluxen. Dit komt verder tot uiting in vergelijkbare respiratie:assimilatie verhoudingen van 40:60 voor zowel grotten als sponzen en de omzetting van opgelost materiaal naar partikels.

Een groot deel van de totale rifrespiratie (27-68%) vindt plaats in grotten. Wat respiratie-activiteit betreft zijn grotten de belangrijkste plekken binnen het totale ecosysteem van het rif. De opnamesnelheden van opgelost organisch materiaal door koraalgrotten zijn van dezelfde ordegrootte, of zelfs hoger, dan de totale productiecapaciteit van het rif. Opgelost materiaal wordt lokaal geproduceerd door algen en koralen op het rif en wordt, in plaats van weggewassen naar de omliggende oceaan, opgenomen door grotten en haar bewoners. Deze bewoners zetten het vervolgens om in partikels die weer gebruikt kunnen worden door de organismen op het rif. Een zeer efficiënte en zichzelf meerdere malen herhalende recycling van materiaal. In de open oceaan wordt dit bewerkstelligd door de zogenaamde 'microbial loop', die ontdekt werd in de jaren '80 van de vorige eeuw. In dit proefschrift wordt geconcludeerd dat in het koraalrif ecosysteem deze functie wordt overgenomen door een zogenaamde 'sponge loop' en moet een koraalgrot als een zogenaamde 'cryptic carbon shunt' worden beschreven. Een dun laagje spons, slechts enkele milimeters dik, zorgt voor een optimale input en minimale output van nutriënten binnen het ecosysteem. Een hoge turnover van biomassa is daarmee een zeer geschikte strategie voor het rif om te overleven in een omgeving die getypeerd kan worden als een woestijn in de oceaan.

ACKNOWLEDGEMENTS

Although producing a thesis is considered to be a very lonely job, many people are involved in the execution of the project and the realisation of this thesis. Almost all also have a very positive effect on the progress and on the mood of the PhD candidate, and those are to be praised.

First, I would like to thank my promotor Gerhard Herndl and co-promotor Fleur van Duyl for giving me the opportunity and the tools to perform this PhD study and guiding me through the process of scientific research.

Eric Epping, you deserve a statue! You've always helped me from the beginning without questions or preconditions. You are the so needed kind of colleague that combines working with passion and knowing that cooperation is a virtue, not a provision. I loved the meetings where you trained (and sometimes overheated) my brains, and thank you for listening to the good and bad. Hope we can work together more in the future, that would be great.

I was very lucky with my five students, who worked and lived with me 24/7 for several months. Martine, Hubert, and Herbert you were the first three students and you helped me a lot in setting up office (underwater and above), and doing great science. Especially when times were a bit rough you all helped me through (thanks for the shoarma, Herbert!). Joost, it was more than fun working with you for six months, you felt more a brother than a student to me. I hope I can visit you in Australia soon to see how your PhD is evolving. Anna, same for you being a PhD in marine science yourself now. I could not wish for a better student to have my last fieldwork period with.

Santiago, Karel, Jan, and Evaline, thanks for analysing thousands of DOC, DIC and NUTS samples and good talks. Edwin, Johan, and Henk, thanks for your immense help in building flow chambers, reef profilers, and optodes. Thanks Harry for teaching me molecular techniques and stupid jokes, Hans for fixing my computer, Erik for giving me a crash course statistics (I HATE STATISTICS, you're my hero) and Bert for helping me with presentations. Without all of you, there wouldn't be science.

I'm very thankfull to Brian Leysner and Carlos Winterdaal, who I have been closely working with on Curaçao. Fixing the boat, fixing the cars, fixing the lab (chasing rabbits, fire, water, insects), fixing the buoy, fixing the rooms, fixing our disputes with customs, fixing the diving gear, but most important of all: working happily together and enjoying life! And definitely, having too less money and tools makes you a lot more creative and is not reflected in the outcome of the work.

Several co-authors have supported me with a lot of their time and energy. Leon Moodley, thanks for your enthusiasm and energy you put in our project, resulting in a very nice paper. The sponge fatty acid story is only starting so I hope to learn from you again. Dear Bert Schutte, Jack Cleutjens, and Ton de Goeij AKA "The Sponge Men", our Maastricht meetings are always a delight; Sponges rock and you know that! I hope that after my presentation on tropical underwater research, the people at Pathology did not take too much diving holidays since. There will definitely be more meetings and more strange scientific alliances coming up, and yes it is a good idea to have a meeting on Curaçao next time.

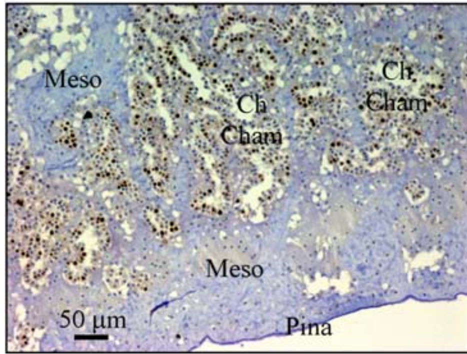
I started working at the NIOZ in March 2003. Without any clue on what to expect with respect to living and working on the island of Texel, I drove my fourstroke Honda C-70 (white, with golden wheels; later also a lot of yellow tape) to my first home in Oudeschild. Conny, thanks to you I didn't have to sleep outside until there was an available room in the Potvis, and letting me stay in the house when I was again homeless (see also subchapter: housing). Thanks also for your friendship; let's say we had a lot of work 'issues' in common. The NIOZ and living on Texel has giving me a lot of friends, laughter, and nice moments. Yann, Phil, Jerome, Theresa, Dennis, Thomas, Anne-Claire and Furu, although I wasn't around that much, you gave me a lot of friendship when I could join your Texel gang. Later on, I will always remember the many great nights especially at the Balcken with Furu, Cees, Lukas, Francien, Cathalina, and Marian, let's keep on annoying Sander, Dirk and Marijn once in a while! There was also this guy on Texel, Curaçao, and Amsterdam who always seemed to follow me… Pedro, you have to go your own way now. But you can always phone me when you need a hair cut or a good talk, you are a dear friend.

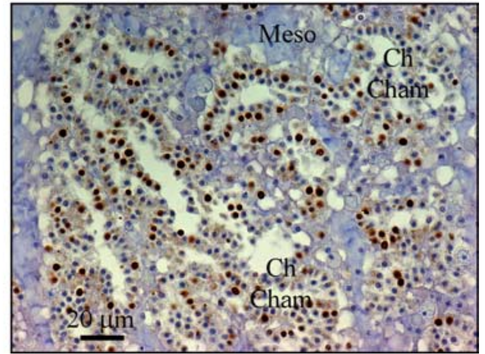
Subchapter the housing problem: I spent the last five years as a parttime homeless tramp. Therefore I would like to pay my gratitude to whom I could spent one or several nights: Conny, Thomas, Marian and Andrea, Joaquin, Pedro, Rooms 2, 3, 5, 7, and 8 of the former Potvis, Rooms 8, 23, and 27 of Tubantia, Rooms 1-9 of CARMABI, Theresa and Anne-Claire, Furu, Lukas and Cees.

On Curaçao, there are too many to thank. But besides my students and Pedro, especially my fellow PhD-student Marieke was a big support and fun dive buddy (especially the 20 m free run underwater). There were constantly people coming and going at CARMABI, but we were always there, struggeling to get the work done, without any direct supervision. And then there is my good friend Vos (Voske, Voskovitsch, "The Face"). Vos, you cannot tell anyone that we once played guitar and smoked in the lab next to the broken flume hood containing sulfuric, phosphoric, and hydrochloric acid. Thanks mate, see you soon. I do also want to mention how valuable I have always found the visiting of friends, some (Tazz) even three times. Guys, that was the most funny holiday I have ever had (I'm laughing now).

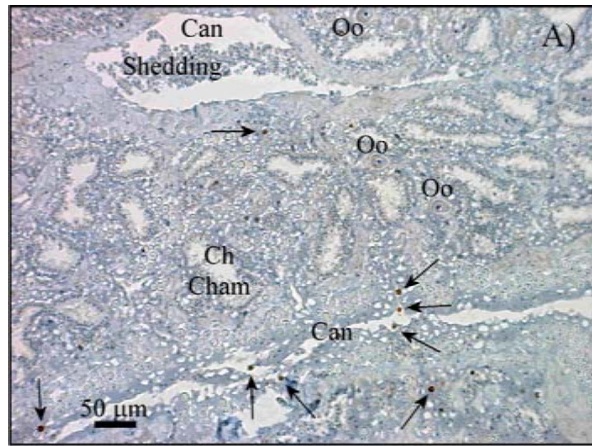
I want to thank my parents Ton and Lucia, my brother Jores and nephew Jip for sticking out with me during the less cheerful last stages of my PhD. Mom, you are for sure for a great deal responsible for this beautiful book, thanks for all your help and dedication. Dad, I'm proud to follow your footsteps (who would have thought). You were my supervisor in many ways and I love you for that.

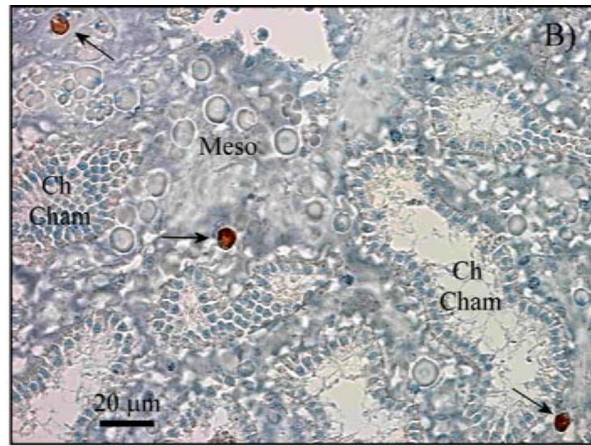
Puk, I saved the last words for you. "I will eat my shoes if I ever kiss her", were the exact words I spoke to my student Hubert in August 2004. I was lucky, while my shoes were stolen after I've kissed you. I'm so proud of you, how you always converted the difficulties of a long distance relation into positive energy, resulting in more and stronger love. How you supported me and in the meantime became an excellent doctor yourself. How you love and aid friends and family when they need it. I will always be there for you and love you from the tip of my little toe to the end of my thinning hair.

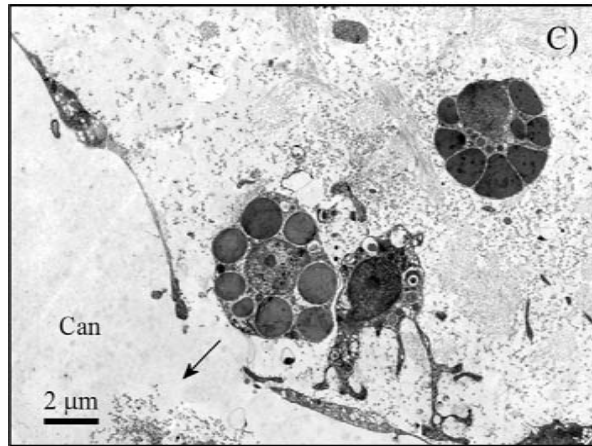


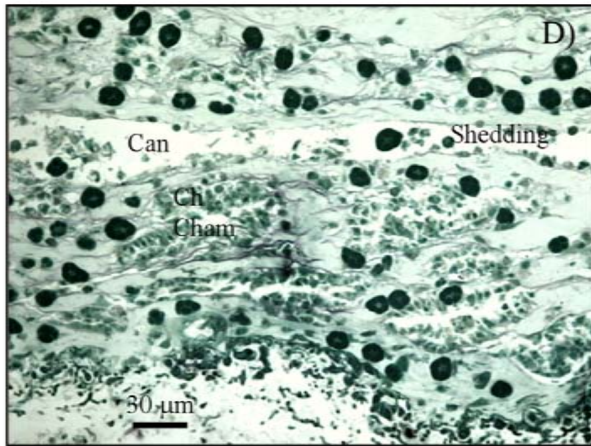


Figuur 6.2

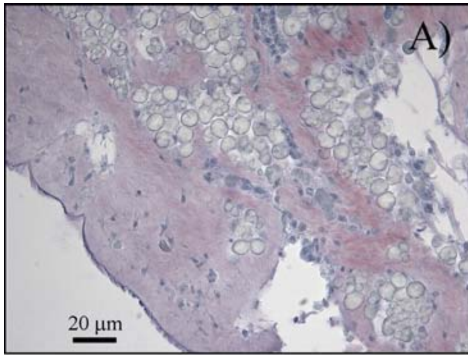


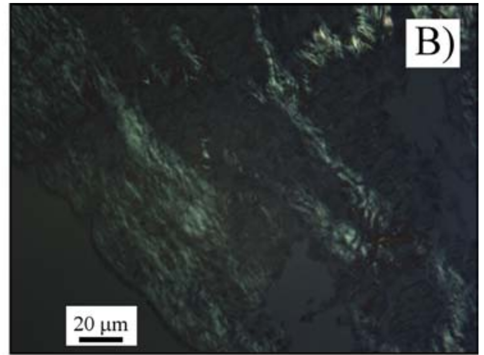


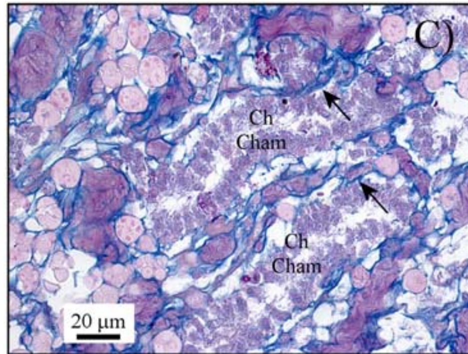


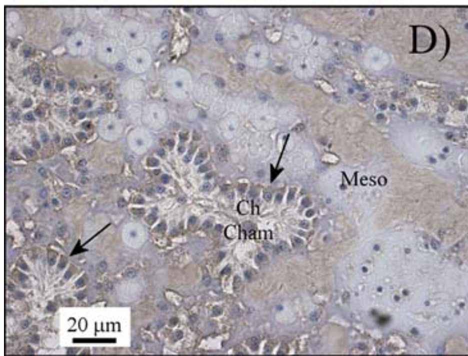


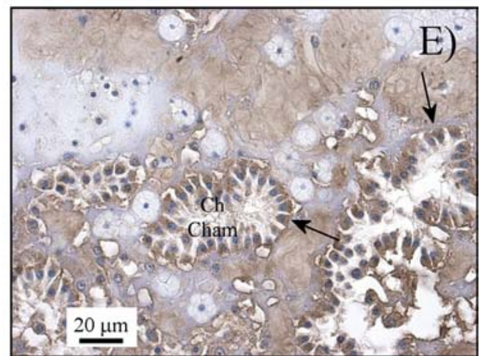
Figuur 6.3



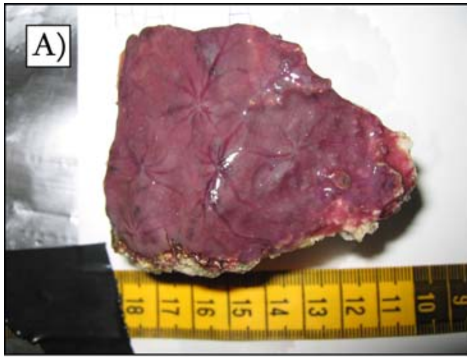


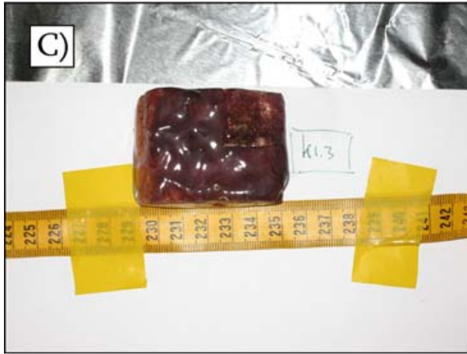




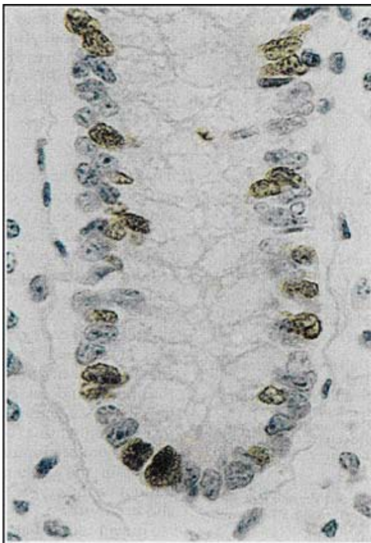


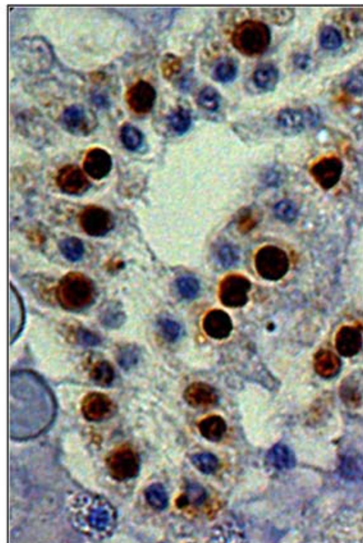
Figuur 6.4





Figuur 4.4





Figuur 8.1