BACTERIAL SUSPENSION FEEDING BY CLIONID SPONGES

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Front cover: *Sidastrea siderea* infested by *Cliona laticavicola*. Several ostia (incurrent papillae) are distinguishable by their orange coloured sievelike appearance. Furthermore two oscula (excurrent papillae) protrude from the coral head surface. Also visible is an epilithic alg and an anemone (*Labrunia coralligens*). Scale: 1: ¼. Picture was taken on Curaçao by J.J.Videler.

This Msc project is achieved in association with the RUG (University of Groningen), CARMABI (Caribbean Marine Biological station) NIOZ (Netherlands Institute for Sea Research) and UvA (University of Amsterdam), under supervision of Prof.dr. R.P.M. Bak.

ABSTRACT

The changes in seawater quality that are associated with eutrophication due to increased antropogenic input can have a major impact on marine communities. It is hypothesised that nutrient enrichment indirectly enhances the growth and production of bacteria, thereby stimulating the growth of organisms that feed on microbes, such as sponges. Increased infestation of corals by boring sponges accelarates bioerosion and may result in a net degradation of the reef.

To gain more insight in the trophic relationship between the microbial community in the water column and benthic reef communities, uptake rates of three species of Clionidae were investigated. Experiments were performed *in situ*, with use of enclosures. Bacterial numbers were determined with a direct count method using acridine orange staining and epifluorescence microscopy.

The results show that *C.lampa*, *C.laticavicola* as well as *C.vermifera* are effective bacterial suspension feeders, with the capability to adapt their feeding strategies to changing densities of food particles over a short time span. We have indications that the optimal clearance rates are different between the species. *C.lampa* is potentially capable of maintaining a higher clearance than *C.laticavicola* and *C.vermifera*. The results furthermore indicate that *C.lampa* and *C.vermifera* have a higher retention efficiency for picoplankton than for nanoplankton. No relationship was found between the clearance rates and biomass of the sponges.

We showed that the species are capable of efficient filtration of enhanced bacterial densities. Considering their responsiveness to changing bacterial densities together with the destructive qualities these species have, we suggest that clionid sponges potentially form a strong link between changes in the water column microbial population and the global deterioration of coral reefs.

SAMENVATTING

De gevolgen van eutrofiëring door een toenemende antropogene toevoer van afvalstoffen naar zee kan een grote impact hebben op mariene levensgemeenschappen. Het wordt verondersteld dat verrijking van nutriënten de groei en aanwas van bacteriën indirect stimuleert. Ook kan mogelijk de groei worden gestimuleerd van organismen die zich voeden met microben, zoals sponzen. Expansie van de boorsponzenpopulatie versnelt het bio-erosie proces en kan resulteren in een netto degradatie van het rif.

Om meer inzicht te verkrijgen in de trofische relatie tussen de microbiële gemeenschap in de waterkolom en bentische rif populaties werden de bacterie-opname snelheden bepaald van drie soorten boorsponzen (*Clionidae*). De experimenten werden *in situ* uitgevoerd met behulp van enclosures. Bacterie-aantallen werden rechtstreeks bepaald door middel van acridine oranje kleuring en epifluoriscentie microscopie.

De resultaten duiden aan dat zowel *C.lampa* als *C.laticavicola* en *C.vermifera* effectief bacteriën uit het zeewater filtreren. Deze sponzen hebben de mogelijkheid om hun voedingsstrategiën op korte termijn aan te passen aan veranderende dichtheden van voedseldeeltjes. Er zijn aanwijzingen dat de optimale clearance snelheden verschillen tussen de soorten. *C.lampa* is mogelijk in staat om een hogere clearance the handhaven dan *C.laticavicola* en *C.vermifera*. De resultaten tonen verder aan dat *C.lampa* en *C.vermifera* mogelijk een hogere retentie hebben voor picoplankton dan nanoplankton. Er is geen relatie gevonden tussen clearance en biomassa van de sponzen.

Op basis van onze resultaten suggereren wij dat clionide sponzen potentieel een belangrijke schakel vormen tussen de microbiële gemeenschap in de waterkolom en de wereldwijde achteruitgang van koraalriffen.

CONTENTS

1. INTRODUCTION

Human expansion and development have caused a worldwide increase in antropogenic input of nutrients to coastal waters. The changes in water quality that are associated with eutrophication can have a major impact on marine ecosystems (Brown 1997, Gabric & Bell 1993, Sebens 1994). Coral reefs are especially vulnerable to increases in nutrient levels since reef communities are adapted to oligothrophic water. It is hypothesised that a high level of nutrient input eventually leads to the erosion of reefs through chemical, mechanical and biological processes (Pastorok & Bilyard, 1985). Several studies have suggested that nutrient enrichment favours the growth and production of benthic filterfeeders, among which sponges are an important component (Table 1). The relationship between changing nutrient levels and infestation of corals by Clionidae is of particular interest in this matter, since bioerosion by boring sponges can substantially attribute to the degradation of coral reefs.

Eutrophication may indirectly enhance the growth and production of pelagic bacteria. (Fig. 1). Microbial populations over reefs are usually in a very dynamic state and respond rapidly to increased levels of nutrients. Measurements on microbial variables show strong horizontal and vertical gradients over reefs. Bacteria are stimulated in growth and at the same time removed from the water column due to mortality and predation (Ducklow & Carlson 1992, Gast 1998, Moriarty *et al* 1985). The overall pattern appears to be that the strongest removal of bacteria on coral reefs takes place in crevices (Gast 1998).
Crevices, the undersides of overhanging corals and dead coral rubble form the habitat of cryptic organisms. The cryptic reef fauna is highly divers and includes suspension feeders such as polychaetes, bryozoans, tunicates, bivalves and sponges (Buss & Jackson 1979, Choi & Ginsburg 1983, Jackson & Winston 1982, Meesters *et al* 1990, Vasseur 1977). Presumably bacterivory by these organisms causes the decline in bacterial densities in cryptic environments as observed by Gast (1998). The cryptic suspension feeding fauna thereby potentially forms an important link in the microbial food web.

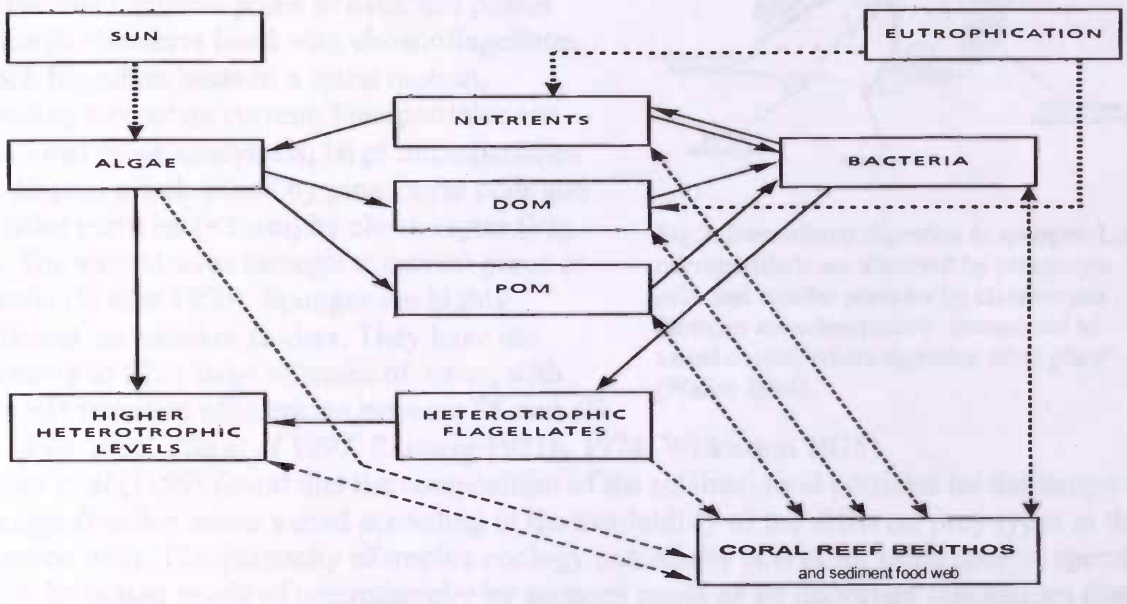

Fig.1. Simplified scheme of trophic linkage between the pelagic microbial foodweb (solid lines) and the coral reef benthic ecosystem (dashed lines). Antropogentic eutrophication can indirectly stimulate the growth of bacteria by means of an extra input of energy and nutrients into the system (dotted lines). DOM: Dissolved Organic Material, POM: Particulte Organic Material (adapted from Gast, 1998).

Many cryptic organisms are bioeroders, which implies that they are capable of chemically or mechanicly breaking down carbonate substrate (Hutchings 1986, Kiene1985). Boring sponges of the family Clionidae (Demospongia: Hadromerida) are among the most abundant and destructive bioeroding organisms. They play a considerable role in processes of calcium carbonate dissolution, sediment production and erosion of coral reefs (Bak 1976, Hein & Risk 1975, Kiene 1985, MacGeachy & Stearn 1976, Neumann 1966, Risk & MacGeachy 1978, Risk & Sammarco 1982, Risk *et al* 1995, Scoffin *et al* 1980).
The substrate of Clionids is mainly formed by dead coral tissue, although there are some species that spread onto and bore into the live surface of coral colonies (MacGeachy 1977). The sponges chemically etch away chips of calcium carbonate, creating caracteristically shaped excavations. This etching detaches a chip of substrate which is then mechanically removed through the sponge tissue and out through the excurrent canal system of the sponge (Pomponi 1980). These calcium carbonate chips form a significant contribution to the silt fraction of reef sediments (Neumann 1966, Rützler 1975, Scoffin *et al* 1980).
The rate at which a coral reef can build depends on the rate of skeletogenisis of the framework, the rate of consolidation and its resistance to erosion (Hein & Risk 1975). Sponge excavations weaken coral colonies, which makes them more susceptible to biological, chemical and physical mechanisms of erosion (McGeachy & Stearn 1976, Neumann 1966). High levels of boring activity by sponges accelerates bioerosion and may result in a net degradation of the coral reef (Hutchings 1986, Rose & Risk 1985, Sammarco & Risk 1990, Stearn & Scoffin 1977).

Sponges can feed on a wide spectrum of food sources, ranging from dissolved organic carbon to phytoplankton. Their diet includes heterotrophic bacteria, cyanobacteria, pico- and nano-eucaryotes and microplankton. (Pile 1996, Pile *et al* 1997, Reiswig 1971b, Ribes *et al* 1999). Water enters the sponge via numerous incurrent pores or ostia and passes through chambers lined with choanoflagellates. Each flagellum beats in a spiral motion, creating a constant current. Foodparticles are absorbed by phagocytosis; large microparticles (5-50 µm) are absorbed by pinacocyte cells and smaller particles (<5 µm) by choanocytes (Fig. 2). The water leaves through excurrent pores or oscula (Waller 1996). Sponges are highly efficient suspension feeders. They have the capacity to filter large volumes of water, with particle retention efficiencies between 75 and 99 % (Pile 1996, Pile *et al* 1997, Reiswig 1971b, 1974, Wilkinson 1978).

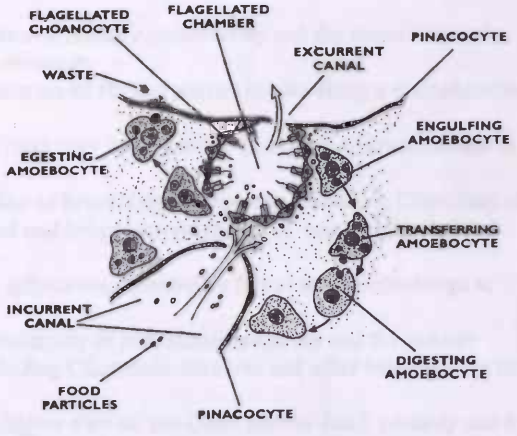

Fig.2. Intercellular digestion in sponges. Large micropartikels are absorbed by pinacocyte cells and smaller particles by choanocytes . Particles are subsequently transported to amoebocytes, where digestion takes place (Waller 1996).

Ribes *et al* (1999) found that the composition of the retained food particles by the temperate sponge *Dysidea avara* varied according to the availability of the different prey types in the watercolumn. This plasticity of trophic ecology potentially counts for other sponge species as well. Increased levels of heterothrophy by sponges could be an important mechanism that couples changing levels of nutrients in the coastal waters to the coral reef system. Several studies have found evidence which indicates a relationship between nutrient enrichment and biomass of clionid sponges (see Table 1). If changes in coastal nutrient levels and bioerosion

by boring sponges are functionally related, this implicates that eutrophication may promote the deterioration of coral reefs.
To gain more insight on the trophic linkage between the microbial population and the degradation of reefs, this study focusses on bacterial uptake rates of Clionidae. An important part of the phytoplankton biomass in shallow tropical waters is composed of picoplankton, among which various cyanobacteria, including *Synechococcus* sp. (Ducklow 1990, Gast 1998, Johnson & Sieburth 1979, Moriarty *et al* 1985). Clearance rates of nano- and picoplankton from ambient sea water will be determined in enclosures. The short term reaction of boring sponges to enhanced densities of bacteria will be tested by adding cyanobacteria (*Synechococcus* sp.) to the enclosures. A comparison will be made between the clearance rates of three different species of clionid sponges.

Table 1. Summary of studies where a relationship between high nutrient levels and the abundance of benthic filterfeeders is suggested. * Denotes a study in which clionid sponges are explicitly mentioned in relation to changing nutrient levels.

Source	Findings
Bak et al (1998)	Efficient linkage between bacterial suspension feeders (*Madracis mirabilis* and *Tridídemnum solidum*) and pelagic microbial communities is suggested as explaination for continued/increased abundance of such benthic organisms on deteriorating Caribbean reefs.
Brock & Smith (1983)	Relationship between nutrient loading due to sewage discharge and elevated biomass of predominantly filter- and suspensionfeeding cryptofauna at Kaneohe Bay (Hawaii).
Cuet & Naïm (1992) *	Relationship between nutrient excess and increase in *Cliona inconstans* infestation at La Réunion Island (Indian Ocean).
Higsmith (1980)	Direct relationship is suggested between primary productivity and the circumtropical abundance of boring sponges and -bivalves.
Holmes (1997) *	Significant increase in clionid infestation of *Porites porites* rubble along a eutrophication gradient at Barbados.
Pastorok & Bilyard (1985)	Nutrient enrichment by sewage effluent may favor benthic filterfeeding invertebrates in coral reef communities.
Risk et al (1995) *	Possible relationship between decline of bioeroding community (including Clionidae) at outer shelf of the Great Barrier Reef and lower concentrations of terrestrially derived organic matter.
Rose & Risk (1985) *	Marked increase in *Cliona delitrix* infestation, affected by faecal sewage discharge at Grand Cayman fringing reef.
Sammarco & Risk (1990) *	Relationship between increased availability of nutricional resources and the inshore abundance of boring sponges (including Clionidae), bivalves and other bioeroders at the Great Barrier Reef.
Wilkinson (1987) Wilkinson & Cheshire (1990)	Sponge biomass on Belize reefs is higher then on the Great Barrier Reef, possibly due to higher nutrient levels in the Caribbean.

2. MATERIALS AND METHODS

Short term *in situ* experiments were carried out between January and June 1999 on the fringing reef near Buoy 1 on the leeward coast of Curaçao, Southern Caribbean (Fig.3). The experiments were performed with SCUBA, at a depth of 4.5 m. Three species of boring sponges; *Cliona lampa* (forma *occulta*, Rützler 1974) *C.laticavicola* and *C. vermifera* were used for the experiments because of their local abundance and for the fact that they frequently occur in relatively small pieces of coral rubble (*Porites porites* a.o.), which makes them fit the experimental enclosures.

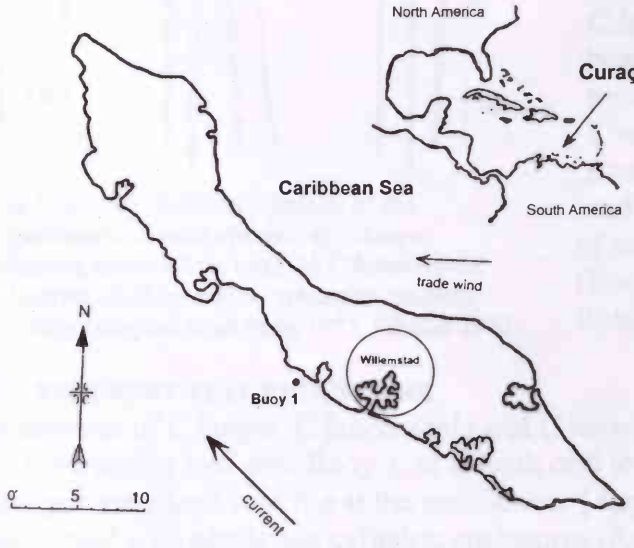

Fig. 3. Map of Curaçao with the study site (Buoy 1), on the leeward side of the island. The bay at the urbanised area of Willemstad (St. Anna Bay) is heavily eutrophied. The general current is west, but effects of eutrophied water from St. Anna Bay are dilluted away at the distance of Buoy 1 (Gast 1998). Water temperature from January to April 1999 at Buoy 1 ranged from 26 to 27°C (M.Vermeij, pers.comm.).

2.1. DETERMINATION

Sponge species were determined by means of several diagnostic elements as described by Pang (1973) and Rützler (1974). *In situ* determination took place on the basis of general shape, colour, surface structure and distribution of the ostia and oscula (respectively in- and excurrent openings of the aquiferous system) (Table 2). Endolithic features as well as sponge skeleton elements were examined in the laboratory. Pieces of rubble with associated boring sponges were chiseled in pieces (Appendix Fig. 1a-b). The size of the excavations was determined and a small amount of sponge tissue was removed and placed on a microscopic slide with a drop of commercial bleach. This causes the calcium carbonate from the substrate to dissolve and leaves siliceous and spongine elements intact (Van Soest pers. comm.).The size, shape and occurrence of species specific sponge-skeleton elements (spicules) was examined under a microscope (Fig. 4).

Table 2. Summary of epi- and endolithic characteristics of the experimental clionid sponges, according to Pang (1973), Rützler (1974) and personal observations.

	Cliona lampa (forma *occulta*)	*Cliona laticavicola*	*Cliona vermifera*
Colour (*in situ*)	dark orange, vermilion	orange	vivid orange-red
Ostial and oscular	0.5 - 1.0	0.2 - 0.3	0.05 - 0.1
perforations (cm)	0.1 - 0.3	0.2 - 1.5	0.1 - 0.15
Shape of ostia and oscula	non fusing, abundant, roughly circular, slightly raised from substrate	some fusing, irregular outline, oscula max. 0.3 cm height	non fusing, numerous, scattered on substrate, oscula shaped like trunkated cones
Colour excavations	dull orange	lighter orange	lighter orange
Excavations (cm)	0.1 – 0.2	1.0 - 2.0	0.2 - 0.6
Shape excavations	small, shallow (max. 0.3 cm deep) spherical to ellipsoidal galleries	very wide galleries, fusing, lobes interconnected by slender stems	rounded to elongated lobes, interconnected by cylindrical stems

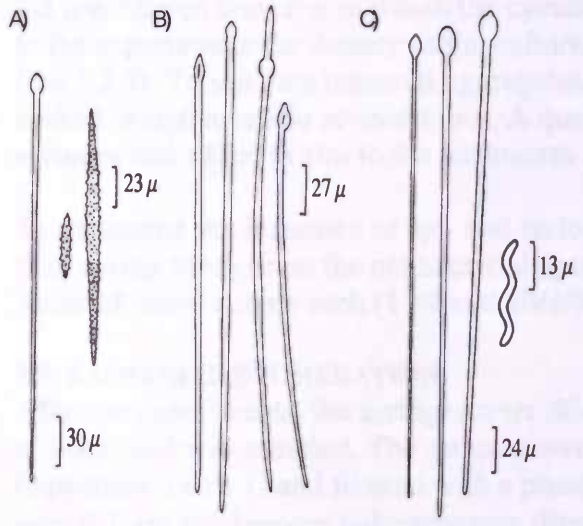

Fig.4. Specific skeleton elements of the experimental clionid species. a) *C.lampa*: tylostyle, microsclere, oxea. b) *C.laticavicola*: tylostyles. c) *C.vermifera*: tylostyles, smooth spiraster (adapted from Pang 1973, Rützler 1974).

Specific skeleton elements include tylostyles (spicules with one end pointed and a globular swelling at the base), oxea (spicules that are pointed at both ends), microscleres (small spicules) and spirasters (spiral shaped microscleres with spines peripherically arranged). *C.lampa* has straight tylostyles with spherical or ovoid heads, oxea which are bent in the centre and provided with minute spines and microscleres which are robustely spined. *C.laticavicola* has long tylostyles of which the heads can have different shapes. No microscleres are observed in this species. *C.vermifera* has mostly straight tylostyles with round, slightly elongated or subterminal heads and smooth spiralled or undulated microscleres of uniform thickness throughout their lenght (Boury-Esnault & Rützler 1997, Pang 1973, Rützler 1974).

2.2. EXPERIMENTAL PROCEDURE

Specimens of *C.lampa*, *C.laticavicola* and *C.vermifera* were collected from a restricted area on the fringing reef near Buoy 1, at a depth of 4 to 8 m. The pieces of rubble with associated sponges were kept on a tile at the reef bottom (Appendix Fig. 2). *In situ* experiments were performed with plexiglass cylindric enclosures (Appendix Fig. 3). Pieces of rubble that were to large for the enclosures where shortened with a hamer and chisel and left for at least two days in order for the sponge to recover (Appendix Table 1). Fouling epibenthos was largely removed from the rubble, without touching the sponge papillae. One day prior to the experiments the sponges were placed inside 6 open cylinders (n = 4 on 13/1 to 10/2/99). In addition 2 empty cylinders were used as control enclosures, to determine if bacterial densities decreased during the experimental period due to factors such as nanoflagellate or ciliate grazing.

To determine the change in natural bacterial abundance in time due to sponge filtration, samples of enclosed sea water were taken at specific time intervals. At the start of the experiment the cylinders were sealed with O-ringed lids, enclosing the experimental sponges in ambient reef water. A sample of c.a. 4.5 ml was taken from each enclosure with a 10cc syringe by inserting the needle through a rubber membrane. Samples were taken at t = 0 and and furthermore at time intervals of 15 min. during one hour. An additional sample was taken at t = 7.5 (25/3, 1/4, 13/4, 28/4/99) and t = 120 (28/4/99). Preceding the experiments the syringes were filled with c.a. 4.5 ml 5% formaldehyde (37% formaldehyde diluted with 0.2 µm filtrated seawater) to immediately fixate the bacteria during the sampling procedure. Subsequently the syringes were weighed.

After completing the experiments the rubble series were transported to the lab. Each piece of rubble was air dried on a paper towel for several minutes before its volume was determined using water displacement. The rubble series were then preserved in a freezer at -20°C. The series from 13/1 to 10/3/99 were dried at 70°C until constant weight prior to being preserved.

Experiments were carried out with either natural occurring bacteria or with blue-green cyanobacteria (*Synechococcus* sp.) added to the seawater in the enclosures. The cyanobacteria

were grown in a batch culture to a dense concentration. An A-medium was added to enrich the 0.2 µm filtered seawater in which the cyanobacteria were cultured (Appendix, Table 2). Prior to the experiments the density of the culture was estimated using epifluorescence microscopy (see § 2.3). To separate bacterial aggregates, the culture was dispersed in a petri dish and sucked trough a needle several times. A quantified amount of cyanobacteria was taken up in syringes and added *in situ* to the enclosures on t = 0.

To determine the influence of epi- and endocryptolithic suspension feeding organisms other than boring sponges on the net bacterial uptake rates, two experiments were carried out with 4 series of 'bare' rubble each (17/2 and 20/4/99).

2.3. BACTERIAL ENUMERATION

After the experiments, the syringes were dried on a towel and weighed to estimate the volume of water that was sampled. The samples were stained for 2 minutes with Acridine Orange (Appendix Table 1) and filtered with a pressure of maximal 10 cm pHg in a Sartorius set up over 0.2 µm Nucleopore polycarbonate filters on top of 0.45 µm cellulose Schleicher & Schuell filters (Hobbie *et al* 1977). The polycarbonate filters were stained in advance with Sudan Black (Appendix Table 1) and rinsed in 0.2 µm filtrated sea water to remove excessive stain. The damp filters where placed on microscope slides with a film of standard immersion oil (Olympus), covered with a cover slip and a drop of oil and immediately stored in a freezer at –20°C.

The numbers of bacteria per ml sample were enumerated using epifluorescence microscopy (Zeiss Axiophot) at 1250 × magnification. After staining with acridine orange 95% of the bacteria fluorescence green and the remainder red or yellow. Other organic particles have a weak red fluorescence (Hobbie *et al* 1977). Per filter a minimum of 200 bacteria was counted in at least 10 randomly selected microscope fields. Total numbers of bacteria per ml of sample were calculated with a special computer programm (Proza4).

Cyanobacteria were enumerated using the same method. Staining with acridine orange was omitted on 13/4 and 28/4/99 (Johnson & Sieburg 1979 and Moriarty *et al* 1985). *Synechococcus sp.* can be distinguished from heterotrophic cells by its red fluorescence in ultraviolet light (Sorokin 1990a).

2.4. BIOMASS

The amount of organic tissue associated with rubble can not be estimated by ashing the rubble itself. The loss of weight trough removal of carbon dioxide from carbonates can rise to 44% of the ash weight, thus leading to a significant underestimation (Holme & McIntyre 1984). Furthermore the abundance and composition of the epi- and endolythic fauna is highly variable (Choi 1984, Choi & Ginsburg 1983, Meesters *et al* 1990), which makes it difficult to estimate the contribution of the boring sponges to the total biomass. It is not possible to mechanically separate sponge tissue from the rubble, since the endolithic parts of the sponges are interwoven with the limestone. An alternative is to dissolve the calciumcarbonate rubble in diluted acid.

By placing the rubble in diluted HCl with EDTA (see Appendix Table 2) for 3 to 6 days (depending on the size of the rubble), the calciumcarbonate substrate is dissolved. The solution was regularly sieved over a 1 mm mesh and renewed. The organic residue was placed in petri-dishes and kept in a fridge until all pieces of rubble per series were dissolved. The residue was seperated in a sponge-, worm-, gastropod- and indefinable fraction. All organic matter was then dried at 70°C to contant weight and, after weighing, ashed for 5 hours at 600°C. Ash-free dry weight (AFDW) was estimated as dry weight minus ash weight.

We assume that the technique of acid dissolution does not result in a significant reduction of organic tissue. Microscopic examination of sponge tissue that was placed in the HCl-EDTA sollution for 24 hours show no changes in cell structure.

To gain insight into the species composition of the endocrypolithic community, additional sponge-infested rubble was gathered near Buoy 1 and chiseled into pieces. Rubble-associated organisms were carefully removed, preserved in 80% alcohol and described.

2.5. STATISTICS

A t-test was applied to determine if there is a statistically significant difference between the total ash free dry weight (AFDW) of *C.vermifera* infested rubble that was dried at 70°C prior to further processing and *C.vermifera* infested rubble that was not dried. A t-test was also applied to determine if the AFDW of *C.laticavicola* differs from that of *C.vermifera*.
The total AFDW of rubble associated organisms per different rubble category (respectively infested with *C.lampa*, *C.laticavicola*, *C.vermifera* and bare rubble) was statistically analysed. Since there is a significant difference between the standard deviations of the samples (Cochran`s C, $P < 0.01$), a non parametric Kruskal-Wallis test was applied to compare the medians within each of the categories. Fisher`s 95.0% least significant difference (LSD) procedure was used to make a multiple comparison between the mean AFDW per category. To test the correlation between rubble volume and total AFDW of rubble associated organisms, a product-moment correlation test ($Df = 65$) was applied.

The bacterial numbers of 13/1/99 are considered to be misrepresentative as a result of problems referring to the counting technique and were therefore not included in any statistical evaluations. T-tests were carried out to determine whether the final densities of bacteria in the enclosures with sponges differ from the average values of the control series. Regression coefficients were determined from the equations for linear regressions between bacterial concentrations on $t = 0$ and $t = 15$. There are significant differences between the standard deviations of the regression coefficients (F-test, $P < 0.01$ for control series, rubble and sponges, all categories respectively tested against each other).Therefore a non parametric Mann-Whitney (Wilcoxon) W test was used to compare the regression coefficients of the different categories.
A paired t-test was carried out for *C.laticavicola* and *C.vermifera* to determine if the slope between $t = 0$ and $t = 7.5$ differs significantly from that between $t = 7.5$ and $t = 15$. An ANOVA was applied to compare the final bacterial densities after 60 minutes for the three sponge species. Fisher`s 95.0% least significant difference (LSD) procedure was used to make a multiple comparison between the mean densities. To find out if there is a functional relationship between the initial bacterial density and the density on $t = 60$, a linear regression was performed. Average values are plotted per experiment to detect general trends. A linear regression was not sufficient to account for the differences among the sample means and therefore a curvilinear regression (expressed as a polynomial function) is fitted trough the averages.

The rate of removal of particles from a known volume of suspension can be expressed as clearance (Coughlan 1969). Clearance (c) of bacteria during the first 15 minutes of the experiments was determined from the exponential reduction in bacterial numbers as a function of time, using the formula:

$$c = (V_w/t) \ln (C_0/C_t)$$

where C_0 and C_t are the bacterial concentrations at t = 0 and t = 15 respectively (according to Riisgård et al 1993). V_w is the volume of enclosed water, calculated as:

$$V_w = V_e - V_r - V_s$$

where V_e is the volume of the enclosure, V_r is the volume of the rubble and V_s is the volume of the samples that were taken.

Data analysis revealed a limiting plateau in bacterial numbers around t = 15. Bacterial uptake after this point was influenced by an unknown factor and is assumed not be representative for the optimal feeding behaviour of the sponges. Therefore clearance rates were calculated for the first 15 minutes of the experiments only.
Negative clearance rates are considered as artefacts due to an apparent increase in bacterial numbers on t = 15 and were therefore not included in any statistical evaluations. The experiments from 13/4 and 28/4/99 were excluded from statistical test since staining with acridine orange was omitted. Uptake rates of ambient bacteria could therefore not be determined which results in an underestimation of the net clearance.
An ANOVA was applied to compare the clearance rates between the three clionid species. A multiple comparison was carried out to determine which mean clearance rates are significantly different from which others (Fisher`s 95% LSD procedure). To determine if the clearance rates on t = 7.5 differ significantly from those on t = 15, a paired t-test was applied. To compare clearance of ambient bacteria with clearance of added cyanobacteria per sponge species, a paired t-test was used. The functional relationship between initial concentration of (cyano)bacteria in the enclosures and clearance was tested by determining the correlation coefficient of a linear regression, as well as a second order polynomial regression on the data points per clionid species.

2.6. BEHAVIOURAL OBSERVATIONS

To determine any possible effects of the enclosures on the filtration of the sponges, observations were made on the behaviour of their oscular papillae during several experiments. An additional *in situ* experiment was carried out on 4/5/99 at a depth of 5 m near Buoy 0, where a comparison was made between the behaviour of sponges on the reef bottom, in an open enclosure, in a closed enclosure and in an enclosure equiped with a stirring device simulating ambient flow. These experiments were carried out simultaneously, with 4 enclosures each. *C. laticavicola* was chosen for this purpose since it has relatively large papillae, which facillitates observations on contraction and re-expanding of the oscula. The number of open, partly contracted and completely contracted oscula was established every 5 minutes during one hour.

3. RESULTS

3.1. BEHAVIOURAL OBSERVATIONS

The oscular papillae of the boring sponges that were enclosed in cylinders during the experiments contracted after 7.5 to 15 minutes (Table 3). After initial partial or complete contraction some oscula were observed to expand and contract again. The moment of sampling (on t = 0, 7.5, etc) occasionally caused a minimal movement of the enclosure. However no direct relationship was observed between the sampling moment and oscular behaviour.

Clionid sponges on the reefbottom and in open cylinders had their oscula expanded continuously during the experimental period (Table 4 a-b). One sponge partially contracted one oscula (t = 15 series 1b), which completely re-expanded after 20 minutes. The use of a stirring device to stimulate ambient flow in closed cylinders had no observable effect on the oscular behaviour compared to enclosures without stirring devices (Table 4 c-d). In all 8 closed cylinders oscula were contracted after 5 to 10 minutes and subsequently started re-expanding and recontracting in a random pattern.

Table 3. Observations on contraction and opening of oscular papillae from clionid sponges in enclosures (n = 6 per experiment). Oscular behaviour was observed during the experiments from 25/3 to 28/4/99, immediately after sampling on t = 0, 7.5, etc. Series 1-4 of 21/4/99 consist of bare rubble. O = oscula wide open, PC = partly contracted, C = completely contracted.

	C.laticavicola, 25/3/99						*C.laticavicola*, 13/4/99						*C.laticavicola*, 7/4/99					
t	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
0	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
7.5	PC	PC	O	PC	PC	O	PC	O	O	O	O	O	O	O	O	PC	O	O
15	C	PC	PC	C	C	PC	C	C	C	PC	C	C	C	C	C	PC	C	C
30	C	PC	C	PC	C	C	PC	PC	PC	O	O	PC	O	O	O	O	C	C
60	PC	PC	O	PC	O	PC	C	C	C	PC	C	C	PC	O	O	PC	C	O
	C.vermifera, 1/4/99						*C.vermifera*, 20/4/99						*C.vermifera*, 28/4/99					
t	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
0	O	O	O	O	O	O					O	O	O	O	O	O	O	O
7.5	O	PC	PC	PC	PC	O					C	O	O	C	O	C	O	O
15	C	C	C	C	C	PC		no data			C	C	C	C	C	C	PC	PC
30	C	C	C	C	C	C					C	C	C	PC	C	C	C	C
60	C	PC	C	PC	C	C					PC	C	C	PC	PC	PC	PC	PC

Table 4 a-c. Observations on contraction and opening of oscular papillae of *Cliona laticavicola* (n = 4 per experiment) a) on the reef bottom, b) in open enclosures, c) in enclosures equiped with a stirring device and d) in enclosures without stirring device. Number of oscula; O = oscula wide open, PC = partly contracted, C = completely contracted.

	A) Reef bottom				B) Enclosure open			
t	1	2	3	4	1	2	3	4
0	O	O	O	O	O	O	O	O
5	O	O	O	O	O	O	O	O
10	O	O	O	O	O	O	O	O
15	O	O	O	O	5 O, 1 PC	O	O	O
20	O	O	O	O	5 O, 1 PC	O	O	O
25	O	O	O	O	5 O, 1 PC	O	O	O
30	O	O	O	O	5 O, 1 PC	O	O	O
35	O	O	O	O	O	O	O	O
40	O	O	O	O	O	O	O	O
45	O	O	O	O	O	O	O	O
50	O	O	O	O	O	O	O	O
55	O	O	O	O	O	O	O	O
60	O	O	O	O	O	O	O	O
	C) Enclosure + stirring device				D) Enclosure			
t	1	2	3	4	1	2	3	4
0	O	O	O	O	O	O	O	4 PC
5	4 PC	O	O	O	O	O	5 PC	4 PC
10	4 PC	4 C	7 PC	3 PC	O	2 C	5 C	4 PC
15	3 C	4 C	1 C	3 C	4 PC	2 C	5 C	4 PC
20	3 C	4 C	7 C	3 C	4 PC	2 C	5 C	4 C
25	3 C	4 C	7 C	3 C	3 C, 1 PC	2 C	5 C	4 C
30	3 C, 1 PC	4 PC	7 PC	2 C,	4 C	2 C	5 PC	3 C, 1 PC
35	3 C, 1 PC	3 PC, 1 O	O	1 PC	2 C, 2 PC	1 C, 1 PC	4 PC, 1 O	4 O
40	2 C, 2 PC	4 O	O	1 C, 2 PC	1 C, 2 PC, 1 O	2 PC	3 PC, 2 O	1 PC, 3 O
45	2 PC, 2 O	2 PC, 2 O	O	2 PC, 1 O	3 PC, 1 O	2 PC	5 PC	4 PC
50	1 C, 3 PC	2 PC, 2 C	7 PC	2 PC, 1 O	3 PC, 1 O	2 PC	2 C, 3 PC	1 C, 3 O
55	2 C, 2 PC	2 PC, 2 C	7 PC	2 C, 1 O	3 PC, 1 O	2 PC	5 C	13 PC, 1 O
60	2 C, 2 PC	2 PC, 2 C	7 HC	2 C, 1 PC	2 PC, 2 O	2 PC	5 C	2C, 1 PC,1 O

3.2. BIOMASS

Ash-free dry weight (AFDW) of the boring sponges and other rubble associated organisms was estimated as dry weight minus ash weight (Appendix Table 3 a-d). The series from 13/01/99 to 10/03/99 were dried at 70°C prior to further processing. This resulted in a large fraction of indefinable organic residue after dissolution of the rubble, from which sponges could not be properly distinguished (Appendix Table 3a and c).

AFDW of *C.laticavicola* ranges from 0.05 to 0.30 g whereas that of *C.vermifera* ranges from 0.07 to 0.21g (Fig.5. See also Appendix Table 3b and d). There is no significant difference between the AFDW of these two sponge species.

Volume of the rubble pieces ranged from 25 to 150 ml. A significant relationship exists between rubble volume and cryptofauna biomass ($r = 0.450$, $P < 0.001$; see Fig 6). Sponges on average made up c.a. 20 - 40% of the total biomass of rubble associated organisms (Fig. 7). The largest fraction exists of organic material that was indefinable after dissolution.This fraction mainly consists of endolithic and encrusting algae, but contains bryozoans, crustaceans, foraminiferans, tunicates and an occasional anemone as well. The smallest contribution to the biomass of rubble-associated cryptofauna is formed by worms, including sipunculids, fire worms (Amphinomidae), feather duster worms (Sabellidae), calcearous tube worms (Serpulidae), sponge worms (*Haplosyllis* sp) and gastropoda, including fuzzy chitons (*Acanthopleura granulata*) as well as boring bivalves.

The total AFDW of rubble associated organisms ranged from 0.60 to 1.65 g (Fig. 8). No significant difference was found between total AFDW of organic material from rubble infested with *C.vermifera* that was dried at 70°C and *C.vermifera* infested rubble that was not dried. A significant difference was found between rubble infested with *C.lampa*, *C.laticavicola* and bare rubble (see multiple comparison, Appendix Table 4). The biomass of organisms associated with bare rubble on average is higher than that of rubble infested with boring sponges (Fig. 9).

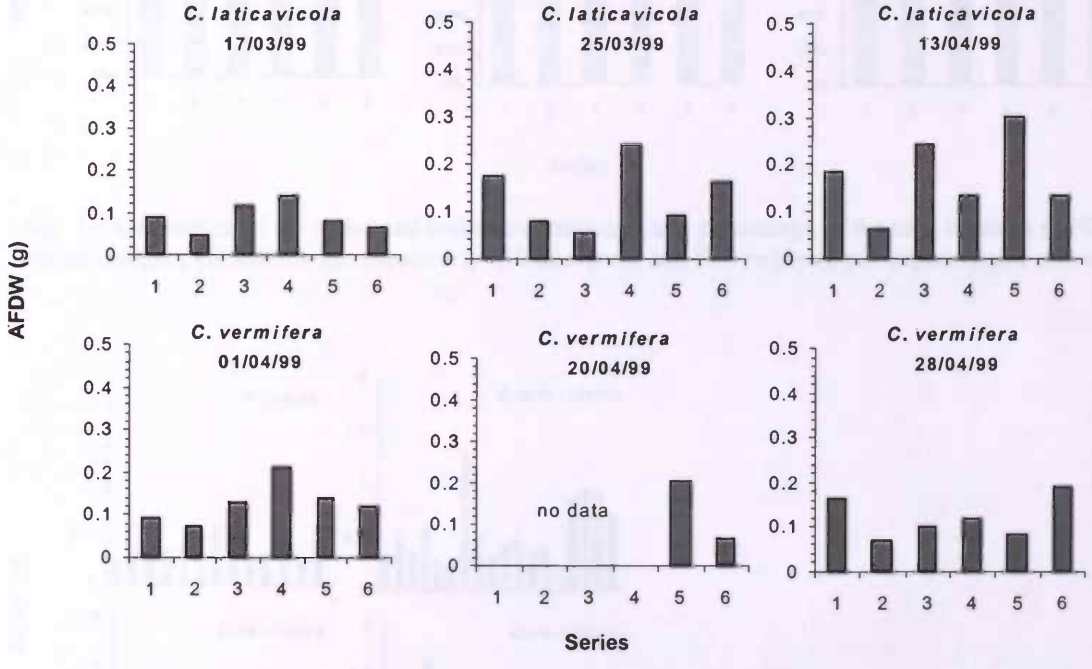

Fig. 5. Ash free dry weight (AFDW) of *C.laticavicola* and *C.vermifera* per experiment, per series. Series 1 - 4 of 20/04/99 consist of bare rubble (i.e. not infested with boring sponges).

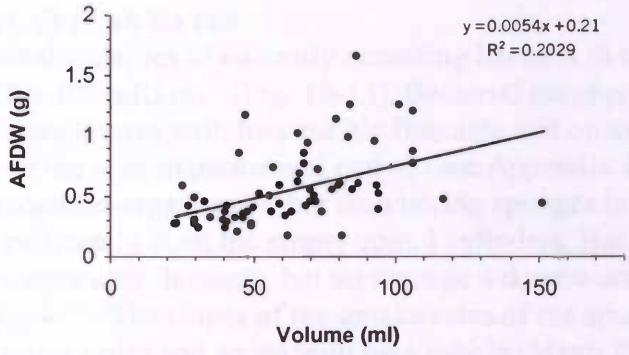

Fig. 6. Total Ash Free Dry Weight (AFDW) of organic material plotted against the volume of the rubble series that were used during the experiments from 13/01/99 to 28/04/99 (n = 67). Linear regression is shown.

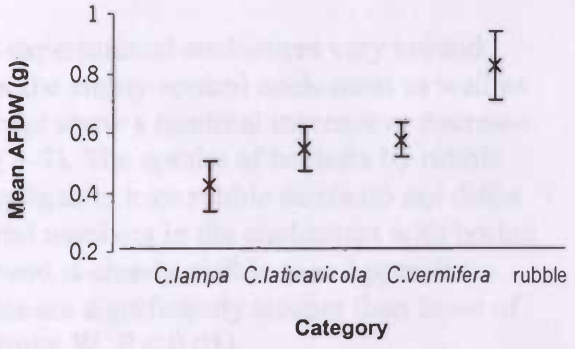

Fig. 9. Mean total ash free dry weight (AFDW) of rubble associated organisms per different rubble category; respectively infested with *C.laticavicola* (n = 17), *C.vermifera* (n = 27) and bare rubble (n = 7). Vertical bars indicate standard errors.

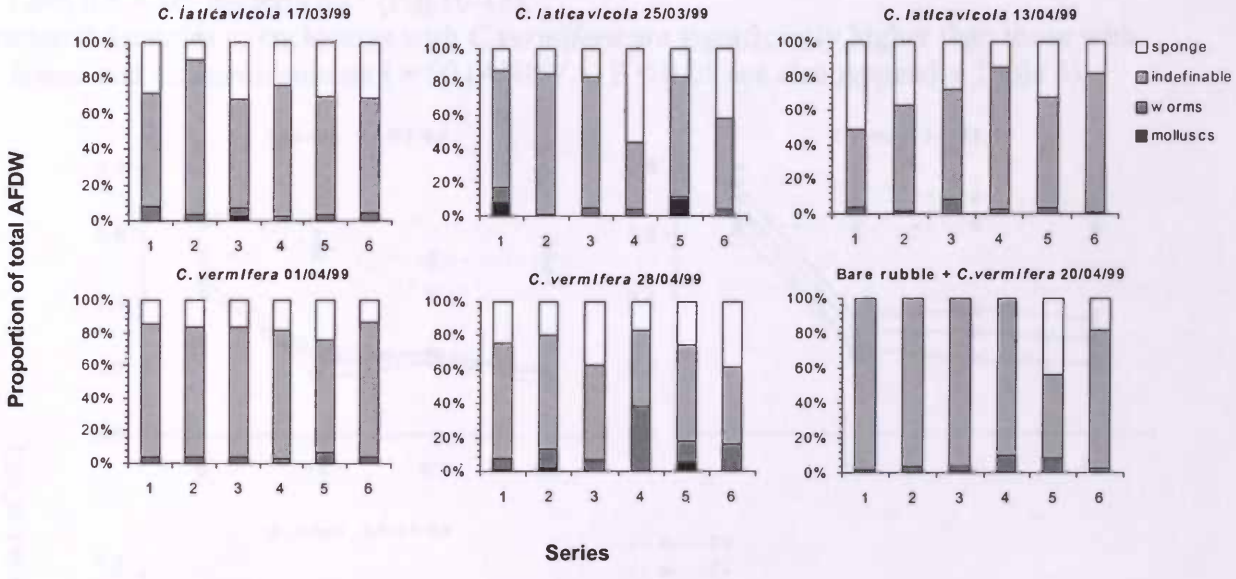

Fig. 7. Composition of the endo- and epilithic community as a percentage of the total biomass (AFDW) per rubble category (infested with respectively *C.laticavicola* and *C.vermifera*), per experimental series.

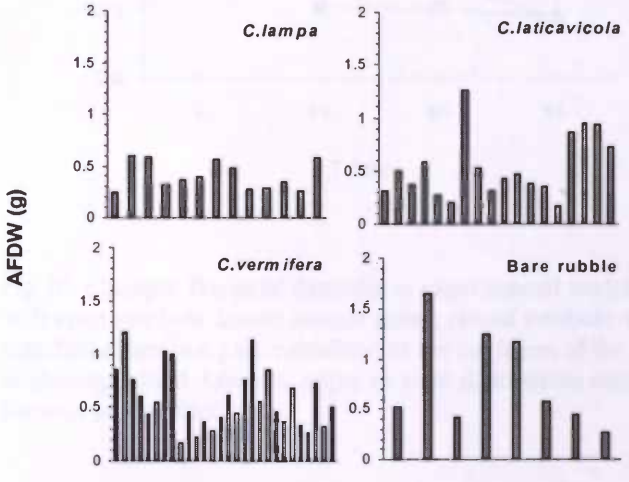

Fig. 8. Total AFDW of the endo- and epilithic community per different rubble category; respectively infested with *C.lampa* (n = 12), *C.laticavicola* (n = 17), *C.vermifera* (n = 27) and bare rubble (n = 7).

3.4. UPTAKE RATES

Initial densities of naturally occurring bacteria in the experimental enclosures vary around 0.6×10^6 cells ml^{-1} (Fig. 10-13). Bacterial numbers in the empty control enclosures as well as the enclosures with bare rubble fluctuate and on average show a minimal increase or decrease over the total experimental period (see Appendix Fig 4-7). The uptake of bacteria by rubble associated organisms other than boring sponges is negligable; bare rubble series do not differ significantly from the empty contol cylinders. Bacterial numbers in the enclosures with boring sponges also fluctuate, but on average a downward trend is clearly visible (see Appendix Fig. 4-7). The slopes of the uptake rates of the sponges are significantly steeper than those of control series and series with bare rubble (Mann Whitney W, $P < 0.01$).

In general the uptake rates of bacteria by the three clionid species were highest during the first 15 minutes of the experiments and subsequently decrease (Fig. 10-13). Slopes of the uptake rates of *C.laticavicola* on t = 7.5 differ significantly from those on t = 15 (paired t-test, $P < 0.05$). Over the total experimental period of 1 hour *C.lampa*, *C.laticavicola* as well as *C.vermifera* caused a significant decrease in bacterial numbers compared to the initial densities on (t-test, $P < 0.01$ for each species). Final densities in the enclosures vary between 0.1 and 0.5×10^6 bacteria ml^{-1} (Fig.10-13).

Bacterial densities in enclosures with *C.vermifera* are significantly higher than those with *C.lampa* and *C.laticavicola* on t = 60 (ANOVA, $P < 0.05$ see also Appendix Table 5).

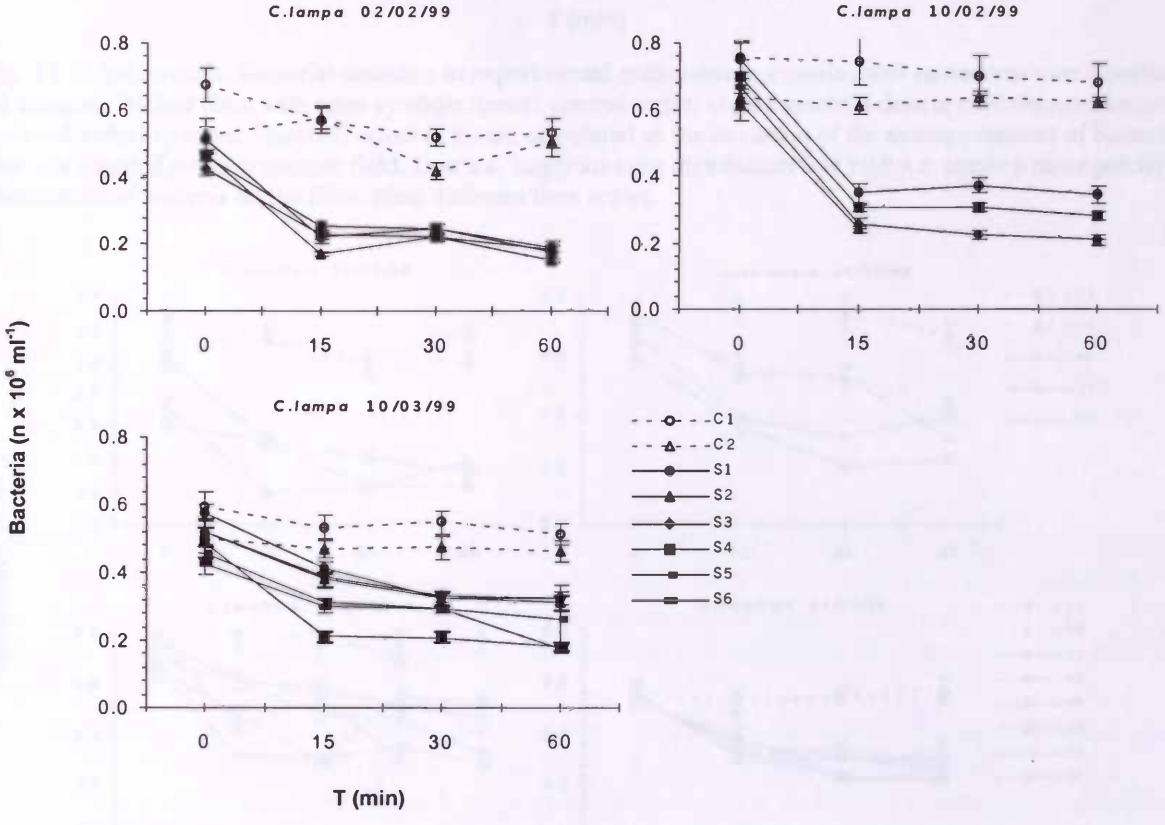

Fig.10. *C.lampa*. Bacterial densities in experimental enclosures over a period of 60 minutes. Dashed lines with open symbols denote control series; closed symbols denote experimental series with individual sponges. Standard errors (s.e.) are calculated as the deviation of the average amount of bacteria that was counted per microscopic field. Low s.e. imply an even distribution and high s.e. imply a more patchy distribution of bacteria on the filter.

However, *C.vermifera* brought about an average decrease of 49% compared to initial densities, which is comparable to values found for *C.lampa* and *C.laticavicola*. Final bacterial densities in enclosures with *C.lampa* and *C.laticavicola* respectively, are on average 44% and 50% below initial densities. A linear relationship exists between the numbers of bacteria counted at t = 60 and the initial bacterial density (Fig.14). The correlation is highly significant for *C.laticavicola* ($r = 0.68$, $P < 0.02$), moderately strong for *C.lampa* ($r = 0.53$, $P < 0.05$) and relatively weak for *C.vermifera* ($r = 0.34$, $P < 0.2$).
No visible effect was found on the uptake rates of rubble pieces that were chiseled to fit the experimental enclosures compared to rubble that was not chiseled.

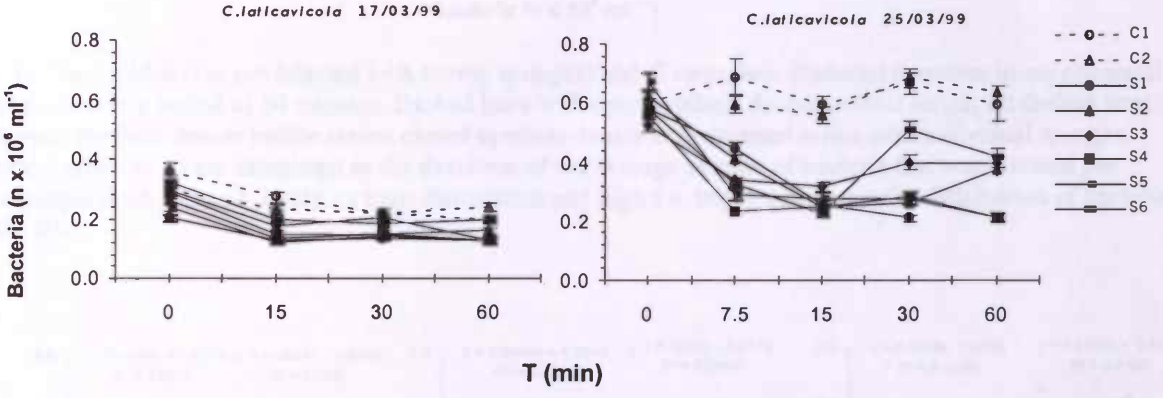

Fig. 11. *C.laticavicola*. Bacterial densities in experimental enclosures in experimental enclosures over a period of 60 minutes. Dashed lines with open symbols denote control series; closed symbols denote experimental series with individual sponges. Standard errors (s.e.) are calculated as the deviation of the average amount of bacteria that was counted per microscopic field. Low s.e. imply an even distribution and high s.e. imply a more patchy distribution of bacteria on the filter. Note different time scales.

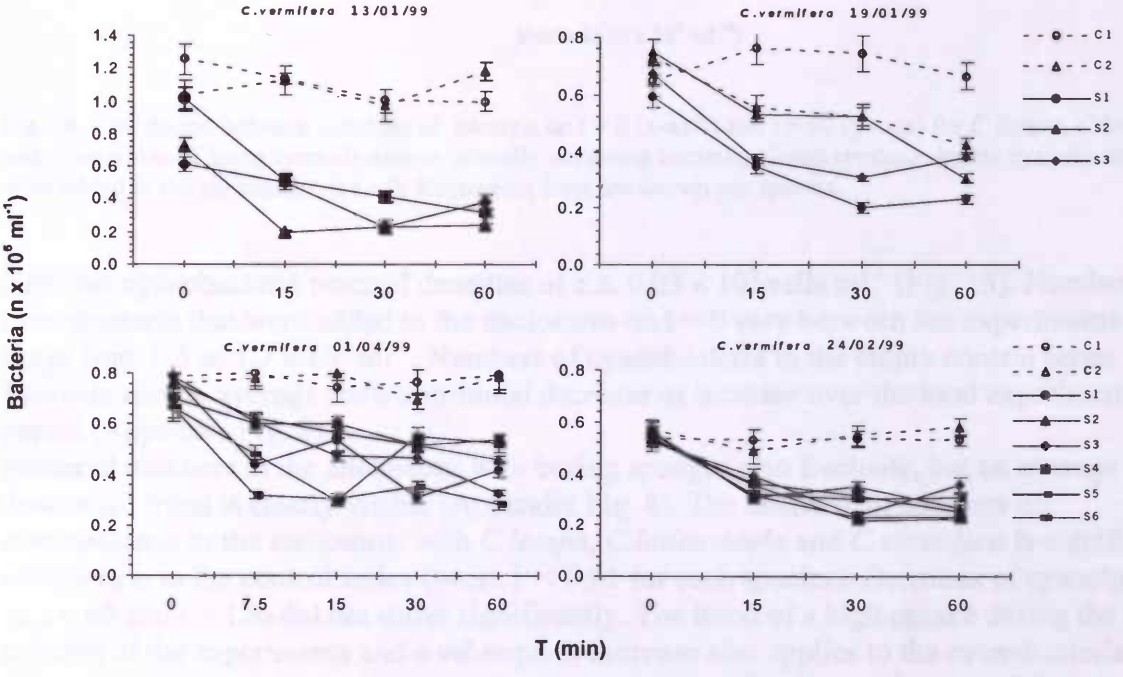

Fig. 12. *C.vermifera*. Bacterial densities in experimental enclosures over a period of 60 minutes. Dashed lines with open symbols denote control series; closed symbols denote experimental series with individual sponges. Standard errors (s.e.) are calculated as the deviation of the average amount of bacteria that was counted per microscopic field. Low s.e. imply an even distribution and high s.e. imply a more patchy distribution of bacteria on the filter. Note different ordinate scales.

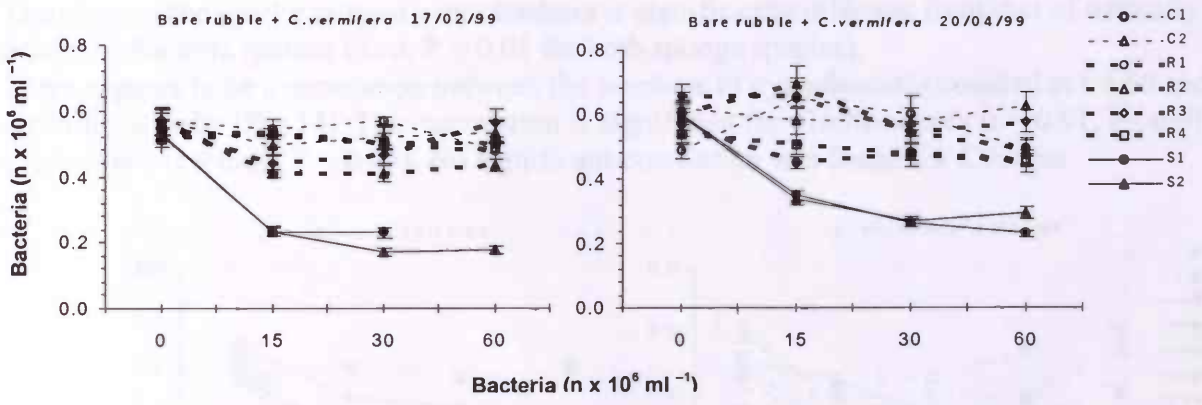

Fig. 13. Bare rubble (i.e. not infested with boring sponges) and *C.vermifera*. Bacterial densities in experimental enclosures over a period of 60 minutes. Dashed lines with open symbols denote control series; fat dashed lines with open symbols denote rubble series; closed symbols denote experimental series with individual sponges. Standard errors (s.e.) are calculated as the deviation of the average amount of bacteria that was counted per microscopic field. Low s.e. imply an even distribution and high s.e. imply a more patchy distribution of bacteria on the filter.

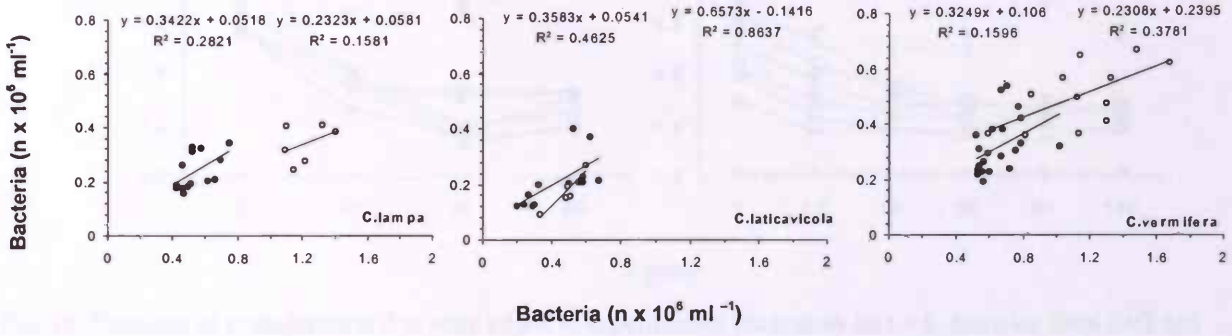

Fig. 14. Correlation between densities of bacteria on t = 0 (x-axis) and t = 60 (y-axis) for *C.lampa*, *C.laticavicola* and *C.vermifera*. Closed symbols denote naturally occurring bacteria, closed symbols denote cyanobacteria that were added to the enclosures on t = 0. Regression lines are shown per species.

Ambient cyanobacteria reached densities of c.a. 0.03×10^6 cells ml^{-1} (Fig. 15). Numbers of cyanobacteria that were added to the enclosures on t = 0 vary between the experiments and range from 0.5 to 1.7×10^6 ml^{-1}. Numbers of cyanobacteria in the empty control series fluctuate and on average show a minimal decrease or increase over the total experimental period (Appendix Fig. 8).

Bacterial numbers in the enclosures with boring sponges also fluctuate, but on average a downward trend is clearly visible (Appendix Fig. 8). The decrease in numbers of cyanobacteria in the enclosures with *C.lampa*, *C.laticavicola* and *C.vermifera* is significant in comparison to the control series (t-test, $P < 0.01$ for each species). Densities of cyanobacteria on t = 60 and t = 120 did not differ significantly. The trend of a high uptake during the first 15 minutes of the experiments and a subsequent decrease also applies to the cyanobacteria (Fig. 15). Slopes of the uptake rates of cyanobacteria by *C.laticavicola* on t = 7.5 are significantly different from those on t = 15 (paired t-test, $P < 0.05$). After 60 minutes the densities of cyanobacteria fluctuate between 0.2 and 0.5×10^6 bacteria per ml.

The experiments with *C.laticavicola* and *C.vermifera* of 10/3 and 24/2 show that the densities of added cyanobacteria decline at a higher rate then those of ambient bacteria (Fig. 16).

The slope of the uptake rates of cyanobacteria is significantly different from that of naturally occurring bacteria (paired t-test, $P < 0.01$ for both sponge species).
There appears to be a correlation between the numbers of cyanobacteria counted at t = 60 and the initial density (Fig.14). The correlation is significant for *C.laticavicola* ($r = 0.91$, $P < 0.01$) *C.vermifera* ($r = 0.62$, $P < 0.05$). No significant correlation was found for *C.lampa*.

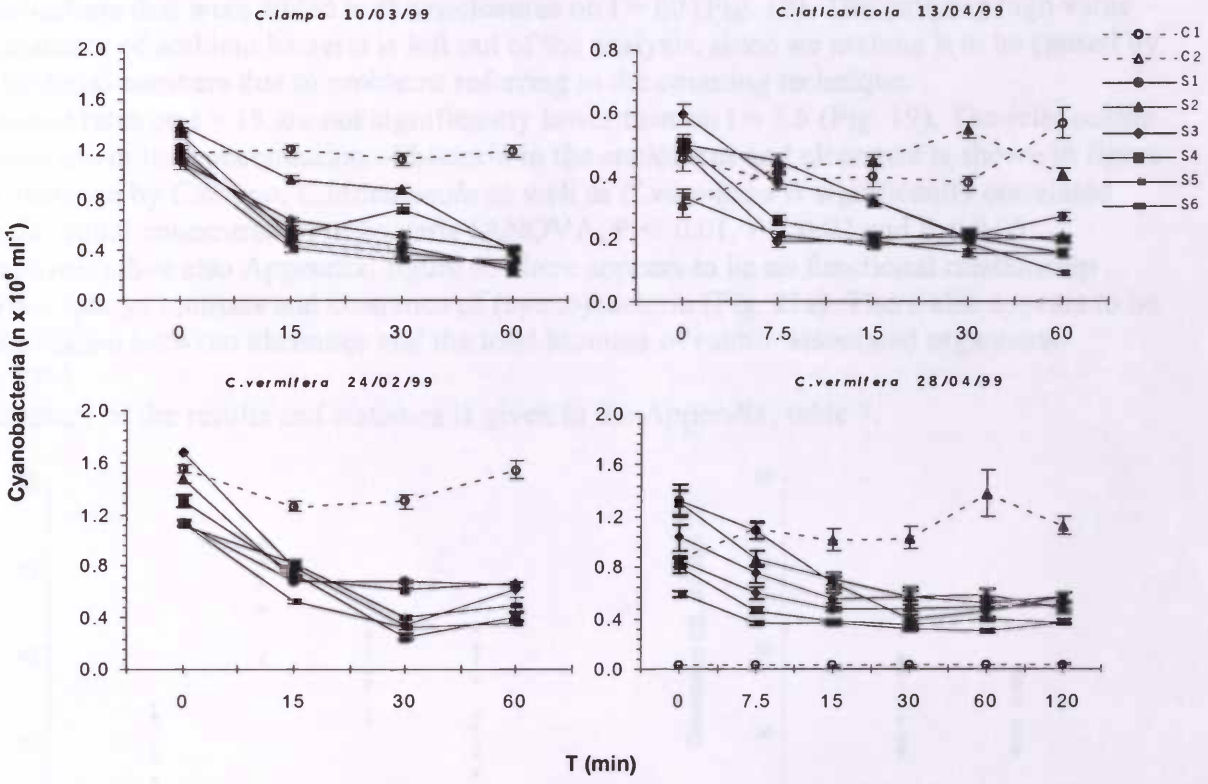

Fig. 15. Densities of cyanobacteria that were added to experimental enclosures on t = 0. Samples from 24/2 and 10/3 were stained with acridine orange, staining was omitted on 13/4 and 28/4. C1 from 28/4 are ambient densities of cyanobacteria. Dashed lines with open symbols denote control series; closed symbols denote experimental series with individual sponges. Standard errors (s.e.) are calculated as the deviation of the average amount of bacteria that was counted per microscopic field. Low s.e. imply an even distribution and high s.e. imply a more patchy distribution of bacteria on the filter. Note different time scales.

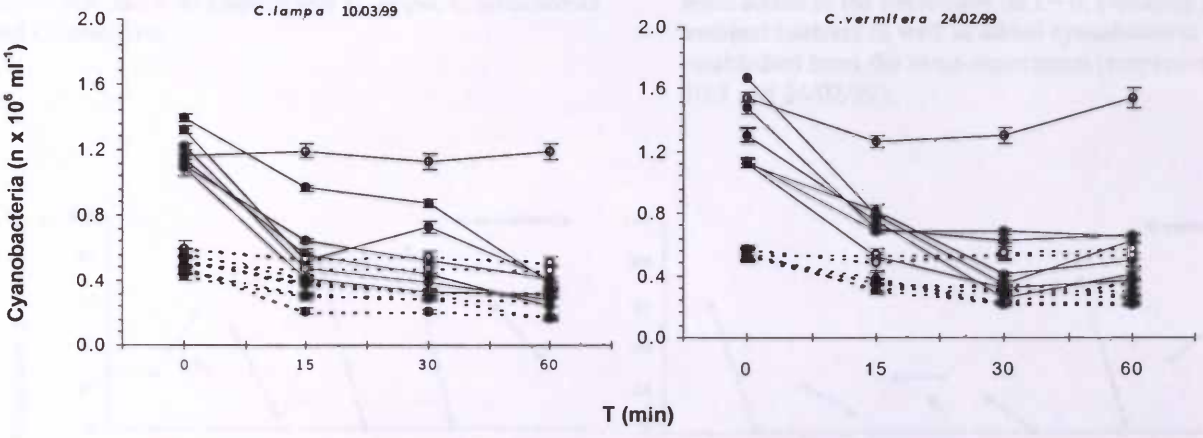

Fig. 16. *C.lampa* and *C.vermifera*. Densities of naturally occurring bacteria as well as cyanobacteria that were added to experimental enclosures on t = 0. Open symbols denote control series, dashed lines denote naturally occurring bacteria, straight denote cyanobacteria.

3.5. CLEARANCE RATES

There is a significant difference in bacterial clearance between the three clionid species (ANOVA, $P < 0.01$). *C.lampa* on average has a higher clearance rate than *C.laticavicola* and *C.vermifera* (Fig.17, see also Appendix Table 6). A significant difference (paired t-test, $P < 0.01$) was found between clearance by *C.lampa* of naturally occurring bacteria and cyanobacteria that were added to the enclosures on t = 60 (Fig. 18). The outlying high value for clearance of ambient bacteria is left out of the analysis, since we assume it to be caused by low bacterial numbers due to problems referring to the counting technique.
Clearance rates on t = 15 are not significantly lower than on t = 7.5 (Fig. 19). The relationship between the initial concentration of bacteria in the enclosures and clearance is shown in figure 20. Clearance by *C.lampa*, *C.laticavicola* as well as *C.vermifera* is significantly correlated with the initial concentration of bacteria (ANOVA, $P < 0.01$, $P < 0.02$ and $P < 0.05$ respectively). See also Appendix, figure 9. There appears to be no functional relationship between sponge biomass and clearance of (cyano)bacteria (Fig. 21a). There also appears to be no correlation between clearance and the total biomass of rubble associated organisms (Fig. 21b).
A summary of the results and statistics is given in the Appendix, table 7.

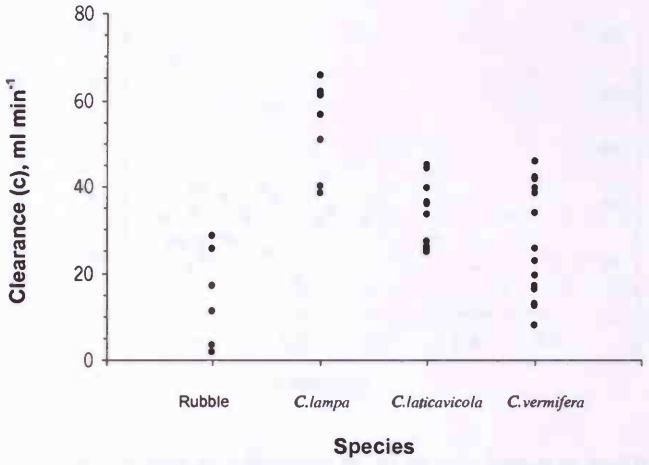

Fig. 17 Clearance of ambient bacteria during the first 15 minutes of the experiments per category; bare rubble and rubble infested with respectively *C.lampa*, *C.laticavicola* and *C.vermifera*.

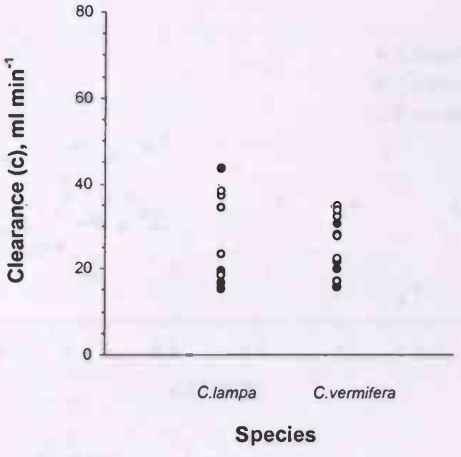

Fig.18. Clearance by *C.lampa* and *C.vermifera* of naturally occurring bacteria and cyanobacteria that were added to the enclosures on t = 0. Densities from ambient bacteria as well as added cyanobacteria were established from the same experiment (respectively 10/3 and 24/02/99).

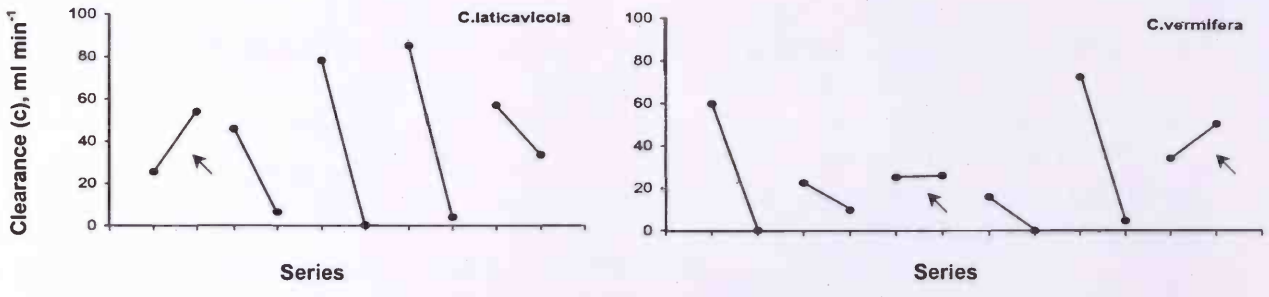

Fig. 19 Clearance of ambient bacteria by *C.laticavicola* and *C.lampa* on t = 7.5 (left points) and t = 15 (right), per experimental series. Arrows denote experiments where clearance on t = 15 is higher than on t = 7.5.

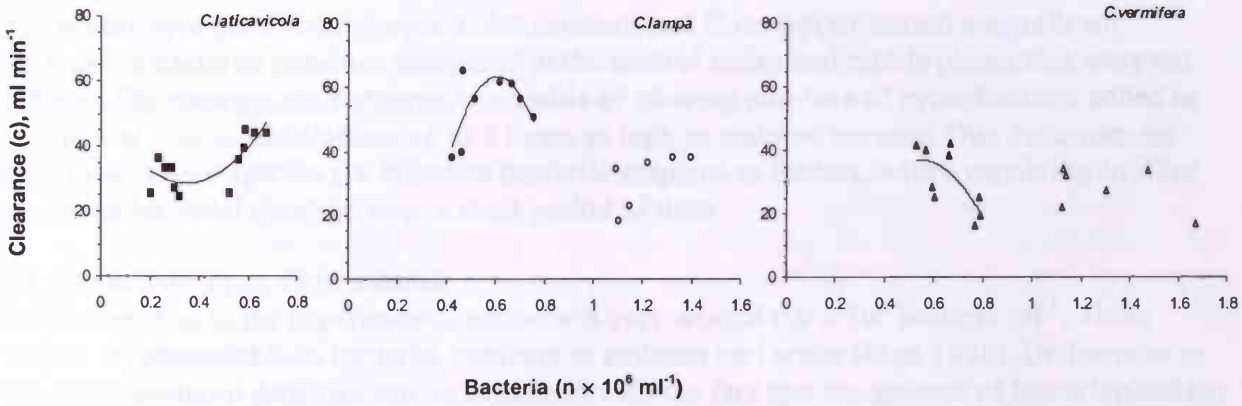

Fig.20 *C.laticavicola, C.lampa* and *C.vermifera*. Clearance as a function of initial bacterial concentration. Filled symbols represent clearance of ambient bacteria, open symbols represent clearance of cyanobacteria that were added to the seawater. Polynomial regression lines are fitted through the data fom ambient bacteria only.

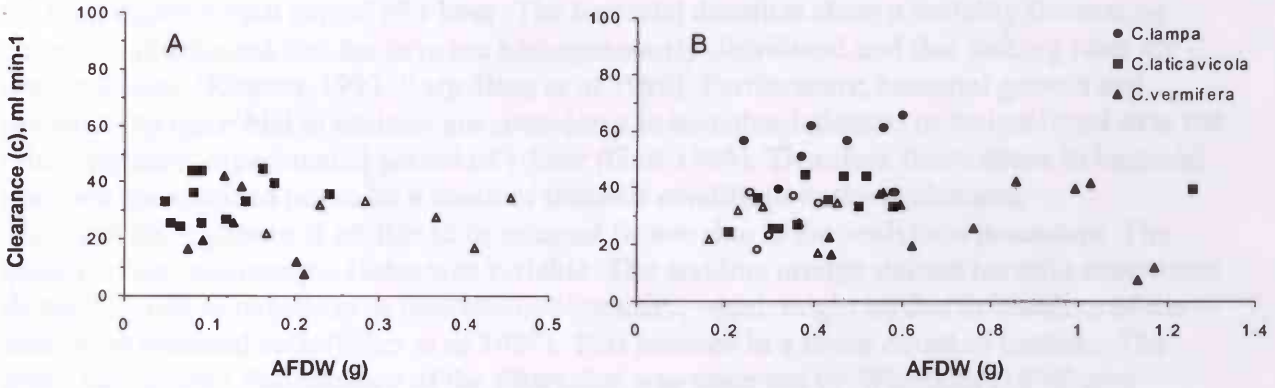

Fig. 21 Clearance as a function of a) sponge biomass and b) biomass of rubble associated organisms (represented as ash free dry weight). Filled symbols represent clearance of ambient bacteria, open symbols represent clearance of cyanobacteria that were added to the sea water.

4. DISCUSSION

The boring sponges *Cliona lampa*, *C.laticavicola* and *C.vermifera* caused a significant decrease in bacterial densities compared to the control series and rubble pieces that were not infested.The sponges are furthermore capable of clearing numbers of cyanobacteria added to the sea water in concentrations up to 3 times as high as ambient bacteria.This demonstrates that these clionid species are effective bacterial suspension feeders, with a capability to filter increased bacterial numbers over a short period of time.

4.1. EXPERIMENTAL PROCEDURE

Initial densities in the experimental enclosures vary around 0.6×10^6 bacteria ml^{-1}. These values are comparable to bacterial numbers in ambient reef water (Gast 1998). Differences in the initial bacterial densities can be explained with the fact that the amount of bacterioplankton in the watercolumn varies within and between days (Gast 1998, Moriarty *et al* 1985, Pile 1997). Furthermore on several occasions closing of the cylinders could not instantly be followed by sampling, allowing the sponges to filtrate bacteria from the enclosed water in the meanwhile. This could result in relatively low numbers of bacteria on $t = 0$, which are not representative of the densities in ambient reef water.
Densities in control conditions did not stay constant, but slighty increased or decreased over the total experimental period of 1 hour. The bacterial densities show a variably fluctuating pattern. It is assumed that bacteria are homogeneously distributed and that sinking rates are not significant (Kiørboe 1993, Karp-Boss *et al* 1996). Furthermore, bacterial growth and predation by microbial organisms are considered to be either balanced or insignificant over the relatively short experimental period of 1 hour (Gast 1998). Therefore fluctuations in bacterial numbers are assumed not to be a result of intrinsic conditions in the enclosures.
The fluctuating pattern is attributed to external factors due to the analytical procedure. The quality of the microscopic slides was variable. The acridine orange stained bacteria sometimes showed a marked reduction in fluorescence intensity, which might be due to bleeding of the stain from bacterial cells (Sherr *et al* 1987). This resulted in a lower count of bacteria. The green background fluorescence of the filters that was observed by Wiegman (1996) also complicated several of our countings. Furthermore some batches of Nucleopore filters are partially hydrophobic (Hobbie *et al* 1977), which results in a more patchy distribution of bacteria on the filter.
Sample errors prior to analysis might also have an influence on the bacterial numbers. Ambient water is potentially able to move by the rubber seal inside the syringe to compensate for compression of small gas bubbles within the syringe neck during descent. The action of drawing back the piston during sample collection also might allow amient water to enter the syringe (Reiswig 1971b). Such occurrences are expected to result in a slightly higher count of bacteria.

The highly diverse fauna that we found associated with coral rubble includes suspension feeders such as polychaetes, bryozoans and tunicates. This corresponds with earlier descriptions of rubble-associated epifauna (Choi 1984, Choi & Ginsburg 1983, Meesters *et al* 1990). Hardly any data are available on the composition of the endocryptolithic fauna. Hutchings & Weate (1978) extracted endolithic organisms from dead coral samples and found that the major part is formed by polychaetes and amphipods. We also found large quantities of worms, ranging from 3 to 56 specimens per rubble piece. Amphipods were scarcely encountered. Boring bivalves were furthermore observed in small quantities. Although we largely removed fouling fauna such as bryozoans from the rubble, it was not possible to remove all epilithic and endolithic organisms without damaging the sponges.

Experiments that were carried out with 'bare' rubble show that the uptake rates of rubble associated organisms other then sponges are minimal. The decrease in bacterial numbers caused by these organisms is negligable and not significantly different from the control series. Therefore we assume that they are not of any substantial influence to the net bacterial uptake in the enclosures.

4.2. INFLUENCES ON FILTER FEEDING

Withstanding the fluctuations in bacterial counts is a general trend of decreasing bacterial densities in the enclosures with boring sponges. The bacterial numbers in the final samples are significantly lower than the initial densities. In general the uptake rates of the *C.lampa*, *C.laticavicola* and *C.vermifera* were highest during the first 15 minutes of the experiments and subsequently decreased.

This corresponds to observations on behaviour of their excurrent papillae. The oscula of sponges that were enclosed in experimental cylinders contracted after 10 to 15 minutes. The only times we observed such behaviour in sponges under natural circumstances was when the oscula were touched. It is known that sponges are sensitive to laboratory conditions (Riisgård *et al* 1993). Attempts made by Reiswig (1974) to maintain sponges in a lab seawater running system show that their activity was reduced by at least 70%. Specimens of *C.laticavicola* and *C.vermifera* that were kept in an aquarium with water running at a minimal velocity contracted their oscula after several days, but seemed to recover again after the velocity of the water flow was increased (pers. obs.).

Sponges are combined passive-active suspension feeders. They are capable of actively inducing a water flow by use of their choanocyte flagellates and at the same time passively make use of ambient velocity induced flow to obtain a constant current of fresh water from which they filter food particles (Waller 1996, Wildish & Kristmanson 1997). However, passive current-induced filtration is considered to be relatively unimportant in sponges (Riisgård *et al* 1993). We assume that the contraction of the oscula was not triggered by a lack of current, since the same behaviour was observed in enclosures equiped with stirring devices simulating ambient flow.

Ribes *et al* (1999) found a drop in the oxygen concentration of experimental enclosures with *Dysidea avara*. The decrease in oxygen concentrations was never above 15% of the initial values and Ribes *et al* therefore considered it not to affect the respiration rate and feeding behaviour of the sponge. Since no data are available on the energetics of clionid sponges, we converted values found by Reiswig (1974) for oxygen consumption in the tropical demosponges *Mycale* sp. (order Ceractinomorpha) and *Tethya crypta* (Hadromerida). These species respectively filter 19.6 and 22.8 liter water for every ml of O_2 that is consumed. *Mycale* has an oxygen consumption of 1.49 ml O_2 h^{-1}, whereas *Tethya* consumes 0.44 ml O_2 h^{-1} per gram dry organic tissue. When these values are reflected on clionid species with an average biomass of 0.2 g dry organic tissue, this implicates that the oxygen supply in an enclosure with a volume of 857 ml whould allow the sponges to filtrate for respectively 9 to 26 minutes. Assuming that the values for clionid sponges are within this range, oxygen deficiency is a likely factor to have induced the contraction of oscula and a subsequent decrease in filtration rates after 10 to 15 minutes.

Water pumping activity of sponges is variable and most likely species dependent (Reiswig 1971a, Pile 1997). We therefore acknowledge that it is arbitrary to draw conclusions by implementing respiration rates of one species on another. On the basis of our estimates however, it seems likely that oxygen depletion does have an influence on the filtering behaviour of sponges in enclosures.

4.3. CLEARANCE RATES

Another factor which possibly influenced the behaviour of the sponges is bacteria depletion. The bacterial densities that are reached after c.a. 15 minutes might represent a plateau beneath which it is no longer energetically favorable for the sponges to continue filtering at a high rate. The difference in decrease of bacterial numbers between the three sponge species is linearly correlated to the initial bacterial density (Fig. 14). If the densities at the plateau are limiting and clearance rates of the sponges stay constant, a linear regression is expected to result in a horizontal line. In other words, final bacterial numbers should be independent of the initial densities. Our data show that higher initial densities result in higher bacterial numbers at termination of the experiments. This indicates that either an external factor such as oxygen deficiency is limiting the filtration of the sponges or clearance rates are not constant in time. Sponges with a low clearance rate are expected to reach the plateau after a longer period of time than sponges with higher clearance rates. This applies to *C.vermifera*, which on average has the lowest clearance rate and generally reaches the plateau at a later point than the other species. The linear correlation between initial and final bacterial densities is relatively weak in this species, whereas it is significantly strong in *C.laticavicola*. Final densities of naturally occurring bacteria found in *C.lampa*, *C.laticavicola* and *C.vermifera* on average fluctuate around 0.2 to 0.3 × 10^6 cells ml^{-1}. Values for individual specimens of *C.laticavicola* show that they are capable of filtering at bacterial densities down to 0.1 × 10^6 cells ml^{-1}. Clearance rates are relatively high at these low densities, compared to clearance of high initial densities by *C.vermifera* (Fig. 20).

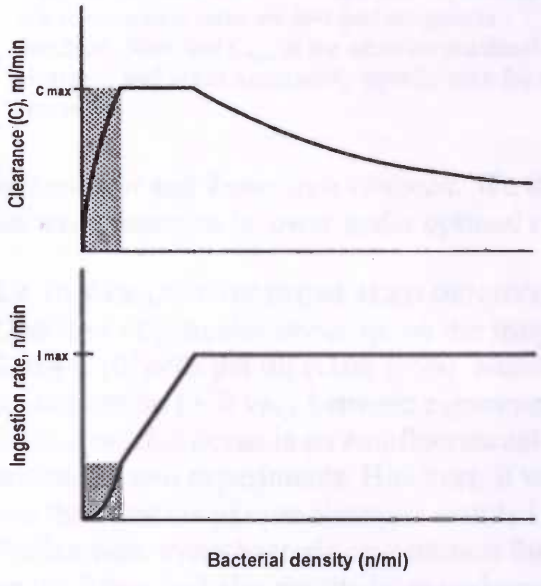

Fig. 22. Relationship between bacterial density, ingestion rate and clearance rates. At the plateau a post-capture step, problably ingestion rate, becomes limiting. Gray shading indicates very low concentrations at which functional forms are not well established. C_{max} = maximal clearance rate. I_{max} = maximal ingestion rate. Adapted from Jumars (1993).

The correlation between initial density and clearance is negative in *C.vermifera* (Appendix, Fig. 9). According to Jumars (1993), clearance in active suspension feeders generally increases with particle abundance, up to a plateau at which particle availability no longer limits the ingestion rate. The volume of water that is required to provide the supply of food particles continues to drop with food concentration, thus reducing the costs of filtering and increasing net gains (Fig. 22). If C_{max} and I_{max} in *C.vermifera* are reached at bacterial densities below the ambient average of 0.6 × 10^6 cells ml^{-1}, an increase in densities leads to a decrease in clearance. This whould explain the negative correlation we observed in *C.vermifera*.

When the clearance rates from the experimental species are plotted according to Jumars, it becomes clear that *C.vermifera* reaches C_{max} at bacterial densities ≤ 0.6 × 10^6 cells ml^{-1} (Fig. 23). This allows the species to increase its net gains when bacterial densities become higher than the daily average. Furtermore, the relationship described by Jumars whould explain the relatively high clearance rates that were observed in *C.lampa*, assuming that this species reaches C_{max} at densities ≥ 0.6 × 10^6 cells ml^{-1}. Clearance rates of *C.laticavicola* are significantly higher on t = 7.5 than on t = 15, indicating that the filtration behaviour of this sponge is already limited before or on t = 15. The relatively high clearance

rates of *C.laticavicola* at initial densities between 0.2 and 0.4 × 10^6 cells ml^{-1} are explained by the fact that with such low densities the chance of encountering particles is relatively low and the volume of water per unit time that is required to provide a minimal food supply is consistantly high. This results in relatively high costs of filtration and a low net gain.

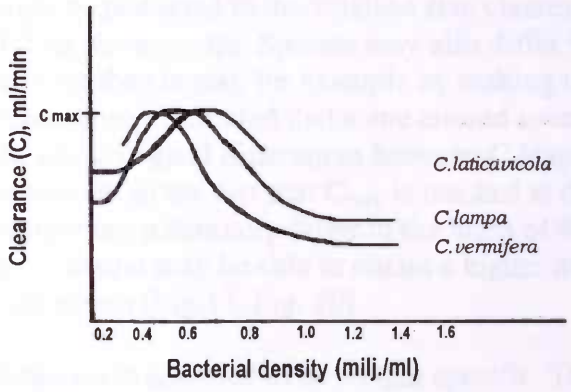

Fig. 23. Hypothetical relationship between bacterial density and clearance rates in *C.lampa*, *C.laticavicola* and *C.vermifera*, on the basis of observed clearance rates in relation with initial bacterial densities in experimental enclosures. Densities of added cyanobacteria are located in right tails of the curves, where clearance rates are low and net gain is maximal. Note that C_{max} is the *absolute* maximal clearance and is not necessarily equally high for each species.

Final densities of cyanobacteria that were added to the enclosures also approach the levels of the plateau that was observed in ambient bacteria. On the basis of these findings we assume bacteria depletion to be the factor wich limits the filtration rates of the sponges rather than oxygen deficiency.

It should be noted that we do not exclude oxygen deficiency as a factor which potentially had a limiting effect on the behaviour of the sponges. The experimental situation potentially effected the hight of the limiting plateau. The plateau in *C.lampa*, *C.laticavicola* and *C.vermifera* was reached around 0.2 to 0.3 × 10^6 bacteria ml^{-1}. Gast (1998) observed a decrease in densities down to 0.02 × 10^6 bacteria ml^{-1} on the reefs of Curaçao. Sorokin (1993) observed a decrease from 0.9 to 0.02 × 10^6 bacteria ml^{-1} in the tropical sponges *Halichordia melandovia* and *Toxadicea violacea*. We therefore find it likely that the plateau in final bacterial densities is lower under optimal circumstances.

4.4. CLEARANCE OF INCREASED DENSITIES

Densities of *Synechococcus* sp. on the fringing reef of Curaçao vary between 0.002 and 0.014 × 10^6 cells per ml (Gast 1998). Numbers of *Synechococcus* that were added to the enclosures on t = 0 vary between experiments and range from 0.5 to 1.7 × 10^6 bacteria ml^{-1}. Since *Synechococcus* is an autofluorescent cyanobacteria, staining with acridine orange was omitted in two experiments. However, it was found that their autofluorescence fades with time and the numbers of cyanobacteria counted on 13/4 and 28/4 are therefore underestimates. Furthermore cyanobacteria on occasion formed aggregates. This leads to a patchy distribution on the filters and also results in an underestimation of the densities.
C.lampa, *C.laticavicola* as well as *C.vermifera* efficiently cleared the increased numbers of cyanobacteria. The experiments were the densities of ambient bacteria as well as added cyanobacteria were established (respectively 10/3 and 24/02/99) show that *C.lampa* and *C.vermifera* generally cleared cyanobacteria at a higher rate than naturally occurring bacteria. This might indicate a higher retention efficiency for picoplankton such as *Synechococcus*. Gast (1998) indicated a consistant specific removal of *Synechococcus* from the reef water column and suggested sponges as likely candidates to cause such a removal. Ribes (1999) found a higher clearance rate for picoplankton than for nanoplankton in *Dysidea avara* and suggested a higher retention efficiency for picoplankton. Our data suggest that this potentially also applies to *C.lampa* and *C.vermifera*.

4.5. DIFFERENCES BETWEEN THE SPECIES

Differences between the species are a reflection of physiological features. Variations in ostia size, length and complexity of the aquiferous system and the size of choanocyte chambers are directly related to filtering efficiencies of sponges (Wilkinson 1978). Reiswig (1971b) furthermore presumed that sponge anatomy reflects size selectivity of particles. Some species might be restricted to the smallest size classes of particles (0.1-1 µm) due to the organisation of their choanocytes. Species may also differ in their capability to gain energy from the food particles they ingest, for example by making use of specific digestive enzymes. Reiswig (pers.comm.) indicated that some clionid sponges indeed have this capacity.
The physiological differences between *C.lampa*, *C.laticavicola* and *C.vermifera* may manifest themselves in the fact that C_{max} is reached at different bacterial densities (Fig. 23). The species furthermore potentially differ in the hight of their maximal clearance. Our findings indicate that *C.lampa* may be able to obtain a higher average clearance than *C.laticavicola* and *C.vermifera* (Fig.17, Fig. 20).

Clearance is assumed to be weight specific. The correlation appears to decrease with larger and older sponges, possibly as a result of fewer living choanocyte chambers per unit of colony volume (Riisgård 1993). The species we studied were all within the same small size range and we therefore expect the density of living choanocytes to be uniform. However we found no significant correlation between clearance and biomass (expressed as AFDW) of the species. Also no correlation was found with the biomass of the total epi- and endolithic rubble associated community. This might be explained by the fact that the amount of organic tissue associated with rubble is relatively small (0.4 to 0.7g total AFDW). Variation between the rubble pieces is high and as a result standard deviations of the total AFDW are high, which makes it difficult to compare the values.
According to Reiswig (1971b), differences in retention rates are caused by variations in the anatomy of the sponges. The differences in the architecture of the sponges that account for the differences between their clearance rates may not be readily detectable by comparing their biomass.

4.7. GENERAL DISCUSSION AND CONCLUSIONS

The relevance of clionids for the coral reef ecosystem is two-sided. When we assume that mean feeding rates of the clionids are comparable to those found by Bak *et al* (1998) for the colonial ascidian *Trididemnum solidum*, the amounts of nitrogen uptake derived from bacteria by the sponges whould probably be similarly high. Bak *et al* calculated a nitrogen uptake rate for *T.solidum* of 4.0 nmol cm^{-2} h^{-1} and concluded that this is a significant ammount, compared to the uptake rates of several coral species. Under natural circumstances the sponges will not be inhibited by oxygen deficiency or depletion of bacteria. We thus expect that their feeding rates per unit of time in their natural habitat will be even higher than under experimental circumstances. This underlines the ecological importance of the sponges in terms of nutrient uptake.
Boring sponges furthermore play an important ecological role as the principal filterfeeding bioeroders on coral reefs (Bak 1976, Hein & Risk 1975, Kiene 1985, MacGeachy & Stearn 1976, Neumann 1966, Risk & Sammarco 1982, Risk *et al* 1995, Rützler 1975, Scoffin *et al* 1980). The plasticity in filtration behaviour we observed in *C.lampa*, *C.laticavicola* and *C.vermifera* enables them to optimally profit from increasing baterial densities. If this behaviour is consistant in time and food is not limiting, this allows the sponges to invest their energy in either competition, reproduction or growth. The potential capability of some clionids to produce digestive enzymes at a high rate, together with suitable defences against attack by specific bacteria (H.M. Reiswig, pers. comm.) makes them likely candidates to profit from

increased baterial densities due to eutrophication, allowing them to extend their niche in the highly competitive community of the coral reef. It appears that this is indeed the matter. Clionid infestation of coral reefs is becoming an increasing global phenomenon and the evidence that this is related to eutrophication is rising (see Table 1).
The limited amount of literature that is available on the areal abundance of boring sponges and the fact that excavation rates are expressed differently per author makes it difficult to determine what the effect of increased clionid infestation on the coral reef will be in terms of carbonate balance (Table 5). It should also be kept in mind that boring rates are not constant in time, but vary depending on factors such as maturity of the organism, substrate limitations, competition for food and space, light levels, currents and temperature (Hutchings 1986, MacGeachy 1977, Rützler 1975, Scoffin *et al* 1980). Several authors have furthermore suggested that boring rates are highest during the initial invasion of new substratum and subsequently decrease (Bak 1976, Neuman 1966, Rützler 1975).

Table 5. Summary of studies where excavation rates of clionids are calculated. Excavated substrate can be either coral, conch shell slabs or Island spar blocks. Note different units of measurement.

Species	Excavation rate year^{-1}	Site	Source
C.delitrix	2.9 g per cm^2 of papillar tissue	Grand Cayman reef	Rose & Risk (1985)
	86.6 g m^{-2} per minimally 4.0 g sponge biomass m^{-2}	Eutrophied site	
	19.0 g m^{-2} per 0.9 g sponge biomass m^{-2}	Non-eutrophied site	
C.lampa	14 mm (initial rates)	Bermuda	Neumann (1966)
	0.22 ×10^6 g m^{-2} (total sponge population)		
	14 - 67 mm (initial rates)		Hudson (1977)
C.lampa +	0.7 g m^{-2} per cm^2 of sponge (optimum long-term rates)	Bermuda	Rützler (1975)
C.aprica	256 g m^{-2} (total sponge population)	Belize	
	0.03 ×10^6 g m^{-2} (areas of particularly high densities)		
C.laticavicola	5.1 - 17.2 g (sponge biomass up to 42 g m^{-1})	Curaçao	De Groot & De Ruyter van Steveninck (1980)
C.peponaca	3 ×10^6 g m^{-2}	Curaçao	Bak (1976)
Cliona sp.	0.42 - 8.66 g (2.5 - 3 years)	Lizard Island, Australia	Kiene (1985)
	24.9 ×10^6 g (whole reef)	Barbados	Scoffin *et al* (1980)
	746 - 4303 mm per coral head	Florida	Hein & Risk (1975)
	12.3% of total coral framework		
	2.5 - 23.0% of *Montastrea annularis* framework	Barbados	MacGeachy (1977)
	0.0 - 22.4% of *Porites astreoides* framework		
	1.1 - 18.9% of *Sidastrea siderea* framework		

De Groot & De Ruyter van Steveninck (1980) estimated that sponge excavation rates (42×10^6 g year^{-1}) do not exceed reef accretion (64×10^6 g year^{-1}). Scoffin *et al* (1980) estimated bioerosion rates of 123×10^6 g year^{-1} and a reef accretion of 206×10^6 g year^{-1}. Hein and Risk (1975) however, found that excavation rates of clionid sponges combined with those of other bioeroders such as spionid polychaetes and mytillid mollusks exceed the rate of coral skeletogenesis. They argued that bioerosion cannot consistantly exceed reef growth and ascibed their findings to the fact that their number of samples was very small. Hein and Risk therefore concluded that the rates of bioerosion and reef accretion possibly are in the same order of magnitude. Bak (1976) found that erosion rates are very small compared to the carbonate production of the coral itself, but that the balance tends to be slightly in favour of the destructional forces. Bak furthermore stated that in many cases enlargement of the coral colony results in a relative weakening of its base. If the coral base is not strengthened by processes such as epigrowth by crustose coralline algae, even a minimum influence of boring sponges will be an important factor.
It can be concluded that under most circumstances sponge excavations do not result in a net degradation of the reef, but in combination with the activity of other bioeroding organisms the balance between erosion and accretion is probably tight. Several authors have therefore suggested that proliferation of the boring sponge community will cause a shift in the carbonate balance and may result in a net degradation of the coral reef (e.g. Hutchings 1986, Rose &

Risk 1985, Sammarco & Risk 1990, Stearn & Scoffin 1977). This emphasizes the fact that although boring sponges are inconspicuous members of the reef community, their role should not be underestimated.

In summary, we showed that *C.lampa*, *C.laticavicola* as well as *C.vermifera* are effective bacterial suspension feeders, with the capability to adapt their feeding strategies to changing densities of food particles over a short period of time. The species are capable of efficient filtration of enhanced bacterial densities. Considering their responsiveness to changing bacterial densities in combination with the destructive qualities these species have, we suggest that clionid sponges potentially form a strong link between changes in the water column microbial population and the global deterioration of coral reefs.

5. RECOMMANDATIONS FOR FURTHER RESEARCH

Several studies suggest a relationship between abundance of clionid sponges and nutrient levels (see Table 1). The evidence that a functional relationship exists between increasing bacterial numbers and infestation by boring sponges is strong. However, this relationship may be explained in two different causal ways.
Type 3 corals, such as *Porites porites*, relay on fragmentation as their primary mode of dispersal. Fragmentation of type 3 corals may increase under eutrophied circumstances and encourage infestment of clionid sponges. In this case the cause of the observed increase in abundance of boring sponges is the increase in available substrate. On the other hand, fragmentation may be caused by an increase in bioeroders such as sponges (Holmes 1997, see references therein).
No research has been done to establish the direct relationship between increased bacterial densities and the long-term effects on clionids and/or other species of boring sponges (e.g. several species of Spirastrellidae and Adociidae; Pang 1973, Rützler 1974). To gain more insight in how this relationship works, long term experiments should be performed where changes in growth and excavation rates of sponges in pristine environments are compared to eutrofied situations. It whould also be interesting to compare present densities of boring sponges with those found by Rose and Risk (1985), to see if the population on the eutrophied site has persisted through time, or if changes in the composition of the community have taken place.
To further test the capacity of clionid sponges to adapt their feeding strategies to changing bacterial densities in an experimental set up, it is recommanded to add bacteria in concentrations that are comparable to high-nutrient situations. Rose and Risk (1985) for example, found bacterial densities of 1.7×10^6 ml^{-1} under eutrophied circumstances. Gast (1998) found bacterial numbers to increase to over 4×10^6 ml^{-1} up to 7×10^6 ml^{-1} under eutrophied circumstances in combination with a reduced mixing of the water column.
For statistical reasons it is furthermore recommanded to perform a number of experiments with the same individual sponge, to eliminate variation that is due to individual differences between specimens.

To resolve unknown effects of refiltration in a limited volume of enclosed water such as the contraction of the excurrent papillae and plateau in bacterial densities we observed, data are needed on the pumping rates and oxygen consumption of the experimental organism. Another option to determine which factor inhibits the filtration behaviour of the sponges after a certain period of time, is to add bacteria at the moment the plateau is reached. If the sponges resume their activity, bacterial densities can be interpreted as the inhibiting factor.
To obtain data on the maximal clearance rates of clionid sponges, it is recommanded to perform experiments under the most optimal circumstances. It might be necessary to aerate the water in the enclosures. Another option is to obtain samples under natural circumstances. Earlier research on particle feeding in demosponges was conducted by collecting samples from the in- and excurrent stream below the margins of respectively the ostia and oscula (Reiswig 1971b). The disadvantage of this method is that a biass occurs, because the samples are slightly contaminated with ambient water that is sucked into the syringe during sample taking. Another disadvantage is that it is not possible to add quantities of bacteria to establish the effect of increased densities. When the possibility of a biass is kept in mind, this method could be used to make a comparison with clearance rates obtained from experiments in enclosures.

Our data demonstrate that clearance rates vary with bacterial density. Comparisons between the weight specific clearance (C_{AFDW}) of individual sponges within a species can therefore only be made between experiments with the same initial bacterial density. To compare C_{AFDW} of different species it is also necessary to obtain clearance rates under circumstances that are equal for each species.
Furthermore a detailed study with a large N is needed to find out whether differences in the anatomy of the sponges that account for the differences between their clearance rates can be detected by comparing their biomass.

Although acridine orange staining is commonly used for direct counts (Bak 1998, Gast 1998, Hobbie *et al* 1977, Mortiarty 1979, Moriarty *et al* 1985, Watson *et al* 1977), it should be kept in mind that this method results in an underestimation of the actual bacterial numbers present.Wiegman (1996) observed significant differences in bacterial numbers between DAPI- and acridine orange staining.To eliminate biasses in bacterial counts due to the analytical procedure, the staining of the filters should be optimal. To prevent bleeding of acridine orange from the bacterial cells, staining should occur only shortly in advance of counting.
Hydrophobic parts of Nucleopore filters (Hobbie *et al* 1977) can be recognised as light coloured spots after staining in Sudan Black (pers.obs.). It goes without saying that such filters should not be used. Moreover it is advised to stain cyanobacteria or to count them immediately after sampling, since their autofluorescence fades within relatively little time.

C.lampa is commonly known as 'red paint encrusting sponge' (Gammill 1997). According to the description of *C.lampa* on Bermuda (Rützler 1974), the Bahamas, Caribbean and Florida (Gammill 1997), the sponge owes its name to the fact that it encrusts its substrate with a continuous layer of tissue. However Pang (1973) observed that the Jamaican specimens of *C.lampa* do not tend to encrust their substrate. The specimens we observed on Curaçao did not exhibit any tendancy to encrust their substrate either. Rützler (1974) refers to the term 'forma' to distinguish between the different types of growth.
However, it is not clear what the status of this 'forma' is. Except for the color and type of growth, there are no morphological differences between the two forma of *C.lampa*. The species belongs to a complex of species that occur all around the tropics and also in some temperate seas. They are all red and have the same set of spicula. The occurrence of forma *occulta* exclusively, on Curaçao (pers. obs.) and Jamaica (Pang 1973) might indicate that this is a separate species. The term subspecies is commonly used to distinguish species that are geographically separated. The fact that both *C.lampa* forma *lampa* as well as forma *occulta* occur on Bermuda (Rützler 1975) rules out the possibility that they are subspecies. Genetical research is needed to solve this puzzle (R.W.M.van Soest, pers.comm.).
The difference between the excavating- or encrusting stage of Clionids is partly caused by the age of the sponge. During the first stages of infestment, the sponges excavate a system of canals and chambers before growing in- and excurrent papillae. Whether or not the sponges expand their tissue on the surface and encrust their substrate during later stages of their life cycle, is probably a matter of energy budget and competition strategies (R.W.M. van Soest, pers. comm.). The encrusting habit has a competitive advantage, which is demonstrated by the large areas of substrate these sponges can occupy (Rützler 1975) More research is needed to find out how the different (clionid) sponges compete in order to maintain their niche.

Although boring sponges are classified as cryptic organisms, they do not occur in specificly cryptic habitats such as cavities and crevices (pers.obs.). This is probably due to the presence of symbiothic zooxanthellae in the tissue of the sponges. It is known that sponges which bear zooxanthellae favor substrate that is exposed to ambient light (Pang 1973). Zooxanthellae

have been observed in *C.aprica*, *C.caribbaea*, *C.langae*, and *C.varians* (Pang 1973, Rützler 1985). Hill (1996) found that absolute boring- and growth rates of *Anthosigmella varians* were significantly higher in sponges with higher zooxanthellae densities. Since the presence of zooxanthellae symbionts has important ecological and life-history consequences for host sponges (Hill 1996), it is recommanded to gain further insight in the proportion of clionid species that bear zooxanthellae.

Sponges are not the initial eroders of newly available coral substrate (Hutchings 1986). Newly available substrates are rather colonized by bacteria, algae, fungi, foraminifers, bivalves and polychaetes (Choi 1984, Davies & Hutchings 1983, Risk & MacGeachy 1978). Boring sponges are found not to invade the substrate until after 2 - 3 years (Kiene 1985). Apparently they depend on other organisms to colonise the substrate and make it suitable for the sponges to invade.
Considering the apparent dependance of boring sponges on other organisms to work the substrate, it is important to gain insight in how suspension feeding bioeroders such as polychaetes react to changes in water quality. Points that also deserve further attention are the interaction between the different bioeroders on the reef and how the species composition changes under influence of eutrophication.

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REFERENCES

Bak RPM (1976) The growth of coral colonies and the importance of crustose coralline algae and burrowing sponges in relation with carbonate accumulation. Neth J Sea Res10:285-337

Bak RPM, Joenje M, de Jong I, Lambrechts DYM, Nieuwland G (1998) Bacterial suspension feeding by coral reef benthic organisms. Mar Ecol Prog Ser 175:285-288

Boury-Esnault N, Rützler K (1997) Thesaurus of sponge morfology. Smithsonian contributions to zoology 595

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

Brown BE (1997) Disturbances to reefs in recent times. In: Birkeland C (ed) Life and death of coral reefs. Chapman & Hall, New York. pp 370-373

Brock RE, Smith SV (1983) Response of coral reef cryptofaunal communities to food and space. Coral reefs 1:179-183

Choi DR, Ginsburg RN (1983) Distribution of coelobites (cavity-dwellers) in coral rubble across the Florida reef tract. Coral Reefs 2:165-172

Coughlan J (1969) The estimation of filtering rate from the clearance of suspensions. Mar Biol 2:356-358

Cuet P, Naïm O (1992) Analyisis of a blatant reef flat degradation in La Réunion Island (l'Etang-Salé fringing reef). Proc 7th Int Coral Reef Symp 1: 313-322

De Groot RA (1980) Excavating rates of the two most common clionid sponges of Curaçao. Proc Assoc Is Mar Labs Carib 14th meeting, Abstract. Jamaican Univ West Indies

De Groot RA, De Ruyter van Steveninck (1980) A quantitative estimate of the extent and nature of reef surfaces off Curaçao. Msc thesis, Univ Groningen

Ducklow HW (1990) Biomass, production and fate of bacteria. In: Dubinsky Z (ed) Coral reefs, ecosystems of the world 25. Elsevier Science Publishers BV, Amsterdam. pp265-289

Ducklow HW, Carlson CA (1992) Oceanic bacterial production. Adv Microb Ecol 12:113-181

Gabric AJ, Bell PRF (1993) Review of the effects of non-point nutrient loading on coastal ecosystems. Aust J Mar Freshwat Res 44:261-238

Gammill ER (1997) Identification of coral reef sponges. Providence marine publishing, Inc. Tampa, Florida

Gast GJ (1998) Microbial densities and dynamics in fringing coral reef waters. PhD thesis, Univ Amsterdam

Hill MS (1996) Symbiothic zooxanthellae enhance boring and growth rates of the tropical sponge *Anthosigmella varians* forma *varians*. Mar Biol 125:649-654

Holme NA, McIntyre AD (1984) Methods for the study of marine benthos. IBP Handbook 16, Blackwell scientific publications, Oxford and Edinburgh p263-265

Holmes KE (1997) Eutrophication and its effect on bioeroding sponge communities. Proc 8th Int Coral Reef Sym 2:1411-1416

Hein FJ, Risk MJ (1975) Bioerosion of coral heads: Inner patch reefs, Florida reef tract. Bull Mar Sc 25:133-138

Highsmith RC (1980) Geographic patterns of coral bioerosion: a productivity hypothesis. J Exp Mar Biol Ecol 46:177-196

Hobbie JE, Daley RJ, Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl environ Microb 33:1225-1228

Holmes KE (1997) Eutrophication and its effect on bioeroding sponge communities. Proc 8th Int Coral Reef Sym 2:1411-1416

Hutchings PA (1986) Biological destruction of coral reefs - a review. Coral Reefs 4:239-252

Hutchings PA, Weate PB (1978) Comments on the technique of acid dissolution of coral rock to extract endo-cryptolithic fauna. Aust Zool 19:315-320

Jackson JBC, Winston JE (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

Johnson PW, Sieburth J McN (1979) Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. Limnol Oceanogr 24: 928-935

Jumars PA (1993) Concepts in biological oceanography. Oxford Univ Press, Oxford. pp 52-53

Karp-Boss L, Boss E, Jumars PA (1996) Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. Oceanogr Mar Biol Annu Rev 34:71-107

Kiene WE (1985) Biological destruction of experimental coral substrates at Lizard Island (Great Barrier Reef, Australia) Proc 5th Int Coral Reef Cong 5:339-344

Kiørboe T (1993) Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Adv Mar Biol 29:1-72

MacGeachy JK (1977) Factors controlling sponge boring in Barbados reef corals. Proc 3rd Int Coral Reef Sym 477-483

MacGeachy JK, Stearn CW (1976) Boring by macro-organisms in the coral *Montastrea annularis* on Barbados reefs. Int Revue ges Hydrobiol 61:715-745

Meesters E, Knijn R, Willemsen P, Pennartz R, Roebers G, van Soest RWM (1991) Sub-rubble communities of Curaçao and Bonaire coral reefs. Coral reefs 10:189-197

Moriarty DJW (1979) Biomass of suspended bacteria over coral reefs. Mar Biol 53: 193-200.

Moriarty DJW, Pollard PC, Hunt WG (1985) Temporal and spatial variation in bacterial production in the water column over a coral reef. Mar Biol 85:285-292

Neumann AC (1966) Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge, *Cliona lampa*. Limnol Oceanogr 11:92-108

Pang RK (1973) The systematics of some Jamaican excavating sponges (Porifera). Postilla 61. Peabody Museum of Natural History, Yale University

Pastorok RA, Bilyard GR (1985) Effects of sewage pollution on coral-reef communities. Mar Ecol Prog Ser 21:175-189

Pile AJ (1997) Finding Reiswig`s missing carbon: quantification of sponge feeding using dual-beam flow cytometry. Proc 8th Int Coral Reef Sym 2:1403-1410

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

Pile AJ, Savarese M, Chernykh VI, Fialkov VA (1997) Trophic effects of sponge feeding within Lake Baikal`s littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnol Oceanogr 24:178-184

Pomponi SA (1980) Cytological mechanisms of calcium carbonate excavation by boring sponges. Int Rev Cyt 65:301-319

Reiswig HM (1971a) In situ pumping activities of tropical Demospongiae. Mar Biol 9:38-50

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

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

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

Ribes M, Coma R, Gili J-M (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

Risk MJ, MacGeachy JK (1978) Aspects of bioerosion of modern Caribbean reefs. Rev Biol Trop 26: 85-105

Risk MJ, Sammarco PW (1982) Bioerosion of corals and the influence of damselfish territoriality: a preliminary study. Oecologia 52:376-380

Risk MJ, Sammarco PW, Edinger EN (1995) Bioerosion in *Acropora* across the continental shelf of the Great barrier Reef. Coral Reefs 14:79-86

Rose CS, Risk MJ (1985) Increase in *Cliona delitrix* infestation of *Montastrea cavernosa* heads on an organically polluted portion of the Grand Cayman fringing reef. Mar Ecol 6:345-363

Rützler K (1974) The burrowing sponges of Bermuda. Smithsonian contributions to zoology no 165: 1-32

Rützler K (1975) The role of burrowing sponges in bioerosion. Oecologia 19:203-216

Rützler K (1985) Associations between caribbean sponges and photosynthetic organisms. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Inst Press, Washington DC, pp 455-466

Scoffin TP Stearn CW, Boucher D, Frydl P, Hawkins CM, Hunter IG, MacGeachy JK (1980) Calcium carbonate budget of afringing reef on the west coast of Barbados. Part II-erosion, sediments and internal structures. Bull Mar Science 30:475-508

Sebens KP (1994) Biodiversity of coral reefs: What are we losing and why? Am Zool 34:115-133

Sherr BF, Sherr EB, Fallon RD (1987) Use of monodispersed Fluorescent Labeled Bacteria to estimate in situ protozoan bacterivory. Appl Environ Microbiol 53:958-965

Sorokin YI (1993) Bacterio plankton. In: Coral Reef Ecology–Ecological studies 102. Springer-Verlag, New York, p83-89

Stearn CW, Scoffin TP (1977) Carbonate budget of a fringing reef, Barbados. Proc 3rd Int Coral Reef Symp 2:471-476

Vasseur P (1974) The overhangs, tunnels and dark reef galleries of Tuléar (Madagascar) and their sessile invertebrate communities. Proc. 2nd Int Coral Reef Symp 2:143-159

Vasseur P (1977) Cryptic sessile communities in various coral formations on reef flats in the vicinity of Tuléar (Madagascar). Proc 3rd Int Coral Reef Symp 1:95-100

Waller G (1996) Sealife. A complete guide to the marine environment. Pica Press, Sussex. pp 135-136

Watson SW, Novitsky TJ, Quinby HL, Valois FW (1977) Determination of bacterial number and biomass in the marine environment. App Environ Microb 33: 940-946

Wiegman S (1996) Ecologische aspecten in het koraalrifecosysteem langs een eutrofiëringsgradient op Curaçao. Aantallen microbiële organismen, bacterivory door heterotrofe flagellaten en diversiteit van boorsponzen op het koraalrif. Msc thesis, Univ Amsterdam.

Wildish D, Kristmanson D (1997) Flow and the physiology of filtration. In: Benthic suspension feeders and flow. Cambridge Univ Press, p129-169

Wilkinson CR (1978) Microbial associations in sponges. I. Ecology, physiology and microbialpopulations of coral reef sponges. Mar Biol 49:161-167

Wilkinson CR (1987) Interocean differences in size and nutrition of coral reef sponge populations. Science 236:1654-1657

Wilkinson CR, Cheshire AC (1990) Comparisons of sponge populations across the Barrier Reefs of Australia and Belize: evidence for higher productivity in the Caribbean. Mar Ecol Prog Ser 67:285-29

Wulff JL, Buss LW (1979) Do sponges help hold the reef together? Nature 281:474-475

APPENDIX

Fig.1. Rubble infested with *C.laticavicola*. Outside appearance with oscula and ostia contracted (top). Endolithic features (bottom). The large excavations are filled with sponge tissue. Also visible are several worms, among which sponge worms (*Haplosyllis sp.*), which feed on sponges by inserting their proboscis into individual cells (Reiswig 1973). Scale: 1:2.

Fig.2. Tile on the reef bottom near Buoy 1 where rubble infested with clionids was kept. Most of the rubble on this site exists of rod shaped pieces of *Porites porites* and other branching coral spieces. Picture taken by R.P.M.Bak.

Table 1. List of clionid infested rubble pieces that were chiseled to fit the experimental enclosures. Date of the experiment, experimental series and time given to the sponge to recover (respectvely in hours or days) are shown.

Species	Date exp.	Series	Recovery time
C.lampa	10/3/99	S1	48 h
C.laticavicola	17/3/99	S1	28 days
		S2	7 days
C.laticavicola	25/3/99	S4	48 h
C.vermifera	19/1/99	S1	48 h

Fig.3. Experimental enclosures on the reef near Buoy 1. On the bottom of the upper three enclosures pieces of rubble are visible. The cylinder on the left is an empty control enclosure. Sampling of enclosed water was done by inserting the needle of a 10cc syringe trough the grey rubber membrane. The volume of the cylinders is respectively 857 ml (all experimental enclosures with rubble) and 1077 ml (control series from 10/2 to 13/4). From 13/1 to 17/3 enclosures were placed vertically in a crate (see picture). From 25/3 to 28/4 enclosures were placed horizontally on the reef, to stimulate the circulation of fresh water during the 24 h the sponges were given to adjust. Picture taken by R.P.M.Bak.

Table 2. Ingredients of the A-medium and chemical substances that were used to respectively stain bacteria (AO), colour filters (SB) and dissolve rubble (HCL-EDTA).

Substance	Constituents
A-Medium	Add 2 ml HCl (1.2 M in bidest) to 1 liter seawater (filtrated over 0,2 µm cellulose acetate). Sterilise in autoclave during 20 min at 1 bar, 120 °C. Add 4ml sterile $NaHCO_3$ in bidest. Add nutrients (sterile): 0.5 ml minor 1 0.5 ml minor 2 0.5 ml nitrate 0.5 ml ammonium 1 ml fosfate 1 ml soil extract 1ml vitamines Re-adjust Ph to 7.9 - 8.1 with respectively sterile HCl or NaOH. Prepare at least one day in advance in order for the medium to stabilise prior to being used.
Acridine Orange (AO)	80 mg AO added to 100 ml H_2O + 5ml 37% formaline. Add 1 ml AO sollution to 9 ml sample (= 4.5 ml sea water sample + 4.5 ml 5% formaline)
Sudan Black (SB)	Dissolve ca 5 mg SB in 1 ml ascidine acid diluted with 0.2 mu filtered sea water. Stain filters with SB sollution for at least 30 min.
HCl – EDTA	Dilute 200 ml 35-38% HCL with 1800 ml H_2O. Add 0.5 g EDTA and place sollution on a stirring device until EDTA is dissolved.

A	SPONGE						INDEFINABLE						WORMS						MOLLUSCS						TOT.
C.lampa	s						s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s							AFDW
02/02/99	1						1	4.275	4.718	4.488	0.443	0.214	0.229	1	4.847	4.862	4.851	0.015	0.003	0.011	1							0.241
	2						2	4.070	5.163	4.593	1.093	0.523	0.571	2	4.886	4.939	4.910	0.053	0.024	0.029	2							0.599
	3						3	4.523	5.557	4.994	1.034	0.471	0.563	3	5.030	5.065	5.044	0.035	0.014	0.021	3							0.584
	4						4	4.984	5.367	5.078	0.383	0.094	0.288	4	5.017	5.051	5.021	0.034	0.004	0.030	4							0.318
C.lampa	s						s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s							AFDW
10/02/99	1						1	4.805	5.507	5.137	0.702	0.333	0.369	1							1							0.369
	2						2	5.091	5.892	5.499	0.801	0.408	0.393	2							2							0.393
	3						3	3.978	4.927	4.369	0.949	0.391	0.558	3							3							0.558
	4						4	4.311	5.052	4.576	0.741	0.266	0.475	4							4							0.475
C.lampa	s						s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s							AFDW
10/03/99	1						1	9.592	10.087	9.675	0.495	0.083	0.412	1							1							0.412
	2						2	4.347	4.886	4.614	0.539	0.267	0.273	2							2							0.273
	3						3	4.224	4.683	4.386	0.459	0.162	0.297	3							3							0.297
	4						4	4.007	4.677	4.323	0.670	0.316	0.354	4							4							0.354
	5						5	4.803	5.362	5.107	0.561	0.305	0.255	5							5							0.255
	6						6	9.838	10.805	10.227	0.967	0.389	0.578	6							6							0.578
Average													0.401							0.023							0.408
SD													0.127							0.008							0.130

B	SPONGE						INDEFINABLE						WORMS						MOLLUSCS						TOT.
C.laticavicola	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	AFDW
17/03/99	1	4.264	4.412	4.322	0.148	0.058	0.090	1	5.195	5.536	5.332	0.341	0.137	0.204	1	4.892	4.926	4.902	0.034	0.010	0.025	1							0.318
	2	4.887	4.975	4.927	0.088	0.040	0.048	2	4.912	5.782	5.346	0.869	0.434	0.436	2	4.768	4.796	4.777	0.028	0.010	0.018	2							0.502
	3	4.069	4.277	4.158	0.208	0.089	0.119	3	5.104	5.497	5.270	0.393	0.166	0.227	3	4.788	4.809	4.792	0.021	0.004	0.017	3	5.031	5.041	5.031	0.010	0.000	0.010	0.363
	4	4.343	4.584	4.443	0.242	0.100	0.141	4	8.901	9.652	9.232	0.751	0.331	0.420	4	4.847	4.868	4.851	0.021	0.004	0.017	4							0.579
	5	4.152	4.293	4.212	0.141	0.060	0.081	5	4.662	4.988	4.809	0.326	0.147	0.180	5	5.018	5.028	5.018	0.010	0.000	0.010	5							0.271
	6	4.759	4.878	4.813	0.118	0.054	0.064	6	4.978	5.228	5.097	0.250	0.119	0.132	6	5.269	5.281	5.272	0.013	0.003	0.010	6							0.205
C.laticavicola	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	AFDW
25/03/99	1	4.743	5.067	4.891	0.324	0.148	0.176	1	9.403	11.264	10.311	1.861	0.908	0.953	1	4.467	4.688	4.560	0.221	0.093	0.128	1	4.146	4.364	4.264	0.218	0.118	0.100	1.257
	2	4.764	4.884	4.806	0.121	0.042	0.079	2	9.540	10.223	9.785	0.683	0.245	0.438	2	4.225	4.229	4.225	0.004	0.000	0.004	2							0.520
	3	5.029	5.123	5.069	0.094	0.041	0.054	3	5.156	5.578	5.339	0.422	0.183	0.239	3	4.226	4.247	4.233	0.021	0.007	0.013	3							0.306
	4	4.548	4.950	4.710	0.402	0.162	0.240	4	4.237	4.547	4.375	0.310	0.138	0.172	4	5.340	5.371	5.353	0.031	0.013	0.018	4							0.430
	5	3.774	3.917	3.827	0.144	0.054	0.090	5	4.736	5.403	5.034	0.667	0.299	0.368	5	4.008	4.023	4.013	0.015	0.005	0.009	5	4.839	4.917	4.869	0.078	0.030	0.048	0.468
	6	4.223	4.498	4.337	0.275	0.114	0.161	6	5.290	5.655	5.451	0.366	0.161	0.205	6	4.804	4.829	4.813	0.025	0.009	0.016	6							0.382
C.laticavicola	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	AFDW
13/04/99	1	4.677	5.024	4.840	0.347	0.163	0.184	1	4.700	5.025	4.861	0.325	0.161	0.164	1	4.378	4.395	4.381	0.017	0.004	0.013	1							0.361
	2	4.681	4.821	4.760	0.140	0.079	0.061	2	4.054	4.273	4.173	0.219	0.118	0.100	2	5.470	5.474	5.470	0.004	0.000	0.004	2							0.165
	3	4.990	5.520	5.278	0.530	0.288	0.242	3	8.672	9.985	9.425	1.313	0.754	0.560	3	5.120	5.244	5.174	0.124	0.054	0.070	3							0.872
	4	4.424	4.837	4.701	0.413	0.276	0.136	4	8.432	10.045	9.263	1.613	0.832	0.782	4	5.248	5.296	5.268	0.048	0.019	0.029	4							0.947
	5	4.296	4.923	4.621	0.628	0.326	0.302	5	9.614	10.946	10.347	1.331	0.733	0.599	5	4.000	4.067	4.028	0.067	0.028	0.039	5							0.940
	6	4.805	5.125	4.990	0.320	0.185	0.135	6	4.542	5.728	5.146	1.186	0.604	0.583	6	5.183	5.188	5.183	0.005	0.000	0.005	6							0.723
Average							0.134						0.376							0.025							0.534
SD							0.073						0.242							0.030							0.299

C	SPONGE						INDEFINABLE						WORMS						MOLLUSCS						TOT.
C.vermifera	s						s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s							AFDW
13/01/99	1						1	8.670	9.804	8.954	1.134	0.284	0.850	1							1							0.850
	2						2	8.208	10.375	9.172	2.167	0.964	1.203	2							2							1.203
	3						3	9.368	10.747	9.987	1.379	0.618	0.761	3							3							0.761
	4						4	9.350	10.210	9.691	0.861	0.341	0.520	4							4							0.520
C.vermifera	s						s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s							AFDW
19/01/99	1						1	8.000	9.408	8.833	1.409	0.833	0.576	1	5.188	5.228	5.206	0.040	0.018	0.022	1							0.597
	2						2	4.714	6.612	6.185	1.898	1.471	0.427	2	4.664	4.686	4.674	0.022	0.011	0.012	2							0.439
	3						3	4.296	4.948	4.413	0.652	0.117	0.535	3	5.106	5.133	5.118	0.026	0.012	0.014	3							0.549
	4						4	5.247	6.259	5.888	1.012	0.641	0.371	4	4.295	4.336	4.305	0.041	0.010	0.031	4							0.402
rubble	s						s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s							AFDW
C.vermifera	1						1	8.916	10.945	9.921	2.029	1.005	1.024	1							1							1.024
17/02/99	2						2	9.224	10.947	9.957	1.723	0.733	0.990	2							2							0.990
r	3						3	4.699	5.746	5.232	1.047	0.532	0.515	3							3							0.515
r	4						4	8.668	10.530	8.883	1.862	0.215	1.647	4							4							1.647
r	5						5	4.808	5.648	5.230	0.839	0.421	0.418	5							5							0.418
r	6						6	9.337	11.361	10.114	2.024	0.776	1.247	6							6							1.247
C.vermifera	s						s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s							AFDW
24/02/99	1						1	5.187	5.501	5.334	0.313	0.147	0.166	1							1							0.166
	2						2	9.248	9.870	9.414	0.621	0.166	0.456	2							2							0.456
	3						3	4.225	4.500	4.271	0.275	0.045	0.230	3							3							0.230
	4						4	8.423	8.893	8.525	0.470	0.102	0.368	4							4							0.368
	5						5	5.128	5.617	5.332	0.489	0.204	0.285	5							5							0.285
	6						6	10.276	10.822	10.410	0.546	0.134	0.412	6							6							0.412
Average													0.650							0.032							0.654
SD													0.392							0.043							0.390

D	SPONGE						INDEFINABLE						WORMS						MOLLUSCS						TOT.
C.vermifera	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	AFDW
01/04/99	1	5.329	5.505	5.412	0.175	0.083	0.092	1	4.151	5.129	4.623	0.978	0.472	0.507	1	4.699	4.735	4.713	0.036	0.014	0.022	1							0.621
	2	5.471	5.583	5.509	0.112	0.038	0.074	2	4.761	5.294	4.942	0.534	0.181	0.353	2	4.681	4.702	4.686	0.021	0.005	0.016	2							0.443
	3	5.120	5.335	5.207	0.216	0.088	0.128	3	4.343	5.338	4.738	0.995	0.395	0.600	3	4.806	4.850	4.820	0.044	0.014	0.030	3							0.758
	4	5.183	5.524	5.312	0.340	0.129	0.212	4	13.762	15.145	14.254	1.383	0.492	0.891	4	4.379	4.418	4.389	0.039	0.011	0.028	4							1.131
	5	4.001	4.248	4.111	0.247	0.110	0.137	5	4.070	4.748	4.365	0.678	0.295	0.383	5	4.284	4.344	4.305	0.060	0.021	0.038	5							0.558
	6	4.692	4.901	4.783	0.209	0.091	0.118	6	4.292	5.100	4.398	0.808	0.106	0.702	6	4.541	4.606	4.569	0.065	0.028	0.037	6							0.857
rubble	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	AFDW
C.vermifera	r	1						1	4.547	5.915	4.762	1.368	0.215	1.153	1	4.467	4.493	4.474	0.026	0.008	0.019	1							1.172
20/04/99	r	2						2	9.332	10.255	9.692	0.923	0.360	0.563	2	5.208	5.318	5.296	0.110	0.088	0.022	2							0.584
	r	3						3	4.310	5.143	4.707	0.834	0.398	0.436	3	4.734	4.746	4.736	0.013	0.003	0.010	3	4.724	4.814	4.810	0.090	0.086	0.005	0.446
	r	4						4	4.808	5.226	4.988	0.418	0.180	0.238	4	4.661	4.695	4.670	0.034	0.009	0.025	4							0.263
		5	4.763	5.136	4.933	0.373	0.170	0.203	5	5.028	5.403	5.181	0.375	0.153	0.223	5	4.838	4.892	4.853	0.054	0.014	0.040	5							0.466
		6	3.773	3.895	3.827	0.122	0.054	0.068	6	4.742	5.262	4.971	0.520	0.230	0.291	6	5.155	5.170	5.159	0.015	0.004	0.011	6							0.370
C.vermifera	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	s	w cup	c dry	c ash	dry w	ash w	AFDW	AFDW
28/04/99	1	4.291	4.529	4.365	0.239	0.074	0.165	1	4.342	5.008	4.538	0.666	0.196	0.470	1	4.758	4.818	4.768	0.060	0.010	0.050	1	4.680	4.707	4.702	0.027	0.022	0.004	0.685
	2	4.888	4.988	4.919	0.099	0.031	0.069	2	4.070	4.380	4.150	0.309	0.080	0.230	2	3.994	4.107	4.069	0.113	0.075	0.038	2	5.181	5.211	5.205	0.030	0.024	0.006	0.336
	3	4.284	4.416	4.315	0.132	0.031	0.101	3	4.377	4.614	4.461	0.237	0.084	0.153	3	5.440	5.489	5.472	0.049	0.031	0.017	3							0.272
	4	4.803	4.984	4.863	0.181	0.060	0.121	4	4.803	5.214	4.882	0.411	0.079	0.332	4	3.977	4.264	3.985	0.288	0.009	0.279	4							0.732
	5	4.005	4.155	4.069	0.149	0.064	0.086	5	5.117	5.388	5.194	0.270	0.077	0.193	5	4.145	4.202	4.158	0.057	0.014	0.044	5	4.293	4.374	4.357	0.082	0.064	0.018	0.323
	6	4.608	4.876	4.686	0.268	0.078	0.190	6	4.691	4.980	4.752	0.289	0.061	0.228	6	5.326	5.438	5.358	0.113	0.033	0.080	6							0.498
Average							0.126						0.441							0.040							0.568
SD							0.049						0.265							0.056							0.246

Table 3a-c. Biomass of sponges and other rubble associated organisms per experiment, per series (s 1, 2 etc.). *C.lampa* (A), *C.laticavicola* (B), *C.vermifera* series that were dried at 70°C prior to being preserved (C) and *C.vermifera* separable series (D). Weight (in grams) of heat-resistant porcelain cups (w cup), cups with dried organic material (c dry), cups with ashed material (c ash), dry weight of organic material (calculated as w cup - c dry), weight of ashed material (calculated as w cup – c ash) and Ash Free Dry Weight (AFDW) per fraction (sponge, indefinable material, worms, molluscs). r = bare rubble series

Table 4. Multiple comparison (Fisher`s 95% LSD test) between the mean total ash free dry weight (AFDW) of rubble associated organisms per different rubble category; respectively infested with *C.lampa*, *C.laticavicola*, *C.vermifera* and bare rubble. Overlap of X denotes homogeneous groups within which there is no statistically significant difference; * denotes a statistically significant difference ($\alpha = 0.05$).

Category	N	Mean	Homogeneous groups
C.lampa	12	0.421	X
C.laticavicola	17	0.546	X
C.vermifera	27	0.575	XX
Bare rubble	7	0.825	X
Contrast		**Difference**	**+ / - Limits**
C.lampa – *C.laticavicola*		-0.125	0.227
C.lampa – Bare rubble		*-0.404	0.286
C.lampa – *C.vermifera*		-0.154	0.209
C. laticavicola – Bare rubble		*-0.279	0.270
C. laticavicola – *C.vermifera*		-0.029	0.186
Bare rubble – *C.vermifera*		0.250	0.255

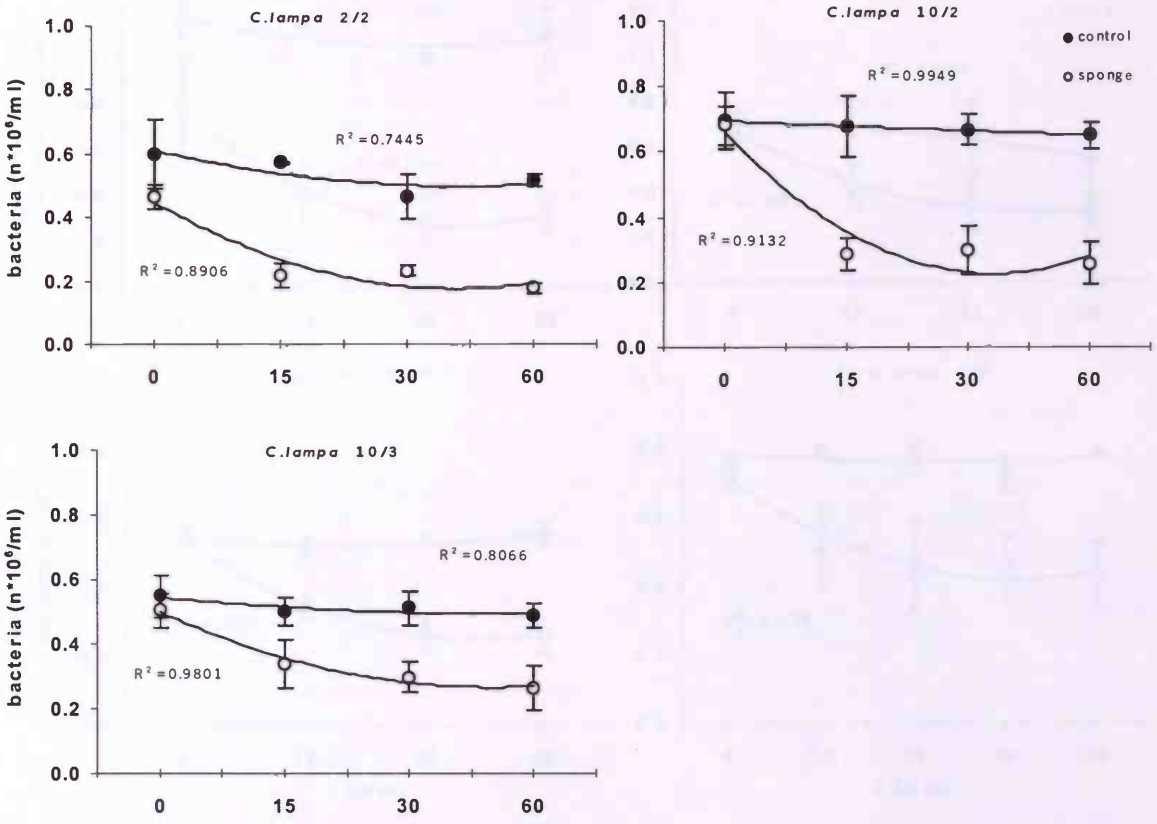

Fig.4. *C.lampa*. Average bacterial density over a period of 1 hour in experimental enclosures. Control n = 2, sponge n = 4 (2 and 10/2), n = 3 (10/3). Polynomial regression lines and R^2 are shown. Vertical bars indicate standard errors.

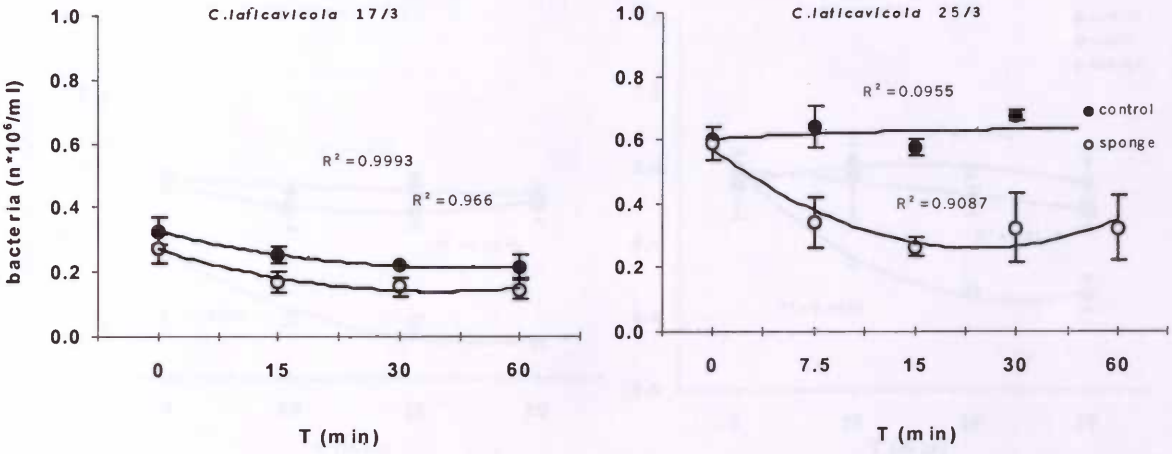

Fig.5. *C.laticavicola*. Average bacterial density over a period of 1 hour in experimental enclosures. Control n = 2, sponge n = 6. Polynomial regression lines and R^2 are shown. Vertical bars indicate standard errors.

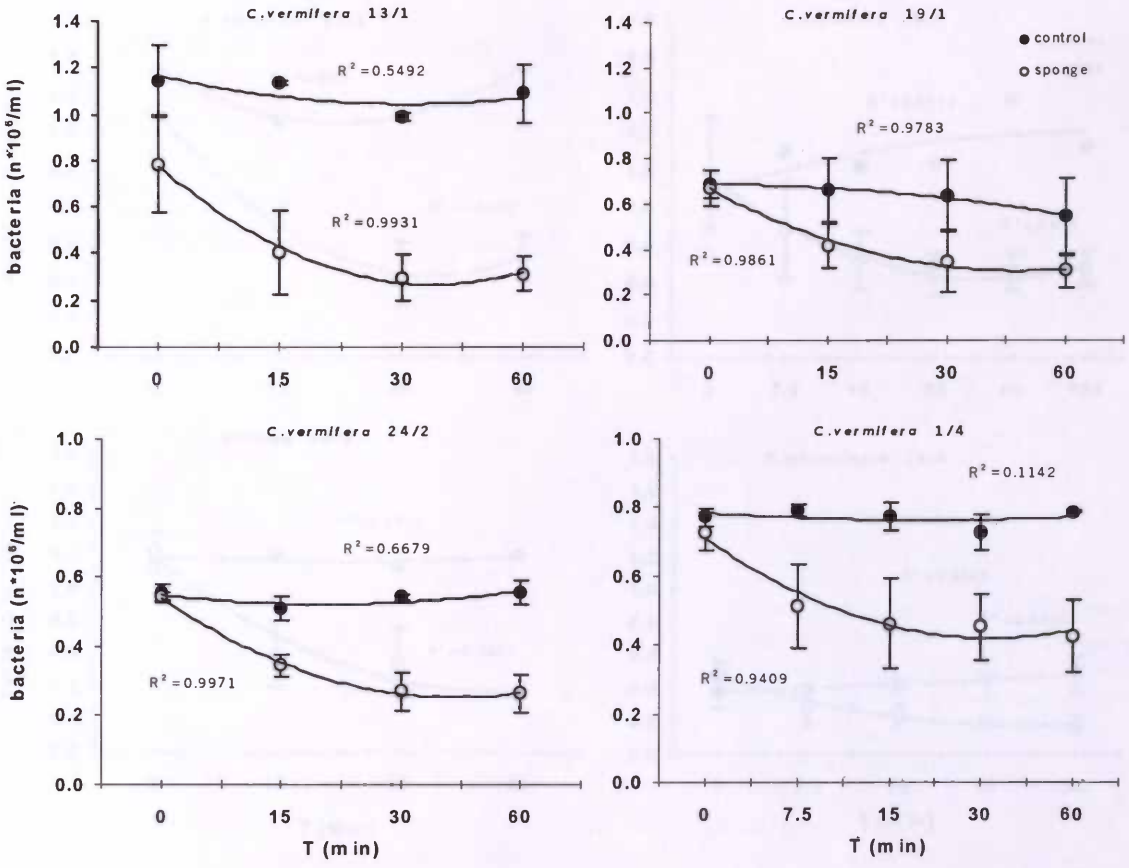

Fig.6. *C.vermifera*. Average bacterial density over a period of 1 hour in experimental enclosures. Control n = 2, sponge n = 3 (13 and 19/1), n = 4 (24/2), n = 6 (1/4). Polynomial regression lines and R^2 are shown. Vertical bars indicate standard errors. Note different ordinate scales.

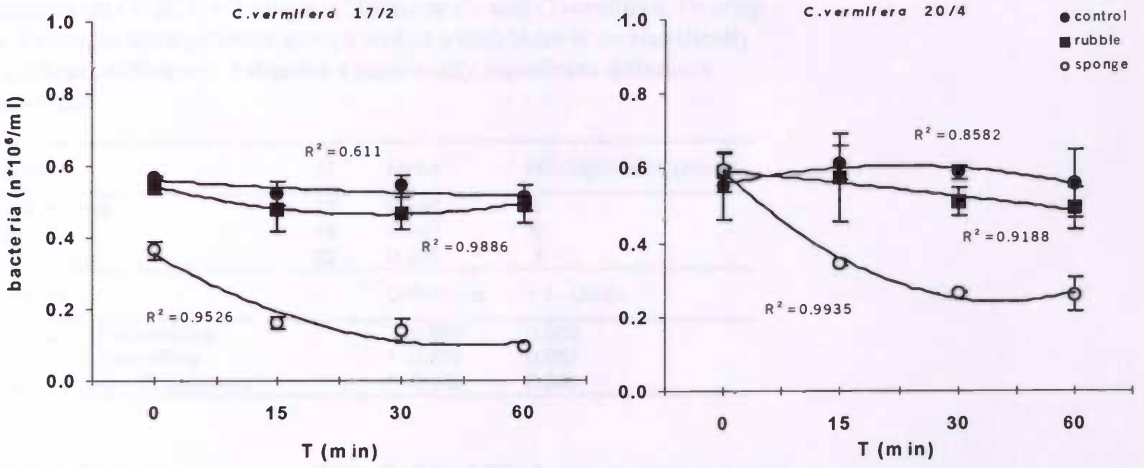

Fig.7. Bare rubble (i.e. not infested with boring sponges) and *C. vermifera*. Average bacterial density over a period of 1 hour in experimental enclosures. Control n = 2, rubble n = 4, sponge n = 2. Polynomial regression lines and R^2 are shown. Vertical bars indicate standard errors.

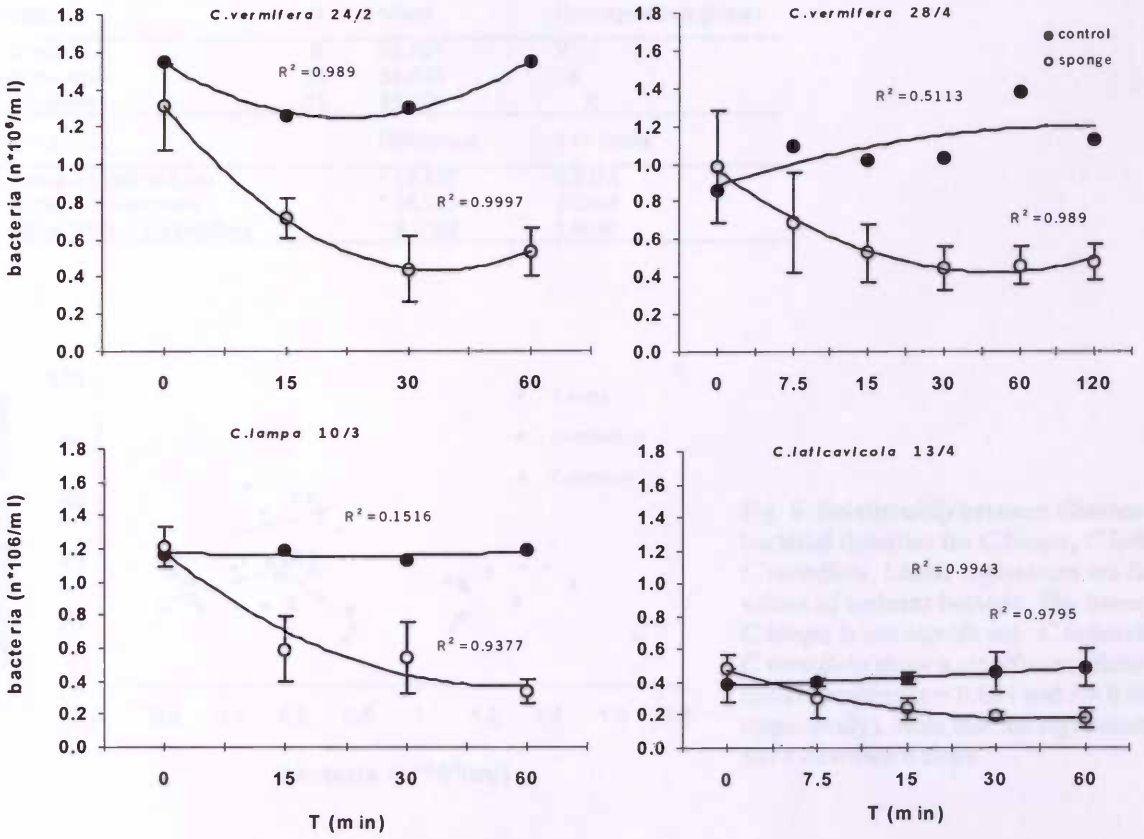

Fig.8. Average densities of cyanobacteria that were added to the seawater in enclosures on t = 0, measured over a period of respectively 1hour (24/2, 13/4, 28/4) and 2 hours (10/3). Control n = 1 (24/2, 10/3, 28/4), n = 2 (13/4), sponge n = 6. Polynomial regression lines and R^2 are shown. Vertical bars indicate standard errors. Note different time scales.

Table 5. Multiple comparison (Fisher`s 95% LSD) between the bacterial densities on t = 60 for *C.lampa*, *C.laticavicola* and *C.vermifera*. Overlap of X denotes homogeneous groups within which there is no statistically significant difference; * denotes a statistically significant difference ($\alpha = 0.05$).

Category	N	Mean	Homogeneous groups
C.laticavicola	12	0.208	X
C.lampa	14	0.237	X
C.vermifera	22	0.306	X
Contrast		Difference	+ / - Limits
C.lampa – C.laticavicola		0.029	0.066
C.lampa – C.vermifera		* -0.070	0.057
C. laticavicola – C.vermifera		* -0.100	0.060

Table 6. Multiple comparison (Fisher`s 95% LSD) between clearance rates during the first 15 min for *C.lampa*, *C.laticavicola* and *C.vermifera*. Overlap of X denotes homogeneous groups within which there is no statistically significant difference; * denotes a statistically significant difference ($\alpha = 0.05$).

Category	N	Mean	Homogeneous groups
C.lampa	8	53.791	X
C.laticavicola	12	34.624	X
C.vermifera	10	25.424	X
Contrast		Difference	+ / - Limits
C.lampa – C.laticavicola		* 19.167	9.2273
C.lampa – C.vermifera		* 28.268	9.5893
C. laticavicola – C.vermifera		* 9.2002	8.6560

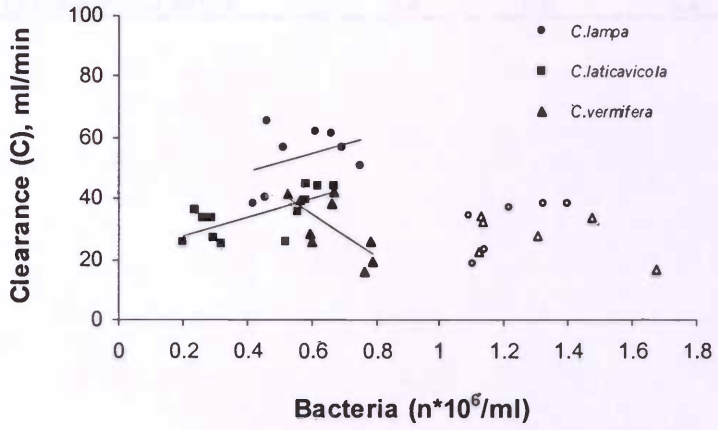

Fig. 9. Relationship between clearance and initial bacterial densities for *C.lampa*, *C.laticavicola* and *C.vermifera*. Linear regressions are fitted through values of ambient bacteria. The linear relationship for *C.lampa* is not significant. *C.laticavicola* and *C.vermifera* show a significant relationship with initial densities ($r = 0.654$ and $r = 0.688$, $P < 0.05$ respectively). Note that the regression on *C.vermifera* has a downward slope.

Table 7. Summary of results. a) Bacterial densities at t = 60 b) Proportional decrease in bacterial numbers relative to t = 0 c) Correlation between densities t = 0 and t = 60 d) Correlation (linear regression and polynomial regression respectively) betweenclearance and initial densities e) Average regression coefficients. RC's of *C.lampa* and *C.vermifera* on t = 15, *C.laticavicola* on t = 7.5 f) Regression coefficients t = 7.5 compared to t = 15 g) Clearance t = 7.5 compared to t = 15 h) Average clearance rates i) Average sponge AFDW j) Average total AFDW k) Correlation between clearance and AFDW nat.bact = naturally occurring bacteria. cy.bact. = cyanobacteria added to enclosures. n.d. = No data available * Denotes a statistically significant difference compared to the other clionid species.

	Species		
Result	*C.lampa*	*C.laticavicola*	*C.vermifera*
a) Bact. densities t = 60 ($n*10^6$bact/ml)	0.2	0.2	0.3*
b) Decr. nat.bact. t = 60 v.s. 0	44%	50%	49%
Decr. Cy.bact t = 60 v.s. 0	28%	36%	41%
c) Correlation # nat.bact. t = 0-60	P < 0.5	P < 0.02	P < 0.2
Correlation # cy.bact. t = 0-60	n.s.	P < 0.01	P < 0.05
d) Correlation C nat. bact. t = 0-60	n.s.	P < 0.05	P < 0.05
Polynomial corr. C t = 0-60	P < 0.01	P < 0.02	P < 0.05
Correlation C cy.bact. t = 0-60	n.s.	n.d.	n.s.
e) RC nat.bact. avg	-0.25	-0.13*	-0.31
RC cy.bact. avg	-0.62	-0.28	-0.59
RC nat. bact v.s. cyanobact.	P < 0.01	n.d.	P < 0.01
f) RC nat bact t = 7.5 v.s. 15	n.s.	P < 0.05	n.s.
RC cyanobact. t = 7.5 v.s. 15	n.s.	P < 0.05	n.s.
g) C nat. bact. t = 7.5 v.s. 15	n.d.	n.s.	n.s.
C. cy.bact t = 7.5 v.s. 15	n.d.	n.s.	n.s.
h) Avg C nat. bact. (ml/min)	54*	35	28*
Avg C cy.bact. (ml/min) A.O. omitted	39	36	31
Avg C cy.bact. (ml/min) A.O. stained	28	n.d.	31
i) Avg AFDW (g)	n.d.	0.13	0.13
j) Avg total AFDW (g)	0.41	0.65	0.57
k) Correlation C AFDW	n.d.	n.s.	n.s.