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The Role of Rock Substratum in the Ecology of Intertidal Epilithic Biofilms

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ABSTRACT

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THE ROLE OF ROCK SUBSTRATUM IN THE ECOLOGY OF INTERTIDAL EPILITHIC BIOFILMS

By Paula Serena Moschella

The overall aim of this thesis was to investigate the role of the underlying rock type on the ecology of epilithic biofilms in relation to other major regulating factors such as season, shore height and grazing. In Chapter 1 the relevant literature has been reviewed covering the development and succession of biofilms and describing their importance to the ecology of rocky shore habitats. The aims and rationale of the thesis are then outlined.

In Chapter 2 a broadscale survey of microbial biomass and assemblage composition was made at sites along the south coast of England from Lands End to the Isle of Wight. This encompassed shores of chalk, limestone, sandstone old red sandstone and granite sampled at a range of spatial scales (10's km to 10's cm) in an hierarchical design with two shores of each rock type and two tidal levels on each shore. Microbiota were quantified using extracted chlorophyll to provide an index of biomass and scanning electron microscopy to visualise microbial assemblages. Microalgal biomass was greater on soft, porous carbonate rocks such as chalk and limestone than on hard, impermeable quartz-based rocks such as granite and old red sandstone. These differences were significant in the early spring and summer. However, they were most apparent in early spring and the in upper eulittoral rather than the littoral fringe.

Local effects of rock type were investigated by comparing microbial films on rocks with contrasting physical and chemical properties that were present at the same shores (Chapter 3). Chalk and flint were compared at Freshwater Bay and Culver Cliff on the Isle of Wight, limestone and chert at Portland Bill, and dolomite and oil shale at Kimmeridge. Microbial biomass tended to be greater on carbonate rocks than on quartz based rock but this effect was only significant contrasts between dolomite and oil shale.

An experiment was run to compare colonisation of virgin surfaces of chalk and dolomite (selected on the basis of work in chapters 2 and 3) using machine cut rock tiles as experimental units (Chapter 4). These were fixed to the shore in randomised blocks at two sites (Wembury and Bovisand) and colonisation recorded for two months in two initiations during both the summer and winter. Grazing activity was monitored in parallel using wax discs recessed into the centre of the tiles. There were major differences in colonisation rates between seasons. Colonisation was suppressed during the summer and differences between rock types were not apparent. However, during winter colonisation was more rapid and differences between rock types were apparent with greater biomass on chalk than dolomite.

Interactions between grazing and rock type were examined in Chapter 5. Colonisation was compared between chalk and dolomite tiles fixed on the shore in areas that were accessible to grazers and in areas were grazers were excluded using fences. After 60 days microalgal biomass was significantly greater in areas where limpets were excluded and was also significantly greater on chalk than on dolomite in the exclusion plots.

In the general discussion (Chapter 6) the methodological constraints of the work are outlined. One of the principal constraints was spatial variability in microbial abundance which was considerable, occurring between shores (10's km), plots (10's m) and replicate samples (10's cm) and being most evident at a microscopic scale (100's of μm) probably as a consequence of grazing. Clear patterns in microbial abundance were still apparent. Season and to a lesser extent grazing and shore level had major influences, but the effects of rock type were apparent when these influences were reduced or removed. For example, the effect of rock type was very clear during the winter when physical stresses associated with emersion are less severe and also when the influence of grazing was removed in experimental exclusion areas. Chalk in particular supported extremely productive microbial communities together with considerable grazer biomass. These findings emphasise the unique nature of intertidal chalk habitats along the English Channel.

CONTENTS

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Chapter 1

General Introduction

1 INTRODUCTION

Biofilms are an essential component of aquatic ecoystems (see Lock, 1993; Cooksey and Wigglesworth-Cooksey, 1995 for reviews) and rocky shores are no exception (Hawkins *et al.*, 1992). In this thesis I have investigated how the physical and chemical properties of rocks themselves affect epilithic biofilms. My work was not only intended to further our knowledge of the ecology of these important microorganisms, but also to provide a better understanding of the processes and interactions which regulate the whole rocky shore community. Interactions between microbial films and macrobiota, both bottom up and top down, are crucial to the functioning of rocky shore ecosystems. Thus, it is likely that any influence of rock type on biofilms will also have consequences for the whole community.

In comparison to the macrobiota many aspects of the ecology of intertidal microbenthic communities are yet to be investigated, in part due to technical difficulties (MacLulich, 1986b). Biofilms consist of a mixed assemblage of micro-organisms (bacteria, cyanobacteria, fungi, protozoans, microalgae and early life history stages of algae), embedded in a matrix of extracellular polysaccharides, which coat every substratum immersed in water (see Wahl, 1989 for marine systems; Lock, 1993 for freshwater). These microbial films play a significant role in aquatic ecosystems in a variety of ways. Primary production (Hawkins *et al.*, 1992; Bustamante *et al.*, 1995; Ahn *et al.*, 1997) and nutrient recycling (Hamilton and Duthie, 1984; Hillebrand, 2000; Frost, 2002) occur in biofilms. They can also lead to bioerosion (Donn and Boardman, 1988; Le Campion-Alsumard, 1989; Peyrot-Clausade *et al.*, 1995). Microbial films also influence and interact with macrobenthic communities. They represent the initial substrate for colonisation by sessile invertebrate larvae and algal propagules, thereby influencing recruitment and settlement of macroorganisms (Crisp, 1974; Keough and Raimondi, 1996; Wieczorek and Todd, 1998; Thompson *et al.*, 1998; Callow, 2000). Biofilms are an important food resource in the diets of many grazers, and thus form the basis of many aquatic food webs (Hawkins *et al.*, 1992; Christian and Luczkovich, 1999).

In contrast to other factors, the underlying substratum, has been little studied, and such investigations have been largely restricted to macrobiota (Richmond and Seed, 1991;

Callow and Fletcher, 1994; Holmes *et al.*, 1997; Kohler *et al.*, 1999).The influence of the rock substratum on intertidal biofilms has been little investigated, and most available knowledge is related to artificial substrata (Pringle and Fletcher, 1986; Taylor *et al.*, 1997; Becker *et al.*, 1997b).

In this introductory chapter, I review the literature on marine intertidal epilithic biofilms. In Section 1 biofilm formation, succession and composition are described. The importance of microbial film in ecosystems is then discussed defining in particular the role of epilithic microalgae in primary productivity and its importance in settlement and recruitment of algae and marine invertebrates (Section 2). Background on the spatial and temporal distribution of intertidal biofilms will be given in Section 3, with particular reference to the physical and biological factors responsible for these patterns. In Section 4 methods for analysing epilithic films will be compared, encompassing sampling techniques and protocols to quantify their biomass and species composition. In Section 5, the limited literature on the influence of geology on rocky shore ecology is briefly reviewed, referring also to macrobiota where most work has been done. This review will concentrate on biofilms on rocky shores, but additional information on freshwaters, biofouling and other aspects of the study of biofilm are incorporated where relevant. The introduction ends with the rationale for the thesis and the specific objectives of the various chapters.

1.1 Biofilm colonisation and succession

Colonisation and succession in epibiotic communities has been widely investigated (e.g. Southward and Southward, 1978; Niell and Varela, 1984; Sousa and Connell, 1992; Anderson and Underwood, 1997; Benedetti-Cecchi, 2000), but little information is available on the early stages of colonisation in the natural environment. The process of primary succession through biofilm formation has been investigated more extensively in studies related to biofouling (Characklis, 1981; Wahl, 1989; Baty *et al.*, 1996; Anderson, 1996) than on natural substrata (but see Niell and Varela, 1984; MacLulich, 1986a; Anderson and Underwood, 1997; Williams *et al.*, 2000). Wahl (1989) has proposed a possible model of temporal sequences of biofilm formation in seawater. The first phase consists of the adsorption of organic compounds (mainly glycoproteins, proteoglucans and polysaccharides) to the substrate just a few seconds

after the substrate has been submerged. This organic film seems to be adsorbed on any kind of substratum which then acquires similar physical and chemical properties at the surface/liquid interface. Within one hour of immersion in seawater bacteria start to colonise the substratum. Colonisation by bacteria involves a reversible process called adsorption, which is governed by physical forces (Brownian motion, electrostatic interactions, Van der Vaal forces), followed by adhesion of bacterial glycocalix to the organic film by means of covalent bonds (Characklis, 1981). After this process the physical and chemical properties of the substratum change again significantly depending on bacterial succession (Marszalek *et al.*, 1979). In general, rod-shaped bacteria arrive first, followed by coccoids and finally by stalked and filamentous forms (Korber *et al.*, 1995). Observations on isolated culture of marine cyanobacteria showed that the production of variable quantities of extracellular polymeric substances (EPS) plays an important role in the adhesion process of these rapid early colonisers (Scott *et al.*, 1996). Colonisation of the substrate continues during the next few days with the arrival and settlement of unicellular eukaryonts such as yeasts, fungi, protozoans and diatoms. Diatoms are usually more abundant than other species and often form a dense mat. Benthic motile diatoms generally precede stalked forms. Diatoms, as well as cyanobacteria, are able to produce exopolymeric substances (EPS) which also enhance the locomotive force in the motile forms (Smith and Underwood, 1998). At this early stage of colonisation biotic interactions may occur within biofilm such as competition for space and food resources (reviewed by Wimpenny, 1996).

The classic successional sequence of conditioning film, bacteria and diatoms, which generally characterises the first phases of microbial colonisation, does not always occur. Tuchman and Blinn (1979) observed diatoms as primary colonisers of artificial substrata, while cyanobacteria followed after the first week of immersion. In marine sediments, Underwood J.G.C. (1997) showed that the colonisation was characterised by a sequence of diatoms, and green algal assemblages, while the occurrence of cyanobacteria was independent of this successional sequence. The type of micro-organisms which first colonise the substrates can be influenced by seasonal variability (Anderson, 1995). In natural waters, exposed surfaces are inoculated with micro-organism by means of physical processes (i.e. advection, diffusion) followed by adsorption and attachment of cells and finally by growth (Wahl, 1989; Characklis *et al.*, 1990). Bacterial adsorption and adhesion is a consequence of interacting processes

involving electrostatic forces, hydrodynamics, chemotaxis surface rugosity, exopolymer production and substratum wettability (Marshall *et al.*, 1971; Marszalek *et al.*, 1979; Lock *et al.*, 1984; Pringle and Fletcher, 1986; Zheng *et al.*, 1994; reviewed in Cooksey and Wigglesworth-Cooksey, 1995; Baty *et al.*, 1996; Taylor *et al.*, 1997).

In general, it takes around three-four weeks for a substrate to be colonised by a mature microbial community. In freshwater systems, after 20-25 days the microalgal community appears to reach a mature state, characterised by no further changes in species composition and cell density (Blinn *et al.*, 1980; Hamilton and Duthie, 1984). In more disturbed and unstable systems, however, such as epipsammic microalgal community, colonisation can be kept in a pioneer state (Miller *et al.*, 1987). On intertidal rocky shores Wimpenny (1996) suggested that the time required for a recolonisation of the rock surface is 3-4 weeks, although a longer period of approximately 6 weeks is more likely to be necessary for a complete recovery of microbenthic communities (Underwood, 1984c).

The colonisation process seems also to be characterised by a diverse and apparently non structured community during the first week, while after this period the assemblages become dominated by one or few species (Niell and Varela, 1984). Furthermore, differences between substrata detected in the initial phases of adhesion and settlement of periphyton gradually disappeared at later stages (Hamilton and Duthie, 1984). After several days to weeks, depending on environmental conditions, the microbial community, evolving continuously in response to abiotic factors and biotic interactions, is enriched by the settlement of larvae and algal propagules. This leads to the establishment of macroscopic assemblages (Characklis, 1981). Grazing (Hawkins and Hartnoll, 1983; Hawkins *et al.*, 1992; Jenkins *et al.*, 1999), and possibly algal sweeping (Hill and Hawkins, 1991) can prevent, however, the establishment of macroflora and fauna and keep a microbial "lawn" on rock surfaces, thereby arresting succession.

1.2 The ecological importance of biofilms

1.2.1 The importance of microbial film in ecosystem functioning

In aquatic environments the microbial film is dominated by the autotrophic component, consisting mainly of diatoms and cyanobacteria. Thus, microalgal communities can significantly contribute to the primary production in a marine system, although their importance has been acknowledged only recently (Bustamante, 1995; Ahn *et al.*, 1997). Microalgal production has been compared to those of phytoplankton and macroalgae (Hawkins *et al.*, 1992; Bustamante *et al.*, 1995).

Microbial films are also an important component of the food web, as a primary source of food in the extended diets of many consumers (Hawkins *et al.*, 1989; Hill and Hawkins, 1991; Hawkins *et al.*, 1992; Christian and Luczkovich, 1999). Invertebrate larvae settling within biofilms may also use them as a primary food resource (Holmstrom and Kjelleberg, 1994).

Hamilton and Duthie (1984) suggested that periphyton community may be considered as a nutrient recycling system in a microhabitat, which may be very important in oligotrophic waters. Nutrients can, however, significantly affect the biomass and species composition of microphytobenthos (Frost, 2002; Gosselin *et al.*, 1990; Hillebrand and Sommer, 1997), although most of the work has been carried out on sediment communities (Sundback and Snoeijs, 1991; Pinckney *et al.*, 1995; Dizon and Yap, 1999). Enrichment experiments showed that nutrients, and in particular nitrogen, strongly limited diatom populations during summer and autumn. In spring and winter, nutrient concentrations were sufficient to stimulate primary production despite low temperature and light (Hillebrand and Sommer, 1997). Similar results were achieved by Dizon and Yap (1999) in tropical sediments, where N and P enrichment produced an increase of chlorophyll content and photosynthetic activity in microphytobenthos. However, in a factorial experiment using nutrient enriched seawater on rocky shores, Thompson (1996) found that increased algal growth occurred only under subdued light conditions and reduced grazing.

1.2.2 The role of microbial films in recruitment and settlement of marine invertebrates

The influence of biofilms on settlement of marine macrobiota has been widely investigated (for reviews see Wieczorek and Todd, 1998; Pawlik, 1992). There can be both positive and negative interactions.

Settlement of macroalgal propagules is influenced by the presence of biofilms (Callow, 2000). Diatoms appear to directly interact with macroalgae by enhancing or inhibiting growth of their sporelings or germlings but, at the same time, diatoms themselves are affected by the presence of algal sporelings (Huang and Boney, 1984). However, the effects of microflora on the algal growth can significantly change when more than one species interact together and competition occurs among diatoms (Huang and Boney, 1985).

Biofilms can provide cues for settlement in many species. Pearce and Bourget (1996) observed a significantly greater abundance of larvae and spat of the giant scallop *Placopecten magellanicus* settling preferentially on various filmed substrata rather than on unfilmed. Films of monocultured bacteria stimulated the settlement of the oysters *Crassotrea gigas* and *Ostrea edulis*, although this effect may vanish when more than one species interact in the biofilm, as happens in nature (Tritar *et al.*, 1992). Competent pediveliger larvae of the mussel *Mytilus galloprovincialis* responded positively to a filmed surface (Satuito *et al.*, 1997). Microbial films can have also an inhibitory effect on settlement of some marine invertebrates, as was observed for bryozoans species and ascidians (Todd and Keough, 1994; Wieczorek and Todd, 1997). In many situations the mechanism of the response to biofilm is still not clear (Keough and Raimondi, 1996). Chemical cues can partially influence settlement on biofilms (e.g. exopolymers of bacteria, Maki *et al.*, 1988).

Most studies have concentrated on interaction between biofilms and barnacle settlement (Maki *et al.*, 1988; Bourget, 1988; Wieczorek *et al.*, 1995; Thompson *et al.*, 1998). Microflora may also represent a cue for settlement of *Semibalanus balanoides* cyprids to guide cyprids to a suitable tidal height on the shore during broad exploration (Le Tourneux and Bourget, 1988). In contrast, *Elminius modestus* larvae settle

preferentially on unfilmed surfaces showing a negative response towards biofilms of different ages (Keough and Raimondi, 1996). The facilitatory or inhibitory effects of biofilms on the settlement of certain species of barnacles can also show a seasonal pattern and in some cases the positive or negative larval response can be reversed (Wieczorek *et al.*, 1996). Thompson (1998) demonstrated in a laboratory study that cyprids of *Semibalanus balanoides* selected surfaces with a mature biofilm and were also able to discriminate between biofilms belonging to different heights on the shore, maybe because of differences in microbial composition. Conversely, in field experiments these results were not so clear, and it appeared that other factors interact in the settlement process and thus may overcome the effect of macrobiota.

Other factors which seem to be involved in the attractive or repulsive effect of biofilm are its age and species composition. Wieczorek *et al.* (1995) observed that *Balanus amphitrite amphitrite* cyprids responded positively to film cues only when exposed to older biofilms and it appeared that the bacterial component is the major cue responsible for influencing barnacle settlement. A similar response to film age was also observed with larvae of the ascidian *Ciona intestinalis* (Wieczorek and Todd, 1997).

1.2.3 Endolithic microbial communities

Some microorganisms including various kinds of bacteria, fungi, algae and protozoa can promote rock erosion by boring the surface and speeding rock weathering by mobilising mineral constituents with inorganic or organic acids or ligands that they excrete (Ehrlich, 1998). Endolithic micro-organisms are an often neglected component of microbial communities in marine environments. A wide variety of organisms are able to bore into rock. These range from the more obvious sponges, barnacles (Donn and Boardman, 1988), bivalves (Trudgill, 1987) to the microalgae (Peyrot-Clausade *et al.*, 1995) and fungi (Schneider, 1976). Most attention has been given to carbonate rocks. Microboring organisms consist primarily of cyanobacteria, although green and red algae and fungi can also actively penetrate carbonate substrates (Perkins and Tsentas, 1976; Schneider, 1976; see for reviews Golubic *et al.*, 1975; Hirsch *et al.*, 1995) and the pore spaces of granite and sandstone (Hirsch *et al.*, 1995).

It is widely recognised that cyanobacteria play an important role in bioerosion, by

dissolving the carbonate and indirectly by increasing the porosity of a substrate which subsequently becomes more vulnerable to attack by macroborers and abrasion by grazers (Le Campion-Alsumard, 1989). It is widely accepted that blue green algae are the most important cause of erosion of limestone coasts (e.g. Purdy and Kornicker, 1968; Schneider, 1976). Potts and Whitton (1980) observed that endolithic blue-green algae grow very rapidly in limestone.

The boring mechanisms and the consequent dissolution of carbonate material is not well known, although it seems endoliths secrete acid substances (Le Campion-Alsumard, 1975). Alexandersson (1975) demonstrated that cyanobacteria can dissolve carbonate by means of specialised organelles. In intertidal systems, cyanobacteria may penetrate rock surfaces up to a depth of 1-2 mm (Schneider, 1976), although maximum density is generally around 0.4 mm depth. On limestone shores blue-green algae are found at all tidal heights, where they can remove up to 50% of the upper 400 μm of rock surface (Donn and Boardman, 1988).

There are significant differences in endolithic microbial assemblages relative to vertical height on the shore and latitude (Perkins and Tsentas, 1976). For example tropical boring microrganisms are different from temperate regions (Perkins and Halsey, 1971; Rooney and Perkins, 1972). Le Campion-Alsumard, (1979, 1989) found a very different vertical distribution of endolithic assemblages between tidal levels. The littoral fringe, which experiences harsh conditions, was dominated by endolithic cyanophytes, possibly because they are better adapted to desiccation and insolation stresses. From the lower shore down the circalittoral zone, chlorophytes and fungi replaced cyanobacteria. A similar pattern was found by Schneider (1976) and Torounsky (1979), who suggested that cyanobacteria are mainly distributed on the mid and upper shore levels.

Colonisation of new substrata by endolithic microalgae is quite rapid; cyanophytes appear after 8-9 days, and after 1 month the endolithic diversity increased significantly and some species penetrated the rock up to 30-50 microns (Le Campion-Alsumard, 1975). On artificial substrates, complete colonisation took at least four months (Perkins and Tsentas, 1976). Surface texture seems to affect colonisation by endoliths. In fact, boring algae infested porous substrata more rapidly and in greater number than

smooth surfaces (Le Campion-Alsumard, 1975).

Le Campion-Alsumard (1989) observed that both biological (the presence of grazers) and physical factors (light and desiccation, wave exposure) might influence the distribution of endolithic microalgae. On the upper intertidal desiccation seems to be a limiting factor as the boreholes were abundant but very shallow. Also, on wave exposed shores the endoliths extend to higher tidal levels. Grazing also stimulates boring activity by the endolithic algae, as the superficial layer of the rock is continuously taken away creating new space and more light penetrates into the already formed galleries. Grazing activity may indirectly influence the endolithic community by removing the epilithic biofilm (Le Campion-Alsumard, 1975) and the growth of macroalgae (Peyrot-Clausade *et al.*, 1995), which otherwise could limit the survival and growth of these microborers by reducing for example light penetration. Boring organisms bore into rock for protection rather than for food (Donn and Boardman, 1988).

Endolithic microalgae also represent an important source of food for many grazers including molluscs, echinoderms and fish, which rasp, bite and scrape away a thin layer of rock using specialised feeding structures (Warme, 1977; Ogden, 1977; Trudgill, 1987; Hawkins *et al.*, 1989).

1.3 Spatial and temporal patterns in intertidal benthic microalgae

Spatial and temporal patterns in the abundance of intertidal epilithic microalgae are not determined by a single factor, but are the consequence of the interaction of physical, chemical and biological factors acting both directly and indirectly.

1.3.1 Spatial patterns

There are large scale geographical differences in species composition of biofilms. In temperate regions many studies describe the intertidal epilithic community as an assemblage dominated by diatoms while, with some exceptions (MacLulich, 1986a), cyanobacteria, algal sporelings and germlings represent a minor component (Nicotri, 1977; Hill and Hawkins, 1991; Hawkins *et al.*, 1992; Anderson, 1995). Tropical shores experience harsher conditions such as very hot temperatures and strong desiccation

which favour more resistant cyanobacteria (Potts and Whitton, 1980; Cubit, 1984; Mak and Williams, 1999).

The vertical distribution of microbial film along the intertidal zone does not follow a common pattern. Microphytobenthos seems to be more abundant, has greater biomass and higher diversity towards the lower shore (Aleem, 1950; Underwood, 1984b; MacLulich, 1987). In contrast, Thompson (1996) observed during winter, maximum standing stock and abundance of diatoms on the upper shore and minimum values in the lower shore but, as the author pointed out, these differences are likely to be more related to seasonal variation, as the inverse occurred during summer.

Greater variability in biofilm distribution can be found also at smaller spatial scales. Many investigators have concluded that these microbial communities are characterised by a high degree of patchiness from scales of a few metres squared down to micrometers squared (Nicotri, 1977; MacLulich, 1987; Hill and Hawkins, 1990; Hill and Hawkins, 1991; Thompson, 1996; Thompson *et al.*, 1996). Differences in biofilm biomass and species composition occur between microhabitats such as barnacles plates, gastropods shells, macroalgae and bare rock, but not in terms of species diversity (Hill and Hawkins, 1991; Thompson, 1996; Thompson *et al.*, 1996).

1.3.2 Temporal patterns

The microalgal community changes significantly with seasons. Many studies have demonstrated that on both tropical and temperate shores microalgae are abundant during winter and spring and then disappear during the warmer summer months (Castenholz, 1961; Nicotri, 1977; Cubit, 1984; Underwood, 1984c; MacLulich, 1987; Dye and White, 1991; Hill and Hawkins, 1991; Williams, 1993; Thompson, 1996). A similar seasonal pattern has been observed for microflora living in sandy intertidal marine sediments (Sundback *et al.*, 1996; Underwood G.J.C., 1997).

Diatoms appear to reach maximum biomass and abundance in spring, followed by a second peak, of lower magnitude, in the autumn (Aleem, 1950; Edyvean *et al.*, 1985; MacLulich, 1987; Hill and Hawkins, 1991). In fact the photosynthetic activity, which determines microalgal primary production, shows a peak in spring, where light intensity, temperature and nutrients are near to the optimal values required by photosynthesis (Blanchard *et al.*, 1997). Cyanobacteria are less affected by seasonal

variation, possibly because of their greater tolerance and resistance to physical stresses such as temperature, desiccation and insolation (Thompson, 1996; Mak and Williams, 1999). Thompson (1996) showed that although the abundance of blue-green algae decreased in summer, a reduced cover still persisted on the shore, while diatoms disappeared completely.

Anderson (1995) found a contrasting seasonal trend, observing that on artificial substrates the greatest cover of microbial film occurred during spring and summer months. She attributed this pattern as being probably due to the very different experimental conditions used.

1.3.3 Abiotic and biotic factors affecting temporal and spatial patterns of microalgae

Physical factors

The intertidal system is characterised by a cycle of high and low tides which in the North East Atlantic are largely semi-diurnal. During low tide, the shore is exposed to the air, and the period of emersion increases towards the upper shore. Therefore, intertidal macrobiota and also the microbiota experience extremely variable environmental conditions, compared to more constant subtidal conditions see (Raffaelli and Hawkins, 1996) for review.

Desiccation, insolation and thermal stresses, appear to be the most important physical factors controlling the growth and abundance of benthic microflora (Underwood, 1984c; MacLulich, 1987; Blanchard *et al.*, 1997; Mak and Williams, 1999; Thompson *et al.*, 2000). In the upper shore these factors have a stronger impact on microbiota, and during the summer they are the main cause of the drastic decline of microalgae (Underwood, 1984b; Williams, 1993; Nagarkar, 1999). In the mid and lower shore physical factors are still very important, but grazing activity may contribute to a greater extent to the control of microbial abundance and species (Underwood, 1984a; Williams, 1994; Thompson, 1996).

Light is also a strong limiting factor for intertidal biofilms, which consist mainly of autotrophic organisms such as diatoms, cyanobacteria and algal germlings (Castenholz, 1963; MacLulich, 1987; Hill and Hawkins, 1991; Thompson, 1996). The effect of light

on microalgae has been known for long time. Aleem (1950) and Castenholz (1961) suggested that the amount of exposure to direct solar radiation during low tide was probably the most important factor in determining the vertical distribution of marine littoral diatoms and the relative species composition. UV radiation, and particularly UV-B effects may also contribute to the significant reduction of microalgae during the summer, but contrasting results have been obtained. Richardson *et al.* (1983) showed that UV radiation caused serious damage to the microalgal photosynthetic system and Bothwell *et al.* (1993) demonstrated that diatoms were directly inhibited by UV-B. Conversely, Hill *et al.* (1997) and Peletier *et al.* (1996) did not find any significant effects of UV radiation on microalgal biomass and diversity. A similar result was also obtained by Thompson (unpublished data). Furthermore, it seems that this factor does not represent a limiting factor during summer time and it has a much less impact than insulation. Experimental reduction of UV-B radiation did not cause an increase in microalgal biomass. The abundance of diatoms, however, increased significantly when the insolation was experimentally reduced by shading (50% or 85%). Insolation seemed to strongly affect diatoms, but not cyanobacteria (Thompson *et al.*, unpublished data).

Another factor which should be considered, especially when comparing microalgal communities between different sites, is the wave exposure of the shore. Aleem (1950) suggested that the degree of exposure might directly affect the distribution of diatoms. Microalgal standing stock and primary productivity can also differ between sheltered and exposed shores (Bustamante *et al.*, 1995; Thompson, 1996; Jenkins and Hartnoll, 2001).

Grazing activity

Grazers appear to play an important role in limiting algal abundance and distribution on rocky shores (for reviews see Lubchenco and Gaines, 1981; Hawkins and Hartnoll, 1983; Sousa and Connell, 1992). Although many studies have demonstrated grazing activity only impacts on macroalgae when they are microscopic stages of sporelings or germlings (i.e. Southward, 1964; Underwood, 1980; Hawkins, 1981; Underwood, 1984a), only a few investigations have been focused on the effects of grazing on microalgal community and, more in general, on microbial films (Castenholz, 1961; Nicotri, 1977; Cubit, 1984; Underwood, 1984a; Hill, 1990; Hill and Hawkins, 1991;

Williams, 1993; Anderson, 1995; Thompson, 1996; Mak and Williams, 1999; Sommer, 1999b). A large variety of grazers have been shown to feed on microflora. In tropical shores herbivorous fish regulate microalgal growth on coral reefs (Edgar and Shaw, 1995). In temperate regions, caddisfly and mayfly larvae in freshwaters (Fuller *et al.*, 1998), isopods (Salemaa, 1987; Schaffelke *et al.*, 1995; Sommer, 1997; Sommer, 1999a), crustaceans (Fleeger *et al.*, 1999) and amphipods (Jernakoff and Nielsen, 1997) also have a microphagous diet.

On rocky shores, molluscan gastropods are the predominant grazers world-wide (see for reviews, Underwood, 1979; Hawkins and Hartnoll, 1983; Norton *et al.*, 1990). A wide variety of gastropods, live in the eulittoral zone. These include prosobranchs and pulmonate limpets, trochids and littorinids (Nicotri, 1977; Hawkins and Hartnoll, 1983; Underwood, 1984a; Underwood, 1984c; Hawkins *et al.*, 1989; Dye and White, 1991; Anderson and Underwood, 1997). Littorinids are particularly well adapted to harsh condition of desiccation and high temperature, and dominate the littoral zone both on tropical and temperate rocky shores (Nicotri, 1977; Underwood and Jernakoff, 1981; reviewed by Norton *et al.*, 1990; Mak and Williams, 1999).

On British rocky shores, prosobranch limpets are the major grazers. They play an important role not only by limiting the abundance and distribution of microalgal assemblages but also by regulating the structure of the whole intertidal community (Southward, 1951; Southward, 1964; Hawkins, 1979; Hawkins, 1981; Hawkins and Hartnoll, 1985; Hill and Hawkins, 1991). In terms of energetics, limpets consume two thirds of the overall microalgal production, although littorinids can also significantly reduce algal film, especially high on the shore (Wright and Hartnoll, 1981; Hawkins *et al.*, 1992).

The feeding apparatus of prosobranch molluscs has been well studied (Hawkins *et al.*, 1989; Fretter and Graham, 1994). Herbivorous gastropods have a specialised feeding apparatus called the radula, which can have various morphologies. The rhipidoglossan radula of trochids consists of many fine teeth, which are used like a broom on the substrate (Steneck and Watling, 1982; Hawkins and Hartnoll, 1983). The taenioglossan radula of littorinids has a reduced number of stronger teeth and a less complex buccal musculature which reflects a change in method of feeding from sweeping to scraping

or rasping the substrate as a rake (Steneck and Watling, 1982; Hawkins *et al.*, 1989). Compared to taenioglossans, docoglossans have few shorter and harder teeth, due to high content in iron compounds. This used to be considered as an advanced feature, but recent phylogenetic work has suggested that the *Patella* gastropods are an ancient lineage. The docoglossans gastropods can use their radula as a shovel for scraping and excavating the substrate (Hawkins *et al.*, 1989).

Different feeding morphology leads to very distinctive methods and abilities of grazing on algae. From an extensive literature review Steneck and Watling (1982) classified molluscs into functional groups based on feeding apparatus and related their different diets to the grazing capabilities. Rhipidoglossan grazers are able to feed mainly on microalgae and filamentous algae on rock substrate or, alternatively, on epiphytic film of macroalgae. Diets of taenioglossans consist mainly of articulated coralline algae and leathery macrophytes where they can leave distinct graze marks, although it has been shown that most Littorinidae feed on epilithic biofilms (Norton *et al.*, 1990). Steneck and Watling (1982) suggested that docoglossans are able to graze on macrophytes, epilithic and endolithic microalgae, but it seems that microalgal film is the principal source of food for many species of these gastropods (Hawkins *et al.*, 1989).

Prosobranchs limpets can remove all algal sporelings, germlings and microalgae down to bare rock, while the pulmonate species can graze only the tops of the thalli leaving a the basal parts of algae on the rock surface (Underwood and Jernakoff, 1981). However the strict relationship between feeding apparatus and diet indicated by Steneck and Watling (1982) has not been confirmed in other studies. Raffaelli (1985) found that the classification of molluscs into functional groups adopted by them does not apply to all the species, as he found dietary differences within the same functional group. For example, most littorinids seem to feed on a variety of macroscopic and microscopic algae, but some of them can be highly selective grazers (Norton *et al.*, 1990; Otero-Schmitt *et al.*, 1997). The diets characterising molluscs can be more dependent on spatial and temporal availability of algae than on radular morphology (Underwood, 1984a).

The differing radula morphology, feeding mechanisms and grazing behaviour of molluscs result in a diverse exploitation of microbial films as food resource. As a

consequence, the spatial and seasonal distribution and abundance of intertidal microflora can be significantly affected by grazers. On tropical shores, during summer Cubit (1984) demonstrated that the exclusion of acmaeid limpets from the high intertidal, produced a thin film consisting of blue-green algae, diatoms and algal sporelings. However, grazing pressure may only exaggerate the seasonal and spatial variation of microalgal primary production (Dye and White, 1991). On high shore, physical stresses can minimise the impact of these grazers on microflora by limiting their feeding activity (Williams, 1994). The effects of grazers on microalgal community can be more or less effective depending also on competition (Underwood, 1984a), density levels (Mak and Williams, 1999) and feeding capabilities (Haven, 1973; Underwood and Jernakoff, 1981).

Mak and Williams (1999) suggested that the impact of grazers can be strong enough to affect the epilithic biofilm, even on the higher shore. When littorinids where excluded from the upper intertidal zone, standing crop of biofilm increased significantly in all the experimental sites. This suggested that littorinids can influence biofilm assemblages at this tidal height, despite the relatively harsh environmental conditions. The influence of grazing becomes much stronger lower on the shore where physical factors are less important and even during the hot season grazers prevented settlement and establishment of algal propagules and reduced algal abundance (Williams, 1993; 1994). Nicotri (1977) showed a great decrease in microalgal biomass at low intertidal rocky shores in presence of grazing. As a general consideration, it appears that grazers may play an important role in regulating the distribution and abundance of microalgae, but the impact is always mediated by physical factors, which determine the seasonal and spatial pattern of the microbial community (Haven, 1973; Underwood, 1984c; Anderson, 1995).

The different radula structure and feeding mechanisms found in herbivorous gastropods may possibly lead to partitioning of the food resource within the microalgal community (Underwood and Jernakoff, 1981). This could affect microalgal diversity and patchiness (Hawkins and Hartnoll, 1983; Sommer, 1999a). Although several researches have attempted to investigate the selectivity of algae by grazers (Nicotri, 1977; Hawkins and Hartnoll, 1983; Hill and Hawkins, 1991; Sommer, 1999a), this is difficult to clarify, mainly due to highly variable algal abundance and to technical

difficulties in analysing the diet of molluscan grazers (for example, the digestive process dissolves rapidly the soft components of the food ingested by grazers such as cyanobacteria, which can be highly underestimated).

Other components of macrobiota can influence the microbial community. Macroalgal canopies can indirectly affect the abundance of microalgae beneath by protecting them from insolation and desiccation stresses (Thompson, 1996). This should be an advantage for microalgal growth during summer, but no differences in abundance and species composition have been detected between areas underneath canopy and open bare rock (Hill and Hawkins, 1991; Thompson, 1996). The negative effect of sweeping the surface by the seaweeds could explain these results (Hill and Hawkins, 1991), but it is likely that limpet grazing has stronger effects on biofilms than macroalgae (Thompson, unpublished data). However, during winter, macrophytes appeared to limit the microalgal growth (Aleem, 1950; Thompson, 1996). The plates of barnacles provide habitat complexity which offers an escape for microflora from limpet grazing, although small littorinids can remove significant quantities of microalgae (Hill and Hawkins, 1991).

1.4 Techniques and methods in assessing microbial films

A wide variety of techniques, methods and protocols have been tested to quantify the biomass, abundance and species composition of the microbenthic community (Jones, 1974; MacLulich, 1986b; Snoeijs, 1991; Nagarkar and Williams, 1997; Norton *et al.*, 1998; Thompson *et al.*, 1999). Some of these techniques and relative methods are described below. Two major approaches have been used: direct identification as enumeration of microbial cells on rock chips and extraction of chlorophyll-a from rock chips or scraping as an index of biomass

1.4.1 Sampling methods

A variety of different methods have been used to collect biofilms from the rock surface. Nicotri (1977) sampled microalgae from the shore by scraping the surface with a toothbrush, and washing it periodically in glass vials filled with seawater and few drops of formalin as fixative. This method provides a sample containing mostly intact cells, which could be used to make direct counts microbial abundance. However,

estimations of microalgal biomass on the shore are very approximate because the endolithic component is not included in the sample (MacLulich, 1986b). A further disadvantage in brushing the rock surface is the efficiency of biofilm removal, which varies with the rock hardness and the capacity of the microorganisms to adhere to the surface (Hill and Hawkins, 1990; Nagarkar and Williams, 1997). Alternatively, the rock surface may be scraped with a sharp knife or blade, which allows the microalgae to be collected with minimal quantities of detritus (Underwood, 1984c). One drawback is that samples must be processed quickly before the chlorophyll degrades (MacLulich, 1986b). Jones (1974) found that the scraping method may seriously underestimate the microalgal population in terms of the number of cells, when compared with direct counts on the rock surface, mainly caused by breakage of cells into uncountable fragments. Both methods, toothbrushing and scraping, need also to be used only in good weather conditions, because in presence of wind, rain or rough sea there is the high possibility of loosing part of biofilm collected (Underwood, 1984c). Chiselling the rock to a thin layer is a very good method for preserving intact microbial film, including the endolithic component (Hill and Hawkins, 1990). Thus it is particularly suitable for the identification of microorganisms, but it can be a very destructive sampling, especially when many replicates must be collected (MacLulich, 1986b).

One method used for detaching microorganisms from marine sediments is to use cation-exchange resin with sodium deoxyxholate combined with sonication. This method recovered up to 98% of bacteria (Lucas *et al.*, 1996). Sonication is a method generally used for removing bacteria from artificial substrata such as steel or glass surfaces, although attention must be paid to the power and duration applied to the sample to avoid possible destruction of the cells and release of chlorophyll (Lindsay and vonHoly, 1997; Claret, 1998). Hirsch *et al.* (1995) adopted a series of physical and chemical methods to detach epilithic and endolithic microorganisms from rock samples. The samples were treated with a solution of NaCl and detergent followed by vortexing and low-power sonication. The solution should minimise charge interactions between cells and substratum and disrupt the protein binding of the cells. The authors suggested also that for organisms producing extracellular glycoproteins such as microalgae the concentration of the reagent and the strength of physical treatments must be increased, although trying not to cause damages to the cells.

The use of artificial substrata for studying microbenthic communities reduces many problems in sampling and processing. They can be easily transported to the laboratory without losing any component of the film and are more suitable for analysis, such as light microscopy and optical density analysis (MacLulich, 1986b). They need, however, a considerable amount of preparation and installation to secure them on the shore and, more important, the microbial community which develops may be very different from the natural substrata (Snoeijs, 1991).

1.4.2 Chlorophyll extraction

Chlorophyll-a concentration has been long used to estimate microalgal standing stock in phytoplankton (Parsons and Strickland, 1963) and on soft shores and subtidal sediments (H.M.S.O., 1983; 1984). It is considered a reliable, repeatable measure of microalgal biomass for epilithic microphytobenthos (Underwood, 1984c). This technique consists basically of immersing samples in solvents which will extract chlorophyll from the algal cells. A variety of different solvents and protocols have been used (e.g. H.M.S.O., 1983). Additional steps during processing such as sonication and centrifuging have also been used in some studies, but without any significant increases in extracted chlorophyll (Thompson *et al.*, 1999).

The concentration of chlorophyll released in the solvent is then determined by spectrophotometric analysis. For chlorophyll-a absorbance is measured at 665 nm and background absorbance at 750 nm is then subtracted (e.g. H.M.S.O., 1983; Thompson *et al.*, 1999). This analysis can give a good estimate of the amount of chlorophyll in the biofilm, although other chlorophyll pigments (*b* and *c*) and degradation products may affect the results if present.

One problem in considering chlorophyll-a as an indirect measure of microalgal biomass is that the concentration of this pigment in the chloroplasts varies from time to time and from place to place, depending upon physiological state of the cells and the environmental conditions (Blanchard *et al.*, 1997).

Additional sources of variation may result from the variety of protocols adopted for the analysis. The state of hydration of epilithic biofilms before the extraction process has been shown to considerably influence the amount of chlorophyll extracted, although

this factor has not been taken into consideration in most studies (e.g Underwood, 1984c; Hill and Hawkins, 1990; Dye and White, 1991). Thompson *et al.* (1999) observed that hydrated samples released at least twice more chlorophyll-a than dry biofilms, and they suggested that very low chlorophyll concentrations during summer season reported by some authors could be the consequence of the extremely dry conditions experienced the microbial community at this time of the year.

Storage might affect the efficiency of extraction. Light is obviously an important factor affecting photosynthesis and it is widely recommended that samples must be kept in the dark prior to and during extraction (H.M.S.O., 1983). Chlorophyll-a concentration appears to decrease if the samples are kept for more than 3 days without being analysed, while there are no significant differences when they are processed fresh or stored frozen for 1 month (Thompson, 1996; Nagarkar and Williams, 1997).

The efficiency chlorophyll extraction depends also on the type of solvent used. Many of studies have concluded that chlorophyll-a extraction with methanol is the most efficient method, although the efficiency depends also on the protocol adopted (Hill and Hawkins, 1990; Simon and Helliwell, 1998; Thompson *et al.*, 1999). The efficiency of this solvent after a single extraction is 100% when heated with the sample for 2 minutes and then cooled for 12 h and is 95% efficient when used cold (Nagarkar and Williams, 1997). The cold solvent is advocated because it is less hazardous. Thompson *et al.* (1999) demonstrated that hot, room temperature and cold methanol extracted over 96% chlorophyll from rock samples, but they suggested the use of 90% methanol at room temperature, as this technique requires less complicated operations in the procedure. Methanol is a hazardous chemical, but is relatively inexpensive, allowing use for routine analyses. For the reasons above described, methanol has been recommended as the best solvent for chlorophyll extraction from epilithic biofilms (Nagarkar and Williams, 1997; Thompson *et al.*, 1999).

Ethanol can be efficient at extracting chlorophyll (up to 95%) from microalgae, but extraction is better when used hot (Nagarkar and Williams, 1997). Thompson *et al.* (1999) found this solvent less efficient than methanol, as it released 88% of the chlorophyll available. Chlorophyll from cyanobacteria seems to be much less released by this solvent (Marker, 1994), maybe because is more difficult to extract. Ethanol is

much less toxic than methanol, but it is also more expensive. Acetone is less efficient solvent than methanol or ethanol, and extracted only half of the total chlorophyll available (Hill and Hawkins, 1990; Thompson, 1996; Thompson *et al.*, 1999). However it can be used to quantify the breakdown products of chlorophyll (Thompson *et al.*, 1999). The efficiency of different solvents also varies according to the algal species present. For example, Nagarkar and Williams (1997) found it more difficult to extract chlorophyll-a from encrusting cyanobacteria than from loosely attached bacterial forms.

In conclusion, the choice of a solvent and the method adopted should consider the efficiency of extraction, the costs and, more important, the potential hazard for operators. Thompson *et al.* (1999) suggested that the optimal method is the cold extraction with methanol 90%, which will be used in this thesis.

1.4.3 Scanning electron microscopy (SEM)

Scanning electron microscopy is an extremely useful technique for *in situ examination* of microscopic communities (MacLulich, 1986b; Hill and Hawkins, 1990) and for determining their three dimensional structure (Veltkamp *et al.*, 1994). SEM offers an excellent resolution of biofilm components, although sample preparation (fixation, dehydration) can cause artefacts (such as condensation of glycocalyx) which obscure cells structures (Surman *et al.*, 1996). Norton *et al.* (1998) pointed out that SEM analysis can exaggerate the diatom component in microbial film, while fungi and bacteria are difficult to visualise. This observation may also explain the difference in the biofilm composition found in limpet gut contents (analysed using contrast phase microscopy after acid cleaning and centrifugation), when compared to the rock surface (analysed using SEM) on which they grazed (Hill and Hawkins, 1991). The abundance of microalgal cells estimated by SEM is generally correlated with chlorophyll-a concentrations, thus these techniques can be reliable for determining microalgal abundance (MacLulich, 1986b; Hill and Hawkins, 1990; Thompson, 1996). Fixation and drying living material prior the SEM examination is an important step in the analysis. Thus different methods have been tested.

Rock chips can be fixed in 2.5% glutaraldehyde solution in filtered seawater for approximately 3 hours and then air dried overnight, before coating with gold palladium

(MacLulich, 1986b; Hill and Hawkins, 1990). This method showed a greater species number and abundance of biofilms (Nagarkar and Williams, 1997). It can be used for routine quantitative analysis because it requires minimal preparation time compared with the other methods. Another advantage is that components of the biofilm are not damaged or detached. One possible drawback is that some species, such as cyanobacteria (Norton *et al.*, 1998) and other fragile microorganisms may not be visualised (MacLulich, 1986b). Hill and Hawkins (1990) observed a high diversity in epilithic diatoms and filamentous algae using this method, but no variation in bacteria.

Critical point drying is more time consuming, as it requires dehydration in an alcohol series followed by critical point drying. Veltkamp *et al.* (1994) suggested a freeze dehydration technique for fixing freshwater epilithic specimens. This technique consists of repeated cycles where the samples are frozen and then cooled in the fridge. The advantages of this technique are less procedural time involved and lower costs than the traditional method for dehydration through an ethanol series. However, critical point drying can cause more damage to the specimens than the air drying method, as Nagarkar and Williams (1997) observed on the alga *Hapalospondigion*. It can also underestimate the biofilm diversity by removing species from the surfaces (Hill and Hawkins, 1990; Norton *et al.*, 1998). However some specimens are more clearly visible and easier to identify using this technique. Hill and Hawkins (1990) observed a greater abundance of algal germlings with this method compared with air dried samples, but fewer diatoms.

Cryo-stage electron microscopy allows samples to be viewed in a hydrated state, revealing the three dimensional structure of the biofilm (Hill and Hawkins, 1990). Samples are freeze-dried and then observed under low temperature criostage SEM. As for critical point drying, this method can cause damage to microorganisms. For instance, Nagarkar and Williams (1997) noticed that the surface morphology of the filamentous cyanobacteria was not well preserved. This technique allows better observation of the bacterial component of biofilms compared with air drying, but reveals fewer species due to the organic matrix (Hill and Hawkins, 1990).

1.4.4 Environmental scanning electron microscopy (ESEM)

Another recently developed technique is environmental SEM (ESEM) which enables

structural analysis of hydrated organisms (Walker *et al.*, 1998). ESEM is a modified version of SEM where the specimen chamber is differentially pumped allowing it to operate with up 10 torr of water vapour, thus normal sample preparation such as fixation, dehydration and staining are not necessary (Surman *et al.*, 1996). Another advantage of ESEM is that the shrinkage and artefacts seen in SEM do not occur, although the electron beam may cause damage after a few minutes of analysis.

1.4.5 Epifluorescence microscopy

Epifluorescence light microscopy has often been used to enumerate microalgae (Nicotri, 1977; MacLulich, 1987), but is difficult for identification of microorganisms. Nagarkar and Williams (1997) observed filamentous and unicellular cyanobacteria while the other specimens were not clear. This technique is also not suitable for thick biofilms, due to lack of light penetration.

This technique, as described by Jones (1974), is based on the property of chlorophyll-a to fluoresce if excited by blue light of a certain wavelength (435) by means of different filters allowing fresh samples to be observed directly under a microscope. Additional use of acridine orange can be useful for differentiating green algae from cyanobacteria, as this stain links the DNA in the prokaryotic forms masking completely the chlorophyll fluorescence, and resulting in a bright green image. While any eukaryotic cells will fluoresce as normal in red, other species like red algae give yellow fluorescence without staining. Samples can also be fixed in glutaraldehyde and then stained (Marszalek *et al.*, 1979). Other components of biofilms can be visualised by using different stains. The mucopolysaccharidic matrix can also be labelled by the lectin concanavalin-A (Norton *et al.* 1998). Another dye used for bacterial counts is fluoresciamine, which reacts specifically with protein and aminoacids of microbial cells (Poglazova *et al.*, 1996).

Wolfaardt *et al.* (1991) tested the use of DAPI (4'-6-diamidino-2-phenylidole), a sensitive fluorescein DNA stain which allows the quantification of sessile bacteria. The stained bacteria fluoresced with a bright blue colour while all the other organisms or materials appeared pale yellow. One advantage is the short time needed for sample preparation and the longevity of fixed samples (stored up to 24 weeks). Unfortunately, the method only visualises bacteria, and it is not possible to discriminate between

living and non-living cells.

Becker *et al.* (1997a) measured fluorescence intensities of epilithic microalgae grown on clay tiles by analysing the surface directly using an epifluorescence microscope photometer. This technique has the advantage of being non-destructive, enabling biofilms to be studied intact and then returned to field or laboratory experiments. This method is very suitable for flat rock surfaces, but high magnification cannot be achieved with rougher substrata, because of the short distance between the lens and the surface (Jones, 1974). As for SEM, this technique gives a good estimate of microalgal abundance, and has been shown to give a good correlation with chlorophyll-a content and *in vivo* fluorescence, although there were more variability among individual measurements in the latter (Becker *et al.*, 1997a). Epifluorescence microscopy is not suitable for species identification of microbial community, as very often it is not possible to identify microorganisms even to generic taxonomic level. In addition MacLulich (1986b) observed that this techniques only works satisfactorily with fresh living microalgal samples, as the fluorescence intensity decreases rapidly 1-2 h after collection and also after short exposure to ultraviolet light it declines to zero. Thus he did not advocate this technique for routine sampling.

1.4.6 Confocal laser scanning microscopy (CLSM)

Confocal microscopy, which has been only recently developed, allows observation of the biofilm together with the evidence of biofilm / substrata interactions (Surman *et al.*, 1996). Biofilms can be observed in detail by using a variety of fluorescent antibodies, lectins and nucleic acid stains which label the different microbial components such as bacteria, microalgae and exopolymers (Lawrence *et al.*, 1998). CLSM seems to have several advantages compared to traditional microscopy. It gives clear images of microorganisms embedded in the mucopolysaccharidic matrix and can discriminate between living and dead cells. Furthermore, the three dimensional structure of thick biofilms can also be observed (Norton *et al.*, 1998).

1.4.7 Transmission electron microscopy (TEM)

Transmission electron microscopy allows observations of biofilms at high resolution through thin sections of samples. The samples, after being fixed and dehydrated in an alcohol series, are then embedded in resin and cut in ultrathin sections before finally

staining for visualization (Surman *et al.*, 1996). This techniques is very useful for looking at the endolithic component of microbial communities.

1.4.8 Atomic Force microscopy (AFM)

AFM has mainly been used for studies of microfouling and it requires hydrated samples as for ESEM. As described in (Surman *et al.*, 1996), it is a form of probe microscopy which scans the surface topography of the samples at high resolution, giving a very detailed structural topology of bacterial cells. This also enables visualisation of the 3-D structure of the biofilm and also enables precise measurements of individual microorganisms to be made. Unfortunately the visualization rapidly disappears with increasing dehydration of the specimens. Although it has been applied for measuring microtopography of a variety of substrata (Mechaber *et al.*, 1996; Hershko *et al.*, 1998), it has not been tested for analysis on epilithic biofilms.

1.4.9 Infrared photography

This technique is based on the phenomenon that chlorophyll-a reflects efficiently in the infrared band (0.7-1.3 μm) and special cameras are used to take infrared photographs. Very little is known about possible application of this technique for studying microalgal communities. However, infrared photography has been used to monitor microalgae and cyanobacteria in old monuments (Van Der Molen *et al.*, 1980).

1.4.10 Electron transport system activity (ETS)

A good system to quantify the relative abundance of the autotrophs (microalgae and cyanobacteria) and heterotrophs (bacteria) of microbial film is the analysis of the electron transport system activity (ETS), as an indirect index of respiration. ETS is the activity of enzymatic system which controls the respiration in mitochondria and microsoms. ETS can be measured quite easily using spectrophotometric analysis (Christensen and Parson, 1980). Aristegui and Montero (1995) demonstrated for oceanic microbial communities a significant positive correlation between respiration and ETS activity. Bhosle *et al.* (1994) analysed the ETS activity of marine biofilms grown on steel panels and found a significant correlation with relative measurements of chlorophyll-a. Relexans (1996) found some possible problems in relating ETS activity to respiration in marine system and suggested the method could be better used as an index of biomass.

1.4.11 Techniques used for analysing endoliths and lichens

Endolithic microorganisms can be studied using several types of microscopy. They have been successfully observed using scanning electron microscopy operating in back scattered electron mode (SEM-BSE). Fixation of these samples is the same as for normal SEM, then the rock substrata are embedded in resin, cut in thin transverse sections, carbon coated and analysed under SEM-BSE (Wierzchos and Ascaso, 1994; Ascaso *et al.*, 1998). Another technique is confocal laser scanning microscopy (CLSM), which is very useful for visualising endolithic microorganisms in their natural undisturbed condition (Ascaso *et al.*, 1998). Samples prepared for the CLSM must be stained with fluorescent stains such as fluorescein isothiocyanate or rhodamine isothiacyanate in order to visualise cyanobacteria.

1.4.12 Standardisation of methods

A major problem in sampling epilithic microbial film is their considerable spatial variability in abundance and biomass on the shore. Microbial distribution is characterised by a high level of patchiness on rocky shores (MacLulich, 1987; Hill and Hawkins, 1991; Thompson *et al.*, 1996). Thus it is really important to determine the best sample size in order to get a good estimate of microbial populations. Hill and Hawkins (1990) suggested that for estimates of microbial diversity and species diversity under SEM a minimum sample area of 2 mm^2 randomised over a replicated number of rock chips should be used. However, for other techniques such as chlorophyll-a extraction the area of each replicate sample can be much larger, generally over 3cm^2. Spatial variability is therefore integrated over larger areas, thus requiring a smaller sample size.

A problem related to the determination of the final chlorophyll-a concentration per unit area is the quantification the total surface area of the sample. Base area can be easily estimated using an image analysis software, but it is accurate only with very smooth and flat substrata, as the measurements are taken in two dimensions. Rock surfaces very often present a complex three dimensional topography, thus the total area can be significantly underestimated, leading to overestimation of the chlorophyll estimated in a sample, particularly when different rock types are compared (Hill and Hawkins, 1990; Thompson *et al.*, 1999). This does not represent, however, a major problem when chlorophyll-a is used for broad scale comparisons of primary productivity.

A recurring problem limiting our ability to compare data on epilithic microbial communities between different studies is that sampling methods, techniques and protocols used for the quantitative and qualitative analyses vary considerably. Attempts have been made by some authors to compare the effectiveness of different techniques and methods and suggest a common protocol (MacLulich, 1986b; Hill and Hawkins, 1990; Nagarkar and Williams, 1997; Thompson *et al.*, 1999).

1.5 The importance of rock substratum on the rocky shore ecology

The physical and chemical nature of the substratum plays an important role in the colonisation and subsequent establishment of the benthic communities (Richmond and Seed, 1991). For example, the surface tension of substrata strongly affects the adhesion of several organisms such as bacteria, algae and invertebrates (reviewed by Callow and Fletcher, 1994; Taylor *et al.*, 1997). However, all surfaces become rapidly modified through the adsorption of "conditioning films" which may influence subsequent adhesive events associated with the permanent attachment of organisms (Wahl, 1989). For macrobiota, various studies have investigated the influence of substratum roughness (Crisp and Barnes, 1954; Crisp, 1974; Le Tourneux and Bourget, 1988; Holmes *et al.*, 1997), porosity (DenHartog, 1972; Caffey, 1982), chemical composition (Anderson, 1996), colour (Holmes *et al.*, 1997) and temperature (Raimondi, 1988). In contrast to the macrobiota, very little is known on the interactions between substrate type and microbenthic communities.

Microalgal standing stock and abundance has been shown to be much higher on rocks characterised by a higher porosity and microrugosity, such as sandstone, than on smoother limestone, basalt or slate (Blinn *et al.*, 1980; Edyvean *et al.*, 1985). Thompson *et al.* (2000) observed the same pattern on differing rugosity of limestone rocks. The remarkable differences shown by Edyvean *et al.* (1985) disappeared completely in the following weeks, suggesting that the typical microtopography and chemical composition of the substrata, which possibly affected the initial colonisation, were soon modified and minimised by a uniform film of organic matter.

Furthermore, other properties of the substratum are likely to be involved in the process, such as the surface tension of the substratum. High surface tension, typical of

hard substrata as granite, can interfere with the adsorption of preconditioning film and the bacterial adhesion, resulting in low colonisation rates (Hamilton and Duthie, 1984). Topography also appears to influence the species composition and diversity of microalgae. Colonisation generally starts in small pits and microcrevices and then propagates on smoother areas (Hamilton and Duthie, 1984; Miller *et al.*, 1987). The general texture of surfaces also appears to influence species composition within microalgal communities. Adnate diatoms, which are tightly attached to the substratum have been found on rougher surfaces, while stalked forms, filamentous colonies and long chain form diatoms dominated smooth substrata (Hamilton and Duthie, 1984; Miller *et al.*, 1987; Snoeijs, 1991). The opposite result was obtained by Sabater *et al.* (1998), who observed that adnate forms were more abundant on the flat surfaces. These contrasting observations may be explained by large differences in the type of substratum considered for the study and in the environmental conditions. The microtopography can also be modified by biological features such as barnacles (Thompson *et al.*, 1996). Further investigations are clearly needed for a better understanding of the factors interacting between the substratum and the microbial film.

1.6 Rationale of the thesis

The overall aim of this study is to unravel the extent that rock substratum influences the abundance and composition of intertidal microbial films, and its relative importance compared to other factors, primarily tidal elevation, season and grazing.

The south coast of England, with its wide range of shores of different geology, provides an ideal site to investigate this type of interactions. With the exception of an excellent qualitative study by Aleem (1950), there is little information on microbial communities of English coasts. Therefore, the first objective of this study (Chapter 2) was to describe epilithic microbial communities on rocky shores of differing geology along the south coast of England. These shores consist of rocks of contrasting physical and chemical features, ranging from soft chalk to hard feldspatic granite. The abundance and composition of biofilms living on each type of substratum was estimated using a nested sampling design. This approach allowed observation of patterns at spatial scales from 100's km to 100's μm. Two different tidal heights were included as an additional factor in the design, to determine if patterns in distribution of

microbial communities on rock types were consistent vertically on the shore. The study was carried out in summer and winter, as seasonal changes can dramatically affect the abundance and composition of microbial films (Aleem, 1950; Hill and Hawkins, 1991; Thompson *et al.*, 2000).

Next, at sites selected on the basis of the broad scale study (Chapter 2), specific comparisons between biofilms from different rock types within the same shore were made (Chapter 3). These comparisons aimed to minimise the effects of other physical and biological variables acting on the shore and enhance the detection of smaller scale patterns associated with rock type. The abundance and composition of microbial films was described and compared for three different pairs of contrasting rock types (chalk vs. flint, limestone vs. chert, oil shale vs. dolomite). Where possible, the sampling was spatially replicated using a nested approach.

The effect of the substratum on microbial colonisation and succession has been widely investigated for biocorrosion and fouling studies using artificial substrata such as alloys, glass, PVC (Marszalek *et al.*, 1979; Edyvean *et al.*, 1985; Anderson, 1995; Kohler *et al.*, 1999), whilst very few studies used natural rock substrata in field experiment (Blinn *et al.*, 1980; Niell and Varela, 1984; MacLulich, 1986a). Colonisation of artificial materials can be very different to natural rock substrata (Edyvean *et al.*, 1985; Cattaneo and Amireault, 1992; Anderson, 1995), therefore the results cannot be easily applied to natural processes. The aim of the work described and discussed in Chapter 4 was to provide further understanding of the processes regulating microbial colonisation and primary succession on rocky shores. The influence of the rock substratum on colonisation by microbial films was formally compared using rock substrata with contrasting physical and chemical features (chalk vs. dolomite, selected on the basis of results from Chapter 2 and 3). This experimental approach allowed a better control and standardisation of the other environmental variables affecting microbial communities. The experiment was spatially and temporally replicated so that interactions between microbial communities and the substratum were analysed taking into account the natural seasonal fluctuations in the abundance and composition of these micro-organisms.

In Chapter 5 further interactions between rock type and grazers were considered.

Gastropod limpets, which are the major grazers on rocky shores (Hawkins and Hartnoll, 1983; Hawkins *et al.*, 1992), can effectively reduce the abundance of epilithic microalgae and modify their distribution (Underwood, 1980; Underwood, 1984a; Underwood, 1984c; Hill, 1990). The type of substratum can modulate the impact of grazers feeding on microalgae. Certain properties of the rock, such as topography and surface hardness, can affect their efficiency in feeding on microalgae by rasping and excavating the rock surface. The aim of this study was therefore to investigate the effect of grazing on biofilms and its interactions with the type of the underlying rock substratum. For this study the same type of rock substrata and methodology described in Chapter 4 was adopted, but with the experimental exclusion of limpets. This design tested the effect of the rock substratum on abundance and composition of microbial films in presence/absence of grazing.

On rocky shores, ecological processes often occur as a result of complex interactions between physical and biological variables rather than a single driving force. Microbial communities might therefore be affected by the rock substratum depending on the presence and strength of the other environmental factors. In Chapter 6 the extent to which several physical and biological factors can combine to influence intertidal microbial communities will be discussed by integrating the outcome of the present study with previous research.

Chapter 2

A broad-scale survey of epilithic biofilms along the south coast of England

2.1 INTRODUCTION

Epilithic biofilms consist of assemblages of different microorganims, including bacteria, microalgae and protozoa, often incorporated in a polysaccharidic matrix, which coat the rock substratum. These assemblages have been well described in terrestrial (Perkins and Tsentas, 1976; Ascaso *et al.*, 1998), freshwater (Blinn *et al.*, 1980; Lock, 1993) and marine (see Cooksey and Wigglesworth-Cooksey, 1995 for a review) ecosystems. In the sea, however, biofilms have been investigated mainly on man-made structures (Baty *et al.*, 1996; Becker *et al.*, 1997; Martin-Cereceda *et al.*, 2001) or on artificial substrata (Anderson, 1995; Walker *et al.*, 1998; Sommer, 1999), due to technical difficulties in sampling and analysing natural communities *in situ* (MacLulich, 1986b). On rocky shores, fewer studies have been carried out on natural microbial films, although their ecological importance has been already widely recognised for some time (Aleem, 1950; Nicotri, 1977; Underwood, 1979; Hawkins and Hartnoll, 1983; Underwood, 1984b; Hill and Hawkins, 1991; Thompson, 1996).

Intertidal epilithic biofilms are dominated by microscopic phototrophs, mainly diatoms and cyanobacteria. These microorganisms play a key role in the ecology of rocky shore communities, being a primary food resource for gastropod grazers (Hawkins and Hartnoll, 1983; Hawkins *et al.*, 1989; Norton *et al.*, 1990) and the site for settlement of macroalgae and marine invertebrates (Keough and Raimondi, 1995; Satuito *et al.*, 1997; Thompson *et al.*, 1998; Callow, 2000). Moreover, they are the major contribution to primary production which is consumed within the community, in contrast to macroalgae whose production is largely exported as detritus (Mann, 1982; Hawkins *et al.*, 1992; Bustamante *et al.*, 1995).

The abundance and distribution of microbial films on rocky shores has been investigated in both temperate and tropical systems, showing clear spatial and temporal patterns. Variation in the abundance and composition of biofilms occur at different scales, between temperate and tropical regions (Thompson, 1996 *versus* Mak, 1999), shores within a region (Thompson, 1996; Jenkins and Hartnoll, 2001) and, at a very small scale, between patches of few cm^2 (Hill and Hawkins, 1990; Hill and Hawkins, 1991). Within a shore, the distribution of biofilms varies also along the vertical

gradient of tidal elevation (Underwood, 1984b; MacLulich, 1987). Strong seasonal patterns in biofilm abundance have been described (Underwood, 1984c; Hill and Hawkins, 1991; Thompson, 1996; Jenkins *et al.*, 2001). In temperate regions during winter-early spring the abundance of microbial films is at its maximum with diatoms dominating, while in summer the overall microalgal standing stock decreases significantly, and becomes dominated by cyanobacteria (Thompson, 1996).

Observed patterns are likely to be caused by complex and dynamic interactions between physical, chemical and biological processes. Several authors have attributed causes of these patterns to specific features such as desiccation and thermal stresses (Castenholz, 1963; Underwood, 1984b; MacLulich, 1987), light (Richardson *et al.*, 1983; Gektidis, 1999), light (Fogg, 1973; Peletier *et al.*, 1996; Hill *et al.*, 1997; Blanchard *et al.*, 1997), wave exposure (Bustamante *et al.*, 1995; Thompson, 1996) and grazing (Underwood, 1984a; Hill, 1990; Mak and Williams, 1999). However, very little research has been addressed to investigate the potential influence of the rock substratum on microbiota.

The effect of some physical and chemical features of the rocks such as surface texture, colour and chemical composition has been already recognised to play an important role in the settlement and distribution of many macroalgae and sessile invertebrates (Caffey, 1982; Raimondi, 1988; Richmond and Seed, 1991; Anderson, 1996; Holmes *et al.*, 1997). Similarly, it is likely that microbial communities are sensitive to specific characteristics of the underlying substratum. Surface roughness and microtopography might provide protection from desiccation, water movement and refuges from grazers. Porous rocks are more permeable to water and retain it for longer. This can reduce considerably the desiccation and thermal stresses, particularly in summer. Blinn *et al.* (1980) and Edyvean *et al.* (1985) found that microalgal standing stock was much higher on rocks characterised by a higher porosity and microrugosity, such as sandstone, than on smoother limestone, basalt or slate. The efficiency of hardness of rocks can modulate the effect of grazing. For example, limpet radula would be expected to graze more efficiently on soft and friable substrata such as chalk and sandstone. The chemical composition of the rocks can be also important for the development and colonisation of endolithic microorganisms, that can only penetrate

carbonate rocks (Le Campion-Alsumard, 1975; Le Campion-Alsumard, 1989; Peyrot-Clausade *et al.*, 1995).

The overall aim of this study was to investigate potential effects of the rock substratum on natural, intertidal microbial communities by comparing shores of different underlying geology. The relative importance of rock type was compared to that of different tidal heights and seasons. Microalgal standing stock, expressed as chlorophyll-a, and species composition were quantified.

Microbial communities were compared on a broad spatial scale, along 300 km of coast in the south of England. This coast offers a large variety of rocky shores of contrasting geology, including chalk, limestone, sandstone, old red sandstone and granite. The effect of the rock substratum on biofilms was described on a range of different spatial scales, from 100's km to cm's, in two tidal zones (upper eulittoral zone, littoral fringe, (*sensu* Lewis, 1964) and in two seasons (summer, early spring). These two seasons were chosen to compare the influence of the rock substratum during the minimum (summer) and maximum (early spring) abundance of biofilms, as observed in previous work (Hill and Hawkins, 1991; Thompson, 1996; Jenkins *et al.*, 2001). The two shore levels were selected because they offer different environmental conditions affecting microbial films, as it was expected that the main limiting factor for these communities is grazing by limpets at lower level, and desiccation stresses at the upper level (Thompson, 1996). Microbial assemblages on these upper shore levels were also likely to be more stressed by physical factors associated with emersion which could be ameliorated by characteristics of some rock types (e.g., chalk) and made worse by others (e.g. granite). Physical and chemical properties of the rocks were also described and examined in relation to microbial communities.

This study also provided for the first time a broad-scale, comprehensive description of intertidal epilithic biofilms, as previous investigations were primarily carried out at local scale (but see Jenkins *et al.*, 2001). Also, very little and largely outdated information on microbial films of the south coast of England was available from literature (Aleem, 1950). Most of the recent studies on epilithic biofilms of British shores have mainly restricted to the Isle of Man (Hill, 1990; Thompson, 1996; Jenkins and Hartnoll, 2001, but see Roberts, 2002 and Jenkins *et al.*, 2001), where

environmental conditions are know to be very different from the English Channel coast. This chapter also helped in selection of sites for more detailed surveys (Chapter 3) and experimental studies (Chapter 4 and 5).

2.2 MATERIALS AND METHODS

2.2.1 Study sites

The study was carried out on several rocky shores of different geology along the south coast of England (Figure 1). The south coast of England was chosen because its coastal geology offers a large variety of rock types of different origin (sedimentary, igneous, metamorphic) and distinctive physical and chemical properties (hardness, porosity, grain size, colour, pH).

Most of the accessible rocky shores from the Isle of Wight to the south of Cornwall were visited during February and March 1999, in order to select a series of sites with differing geological nature and approximately similar exposure (moderately exposed to exposed), orientation and macrobiota. Five different rock types were considered: chalk, limestone, sandstone, old red sandstone and granite. For each type of substratum two shores were selected. The distance between them was generally between 4 and 10 km. Only chalk shores were further apart (approximately 20 km). The main physical and biological properties of the rocky shores sampled are summarised in Table 2.1 and 2.2.

Chalk shores (Freshwater Bay and Culver Cliff, Isle of Wight)

The rocky shores in Freshwater Bay and Culver Cliff have very similar physical and biological features. They consist of extensive rocks that have fallen from a steep cliff into the sea. The rocks of the intertidal zone are mainly Cretaceous fossiliferous chalk with flint inclusions. Chalk is a very porous, soft and friable rock, subjected to a high degree of physical and biological erosion. On these shores the surface is irregular, often exposing virgin substratum after slabs are eroded off by the waves during stormy weather. There are also inclusions of flint, which is an extremely hard, black

metamorphic rock, similar to quartz and showing a very smooth and compact surface. A thin, brownish porous layer can sometimes cover the surface.

Table 2.1 – General physical features of the study sites (see Figure 1 for locations).

Shore location	Rock type	Orientation	Wave exposure	Slope
Freshwater Bay	Chalk	S	Exposed	10°
Culver Cliff	Chalk	S	Exposed	10°
Lulworth Cove	Limestone	S-SW	Exposed	20°
Portland Bill	Limestone	SW	Exposed	10°
Salterton Cove	Red sandstone	S-SE	Moderately exposed	10°
Otterton Cove	Red sandstone	S-SE	Moderately exposed	10°
Wembury Bay	Old red sandstone	S-SW	Moderately exposed	20°
Heybrook Bay	Old red sandstone	S-SW	Moderately exposed	20°
Lamorna Cove	Granite	S	Exposed	20°
Portgwarra	Granite	S	Exposed	20°

Table 2.2 – Macrobiota at the study sites: mean abundance of limpets, trochids and barnacles on study sites. Values were averaged across plots and sampling occasions. Standard errors are shown in brackets.

Shore location	Limpet density (N/m^2)		Trochid density (N/m^2)		Barnacle cover (%)	
	Littoral fringe	Upper eulittoral	Littoral fringe	Upper eulittoral	Littoral fringe	Upper eulittoral
Freshwater Bay	1.2 (0.4)	91.2 (9.2)	0	0	0	0
Culver Cliff	0.8 (0.4)	41.2 (2.4)	0	0	0	2.3 (0.5)
Lulworth Cove	23.8 (3.7)	142 (8.8)	0	0	15.2 (2.4)	21.2 (1.8)
Portland Bill	0	139.6 (11.6)	0	0	7.6 (1.7)	25.2 (3.2)
Salterton Cove	2.6 (0.2)	44 (6)	0	9.2 (2)	9.3 (1.5)	27.1 (9.9)
Otterton Cove	1 (0.5)	99.2 (11.2)	0	8.4 (2)	6.5 (1.1)	32.8 (2.8)
Wembury Bay	0	30.8 (3.4)	0	6 (1.6)	1.1 (0.4)	28.4 (2.4)
Heybrook Bay	0	24.4 (7.2)	0	0	2.4 (0.6)	15.4 (2.1)
Lamorna Cove	1.8 (.9)	36.4 (4)	0	0	0.6 (0.3)	11.3 (1.6)
Portgwarra	6.8 (1.6)	35.2 (4)	0	0	0.9 (0.3)	14.7 (1.5)

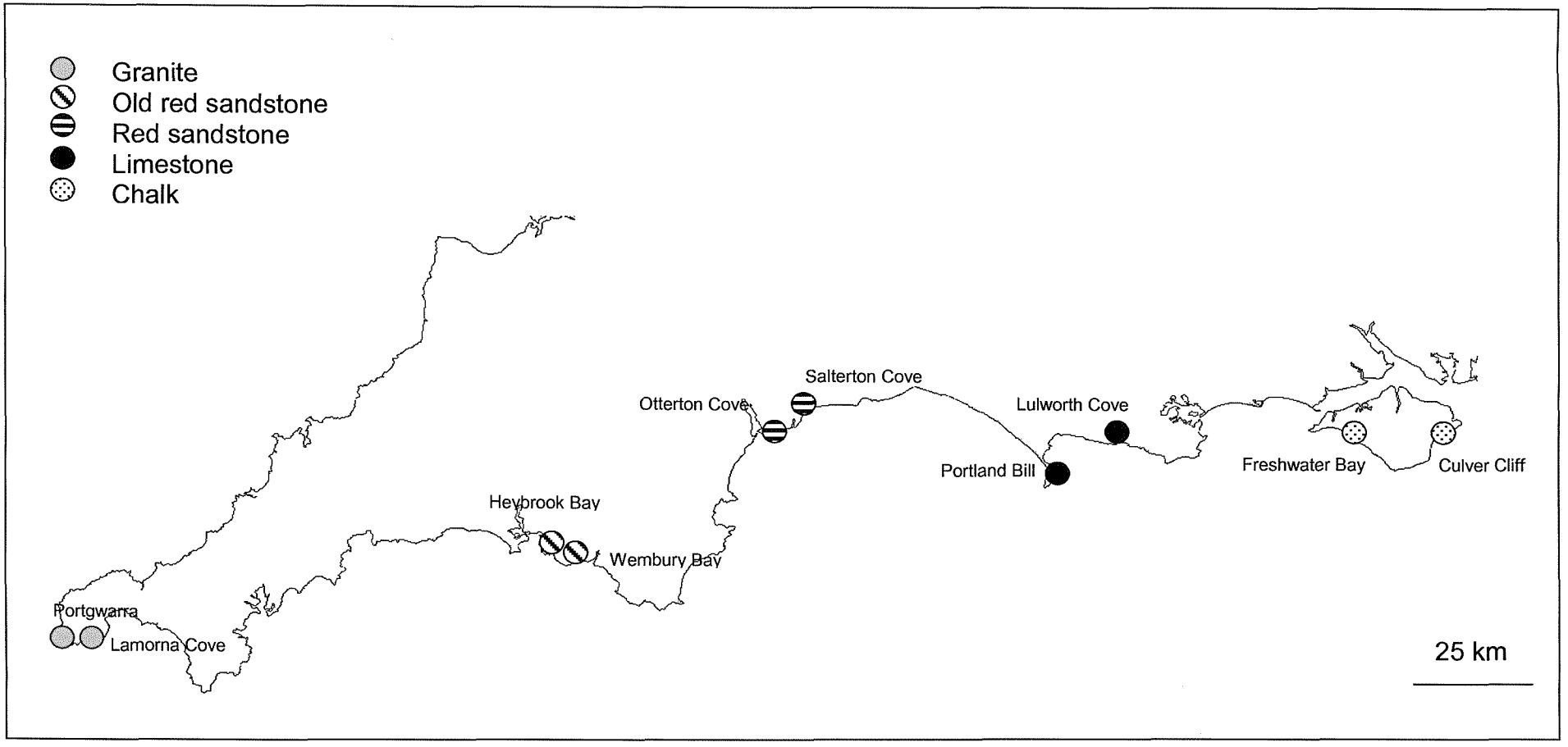


Figure 2.1 - Map of the study sites selected for the broad scale survey along the south coast of England.

The macrobiota on the eulittoral zone (Lewis, 1964) showed typical features of exposed British shores (Lewis, 1964; Raffaelli and Hawkins, 1996), being dominated by relatively high density of limpets (*Patella vulgata*; *P. depressa*). Barnacles (mainly *Chthamalus* spp.) were very rare, however, often only found on flint nodules. Higher on the shore periwinkles (*Littorina saxatilis*) were very abundant. Macroalgae (fucoids and kelps) were present only lower on the shore, below the areas sampled.

Limestone shores (Lulworth Cove and Portland Bill, Dorset)

The shore at Lulworth Cove faces south and consists of moderately sloping bedrock. The surface is very rough, with numerous pits of various diameter and depth. Portland Bill is characterised by various rock platforms facing south-west and connected by gentle slopes. The topography of the rock is less complex than at Lulworth Cove.

Both shores consist of a late Jurassic oolithic limestone (ooids cemented by calcite), named Portland stone. This sedimentary rock is porous but relatively hard and resistant to erosion. In some areas numerous inclusions of chert rock are exposed to the surface. This is a metamorphic rock much harder and less porous than limestone. The biota was typical of that predicted for exposed shores, with a limpet/barnacle dominated community (*P. vulgata, P. depressa*, *Chthamalus* spp.) on mid-shore and a broad zone in the high intertidal dominated mainly by the red alga *Porphyra* sp. and periwinkles (*L. saxatilis*).

Sandstone shores (Salterton and Otterton Cove, South East Devon)

The site at the east side of Salterton village consists of large boulders (1.5-2 m diameter) alternating with bedrock. It is gently sloping and faces south. The rock is a very soft devonian red sandstone. The surface, easily disintegrating, has a rough and irregular aspect. Despite the relatively friable nature of this rock type, the shore supported a high cover of barnacles and limpets (*P. vulgata, P. depressa*), and numerous topshells (*Gibbula umbilicalis*, *Osilinus lineatus*). The presence of small patches of macroalgae (*Fucus* spp.) indicated moderate exposure to wave action.

The shore at Otterton Cove consists of the same type of rock as at Salterton, and has similar exposure, topography, orientation and slope. Limpets (*P. vulgata, P. depressa*) and barnacles were present in relatively high densities; topshells (*G. umbilicalis*; *O. lineatus*) were particularly abundant. Characteristic round pits (5-10 cm diameter) of different depths (5-7 cm) hosted a more diverse biota, typical of lower shores.

The littoral fringe at both shores at Salterton and Otterton Cove was characterised by a few patches of barnacles and periwinkles (mainly *L. saxatilis* aggregate).

Old red sandstone (Wembury and Heybrook Bay, South West Devon)

Wembury and Heybrook Bays have extensive and variable rocky shores consisting of platforms and rocky outcrops and boulders with different slopes and orientations. The sites selected were both facing south, with very gentle slopes. These shores are made of old red sandstone, a very fine grained and stratified sedimentary rock, with a flaky structure. The surface texture is very smooth, with very few crevices and pits.

Macrobiota at Wembury and Heybrook sites were typical of a moderately exposed shore and have been well described (Colman, 1933; Evans, 1947). Limpets (*P. vulgata, P. depressa*) and barnacles (*Chthamalus montagui, C. stellatus, Semibalanus balanoides*) were the dominant species on mid intertidal, but topshells (*G. umbilicalis, O. lineatus*) were also present, restricted mainly to rock pools. Periwinkles (*L. saxatilis*) were abundant in the littoral fringe at both sites.

Granite shores (Lamorna Cove and Portgwarra, South Cornwall)

Lamorna Cove and Portgwarra are two extensive rocky shores located near Land's End and thus exposed to the Atlantic Ocean. Lamorna Cove is, however, less exposed to wave action. They both consist of natural boulders (2-3m diameter) which have different slopes and orientations. For the study boulders with gentle slopes facing south were selected. These shores are composed of igneous granite, containing several quartz feldspars. Granite is one of the hardest rocks and is characterised by very low porosity. The surface of the rocks in Portgwarra is rougher and more irregular than in Lamorna Cove.

Macrobiota of these sites was typical of exposed shores in the south west of England (Lewis, 1964; Hawkins *et al.*, 1992), being dominated by just barnacles (*C. montagui, C. stellatus*) and limpets (*P. vulgata*) in the eulittoral zone, while periwinkles (*L. saxatilis*) and small patches of *Verrucaria mucosa* dominated the littoral fringe.

2.2.2 Survey design

In July 1999 and March 2000 microbial communities of five different rock substrata (chalk, limestone, sandstone, old red sandstone, granite) were described and compared. On each occasion all sites were sampled within 2-3 weeks. Visits to each site were randomly chosen within this period, to ensure temporal interspersion of sampling.

In order to guarantee a good representation of their natural spatial variability sampling of microbial communities was carried out at a range of scales using a hierarchical approach. For each rock type two shores were selected and within each shore two patches (approx. 8m x 8m) were randomly chosen. Two tidal levels were also included in the design to investigate the effect of the substratum on biofilms under different environmental conditions. The tidal heights were defined on the basis of their biological features: the littoral fringe, dominated by periwinkles and lichens, and the upper eulittoral, characterised by the upper limit of the zone dominated by limpets and barnacles.

At each of the two tidal levels replicate samples for microalgal biomass, by chlorophyll extraction (n=15), and species composition, by SEM examination (n=6), were taken from each patch within each shore. In total, 1680 rock chips were collected on the two sampling occasions. Boulders of each rock type were also collected from the same sites on one occasion for the determination of the physical and chemical properties of the rocks by subsequent laboratory analysis.

Densities of grazers and other macrobiota were also recorded at each site and on each sampling occasion using 10 replicate quadrats of 50 x 50 cm.

2.2.3 Physical and chemical properties of rock types

The chemical composition, hardness, porosity and surface roughness of each rock type considered in this study were experimentally measured. General information such origin, colour and grain size was collected from the literature (House, 1993; Insole *et al.*, 1998).
A diamond corer drill (32 mm) inserted on a mechanical column drill was used to prepare 10 cores for each rock type to measure hardness and porosity. Chemical composition and surface roughness were measured on rock chips collected from the shore with hammer and chisel.

Chemical composition (principal components) of the rocks was determined using the X-Ray Diffraction analysis (Philips Systems) in the Geochemistry Laboratory at the Southampton Oceanographic Centre.

Rock hardness was initially measured using Rockwell testing machine in the Engineering Material Laboratory at the University of Plymouth. However, the large range of soft and hard rocks considered made it difficult to determine hardness on the same scale. Also, hardness of chalk and limestone could not be measured because it was too soft to be tested with the machine. Therefore, a method commonly used by mineralogists to estimate the relative hardness of rocks, based on Moh's scale, was adopted. This method is based on the principle that if a specimen can be scratched by a mineral of known hardness listed on Moh's scale, then it is softer than that mineral. If it in turn will scratch another known mineral, it is harder than that mineral. Hardness was estimated using the kit of minerals classified on Moh's scale and provided by the Geology Laboratory at the University of Plymouth.

Porosity was indirectly estimated by calculating the rate of water loss through time. Ten cores were used for each rock type, except for flint, which could not be cut due to its extreme hardness. Ten samples of approximate similar volume were taken instead. The samples were first dried in the oven for 24 hours at 110 °C, to remove any moisture, then soaked in filtered seawater for 3 hours and finally dried again at 30°C. This temperature was chosen in order to simulate the temperature of the rock surface in

hot summer days. The rocks were weighed before and after soaking, and at 30 minute intervals during oven drying until constant weight.

Natural surface roughness of rock types was measured using the Talysurf system, (Taylor-Hobson system) in the Engineering Laboratories at the University of Plymouth. The machine was specifically set up (traverse length, 3.5 mm, cut-off 0.25; magnification X100) to measure surface roughness at a scale of resolution appropriate to the biofilm.

2.2.4 Field collection and laboratory analysis

Microbial communities were characterised in terms of microalgal standing stock and species composition, determined by chlorophyll-a extraction (H.M.S.O., 1983) and scanning electron microscopy (SEM) respectively. These techniques were selected as they provide a good estimation of microalgal abundance and diversity (see comments in MacLulich, 1986; Hill and Hawkins, 1990). Chlorophyll-a has been widely recognised as a valid index to estimate microalgal standing stock in planktonic (Parsons and Strickland, 1963) and benthic systems (H.M.S.O., 1984). Scanning electron microscopy is very useful technique to identify single components of microbial films, as it offers an excellent resolution and allows observation of their three dimensional structure (MacLulich, 1986; Hill and Hawkins, 1990; Veltkamp *et al.*, 1994).

Microalgal standing stock

Epilithic microorganisms were collected from each sampling area by chipping with hammer and chisel 15 thin layers of rock (~2cm x 2cm) and placing them carefully into separate labelled plastic bags. Care was taken to avoid areas colonised by macroalgae or barnacles as they can cause microbial biomass to be overestimated. In the laboratory rock samples were rehydrated in filtered seawater for 30 min., blotted and stored in a freezer at -18 °C until processed.

The cold methanol extraction protocol was followed (see Thompson, 1996; Thompson *et al.*, 1999 for details). Before processing, samples were defrosted in filtered seawater for ~30 minutes and blotted to remove any excess of water. Chlorophyll-a was extracted by immersing each rock sample in 100% methanol Analar® grade at room

temperature overnight. The concentration of chlorophyll-a released in the solvent was determined by spectrophotometric analysis, by reading absorbance at 665 nm and then subtracting background absorbance at 750 nm. In order to obtain values of chlorophyll-a concentration per unit area, the surface of each sample was estimated using IBAS image analysis software, which provides a 2D measurement of the projected area.

Microalgal composition

To assess the species composition and relative abundance of the microbial assemblages 6 small rock chips (approximately 1cm x 1cm) were chiselled from the shores at each sampling site and placed separately in Petri dishes. In the laboratory samples were rinsed in filtered seawater, fixed in glutaraldehyde (2.5 % in filtered seawater) for 3 hours and air dried. They were stored at room temperature in a desiccator until processed under SEM (Hill and Hawkins, 1990). Preserved rock chips were mounted on aluminium stubs and coated in a gold/palladium mixture. They were observed under SEM at fixed magnification (250x) and three random fields were photographed from each rock chip. Negatives were observed on a light box or under a light microscope (10x). Percentage cover and direct counts from the negatives were used to estimate the abundance of cyanobacteria and diatoms respectively. Species level identification of diatoms is very difficult when based only on SEM analysis, as some of the distinctive characters like the shape and number of chloroplasts are visible only under light microscopy. However, some of the taxa could be identified at more generic level on the basis of morphological features of the frustules.

2.2.5 Data analysis

The effect of the rock type on microalgal standing stock and relative abundance of diatoms and cyanobacteria were tested using analysis of variance (ANOVA, G-Mav software programme). A mixed model with four factors was used: 1) Rock type (5 levels), fixed and orthogonal; 2) Shore (2 levels), random and nested in Rock type: 3) Tidal level (2 levels), fixed and orthogonal factor; 4) Plot (2 levels), random and nested in Rock type, Shore and Tidal level.

Separate analyses of variance were performed for the summer and early spring sampling dates, as doing one overall analysis proved unnecessarily complex and led to many relevant terms not being testable.

Data were tested for heterogeneity of variance using Cochran's test and where necessary appropriately transformed (Underwood, 1997). In cases when the test was significant ANOVA was still performed, as the large number of samples analysed was sufficient to guarantee the validity of the analysis (Underwood and Jernakoff, 1981). Spatial variability of microalgal biomass on each rock type was assessed by comparing with ANOVA the variances estimated on each plot. The type of spatial distribution of biofilms was also determined using the Index of Dispersion based on variance/mean ratio (Fowler and Cohen, 1990).

A multivariate approach was also used to compare the composition of microbial communities on the different types of rock substratum. MDS plots, ANOSIM and SIMPER procedures (PRIMER software programme) were used to test differences between communities and identify the components of biofilm which mostly contributed to these differences.

Correlations between microalgal standing stock, diatoms, cyanobacteria and abundance of grazers were made using Pearson's correlation coefficient.

2.3 RESULTS

2.3.1 Physical and chemical properties of the rock substrata

The various rock types examined showed marked differences in the physical and chemical properties (Table 2.3). Mineral composition varied considerably among rock types. Chalk and limestone consist of calcium carbonate. Quartz is the principal mineral in the old red sandstone and granite. Sandstone consists mainly of a mixture of calcite and quartz. Chemical composition affects rock weathering, which is generally high on calcite and low on quartz (Press and Raimond 1997).

Rock hardness, which depends on mineral composition and structure, is very low for chalk and limestone, intermediate for the two types of sandstone and high for granite. The size of grains and the way these are cemented together also varies greatly among rock types. Old red sandstone consists of extremely fine grains (less than 0.1 mm) closely cemented and structured in thin layers. Chalk has a relatively fine grained structure (less than 0.5 mm), although large spaces are interspersed with the calcite grains. Limestone and sandstone are characterised by larger grains (up to 2 mm), which are loosely cemented together. Granite has a very coarsely crystalline structure in which crystals of quartz and feldspars can exceed 10 mm in length.

Porosity differed greatly between rocks (Figure 2.2). Chalk absorbed the greatest amount of seawater and retained it for a few hours. Porosity was still relatively high on limestone, although this rock dried out after 90 minutes. Sandstone absorbed a smaller amount of seawater than chalk and limestone. The water, however, did not evaporate from the rock for relatively long time as observed for chalk. Granite and old red sandstone showed minimal porosity, as the little amount of water absorbed dried out after only few minutes.

Surface roughness differed significantly between rock types (Table 2.4; Figure 2.3 and 2.4). Despite the failure of the SNK test in statistically ranking these differences (Table 2.4), there was clear evidence from the results of higher surface roughness on

sandstone, chalk and limestone than on old red sandstone. Granite was rougher than old red sandstone, but smoother than the other three rock types.

Table 2.3 – Summary of the physical and chemical properties for each rock type.

	Origin	Chemical composition	Colour	Hardness	Grain size
Chalk	sedimentary	calcite (calcium carbonate)	white	3	fine grained
Limestone	sedimentary	mainly calcite, traces of quartz	yellow	3.5	coarse grained
Sandstone	sedimentary	mainly quartz, calcite, traces of plagioclase feldspar and potassium feldspar	red, brown	4-5	coarse grained
Old red sandstone	sedimentary	mainly quartz, traces of plagioclase feldspar, mica, possibly chlorite	red	4-5	very fine grained
Granite	metamorphic	mainly quartz, traces of potassium feldspar, plagioclase feldspar and mica	yellow - black	feldspar, 6-7; mica, 3.5; quarts, 7	very coarse crystals

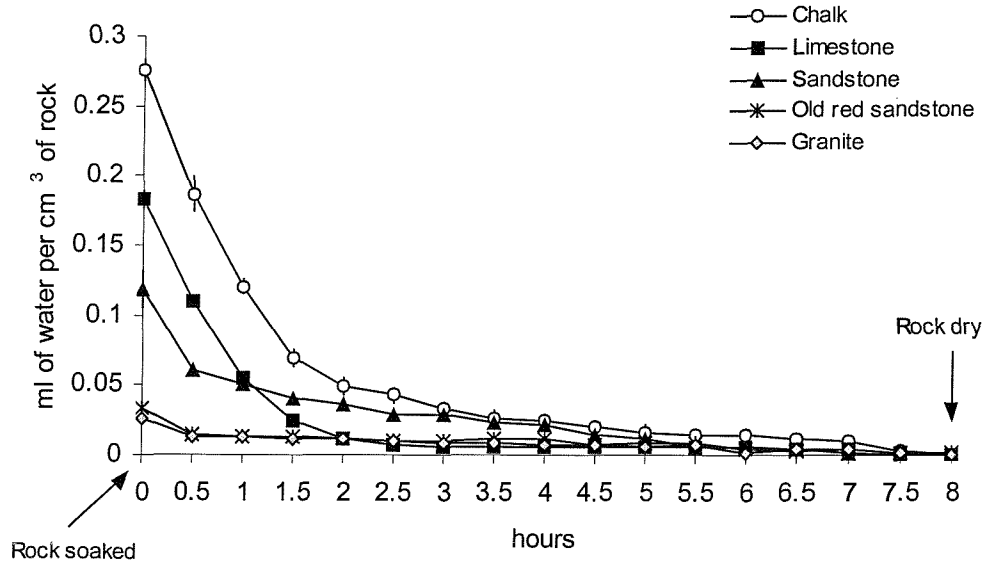


Figure 2.2 – Porosity, expressed as seawater loss (ml) per cm^3 of rock, in chalk, limestone, sandstone, old red sandstone and granite rocks after soaking in seawater for three hours and oven drying at 30°C. Values were averaged from 10 replicate cores of each rock type. Vertical bars indicate standard error.

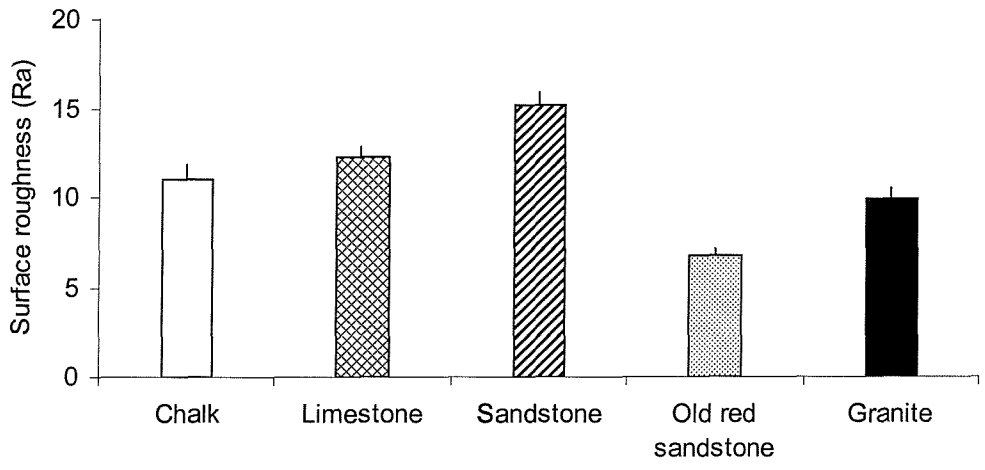


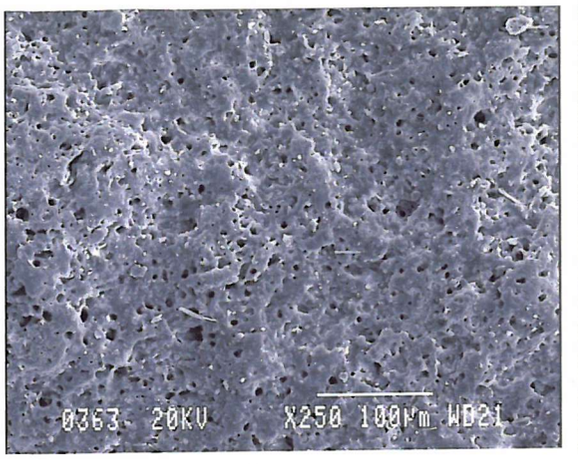
Figure 2.3 – Surface roughness measured on 10 cores of each rock type. Bars indicate standard errors.

Table 2.4 – Analysis of variance and SNK test for differences in surface roughness between rock types. Rocks are ranked in ascending order.

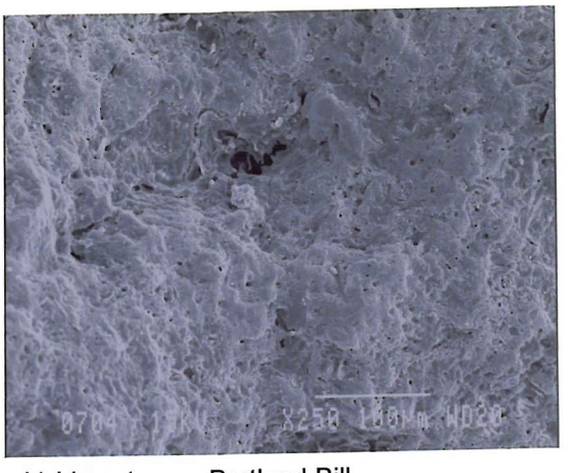
Source	df	MS	F	F versus	P
Rock	4	23.03	95.21	RES	< 0.001
RES	45	0.13	4.38		
TOT	49				

SNK test

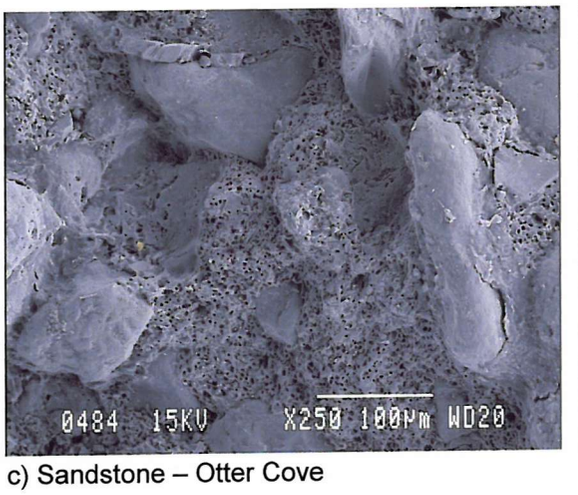
2	3	4	5	1
granite	chalk	limestone	sandstone	old red sandstone



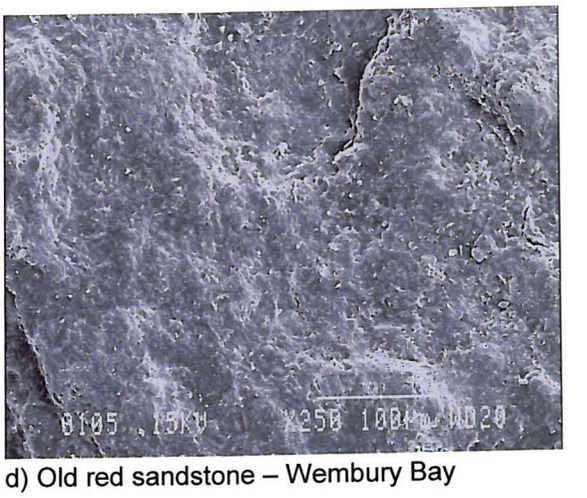
a) Chalk– Culver Cliff



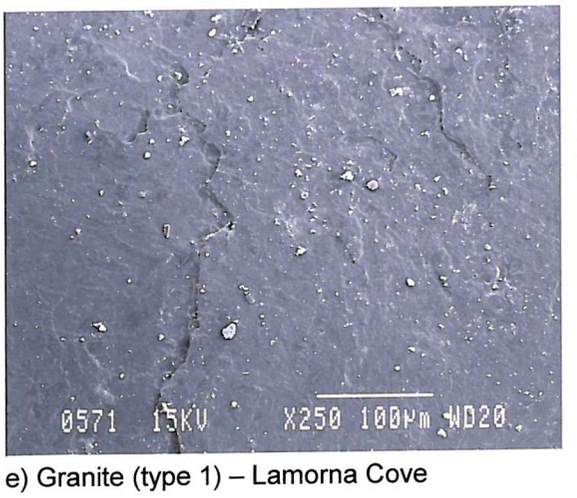
b) Limestone – Portland Bill



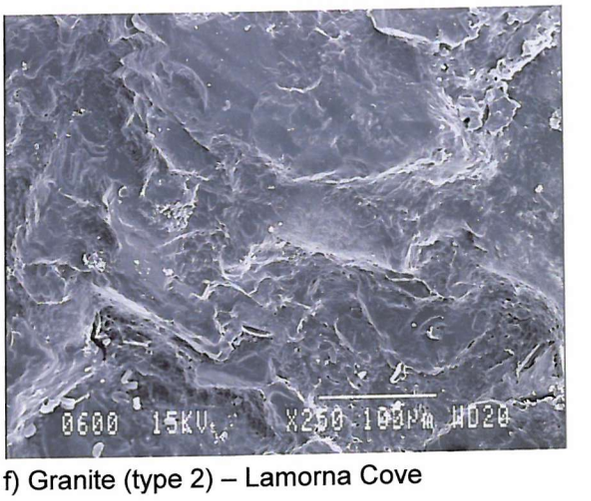
c) Sandstone – Otter Cove



d) Old red sandstone – Wembury Bay



e) Granite (type 1) – Lamorna Cove



f) Granite (type 2) – Lamorna Cove

Figure 2.4 – SEM microphotographs (X250) of 5 different rock types. a) chalk, Culver Cliff; b) limestone, Portland Bill; c) sandstone, Otter Cove; d) old red sandstone, Wembury Bay; e) and f) granite, Lamorna Cove.

2.3.2 Microalgal standing stock

In summer microalgal biomass (expressed by chlorophyll-a content) ranged, on average, between 2.8 on old red sandstone and 8.4 μg cm^{-2} on chalk in the littoral fringe and between 1.6 on old red sandstone and 9.7 μg cm^{-2} on chalk in the upper eulittoral zone (Figures 2.5). In general, microalgal standing stock was higher on the lower tidal level, except for old red sandstone shores where higher values were obtained for the upper shore. Considerable differences in microalgal abundance between rock types could be observed at both tidal levels (Figure 2.5). Chalk shores showed the highest concentration of chlorophyll-a, whilst limestone and sandstone shores had intermediate values. Microalgal standing stock was much scarcer on granite and old red sandstone, with values two-three times smaller than on chalk. Detailed analysis of each type of substratum, however, showed more complex patterns, as high variability in the abundance of microalgae was observed between shores and plots on all the rock types considered (Figures 2.6a, b).

The analysis of variance confirmed these observations (Table 2.5). Overall, microalgal abundance differed significantly between rock types. These differences were also consistent between the two tidal zones, as no significant interaction between the factors rock type and tidal level was observed (Table 2.5). Thus shores as a whole differed and tidal level was not important. The *a posteriori* multiple comparisons (SNK tests) failed to statistically rank the differences found between specific rock types (Figure 2.7). This can be attributed either to the great variability of microbial films within each rock types or to lack of sensitivity of the SNK procedure in separating different means. Chalk, however, had the greatest amount of microalgal biomass in all pairwise comparisons with the other rock types, although significant differences were detected only between chalk and the old red sandstone and the granite shores. Conversely, microalgal biomass was significantly lower on old red sandstone when this substratum was compared with chalk, limestone and sandstone.

In early spring (March 2000), overall, microalgal abundance was greater than in July 1999 on all types of rock, particularly on chalk and limestone shores (Figures 2.8). Chlorophyll-a values ranged, on average, between 4.3 on granite and 12.9 μg cm^{-2} on

limestone in the littoral fringe and between 1.7 on granite and 18.2 μg cm^{-2} on chalk at the upper eulittoral zone. As in the summer survey, the upper eulittoral supported greater microalgal biomass, with the only exception of old red sandstone and granite, were lower values could be observed on one plot at Heybrook Bay and Lamorna Cove respectively. Microalgal standing stock was considerably higher on chalk and limestone, intermediate on sandstone and relatively low on old red sandstone and granite. High spatial variability was apparent on almost all types of substratum, as shown in Figures 2.9a, b.

The analysis of variance detected more marked differences between rock types in early spring than in summer and clear groupings could be identified overall by the SNK procedure (Table 2.6, Figure 2.10). Chalk and limestone showed similar higher microalgal biomass than the other three types of rocks. Old red sandstone and granite could be grouped together, both having significantly lower microalgal biomass. Sandstone differed from all other substrata, being in an intermediate position between the two groups. These differences were not consistent in the two tidal zones, as a significant interaction between the factors rock type and tidal level was detected by the analysis of variance (Table 2.6). This suggested that differences in microalgal biomass found among rock types can vary depending on shore level considered. In the early spring the SNK test failed to identify and rank differences between the rock substrata at each tidal level, although a general pattern was observed in both tidal zones, where microalgal biomass was greater on chalk and limestone, intermediate on sandstone and very low on granite and old red sandstone. These differences were more marked at the upper eulittoral than in the littoral fringe, where microalgal biomass varied significantly only in few comparisons. As in the summer sampling date microalgal standing stock accounted for a considerable amount of spatial variation within rock types (Table 2.6).

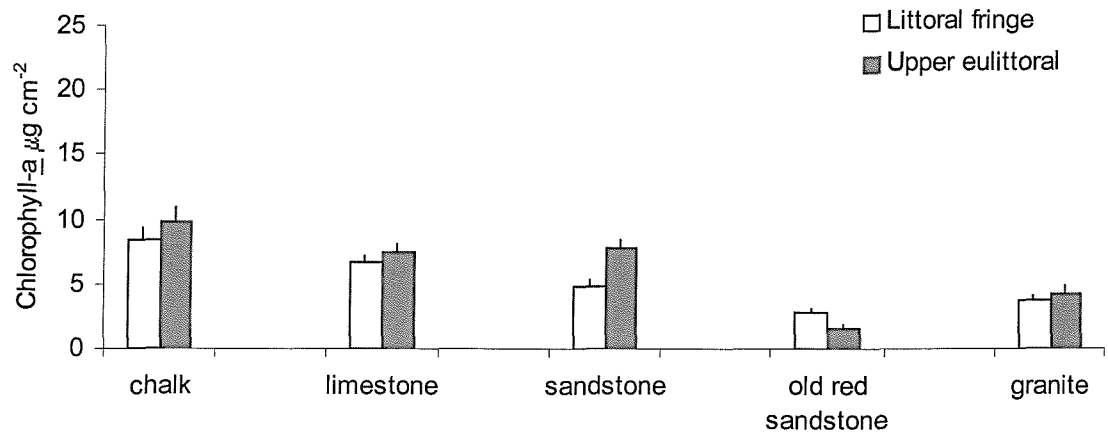


Figure 2.5 - Microalgal standing stock (values averaged across shores and plots) on five different rock types. Summer survey, July 1999. Bars indicate standard errors.

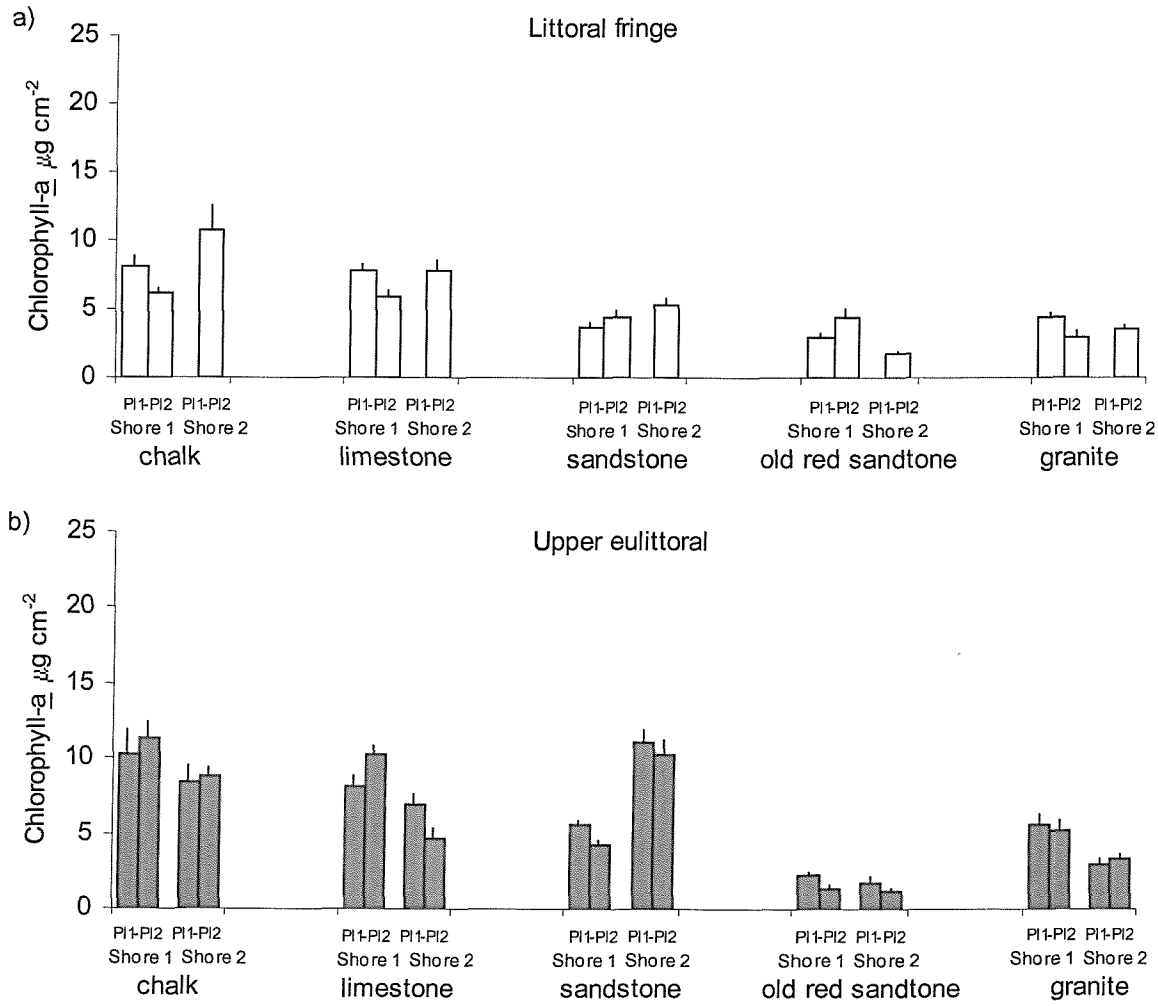


Figure 2.6 - Microalgal standing stock on shores of different geology along the south coast of England. Summer survey: July 1999. a) littoral fringe; b) upper eulittoral. Pl 1 and Pl 2 indicate plot 1 and plot 2 on each shore. Chalk : Shore 1 is Freshwater Bay, Shore 2 is Culver Cliff; Limestone: Shore 1 is Portland Bill, Shore 2 is Lulworth Cove; Sandstone: shore 1 is Otter Cove, Shore 2 is Salterton; Old red sandstone: Shore 1 is Wembury Bay, Shore 2 is Heybrook Bay; Granite: Shore 1 is Lamorna Cove, Shore 2 is Portgwarra. Bars indicate standard errors.

Table 2.5 - Analysis of variance for differences in microalgal standing stock between five different rock types. Summer survey (July 1999). Data were Ln (X+1) transformed to remove heterogeneity of variances. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock type	4	23.03	10.98	Sh (Ro)	**0.011**
Shore (Ro)	5	2.09	5.36	Pl (Ro x Sh x Ti)	**0.003**
Tidal level	1	0.28	0.38	Ti x Sh (Ro)	0.565
Plot (Ro x Sh x Ti)	2	0.39	2.92	RES	**<0.001**
Ro x Ti	4	2.22	3.03	Ti x Sh (Ro)	0.128
Ti x Sh (Ro)	5	0.73	1.87	Pl (Ro x Sh x Ti)	0.145
RES	560	0.13			
TOT	599				

Table 2.6 - Analysis of variance for differences in microalgal standing stock between five different rock types. Early spring survey (March 2000). Data were Ln (X+1) transformed to remove heterogeneity of variances. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock type	4	45.49	44.25	Sh (Ro)	**<0.001**
Shore (Ro)	5	1.03	2.11	Pl (Ro x Sh x Ti)	0.106
Tidal level	1	0.07	0.10	Ti x Sh (Ro)	0.769
Plot (Ro x Sh x Ti)	2	0.49	5.35	RES	**<0.001**
Ro x Ti	4	7.41	9.72	Ti x Sh (Ro)	**0.014**
Ti x Sh (Ro)	5	0.76	1.57	Pl (Ro x Sh x Ti)	0.215
RES	560	0.09			
TOT	599				

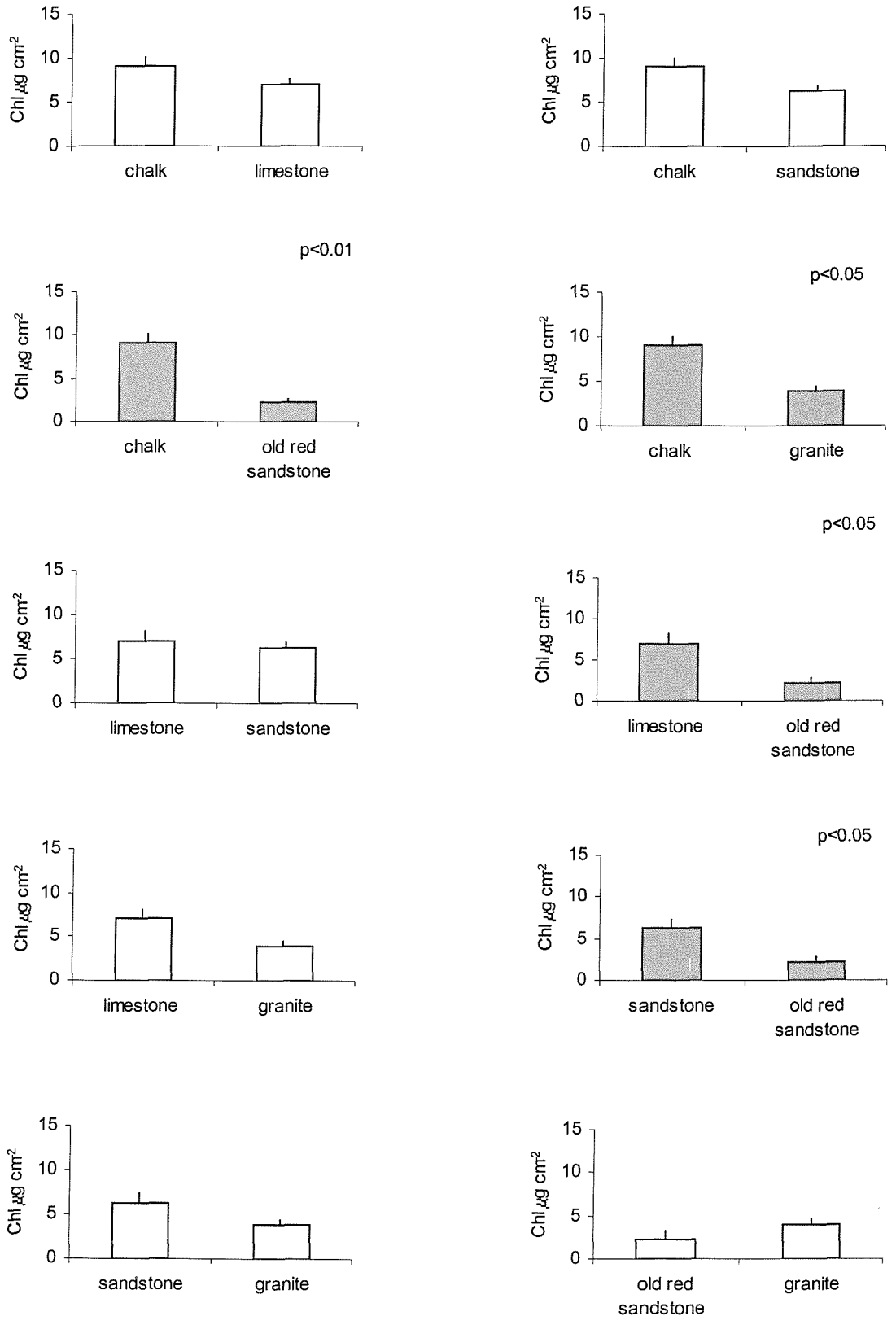


Figure 2.7 - *A posteriori* multiple comparison (SNK tests) after analysis of variance in Table 2.5. Plots show pairwise comparisons of average value of microalgal standing stock on different rock types. Plots coloured in grey show significant differences between each pair of rock types. Bars indicate standard errors.

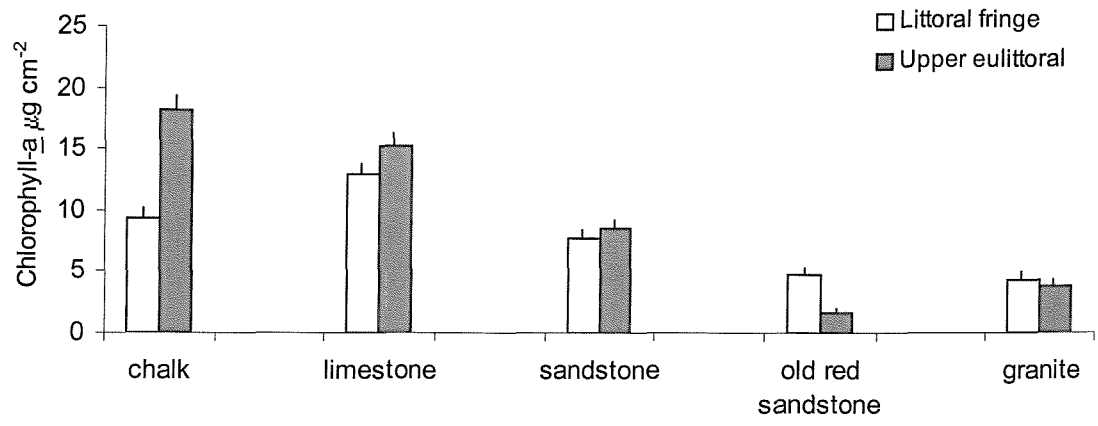


Figure 2.8 - Microalgal standing stock (values averaged across shores and plots) on five different rocks. Early spring survey, March 2000. Bars indicate standard errors.

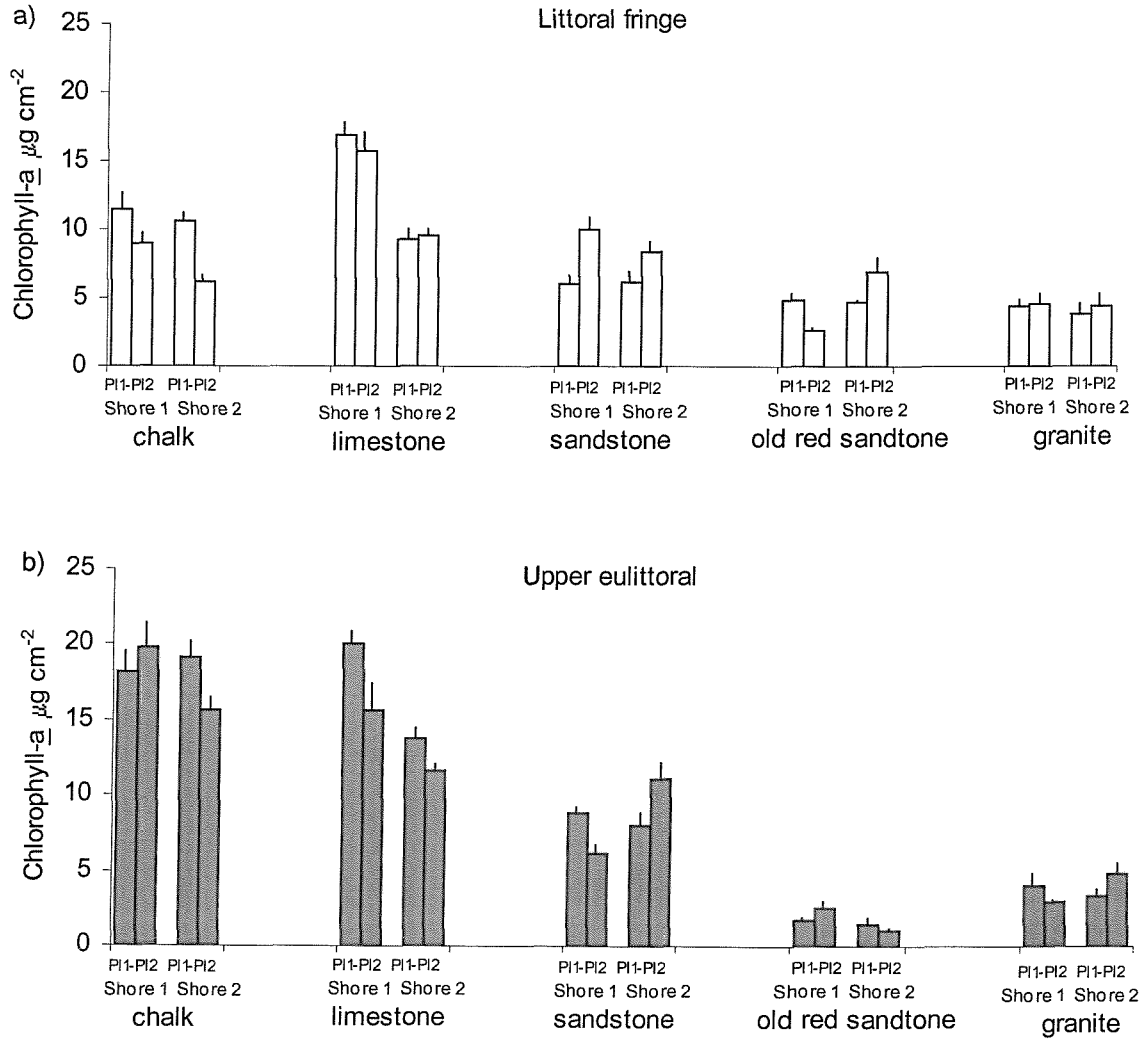


Figure 2.9 - Microalgal standing stock on shores of different geology along the south coast of England. Early spring survey: March 2000. a):littoral fringe; b): upper eulittoral. Bars indicate standard errors.

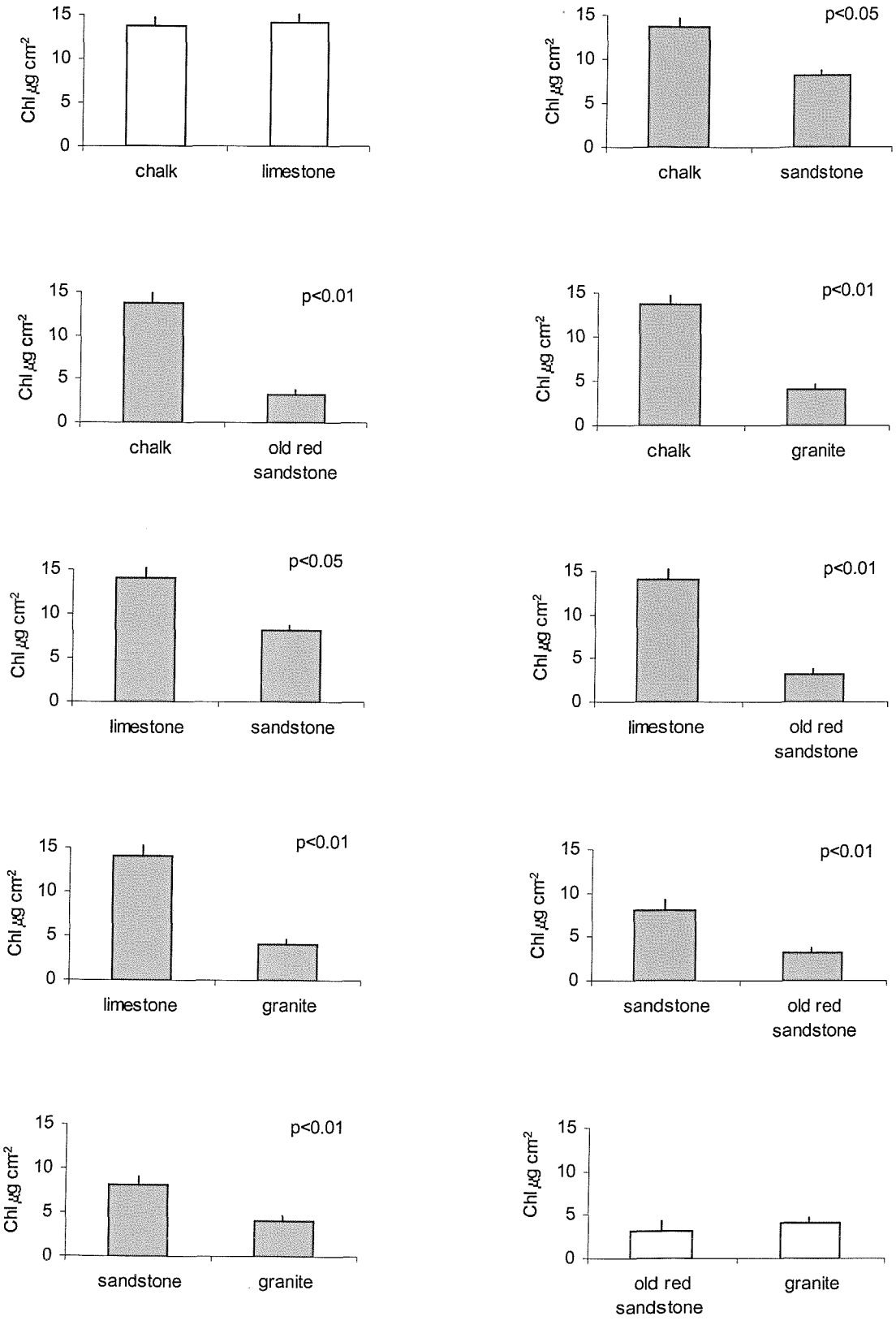


Figure 2.10 - *A posteriori* multiple comparison (SNK tests) after analysis of variance in Table 2.6. Plots show pairwise comparisons of average value of microlagal standing stock on different rock types. Plots coloured in grey show significant differences between each pair of rock types. Bars indicate standard errors.

2.3.3 Spatial variability

The great variability observed on microbial communities made necessary a separate analysis to estimate the magnitude and the scales at which spatial variation occurred within each rock type. The spatial replication used in the sampling design allowed an estimate of variation in microalgal abundance at scales of 10's of km, 10's of m , and cm's. The analysis of variance was used to compare means and variances among plots within each rock type.

Spatial variation in each rock type was estimated using SNK tests for the means between plots, shores and tidal levels (see Table 2.7). It was clear that the two tidal zones sampled did not vary in terms of microalgal biomass, particularly in summer, as none of the rock substrata showed significant differences. Higher variation occurred between plots and secondarily between shores. Limestone, sandstone and old red sandstone contributed mostly to the overall spatial variability, while chalk and granite showed the least variation, as no significant differences were detected between shores and plots.

Spatial variability was also estimated comparing variances instead of means. Variance is a statistical parameter which is used as an index of variability. When variances of plots within each rock type were compared, the pattern obtained was markedly different from the previous analysis of the means. In the summer, chalk showed significantly higher variability than all the other rocks (Figure 2.11a, Table 2.8). No overall variation was shown at shore level and between the two tidal zones. SNK multiple comparisons showed significant differences in the variances between shores only for chalk, in the littoral fringe, and on sandstone, at the upper eulittoral (Figure 2.11b). In early spring variances between rock types did not differ significantly, although higher values were observed for chalk and limestone (Figure 2.12a, Table 2.9). Also some variation occurred only on these rock substrata at shore level, as no significant differences were detected for the other rocks (Figure 2.12b).

These contrasting results could be due to an effect of the size of the means compared. It is known that variance is tied to the mean by a linear relationship (Fowler and Cohen, 1990). Hence comparing variances of samples with very different means might

be of dubious validity. Therefore plotting variances against means could provide a better estimate of potential differences in the variability within each rock type (Figure 2.13). Microbial communities on chalk and limestone shores still appeared to be the most variable, as data points are considerably more spread.

An Index of Dispersion based on the standardised ratio between the variance and the mean was also used to investigate if the type of spatial distribution of microbial communities was similar on all different substrates (Table 2.10). Overall, all rock substrata were characterised by a random distribution where the variance did not differ significantly from the mean.

Table 2.7 – Differences in microalgal standing stock between plots, shores and tidal levels on each rock type. Data were obtained from ANOVA and SNK tests performed on means. Number of asterisks refers to level of significance: *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$.

	Summer			Early spring		
	Plots	Shores	Tidal levels	Plots	Shores	Tidal levels
Chalk	n.s.	n.s.	n.s.	mainly n.s.	n.s.	*
Limestone	mainly **	n.s.	n.s.	mainly n.s.	**	n.s.
Sandstone	n.s.	**	n.s.	**	n.s.	n.s.
Old red sandstone	mainly n.s.	n.s.	n.s.	mainly *	n.s.	**
Granite	mainly n.s.	n.s.	n.s.	mainly n.s.	n.s.	n.s.
Overall variation	***	**	n.s.	***	n.s.	n.s.

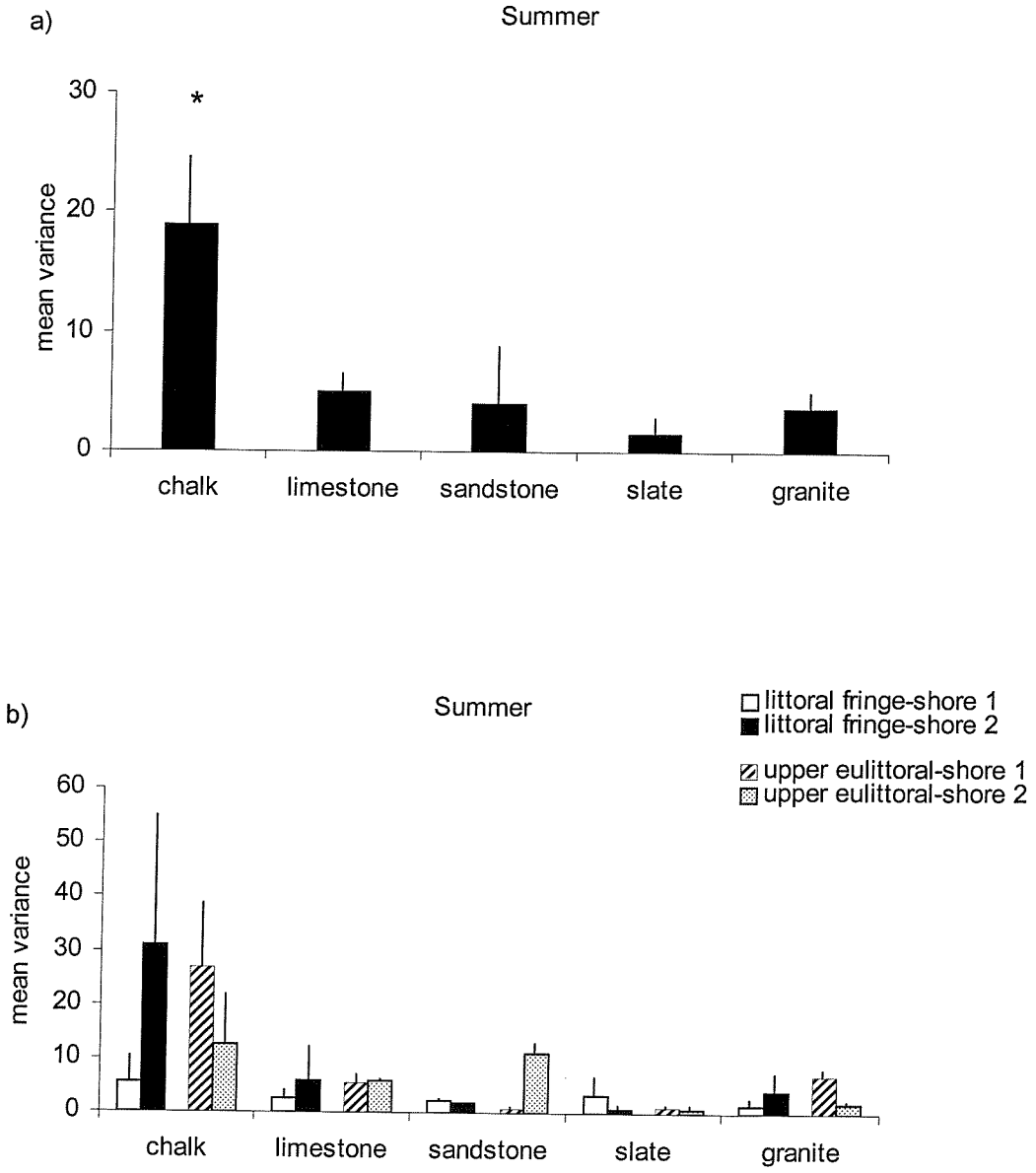


Figure 2.11 – Mean variance among plots on different rock types. a) averaged values; b) variances at shore and tidal levels. Summer survey, July 1999. Bars indicate standard errors.

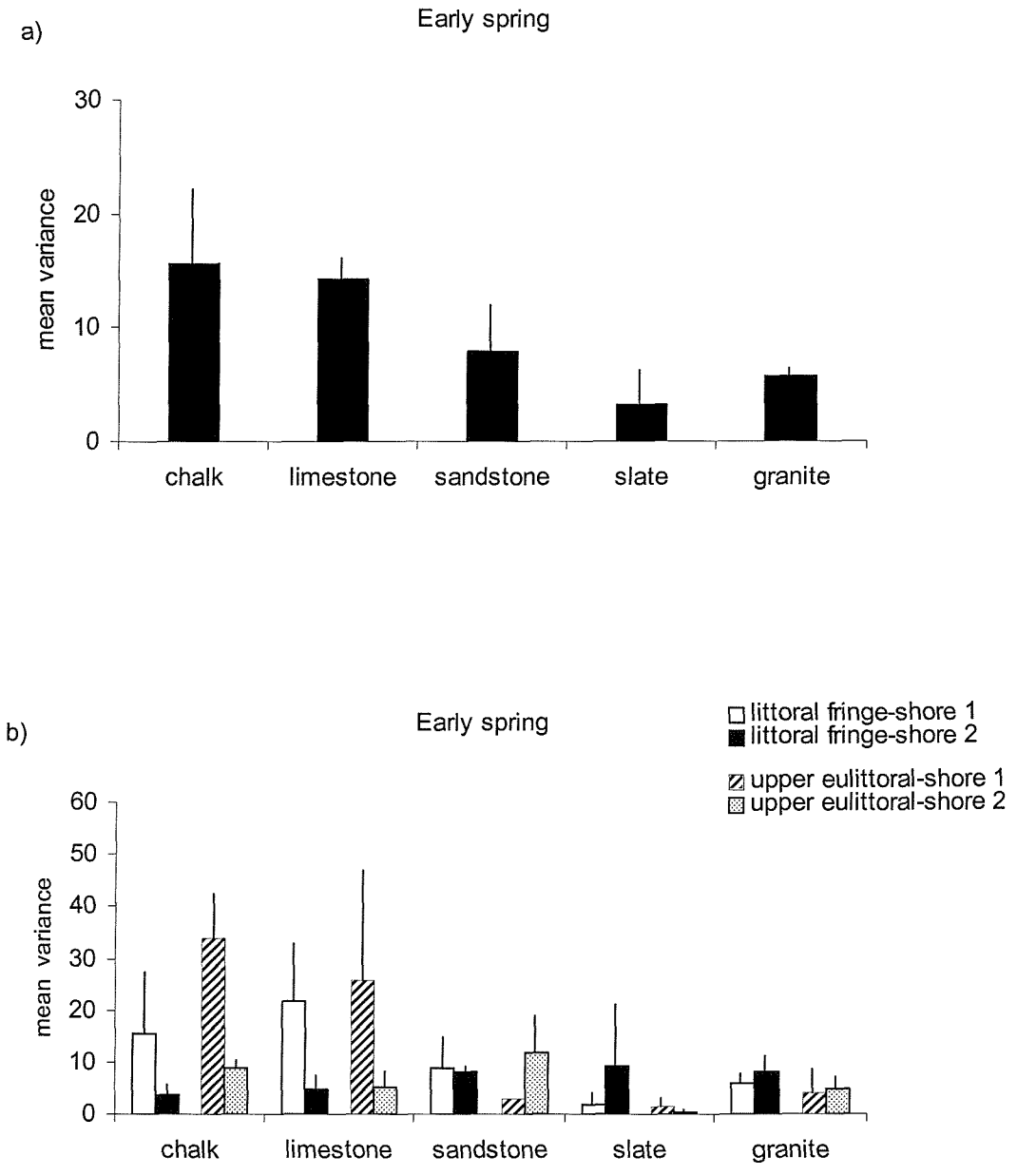


Figure 2.12 – Mean variance among plots on different rock substrata. a) averaged values; b) variances at shore and tidal levels. Early spring survey, March 2000. Bars indicate standard errors.

Table 2.8 - Analysis of variances for differences in variance among plots for microalgal standing stock between five different rock types. Summer survey (July 1999). Data were Ln (X+1) transformed to remove heterogeneity of variance. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock	4	3.97	7.89	Sh (Ro)	**0.022**
Shore (Ro)	5	0.50	1.91	Pl (Ro x Sh x Ti)	0.138
Tidal level	1	0.72	0.58	Ti x Sh (Ro)	0.480
Ro x Ti	4	0.22	0.17	Ti x Sh (Ro)	0.943
Ti x Sh (Ro)	5	1.24	4.71	Pl (Ro x Sh x Ti)	**0.005**
RES	20	0.26			
TOT	39				

Table 2.9 - Analysis of variances for differences in variance among plots for microalgal standing stock between five different rock types. Early spring survey (March 2000). Data were Ln (X+1) transformed. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock	4	3.24	2.14	Sh (Ro)	0.213
Shore (Ro)	5	1.52	3.44	Pl (Ro x Sh x Ti)	**0.021**
Tidal level	1	0.20	0.65	Ti x Sh (Ro)	0.455
Ro x Ti	4	0.79	2.58	Ti x Sh (Ro)	0.164
Ti x Sh (Ro)	5	0.31	0.70	Pl (Ro x Sh x Ti)	0.632
RES	20	0.44			
TOT	39				

Table 2.10 – Percentages of samples characterised by clumped, random or regular distribution on the different rock types assessed using Index of Dispersion. Values were based on 95 % confidence interval.

	Clumped	Random	Regular
Chalk	19	69	13
Limestone	0	75	25
Sandstone	0	69	31
Old red sandstone	6	44	50
Granite	6	81	13

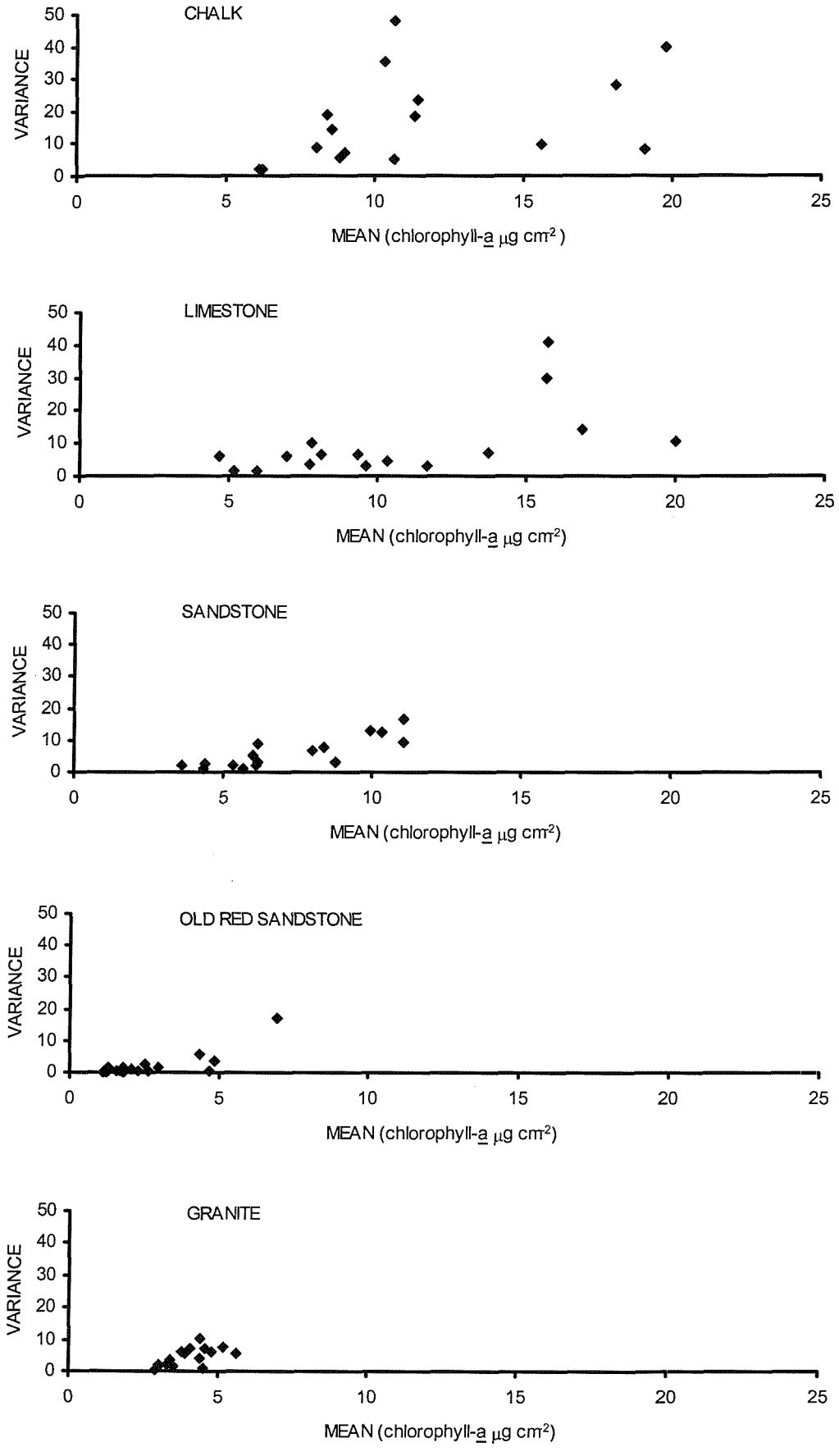


Figure 2.13 – Variance against mean of microalgal standing stock on each plot and for each rock type. Data from summer and early spring sampling and shore level were pooled.

2.3.4 Microbial composition

The analysis of biofilms under scanning electron microscope revealed a community dominated, although in different proportions, by diatoms, cyanobacteria and microscopic forms of coralline algae. Minor components of the biofilms analysed were round shaped bacteria, protozoa, mainly coccoliths, algal germlings.

Several species of diatoms were observed, although the scanning electron microscopy allowed identification of only a few taxa. Taxa most frequently observed were *Achnanthes*, *Cocconeis* and *Fragilaria*. Rarer taxa included *Melosira*, *Tabularia, Synedra, Thalassionema*, *Psammodyction*, *Lyrella, Grammatophora* and *Amphora*. There were no apparent differences in the composition of diatom assemblages among rock types. Cyanobacteria were observed on all rock substrata and consisted of long colonies. Coccoliths were very common on chalk and limestone, but very scarce on old red sandstone. Coralline algae were very common on all rock substrata whilst algal germlings, probably *Fucus* spp. , were found sporadically.

Diatoms and cyanobacteria

In the summer the number of diatoms observed was extremely low or absent from all the rock types considered (Figure 2.14 a, b). Diatoms were absent from at least two the plots on each type of shore. Maximum abundance was just over 200 diatoms cm^{-2} on limestone in the littoral fringe, but in almost all substrata density was not higher than 100 diatoms cm^{-2} in both zones. The number of diatoms was also very similar in the littoral fringe and the upper eulittoral zone.

The analysis of variance showed no significant differences either between rock types or zones (Table 2.11). Some variation was instead found between shores within each rock type. This was mainly due to differences between the two limestone shores, at Portland Bill and Lulworth Cove (SNK test, $p<0.05$). These results were expected, as the sensitivity of univariate statistical analysis is relatively low when data contains several zero values and high variability.

In the early spring the range of values for density of diatoms increased by two orders of magnitudes (Figure 2.14 c, d). The abundance of diatoms was higher in the spring

than in the summer when matched pairs of the same plots were compared across all the rock types. The only exception was one plot out of 40 in the littoral fringe on a limestone shore, where the density of diatoms was the same as in summer. Not surpisingly this was highly significant when tested with a Sign test for matched pairs ($z = -6.08$, $p<0.001$). In particular, the number of diatoms on chalk and limestone shores increased dramatically in the spring, reaching peaks of 139800 cells cm^{-2} in the upper eulittoral and 86400 cells cm^{-2} in the littoral fringe respectively (Figure 2.14c, d).

The abundance of diatoms on chalk and limestone appeared to be also considerably higher than on other rock types, particularly on old red sandstone, where numbers of diatoms were approximately only 1000 cells cm^{-2} at both tidal levels. High spatial variability within each rock type occurred at shore level, as shown by ANOVA (Table 2.12), although considerable variation was also observed within plots. Spatial variation between shores was mainly observed, however, on chalk and limestone.

The ANOVA did not detect any significant difference in the abundance of diatoms on the different rocky shores, probably due to the high variation within plots, as variances where highly heterogeneous before transformation.

Within each rock type, there was a significant interaction between tidal level and shore (Table 2.12). The SNK multiple comparison tests showed that the abundance of diatoms was significantly higher in the upper eulittoral on old red sandstone, at Wembury Bay, and on chalk, at Freshwater Bay. In contrast, the use of non parametric Kruskal-Wallis test , which is not based on the assumptions of normality of data and homogeneity of variances, detected significant differences in the abundance of diatoms between rock types (Table 2.13).

Cyanobacteria did not show a clear pattern in terms of abundance and distribution among the rocks in either summer or early spring (Figure 2.15a-d). Percentage cover of cyanobacteria rarely reached 50 %, while the average value was generally much lower, around 10 %. High spatial variation within each type of rock was observed: for example, in the spring, in the littoral fringe on old red sandstone, the abundance of cyanobacteria in the plots could vary from 1 % to almost 50 %. The analysis of variance did not detect any difference between rock types, but spatial variability was

highly significant in both seasons (Table 2.14-15). Interestingly the scale at which spatial variability occurred shifted locally from the plot level in the summer to whole shore in the early spring (Table 2.14-15). Significant differences in abundance of cyanobacteria between plots were limited to limestone and old red sandstone in the summer, and only to old red sandstone in the early spring.

The abundance of cyanobacteria did not differ between tidal zones, although in the spring, percentage cover was on average slightly higher in the littoral fringe than at the upper eulittoral zone on all rock types but chalk (Figure 2.15c, d). In summer, this pattern was observed only on sandstone, old red sandstone and granite shores (Figure 2.15a, b).

Whole community

Despite the lack of patterns detected by univariate approaches in the abundance and distribution of diatoms and cyanobacteria between rock types, the multivariate analysis of the microbial community as a whole provided clearer results. In the analysis all the components of the microbial film were included, except for the coccoliths. These protozoans are known to be present also as fossils in carbonate rocks such as chalk and limestone, thus results from comparisons of their abundance with other types of rock could be misleading.

In both summer and early spring microbial communities varied considerably between rock types, as it was observed in the 2-d ordination plots (Figure 2.16a, b). These plots, which are spatial representations of the relationships between groups of samples, showed a marked separation between the microbial community on old red sandstone and the assemblages on chalk and limestone. Microbial films on the other substrata were also relatively far apart, although some of the samples overlapped, particularly in the early spring sampling (Figure 2.16b). The patterns observed in the MDS plots were tested using the ANOSIM procedure. The analysis showed overall significant differences in the microbial community between rock types in summer and early spring.

In the summer, these differences were marked in the pairwise comparisons of chalk *versus* old red sandstone and limestone *versus* old red sandstone, with a coefficient of

dissimilarity of 0.844 and 0.631 respectively (Table 2.16). The microbial community on chalk showed considerable dissimilarities when compared with sandstone and granite (R=0.518; R= 0.489 respectively), this being different also from limestone. Interestingly, there were no significant differences in the microbial community in the comparison of chalk with limestone. Similarly, in the comparisons involving old red sandstone, sandstone and granite very low dissimilarities were also observed between the microbial assemblages. These results suggested a putative pattern where microbial films on rock substrata could be segregated into two groups; the first represented by chalk and limestone, sharing very similar assemblages, and the second formed by old red sandstone, sandstone and granite whose communities differed only slightly.

In the early spring results from the ANOSIM test were almost consistent with the summer. The coefficient of dissimilarity between microbial communities on chalk and old red sandstone was still high and increased considerably in the comparisons of chalk with both sandstone and granite (Table 2.16). The two soft rocks chalk and limestone had relatively similar assemblages as in the summer. Also, differences between the other rock types were further reduced except for limestone compared with sandstone, where these slightly increased. The pattern observed in summer was therefore reinforced in early spring, although the microbial community on limestone became more similar to those on the other rock substrata.

The biofilm components which appeared to contribute most to the strongest differences between rock types were diatoms, cyanobacteria and the early stages of coralline algae, as shown in the results from the SIMPER analysis (Table 2.17 and 2.18). However, the relative importance of each component in the discrimination between rock substrata varied considerably between summer and early spring. Corallines contributed almost 50% to differences in the comparisons of chalk and limestone with old red sandstone in summer, and chalk *versus* old red sandstone, sandstone and granite in early spring. In both season, the abundance of these algae was in fact much lower on chalk and limestone than on the other substrata. Cyanobacteria enabled discrimination between the microbial communities on chalk and the assemblages on granite and sandstone in summer, whilst no major differences were detected in the abundance of this component in early spring. These microorganisms were almost absent from the two types of sandstone and granite. Diatoms were

generally more abundant on chalk and limestone than on other substrata. The differences were particularly evident in early spring, probably because of their increased abundance in this season. In conclusion, the main components of the microbial communities on chalk and limestone were diatoms and cyanobacteria, whilst the assemblages on the other substrata appeared to be dominated by coralline algae.

2.3.5 Correlation between biofilm components and limpets

The grazers observed on the rocky shores were limpets (*Patella vulgata* and *P. depressa*), top shells (*Osilinus lineatus, Gibbula umbilicalis*) and littorinids (*Littorina saxatilis, Melaraphe neritoides*). Topshells and littorinids were present only at a few sites and at very low densities, therefore the effect of these grazers on biofilm was considered negligible. Limpet densities varied considerably on the different types of shore, but were consistent between sampling dates (Figure 2.17). Limestone and sandstone shores had relatively high densities of limpets, being three times more abundant than on granite and old red sandstone. Chalk shores showed more similar numbers of limpets to sandstone shores.

In total, total limpet densities on all rocky shores were positively correlated to chlorophyll-a but not with diatoms or cyanobacteria (Table 2.19). A significant correlation between limpets and chlorophyll-a was also observed within each type of rock. On old red sandstone shores limpet density was negatively related to chlorophyll-a, whilst on chalk shores limpets were positively correlated with cyanobacteria.

Chlorophyll-a was highly correlated with the abundance of diatoms on all types of shores (Pearson's $r = 0.43$, $p<0.01$). Within each rock type, however, a positive correlation was observed only on chalk. Chlorophyll-a was also positively correlated with cyanobacteria, but only on old red sandstone shores (Pearson's $r = 0.85$, $p<0.01$).

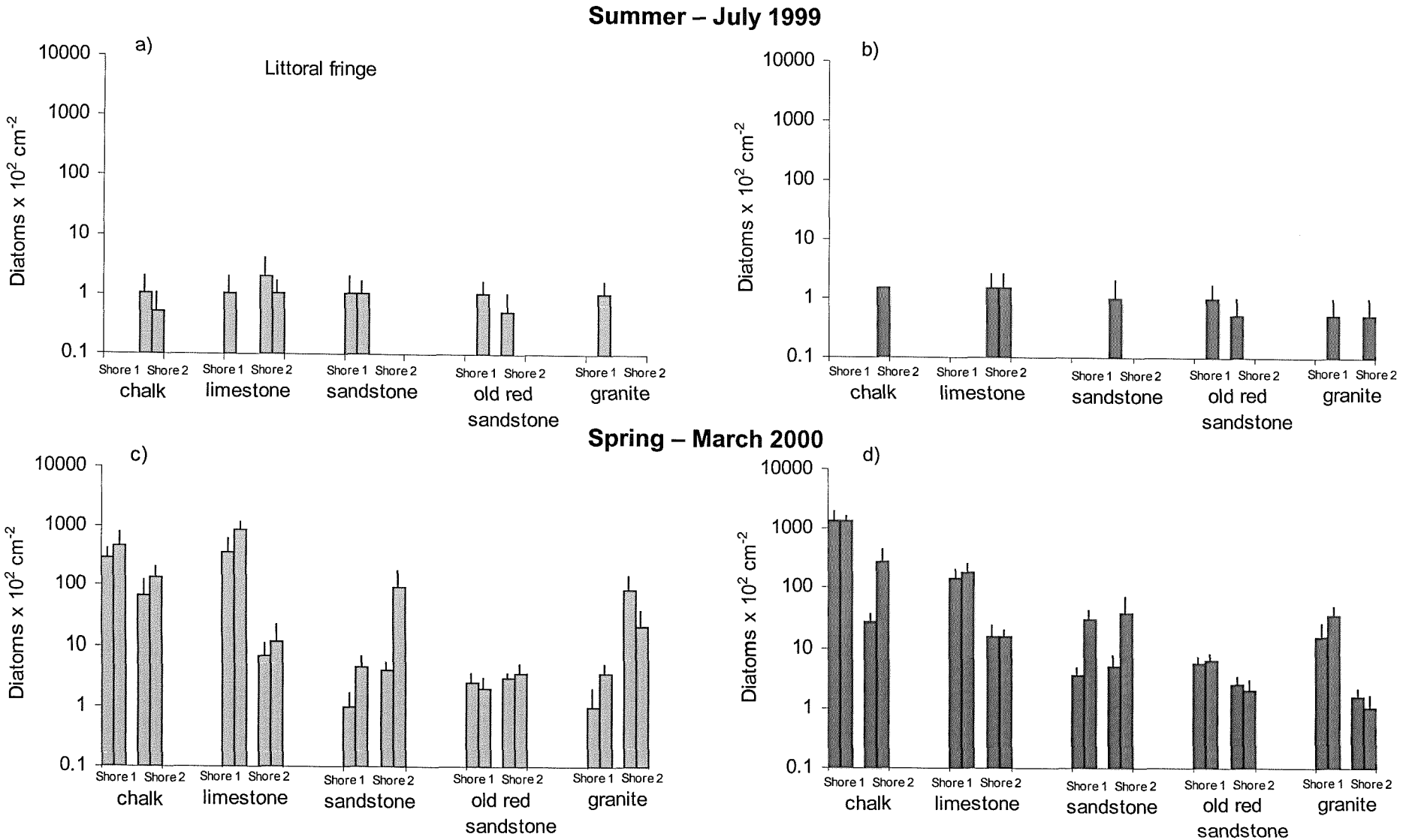


Figure 2.14 - Abundance of diatoms in the littoral (a, c) and upper eulittoral (b, d) zones in the summer (a, b) and early spring (c, d). Bars = standard errors.

Table 2.11 - Analysis of variance for differences in the abundance of diatoms on shores of different geology in July 1999. Data were ln (X+0.1) transformed to remove heterogeneity of variance. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock	4	0.34	0.39	Sh (Ro)	0.809
Shore (Ro)	5	0.86	3.47	Pl (Ro x Sh x Ti)	**0.020**
Tidal level	1	0.11	0.31	Ti x Sh (Ro)	0.603
Plot (Ro x Sh x Ti)	20	0.25	0.75	RES	0.765
Ro x Ti	4	0.04	0.10	Ti x Sh (Ro)	0.976
Ti x Sh (Ro)	5	0.35	1.43	Pl (Ro x Sh x Ti)	0.258
RES	160	0.33			
TOT	199				

Table 2.12 - Analysis of variance for differences in the abundance of diatoms on shores of different geology in March 2000. Data were ln (X+0.1) transformed to remove heterogeneity of variance. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock	4	108.8	3.49	Sh (Ro)	0.102
Shore (Ro)	5	31.21	13.03	Pl (Ro x Sh x Ti)	**<0.001**
Tidal level	1	9.43	1.03	Ti x Sh (Ro)	0.357
Plot (Ro x Sh x Ti)	20	2.39	1.22	RES	0.242
Ro x Ti	4	2.28	0.25	Ti x Sh (Ro)	0.899
Ti x Sh (Ro)	5	9.18	3.83	Pl (Ro x Sh x Ti)	**0.013**
RES	160	1.96			
TOT	199				

Table 2.13 – Kruskal-Wallis test for differences in the abundance of diatoms between rock types.

	N	Mean Rank of abundance of diatoms
chalk	8	33.50
limestone	8	27.31
sandstone	8	17.13
old red sandstone	8	9.88
granite	8	14.69
	40	
Chi-Square	21.885	
df	4	
Asymp. Sig.	<0.001	

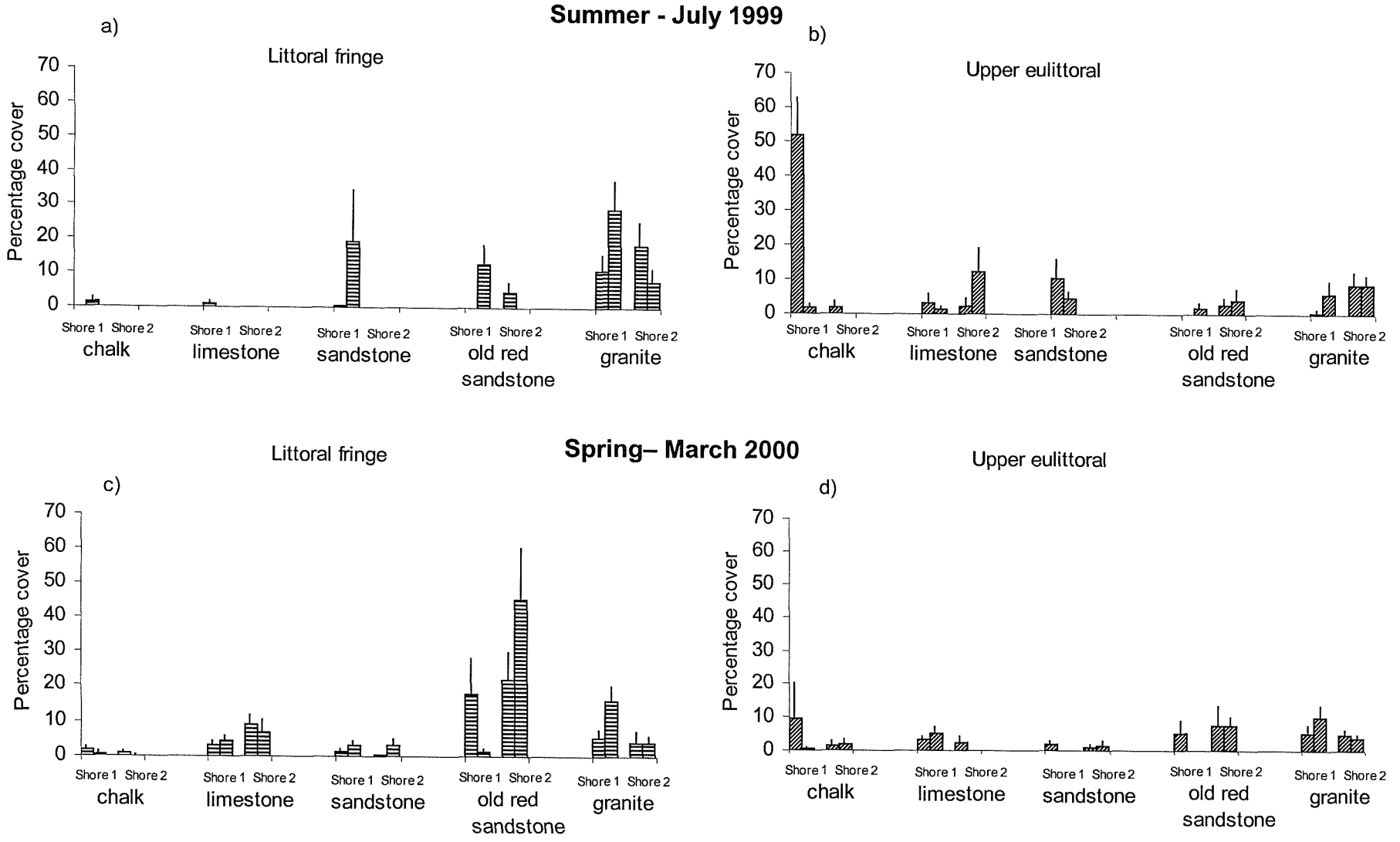


Figure 2.15 - Abundance of cyanobacteria in the littoral (a, c) and upper eulittoral (b, d) zones in the summer (a, b) and early spring (c, d). Bars = standard errors.

Table 2.14 - Analysis of variance for differences in the abundance of cyanobacteria on shores of different geology in July 1999. Data were ln (X+0.1) transformed to remove heterogeneity of variance. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock	4	43.92	2.50	Sh (Ro)	0.172
Shore (Ro)	5	17.60	2.15	Pl (Ro x Sh x Ti)	0.100
Tidal level	1	13.64	1.05	Ti x Sh (Ro)	0.353
Plot (Ro x Sh x Ti)	20	8.17	3.10	RES	**<0.001**
Ro x Ti	4	23.59	1.81	Ti x Sh (Ro)	0.265
Ti x Sh (Ro)	5	13.05	1.60	Pl (Ro x Sh x Ti)	0.207
RES	160	2.63			
TOT	199				

Table 2.15 - Analysis of variance for differences in the abundance of cyanobacteria on shores of different geology in March 2000. Data were ln (X+0.1) transformed to remove heterogeneity of variance. Significant values are highlighted in bold.

Source	df	MS	F	F versus	P
Rock	4	11.67	2.62	Sh (Ro)	0.159
Shore (Ro)	5	4.45	3.59	Pl (Ro x Sh x Ti)	**0.018**
Tidal level	1	6.11	4.03	Ti x Sh (Ro)	0.100
Plot (Ro x Sh x Ti)	20	1.24	1.31	RES	0.180
Ro x Ti	4	2.86	1.89	Ti x Sh (Ro)	0.251
Ti x Sh (Ro)	5	1.52	1.22	Pl (Ro x Sh x Ti)	0.335
RES	160	0.95			
TOT	199				

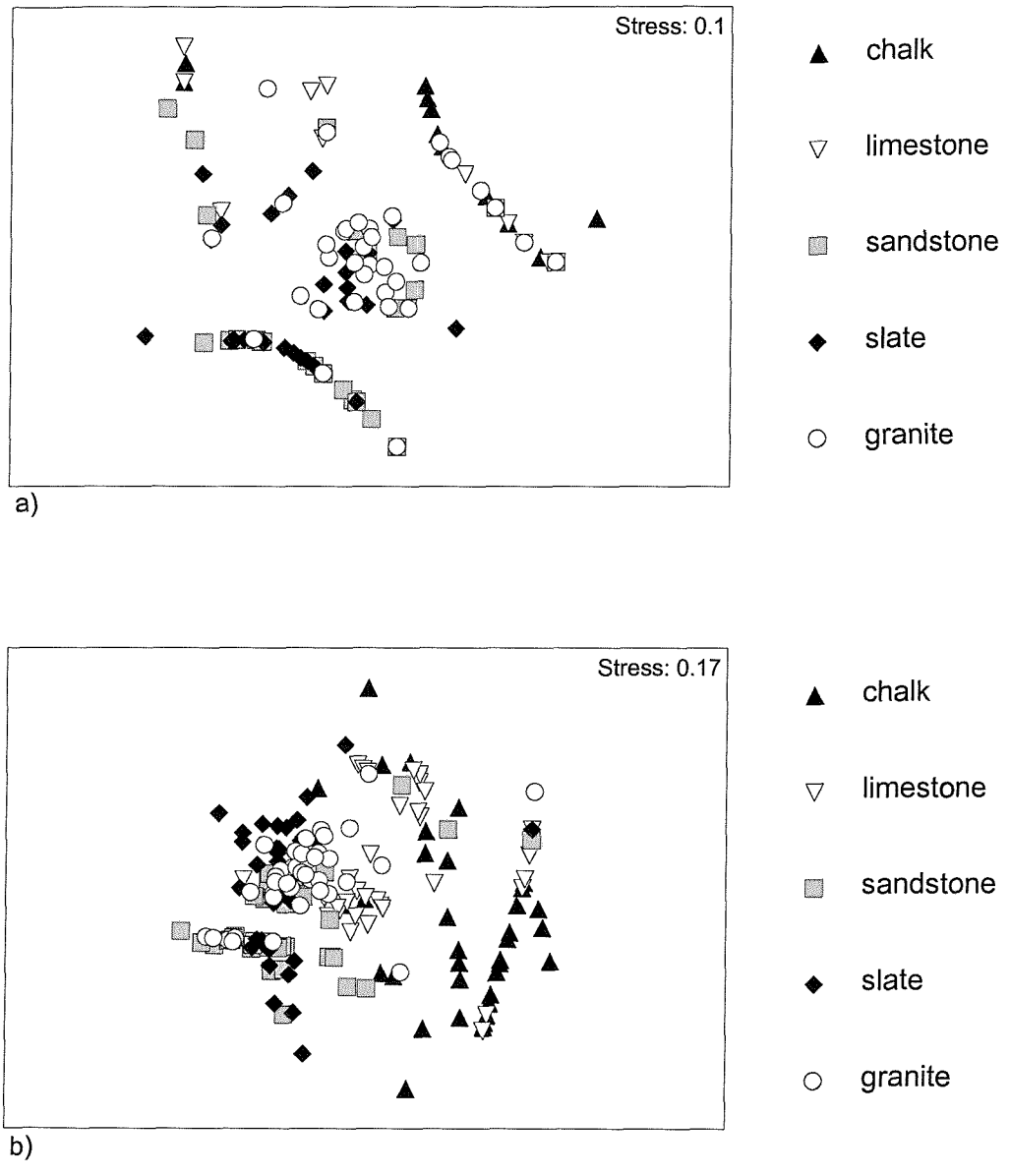


Figure 2.16 – MDS plots of microbial community on different rock types in the summer (a) and early spring (b).

Table 2.16 - ANOSIM tests for microbial communities on different rock types and between tidal levels. R is the coefficient of dissimilarity; P is the significance of the test.

	July 1999		**March 2000**	
Differences between rock types	Global R: 0.322, p=0.001		Global R: 0.362, p<0.001	
Pairwise tests	**R**	**P**	**R**	**P**
chalk, limestone	0.006	0.373	0.284	0.001
chalk, sandstone	0.489	0.001	0.715	0.001
chalk, old red sandstone	0.844	0.001	0.752	0.001
chalk, granite	0.518	0.001	0.662	0.001
limestone, sandstone	0.292	0.001	0.362	0.001
limestone, old red sandstone	0.631	0.001	0.373	0.001
limestone, granite	0.444	0.001	0.238	0.001
sandstone, old red sandstone	0.104	0.006	0.067	0.010
sandstone, granite	0.203	0.001	0.172	0.001
granite, old red sandstone	0.199	0.001	0.139	0.001
Differences between tidal zones	Global R: 0.058, p=0.028		Global R: 0.082, p=0.001	

Table 2.17 - SIMPER analysis of taxa which mostly contributed to differences in microbial communities between rock types for the summer sampling date. Results are shown for comparisons of rock types where R>0.4.

	Average abundance		Contribution %	Cumulative %
	chalk	**old red sandstone**		
Corallines	0.00	21.11	49.93	46.93
Cyanobacteria	20.21	3.47	32.04	78.96
	limestone	**old red sandstone**		
Corallines	1.57	21.11	49.38	49.38
Diatoms	20.59	4.05	32.29	81.67
	chalk	**granite**		
Cyanobacteria	20.21	12.44	47.00	47.00
Corallines	0.00	11.62	31.08	78.09
	chalk	**sandstone**		
Cyanobacteria	20.21	5.86	42.65	42.65
Corallines	0.00	13.48	34.52	77.17
	limestone	**granite**		
Diatoms	20.59	2.78	36.00	36.00
Cyanobacteria	5.96	12.44	32.62	68.62

Table 2.18 - SIMPER analysis of taxa which mostly contributed to differences in microbial communities between rock types for the early spring sampling date. Results are shown for comparisons of rock types where R>0.4.

	Average abundance		Contribution %	Cumulative %
	chalk	**old red sandstone**		
Corallines	0.64	23.25	43.89	43.89
Diatoms	17.02	0.11	26.3	70.19
	chalk	**sandstone**		
Corallines	0.64	18.21	49.71	49.71
Diatoms	17.02	0.73	34.38	84.09
	chalk	**granite**		
Corallines	0.64	11.97	36.96	36.96
Diatoms	17.02	0.75	35.27	72.23

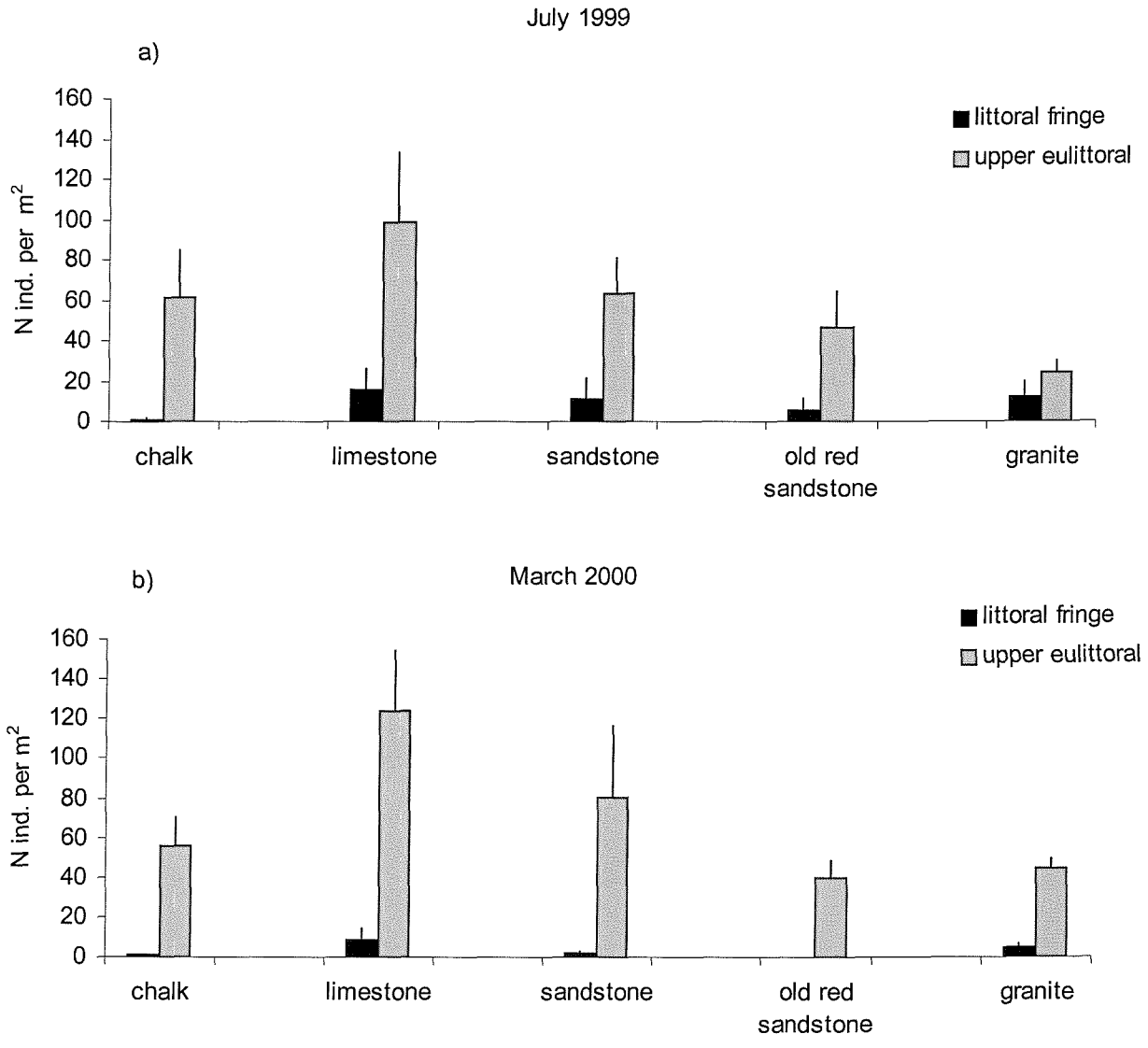


Figure 2.17 - Limpet density on different rocky shores in the summer (a) and early spring (b) sampling date. Data were averaged across shores and plots. Bars indicate standard errors.

Table 2.19 - Correlations between chlorophyll-a, diatoms, cyanobacteria and limpets (Pearson r). Data for each rock type refer to each plot sampled from each shore, tidal levels and sampling dates. Sample size is given after list-wise deletion of missing values. Significant values are in bold. *=p<0.05; **=p<0.01.

	n	Chalk	n	Limestone	n	Sandstone	n	Old red sand.	n	Granite	n	All
Limpets *versus*:												
Chlorophyll-a	15	0.50	16	0.19	15	-0.07	14	**-0.51***	16	-0.07	76	**0.27***
Diatoms	15	0.30	16	-0.45	15	-0.35	14	0.29	16	0.28	76	-0.02
Cyanobacteria	15	**0.79 ****	16	-0.08	15	-0.15	14	-0.31	16	-0.07	76	0.03

2.4 DISCUSSION

2.4.1 Limitations of the study and sources of variation

The geology of the south coast of England did not allow interspersion of the study sites (see Figure 2.1). For example, chalk shores were located in the Isle of Wight, while granite shores were restricted to the south west coast of Cornwall. Lack of spatial interspersion makes it very difficult to separate the effect of the rock substratum on microbial films from all the other environmental variables. The different geographical locations cause variation in factors such as tidal range and the time at which diurnal low tides occur, climate, currents and nutrients. These factors vary considerably in the English Channel from west to east and are known to affect macrobiota. Similarly, they are likely to influence the abundance and composition of microbiota and also modulate the effect of more local factors such as the rock type. For example, weathering, which mainly depends on climate and wave exposure, is likely to be of differing importance in the locations investigated. Higher weathering causes an increase in the surface roughness and porosity of the rocks, which could facilitate development of biofilms.

Spatial variability was very evident occurring at all scales, at shore level (range of km's), plot level (range of m's) and particularly among replicate rock chips (cm's to mm's). Despite the attempt of selecting shores of similar physical and biological features, some local factors such as wave exposure and macrobiota differed. For example, the site at Portland Bill is particularly exposed to waves from the west, whilst Freshwater Bay in the Isle of Wight is more sheltered. Densities of limpets varied greatly within and between shores of different geology. Several authors showed that the abundance and composition of grazers can strongly control microbial community (Underwood, 1980; Cubit, 1984; Underwood, 1984a; Hill, 1990). In contrast, in my study, however, there were no negative correlations between these and microalgal standing stock. However, variation in the number of limpets and other grazers such as topshells, which were present only on sandstone and old red sandstone shores, or periwinkles, which were particularly abundant at Portland, might have contributed to this spatial variability, particularly at a small scale. Patchiness did not appear to be a

consequence of the natural dispersion of biofilms, as a random distribution in microalgal biomass was observed on most shores of differing geology.

2.4.2 Abundance and composition of epilithic biofilms in relation to seasonal patterns and vertical distribution

In the south coast of England epilithic biofilms have been investigated in a very early study, which was restricted to one shore (Aleem, 1950). More detailed and extensive work on the ecology of epilithic biofilms has only been carried out on the Isle of Man (Hill, 1990; Hill and Hawkins, 1991; Thompson, 1996). My study provided, for the first time, a large scale description of the abundance, composition and spatial distribution of epilithic microbial communities along the south coast of England.

Microbial communities sampled along the different rocky shores showed a substantially similar composition, consisting mainly of diatoms and cyanobacteria. Other components observed were round bacteria, microscopic forms of coralline algae, algal germlings and coccoliths. This type of community has been described in other studies on rocky shores (Hill, 1990; Thompson, 1996). Microscopic phototrophs, which consist of diatoms and cyanobacteria, were the main component of intertidal epilithic biofilms (Thompson, 1996). The most common diatom taxa observed on the different substrata were *Achnantes*, *Fragilaria* and *Cocconeis*. These diatoms are common in marine and brackish environments and have been already observed on rocky shores also by other authors (Aleem, 1950; Castenholz, 1963; Nicotri, 1977; Hill, 1990).

Microbial communities varied considerably between the two sampling dates, in July 1999 and March 2000. Diatoms were almost absent in summer, whilst their abundance increased dramatically in early spring, reaching peaks of over 100000 cm^{-2} on chalk shores. A similar pattern was observed by Hill and Hawkins (1991) and Thompson (1996) on the Isle of Man and throughout Europe by (Jenkins *et al.*, 2001), where abundance of diatoms fluctuated from very low values in summer to maximum values in late winter-early spring and by . Seasonal variability of biofilm has been also described in other temperate regions, always showing a decline of the diatom component in the summer (Castenholz, 1963; Underwood, 1984c). Diatoms are known

to be very sensitive to insolation and desiccation stresses (Richardson *et al.*, 1983; Blanchard *et al.*, 1997), which can become a limiting factors for these organisms during the summer.

Cyanobacteria did not show a distinct pattern between the summer and early spring sampling dates, as they varied considerably in all types of rocky shores considered. The abundance was low also in early spring, generally below 30% of cover. Thompson (1996) and Hill and Hawkins (1991) observed a similar pattern for cyanobacteria.

Differences in microalgal standing stock between summer and early spring were more evident than in the single components of microbial communities. In the summer sampling date microalgal biomass was considerably lower than in the early spring on almost all the rocky shores sampled. Although lack of replication within seasons precluded formal analysis of variance, the matched Sign test showed an unequivocal difference. The largest difference between summer and early spring was observed on chalk and limestone shores, where the amount of estimated chlorophyll-a doubled in early spring in more than one plot sampled. On the other rocky shores these differences were less apparent, particularly on old red sandstone. These results are consistent with the seasonal pattern observed in previous studies worldwide (Underwood, 1984c; MacLulich, 1987; Hill and Hawkins, 1991; Williams, 1993; Thompson, 1996). On limestone shores in the Isle of Man, Thompson (1996) and Hill and Hawkins (1991) observed a similar peak in February on limestone shores, followed by an abrupt decline in the summer months. Thompson (1996) also suggested that microalgal standing stock varied between season to a lesser extent than observed in the abundance of diatoms and cyanobacteria. In the present study seasonal variation in the abundance of diatoms and cyanobacteria was not as evident as in the microalgal standing stock even for carbonate rocks, where the endolithic component cannot be estimated by SEM analysis. A possible explanation for the different observation might lie in the extreme variability in the number of diatoms and cyanobacteria. In the early spring, counts of diatoms on replicate rock chips could vary from zero to over one hundred thousand cell per cm^2. It is likely therefore that the number of replicate samples was not always sufficient to cover the spatial variability. This could particularly true on the upper eulittoral, where limpet grazing can contribute to the

small scale patchiness of microbial film (Hill and Hawkins, 1990; Hill and Hawkins, 1991).

Epilithic communities did not differ between the littoral fringe and the upper eulittoral. The abundance of diatoms and cyanobacteria was similar in the two tidal zones, despite the large spatial variability among sampling units. Also, microalgal standing stock did not differ significantly between the two tidal zones considered, although, on average, higher values were observed at the upper eulittoral on chalk, limestone and sandstone shores. This is in apparent contrast with results on microalgal vertical distribution described by previous authors (Castenholz, 1963; Underwood, 1984b; MacLulich, 1987). MacLulich (1987) found that the abundance and diversity of diatoms decreased along a vertical gradient from lower to the upper shore. However, in the present study the two tidal zones chosen were not spatially separated as the tidal levels considered in the other studies. It is likely therefore that physical stresses, which are a limiting factor for biofilms on the upper shore (Underwood, 1980; Cubit, 1984), were very similar. The effect of grazing in the upper eulittoral zone should have caused, in theory, differences in the abundance of microalgae, as observed by Thompson (1996). However, no negative correlation was found between the density of limpets and diatoms or cyanobacteria, suggesting that if there is an influence of grazing on biofilms, than this is not as important as the effect of the physical factors. Interestingly, there was a significant positive correlation between limpet densities and chlorophyll-a, suggesting that microalgal biomass might promote grazing.

2.4.3 Considerations on the effects of the physical and chemical properties of the rock substratum on intertidal biofilms

Microalgal standing stock varied significantly among the different rock types on both sampling dates. The highest values of chlorophyll-a were estimated on chalk and limestone, intermediate on sandstone and low on old red sandstone and granite. This pattern was almost consistent between summer and spring sampling occasions, and tidal level. When differences in the physical and chemical properties of the rock types examined were ranked and compared with the differences in microalgal biomass, a strong match could be observed between microalgal standing stock and porosity. Porosity was higher on chalk and limestone, intermediate on sandstone, and negligible

on granite and old red sandstone. Ranked differences in microalgal biomass between rock types perfectly mirrored those in porosity in summer, at both tidal levels and in winter, in the upper eulittoral. Surface roughness and to a lesser extent rock hardness, appeared to be related to patterns observed in microalgal biomass.

Biofilms on porous rocks are likely to be more protected by desiccation stresses during low tide, particularly during summer (Edyvean *et al.*, 1985). A complex microtopography would also be expected to enhance microbial communities by providing refuges from grazers and from exposure to water movement. Hardness might indirectly influence the composition of biofilms, as soft rocks are generally made of calcite which can be easily colonised by the endolithic cyanobacteria. Thus the total microalgal standing stock will be higher on chalk and limestones than on hard, quartz-based rocks, where only epilithic microalgae can survive.

Significant differences were also observed in whole assemblage composition biofilms between the rock substrata. These differences were not apparent when the single components of the biofilm, diatoms and cyanobacteria, were analysed with the parametric univariate approach. In this case, the large spatial variability and the lack of normality of the data lessened the likelihood of detecting differences between the rock types considered. Using non parametric analysis and a multivariate approach, clearer patterns could be observed. In both summer and early spring microbial communities significantly differed between rock types but at different levels. Chalk and limestone appeared to have similar communities, which strongly differed from the biofilms on old red sandstone. Granite and sandstone occupied an intermediate position with communities overlapping in both directions but closer to old red sandstone. However, different components of the biofilm contributed to differences between rock types. These were mainly coralline algae, cyanobacteria and diatoms. Coralline algae were very scarce on chalk and limestone, but relatively common on old red sandstone, sandstone and granite. Cyanobacteria were also more abundant on old red sandstone and granite, than chalk and limestone. The abundance of diatoms followed an opposite pattern; they were very scarce on granite and old red sandstone and high on chalk and limestone. Very little information is available from the literature, as the influence of the rock substratum on biofilm has been poorly investigated on only two or three rock types (Blinn *et al.*, 1980; Edyvean *et al.*, 1985). However, several authors suggested a

potential effects of the substratum on the diversity of microbial community (Hamilton and Duthie, 1984; Pringle and Fletcher, 1986; Miller *et al.*, 1987; Snoeijs, 1991; Becker *et al.*, 1997)

2.4.4 Conclusions

There were major differences between seasons, but not between shore levels in the overall microbial biomass and community composition. There was also considerable variation at different scales, particularly between plots in both summer and spring. Despite this variability some differences in the patterns observed were discernible and, with suitable caution, can be attributed to rock type, although other broad-scale factors cannot be excluded. Microbial biomass was highest on chalk and least on old red sandstone, with intermediate values being found on limestone, sandstone and granite. Rock type appeared also to affect microbial composition, as this differed significantly between soft and hard rocks, in particular between chalk and old red sandstone, granite and sandstone.

Chapter 3

Within shore effects of rock type on biofilms

3.1 INTRODUCTION

In Chapter 2 the effect of rock type on the abundance and composition of microbial communities was investigated at a broad spatial scale, encompassing a variety of shores of differing geology along the south coast of England. This provided evidence for an effect of rock type on biofilms, which varied significantly between the different rock substrata. Unfortunately, natural geological patterns did not allow proper interspersion of the study sites. These therefore were subjected to different environmental conditions which may also have affected the patterns previously described. In this chapter comparisons between different rock types present on the same shores and less than 100s of metres apart were made, to provide comparisons which were free of all other potential confounding factors.

The substratum is not the only variable acting on rocky shore communities. Microbial films are known to be influenced by interaction of a variety of physical and biological factors whose relative importance may vary in time and space. Desiccation and thermal stresses can be important limiting factors during summer, leading to a significant decline in the microalgal abundance and dramatic changes in the species composition (Thompson, 1996; Thompson *et al.*, 2000; Jenkins and Hartnoll, 2001; Jenkins *et al.*, 2001). The effects of physical stresses on microbial communities also vary vertically on the shore. On the upper shore, harsh desiccation and insolation stresses strongly control the survival of microbial communities (Underwood, 1984; Williams, 1993). At mid and low shore, the abundance of epilithic microalgae seems to be more affected by grazers (Underwood, 1980). Grazing on biofilms also varies with season, as limpet grazing appears to be more intense during summer and autumn than in winter (Thompson, 1996; Jenkins and Hartnoll, 2001; Jenkins *et al.*, 2001). Wave exposure may also affect microalgal standing stock, which is apparently higher on sheltered shores (Jenkins and Hartnoll, 2001). These biotic and abiotic factors contributes to the spatial variability in microbial films, which occurs at a range of spatial scales, among geographical locations (Jenkins *et al.*, 2001), between shores (Thompson, 1996) and patches within shores (Hill and Hawkins, 1990; Thompson *et al.*, 1996). This variability often makes it difficult to generalise about the effect of a single factor on intertidal microbial communities.

The overall aim of this chapter was to assess the relative importance of the type of rock substratum on natural mature microbial communities compared with seasonal variation and other environmental factors such as tidal level. The first objective was to assess the effect of substrata with contrasting physical and chemical features. The following hypotheses were examined: 1) biofilms are more abundant on rock types characterised by high surface roughness and a porous structure; 2) the composition of microbial communities differs between substrata of contrasting geology. Secondly, the consistency of the patterns observed was compared between different sampling dates and tidal levels. It was anticipated that the effect of substratum would be more evident higher on the shore and in summer, where desiccation and thermal stresses have their greatest effect on microbial communities.

The first objective was achieved by comparing rock types of contrasting geology which were naturally interspersed within the same shore, and thus subjected to similar physical and biological conditions. Shores with such geological features are rare, and local comparisons were linked to the shores available on the English south coast. Three pairs of rock types were compared: chalk *versus* flint, which both occurred on two shores in the Isle of Wight, limestone *versus* chert, which co-occurred on only one shore at Portland Bill and shale *versus* dolomite, both adjacent on a rock ledge at Kimmeridge Bay. Sampling was carried out in summer and winter and at two different tidal levels, in the littoral fringe and the upper eulittoral.

3.2 MATERIAL AND METHODS

3.2.1 Study sites

Chalk – Flint comparison

Freshwater Bay and Culver Cliff located on the Isle of Wight (Figure 3.1a, b) were selected to compare chalk and flint rocks. These shores have very similar wave exposure, orientation and macrobiota (for more detailed descriptions of these sites, see Chapter 2). More importantly, they consist of the same geological type of chalk and flint, these being part of a unique large ridge diagonally crossing the Isle of Wight from west to east. These two shores are mainly made of chalk together with rounded lumps of flint randomly interspersed. Chalk and flint have contrasting physical and chemical properties.

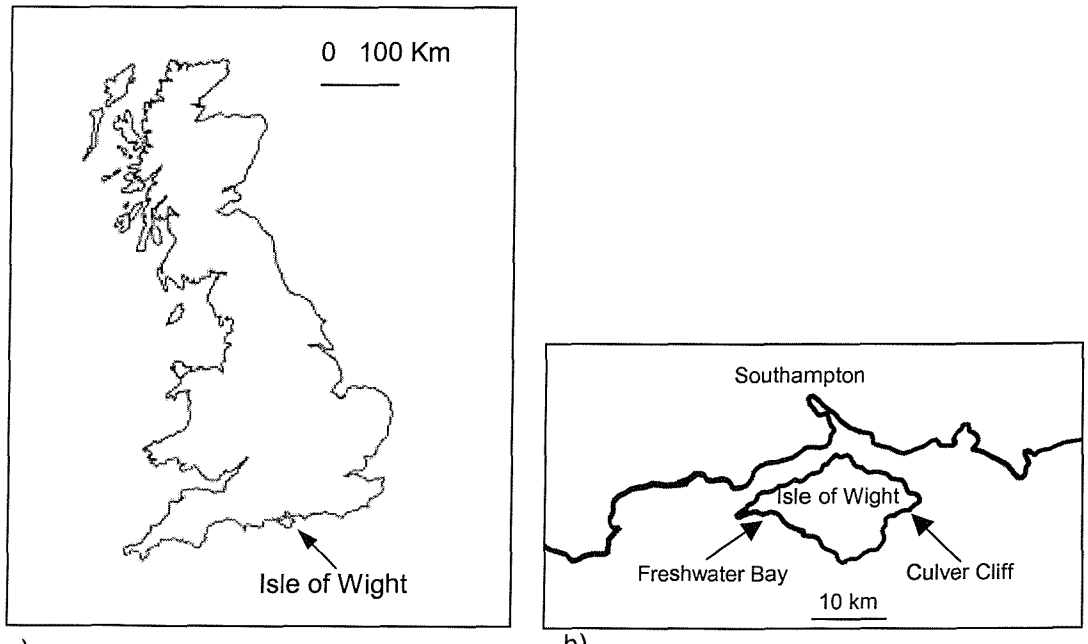


Figure 3.1 - Location of the study site; a): in the UK context; b): detailed map.

Chalk is a form of calcite, a mineral which consists of calcium carbonate. Its colour is white and its texture is finely grained. At higher resolution, using scanning electron microscopy, a mixed composition of calcite grains, fragments of fossil shells and coccolithophorids can be seen. The loose texture of chalk makes it very permeable to

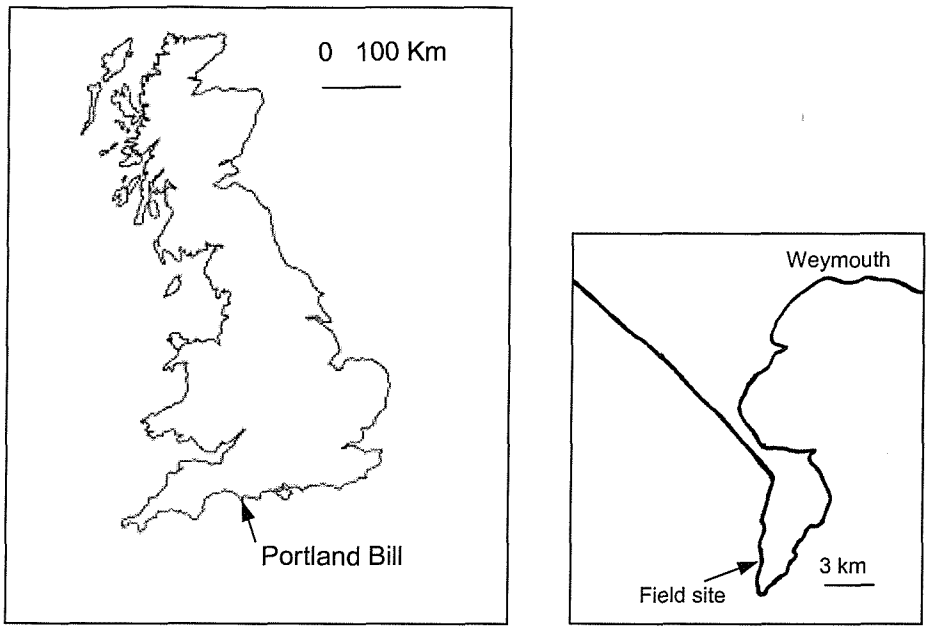
water; hence its surface remains damp when emersed at low tide. Chalk is extremely soft and therefore its surface is often deeply weathered, sometimes showing new substrate, as flakes of rock are easily broken off by wave action. Additionally, bioerosion often occurs on chalk shores as a consequence of grazing. In the UK this is mostly caused by limpets, which have hard teeth that can leave deep marks on the surface, clearly visible under the scanning electron microscope (Hawkins *et al.*, 1989). Endolithic microorganisms are also able to bore in the surface layer of soft carbonate rocks, contributing considerably to bioerosion (Schneider *et al.*, 1999; Donnand Boardman, 1988; Le Campion-Alsumard, 1989; Peyrot-Clausade *et al.*, 1995; Donn and Boardman, 1988; Le Campion-Alsumard, 1989; Peyrot-Clausade *et al.*, 1995).

Flint differs in composition and appearance. It has an organic origin as does chalk, although it consists of silica in a non-crystalline state. It is deep black or dark grey and the surface is very smooth. The surface has a porous layer of particles of silica, although permeability is very low. Its hardness is similar to that of quartz, and it is very difficult to fracture. Erosion of flint is therefore negligible compared with chalk.

A striking difference in macrobiota was observed between the two types of rocks. For example, at Freshwater Bay and Culver Cliff barnacles settled mainly on flint nodules, whilst very few individuals were observed on chalk.

Limestone – Chert comparison

Portland Bill (Dorset) was the only location available in the study area where limestone and chert co-occurred. Portland Bill is a peninsula extending south of the Dorset coast (Figure 3.2a, b). The area selected for this comparison was on the south west shore of Portland Bill, consisting of limestone with small inclusions of chert. The macrobiota was typical of exposed shores (for a more detailed description of the physical and biological features of the shore, see Chapter 2). The physical and chemical characteristics of limestone and chert are very similar to chalk and flint respectively.



a) b)

Figure 3.2 - Location of the study site; a): in the UK context; b): detailed map.

Limestone is different from chert in colour and hardness, although both have similar chemical composition. They both consist of calcite, although chert is in a less pure form. Limestone can vary greatly in hardness, colour and grain size and that present in the intertidal area of Portland Bill is oolithic. This type of limestone has a yellow colour and consists of coarse grains (oolites), up to 2 mm in diameter. Limestone is harder than chalk, but still sufficiently soft to be easily eroded leading to a very rough rock surface. Also, the coarse grains are not well cemented in the calcite, making the surface layer relatively friable. Similarly to chalk, this texture makes limestone permeable to water which can be retained on the surface.

Chert is a much harder rock than limestone and is made of silica in a non crystallised state like flint. Its appearance, however, differs considerably from flint. The colour is grey to light grey and the surface texture is rougher than flint. Porosity is also very low, especially when compared with the limestone.

Qualitative observations on macrobiota, which consisted mainly of ephemeral algae and barnacles, suggested that chert was more densely colonised than on limestone.

Shale – Dolomite comparison

Kimmeridge Bay is characterised by natural marine oil seeps emerging in the intertidal and subtidal zones (Figure 3.3a, b). In some areas of the intertidal shore oil shale and

non-oil bearing dolomite can be found together at the same tidal level. These two rocks have very different features.

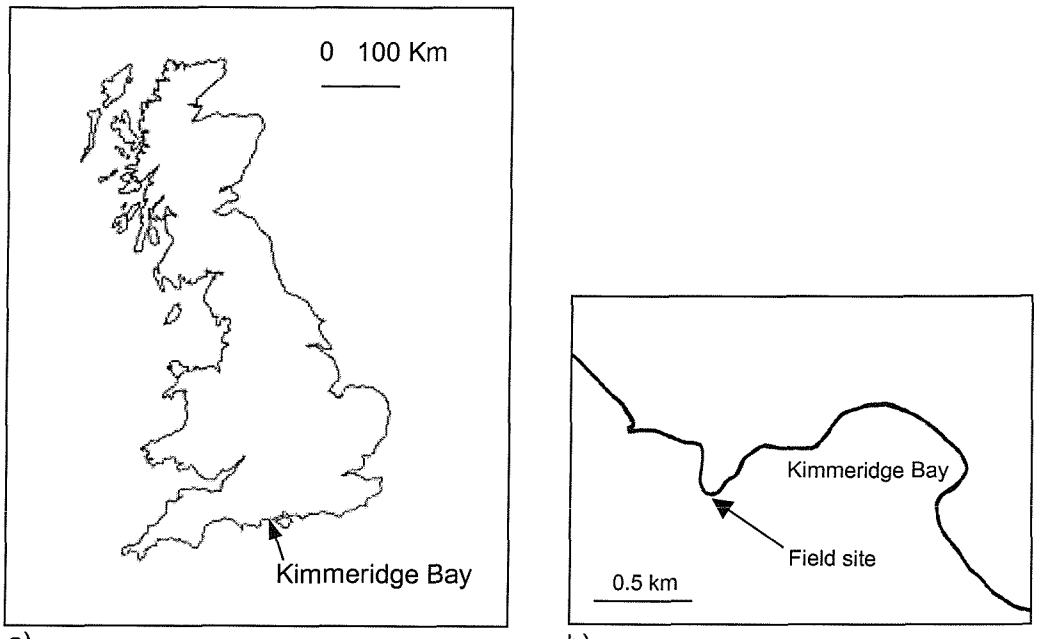


Figure 3.3 - Location of the study site; a): in the UK context; b): detailed map.

Oil shale is a type of clay, consisting of very fine grains immersed in matrix of aliphatic, aromatic and heterocyclic compounds. This rock, deep black in colour, is structured in thin layers, which easily flake off from the surface. The surface texture is extremely smooth.

Dolomite, also called cementstone, is a type of limestone which contains mainly calcium-magnesium carbonate. It is a compact rock, harder than other limestones. Dolomite is finely grained, without any layered structure and its colour can vary from off-white to light grey.

The effect of these two rock types on macrobiota has already been investigated by Holland *et al.* (1986), who found higher abundance and distribution of fucoid algae, barnacles and limpets on dolomite.

3.2.2 Sampling design

For chalk *versus* flint and limestone *versus* chert comparisons, sampling was carried out at the same time as the broad scale surveys. The shores selected for the specific

comparisons between these rock types were the same as considered in the broad scale study, thus a single set of samples from chalk and limestone rocks was collected to accomplish the purpose of both studies. Temporal replication was not included in the summer and early spring sampling, therefore no formal statistical comparison between seasons could be made. However, the terminology of summer and early spring was used here to refer to July and March sampling dates. The two tidal zones selected, the littoral fringe and upper eulittoral, were defined on the basis of the biological features of the shore (Lewis, 1964).

Sampling design differed considerably between each local scale comparison, depending on the availability of rock types and access to the shore. Spatial replication and sampling dates are summarised in Table 3.1. The number of replicate samples was the same in all comparisons (see paragraph 3.2.3.1 for details).

In the chalk *versus* flint comparison two shores were selected (Freshwater Bay and Culver Cliff, Isle of Wight). On each shore and for each type of substratum replicate rock chips were collected from two random areas at each of two tidal levels (littoral fringe and upper eulittoral). Sampling was carried out on one occasion in both July 1999 and March 2000.

The limestone *versus* chert comparison was limited to one shore (Portland Bill), as chert inclusions were not found at other sites. On this shore, sampling was carried out in the littoral fringe and at the upper eulittoral. Replicated rock chips for each rock type were collected in two random areas within each tidal zone. However, chert could be found only in one area in the upper eulittoral. The sampling carried out in July 1999 and March 2000.

In the shale *versus* dolomite comparison, five random areas along the edge of a wide rocky platform extending on the west side out of the Kimmeridge Bay (Dorset) were selected. Within each area, located in the upper eulittoral, equivalent sets of rock chips were chiselled from the oil shale and from dolomite. Sampling was carried out on one occasion only, at the end of August 1999, as the access to the area was subsequently forbidden due to military training.

As grazing represents an important factor controlling intertidal algae including biofilms (Hill, 1990), estimates of density of grazer (*Patella vulgata*) were also made on each shore using 10 replicate quadrats 50x50 cm.

Table 3.1 – Summary table of the sampling design carried out in each local comparison. See text for details on shore location and sampling dates.

Rock type	Shore		Littoral fringe		Upper Eulittoral		Sampling occasion	
	Sh 1	Sh 2	Area 1	Area 2	Area 1	Area 2	Summer	Winter
Chalk	√	√	√	√	√	√	√	√
Flint	√	√	√	√	√	√	√	√
Limestone	√		√	√	√	√	√	√
Chert	√		√	√	√		√	√
Dolomite	√				5 areas		√	
Shale	√				5 areas		√	

3.2.3 Laboratory analysis

Microalgal biomass and biofilm composition

Chlorophyll-a extraction was used to obtain an index of microalgal biomass. On each occasion 15 replicate rock chips (10-15 cm^2 each) were collected with hammer and chisel from each type of rock and in each sampling area. Film composition was assessed using scanning electron microscopy in air dried samples (Hawkins *et al.*, 1989). For this purpose 6 small rock chips (approximately 1 cm^2) were also collected from the same sampling areas. Field sampling techniques and laboratory protocols were the same used for the broad scale study (for a more detailed description of the methods, see Chapter 2).

Physical and chemical characteristics of the rocks

The physical and chemical properties of flint, chert, dolomite and shale were measured on cores which were obtained from large samples collected on the shores. Chemical

composition, porosity, surface roughness and hardness were experimentally determined using the methodologies described in Chapter 2 and compared between each pair of rock types. The properties of chalk and limestone were already measured in the broad scale survey, therefore the same set of data was used.

3.2.4 Statistical analysis

Comparison of microbial films between rock types was made using, whenever possible, both univariate and multivariate approach. Separate analyses of variance were performed for each sampling date, as doing one overall analysis proved unnecessarily complex and led to many of the relevant terms being untestable.

Differences in microalgal standing stock and in the abundance of diatoms and cyanobacteria were analysed using ANOVA (G-Mav software package). This technique is, however, relatively weak in detecting significant differences when applied to data which are highly variable, as observed in previous studies. The multivariate analysis (PRIMER software package) was therefore also used to compare relative abundance of diatoms, cyanobacteria and other taxa between the two different rock types.

The experimental design used for each comparison had to be slightly different, as spatial replication varied according to the availability of areas where the rocks were properly interspersed. However, an equivalent number of replicate samples was examined in all comparisons: n=15 for estimating microalgal standing stock and n= 5 for the analysis of species composition.

For the comparison between chalk and flint, the following factors were considered: 1) Shore, 2 levels, random and orthogonal; 2) Tidal level, 2 levels, fixed and orthogonal, 3) Plot, 2 levels, random and nested in shore and tidal level; 4) Rock type, 2 levels, fixed and orthogonal.

For the comparison of limestone and chert only one shore was available. As spatial replication was possible only at the littoral level, the experimental design involved just two factors: 1) Plot, 2 levels, random and orthogonal; 2) Rock type, 2 levels, fixed and orthogonal.

One shore and five plots were considered in the comparison between oil shale and dolomite. The factors included in the design were: 1) Plot, 5 levels, random and orthogonal; 2) rock type, 2 levels, fixed and orthogonal.

For parametric analyses, data were tested for heterogeneity of variance using Cochran's test and transformed where necessary. When the test was still significant, ANOVA was performed anyway, as the number of replicate samples being examined was sufficiently large to reduce problems with heterogeneity of variances (Underwood, 1981). Also, heterogeneity of variances are likely to only cause a problem in the analysis only if results are significant, due to an increased risk of Type I Error (Underwood, 1997). Multiple comparisons of levels within significant factors were made using Student Newman Keuls (SNK) tests.

Pearson's correlation coefficient was used to relate limpet density to microalgal biomass, diatom and cyanobacteria abundance.

3.3 RESULTS

3.3.1 Physical and chemical properties of the rock types compared

Chalk *versus* Flint

The experimental analysis of chemical composition, hardness, porosity and surface roughness, confirmed the contrast in the physical and chemical properties of chalk and flint previously mentioned in the description of the study sites (see Table 3.2, for a summary of the properties). X-Ray analysis showed that chalk consists mainly of calcite (calcium carbonate) with minimal traces of quartz (silicon dioxide) whilst flint is uniquely made of quartz. The hardness of these rocks, which depends on their mineral composition, is very different. The hardness of the chalk cores examined was grade 3 on the Moh's scale, which is attributed to very soft minerals. Flint hardness was between 8 and 9 grade, which corresponds to the hardest rocks, other than diamond. Porosity, expressed indirectly as the amount of water adsorbed and evaporated from the rock, also differed greatly between chalk and flint (Figure 3.4). Flint cores adsorbed a minimal amount of seawater, which evaporated completely after 30 minutes. In contrast, chalk, absorbed a greater amount of seawater, retaining it for a relatively long time. Surface roughness on natural weathered chalk samples was significantly higher than flint (ANOVA, $F_{1,18} = 30.44$; $p<0.001$). On average, surface roughness on chalk was the double that of flint (Figure 3.5). Also, rugosity of chalk was much more variable than flint, as also indicated by the larger standard error.

Table 3.2 – Summary of the physical and chemical properties of chalk and flint.

	Chalk	Flint
Origin	sedimentary	sedimentary
Chemical composition	calcite	quartz
Colour	white to off-white	black with a brownish surface
Grain size	fine grained	very fine grained
Porosity	very high	very low
Hardness (Moh's scale)	3	8-9

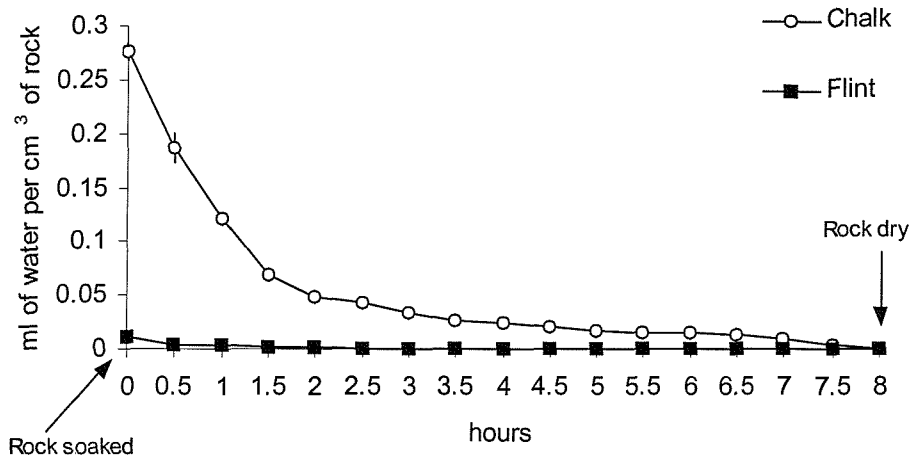


Figure 3.4 – Porosity, expressed as seawater loss (ml) per cm^3 of rock, in chalk and flint rocks after soaking in seawater for three hours and oven drying at 30°C. Values were averaged from 10 replicate cores of each rock type. Vertical bars indicate standard error.

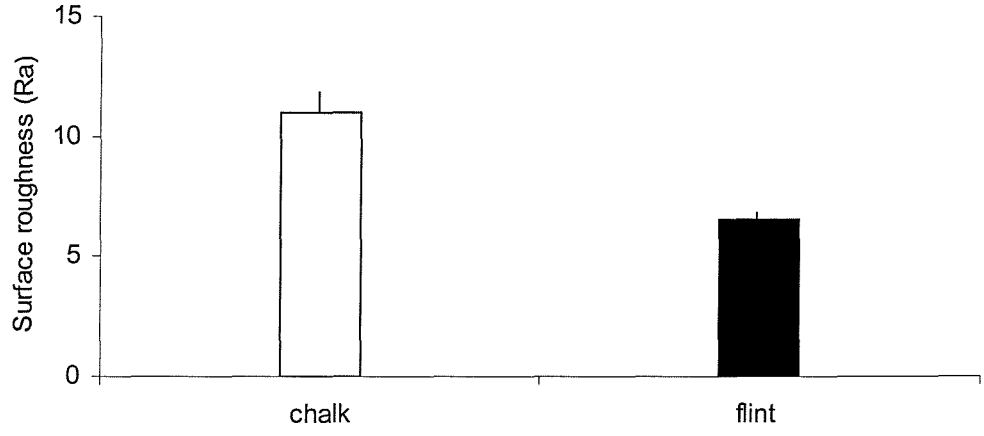


Figure 3.5 – Surface roughness measured with a Talysurf profiler (Taylor Hobson) on 10 chalk and flint cores. Bars indicate standard errors.

Limestone *versus* Chert

Limestone and chert showed contrasting properties in terms of chemical composition and hardness and, to a lesser extent, in porosity and surface roughness (Table 3.3). The chemical composition of limestone and chert is very similar to chalk and flint respectively. Calcite was the main component of the limestone examined, although there were also small quantities of quartz. Chert, as flint, consisted mainly of quartz, but with some traces of calcite. The relative hardness was 3.5 on Moh's scale for limestone and 8-9 for chert. Limestone appeared to be more porous than chert, as it absorbed more water than chert (Figure 3.6). Limestone, however, dried out quickly,

after approximately 90 minutes. In contrast, a small amount of water was kept inside the chert for over six hours. Limestone is made of oolithic grains interspersed with large pores. Water can therefore be easily absorbed in such a loose structure but also evaporates faster. Chert is probably less permeable to water, but this can be retained for longer. Both limestone and chert showed very rough surfaces (Figure 3.7). Surface roughness, however, was significantly higher on limestone than on chert (ANOVA, $F_{1,18} = 4.98$, $p<0.05$).

Table 3.3 - Summary of the physical and chemical properties of limestone and chert.

	Limestone	**Chert**
Origin	sedimentary	sedimentary
Chemical composition	mainly calcite	mainly quartz
Colour	yellow	grey to dark grey
Grain size	coarse grained	fine grained
Porosity	high	low
Hardness (Moh's scale)	3.5	8-9

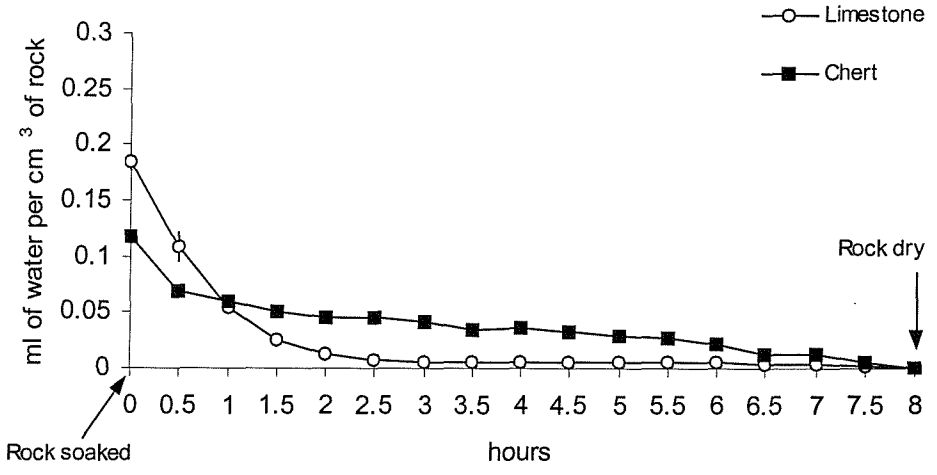


Figure 3.6 – Porosity, expressed as seawater loss (ml) per cm^3 of rock, in limestone and chert rocks after soaking in seawater for three hours and oven drying at 30°C. Values were averaged from 10 replicate cores of each rock type. Vertical bars indicate standard error.

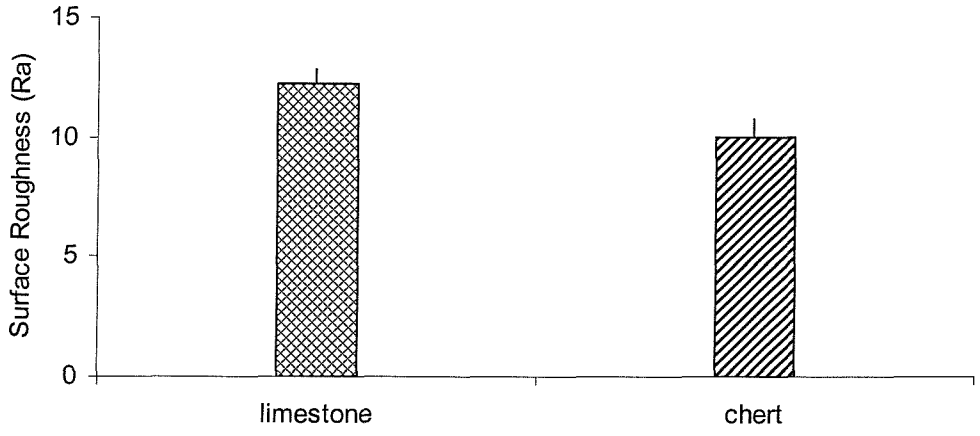


Figure 3.7 – Surface roughness measured on 10 limestone and chert cores. Bars indicate standard errors.

Oil shale *versus* Dolomite

The physical and chemical properties of oil shale and dolomite are markedly different (Table 3.4). Oil shale is a peculiar type of rock, as it mainly consist of organic matter mixed with traces of calcite and quartz. Results from X-Ray analysis of the dolomite used in the study showed that ferroan dolomite (calcium magnesium carbonate) is the main component of this type of limestone, although a small quantity of calcite was observed. The yellowish colour of the dolomite surface is the result of the oxidation of the iron. The shiny black colour of shale depends probably on its bituminous components. Porosity differed considerably between shale and dolomite (Figure 3.8). Oil shale and dolomite have both have a medium hardness compared to Moh's scale (4 for the oil shale and 5 for the dolomite). The characteristic flaky structure of the oil shale, however, makes this more vulnerable to breakage than dolomite. Dolomite is a compact rock and therefore very little permeable to water. Oil shale appeared to absorb and retain more water than dolomite, despite its finely grained structure. It is likely, however, that water was not absorbed, but drained in the spaces between the thin layers of rock characterising the oil shale at Kimmeridge. Clear differences in surface roughness between the two rock types were also observed (Figure 3.9). The natural surface of dolomite was significantly rougher than the smooth oil shale (ANOVA, $F_{1, 18} = 81.56$; $p<0.001$).

Table 3.4 – Summary of the physical and chemical properties of oil shale and dolomite.

	Oil shale	**Dolomite**
Origin	sedimentary	sedimentary
Chemical composition	mainly carbonaceous matter	mainly ferroan dolomite
Colour	black	dark grey, yellowish
Grain size	very fine grained	fine grained
Porosity	high	very low
Hardness (Moh's scale)	4	5

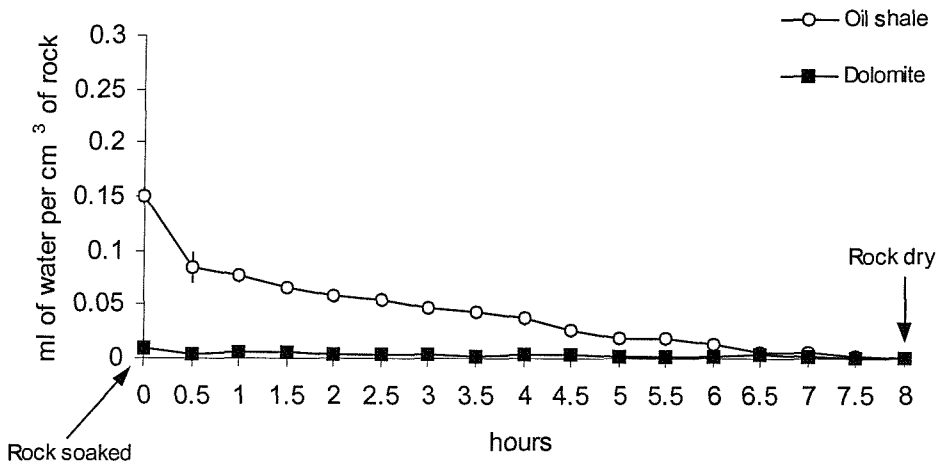


Figure 3.8 – Porosity, expressed as seawater loss (ml) per cm^3 of rock, in oil shale and dolomite rocks after soaking in seawater for three hours and oven drying at 30°C. Values were averaged from replicate cores of each rock type (8 for oils shale and 10 for dolomite). Vertical bars indicate standard error.

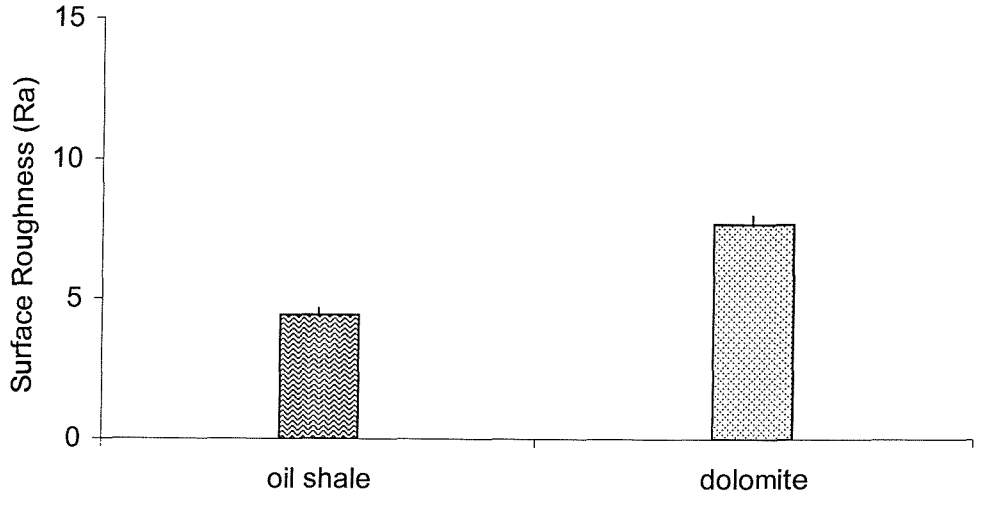


Figure 3.9 – Surface roughness measured on 8 oil shale and 10 limestone cores. Bars indicate standard errors.

3.3.2 Microbial films on chalk and flint rocks

Microalgal standing stock

Differences in the microalgal biomass, expressed as chlorophyll-a, between chalk and flint rocks were apparent on both sampling dates and for most of the plots sampled, independently of the shore location, tidal level or sampling date (Figure 3.10a-d). Ten out of sixteen plots sampled showed significantly higher microalgal biomass on chalk than flint (SNK's, Table 3.7). Five plots showed no differences between the two rock types; microalgal biomass was significantly greater on flint than chalk in only one plot during the winter sampling at Freshwater Bay. It was also apparent that microalgal biomass on both types of rock was highly spatially variable both between shores and plots and between dates. The observed patterns were analysed in detail and differences between chalk and flint were formally tested.

Summer (July 1999)

In the summer mean values of chlorophyll a per plot were higher on chalk, ranging between 6.12 μg cm^{-2} to 11.33 μg cm^{-2} and in contrast to values between 2.95 μg cm^{-2} and 8.49 μg cm^{-2} on flint (Figure 3.10a, b). On both shores large differences between the two rock substrata were observed, although there was considerable variation between the two tidal levels and plots. The analysis of variance confirmed the pattern observed (Table 3.5). Significant differences in microalgal biomass were found between chalk and flint, although these were not consistent across the plots (Rock X Plot (Shore X Tidal level); Table 3.5, 3.7).

At Freshwater Bay, microalgal standing stock was significantly higher on chalk than on flint rock except for plot 1, located in the littoral fringe (Figure 3.10a; Table 3.7). At the upper eulittoral level differences between rock types were particularly evident, with chlorophyll a being more than three times greater on chalk. At Culver Cliff, differences between the two rock types were less marked but still microalgal biomass was significantly higher on chalk in both plots located in the littoral fringe and in one plot in the upper eulittoral (Figure 3.10b;Table 3.7).

Microalgal biomass on chalk and flint differed between the two tidal levels, although the pattern was not consistent between shores. At Freshwater Bay (Figure 3.10a; Table 3.7), there was higher biomass on chalk at the upper eulittoral level. Conversely, at Culver Cliff microalgal biomass was higher on chalk in the littoral fringe (Figure 3.10b; Table 3.7).

Spring (March 2000)

In the spring, microalgal biomass estimated on both rocks was considerably higher than during the summer: chlorophyll-a values (mean per plot) ranging from 6.56 μg cm^{-2} to 19.79 μg cm^{-2} on chalk and from 9.07 μg cm^{-2} and 14.38 μg cm^{-2} on flint (Figure 3.10c, d). The analysis of variance showed a significant interaction between rock type and plot (Rock X Plot (Shore X Tidal level); Table 3.6), suggesting that there were differences in microalgal biomass between chalk and flint, although these were not consistent across all the plots. On both shores, there were no differences between the two rock types in the plots located in the littoral fringe, except for one, where flint had significantly higher microalgal biomass (SNK's, Table 3.7). By contrast, well defined, consistent differences were shown on both shores at the upper eulittoral level, where microalgal standing stock was consistently greater on chalk than on flint in all plots (Figure 3.10c, d; SNK's, Table 3.7).

Tidal level appeared to influence the effect of rock type on microalgal biomass, as differences between chalk and flint were evident only in the upper eulittoral zone. Microalgal biomass appeared also to vary considerably among plots (Plots (Shore X Tidal level); Table 3.6).

Biofilm composition

On both chalk and flint substrata biofilms consisted mainly of diatoms and cyanobacteria. Other components included protozoans, ephemeral algae, macroalgal germlings and encrusting corallines.

The total number of diatoms on both rock types differed greatly between the summer and spring (Figure 3.11a-d). In summer diatoms were almost absent on both shores, with numbers not exceeding 0.1 x 10^3 cells cm^{-2} (Figure 3.11a, b). In spring, abundance of diatoms was much higher than in summer, particularly at Freshwater

Bay, where densities of diatoms reached 113740 cm^{-2} in the upper eulittoral (Figure 3.11c, d). There was significant variation between shores during the spring in the overall abundance of diatoms with more at Freshwater Bay than at Culver Cliff (Table 3.9; SNK $p<0.01$; Figure 3.11c, d).

Rock type did not show a significant effect on the abundance of diatoms on either sampling date (Tables 3.8 and 3.9; Figure 3.11 a-d). In the spring, however, the number of diatoms appeared to be generally higher on chalk than on flint on both shores, although significant differences were found only in single plots, in each of the littoral fringe and the upper eulittoral at Culver Cliff (SNK's, $p<0.01$; Figure 3.11c, d).

The abundance of cyanobacteria varied little between the two sampling dates (Figures 3.12a-d). The percentage cover of cyanobacteria was always low, generally under 20%, on both types of rocks. The abundance of cyanobacteria varied between plots, particularly in the summer and at plot level (Table 3.10; Figure 3.12a, c).

Some differences in the abundance of cyanobacteria between the two types of rock could be observed in few plots at Freshwater Bay in both summer and early spring, but there was not a clear pattern, as also shown by ANOVA (Table 3.10 and 3.11; Figure 3.12a-d).

Multivariate analysis of the microbial community as a whole showed no significant differences in the composition between chalk and flint in the summer, in the littoral fringe (ANOSIM, $R = 0.26$, $p = 0.09$; Figure 3.13a). In the upper eulittoral zone, microbial communities on chalk and flint differed considerably (ANOSIM, $R = 0.49$, $p = 0.001$; Figure 3.13b). The cyanobacteria component and coralline algae accounted for over 70 % of the difference betweeen the two rock types (from SIMPER analysis). Cyanobacteria were more abundant on chalk, whilst corallines were present only on flint. In the spring, microbial communities on chalk and flint were different at both tidal levels. For the littoral fringe there was greater dissimilarity in assemblages between the rock types (ANOSIM, $R = 0.53$, $p = 0.001$; Figure 3.14a) than that found for the upper eulittoral (ANOSIM, $R = 0.25$, $p = 0.001$; Figure 3.14b). In the spring, diatoms and corallines mostly contributed to the differences between chalk and flint. As in the summer sampling date, results from SIMPER analysis showed that the

abundance of diatoms was higher on chalk than on flint, whilst corallines were almost absent on chalk.

Spatial variability in the microbial film and abundance of grazers

A high level of variability at different spatial scales typified all the results presented so far. Spatial variability influenced microalgal biomass on both rock types, although this was more evident for chalk substrata. When variances among plots were formally compared between the two rock types, chalk showed higher variation in biomass than flint during the summer. This was also shown by nearly significant result from ANOVA (Table 3.12, Figure 3.15a); but this pattern was not consistent over time. In spring flint appeared to have more variability in biomass than chalk, particularly in Culver Cliff, although this difference was not significant (Table 3.13, Figure 3.15b).

At Freshwater Bay and Culver Cliff the most abundant grazers in the eulittoral zone were the limpets *Patella vulgata*, while in the littoral fringe these were almost absent. At this level, only a few littorinids, mainly *Littorina saxatilis*, were present. In the littoral fringe, limpet density was very low on both sampling dates, while in the upper eulittoral level number of grazers varied considerably between the shores and among the plots (Table 3.14). In the summer, no significant correlation was shown between limpet densities and chlorophyll-a on both rock types (Table 3.15; Figure 3.16a). In contrast, in spring, there was a significant positive correlation between limpet density and chlorophyll-a on chalk alone (Table 3.15, Figure 3.16b).

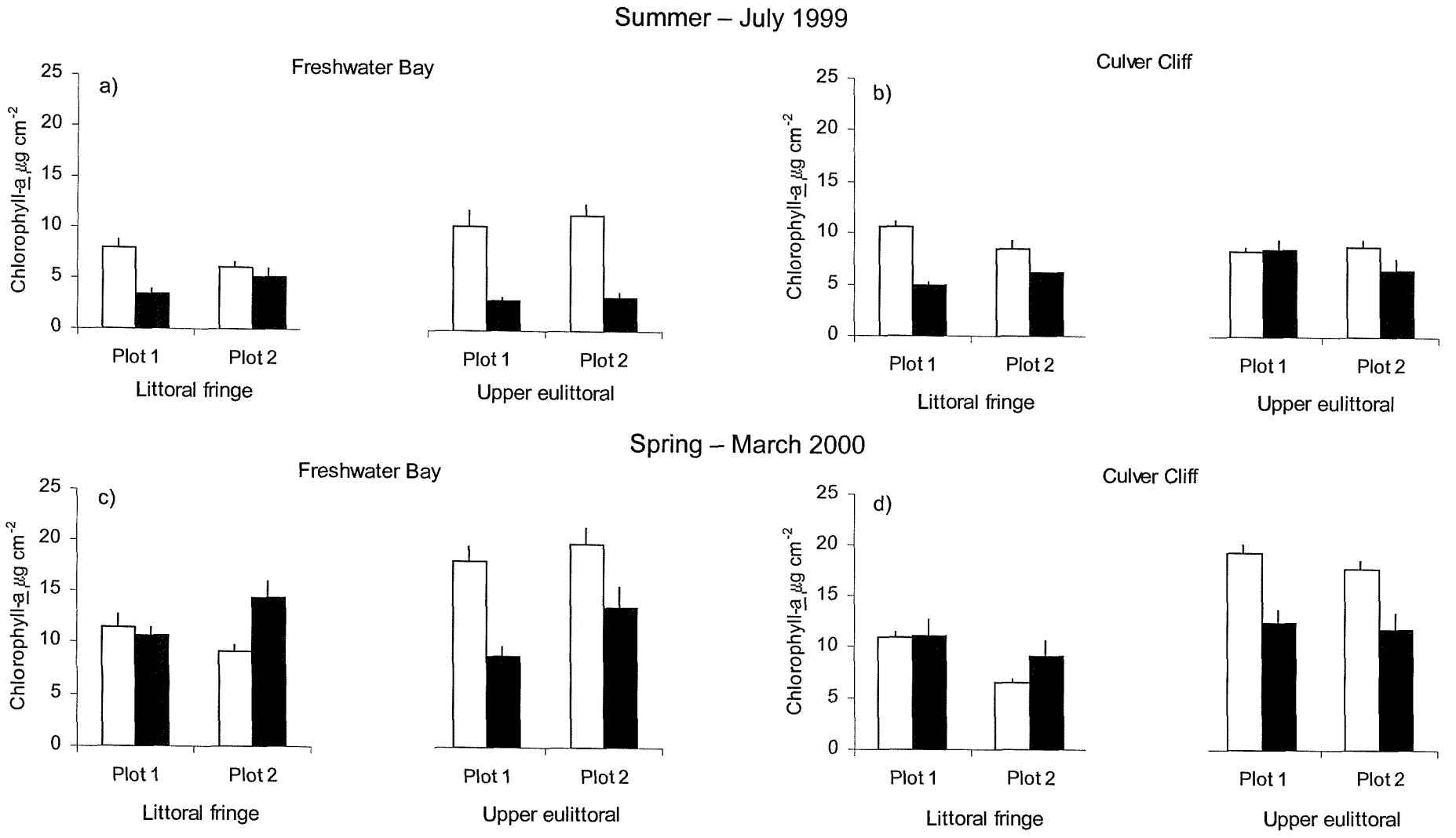


Figure 3.10 - Microalgal standing stock,(chlorophyll-a), on chalk (white bars) and flint (black bars), at Freshwater Bay (a, c) and Culver Cliff (b, d) in July 1999 (a, b) and March 2000 (c, d). Bars indicate standard errors.

Table 3.5 – Analysis of variance to test differences in microalgal standing stock between chalk and flint in July 1999. Data were Ln (X+1) transformed. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Shore	1	2.96	27.92	Plot (Sh X Ti)	**0.006**
Tidal level	1	0.67	7.10	Sh X Ti	0.229
Plot (Sh X Ti)	4	0.11	0.60	RES	0.660
Rock type	1	16.94	5.99	Sh X Ro	0.247
Sh X Ti	1	0.09	0.88	Plot (Sh X Ti)	0.400
Sh X Ro	1	2.83	6.04	Ro X Pl (Sh X Ti)	0.070
Ti X Ro	1	0.09	0.03	Sh X Ti X Ro	0.893
Ro X Pl (Sh X Ti)	4	0.47	2.67	RES	**0.033**
Sh X Ti X Ro	1	3.21	6.84	Ro X Pl (Sh X Ti)	0.059
RES	224	0.18			
TOT	239				

Table 3.6 – Analysis of variance to test differences in microalgal standing stock between chalk and flint in March 2000. Data were Square root of (X+1) transformed. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Shore	1	1.57	0.64	Plot (Sh X Ti)	0.468
Tidal level	1	20.63	7.83	Sh X Ti	0.219
Plot (Sh X Ti)	4	2.46	5.4	RES	**<0.001**
Rock type	1	3.54	21.23	Sh X Ro	0.136
Sh X Ti	1	2.63	1.07	Plot (Sh X Ti)	0.359
Sh X Ro	1	0.17	0.15	Ro X Pl (Sh X Ti)	0.718
Ti X Ro	1	20.19	15.44	Sh X Ti X Ro	0.159
Ro X Pl (Sh X Ti)	4	1.11	2.43	RES	**0.048**
Sh X Ti X Ro	1	1.31	1.18	Ro X Pl (Sh X Ti)	0.338
RES	224	0.45			
TOT	239				

Figure 3.7 – SNK tests for differences between chalk (Ck) and flint (Fl) within plots in summer 1999 and winter 2000.

	Freshwater Bay				Culver Cliff			
	Plot 1	**p**	**Plot 2**	**p**	**Plot 1**	**p**	**Plot 2**	**p**
July 1999								
Littoral fringe	Ck > Fl	<0.01	Ck = Fl	n.s.	Ck > Fl	<0.01	Ck > Fl	<0.01
Upper eulittoral	Ck > Fl	<0.01	Ck > Fl	<0.01	Ck = Fl	n.s.	Ck > Fl	<0.05
March 2000								
Littoral fringe	Ck = Fl	n.s.	Fl>Ck	<0.01	Ck = Fl	n.s.	Ck = Fl	n.s.
Upper eulittoral	Ck > Fl	<0.01	Ck > Fl	<0.01	Ck > Fl	<0.01	Ck > Fl	<0.01

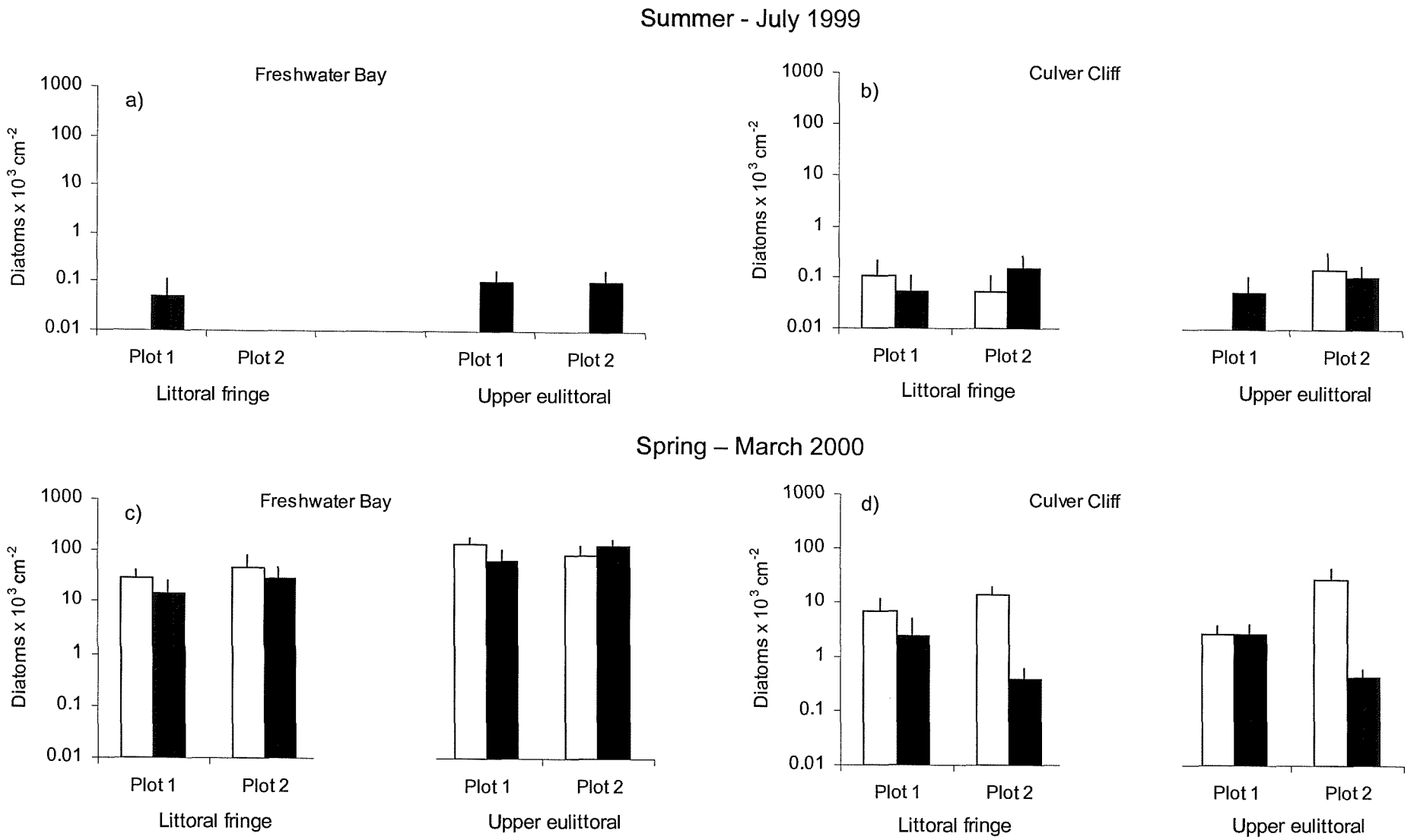


Figure 3.11 a-d - Abundance of diatoms, in chalk (white bars) and flint (black bars), at Freshwater Bay (a, c) and Culver Cliff (b, d) in July 1999 (a, b) and March 2000 (c, d). Data are represented on a log scale to allow graphic comparison between the two dates. Bars indicate standard errors.

Table 3.8 – Analysis of variance to test differences in abundance of diatoms between chalk and flint in July 1999. Data were Ln(X+0.1) transformed. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Shore	1	0.79	3.28	Plot (Sh X Ti)	0.145
Tidal level	1	0.09	0.20	Sh X Ti	0.732
Plot (Sh X Ti)	4	0.24	0.58	RES	0.676
Rock type	1	1.46	7.81	Sh X Ro	0.219
Sh X Ti	1	0.46	1.92	Plot (Sh X Ti)	0.238
Sh X Ro	1	0.19	1.52	Ro X Pl (Sh X Ti)	0.285
Ti X Ro	1	0.30	1.63	Sh X Ti X Ro	0.423
Ro X Pl (Sh X Ti)	4	0.12	0.30	RES	0.878
Sh X Ti X Ro	1	0.19	1.52	Ro X Pl (Sh X Ti)	0.285
RES	64	0.41			
TOT	79				

Table 3.9 – Analysis of variance to test differences in abundance of diatoms between chalk and flint in March 2000. Data were Ln (X+0.1) transformed. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Shore	1	189.33	47.97	Plot (Sh X Ti)	**0.002**
Tidal level	1	40.04	12.31	Sh X Ti	0.177
Plot (Sh X Ti)	4	3.95	1.64	RES	0.174
Rock type	1	33.62	2.51	Sh X Ro	0.358
Sh X Ti	1	3.25	0.82	Plot (Sh X Ti)	0.415
Sh X Ro	1	13.39	2.54	Ro X Pl (Sh X Ti)	0.186
Ti X Ro	1	1.50	47.31	Sh X Ti X Ro	0.092
Ro X Pl (Sh X Ti)	4	5.26	2.19	RES	0.080
Sh X Ti X Ro	1	0.03	0.01	Ro X Pl (Sh X Ti)	0.942
RES	64	2.40			
TOT	79				

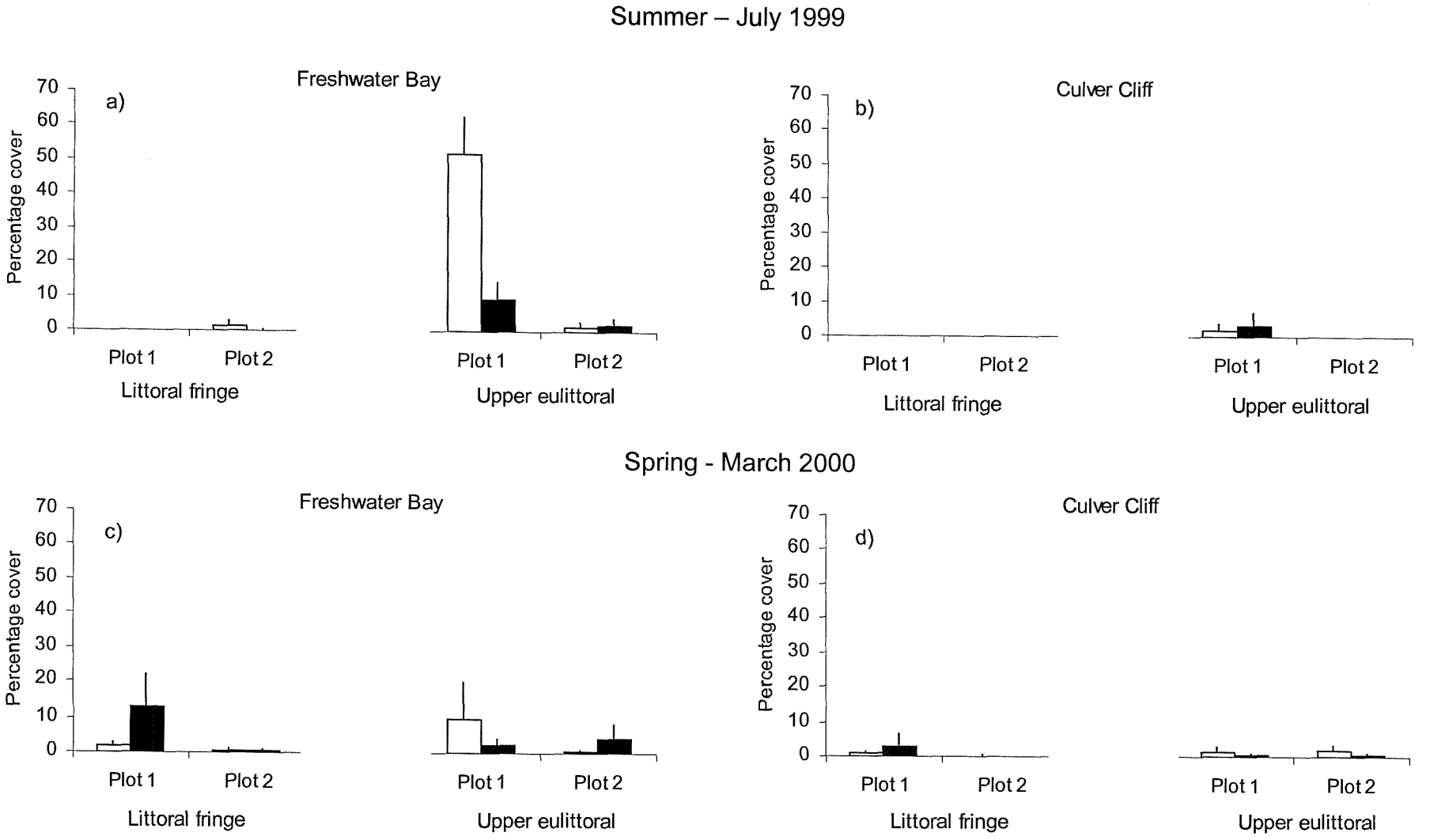


Figure 3.12 – Abundance of cyanobacteria, in chalk (white) and flint (black), at Freshwater Bay (a, c) and Culver Cliff (b, d) in July 1999 (a, b) and March 2000 (c, d). Bars indicate standard errors.

Table 3.10 – Analysis of variance to test differences in abundance of cyanobacteria between chalk and flint in July 1999. Data were Ln (X+0.1) transformed. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Shore	1	35.18	2.33	Plot (Sh X Ti)	0.201
Tidal level	1	53.47	2.60	Sh X Ti	0.353
Plot (Sh X Ti)	4	15.07	8.30	RES	**< 0.001**
Rock type	1	7.80	1.47	Sh X Ro	0.439
Sh X Ti	1	20.55	1.36	Plot (Sh X Ti)	0.308
Sh X Ro	1	5.31	3.05	Ro X Pl (Sh X Ti)	0.156
Ti X Ro	1	5.32	1.61	Sh X Ti X Ro	0.425
Ro X Pl (Sh X Ti)	4	1.74	0.96	RES	0.437
Sh X Ti X Ro	1	3.30	1.90	Ro X Pl (Sh X Ti)	0.240
RES	64	1.82			
TOT	79				

Table 3.11 – Analysis of variance to test differences in abundance of cyanobacteria between chalk and flint in March 2000. Data were Ln (X+0.1) transformed. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Shore	1	6.74	1.17	Plot (Sh X Ti)	0.340
Tidal level	1	0.61	5.90	Sh X Ti	0.249
Plot (Sh X Ti)	4	5.76	1.64	RES	0.175
Rock type	1	0.05	0.02	Sh X Ro	0.917
Sh X Ti	1	0.10	0.02	Plot (Sh X Ti)	0.900
Sh X Ro	1	3.16	4.10	Ro X Pl (Sh X Ti)	0.113
Ti X Ro	1	0.22	0.39	Sh X Ti X Ro	0.644
Ro X Pl (Sh X Ti)	4	0.77	0.22	RES	0.927
Sh X Ti X Ro	1	0.57	0.73	Ro X Pl (Sh X Ti)	0.440
RES	64	3.51			
TOT	79				

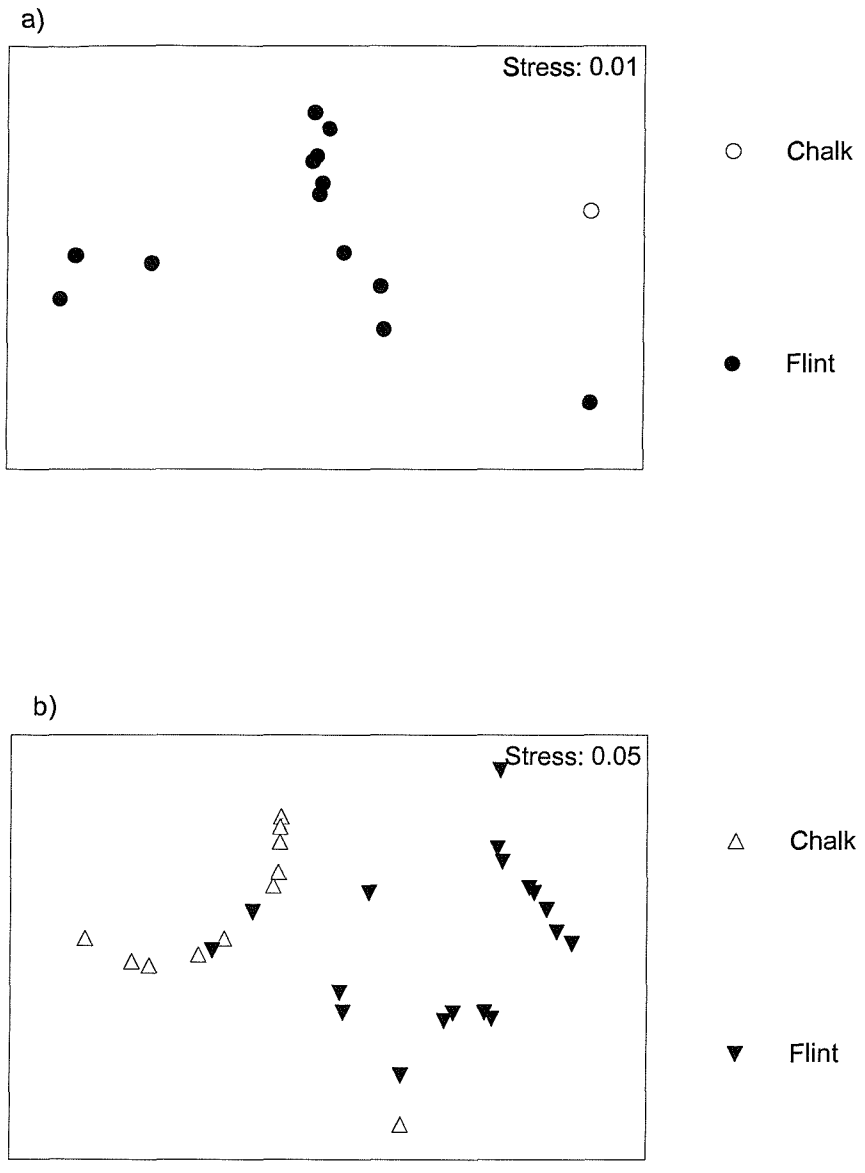


Figure 3.13 – MDS plots of microbial communities on chalk and flint in the littoral fringe (a) and at the upper eulittoral (b) in July 1999.

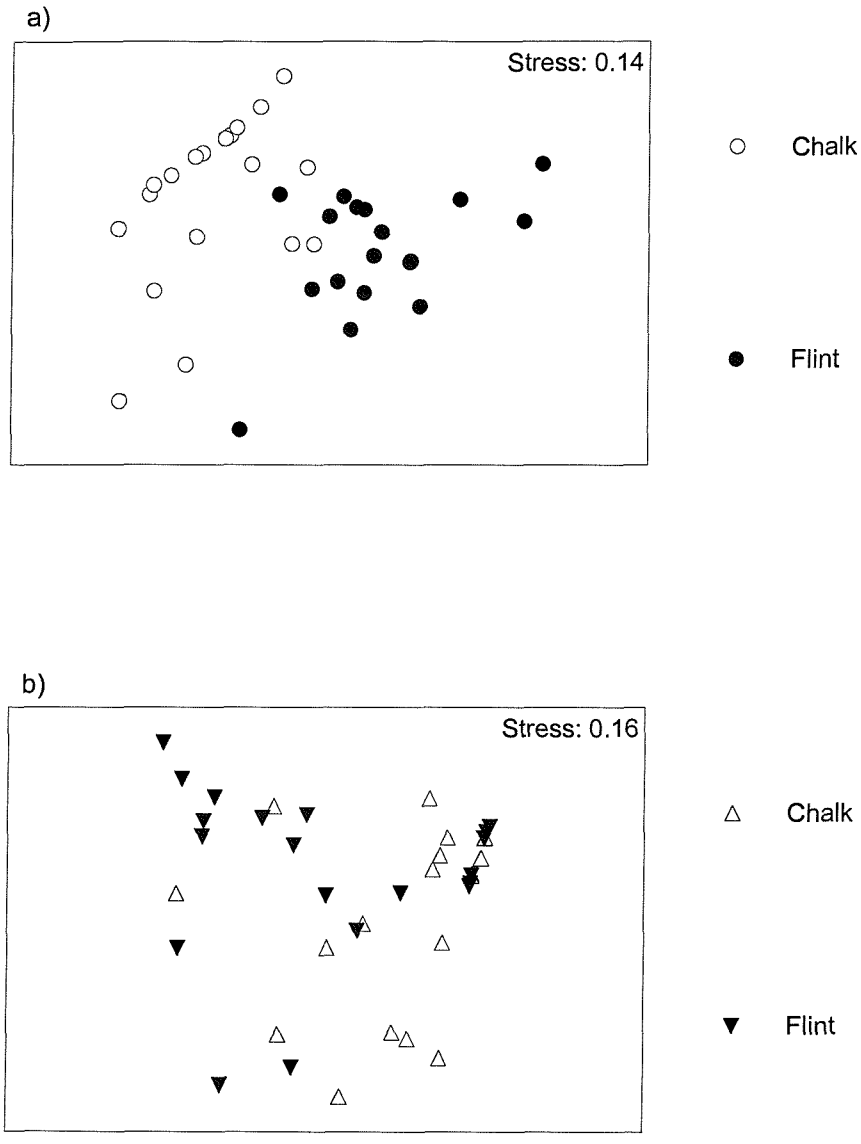


Figure 3.14 – MDS plots of microbial communities on chalk and flint in the littoral fringe (a) and at the upper eulittoral (b) in March 2000.

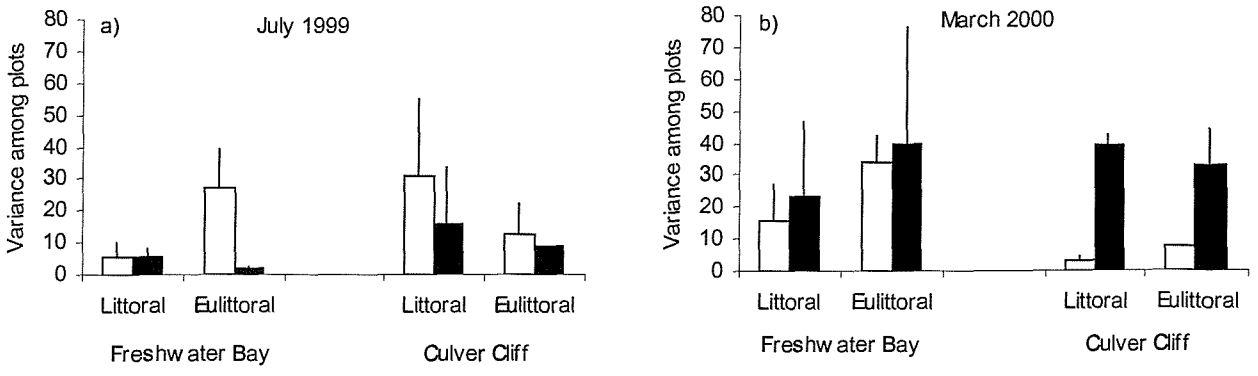


Figure 3.15 – Variance among plots in chalk (white bars) and flint (black bars) rocks in July 1999 (a) and March 2000 (b).

Table 3.12 – Analysis of variance to test differences in variances among plots for microalgal standing stock between chalk and flint in July 1999.

Source	df	MS	F	F versus	P
Shore	1	0.79	1.40	RES	0.271
Tidal level	1	0.09	0.03	Sh X Ti	0.883
Rock type	1	1.46	54.65	Sh X Ro	0.086
Sh X Ti	1	0.46	3.39	RES	0.103
Sh X Ro	1	0.19	0.06	RES	0.810
Ti X Ro	1	0.30	0.14	Sh X Ti X Ro	0.775
Sh X Ti X Ro	1	0.19	2.31	RES	0.167
RES	8	0.41			
TOT	15				

Table 3.13 – Analysis of variance to test differences in variances among plots for microalgal standing stock between chalk and flint in March 2000.

Source	df	MS	F	F versus	P
Shore	1	234.80	0.85	RES	0.384
Tidal level	1	269.66	0.82	Sh X Ti	0.531
Rock type	1	1387.46	2.46	Sh X Ro	0.361
Sh X Ti	1	327.50	1.19	RES	0.308
Sh X Ro	1	563.84	2.04	RES	0.191
Ti X Ro	1	45.13	2.52	Sh X Ti X Ro	0.358
Sh X Ti X Ro	1	17.91	0.06	RES	0.805
RES	8	276.27			
TOT	15				

Table 3.14 – Mean densities of limpets (N/m^2) in July 1999 and March 2000.

	July 1999				March 2000			
	Freshwater Bay		Culver Cliff		Freshwater Bay		Culver Cliff	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Littoral fringe	1.2 (0.8)	0.8 (0.5)	2.0 (1.0)	0	0.4 (0.4)	2 (1.6)	0.4 (0.4)	0.8 (0.5)
Upper eulittoral	94.4 (11.3)	46 (6.0)	32.8 (3.7)	37.2 (3.5)	98 (8.9)	--	42.4 (2.8)	51.6 (5.5)

Table 3.15 – Pearson's correlation coefficient for relationship between limpet density and microalgal biomass (chlorophyll-a) on chalk and flint in each plot. Data from shores and tidal levels were pulled. Significant values are highlighted in bold.

	Chalk	p	Flint	p
July 1999	0.50	0.20	-2.83	0.49
March 2000	**0.82**	**0.02**	-3.12	0.48

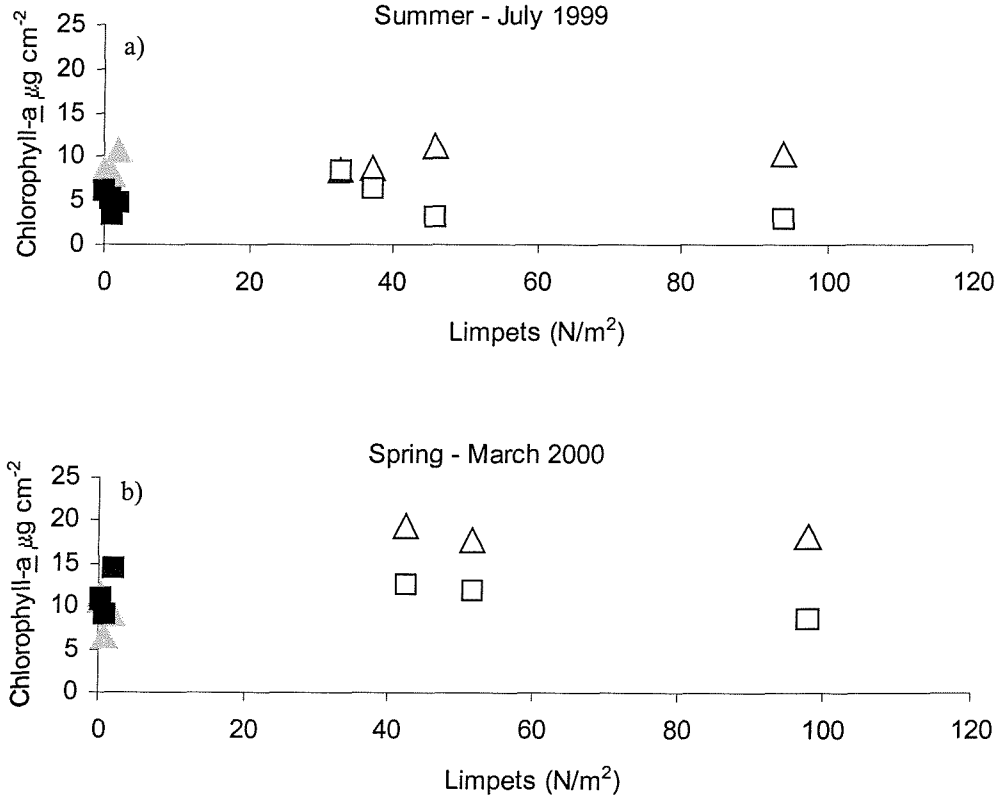


Figure 3.16 – Correlation between limpet densities and chlorophyll-a on chalk (triangles) and flint (squares) in the littoral fringe (plain symbols) and in the upper eulittoral zone (empty symbols); a): July 1999; b): March 2000.

3.3.3 Microbial films on limestone and chert rocks

The univariate statistical analysis for these two types of rock was carried out only for the plots located in the littoral fringe, where limestone and chert were properly interspersed. Multivariate analysis also included data from the littoral fringe.

Microalgal standing stock

In the summer microalgal biomass on limestone and chert was very similar in all the plots sampled, with chlorophyll-a ranging between 5.9 to 10.3 μg cm^{-2} on flint and between 5.5 and 10.3 μg cm^{-2} on chert (Figure 3.17a). In the littoral fringe on one plot microalgal biomass was slightly higher on limestone than chert, although this difference was not significant (Figure 3.17a; Table 3.16a). In the only plot sampled in the upper eulittoral level microalgal standing stock was almost equally distributed between the two types of rock.

In the early spring, microalgal biomass was much higher on both types of substratum, with chlorophyll-a ranging between 15.7 μg cm^{-2} and 20.01 μg cm^{-2} on limestone and between 16.7 μg cm^{-2} and 26.3 μg cm^{-2} on chert (Figure 3.17b). In the littoral fringe microalgal standing stock appeared to differ between limestone and chert more markedly than in summer, although no significant differences were detected by ANOVA (Table 3.16b).

In both summer and early spring, microalgal biomass on limestone and chert was characterised by small scale spatial variability, particularly within plots, where variances were highly heterogeneous.

Biofilm composition

Figures 3.18a-d depict results from the comparison of the abundance of diatoms and cyanobacteria between limestone and chert in summer and spring. Univariate statistical comparison was carried out only in the spring, as rock samples from the summer sampling were almost bare.

Microbial communities on limestone and chert consisted mainly of diatoms and cyanobacteria, although encrusting microalgae (probably corallines) and algal

germlings were frequently observed covering the surface. There were no apparent differences between the two rock types in the species composition of diatoms. These consisted mostly of *Fragilaria*, *Melosira*, *Cocconeis* and *Achnanthes.*

In the summer, the abundance of diatoms was extremely low (Figure 3.18a) on both rock types. In the spring the number of diatoms increased strongly on both limestone and chert, particularly in the littoral fringe, where density on limestone reached 86,410 cells cm^{-2} (Figure 3.18c). However, no significant differences in the abundance of diatoms were detected by ANOVA (Table 3.17a). Variances of the abundance of diatoms within plots was, as observed for chlorophyll-a, were generally very high.

In the summer, abundance of cyanobacteria was very low, with percentage cover being less than 10% (Figure 3.18 b). In the littoral fringe cyanobacteria were almost absent. In the eulittoral zone, cover of cyanobacteria was generally higher than in the littoral fringe, but there were not apparent differences in the abundance of cyanobacteria between limestone and chert (Figure 3.18b). In the spring cyanobacteria were present in all the plots, although the percentage cover was still very low, being less than 10% (Figure 3.18d). On this sampling date, however, cyanobacteria were consistently more abundant on limestone than on chert at both tidal levels. In the littoral fringe, this trend was also confirmed by the ANOVA, that showed nearly significant differences in the abundance of cyanobacteria between limestone and chert (Table 3.17b).

The multivariate analysis of the whole community, which included also data from the upper eulittoral level, showed significant differences between limestone and chert on both sampling dates (Figures 3.19a, b). The coefficient of dissimilarities was, however, relatively low in summer (ANOSIM, $R = 0.28$, $p = 0.018$) and spring (ANOSIM, $R = 0.24$, $p = 0.002$). SIMPER analysis showed that in both sampling occasion corallines accounted for almost 60% of the differences between limestone and chert. These algae were rarely found on limestone, as previously observed for the chalk.

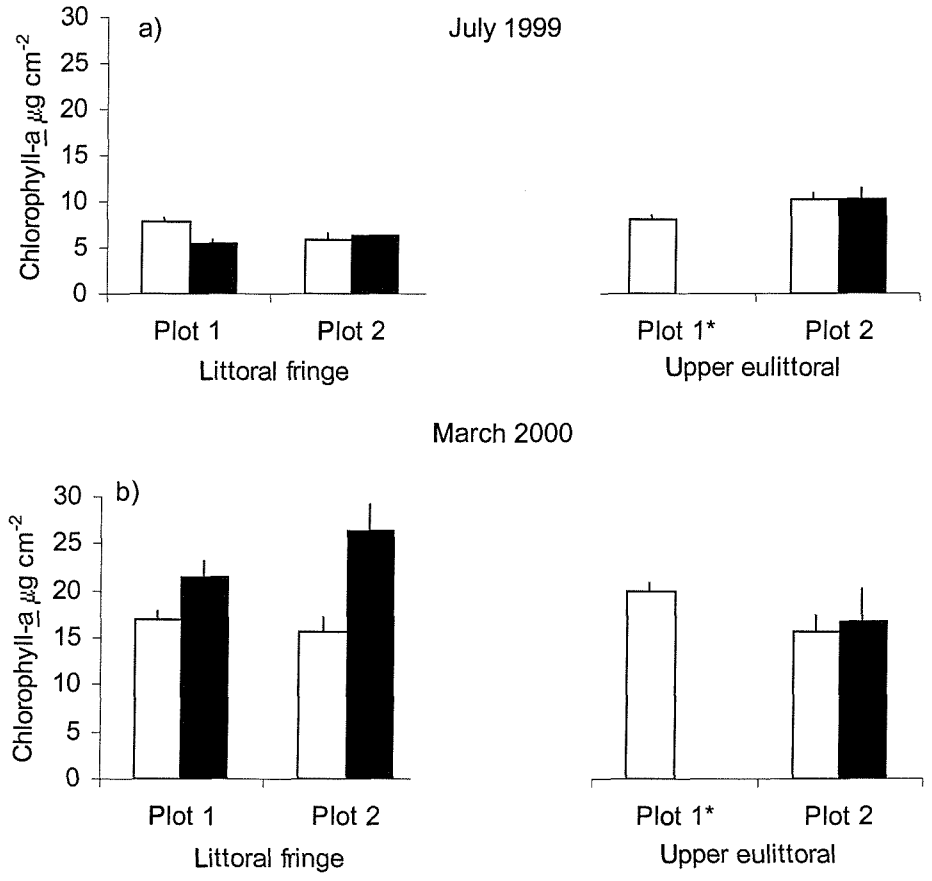


Figure 3.17 - Microalgal standing stock, (chlorophyll-a), in limestone (white) and chert (black), in Portland Bill in July 1999 (a) and March 2000 (b). In the plot with an asterisk (*) chert was absent. Bars indicate standard errors.

Table 3.16 – Analysis of variance to test differences in microalgal standing stock in the littoral fringe between limestone and chert in July 1999 (a) and March 2000 (b).

a)

Source	df	MS	F	F versus	P
Plot	1	0.14	1.53	RES	0.221
Rock type	1	0.43	1.83	Pl X Ro	0.406
Pl X Ro	1	0.24	2.61	RES	0.112
RES	56	0.09			
TOT	59				

b)

Source	df	MS	F	F versus	P
Plot	1	0.01	0.07	RES	0.795
Rock type	1	1.51	7.99	Pl X Ro	0.217
Pl X Ro	1	0.19	1.64	RES	0.206
RES	56	0.12			
TOT	59				

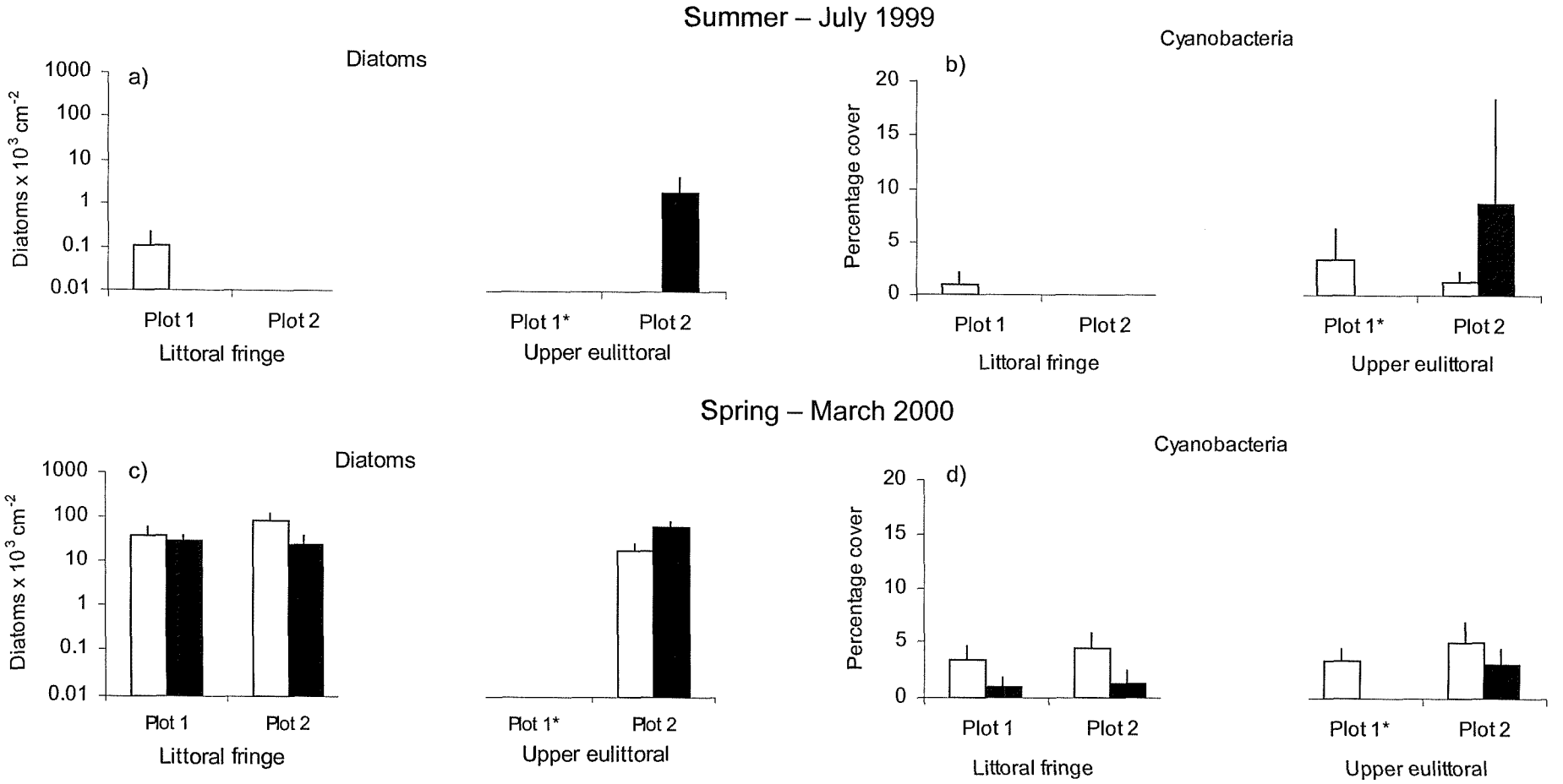


Figure 3.18a-c – Abundance of diatoms (a, c) and cyanobacteria (b, d) on limestone (white) and chert (black) in July 1999 (a, b) and March 2000 (c, d). In the plot with asterisk (*) chert was absent. Data for diatoms are represented on a log scale. Bars indicate standard errors.

Table 3.17 – Analysis of variance to test differences in the abundance of diatoms (a), and cyanobacteria (b) in the littoral fringe between limestone and chert in March 2000.

a) Diatoms

Source	df	MS	F	F versus	P
Plot	1	4671.61	1.38	RES	0.257
Rock type	1	10260.45	1.74	Pl X Ro	0.413
Pl X Ro	1	5882.45	1.74	RES	0.206
RES	16	3386.30			
TOT	19				

b) Cyanobacteria

Source	df	MS	F	F versus	P
Plot	1	2.22	0.41	RES	0.531
Rock type	1	35.56	64.00	Pl X Ro	0.079
Pl X Ro	1	0.56	0.10	RES	0.753
RES	16	5.42			
TOT	19				

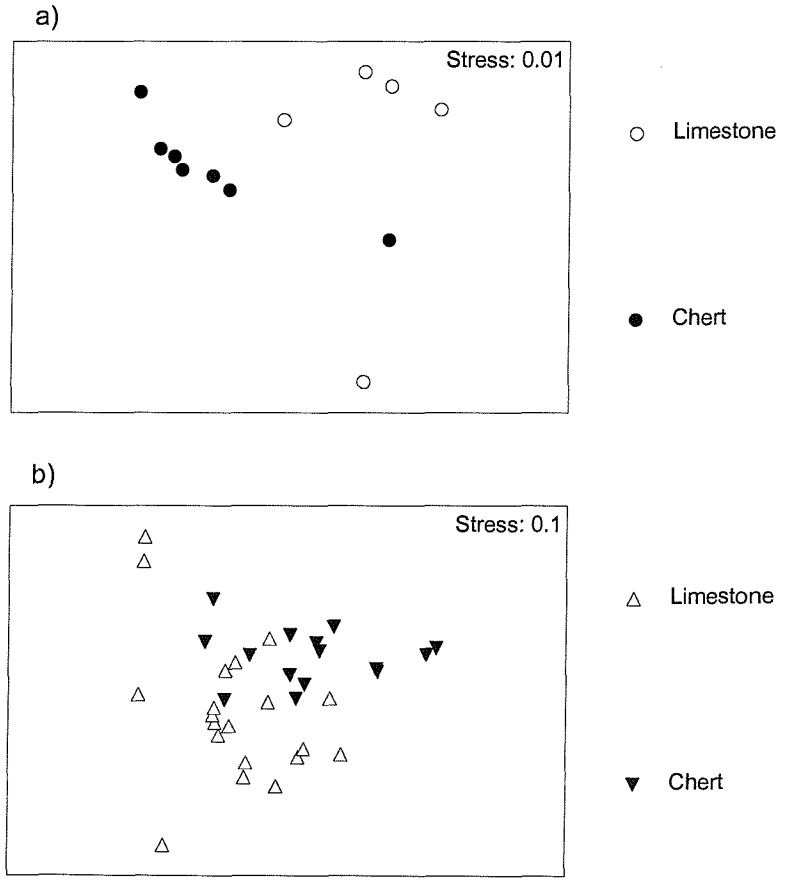


Figure 3.19 - MDS plot of microbial communities on limestone and chert in July 1999 (a) and March 2000 (b).

3.3.4 Microbial films on oil shale and dolomite rocks

Microalgal standing stock

Microalgal standing stock on both oil shale and dolomite was in general very low (Figure 3.20), usually under 1 μg cm^{-2} of chlorophyll-a. This was probably a consequence of strong desiccation and thermal stresses to which biofilms were subjected in August 1999, when sampling was carried out. Differences in microalgal abundance between shale and dolomite were highly significant (Table 3.18). The amount of chlorophyll-a on dolomite was three-four times higher than on shale. In contrast with the results obtained from comparisons of other types of rock, differences between oil shale and dolomite were highly consistent between replicate plots, due to small scale spatial variability. The analysis of variance confirmed that there was no significant interactions between the factors rock type and plot (Rock type X Plot, Table 3.18).

Biofilm composition

The microbial film on oil shale and dolomite consisted mainly of cyanobacteria, whilst diatoms were extremely rare. Diatoms appeared to be slightly more abundant on shale than dolomite in some of the plots, although no significant differences were observed in the abundance of diatoms between the two rock types (Figure 3.21, Table 3.19). Spatial variation in the abundance of diatoms between plots characterised both substrata and appeared to be more important than rock type (Table 3.19). Some variability was observed also within plots, particularly in the shale substrata (Figure 3.21).

Cyanobacteria were much less common on shale than on dolomite, although this pattern was not always consistent across the plots (Figure 3.22, Table 3.20). Cyanobacteria were almost absent on oil shale, while percentage cover on dolomite was high, generally over 45%. The only exception was in plot 4 where the abundance of cyanobacteria was low and similar on both substrata, reaching a percentage cover of only up to 10%.

The multivariate analysis of biofilm composition confirmed the results obtained with the univariate analysis, showing significant differences in the microbial community

between oil shale and dolomite (ANOSIM, R= 0.654, p = 0.001). As expected, the cyanobacterial component explained almost 50% of the difference between the substrata, as shown by the SIMPER procedure (Figure 3.23).

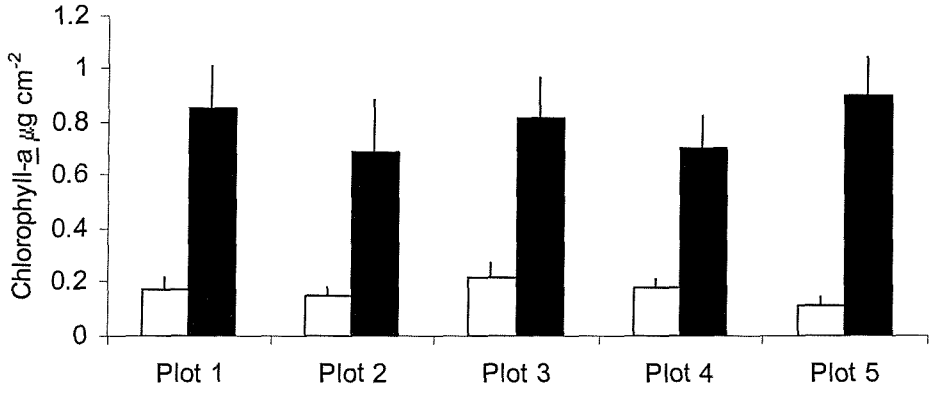


Figure 3.20 – Microalgal standing stock on oil shale (white bars) and dolomite (black bars) at the upper eulittoral level at Kimmeridge Bay in August 1999. Bars indicate standard error.

Table 3.18 – Analysis of variance to test differences in microalgal standing stock between oil shale and dolomite in August 1999. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Plot	4	0.21	0.37	RES	0.827
Rock type	1	43.89	114.17	Pl X Ro	**< 0.001**
Ro X Pl	4	0.38	0.69	RES	0.599
RES	140	0.56			
TOT	149				

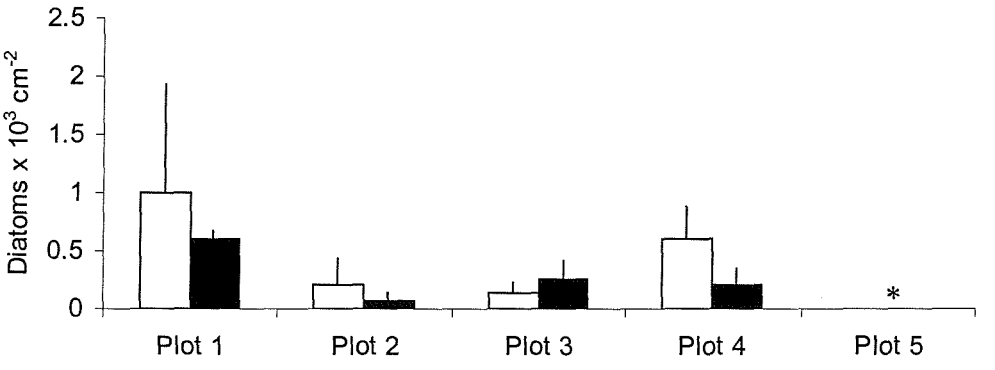


Figure 3.21 – Abundance of diatoms on oil shale (white) and dolomite (black) at Kimmeridge Bay in August 1999. Asterisk indicates that no diatoms were observed in plot 5 on both rock types. Bars indicate standard errors.

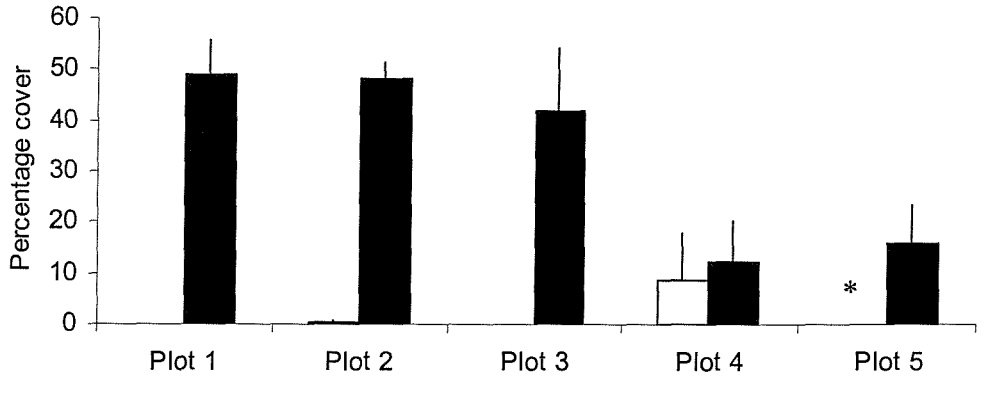


Figure 3.22 – Abundance of cyanobacteria on oil shale (white) and dolomite (black) at Kimmeridge Bay in August 1999. Asterisk indicates that no cyanobacteria were observed in plot 5 on oil shale. Bars indicate standard errors.

Table 3.19 – Analysis of variance to test differences in abundance of diatoms between oil shale and dolomite in August 1999. Significant values of P are highlighted in bold

Source	df	MS	F	F versus	P
Plot	4	4.07	5.34	RES	**0.002**
Rock type	1	0.00	0.01	Pl X Ro	0.943
Ro X Pl	4	0.84	1.10	RES	0.371
RES	40	0.76			
TOT	49				

Table 3.20 – Analysis of variance to test differences in abundance of cyanobacteria between oil shale and dolomite in August 1999. Significant values of P are highlighted in bold.

Source	df	MS	F	F versus	P
Plot	4	1.62	1.75	RES	0.157
Rock type	1	97.46	22.55	Pl X Ro	**0.009**
Ro X Pl	4	4.32	4.69	RES	**0.003**
RES	140	0.92			
TOT	149				

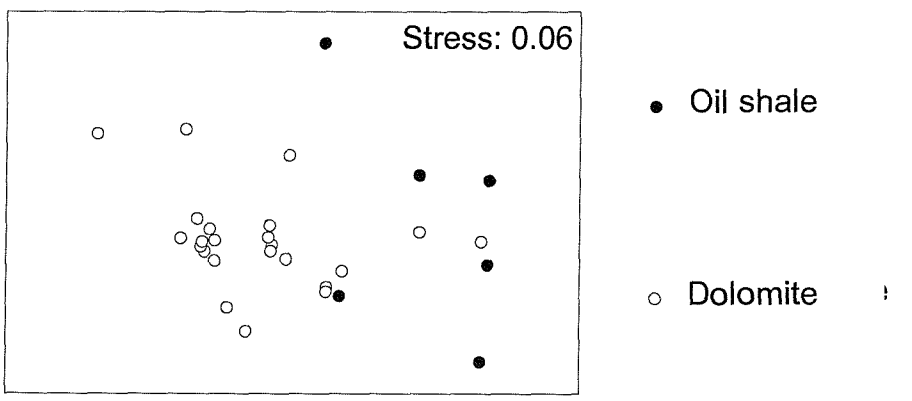


Figure 3.23 – MDS plot of microbial communities on oil shale and dolomite at Kimmeridge Bay in August 1999.

3.4 DISCUSSION

This study showed how the effect of rock type on biofilms was modulated by different factors, such as spatial variability and seasonal patterns. These will be considered before the detailed discussion on the influence of rock type on microbial communities.

3.4.1 Spatial variability

The microbial communities sampled were highly variable in terms of abundance on all rocks (see also Hill, 1990; Hill and Hawkins, 1991). Spatial variation occurred on different scales: between shores (10's km), plots (10's m) and samples (10's cm), depending on the type of rock substratum.

In the chalk vs. flint comparison microalgal biomass and species composition differed between shores on both substrata, although variability was greater on flint. Differences in microalgal standing stock between shores have been already observed in previous studies (MacLulich, 1987; Thompson, 1996; Jenkins and Hartnoll, 2001). Variation at this scale was attributed by these authors to differences in wave exposure, as this can indirectly affect biofilms by modifying desiccation, thermal stresses and nutrient availability. This explanation does not seem to apply to the shores considered in my study. Freshwater Bay and Culver Cliff are respectively located on the east and west coast of the Isle of Wight, more than 30 km apart. Despite the relative distance between the two shores, they have very similar wave exposure and orientation. This is also demonstrated by a similar distribution and composition of the macrobiota, typical of moderately exposed shore (Lewis, 1964; Raffaelli and Hawkins, 1996, Ballantines, 1961). It is likely therefore that other variables might have contributed to the variation observed.

The numbers of grazers varied considerably between the two shores: at Freshwater Bay, limpet densities were almost the double than at Culver Cliff on both dates sampled. Despite this stark difference in limpet density, microbial biomass was similar on both shores. Also, no correlation has been observed between grazers and microalgal biomass, except for the chalk substrata in the early spring. Lack of correlation between grazers and microalgal standing stock was observed also by Thompson (1996). This

suggests that grazing is probably more important at small spatial scale, by increasing biofilms patchiness.

At a smaller scale, between and within plots, spatial variation increased considerably. Variability among plots was observed in microbial communities on all the rocks considered in the comparisons, although to different extents. In the comparison of chalk vs. flint, microalgal standing stock did not vary much between plots on chalk, whilst variability was higher on flint, particularly in spring. Biofilms on chert and limestone appeared to be less variable at this scale, showing no big differences between plots. The only exception was for shale and dolomite, where microalgal biomass was very consistent in all the five plots sampled. However, higher variation in the abundance of diatoms and cyanobacteria was shown between plots on both shale and dolomite. Within plots spatial variability was extremely high. Large and highly heterogeneous variances were obtained in all types of rock, showing microbial communities are extremely patchly distributed on the shore. This spatial heterogeneity varied considerably with the sampling date, as in spring, biofilms seemed to be more patchily distributed than in summer. Natural patchiness in microbial communities has been widely described by several authors (Castenholz, 1961; Nicotri, 1977; MacLulich, 1987; Hill and Hawkins, 1990; Sommer, 2000).

Patchiness in the distribution of biofilms occurs at a very small scale, between samples (10's cm) and within samples (100's μm). At this spatial scale, grazing by limpets might have a strong effect in microbial patchiness. This was evident on chalk substrata, where limpet radula can easily penetrate the surface leaving distinctive marks (Hawkins *et al.*, 1989). Analysis of several samples under scanning electron microscope revealed a mosaic of areas heavily colonised by cyanobacteria and diatoms and bare rock with the presence of clear grazing marks. The effect of grazers on microalgal standing stock has been well documented (Nicotri, 1977; Underwood, 1984a; Hill, 1990; Mak and Williams, 1999). On British rocky shores, littorinids and limpets are the major grazers of microbenthic communities (Hawkins and Hartnoll, 1983; Hawkins *et al.*, 1989; Norton *et al.*, 1990). Their different feeding apparatus often leads to different abilities and ways of grazing microalgae, causing different spatial patterns on the rock surface (Sommer, 2000). Moreover, several authors described a distinctive food selectivity by different species of grazers, which therefore

could strongly affect microalgal diversity (Nicotri, 1977; Kitting, 1980; Hill and Hawkins, 1991; Sommer, 1997). This could explain the large variability within plots observed in the composition of biofilms of all rock types sampled.

Microtopography is another potential factor which can cause microalgal variation. The effect of surface roughness on distribution and abundance of intertidal organisms has been well described (Caffey, 1982; Dudley, 1991; Holmes *et al.*, 1997; Camus *et al.*, 1999). A complex topography seems to facilitate settlement and growth of macrobiota, by providing protection from physical stresses, greater habitat heterogeneity and more refuges from grazing (Crisp and Barnes, 1954; Le Tourneux and Bourget, 1988; Hills and Thomason, 1996; Holmes *et al.*, 1997). Similarly, at microscopic level, a complex surface might provide more niches for the settlement and development of microbial communities (Thompson *et al.*, 1996). Various authors observed that species composition appears to vary between smooth and rough surfaces. For example, adnate forms of diatoms appeared to prefer rough substrata, while stalked forms and long chain colonies were more abundant on smooth surfaces (Miller *et al.*, 1987; Snoeijs, 1991). The microbial film is more likely to be patchily distributed on rough surfaces, as these consist of areas easily exposed to grazing and other environmental stresses and protected areas located in small pits and microcrevices. Therefore microtopography could explain the degree of patchiness of the different rock substrata considered in this study. Microbial communities on chalk and limestone were more patchily distributed than smoother rocks such as shale and dolomite. Spatial heterogeneity was even higher on flint and chert, which are relatively smooth rocks. This contrasting pattern can be explained by the fact that both flint and chert occurred in small nodules interspersed in the chalk and limestone shores respectively. These inclusions were very small, often the same size of a replicate sample, therefore spatial variation of microbiota between such small areas is exaggerated.

3.4.2 Vertical patterns

Microbial communities did not show a marked difference between the two tidal heights considered in the study. No significant differences in microalgal abundance and microbial composition were found between the two tidal levels on most of the rock types sampled. Only on chalk and limestone was it possible to observe a marked increase in the microalgal standing stock at the upper eulittoral level, particularly in the

early spring. On the other rock types there was some vertical variation, but this was not consistent between the two tidal heights. Complex interactions of abiotic and biotic factors appear to affect the vertical distribution of biofilms. Desiccation and insolation stresses appear to be much stronger on the upper shore, as the period of emersion is longer than lower on the shore (Castenholz, 1963; MacLulich, 1987). By contrast, grazing pressure on microalgae tends to increase towards mid and lower shore (Underwood, 1984). However, the relative importance of these factors at different tidal levels can vary considerably between locations. On British rocky shores Thompson (1996) observed that vertical distribution patterns of microalgae were not consistent between shores. In my study lack of clear patterns can be explained by the relative nearness of the littoral fringe and the upper eulittoral level, both located above mid shore. It is likely that these two areas were therefore influenced by similar levels of desiccation, insolation and temperature; these are known to be important factors in limiting the microalgal abundance higher in the upper shore especially during summer (Cubit, 1984; Underwood, 1984; MacLulich, 1987). Density of grazers was higher on the upper eulittoral zone, as only a few limpets were present in the littoral fringe. The presence of littorinids at this level can have a significant effect, as observed by Hawkins and Hartnoll (1983) and Mak and Williams (1999). At Portland obvious grazed haloes occur around crevices and pits sheltering littorinids (Hawkins and Jones, 1997, pers. obs.).

3.4.3 Temporal patterns

There were considerable differences among rock types between early spring and summer. Microalgal standing stock on chalk and flint was generally higher in spring than in summer. This difference was even more marked on chert and limestone, where values of chlorophyll a in spring were almost twice those in the summer. A similar pattern was observed for the abundance of diatoms. These were almost absent during the summer, while in spring the number of cell per cm^2 reached peaks of over 10000 on chalk. Less clear patterns were observed for cyanobacteria, which did not vary much between the two sampling dates. These results reflected the previous studies on seasonal patterns of microbial communities.

Temporal variation in microbial communities is well known worldwide (Underwood, 1984c; MacLulich, 1987a; Dye and White, 1991; Hill and Hawkins, 1991; Williams,

1993; Thompson, 1996). Microbial films are very abundant in winter and spring, when diatoms appear to reach a peak (Aleem, 1950; Edyvean *et al.*, 1985; MacLulich, 1987; Hill and Hawkins, 1991). This effect is mainly due to more favourable conditions of light intensity and temperature. Cyanobacteria seem to be less affected by seasonal variation, possibly because of their greater tolerance and resistance to physical stresses such as temperature, desiccation and insolation (Thompson, 1996; Mak and Williams, 1999). In the present study, it is also likely that any pattern in cyanobacteria were masked by spatial variation, as considerable differences in the abundance of these organisms between shores and between plots were observed in all the four substrata considered.

3.4.4 The effect of the rock substratum

Microbial films on all the rock types considered in this study strongly exhibited marked spatial and temporal patterns and considerable small scale variability. Despite the high level of variability, microalgal biomass often differed considerably between the rock types. These differences became more evident when comparing each pair of substrata at plot level. In the shale vs. dolomite comparison, all the plots sampled showed significantly higher microalgal biomass on the dolomite substratum. In the chalk vs. flint comparison, microalgal standing stock was higher on chalk substrata in ten out of sixteen plots tested using SNK's on the different occasions. Only in the comparison between chert and limestone were differences in microalgal abundance still present but less obvious. Differences were only apparent in the spring. The harsh conditions which generally occur during summer can dramatically reduce the abundance of microbial communities, independently of the type of the underlying substratum. Microalgal biomass and the abundance of diatoms and cyanobacteria were very low during summer on almost all rocks sampled, and large numbers of zero values decreased the power of ANOVA in detecting significant differences between the substrata. Shale and dolomite, which were sampled only in one occasion in summer, were the exception. Despite very low values of microalgal standing stock, there were significantly higher values on dolomite. These clear differences were not marked by high levels of small scale variability and hence were detectable by ANOVA.

In this study microbial communities appeared to be more abundant on limestones (chalk, dolomite) than on shale or flint. Limestones differ from shale and flint in several physical and chemical properties. Limestones consist mainly of calcium carbonate which makes these rocks particularly porous and permeable to water and more subject to weathering and bioerosion. As a result, this type of rock tends to have a relatively rough surface, often characterised by several microcrevices, small pits and microholes. These two aspects of the limestone rocks could facilitate the settlement and development of microbial films. Diatoms which are very sensitive to desiccation and thermal stresses will survive better on substrata retaining water at the surface for longer during emersion at low tide. Surface roughness can provide microniches for diatoms and cyanobacteria which therefore cannot easily be reached by grazers nor dislodged by waves (Edyvean *et al.*, 1985). Similar observations were made in previous studies carried out mainly in freshwater systems. Blinn *et al.* (1980) found higher microalgal standing stock on rocks characterised by higher porosity and microrugosity. Thompson (1999, unpublished data) observed a similar pattern on limestones with differing surface roughness.

The higher microalgal biomass estimated on limestone substrata considered in this study could be also the result of the presence of an another component of microbial communities, the endolithic cyanobacteria. These organisms are microborers which penetrate the rock surface using acidic secretions, therefore they are only present in carbonate rocks (Le Campion-Alsumard, 1975; Le Campion-Alsumard, 1989; Peyrot-Clausade *et al.*, 1995).

3.4.5 Conclusions

Despite considerable spatial and temporal variability, however, differences between rock types were still apparent; especially in paired comparisons of plots made at the same time or place, thereby reducing other variables. The causes of such differences are explored by planned experiments controlling various factors. These have been described in chapter 4 and 5.

Chapter 4

The effect of rock type on initial colonisation and succession in microbial films

4.1 INTRODUCTION

The studies described in Chapter 2 and 3 provided evidence for the influence of the geology of the shore on epilithic biofilms. The microbial communities previously described were at a mature stage, with further successional events arrested (MacLulich, 1986), primarily by grazing (Underwood, 1984b; Hill, 1990). Rock type, however, is also likely to affect the early stages of colonisation by microbial films and the subsequent rate and trajectories of early successional processes.

Colonisation in biofilms has been largely investigated in freshwater (Lock, 1993) and marine subtidal environments , especially in relation to fouling (Marszalek *et al.*, 1979; Becker *et al.*, 1997b). On rocky shores, secondary succession of macrobiota has been studied much more than the early microbial phases (Connell and Slatyer, 1977; Sousa and Connell, 1992). Microbial primary colonisation on rocky shores appears very similar to that described for any substratum immersed in water (MacLulich, 1986 *cf* Wahl, 1989). After adsorbtion of organic molecules, the first stage of colonisation consists of adhesion of bacteria to the substratum, generally after a few hours of immersion (Cooksey and Wigglesworth-Cooksey, 1995). Subsequently, diatoms colonise, followed by cyanobacteria (Tuchman and Blinn, 1979). With the appearance of macroalgal propagules and invertebrate larvae in the film, microbial colonisation switches to macrobiotic succession (Characklis, 1981). Mature biofilm communities are also characterised by a limited suite of dominant taxa, primarily *Achanthes*, *Fragilaria*, *Cocconeis* (Aleem, 1950; Castenholz, 1963; MacLulich, 1986; Hill, 1990; Hill and Hawkins, 1991).

Succession of microbial species within biofilms depends on factors such as the type of the substratum (Hamilton and Duthie, 1984; Miller *et al.*, 1987), grazing and seasons (Castenholz, 1961; Castenholz, 1963; Nicotri, 1977). There are also differences between habitats and ecosystems. For example in freshwaters, epilithic colonisation starts with adnate diatoms, followed by stalked or pennate forms (Hoagland *et al.*, 1982; Korte *et al.*, 1983). In contrast, the opposite sequence has been observed in the epipsammic communities (Miller *et al.*, 1987).

The time taken for microbial films to reach a mature stage also varies, being two to four weeks in freshwaters systems (Blinn *et al.*, 1980; Hoagland *et al.*, 1982; Korte *et al.*, 1983) and up to eight weeks on rocky shores (Leskinen and Sarvala, 1988). This mature stage remains where physical disturbance and biological factors keep microbial communities permanently at a pioneer stage (MacLulich, 1986). Microbial recolonisation can also take longer in unstable environments. For example, in epipsammic systems, where microbial communities experience continuous physical abrasion, colonisation can take approximately five weeks (Miller *et al.*, 1987; Bergey, 1999). Grazing is, however, the main factor of disturbance in the colonisation and successional processes on hard substrata in both freshwater and marine systems (Hill, 1990; Dudley, 1991; Sousa and Connell, 1992; Sommer, 1999). On rocky shores, it also prevents the settlement and growth of algal germlings within the biofilms and consequently the development of macroalgae (Hawkins and Hartnoll, 1983; Underwood and Jernakoff, 1984; Hawkins *et al.*, 1992)

Colonisation and successional processes also vary between season (Belangér and Cardinal, 1977; Hudon and Bourget, 1981). In spring, when light and temperature are at the optimal level for phototrophs, diatoms and cyanobacteria are more likely to develop faster (Hill and Hawkins, 1991; Thompson, 1996). In summer, harsher conditions such as desiccation and insolation stresses limit diatom colonisation, by favouring more resistant and adaptive microorganisms such as cyanobacteria (Fogg, 1973; Birke, 1974). Reproduction of macroalgae is also strongly dependent on seasons, therefore the presence of algal propagules in biofilms will vary accordingly (Hill, 1990).

A few studies have suggested that the characteristics of the underlying substratum can also affect colonisation of microbial communities. Physical features such as surface tension are important during the early stages of microbial colonisation, by bacteria (Becker *et al.*, 1997a). Marszalek *et al.* (1979) suggested that the chemical composition and the degree of surface inertness could influence colonisation. Total microbial abundance and diversity of species also appears to increase with surface roughness (Edyvean *et al.*, 1985; Dudley, 1991). However, the effect of rock type on colonisation and succession of intertidal epilithic biofilms has been little investigated. Hamilton and Duthie (1984) showed that microtopography plays an important role in

the development of microbial films with initial settlement in microcrevices and small pits, followed by colonisation of smoother areas. Differences in initial colonisation between smooth and rough surfaces have also been observed by Miller *et al.* (1987), but these disappeared after few weeks.

On rocky shores microbial colonisation processes have been studied using areas previously cleared by various chemical and mechanical methods (Castenholz 1961; Hill, 1990; Edyvean *et al.*, 1985). It has proved difficult, however, to completely clear a filmed surface without damaging or modifying its physical and chemical properties. Therefore microbial colonisation has been studied more extensively on artificial substrata including glass, metal plates, clay tiles, wood, plastic and rock (Hudon and Bourget, 1981; Niell and Varela, 1984; MacLulich, 1986; Danilov *et al.*, 2001) . The use of artificial substrata for monitoring colonisation of benthic communities has stimulated debate about their effectiviness (Cattaneo and Amireault, 1992 *cf* Costello and Thrush, 1991). Experimental panels are generally easy to prepare and deploy in the field and to retrieve for examination in the laboratory; hence they make sampling and monitoring less difficult (Costello and Thrush, 1991). For example, glass slides and transparent plastic sheets allow observations of microbial communities directly under light microscopy (Belanger and Cardinal, 1977; Anderson, 1995, Sommer, 2000). Artificial substrata also provide standard surfaces, which are true replicates thereby reducing background variability. Conversely, many authors have shown that the artificial substrata used affected colonisation and succession processes. For example, in freshwater systems colonisation time, biomass and composition of epilithon investigated on artificial substrata appeared to be underestimated when compared to natural rock surfaces (for review of work in freshwater see, Cattaneo and Amireault, 1992; Snoeijs, 1991; Edyvean *et al.*, 1985). Edge effects are another well known problem of using panels, with colonisation near the edges of the substrata differing considerably from the central area (Hamilton and Duthie, 1984; Sekar *et al.*, 1998). Artificial substrata can also be lost in the field as they are more susceptible to breakage or dislodgement under severe weather conditions.

The overall aim of this chapter was to investigate the effect of the rock substratum on early colonisation and establishment of intertidal epilithic biofilms and interactions with season. Chalk and dolomite rock were specifically chosen on the basis of work

described in Chapter 2 and 3, to represent two extremes in the physical and chemical properties of the rocks. Chalk is very porous with a rough surface texture, whilst dolomite is a highly compact, smooth rock. To reduce the effects of spatial variability due to microtopography, I used an experimental approach deploying machine-cut tiles made from natural substrata.

Chalk and dolomite tiles were employed in the intertidal in a multifactorial experiment to test the following hypotheses. First it was predicted that, colonisation and development of microbial communities would occur faster and to a greater extent on the soft and porous chalk than on hard, smooth dolomite. In consequence, mature microbial communities would be more abundant and diverse on chalk than on dolomite. Secondly, it was predicted that microbial colonisation and abundance would be greater in winter than in summer. Hence the effect of substratum would be expected to be stronger in summer, when porous, damper rocks such as chalk might mitigate the harsher desiccation stresses experienced by microbial organisms. Therefore differences in colonisation trajectories between the two rock substrata would be modified by the seasonal effect.

The experiment was spatially replicated within and between two rocky shores. It was also temporally replicated using two separate initiation times within each season. Following each initiation, microbial assemblages on both chalk and dolomite tiles were sampled every 10-15 days over a two month periods. A full formal comparison of microbial biomass was made at the end of the experiment at 60 days.

4.2 MATERIAL AND METHODS

4.2.1 Study sites

Wembury and Bovisand Bay, east of Plymouth, in Devon, were selected because of their proximity to the Marine Biological Association Laboratory (Figure 4.1a-b). This easy access made possible frequent sampling visits to the experimental plots. More importantly, they had similar physical and biological features. They are approximately 4 km apart, have similar exposure, orientation, slope and the same underlying rock type: old red sandstone, which is a very fine grained sedimentary rock structured in thin, flaky layers. Its different geology from the types of rock used in the experiment avoided confounding effects of the background microflora on colonisation processes occurring on the virgin substrata.

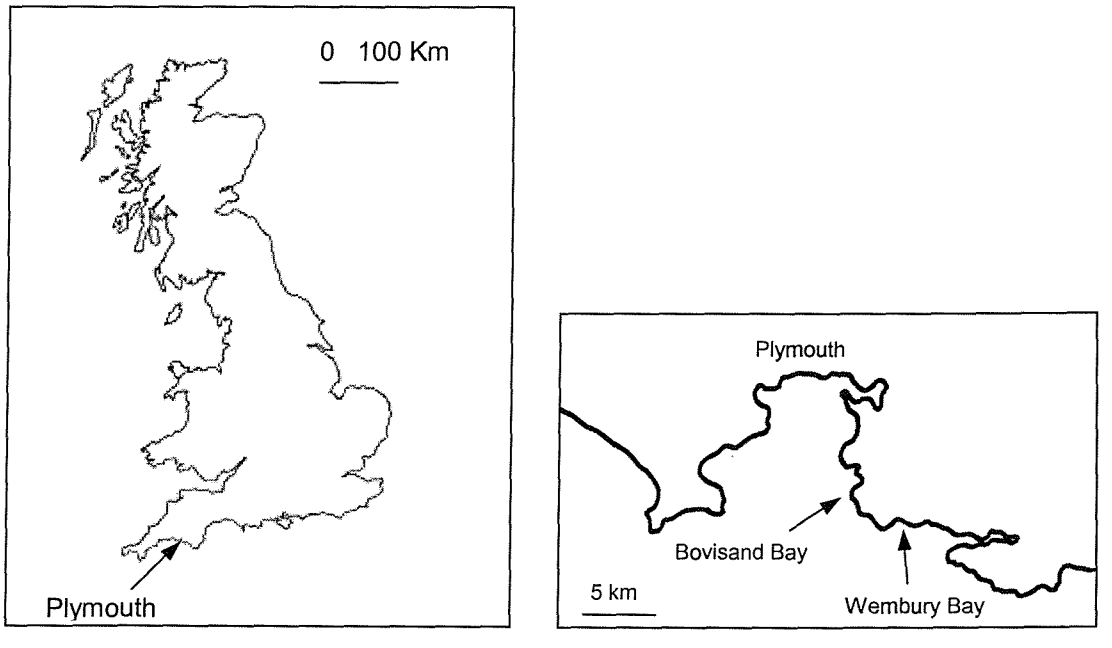


Figure 4.1 – Location of the study sites. a): in the UK context; b): detailed map.

The macrobiota on the mid-shore consisted mainly of barnacles (*Chthamalus* spp., *Semibalanus balanoides*) and limpets (*Patella vulgata, P. depressa*) and top shells (*Osilinus lineatus*, *Gibbula umbilicalis*). Macroalgae were rare, as a consequence of the high density of limpets which control macroalgae in this region (Southward and Southward, 1978; Roberts, 2002). *Fucus* spp. and *Enteromorpha* spp. were generally

restricted to crevices or small patches where they had escaped from limpets. The abundance of limpets was relatively similar among areas selected for the experiment, except for one of the plots located at Bovisand (Table 4.1). Barnacles were very patchy and cover generally did not exceed 40% (Table 4.1).

Table 4.1 – Densities of limpets and percentage cover of barnacles in the plots at the beginning of the first (20/05/2000) and second initiation (1/07/2000) during the summer experiment.

	1st summer initiation		2nd summer initiation	
	Wembury Bay	Bovisand Bay	Wembury Bay	Bovisand Bay
Limpets (N/m^2)				
Plot 1	134.4 (12.2)	196.0 (7.4)	106.0 (5.9)	154.8 (2.9)
Plot 2	137.2 (9.0)	110.4 (17.0)	130.4 (3.0)	145.6 (4.7)
Barnacles (%)				
Plot 1	38.3 (3.9)	37.2 (2.4)	26.1 (0.6)	25.8 (0.7)
Plot 2	30.6 (3.3)	32.9 (3.4)	38.8 (0.5)	30.7 (1.0)

4.2.2 Physical and chemical properties of the rocks

Porosity, hardness and chemical composition of chalk and dolomite were measured experimentally in the studies described in Chapter 2 and 3 (see material and methods and results sections). Surface roughness of the machine cut surface of the rocks was determined using the Talysurf System (Taylor-Hobson). Ten measurements were made on each rock type at high resolution (X1000) and with a traverse length of 7.2mm (cut-off 0.8mm). Measurements of surface temperature of chalk and dolomite were also taken in the field during summer 2000.

Chalk and dolomite have very different chemical and physical characteristics (Table 4.2). They are both sedimentary rocks, but chalk is made of calcium carbonate and dolomite mainly consists of calcium magnesium carbonate. Porosity, estimated indirectly as the amount of water loss per gram of rock, was much greater in chalk than dolomite (Figure 4.2). The amount of seawater absorbed by dolomite was minimal and hence the rock dried out in less than 30 minutes. In contrast, chalk absorbed more seawater, and its surface remained damp for up to 90 minutes. A small quantity of

water did remain in the rock for several hours before it dried out completely. This marked difference between the rocks in the capability of adsorbing and retaining water is likely to be caused by differing structure of the two rock types. Dolomite is a very finely grained, compact rock, whilst chalk has a loose structure, made of fine grains of calcite interspersed with relatively large spaces. Therefore the water can easily penetrate into the chalk, whilst it is probably not absorbed by the dolomite. The intrinsic surface roughness, which was estimated on the machine cut surface of the rocks at scale of 100's microns, was almost double on chalk (*t* test, $p<0.001$; Figure. 4.3). In addition, the surface temperature of the rocks, once deployed in the field, differed considerably, particularly in warm weather. During the summer, the white surface of chalk was significantly cooler than the black dolomite (ANOVA, $F_{1,5} = 43.13$; $p<0.01$; Figure 4.4). Differences between the two rock types were greater, as expected, on the second date, when the mean air temperature was also higher (Figure 4.4). On this date, surface temperature on chalk and dolomite differed up to 7 °C, with dolomite reaching 27°C.

Table 4.2 – Summary table of the main chemical and physical properties of chalk and dolomite. The colour and surface roughness refer to the machine cut surface of the tiles. Values in brackets refer to standard error.

	Chalk	**Dolomite**
Origin	sedimentary	sedimentary
Chemical composition	calcite	mainly ferroan dolomite
Colour	white to off-white	black
Grain size	fine grained	fine grained
Porosity	very high	very low
Hardness (Moh's scale)	3	5

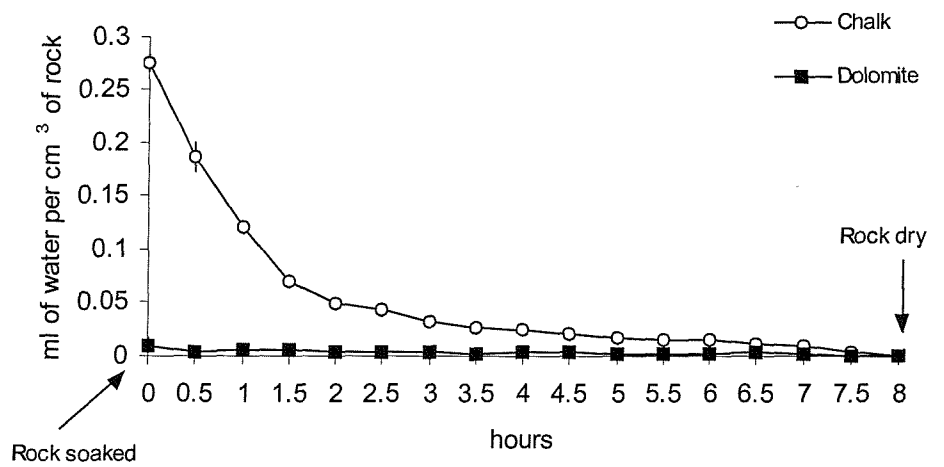


Figure 4.2 – Porosity, expressed as seawater loss (ml) per cm^3 of rock, in chalk and dolomite rocks after soaking in seawater for three hours and oven drying at 30°C. Values were averaged from 10 replicate cores of each rock type. Vertical bars indicate standard error.

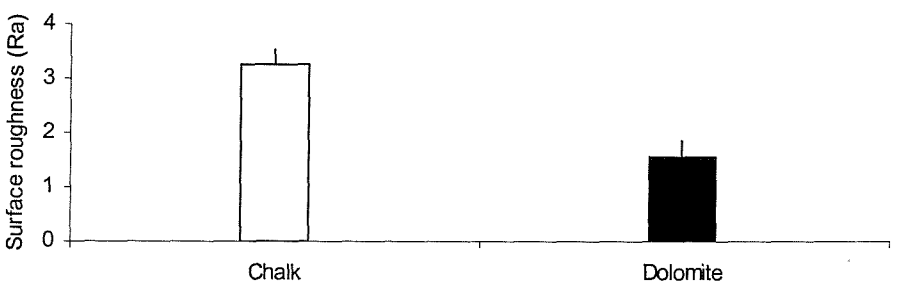


Figure 4.3 – Surface roughness determined with the Talysurf System (Taylor Hobson) on the machine cut surface of chalk and dolomite tiles. Traverse length = 7.2 mm; Cut off = 0.8; magnification X 1000. Bars indicate standard error.

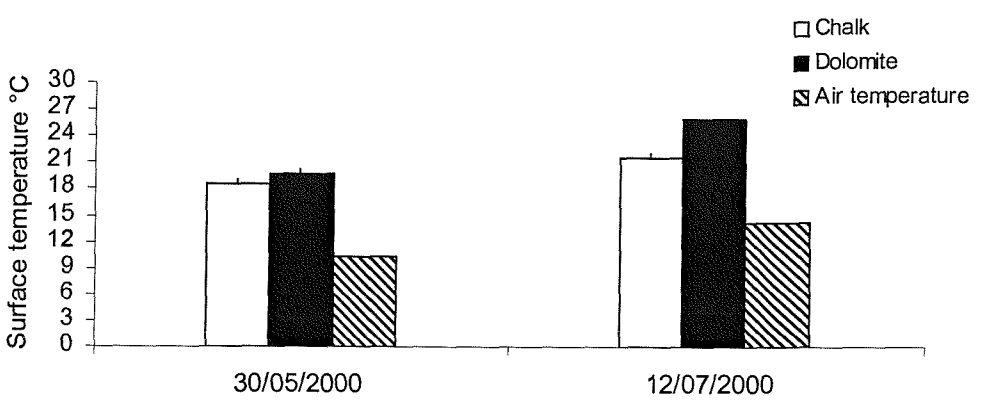


Figure 4.4 – Average surface temperature on chalk and dolomite tiles (n=15) measured at low tide on two different summer days. Air temperature data were recorded by Institute of Marine Studies at the University of Plymouth, about 5 km away from Bovisand (Metereological Data Archive); no local meteo data were recorded at the site. Bars = standard error.

4.2.3 Preparation of the substrata and experimental design

Chalk and dolomite boulders were collected from the mid shore zone at Freshwater Bay (Isle of Wight) and Kimmeridge Bay (Dorset). In order to avoid confounding effects of surface weathering and the presence of organic material on microbial colonisation, squared tiles (8x8x1 cm) were machine cut from the boulders with a 35cm continuous rim diamond saw (Diamond Boart®) in the rock sectioning laboratory (Southampton Oceanographic Centre) so that all the sides had virgin surfaces. The use of machine cut rock substrata was considered a useful method to monitor colonisation, as it allows sampling and observation of microbial communities on natural rock with the advantages of artificial substrata (see discussion in the introduction to this chapter). The edges of the tiles were also planed at an angle to facilitate access to the surface by limpets and therefore allow grazing pressure equivalent to that on the surrounding shore. Tiles were cleaned of rock dust in distilled water, air dried and fixed to the shores with stainless steel (grade A2) screws and a rubber washer. Shallow pits of 13mm in diameter were also made on a set of rock tiles, to allow recessing of a wax disc to assess grazing (see Thompson *et al.*, 1997, for detailed methods).

The study was carried out in summer 2000, from June to August, and in winter 2001, from January to early April. This experiment was spatially and temporally replicated (Table 4.3a-b). On each of the two shores selected at Wembury and Bovisand Bay, two plots (approx. 3x3m) were randomly chosen on the mid-shore and within each plot replicate tiles of each rock type were randomly interspersed (Figure 4.5). The tiles were harvested after 10, 20 30, 45 within 60 days. This sampling scheme was repeated twice within season (two random periods) in summer and winter, using replicate tiles on each occasion.

To optimise sampling, the number of replicate tiles sampled differed on the various sampling dates. In the first summer initiation, 9 replicate chalk and dolomite tiles were used in all plots on both shores at 10, 30 and 60 days, to allow formal statistical comparison. Only four replicate tiles were also used in one plot per shore for 20 and 45 days sampling, to obtain intermediate data points to describe colonisation trajectories. The preliminary analysis of a few samples, however, showed very little colonisation at 10 and 30 days, therefore in the following initiations it was decided to restrict the

formal comparison, which requires full spatial and temporal replication, only to the end of the experiment, after 60 days. At 10, 20, 30 and 45 days, colonisation trajectories were monitored on 4 replicate tiles from only one plot per shore.

Table 4.3 – Experimental design for the summer (a) and winter (b) initiations. The number of replicate tiles used on each sampling occasion and site are shown. The 60 days end date (in bold) was selected for the formal comparison between rock types and seasons.

a)

Summer experiment								
	1st initiation				2nd initiation			
	Wembury Bay		Bovisand Bay		Wembury Bay		Bovisand Bay	
	Piot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Date								
10 days	9	9	9	9	4	--	4	--
20 days	4	--	4	--	4	--	4	--
30 days	9	9	9	9	4	--	4	--
45 days	4	--	4	--	4	--	4	--
60 days	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**

b)

Winter experiment								
	1st initiation				2nd initiation			
	Wembury Bay		Bovisand Bay		Wembury Bay		Bovisand Bay	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Date								
10 days	4	--	4	--	4	--	4	--
20 days	4	--	4	--	4	--	4	--
30 days	4	--	4	--	4	--	4	--
45 days	4	--	4	--	4	--	4	--
60 days	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**

On the selected sampling dates, rock chips were also collected from the rocky shore in vicinity of the experimental plots, to obtain reference information on natural microbial communities. The density of limpets was also recorded in 10 replicate quadrats (50x50cm) at the beginning of the experiment.

During the first summer initiation, four tiles of virgin machine cut and unfilmed weathered old red sandstone tiles were compared with the natural filmed old red sandstone rock on the shore and with dolomite and chalk tiles. This comparison, which was made after 60 days of exposure, allowed a procedural control for the use of substrata and machine cut surfaces.

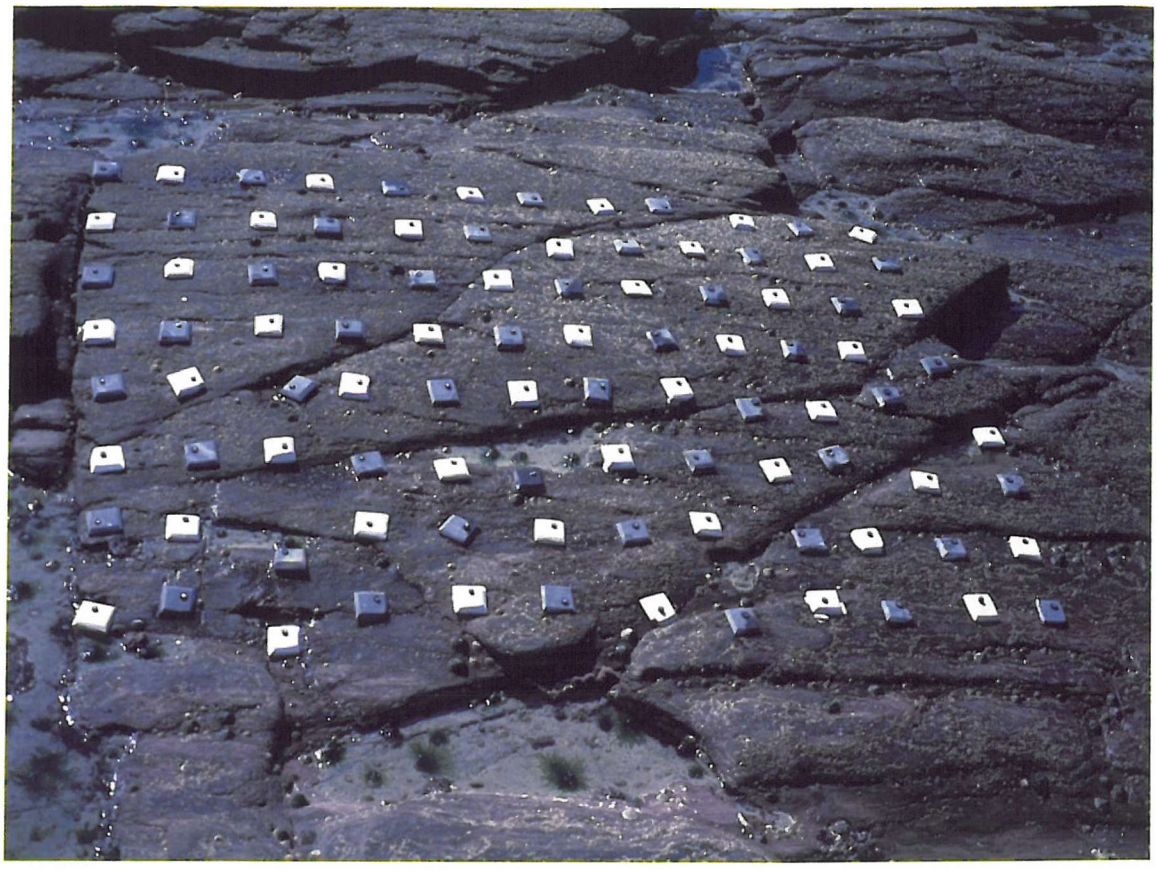


Figure 4.5 – Chalk and dolomite tiles in one experimental plot at Bovisand Bay.

4.1.1 Sampling and laboratory analysis

On each sampling occasion chalk and dolomite tiles were unscrewed from the shore at low tide and broken in pieces with hammer and chisel. From each tile one piece of approximately 9 cm^2 was put in a sealed plastic bag for chlorophyll-a analysis and a smaller rock chip (1 cm^2) was stored in a sterilised multi-compartment plastic dish (Sterilin®) for observation using scanning electron microscopy. Care was taken to collect parts of the surface located at least 1 cm from the edges and the screw hole, to avoid potential differences in the microbial colonisation between inner and outer areas of the tiles. Rock samples were then transported, on the day of collection, to the laboratory of the Marine Biological Association of the UK (Plymouth) for preliminary treatments and storage. Chlorophyll-a analysis was carried out at the University of

Southampton whilst samples for scanning electron microscopy were processed at the University of Plymouth. Rock samples for chlorophyll-a were frozen and analysed within a month with a cold methanol extraction, followed by spectrophotometric analysis (see chapter 2 for details). Rock chips for SEM were cleaned from debris and rock dust in filtered seawater, fixed for 3 hours in glutaraldehyde 5%, and air dried. Before analysis under SEM, rock chips were glued onto aluminium stubs and sputter-coated with gold/palladium. Four fields of view at X300 magnification were recorded on each stub using a digital image software (ISIS®, Oxford Instruments). The level of magnification and the number of fields of view were chosen to cover a representative sampling area on the rock and identify the main biofilm taxa present.

Microalgal biomass, expressed as chlorophyll-a concentration, was estimated for all sampling occasions (10, 20, 30, 45, and 60 days). Analysis of microbial composition using SEM was limited to 10, 30 and 60 days only. Microalgal biomass was also estimated on old red sandstone rock chips from the shore used for the experiment.

4.2.5 Statistical analysis

Colonisation trajectories for each rock type were described in terms of microalgal biomass on both rock types. For each trajectory, regression lines of chlorophyll-a against time were calculated after square root transformation and a *t* test was applied to compare the slopes. Microbial composition was analysed only at 60 days, as on the other sampling dates colonisation was scarce and rock samples were almost bare.

The effect of rock type on microalgal biomass after 60 days of exposure was formally tested using analysis of variance (G-Mav software programme). The following factors were included in the analysis: 1) Season (2 levels, summer and winter) fixed, orthogonal 2) Initiation (2 levels), random, nested in season 3) Shore (2 levels), random, orthogonal 4) Plot (2 levels), random, nested in Season, and Initiation and Shore 5) Rock type (2 levels), fixed and orthogonal. Cochran's test was used to verify the homogeneity of variances and data were transformed to remove heterogeneity. The complexity of the design, which involved interactions between fixed and random factors, meant that some factors could not be tested due to the absence of a

denominator for the calculation of F values. Therefore, a pooling procedure was applied where possible (Winer *et al.*, 1991).

The effect of rock type on abundance of diatoms was analysed with ANOVA in only one initiation for each season. Separate analyses were carried out for the summer and winter initiations. Three factors were therefore included in the analysis: 1) Shore (2 levels), random, orthogonal 2) Plot (2 levels), random, nested in Season, and Initiation and Shore 3) Rock type (2 levels), fixed and orthogonal. The same analysis was used to compare the abundance of cyanobacteria, during the summer only, as they were almost absent during winter.

The composition of microbial communities was assessed using the multivariate analysis (PRIMER 5, software programme). Differences in microbial composition on chalk and dolomite tiles at the end of the summer and winter experiment, after 60 days of exposure, were compared using the ANOSIM procedure. Only one initiation was considered in summer and winter. SIMPER analysis was applied to identify the taxa that mainly contributed to the differences observed.

Wilcoxon Signed ranks test for matched pairs was used to compare the average amount of grazing on chalk and dolomite tiles in each plot. Pearson's correlation test was used to relate the amount of chlorophyll-a in each plot to average grazing pressure for both chalk and dolomite tiles.

4.3 RESULTS

4.3.1 Description of trajectories of microbial colonisation on different rocks

Microalgal biomass

In both seasons, colonisation by microorganisms always proceeded very slowly (Figure 4.6a-h). Microalgal biomass was negligible for the first month of exposure, independent of the location or the time of the year colonisation started. Colonisation became apparent after 30 days, although maximum values observed occurred between 45 and 60 days of exposure.

During summer, microbial colonisation on chalk and dolomite tiles was similar, being almost absent during the first month of exposure and then increasing gradually in the following month (Figure 4.6a-d). Colonisation trajectories differed slightly between the two summer initiations. In the first initiation, started at the end of May 2000, maximum values of microalgal standing stock, expressed as chlorophyll-a, were not higher than 0.3 $\mu g\ cm^{-2}$, even after 60 days of exposure (Figure 4.6a, b). In the second initiation, started a month later, microbial colonisation increased more markedly after 30 days to 60 days of exposure, where microalgal biomass was twice that of the first initiation (Figure 4.6c, d). These patterns were also very consistent between the two shores.

During winter, colonisation of chalk and dolomite tiles was very similar during the first month of exposure, characterised by extremely low values of microalgal biomass (Figure 4.6e-h). In contrast, microbial colonisation in the following month was greater than in summer. During the two winter initiations, started in early and mid February 2001, microalgal biomass reached values equivalent to maximum summer levels after only 45 days of colonisation. After 60 days of exposure, microalgal colonisation increased more steeply, with values of chlorophyll-a up to 3.44 $\mu g\ cm^{-2}$. Marked differences in the colonisation processes were also observed between chalk and dolomite substrata after the first month of exposure. Microbial communities developed

much more rapidly on chalk. This was particularly evident in the first initiation at Bovisand Bay, where colonisation on chalk increased after just 20 days of exposure (Figure 4.6f). In both initiations, microalgal biomass was at least three times higher on chalk than on dolomite after 45 and 60 days of exposure. Trajectories diverged more markedly after 45 days, except for the second initiation, when microalgal biomass on chalk slightly decreased towards the end of exposure at Bovisand Bay. Furthermore, microalgal abundance appeared to be more variable on chalk than on dolomite, as shown by the larger standard errors associated with this type of rock.

Comparison of the slopes of the regression lines drawn for each colonisation trajectory showed, as expected, no significant differences between chalk and dolomite during summer (Figure 4.7a-d). There were significant differences in colonisation trajectories between chalk and dolomite during winter, but only in the first initiation (*t* test: Wembury Bay, $p<0.01$; Bovisand Bay, $p<0.05$; Figure 4.7e, h).

Microbial composition

Preliminary observation of a subset of rock chips from chalk and dolomite tiles exposed for 10 and 30 days showed very little colonisation by the main biofilm taxa considered in the study, mirroring the chlorophyll-a estimates. Most of the samples were virtually bare. Therefore, these two sampling occasions were not included in the statistical analysis which was restricted to the end of the experiment, at 60 days (results are described in paragraph 4.3.2 below).

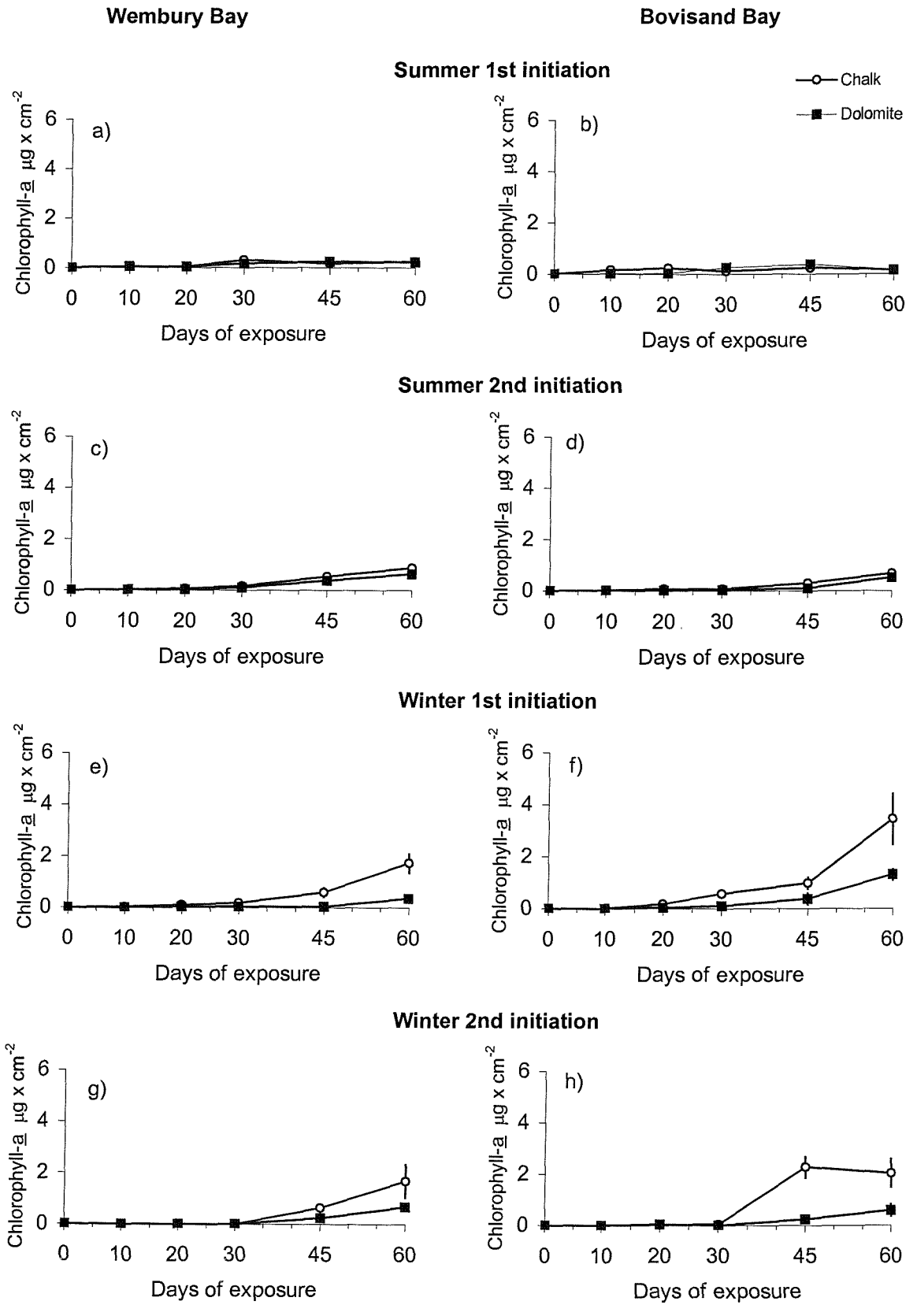


Figure 4.6 - Colonisation trajectories on chalk and dolomite in Wembury and Bovisand Bay; a – d: summer initiations (20/5/2000; 12/7/2000); e – h: winter initiations (31/1/2001; 9/02/2001). n = 4 except for 60 days, where n = 8. Vertical bars represent the standard error.

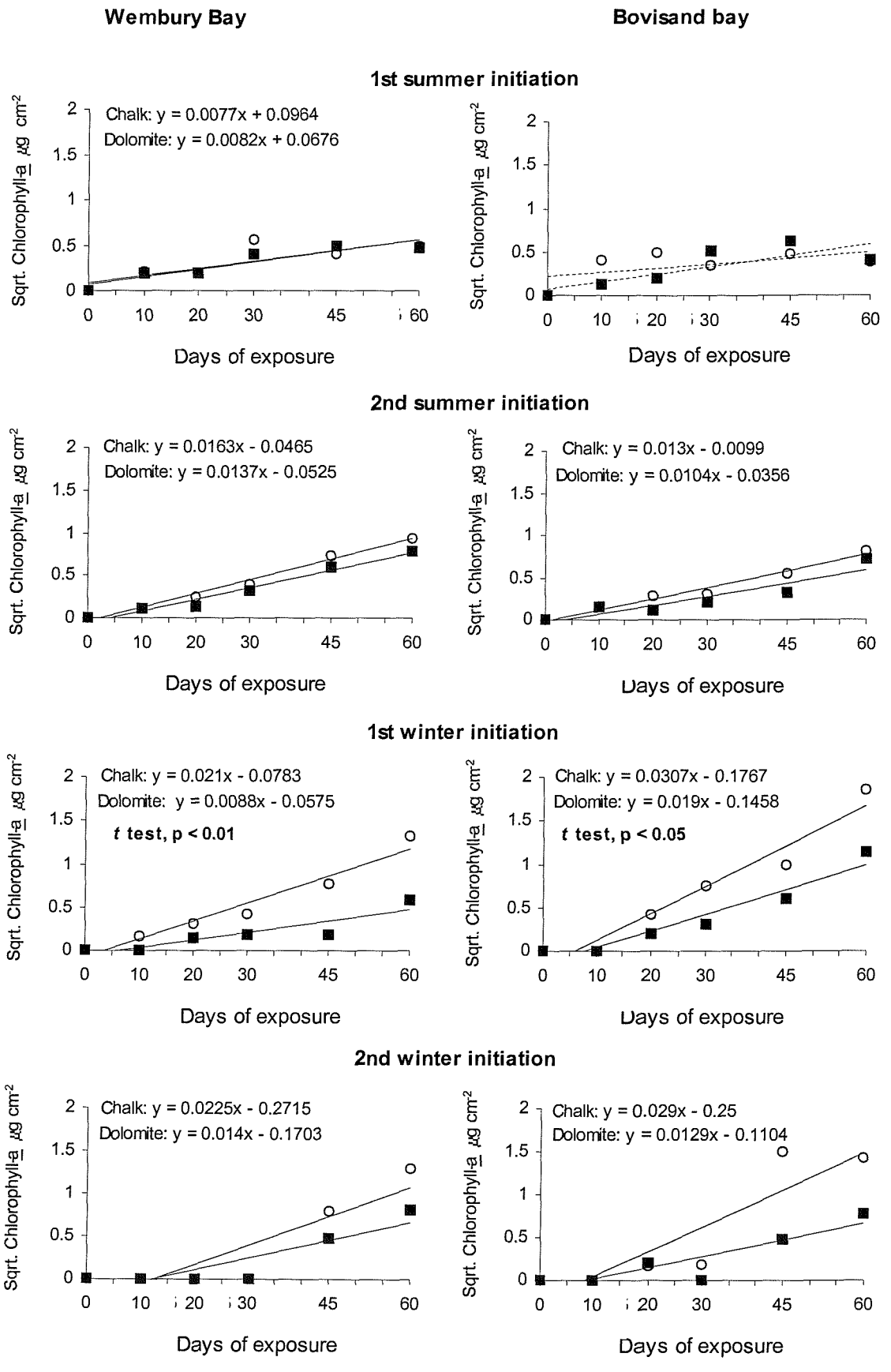


Figure 4.7 – Regression lines for microalgal colonisation on chalk and dolomite (see Figures 4.6a-h). Dotted lines were drawn when regression line was not significant. P values were given for slopes which differed significantly.

4.3.2 Analysis of mature microbial communities after 60 days on chalk and dolomite rocks

Procedural controls

The amount of chlorophyll-a estimated on chalk and dolomite tiles after 60 days of exposure was considerably lower than the background level measured on the shore near the plots, although no formal comparison was made (Figure 4.8a-d). Microalgal standing stock on the old red sandstone rock was at least double that on rock tiles, particularly during summer. This effect was, as expected, less on chalk than on dolomite, which in winter reached similar levels of microalgal biomass as on the filmed old red sandstone rock (Figure 4.8c, d). In the first winter initiation at Bovisand Bay, microalgal biomass on chalk tiles was higher than on the old red sandstone on the surrounding shore (Figure 4.8c). Microalgal abundance on naturally filmed old red sandstone was also higher than on tiles made of the same rock with the surface machine cut or naturally weathered, but cleared of biofilm (Figure 4.9). Old red sandstone tiles were, however, more markedly colonised than chalk and dolomite substrata. No relevant differences were observed between machine cut and weathered surfaces, although the latter showed, on average, a slightly greater amount of microalgal biomass.

Microalgal biomass

Microalgal biomass on tiles exposed for 2 months showed a marked seasonal pattern (Figure 4.10 a, b compared to c, d). During summer both chalk and dolomite tiles were poorly colonised, as shown by values of chlorophyll-a which were always lower than 1 $\mu g\ cm^{-2}$ (Figure 4.10a, b). A slight difference was observed between the two summer initiations. Tiles exposed from late May to late July were almost bare in all the plots at Wembury and Bovisand, with little difference between chalk and dolomite. In the second summer initiation, during July and August, microalgal biomass was relatively higher on chalk than dolomite. During winter there was a considerable increase in microalgal standing stock in all the plots, irrespective of the date tiles were deployed (Figure 4.10c, d). The increase was more marked on chalk, where microalgal biomass was several times the amount recorded in summer. Clear differences between chalk and dolomite were apparent in the winter initiations. Microalgal standing stock was

always higher on chalk than on dolomite, particularly at Bovisand Bay during the first winter initiation.

The patterns observed were confirmed by the analysis of variance. Pooling procedure allowed detection of a highly significant interaction between season and rock type (Se X Ro), suggesting that there were differences between rock types, but these were not consistent with season (Table 4.4). During summer, there were no significant differences in microalgal biomass between rock types; whilst in winter, chalk had significantly higher microalgal standing stock than dolomite. This pattern did not vary within season, as no significant differences were observed between initiations in either winter or summer (Ro X In (Se); Table 4.4)).

Analysis of grazing marks on wax discs showed that radula marks were generally more abundant on chalk tiles (Table 4.5). In the summer grazing on chalk was higher than dolomite in 6 out 8 plots. During winter, the grazing index was always higher on chalk tiles than on dolomite, except for one tie. When tested using a matched pair approach, these were significant differences between rock types in the winter, but not in the summer (Table 4.6). There was no apparent relationship between the mean amount of chlorophyll-a and the mean percentage cover of grazing marks in each plot when both rock types were pooled in the summer (Figure 4.11 a). A positive correlation was found, however, between microalgal biomass and grazing pressure in winter (Pearson's $r = 0.55$; $p<0.05$; Figure 4.10b). The bivariate plot indicates that during winter grazing appeared to be greater on chalk, where higher microalgal biomass was also measured (Figure 4.11b).

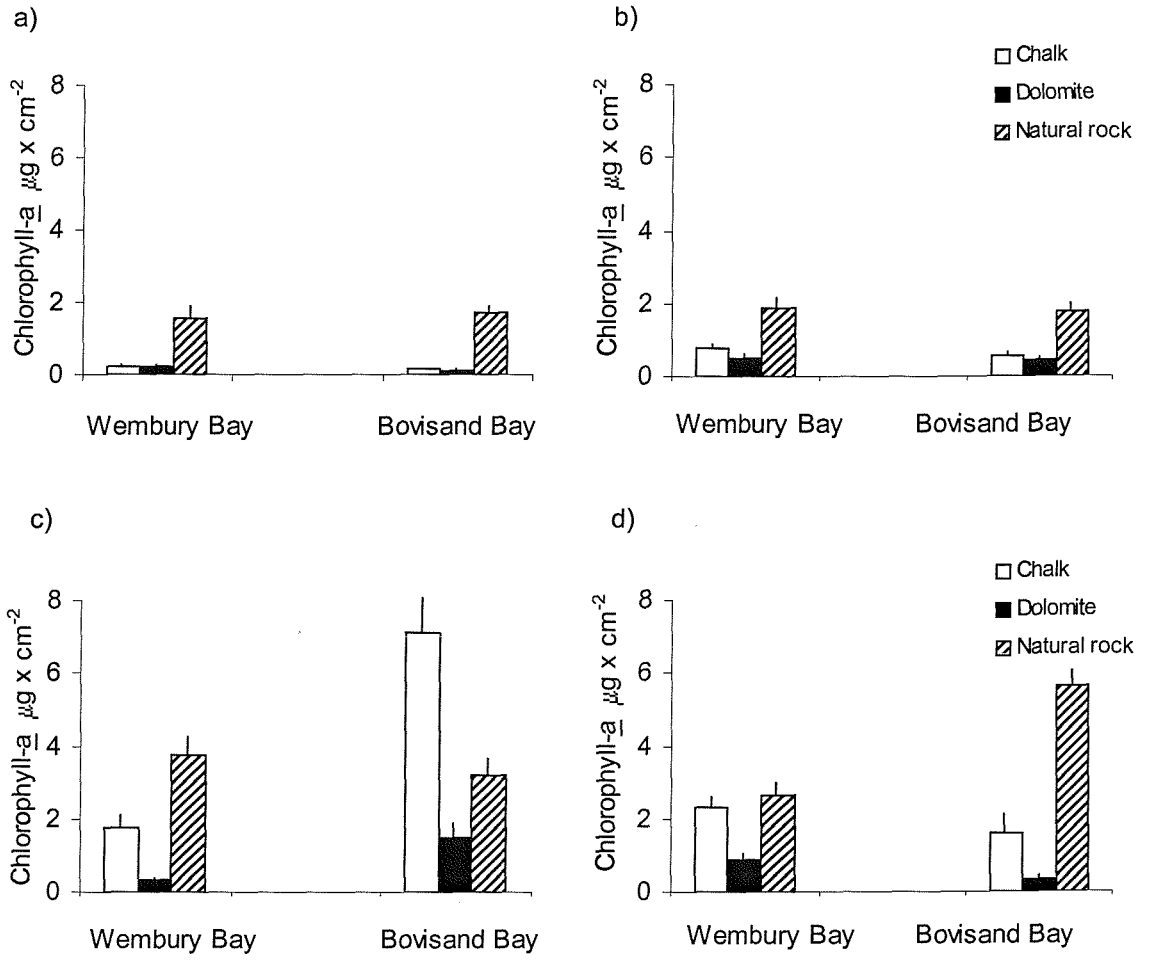


Figure 4.8 –Microalgal standing stock (averaged between plots) at Wembury and Bovisand Bay on chalk, dolomite and natural filmed rock (old red sandstone). a) and b): summer initiations. c) and d): winter initiations. Data for the winter initiations are obtained from one plot only. Vertical bars indicate standard errors.

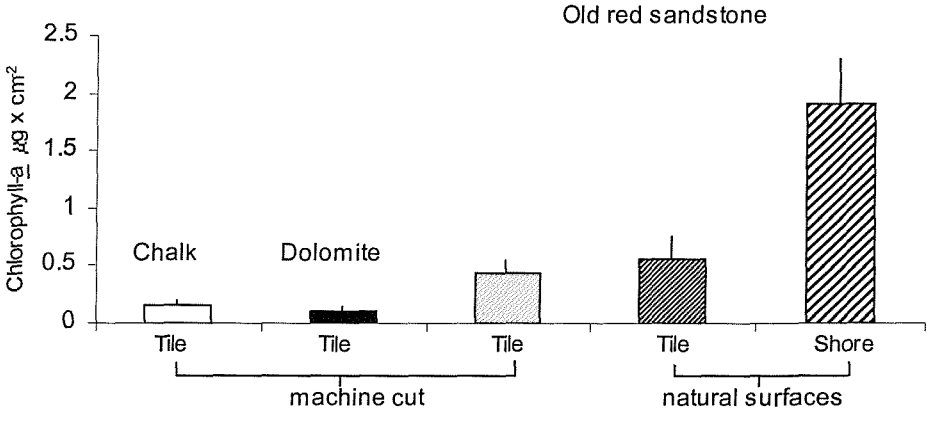


Figure 4.9 – Comparison of the effect of different substrates on microalgal standing stock. Data were obtained from one plot (n = 4) at Bovisand Bay, in summer 2000 (1st initiation). Tiles were exposed for 60 days. The old red sandstone substrata are, from left to right, unfilmed and machine cut tiles, unfilmed and weathered tiles and filmed natural rock (shore).

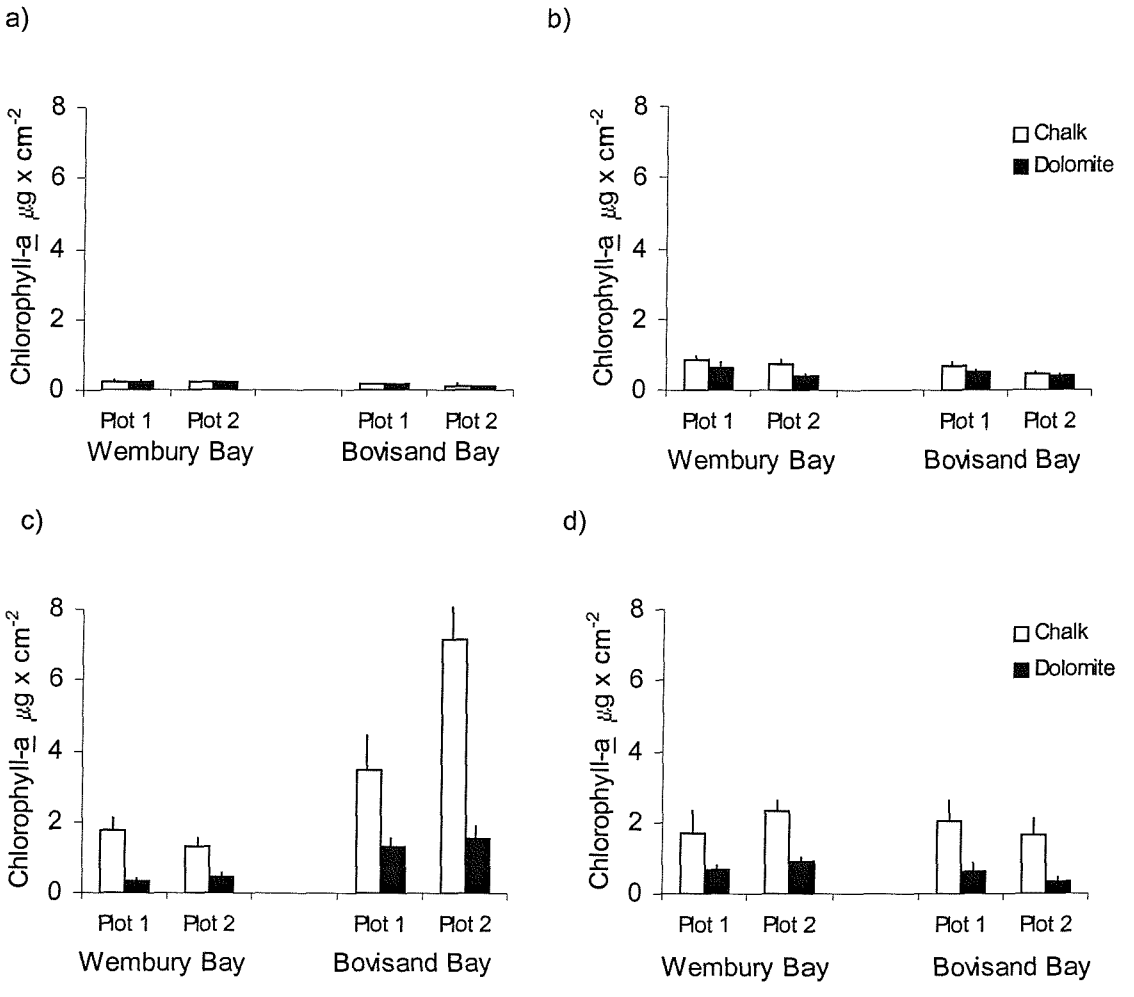


Figure 4.10 - Microalgal biomass (expressed as chlorophyll-a) on chalk and dolomite tiles exposed for 60 days. a: 1st summer initiation (20/05/2000); b: 2nd summer initiation (1/07/2000); c: 1st winter initiation (31/01/2001); 2nd winter initiation (9/02/2001). Vertical bars indicate standard errors.

Table 4.4 – Analysis of variance for differences in microalgal biomass between chalk and dolomite. Comparison was made between season and initiation within season. Data were Ln (X) transformed to remove heterogeneity of variances.

Source	df	MS	F	F versus	P
Season	1	104.94	--	no test	--
Initiation (Se)	2	25.96	2.33	Sh x In (Se)	0.30
Shore	1	0.02	0.01	Sh x In (Se)	0.97
Plot (Se x In x Sh)	8	0.83	3.00	Res	**<0.005**
Rock type	1	31.76	89.05[1]	Pooled data	**<0.001**
Se x Sh	1	7.79	0.70	Sh x In (Se)	0.49
Se x Ro	1	16.65	46.69[1]	Pooled data	**<0.001**
Sh x In (Se)	2	11.15	13.41	Pl (Se x Ti x Sh)	**<0.005**
Ro x In (Se) [a]	2	0.17	0.60[2]	Res	0.55
Sh x Ro [b]	1	0.02	0.06[2]	Res	0.81
Ro x Pl (Se x In x Sh)	8	0.33	1.21	Res	0.29
Se x Sh x Ro [c]	1	0.60	2.17[2]	Res	0.14
Ro x Sh x In (Se) [d]	2	0.60	2.15[2]	Res	0.12
Res	224	0.28			
TOT	255				

[1] F calculated using pooled MS (a+b+c+d=0.3566 with 6 df)

[2] tested over Res after pooling

Table 4.5 – Percentage cover of grazing marks recorded on wax discs on chalk and rock tiles. The number of replicate wax discs scored in each plot is shown.

	Wembury Bay				Bovisand Bay			
	Plot 1		Plot 2		Plot 1		Plot 2	
	chalk	dolomite	chalk	dolomite	chalk	dolomite	chalk	dolomite
1st summer initiation	10.0	13.8	31.9	41.3	41.7	36.4	20.0	12.1
n	6	4	8	8	6	7	7	7
2nd summer initiation	28.5	15.0	43.1	25.0	45.0	20.6	33.9	25.0
n	10	8	8	9	9	9	9	9
1st winter initiation	58.8	10.0	12.2	3.8	47.8	9.6	32.4	9.1
n	8	6	9	8	6	7	8	7
2nd winter initiation	3.8	3.8	22.3	6.1	9.5	4.1	6.3	0.6
n	9	9	7	8	8	8	9	9

Table 4.6 – Wilcoxon signed ranks test for mean percentage cover of grazing marks in each plot in summer and winter.

	Chalk >Dolomite	Chalk = Dolomite	Chalk< Dolomite	p
summer	6	0	2	0.09
winter	7	1	0	0.02

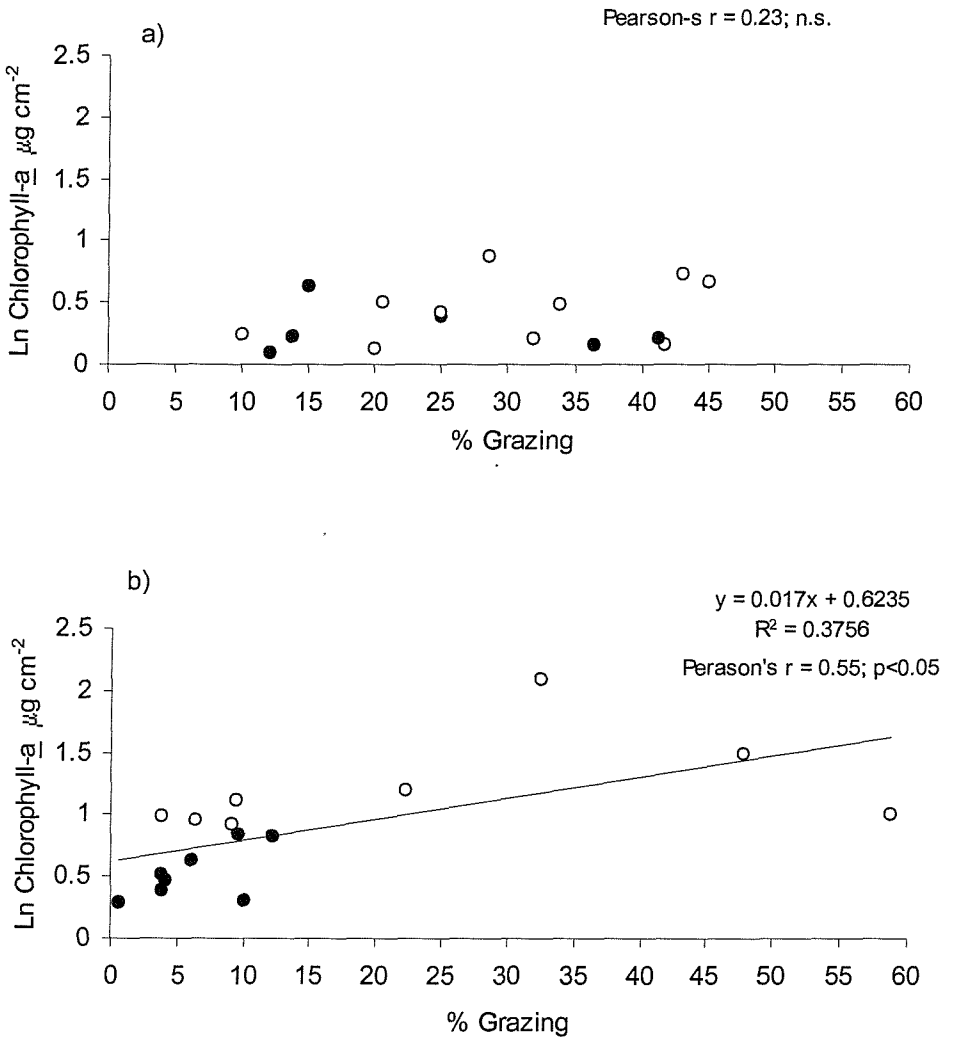


Figure 4.11 – Relationship between percentage of grazing and microalgal standing stock on chalk (white circles) and dolomite (black circles) tiles in summer 2000 (a) and winter 2001 (b). Both sets of data have been pooled for the analysis.

Microbial composition

Microbial communities on chalk and dolomite tiles were relatively similar, consisting mainly of diatoms, cyanobacteria and encrusting microalgae. Algal germlings were rare, as were ephemeral algae; both were only observed in the winter. The most abundant species of diatoms were *Fragilaria* spp. and *Achnanthes* spp., whilst *Cocconeis* and *Synedra* were rarer. The abundance of diatoms varied considerably between the two initiations (Figure 4.12 a, b). In the summer diatoms were very scarce, being less than 1000 cells cm^{-2} with no significant differences being detected between chalk and dolomite (Table 4.7). The number of diatoms increased markedly in the winter, reaching over 100000 cells cm^{-2} on chalk tiles. In the winter there were significantly more diatoms on chalk than on dolomite (Table 4.8). In contrast, cyanobacteria were almost absent from the rock tiles exposed during winter, whilst they dominated the substrata during the summer initiation (Figure 4.13a, b). Their abundance did, however, vary considerably between shores (Figure 4.13b), with no clear differences in abundance between chalk and dolomite (Table 4.9).

Multivariate analysis of the main taxa within biofilm did not show any significant differences between chalk and dolomite (Figure 4.14, Table 4.10). A high coefficient of dissimilarity was, however, obtained in the comparison of microbial communities between summer and winter (Figure 4.15; Table 4.10). Results from SIMPER analysis suggested that differences in the abundance of diatom were mostly responsible for these differences.

Microalgal biomass, expressed as chlorophyll-a, was significantly correlated with the number of diatoms on chalk, but not on dolomite (Table 4.11). However, the abundance of cyanobacteria was not correlated with chlorophyll-a on either rock type. The abundance of diatoms and cyanobacteria at plot level was not significantly related to the amount of grazing marks observed on the tiles.

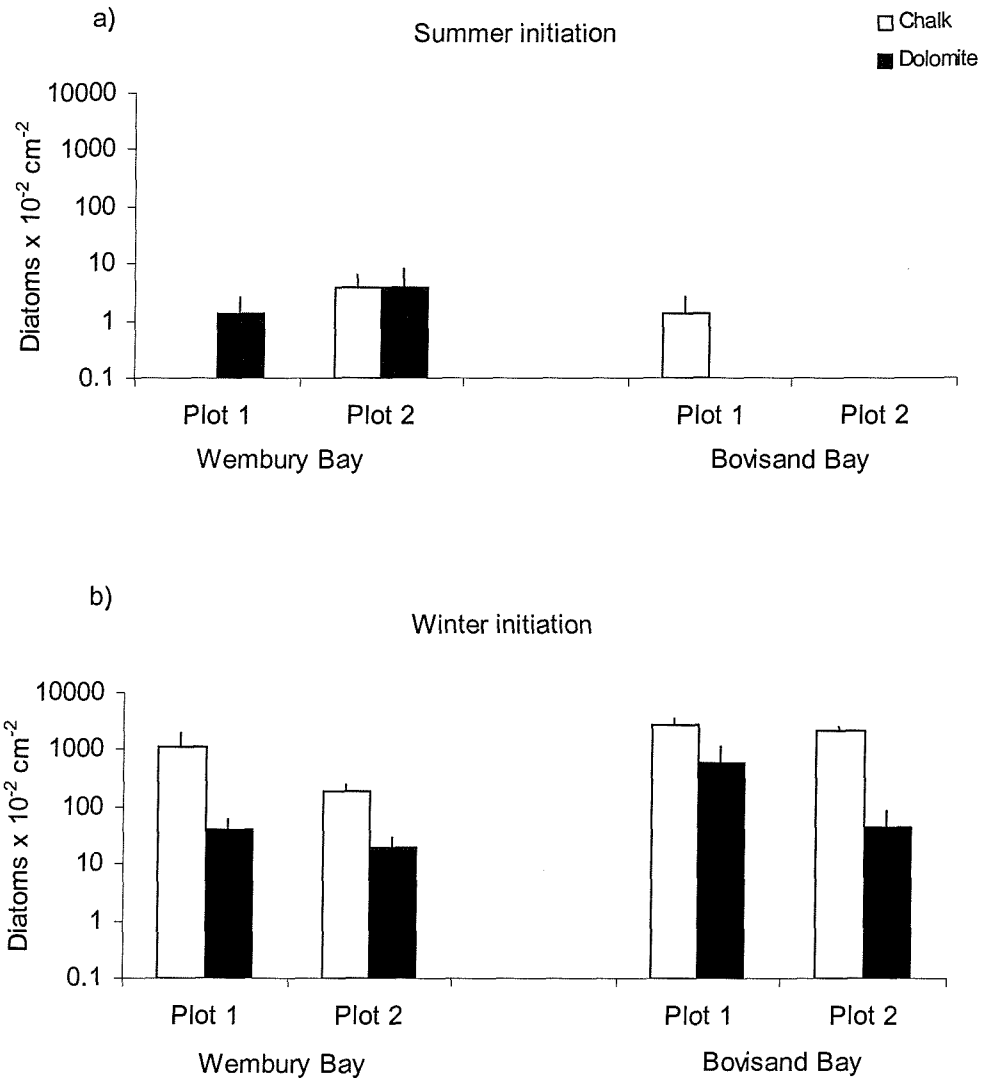


Figure 4.12 – Abundance of diatoms on chalk and dolomite tiles exposed for 60 days at Wembury and Bovisand Bay. a: summer initiation, from the 1st July to 30th August 2000; b: winter initiation, from 31st January to 28th March 2001. Vertical bars indicate standard errors.

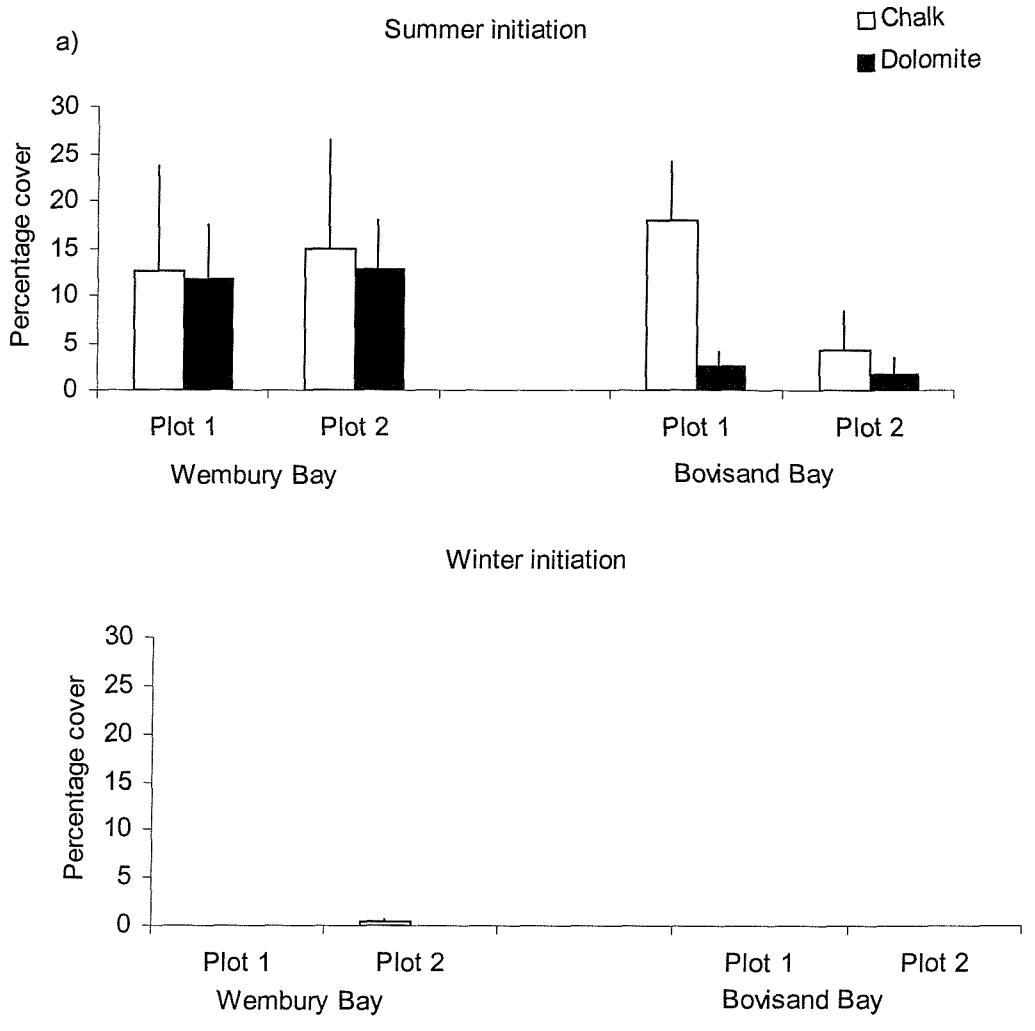


Figure 4.13 – Abundance of cyanobacteria on chalk and dolomite tiles exposed for 60 days at Wembury and Bovisand Bay. a: summer initiation, from the 1st July to 30th August 2000; b: winter initiation, from 31st January to 28th March 2001. Vertical bars indicate standard errors.

Table 4.7 – Analysis of variance for differences in the abundance of diatoms between chalk and dolomite rock in summer 2000. Data were Ln (X+0.1) transformed to remove heterogeneity of variance.

Source	df	MS	F	F versus	P
Shore	1	2.02	1.62	Plot (Sh)	0.33
Plot (Sh)	2	1.25	1.60	RES	0.23
Rock type	1	0.08	0.19[1]	Pooled data	0.69
Sh x Ro [a]	1	0.20	0.44[1]	Pooled data	0.55
Ro x Pl (Sh) [b]	2	0.56	0.72	RES	0.50
RES	16	0.78			
TOT	23				

[1] F calculated using pooled MS (a+b).

Table 4.8 – Analysis of variance for differences in the abundance of diatoms between chalk and dolomite rock in winter 2001.

Source	df	MS	F	F versus	P
Shore	1	99781.0	7.28	Plot (Sh)	0.11
Plot (Sh)	2	13700.0	1.33[1]	Pooled data	0.29
Rock type	1	176216.3	3.47	Sh x Ro	0.31
Sh x Ro	1	50738.0	4.92[1]	Pooled data	**0.04**
Ro x Pl (Sh) [a]	2	5151.5	0.50[1]	Pooled data	0.61
RES [b]	16	10956.6			
TOT	23				

[1] F calculated using pooled MS (a+b).

SNK test for (Sh x Ro):	Wembury Bay	Bovisand Bay
	Chalk = Dolomite	Chalk > Dolomite **p<0.01**

Table 4.9 – Analysis of variance for differences in the abundance of cyanobacteria between chalk and dolomite rock in summer 2001.

Source	df	MS	F	F versus	P
Shore	1	250.26	2.98	Plot (Sh)	0.23
Plot (Sh)	2	83.88	0.70	RES	0.51
Rock type	1	162.76	2.35[1]	Pooled data	0.22
Sh x Ro [a]	1	82.51	1.19[1]	Pooled data	0.35
Ro x Pl (Sh) [b]	2	62.43	0.52	RES	0.60
RES	16	119.61			
TOT	23				

[1] F calculated using pooled MS (a+b).

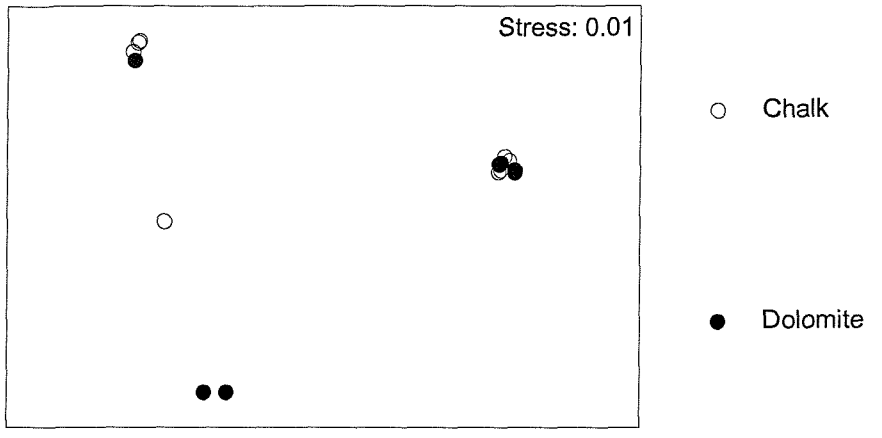


Figure 4.14 – MDS plot of microbial communities on chalk and dolomite tiles after 60 days of exposure.

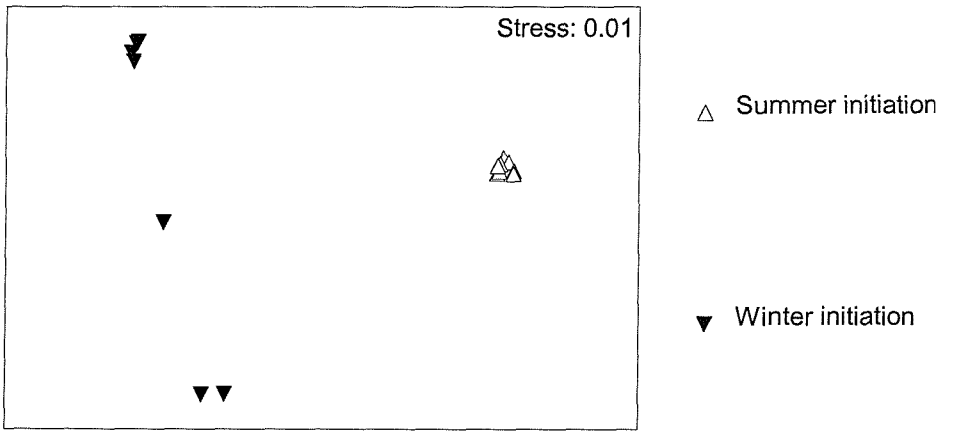


Figure 4.15 - MDS plot of microbial communities between the summer and winter initiation.

Table 4.10 – ANOSIM for differences in biofilm composition between chalk and dolomite and between the summer and winter initiation. R is the coefficient of dissimilarity, P is the relative level of significance. Contribution of the major taxa groups to differences between initiations obtained from SIMPER procedure.

	R	P
Differences between rock types:	0.24	0.11
Differences between initiation	0.99	0.001

Main taxa contribution to differences between summer and winter initiations:

	Average abundance		Contribution %	Cumulative %
	summer	winter		
Diatoms	0.17	107.94	61.72	61.72
Cyanobacteria	0.04	21.66	22.09	83.81
Corallines	6.21	0.15	14.76	98.57

Table 4.11 – Pearson's correlation indexes showing relationships between chlorophyll-a and the abundance of diatom and cyanobacteria. Correlations between the grazing index, expressed as percentage cover of grazing marks, and the two biofilm components are also shown. * Denotes significance at $P < 0.05$.

	Chlorophyll-a		Grazing index	
	Chalk	Dolomite	Chalk	Dolomite
Diatoms	**0.81***	0.58	0.32	-0.32
Cyanobacteria	-0.54	-0.33	0.09	0.54

4.4 DISCUSSION

4.4.1 Using rock tiles as experimental substrata

Microbial communities colonising chalk and dolomite substrata were less abundant than natural biofilms coating old red sandstone constituting the shore. Microalgal biomass on the natural old red sandstone was at least twice that on the rock tile after 60 days of exposure. This difference was not apparent during summer. This difference was unexpected, as 60 days was thought to be a sufficiently long period for the formation of a mature community similar to natural assemblages on rocky shores based on previous work (Underwood, 1984a; MacLulich, 1986; Hill, 1990). The higher levels of chlorophyll-a on the shore during summer and winter were within the observed range of values recorded for that part of the coast (Jenkins *et al.*, 2001) and comparable to those recorded in Chapter 2.

The above discrepancy between natural rock and the experimental tiles could be an effect of the different types of rock. However, comparison of chalk and dolomite tiles with equivalent machine cut tiles of old red sandstone did not show any significant difference in microalgal biomass. The slightly higher but non significant values of chlorophyll-a estimated on the old red sandstone tiles hints at an influence of the surrounding rocks; microbial communities would colonise to a greater extent substrata made of the same rock as that the shore. This was also observed in a previous pilot study at Lyme Regis, where tiles made of the Blue Lias limestone, of which the shore is made of, showed a higher number of diatoms than two other limestones (unpublished observations). Early microbial colonisation is known to be influenced by specific physical and chemical properties of the substratum (Marszalek *et al.*, 1979; Taylor *et al.*, 1997; Becker *et al.*, 1997b). It could be possible therefore that microbial communities tend to colonise types of substrata to which they have already adapted. This is speculation only, as no information is available on mechanisms of site selectivity operating in biofilms. Moreover, unfilmed machine cut or scraped, weathered tiles made of old red sandstone were also less colonised than the filmed

rock of the shore. This indicates that the use of tiles interferes in some way with the mechanisms of colonisation and further development of biofilm communities.
One explanation could be simply mechanical. The bottom surface of the rock tiles rarely is in full contact with the background rock of the shore. This discontinuity could slow down sideways colonisation by certain components of biofilm such as diatoms, which are known to reproduce mainly by multiple vegetation from the surrounding areas (Round, 1981). Although 60 days was sufficiently long period for the purpose of this experiment, it is also possible that development of a mature biofilm might take longer.

Weathering processes might also contribute to the observed differences between the machine cut and natural substratum. For example physical conditioning might include changes in the surface tension and superficial electrostatic forces, which can influence the attachment of early colonising bacteria (Becker *et al.*, 1997b). Chemical weathering of rock such as dissolution of minerals may be also important, particularly for carbonate rocks such as chalk, which start releasing ions (Ca^{+}, Na^{+}) when they are first immersed in water (Press, 1997). Bacteria might contribute to the biological conditioning by producing mucopolysaccharids. These are likely to facilitate the subsequent attachment of diatoms and cyanobacteria on the substratum (Marszalek *et al.*, 1979). These processes probably occur faster on weathered surfaces than on smooth, virgin substrata.

The physical and chemical transformations of the rock surface caused by weathering could make the substratum more suitable for microbial colonisation by increasing the surface roughness, since naturally weathered rocks are rougher than machine cut surfaces. Colonisation by microbial communities is known to be affected by greater rugosity, which provides more protection from physical disturbance and grazing (Edyvean *et al.*, 1985). This probably accounts for the higher amount of microalgal biomass on naturally weathered rather than machine cut old red sandstone tiles. Formal experiments testing specifically the effect of surface roughness on biofilms are, however, needed before any conclusion can be drawn.

The use of rock tiles for assessing biofilms seems therefore to underestimate the abundance of the natural communities. This has been already observed for epilithic

biofilms in freshwaters systems, where the use of artificial substrata also appeared to affect also microbial diversity (Cattaneo and Amireault, 1992). Despite this, tiles are a powerful and valid method to compare microbial communities between rock types, provided absolute estimates are not required.

In the present study the use of rock tiles allowed a direct comparison of biofilm colonisation on chalk and dolomite rock, to test for potential effects of the rock substratum. The geographical separation of the chalk and dolomite shores, located in the Isle of Wight and Dorset respectively, made it difficult to separate the effect of the substratum from other environmental variables. Standard rock substrata also reduced spatial variability due to surface topography in microbial communities, which might also have obscured patterns. The use of machine cut substrata instead of scraped natural surfaces was also made to overcome the confounding effect of the rock erosion, which varies accordingly to wave action, with exposed shores weathering more than sheltered shores. Furthermore, scraping filmed surfaces, does not always guarantee the complete removal of microorganisms and other organic compounds. The decision to set up the experiment on a shore of different rock type was also supported, as there was also evidence of an effect of the background rock type.

4.4.2 Microbial colonisation and effects of season

Microbial colonisation of rock tiles was extremely slow, and microalgal biomass was present in sufficient quantities to measure after one month. Also, the mature stage of microbial communities, characterised by the settlement of algal germlings and ephemeral algae, was observed on tiles which have been exposed for 60 days. Previously described colonisation trajectories for freshwater (Blinn *et al.*, 1980; Hoagland, 1982; Korte, 1983) and marine systems (Marszalek *et al.*, 1979; Edyvean *et al.*, 1985; Bhosle *et al.*, 1994; Anderson, 1995) generally showed faster rates of colonisation, taking approximately four weeks for the formation of mature assemblages. This discrepancy is probably a consequence of previous work being carried out on surfaces which were continuously immersed freshwater epilithon and marine microfouling forming on submerged man-made structures. Assemblages on these surfaces do not normally experience desiccation and insolation stresses occurring in the intertidal environment. Desiccation stresses resulting from the exposure to air

during low tide is likely to reduce the rate of colonisation on the substratum. This might also explain the poorer colonisation on rock tiles during summer, where harsher thermal and desiccation stresses are likely to occur (Castenholz, 1963; Cubit, 1984; Underwood, 1984a; MacLulich, 1987).

Work on rocky shores has suggested, however, relatively shorter periods for a complete development of natural biofilms than those observed in the present study (Underwood, 1984a; Hill, 1990). These studies were made on areas of the shore from which biofilms were previously scraped and the rock sterilised and may result from differences between the natural rock surface and machine cut tiles, as discussed above. Colonisation is likely to be enhanced on natural substrata compared to machine cut surfaces, as discussed in the above paragraph. The results obtained from this experiment suggest that a longer period is required for biofilm formation when freshly machine cut rock surfaces are used, as tiles probably require a period of physical, chemical and biological conditioning before microbial colonisation by diatoms and cyanobacteria.

Colonisation was markedly seasonal. During the first month of exposure very little colonisation occurred during both summer and winter and the rock tiles were almost bare. After 30 days, differences in colonisation trajectories were apparent between seasons: rock tiles remained uncolonised during summer, but during winter microalgal abundance increased steeply.

Seasonal variation seems therefore to control the later stages of microbial colonisation and hence the development of mature communities. Consistent patterns were observed within season, although biomass slightly differed between the two summer initiations. During the first initiation, colonisation increased little with time and appeared arrested after 30 days of exposure. This difference might be explained by variations in weather conditions, such as insolation, air temperature and rainfall. Only a slight variation in these variables could, in summer, have a strong impact on microalgal communities.

Species composition was also influenced by season. The poorly developed biofilm on tiles exposed for 10 and 30 days did not allow the analysis of changes in species

succession. At 60 days, however, there were stark differences in microbial composition between summer and winter. Cyanobacteria dominated in summer and the abundance of diatom was very low. While in the winter diatoms dominated and cyanobacteria were almost absent from the substrata. Cyanobacteria are more resistant to desiccation, insolation and thermal stresses than diatoms and this may explain the seasonal shift in biofilm composition (Fogg, 1973; Birke, 1974).

Diatoms, in particular *Fragilaria*, often completely covered the rock substratum during the winter and so cyanobacteria could therefore be present, but hidden beneath the layer of diatoms. The presence of cyanobacteria, however, was minimal even on tiles with few diatoms. Alternatively, it could be possible that colonisation by cyanobacteria in the presence of the more opportunistic diatoms is delayed during winter. Successional sequences have been classically described as the arrival on the substratum of bacteria, followed by diatoms and, subsequently, by cyanobacteria (Tuchman and Blinn, 1979; Cooksey and Wigglesworth-Cooksey, 1995).

4.4.3 Effect of rock type

Differences in microalgal biomass between chalk and dolomite were dependent on season and only evident after 45 days of colonisation. In the summer microalgal biomass was similar on both types of rock during the whole period of colonisation. In contrast, during winter, colonisation trajectories on chalk and dolomite diverged considerably after 30 days, with a more marked increase in microalgal standing stock on chalk. This effect was highly significant when formally compared after 60 days in the winter. Differences between rock types in diatom numbers were also apparent in the winter only, but multivariate methods did not detect any difference in the community as a whole.

The effect of rock substratum on microbial communities appears therefore to be modulated by natural seasonal variation, as anticipated at the beginning of the experiment. The direction of the seasonal differences between chalk and dolomite are, however, contrary to the *a priori* prediction. In summer, physical stresses appear to be more important than the substratum in controlling the abundance of microbial films. In

winter, more favourable conditions reduce physical stresses and the effect of the substratum becomes evident.

The results obtained from the present experiment and the previous investigations described in chapter 2 and 3, seem to reinforce the hypothesis that surface roughness and porosity are the rock properties which are more likely to affect microbial communities. This idea is supported by other authors, although few investigations have been made of the effect of the rock substratum on microbial colonisation in intertidal systems (Blinn *et al.*, 1980; Edyvean *et al.*, 1985). In contrast with my results, Edyvean *et al.* (1985) showed that differences in biofilm formation on rock types tended to disappear during later stages of colonisation. This discrepancy could be explained, however, by the large differences in the experimental protocols followed.

Grazing appeared to interact with the rock substratum in modulating microbial communities. Grazing by limpets was more intense on chalk than on dolomite, in both seasons. Also the index of grazing positively correlated with chlorophyll-a, showing a greater grazing pressure on chalk. It could be possible that higher microalgal abundance on this substratum attracted more grazers. Despite the lack of experimental verification, it is a common opinion that limpets are attracted to graze areas with higher microalgal biomass (Della Santina and Thompson, unpublished obs.). The influence of the rock substratum could be therefore obscured by a difference in grazing pressure between substrata. A specific experiment is needed to separate the effects of rock substratum and grazing and their possible interactions. Chapter 5 describes this experiment.

4.4.4 Conclusions

This experiment tested for the first time the effect of rock substratum on colonisation of microbial communities in relation to seasonal patterns. The use of rock substrata proved to be an efficient method for studying colonisation of microbial communities and testing the effect of specific factors, but care must be taken when results are compared with studies using different methodologies.

This study established for the first time clear evidence for the influence of rock substratum on the biofilm: as predicted the biofilm biomass was greater on chalk than dolomite . Interestingly, those differences were only evident in the winter which was contrary to the prediction made at the design stage. This counterintuitive result implies that microbial colonisation was suppressed by stresses in summer to such an extent that differences could not be expressed. Grazing pressure showed no relationship with microbial biomass in summer, but during winter limpet densities were positively correlated with grazing pressure, suggesting that these grazers are attracted to higher microalgal biomass.

Chapter 5

The effect of limpet grazing and rock substratum on microbial colonisation

5.1 INTRODUCTION

Gastropod grazers are an important component of rocky shore communities world-wide, as they are able to control the abundance of macroalgae (reviewed by Southward, 1964; Lubchenco and Gaines, 1981; Gaines and Lubchenco, 1982; Hawkins and Hartnoll, 1983). On British and European rocky shores, the main grazers consist of littorinids, trochids and limpets. Small littorinid species dominate the littoral fringe, as they are well adapted to the high desiccation and thermal stresses typical of this zone (Norton *et al.*, 1990). Limpets, trochids and *Littorina littorea* are mainly found in the eulittoral zone, with limpets being the dominant grazer (Hawkins *et al.*, 1992).

Herbivorous gastropods have evolved different feeding systems which lead to distinctive diets (Steneck and Watling, 1982; Hawkins and Hartnoll, 1983; Hawkins *et al.*, 1989; Fretter and Graham, 1994). Some species of littorinids are able to feed directly on macroalgae, although their radula system can also scrape and rasp microalgae from the substratum. The feeding system in trochids consists of many finer, softer teeth which are used to sweep filamentous algae, epilithic or epiphytic microalgae. Limpet radulae are comprised of few short and extremely hard teeth instead. This feeding system can effectively scrape and excavate the rock substratum. Limpets can therefore have a much wider diet, including macrophytes, epilithic, epiphytic biofilms and possibly endolithic algae, but biofilms are probably the main food resource for limpets, including *Patella vulgata* and *P. depressa* found on the shores of south west of England (Hawkins *et al.*, 1989; Hill and Hawkins, 1991). They can consume up to two thirds of the overall microalgal production (Wright and Hartnoll, 1981). The effects of other microphagous grazers, in particular small littorinids, on microbial films are much less, except in haloes around rock crevices high on the shore (see Hawkins and Hartnoll, 1983).

Removal of algal spores and germlings settling in biofilms by limpet grazing, has dramatic effects on the establishment of macroalgal assemblages (Southward, 1964; Underwood, 1980; Underwood and Jernakoff, 1981; Hawkins and Hartnoll, 1983; Sousa and Connell, 1992; Williams, 1993; Boaventura *et al.*, 2002b). Limpets are able

to significantly reduce the abundance of macroalgae, and at mid-shore in Europe, they can prevent completely the development of fucoids (Southward, 1964; Southward and Southward, 1978; Hawkins, 1981). Similarly, limpets have been shown to control the abundance and distribution of epilithic microalgae (Nicotri, 1977; Underwood, 1984c; Hill, 1990; Hill and Hawkins, 1991; Thompson *et al.*, 2000). In the presence of grazers microbial colonisation is kept permanently in the early stages of succession preventing colonisation by macroalgae. Exclusion of limpets from experimental areas can cause an increase in the microalgal standing stock in the North East Atlantic (Hill, 1990). Whilst limpets have been thought of as unselective feeders, there is some evidence of selectivity (Nicotri, 1977; Hill and Hawkins, 1991) with likely consequences for the diversity and small scale spatial heterogeneity of microbial films (Hill and Hawkins, 1991).

The impact of grazing on intertidal biofilms varies depending on the density and feeding activity of limpets which in turn vary with seasons (Jenkins and Hartnoll, 2001; Jenkins *et al.*, 2001). Physical and biological factors are also known to influence the abundance and activity of grazers and can mediate the effects of grazing on microalgae. These factors include wave exposure (Thompson, 1996; Jenkins and Hartnoll, 2001), desiccation and thermal stresses (Cubit, 1984; Underwood, 1984c), intra- and inter-specific competition (Underwood, 1984a; Underwood, 1984c; Boaventura *et al.*, 2002a) and predation (Menge *et al.*, 1999; Coleman, 1999).

The underlying rock substratum has not been investigated as an additional factor that might also interact with grazing on microbial films. For example, surface roughness of substrata might reduce the amount of biofilms accessible to the limpet radula. Conversely, limpets are able to penetrate and excavate very soft rocks such as chalk and limestone, scraping the filmed surface back to virgin rock. On these carbonate rocks, limpets will be able to graze on both epilithic and endolithic microrganisms. Moreover, as limpet grazing seems to be influenced by the abundance and composition of microalgae available (Nicotri, 1977), this could be more intense on rocks which facilitate the colonisation and development of microbial communities. In the previous chapter I suggested this as a possible explanation, of the patterns observed in microbial communities colonising chalk and dolomite tiles.

The overall aim of this chapter was therefore to investigate the relative importance of limpet grazing and rock type on abundance (measured by chlorophyll-a) and composition of microbial films. The main effects of these two factors and their putative interactions on epilithic biofilms were tested in a factorial experiment set-up on a mid shore in Wembury Bay (Devon). Limpet exclusion plots and experimental rock tiles of different composition (chalk and dolomite) were used to assess the impact of grazing and rock types on the abundance and composition of microbial film. It was predicted that the exclusion of limpets from the experimental plots would cause an increase in microalgal abundance, measured as chlorophyll-a. The effect of rock type on microalgal abundance was also assessed. The formal hypothesis tested was that microbial communities are more abundant on soft porous chalk than on hard, less permeable dolomite. Third, it was predicted that there would be a significant interaction between the effect of grazing and rock type on microalgal abundance. The hypothesis tested was that differences in abundance of microalgae between these two rock types are reduced by limpet grazing, as this is thought to be stronger on chalk than on dolomite. Therefore the magnitude of the effects of limpet grazing on biofilms depends on the type of rock substratum and *vice versa*. Finally, the potential influence of limpet grazing and rock type on the composition and diversity of the microbial community was investigated. Two hypotheses about the composition of films were tested: firstly, limpet grazing causes significant changes in the relative abundance of the species present; second, the effect of limpet grazing varies between chalk and dolomite substrata.

Microalgal abundance and composition were assessed in the experimental plots on chalk and dolomite rock tiles in presence/absence of limpets after approximately two months of exposure on the shore, to allow complete formation of a mature community based on observations in chapter 4.

5.2 MATERIAL AND METHODS

5.2.1 Study sites

The experiment was set up on the mid shore at Wembury Bay. This site was chosen to provide information to help explain the patterns observed in the previous experiment described in Chapter 4. The shore consists of rock ledges gently sloping towards the sea and south facing. The rock type is very finely grained old red sandstone, structured in multiple layers. At mid-shore, where the experiment was located, the macrobiota were relatively homogeneous, consisting of sheets of barnacles interspersed with limpets, (*Patella vulgata* and *P. depressa*). In the areas selected for the experiment, density of limpets was relatively similar whilst barnacle cover (mainly *Chthamalus* spp.) varied slightly (Table 5.1). Trochids (*Gibbula umbilicalis*, *Osilinus lineatus*) were also present, but at very low densities and generally restricted to areas near to pools and higher on the shore.

Table 5.1 – Mean densities of limpets (n = 10) and barnacle percentage cover (n = 10) in the experimental areas at the beginning of the experiment. Standard errors are shown in brackets.

	Exclusion plots		Open plots		Control plots	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Limpets (N/m^2)	48.0 (3.4)	49.2 (4.6)	61.2 (5.3)	54.0 (4.3)	56.0 (6.2)	56.0 (4.2)
Barnacles (%)	25.5 (4.5)	41 (4.4)	25.0 (4.3)	38.0 (4.2)	37.0 (2.5)	16.5 (3.2)

5.2.2 Experimental layout and laboratory analysis

Six plots, each approximately one metre square, were randomly selected on the shore. Care was taken to exclude areas with pools and crevices which could affect the microhabitat. Fences were constructed to exclude limpets from some experimental areas. This method has been proven effective in numerous previous studies (for example: Hill, 1990; Boaventura *et al.*, 2002b; Roberts, 2002; Underwood, 1984a) and does not require frequent maintenance nor cause excessive disturbance to microbial communities as might be caused by the use of chemical barriers. Three different treatments were randomly assigned to these areas, which were replicated twice:

limpets excluded (fenced plots), limpets present (open plots), and experimental procedural control (semi-fenced plots). In fenced plots plastic covered wired mesh approximately 10 cm high was fixed to the rock substratum with 6mm screws. In control plots the same type of fences were installed but these were open along one side. Machine cut rock tiles of chalk and dolomite (8x8x2.5 cm) were fixed on each experimental area with 6mm screws and a rubber washers (for detailed description of the preparation of tiles see Chapter 4). Six replicate tiles for each type of rock were randomly placed in each treatment area.

Tiles were fixed at the end of January 2001 and harvested at the beginning of April, after approximately 2 months of exposure on the shore. After harvesting each rock tile was broken to obtain a sample of approximately 10 cm^2 for the determination of chlorophyll-a, used as an index of microalgal biomass, and a smaller rock chip (approx. 1cm^2) for scanning electron microscopy, to analyse microbial composition. Detailed description of methods used for collection and preservation of samples and protocols for laboratory analysis can be found in Chapter 2.

At the beginning and the end of the experiment nine rock chips for chlorophyll-a were collected from the natural background rock in each plot, to compare the effect of limpet exclusion on natural filmed surfaces and on chalk and dolomite tiles. Rock chips were also collected for SEM but have not yet been analysed. Densities of limpets were estimated in each experimental area using 10 replicate quadrats (50x50cm).

5.2.3 Experimental design and statistical analysis

A mixed design was used for this experiment. Three factors were involved in the statistical analysis: 1) experimental treatment, fixed and orthogonal, with 3 levels (+limpets, -limpets, control); 2) plot , random and nested in treatment, with 2 levels; 3) rock type, fixed and orthogonal, with 2 levels (chalk and dolomite).

Microalgal standing stock, abundance of diatoms and cover of cyanobacteria were separately compared between treatments using ANOVA (G-Mav software programme). Data were checked for heterogeneity of variances using Cochran's test and where necessary transformed. A similar approach was used to compare microalgal biomass on the natural background rock in the three different treatments at the start

and end of the experiment. Biofilm composition was also compared using a multivariate approach, using MDS, ANOSIM and SIMPER procedures (PRIMER software programme).

Pearson's correlation index was used to correlate microalgal standing stock with the abundance of diatoms and cyanobacteria on chalk and dolomite tiles. On the natural "background" rock the amount of chlorophyll-a was also correlated with densities of limpets in the experimental plots at the beginning of the experiment.

5.3 RESULTS

5.3.1 Microalgal biomass

The use of fences did not affect the outcome of the results in this experiment, as no significant differences were detected between open and control treatments (Figure 5.1, SNK test, $p>0.05$). However, there was a considerable difference between the microbial communities on the rock tiles and those on the natural rock surface of old red sandstone. At the end of the experiment, the background level of microalgal biomass was higher on the natural rocky shore in the experimental plots than on chalk and dolomite tiles, except on chalk tiles located in the limpet exclusion treatment (Figure 5.2). The level of microalgal biomass on chalk in the open unfenced and half fenced controls at the end of the experiment, was as high as on filmed old red sandstone at the beginning of the experiment. In contrast, on dolomite tiles microalgal standing stock was always lower than the other two types of rock (see discussion for methodological implications).

Microalgal standing stock was affected by grazing, as considerable differences in the amount of chlorophyll-a were observed between plots where limpets were excluded and open control plots (Figure 5.1). Chlorophyll-a was, on average, 5.86 $\mu g\ cm^{-2}$ in limpet exclusion plots, and 1.61 and 1.59 $\mu g\ cm^{-2}$ in open, unfenced control and half fenced control plots respectively. The effect of limpet exclusion from plots also varied between the chalk and dolomite tiles. The exclusion of grazers in the fenced plots caused a great increase in chlorophyll-a on chalk, with values up to four times greater

than in the open and control plots (Figure 5.3). On dolomite, microalgal biomass was still higher in the fenced plots than the other treatments, but the difference was much reduced. The analysis of variance detected an overall significant effect of limpet exclusion on microalgal standing stock (Figure 5.1, Table 5.2). Microalgal biomass was significanty higher in the fenced plots than in the open and control plots, whilst but did not differ between the latter two treatments (SNK test, Figure 5.1).

The effect of rock type on microalgal biomass was less evident. Microalgal biomass on chalk was higher than on dolomite in all the six experimental plots, although the difference between the two substrata was greater in the fenced plots (Figure 5.3). In the limpet exclusion treatment, the amount of chlorophyll-a estimated on chalk tiles was at least twice than at on dolomite substrata, although great variation was observed between the two plots with chalk substrata (Figure 5.3). This difference was likely to be caused by the intrusion, towards the end of the experiment, of one limpet in plot 1, where some of the tiles were heavily grazed (Figure 5.4). The pattern observed was reflected in the analysis of variance, which showed, overall, a nearly significant effect of rock type on microalgal biomass (Table 5.2). Moreover, a significant interaction between the factor rock type and plot was also detected by ANOVA, suggesting that there probably were differences between chalk and dolomite.

Figure 5.4 – Grazing on microbial film (visible on the tile as brownish colour) on a chalk tile in the fenced plot 1, after 5 weeks of exposure on the mid-shore at Wembury Bay.

There was not a significant interaction between the factor rock type and treatment, showing that the effects of grazing and rock type on microalgal standing stock were independent (Figure 5.5, Table 5.2). The presence of grazers significantly reduced microalgal biomass but did not affect the differences observed in microalgal biomass between chalk and dolomite. If plots were considered separately, however, an interaction between rock type and treatment was evident (Table 5.2). The SNK test for differences in microalgal standing stock between the two substrata in each plot showed significantly higher biomass on chalk than on dolomite in both fenced plots. No differences were apparent in the open plots; whilst in the control areas microalgal biomass on the two substrata was significantly different only in plot 1 (Table 5.3). In the exclusions, differences were more marked in the plot 2 ($p<0.01$) than in plot 1 ($p>0.05$), where the unexpected grazing by an intruding limpet occurred.

The impact of grazing was also apparent on the filmed old red sandstone. The amount of chlorophyll-a estimated at the beginning of the experiment on the natural filmed rock (old red sandstone), in each experimental plot, was similar in all the treatments (Figure 5.6a). At the end of the experiment (after two months), microalgal standing stock increased in all the three treatments, but to a greater extent in the exclusion plots (Figure 5.6b). Analysis of variance confirmed that there were no significant differences in the microalgal biomass between the treatments at the start of the experiment (Table 5.4). These differences were, however, still not significant at the end of the experiment, although the probability value (0.06) was very close to the significance level (Table 5.4). The low number of degrees of freedom used to test the factor treatment could have explained the weakness of the analysis in detecting differences between treatments. In fact, if the factor "plot" nested in treatment ($P = 0.1428$) is pooled, "treatment" can be tested over the residual, with a much higher number of degrees of freedom. Then differences between treatments become highly significant ($p<0.001$). This procedure, however, should be applied with caution, as the significance of the factor plot was under the level of $P = 0.25$, recommended to avoiding the risk of Type II error (Winer, 1991).

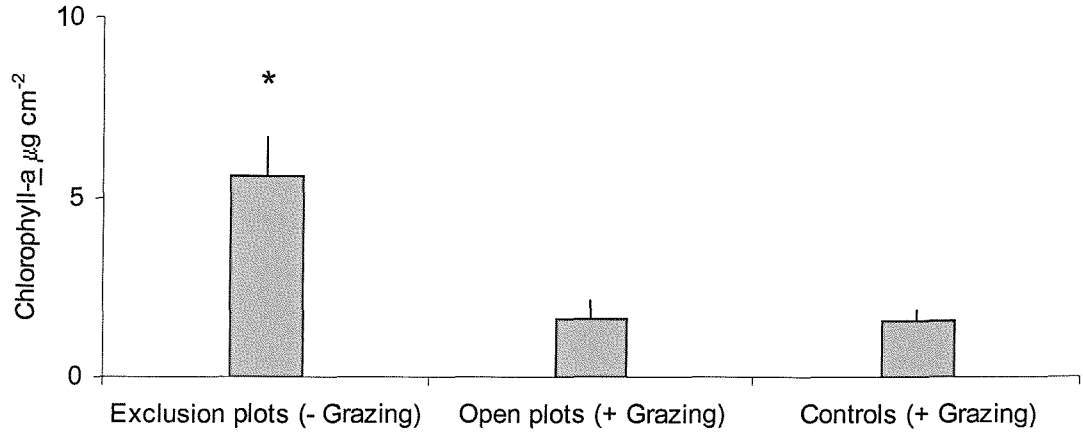


Figure 5.1 - Microalgal standing stock, expressed as chlorophyll-a, in fenced plots (– grazing), open areas (+ grazing) and procedural controls (+ grazing). Values were averaged across plots and rock types. Multiple comparison SNK test showed significantly higher microalgal standing stock in fenced plots than in open and control areas. Bars indicate standard errors.

Figure 5.2 – Microalgal standing stock on the background rock (old red sandstone) at the beginning of the experiment, before treatments were assigned to the plots, and at the end of the experiment, after 2 months. Values are compared with the microalgal standing stock estimated on chalk and dolomite tiles at the end of the experiment, after 2 months of exposure. Values were averaged across the two plots in each treatment. Bars indicate standard errors.

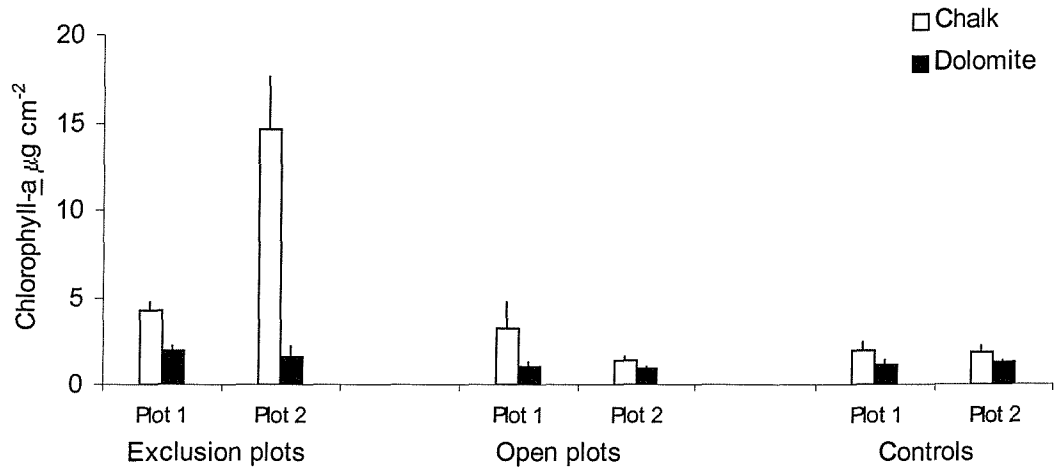


Figure 5.3 - Microalgal standing stock, expressed as chlorophyll-a, on chalk and dolomite tiles in fenced plots (limpets absent), open plots (limpets present) and semi-fenced plots (control areas). Bars indicate standard errors.

Table 5.2 - Analysis of variance for difference in microalgal standing stock between chalk and dolomite rock in exclusion, open and controls plots. Data were Ln X transformed.

Source	df	MS	F	F versus	P
Treatment	2	5.63	23.91	Plot (Tr)	**<0.02**
Plot (Tr)	3	0.24	0.70	RES	0.55
Rock	1	12.02	5.57	Ro X Pl (Tr)	**0.09**
Tr X Ro	2	2.96	1.37	Ro X Pl (Tr)	0.38
Ro X Pl (Tr)	3	2.16	6.45	RES	**<0.001**
RES	60	0.33			
TOT	71				

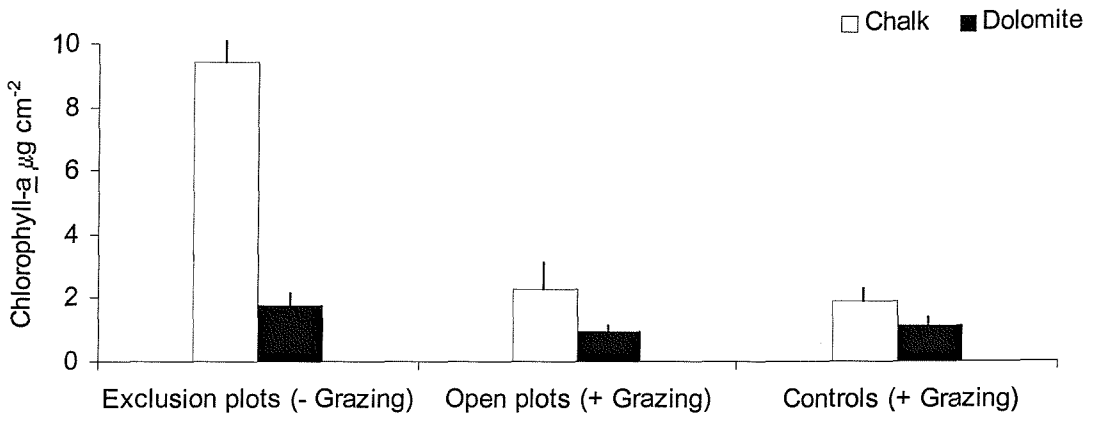


Figure 5.5 – Effects of rock type and grazing on microalgal standing stock, expressed as chlorophyll-a. The graph shows a marked increase in microalgal standing stock on chalk in the exclusion plots (– grazing) than in the open (+grazing) and control areas. Bars indicate standard errors.

Table 5.3 – Results from SNK tests for differences in microalgal standing stock between chalk and dolomite in each plot within treatment.

	Fenced plot	p	Open plots	p	Controls	p
Plot 1	Chalk>Dolomite	<0.05	Chalk>Dolomite	<0.05	Chalk=Dolomite	n.s.
Plot 2	Chalk>Dolomite	<0.01	Chalk=Dolomite	n.s.	Chalk=Dolomite	n.s.

Table 5.4 –Analyses of variance for differences in microalgal standing stock on natural filmed rock (old red sandstone) between in exclusion, open and control plots, at the beginning and end of the experiment.

Source	**df**	**MS**	**F**	**F versus**	**P**
Start of the experiment					
Treatment	2	2.18	3.24	Pl (Tr)	0.18
Plot (Tr)	3	0.67	0.84	RES	0.48
Res	48	0.81			
TOT	53				
End of the experiment					
Treatment	2	33.56	7.65	Pl (Tr)	0.07
Plot (Tr)	3	4.39	1.90	RES	0.14
Res	48	2.31			
TOT	53				

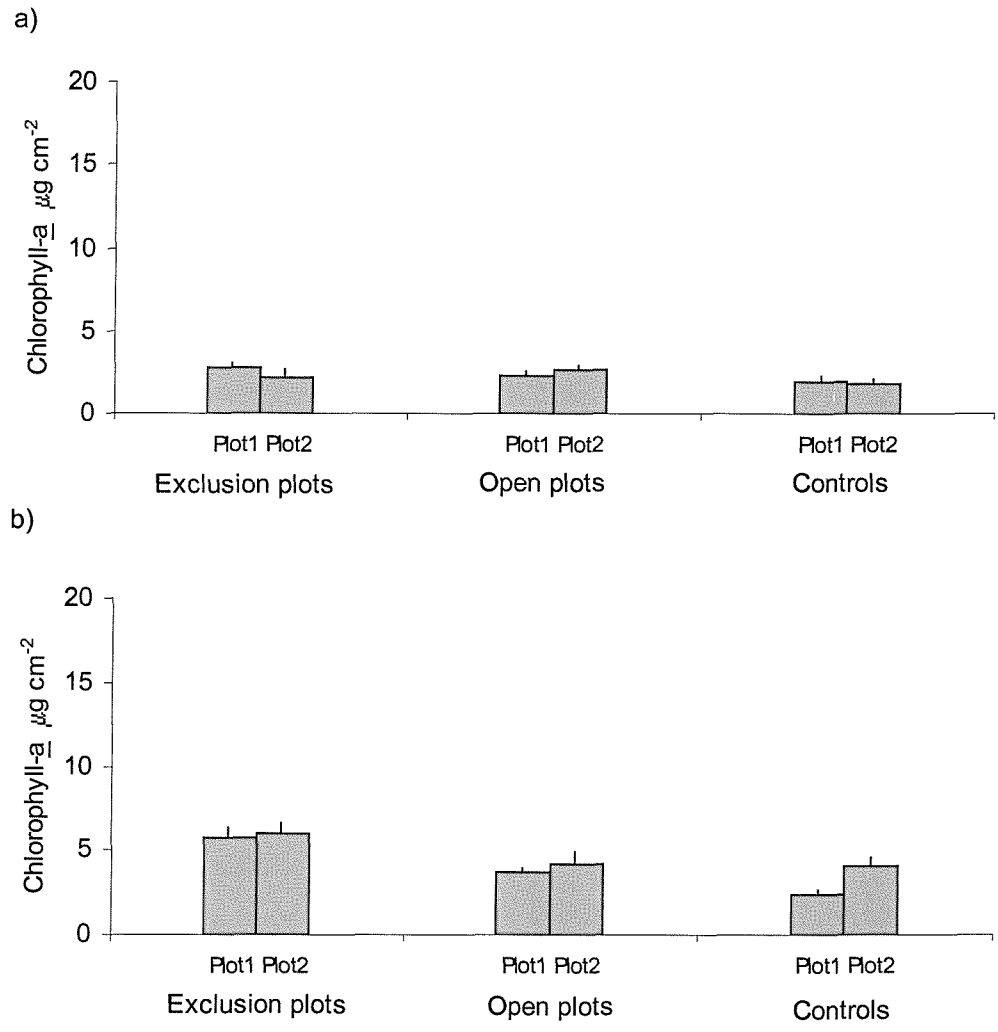


Figure 5.6 – Microlagal standing stock of the natural rock in the experimental plots at the start (a) and the end of the experiment (b), after removal of limpets. Bars indicate standard errors.

5.3.2 Microbial composition

Univariate analysis of abundance of diatoms and cyanobacteria between the different treatments did not show strong patterns. The abundance of diatoms was on average, higher in exclusion plots than in the open and control areas (Figure 5.7). These differences, however, were obscured by a high level of variability between plots (Table 5.5). The highest variability was observed among plots in the exclusion treatment. The abundance of diatoms on chalk and dolomite substrata did not follow the same pattern observed for microalgal standing stock. Diatoms were considerably more abundant on chalk in plot 2 of the exclusion and open treatments whilst in all the other plots variation between rock type was minimal. Finally, no apparent interaction between the factor rock type and treatment was found, showing that grazing did not influence the abundance of diatoms on the two types of rocks (Table 5.5).

The abundance of cyanobacteria also did not show a clear pattern (Figure 5.8); they were very scarce or even absent in the experimental plots, independently of the treatment or rock type considered. Cover of cyanobacteria varied between 0.3% on dolomite in the control plot 2 and 1.7% on chalk in the exclusion plot 1. Cyanobacteria were in general slightly more abundant on chalk than dolomite rock, although the amount of variation within and between plots was much higher than between the two rock types. This was confirmed by the analysis of variance which showed, as for the diatom component, a considerable variability between the experimental plots and in particular between exclusion areas (Table 5.6).

Microalgal standing stock, expressed as chlorophyll-a, was correlated with the abundance of diatoms, although a significant positive relationship was shown only within the exclusion treatment (Table 5.7). In contrast, no significant correlation was observed between microalgal standing stock and the abundance of cyanobacteria in any of the treatments or rock types considered, although the correlation coefficient always indicated a low, negative relationship.

Multivariate analysis of the composition of epilithic biofilms across treatments showed slight differences (Figure 5.9). The components considered in the analysis were diatoms, cyanobacteria, ephemerals, coralline algae and algal germlings. The

coefficients of dissimilarity obtained from ANOSIM pair-wise comparisons of the treatments were very low (Table 5.8). A slightly higher coefficient, just under 0.3, was obtained in the test for differences in the microbial community between exclusion and control plots. The two taxa that mainly contributed to these differences were shown by the SIMPER analysis to be the abundance of diatoms followed by coralline algae. However, although significant, the actual level of this difference was almost negligible. The composition of microbial communities on rock types did not differ significantly (Figure 5.10, Table 5.8). The separate analysis of each rock type showed that microbial communities on dolomite varied across treatments slightly more than on chalk, particularly between the exclusion and control plots, as indicated by a higher dissimilarity coefficient (Table 5.8). The relative proportion of diatom taxa in the communities between the different treatments appeared also to vary more on dolomite than on chalk tiles (Figure 5.11a, b; Table 5.9). In the exclusion plots, in absence of grazing, *Achnanthes* was the dominant taxa on both substrata. On chalk tiles, the relative composition of the diatom assemblage varied between the exclusion and the other two treaments, but these differences were not consistent (Figure 5.11a). *Achnanthes* was still abundant in the open plots, but it almost disappeared in the control areas, where *Cocconeis* was dominant. On dolomite substrata, in both unfenced and half fenced plots, the proportion changed markedly from the exclusion plots (Figure 5.11b). The abundance of *Achnanthes* was considerably reduced, whilst the abundance of *Fragilaria* and *Cocconeis* taxa and other minor species increased, suggesting a positive effect of grazing on diversity of the diatom assemblages.

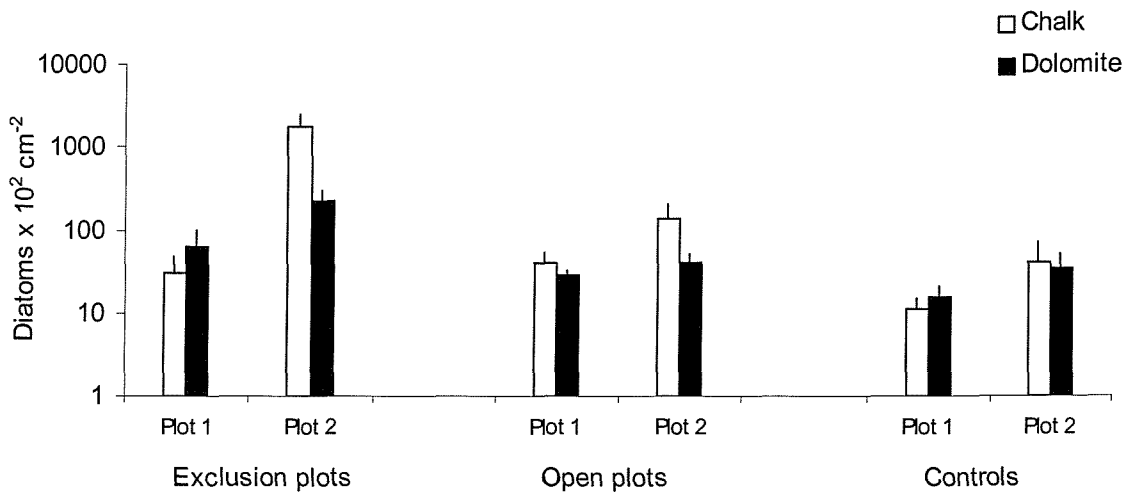


Figure 5.7 – Colonisation by diatoms on chalk and dolomite tiles in exclusion, open and control plots after 2 months of exposure. Abundance of diatoms is represented on a logarithmic scale as density per cm^{-2}. Bars indicate standard errors.

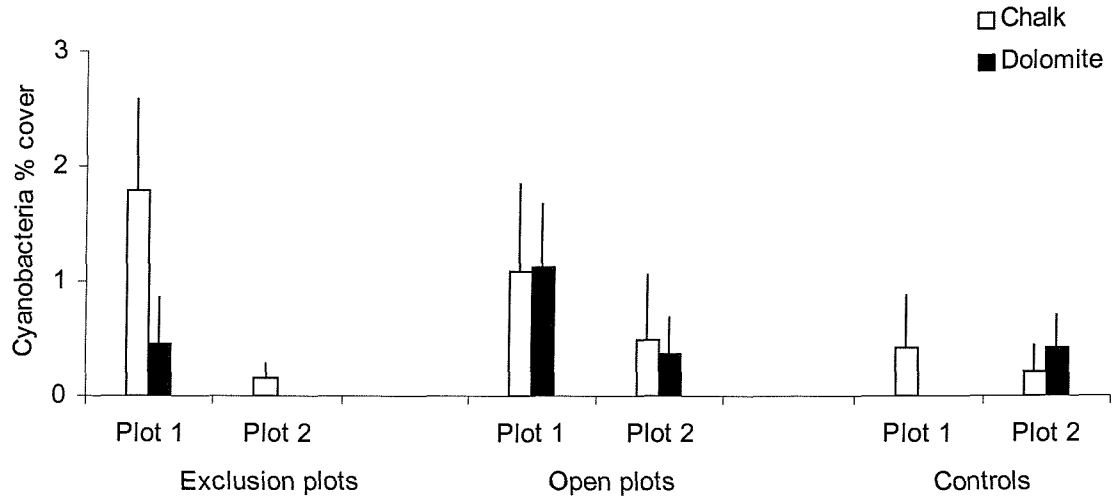


Figure 5.8 - Colonisation by cyanobacteria on chalk and dolomite tiles in exclusion, open and control plots after 2 months of exposure. Abundance of cyanobacteria is expressed as percentage cover. Bars indicate standard errors.

Table5.5 - Analysis of variance for differences in the abundance of diatoms between chalk and dolomite rock in exclusion, open and controls plots. Data were Ln (X+0.1) transformed to remove heterogeneity of variances.

Source	df	MS	F	F versus	P
Treatment	2	20.18	1.09	Plot (Tr)	0.44
Plot (Tr)	3	18.54	12.38	RES	**<0.001**
Rock	1	0.17	0.05	Ro X Pl (Tr)	0.83
Tr X Ro	2	1.83	0.59	Ro X Pl (Tr)	0.61
Ro X Pl (Tr)	3	3.10	2.07	RES	0.11
RES	60	1.50			
TOT	71				

Table 5.6 - Analysis of variance for differences in the abundance of cyanobacteria between chalk and dolomite rock in exclusion, open and controls plots.

Source	df	MS	F	F versus	P
Treatment	2	1.63	0.53	Plot (Tr)	0.64
Plot (Tr)	3	3.08	3.08	RES	**0.03**
Rock	1	1.61	1.8	Ro X Pl (Tr)	0.27
Tr X Ro	2	0.92	1.04	Ro X Pl (Tr)	0.45
Ro X Pl (Tr)	3	0.89	0.89	RES	0.45
RES	60	1.00			
TOT	71				

Table 5.7 – Correlation coefficients (Pearson's) for chlorophyll-a, diatoms and cyanobacteria on chalk and dolomite tiles in the different treatment plots. Significant correlations are marked in bold. For each rock type, n= 12 for correlation within treatments and n=36 for correlation involving all treatments.

	Chalk				**Dolomite**			
	Exclusion	Open	Control	All	Exclusion	Open	Control	All
Chlorophyll-a *vs.*:								
Diatoms	**0.54****	-0.150	-0.078	**0.58****	-0.387	-0.126	-0.268	-0.128
Cyanobacteria	-0.374	-0.112	-0.078	-0.091	-0.088	-0.228	-0.142	-0.128

Table 5.8 – Results from ANOSIM procedure for differences in community composition between treatments and rock types. SIMPER analysis for taxa contribution to differences between exclusion and control plots is also shown. Significant values are highlighted in bold.

Differences between treatments: (averaged across rock types)			Difference between rock types: (averaged across treatments)
Global R = 0.168 p<0.001			**Global R = 0.069 p>0.05**
	R	**p**	
Exclusion vs. open plots	**0.13**	**0.013**	
			no differences
Exclusion vs. control plots	**0.27**	**0.001**	
Open vs. control plots	**0.11**	**0.029**	

SIMPER analysis for species contribution to differences between treatments

	Exclusion plots Average abundance	Control plots Average abundance	%Contribution	% Cumulative
Diatoms	12.48	0.57	32.87	32.87
Corallines	3.66	1.87	28.31	61.18

Differences between between treatments within rock types:

	Global R = 0.125 p<0.05 Chalk		**Global R = 0.211 p<0.01** Dolomite	
	R	**p**	**R**	**p**
Exclusion vs. open plots	0.08	0.113	**0.191**	**0.016**
Exclusion vs. control plots	**0.21**	**0.009**	**0.316**	**0.003**
Open vs. control plots	0.09	0.07	**0.124**	**0.033**

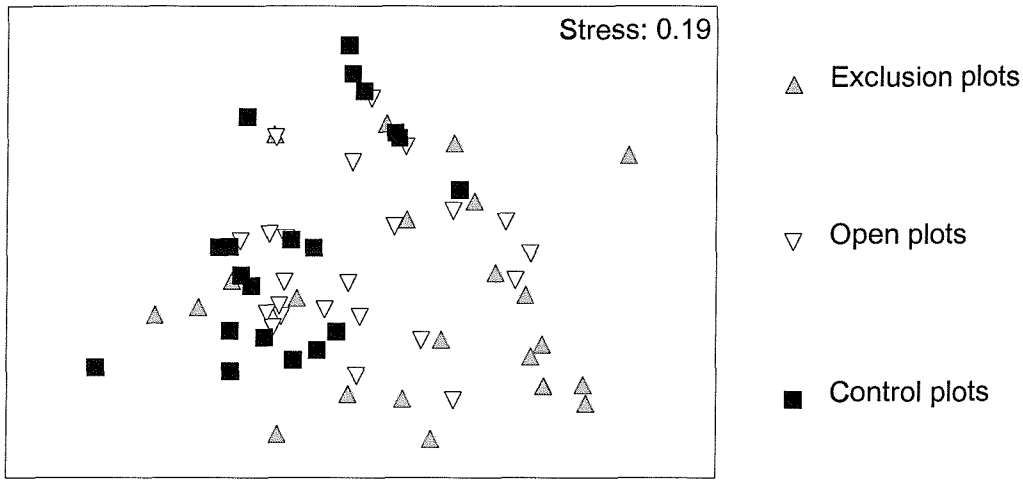


Figure 5.9 – MDS plot representing microbial communities in the different treatments across chalk and dolomite tiles.

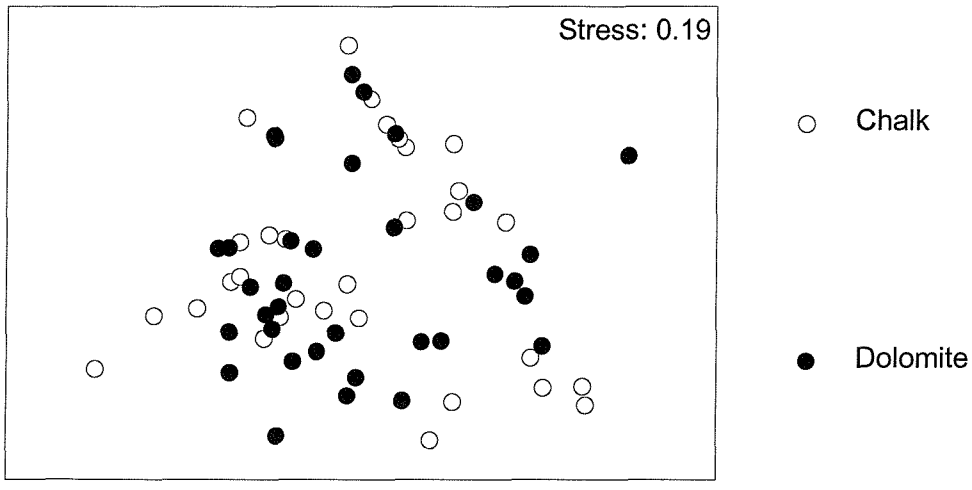


Figure 5.10 – MDS plot representing microbial communities on chalk and dolomite substrata across the treatments.

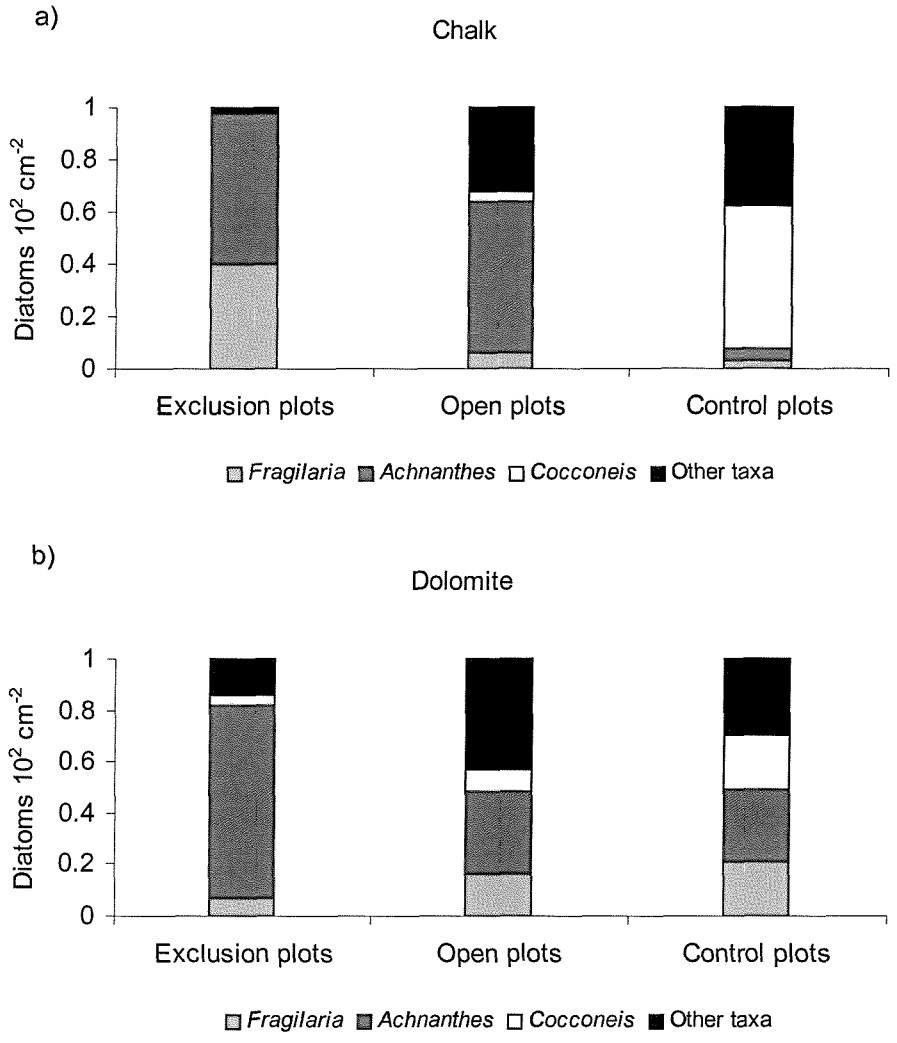


Figure 5.11 - Variation between treatments in the relative proportion of main diatom taxa on chalk (a) and dolomite tiles (b).

Table 5.9 – Relative abundance of diatom taxa, expressed as individual counts, on chalk and dolomite substrata in the different treatments.

	Exclusions			
	Fragilaria spp.	*Achnantes* spp.	*Cocconeis* spp.	Other taxa
Chalk	46.1	66.4	0.3	2.5
Dolomite	1.3	13.6	0.6	2.6
	Open controls			
	Fragilaria spp.	*Achnantes* spp.	*Cocconeis* spp.	Other taxa
Chalk	0.7	6.5	0.4	3.6
Dolomite	0.7	1.4	0.4	1.9
	Half fenced control			
	Fragilaria spp.	*Achnantes* spp.	*Cocconeis* spp.	Other taxa
Chalk	0.1	0.1	1.7	1.1
Dolomite	0.8	1.1	0.8	1.2

5.4 DISCUSSION

5.4.1 Limitations of the study

The fences did not completely prevent grazing by limpets, as one of the exclusion plots was invaded towards the end of the experiment. This was probably caused by a gap between the fence and the rock surface created by stormy weather. After that time some detritus, consisting mainly of algal debris and gravel washed off from the near beach, was also trapped at the base of some fences, potentially increasing moisture and abrasion on the rock tiles within the plots. However, these problems did not persist for long, as plots were checked and maintained every 10 days, therefore any possible effect was reduced to the minimum. This was confirmed by results from the formal comparison between unfenced open plots and half fenced plots, the latter used as procedural control for the use of fences. Open and control plots did not differ, showing therefore no significant effect of fences on biofilm communities. The use of fences proved to be effective, although a higher number of replicate plots for each treatment would have been more appropriate to counteract the odd incident like limpet intrusion. Also, a higher number of replicate tiles would have probably overcome problems with intrinsic spatial variability of biofilms and increase the power of statistical test to detect differences among factors.

This experiment was carried out only on one shore therefore the patterns observed can not be generalised to a larger scale, as spatial variability is known to be a strong factor influencing the ecology of biofilm communities (see chapter 2, 3 and 4 for discussion). Also, colonisation by microbial films was investigated only during one period, from late winter (end of January 2001) to early spring (beginning of April 2001), although this is the time of maximum abundance (Underwood, 1984b; Hill and Hawkins, 1991; Thompson, 1996; Jenkins *et al.*, 2001). In terms of composition, biofilm colonisation and succession of species follows different pathways depending on the seasonal patterns of diatoms and cyanobacteria (Hill, 1990), and recruitment of macroalgae (Benedetti-Cecchi and Cinelli, 1993). In the present experiment, the impact of grazing and rock type on biofilms, might have been different in another season. Nevertheless, the factors investigated here appear to be relatively independent from temporal

patterns. Several studies on the mid and upper shores showed that the impact of grazing decreased microalgal standing stock throughout the year and the effect was independent of the season, although the magnitude of this effect could vary with time (Cubit, 1984; Underwood, 1984b; Hill, 1990). Apart from the results presented in Chapter 4 there is no information available for the seasonal effect of rock type on biofilms. Chapter 4 provided evidence of an effect of rock type on biofilms during winter, when the current experiment was carried out.

Despite limited scope for generalisation in space and time, this study provides for the first time an insight into the potential interaction between biotic (grazing) and abiotic (rock type, small scale spatial variability) factors influencing the abundance and composition of microbial communities.

5.4.2 Comparison between primary and secondary colonisation

A formal comparison between primary succession (virgin, unfilmed rock) and secondary succession (filmed rock, maintained free of macroalgae by grazing) was not the purpose of this experiment, however, some observations could be made. These refer only to microalgal standing stock, as no SEM data on microbial composition were available for the natural old red sandstone. In the presence of grazing, microalgal biomass on old red sandstone rock was higher than on chalk and dolomite tiles, after exposure on the shore for two months. This effect was also evident between dolomite and natural old red sandstone in the exclusion plots. It appears therefore that, despite the long period left for colonisation and the favourable conditions of winter-spring, microalgal abundance was still far from reaching a level similar to natural microbial communities. Several authors indicated a period of three to four weeks for a complete recovery of intertidal epilithic biofilms (Castenholz, 1963; Underwood, 1984c; Edyvean *et al.*, 1985; Taylor *et al.*, 1997). The results of this chapter back up those found in the previous chapter (see discussion).

These observations highlight the importance of carefully choosing the appropriate methodology to study primary and secondary succession and the problem in comparing results when different techniques are used. Future experiments expressively aimed to test differences between primary and secondary succession are therefore needed.

5.4.3 The effect of limpet grazing

Grazing by limpets clearly affected epilithic microbial communities: it reduced the abundance of established microbial communities on old red sandstone and significantly affected microbial colonisation on chalk and dolomite substrata.

On old red sandstone, microalgal standing stock was greater in the fenced exclusion plots than in the control open and half fenced areas which had similar standing stock. The effect of grazing on established biofilm communities has been investigated in both tropical (Mak and Williams, 1999) and temperate rocky shores (Cubit, 1984; Hill, 1990). There, removal or exclusion of limpets from areas of filmed rock, significantly increased microalgal standing stock and allowed succession of species which eventually led to a macroalgal growth (Hill, 1990; Roberts, 2002). In Britain, detailed work by Hill (1990) showed a similar pattern in winter-spring, with a significant increase in the microalgal density followed by colonisation by filamentous algae and fucoids in plots unscraped from biofilm where limpets were excluded. In the grazed control plots macroalgal growth was minimum or even absent. The similarity between these common results reinforces the theory that grazing acts as continuous disturbance factor on microbial films, which therefore never reach an end point but are permanently kept in the earlier stages of succession (MacLulich, 1986). This has important consequences for macroalgal assemblages, which strongly depend on settlement and subsequent development of sporelings and germlings within the biofilm. The early life stage of macroalgae such as fucoids are most susceptible to grazing by limpets, as their radula cannot feed on larger algae (Hawkins *et al.*, 1989). The abundance of sessile marine invertebrates such as barnacles can also be indirectly affected by grazing on biofilm. It has been shown that during the settlement phase to the substratum the invertebrate larvae of several species show a site-specific behaviour, which strongly depends upon biofilm cues (Bourget, 1988; Keough and Raimondi, 1995; Wieczorek and Todd, 1998).

Microalgal biomass on filmed old red sandstone increased significantly from the beginning of the experiment, in late January, to early April. This was observed in exclusions and to a lesser extent in control plots. The increase in microalgal biomass from winter to spring conforms to the seasonal pattern of microbial communities

observed in temperate systems and particularly on British rocky shores. In a detailed study in Port St. Mary (Isle of Man), Hill and Hawkins (1991) observed the highest values in microalgal standing stock and diatom abundance in February and March. On the same shore, but a few years later, Thompson (1996) recorded a steep increase in microalgal abundance from January to February-March. Jenkins *et al.* (2001) confirmed once again these patterns in the Isle of Man but also observed, during the same months, even higher values of microalgal biomass on rocky shores in Heybrook Bay and Prawle Point, the former being very close to Wembury Bay. In the present study, however, the increase between late January and early April was not so evident as in that study. This can be explained by a change, at the end of the experiment in the seasonal conditions, shifting from spring to summer, possibly causing an initial decline in the abundance of microbial communities.

Primary colonisation on chalk and dolomite tiles was negatively affected by grazing. After approximately two months of exposure, microalgal biomass on chalk and dolomite tiles in plots subject to grazing was significantly reduced compared to fenced, ungrazed plots. The effect of grazing on microbial colonisation and succession has been described on natural cleared rock surfaces and artificial substrata (Nicotri, 1977; Hill, 1990; Anderson and Underwood, 1997). Despite the differences due to the experimental procedures used, these experiments led to a common outcome, showing a marked inhibitory effect of grazing on microalgal abundance. Microalgal abundance, expressed in terms of standing stock, primary production rates and density of cells, significantly increased in the ungrazed areas. On the mid and high shore, greater differences in primary production and microalgal density between grazed and ungrazed treatments were observed during the winter and early spring (Nicotri, 1977; Hill, 1990; Anderson and Underwood, 1997). Although no seasonal comparison could be made in the present study, the amount of variation in microalgal biomass was similar; chlorophyll-a, averaged across chalk and dolomite substrata in fenced plots, was more than threefold the amount estimated in the open and partly fenced control areas.

In contrast, the effect of grazing on the composition of the microbial community was much less clear and did not reflect the patterns described for microalgal standing stock. After two months of exposure, tiles were colonised by a microbial film consisting of diatoms, cyanobacteria, filamentous algae (probably *Blidingia* and *Enteromorpha*

spp.), encrusting macroalgae (probably corallines) and algal sporelings and germlings (probably *Fucus* sp.). The relative abundance of these major taxonomic groups within the biofilms was very similar among treatments. Microbial communities differed only slightly between exclusion and procedural control plots, mainly because of the difference in abundance of diatoms and corallines. Grazing did not show obvious effects on the composition of biofilms even when the components were considered separately. The abundance of diatoms was higher in the exclusions, but did not differ significantly from the other two treatments. This is in contrast with the findings of other experiments, where a significant decline in diatom density in the ungrazed areas was observed (Nicotri, 1977; Hill, 1990). A possible explanation for this discrepancy is the high spatial variation between and within plots, which probably obscured the overall effect of grazing. Within identical treatment, the abundance of diatoms was up to three times greater in one of the two plots. The degree of patchiness was even higher within the same plot, where diatoms could vary from 3 to 293 cells per replicate chip.

Spatial variability and small scale patchiness is a factor observed frequently in ecological studies of microbial communities. Various authors have suggested that patchiness presents serious problems in quantifying the abundance of diatoms and investigating their distribution patterns (Nicotri, 1977; Hill and Hawkins, 1991). One of the main causes for microalgal patchiness has been attributed to grazing. Sommer, (1999a, b) demonstrated that spatial patterns of grazing in *Littorina* enhanced spatial heterogeneity and consequently diversity of biofilms. The radulae of limpets are able to reduce a filmed surface to bare rock, resulting in a great variation in microalgal abundance between the grazed and surrounding areas. In the present study, however, microalgal patchiness was also present in limpet exclusion plots, showing therefore that grazing had little influence on small scale spatial heterogeneity. Surprisingly, the degree of variability in the diatom component observed in this experiment was very similar to those estimated in the investigations on natural rock surfaces described in the chapters 2 and 3. These results suggest that a higher number of replicates was needed to overcome patchiness when the single components of biofilms are analysed. The influence of grazing was more evident in the relative abundance of diatom taxa within each treatment. *Achnanthes* and *Fragilaria* dominated in the exclusion plots, but in the open and half fenced plots these taxa were greatly reduced. In contrast *Cocconeis* and other minor taxa remained fairly constant or, in some cases increased.

Thus this result suggests a possible selective feeding by limpets. Various factors might explain this feeding behaviour. *Achnanthes* and *Fragilaria* are known to be dominant species in the mid-high shore zone, as both are relatively resistant to light intensity and desiccation stresses (Aleem, 1950; Castenholz, 1963). Some authors have suggested that limpet grazing has a preference for dominant taxa, which are probably more readily available (Nicotri, 1977). Hill and Hawkins (1991), however, suggested that limpets are preferentially feeding on rarer taxa. Selective feeding is likely to depend on other factors, such as "accessibility" to food resources and digestibility. The large chain morphology of genera such as *Fragilaria* and *Melosira* can facilitate ingestion by limpets (Nicotri, 1977). Also, *Fragilaria* is one of the species better digested by limpets. Such criteria for food selectivity are supported by the increase of *Cocconeis* spp in the grazed plots. These are isolated adnate diatoms firmly attached to the substratum and poorly digested by limpets. The increase of these species could also be the result of the marked decline in the abundance of the dominant taxa, reducing interspecific competition. No clear explanation, however, is available for variation in the abundance of *Achnanthes,* which consists of stalked diatoms firmly attached to the substratum. Digestibility in *Achnanthes* is also very low, making these diatoms less attractive to limpets (Nicotri, 1977). Thus, preference for abundant taxa or stochastic successional events appear to be the most likely explanations for the steep decline in the abundance of *Achnanthes*.

The abundance of cyanobacteria on chalk and dolomite tiles, was very low in all the plots considered. Percentage cover was generally not greater than 5%, independently of the presence or absence of grazing. Abundance of cyanobacteria estimated on the filmed old red sandstone in early spring (see details in Chapter 2) was also relatively low, but still twice that on chalk and dolomite. One possible explanation is that colonisation by cyanobacteria needs to be preceded by diatoms. Classical successional processes in biofilms often describe the colonisation by diatoms and cyanobacteria as a simultaneous event. It has been suggested (Underwood G.J.C., 1997) that the occurrence of cyanobacteria is independent of the successional sequence involving bacteria, diatoms and green algae. There is also no evidence for an impact of grazing on cyanobacteria (but see Tuchman, 1991).

On the basis of these observations, it appears that grazing affects species diversity rather than influencing composition of higher taxonomic of functional groups. However, the limited identification work possible in this study does not allow any clear conclusions to be reached.

5.4.4 The effect of rock type and interactions with grazing

The hypothesis that biofilm formation would be greater on chalk than on dolomite, was only partially supported. Chalk tiles had more colonisation than dolomite tiles in all treatments, but these patterns were not statistically significant. The high degree of spatial variability and patchiness in microbial communities on chalk and dolomite probably contributed to the lack of statistical significance. This is in contrast with results obtained in chapter 4, where spatial variability and patchiness appeared to be of less importance compared to other factors such as the rock substratum or seasonal variation. In the experiment in chapter 4, significant differences in microalgal abundance between the two rock types could be detected despite the observed spatial variability. The different outcome obtained from the two studies is likely to be explained by the different the size of the experiment. The size of experiment described in the present chapter was much reduced, both in terms of spatial replication, restricted to one shore, and sample size, which consisted of 6 instead of 8 replicates thereby reducing statistical power, particularly in parametric univariate analyses such as ANOVA (Underwood, 1991).

The effect of substratum on microalgal abundance was, however, more evident in the limpet exclusion treatment. In these plots, chalk had significantly higher microalgal biomass than dolomite, whilst no significant differences were found in the other treatments, except for one plot in the procedural controls. This suggests that in absence of grazing, microalgal abundance would be high on chalk and low on dolomite, showing therefore that rock type does affect epilithic biofilms. The interaction between the effect of the substratum and the impact of grazing on microbial communities can be explained in two ways. First that grazing is facilitated on chalk, as this is a much softer rock than dolomite. On the rock substratum microalgae often accumulate in microcrevices or small pits, as these can give more protection from physical stresses. These micro-niches can be easily destroyed by the strong limpet radula in the chalk, which therefore might collect more microalgae with the same stroke. In this case, more

efficient grazing would reduce microalgal abundance on chalk and dolomite to similar levels.

An alternative explanation may be given in terms of food availability and grazing preference. Several authors have shown how limpet density, growth and distribution depends on food availability (Underwood, 1984a; Underwood, 1984c; Jenkins *et al.*, 2001). Limpets also seem to actively prefer grazing on areas with a rich cover of microalgae, although this has not been demonstrated experimentally (Della Santina and Thompson, unpublished obs.). In this context, microalgal production rates and turnover could be of a certain importance. In the experiment described in Chapter 4, colonisation in the presence of grazers occurred faster on chalk than on dolomite, suggesting that microbial communities on the former substrata develop more easily. Chalk would be therefore more exploited as it represents a greater source of food than dolomite.

Previous studies have shown a significant variations in the composition of biofilms on substrata with different physical and chemical properties (Hamilton and Duthie, 1984; Snoeijs, 1991; Sabater *et al.*, 1998). The composition of microbial communities on chalk and dolomite were in fact very similar. Some differences were only observed in the proportion of common taxa in the diatom component. The abundance of *Fragilaria*, an easily ingested and digested diatom, was much higher on chalk than on dolomite in the exclusion treatment. In the control treatments, limpets grazed efficiently on these species, reducing their abundance to the same level in both rock types. This apparent species selectivity by limpets is difficult to demonstrate, as the grazing on *Fragilaria* could be just the result of its greater availability. Further investigations would be needed to validate these observations, for example through the analysis of the gut content (e.g. Hill and Hawkins, 1991) to estimate the relative proportion of rock and diatoms ingested by limpets.

Differences in the relative abundance of endolithic microorganisms might also represent an alternative explanation for the selective grazing by limpets on chalk substrata. Chalk, as other calcium carbonate-based rocks, can be readily be penetrated by microborers, which generally consist of phototrophic microroganisms (Le Campion-Alsumard, 1979; 1989). Grazing on chalk substrata would mean thus the

access to a further food resource not available on other rocks such as dolomite. The endolithic component was not considered in the present study because it requires different sampling techniques and methods of analysis (Le Campion-Alsumard, 1979; 1989; Surman *et al.*, 1996; Ascaso *et al.*, 1998), which were beyond the time and resources available. However, the previous considerations highlighted the importance of including this often neglected component in future ecological investigations of microbial communities.

5.4.5 Conclusions

This study provided evidence for the validity of the hypotheses, proposed in the previous chapters, and particularly chapter 4, on the effects of the rock substratum and grazing. Limpet grazing reduced microalgal abundance on rocky shores, but also modulated the effect of the rock substratum on microbial communities. Chalk supported in general more microalgal biomass than dolomite, suggesting that porosity and surface roughness can facilitate colonisation and development of microbial communities. This consideration, however, cannot be extrapolated further unless a wider range of rocks with contrasting physical and chemical properties is compared. Moreover, the use of machine cut rock substrata might not be representative of the ecological processes on natural rock surfaces where colonisation occurs faster than on rock tiles. Such an experimental approach is useful to investigate the effects of specific physical or biological factors and their interactions, but care must be taken in generalising from the results obtained, as in natural systems patterns observed in microbial communities are often the results of more complex interactions involving several variables.

Chapter 6

General discussion

6 GENERAL DISCUSSION

The overall aim of this work was to investigate the importance of the rock substratum in the ecology of intertidal communities. For the first time, the effects of the underlying substratum were assessed in relation to broad-scale and local variability, tidal elevation and season, colonisation and successional processes. In this final chapter, results from whole thesis are interpreted and synthesised. Three themes will be considered: the problems of studying intertidal epilithic biofilms; factors regulating microbial communities, especially the rock substratum; and patterns of variability and homogeneity of intertidal biofilms. Conclusions and suggestions for further work are then made.

6.1 Limitations of the study

6.1.1 Quantification of epilithic biofilms

In the present study epilithic biofilms were always analysed on the rock substratum, using both chlorophyll-a extraction and scanning electron microscopy. Chlorophyll-a provided an index of the microalgal biomass (Underwood, 1984b; Nagarkar and Williams, 1997; Thompson *et al.*, 1999), as intertidal biofilm consist mainly of microphototrophs (Thompson, 1996). SEM analysis was used to estimate the abundance of single components within the microbial films. This technique complemented the chlorophyll-a extraction, which does not provide any information on the relative contribution of diatoms and cyanobacteria to the total microalgal biomass (see comments in Hill and Hawkins, 1990).

Results obtained from the two techniques, however, were rarely consistent. The abundance of diatoms and cyanobacteria did not correlate with the concentration of chlorophyll-a on most of the sampling occasions. The discrepancy in quantification of microbial communities is probably due to the different scale of resolution of these two techniques. Cold extraction of chlorophyll-a is a quick and flexible technique which allows analysis of relatively large rock samples (in this study approximately 10 cm^2) and a high number of replicates. In contrast, the procedures for scanning electron

microscopy are considerably more time consuming, expensive and restrictive, because only small rock chips (approximately 1 cm^2) can be inserted into the microscope vacuum chamber. These technical constraints strongly limited the number of samples which can be processed under SEM within the time and resources available. In consequence, total estimates of chlorophyll-a were obtained from a much larger sampling area than that analysed under SEM. The estimates of chlorophyll-a better integrate the small scale spatial variability which often characterises microbial films. Conversely, spatial variability of diatoms and cyanobacteria was exaggerated; variation among replicates was very high due to the intrinsic patchiness of the system and the small size of rock chips in relation to fields of view used. The number of diatoms significantly correlated with the amount of chlorophyll-a only on a few occasions, especially where spatial variability was reduced by the use of standard rock substrata (see chapter 4 for example).

SEM does not allow observation of the biofilm across its 3-dimensional structure as with other techniques (Norton *et al.*, 1998). This was a problem particularly during winter, when biofilms were very thick and dense and only the top layer of microorganims could be visualised. Thus the numbers of diatoms and cover of cyanobacteria might have been underestimated. Also, endolithic phototrophs, which are likely to contribute considerably to the amount of chlorophyll-a in carbonate rock such as chalk and limestone, could not be estimated under SEM, as different methodologies based on thin sectioning (Ascaso *et al.*, 1998) or dissolution (Le Campion-Alsumard, 1989) must be used.

Epilithic biofilms were viewed under SEM at a relatively low magnification (between X250 and X300) to increase the sampling area and therefore to overcome spatial variability. The low level of resolution restricted the identification to major categories such as cyanobacteria and diatoms, whilst smaller microorganisms such as bacteria and fungi could not be visualised. This also limited taxonomic discrimination to a few conspicuous genera, as species identification requires higher resolution of the frustules. This limited the study of microbial colonisation, where pioneer organisms such as bacteria or single species of diatoms can have an important role in the successional sequences of microbial communities. However, seasonal variation and factors such as the rock substratum and grazing did not appear to affect biofilm communities at such

fine resolution, as very little variation was observed in the composition of the diatom assemblages investigated throughout the study (see also Hill and Hawkins, 1991).

These observations demonstrated the limitation of using SEM in assessing the abundance of microbial communities. SEM should perhaps be employed for qualitative work aimed at describing microbial composition, using a smaller sample size but at higher resolution. This would better complement chlorophyll-a extraction in the assessment of biofilms.

Estimates of chlorophyll-a per unit area were obtained from the measurement of the two dimensional projected area of the rock samples. The amount of chlorophyll-a estimated on rocks with a complex microtopography could thus be overestimated, as the actual three dimensional surface area is greater than the base area. This does not represent a major limitation in broad scale comparisons of microalgal standing stock and productivity, especially in terms of what may be available to grazers. It could, however, confound detailed comparisons of rock types (see chapter 2 and 3), as different microtopographic features could explain higher biomass. The use of standard machine cut surfaces in chapter 4 and 5 showed that higher biomass on rocks such as chalk did not depend on the higher natural surface roughness which characterise chalk shores.

6.1.2 Descriptive versus experimental approach

One of the major problems encountered in the broad scale survey of epilithic biofilms along the south coast of England was the lack of interspersion of the different types of shores investigated. Due to the natural geology of this stretch of coast, distances between each type of rock were at the scale of 10's to 100's km. Rocky shores were located from the Isle of Wight to the south west of Cornwall. Therefore such widespread geographical locations may have confounded the effect of the rock substratum on microbial communities with the influence of several environmental variables which are likely to differ from place to place. These include local factors such as wave exposure, orientation, local nutrient availability, grazing pressure and larger scale variables, including eutrophication, tidal range and microclimate. Despite choosing sites with similar physical and biological features, some differences between the shores were observed. For example density of limpets varied considerably between

differing shores. Different grazing pressures can lead to strong differences in the estimates of microalgal abundance (Cubit, 1984; Underwood, 1984a; Hill, 1990). Tidal range varied from 2 metres on the Isle of Wight to 4-5 metres towards the South West of England. It is also likely that the period of exposure of the substratum during low tide varied slightly and the timing of low water would certainly be different (midday springs in the west, evening and mornings in the east). This might have been of some importance in the summer, where longer exposure to desiccation and thermal stresses could have reduced microbial film biomass. Weather conditions are also known to vary along the south coast of England. The South West of England is generally wetter, cooler and windier than the central southern coast in summer. During winter the west is milder and much less frost-prone. These differences in local climate might have again affected intertidal biofilms.

The problem of geographical variation was partially solved by the local comparisons of microbial communities on different rock types interspersed within the same shore (chapter 3). In this study, however, lack of spatial replication did not allow the generalisation of the results, as in the comparison between shale and dolomite on one single site at Kimmeridge Bay. Also, the two rock types interspersed were not equally abundant. For example in the comparisons of chalk *versus* flint and limestone *versus* chert the harder rock type only consisted of very small inclusions surrounded by the dominant softer rock of the shore.

These natural constraints were overcome when an experimental approach was adopted (chapter 4 and 5). The use of rock substrata allowed local comparisons of different rock types within the same shore, thus under the same environmental conditions using a standard tile size. Rock tiles could also be spatially and temporally replicated to establish generality of the patterns observed. This approach greatly increased the power in detecting differences between rock types, as background variability was much reduced. However, tiles appeared not to mimic the natural environment entirely due to their machined nature and slight elevation above the rock surface. These are considered minor problems compared with the advantages of replicate units in which all variables other then rock type were controlled.

6.2 Factors regulating microbial communities

6.2.1 Physical features and biological interactions

My work showed clear evidence for a seasonal fluctuation of microbial communities operating on broad scales. Microalgal standing stock and abundance of diatoms on natural biofilms along the south coast of England were always high in the early spring and very low in summer. In contrast, cyanobacterial abundance did not show seasonal patterns, as their abundance was highly variable in both summer and early spring. The early stages of colonisation were also affected by season, being much faster in winter. During summer, microbial colonisation on virgin rock substrata was very slow and microalgal biomass never reached the levels observed during winter, even after two months. Microbial composition was also influenced by season: diatoms were almost absent during the summer, but dominated microbial assemblages during the more favourable winter season.

These seasonal patterns have been widely documented world-wide (Cubit, 1984; Underwood, 1984c; Dye and White, 1991; Hill and Hawkins, 1991; Williams, 1993; Thompson, 1996). On British shores, seasonal variation in epilithic biofilms was first described on the central south English coast at Swanage by Aleem (1950), who observed that the abundant diatom mats developed during winter disappeared from both upper and lower shore. In the Isle of Man, Hill and Hawkins (1991) showed strong seasonal patterns in total abundance and species composition during primary succession. Seasonal fluctuations in microalgal standing stock were apparent at all tidal levels and shores described by Thompson (1996) and Jenkins *et al.* (2001).

Physical stresses are the factors mostly responsible for seasonal effects on microbial communities. Intertidal epilithic biofilms consist of microphototrophs which are sensitive to variation in light, including the effects of UVA, UVB, temperature and desiccation (Williams, 1994; Thompson, 1996; Blanchard *et al.*, 1997; Blanchard and Guarini, 1998). In the summer, the more sensitive diatoms are mostly affected by high levels of insolation and thermal stresses and tend to disappear from the biofilms (Castenholz, 1963; Cubit, 1984; Hill, 1990). Cyanobacteria, which are more resistant and adapted to high temperature and light (Fogg, 1973; Birke, 1974), do not appear to

be limited by physical stresses as they persist also during the summer. In my work, cyanobacteria were more abundant in summer than winter-spring on various occasions. In the hot weather, competition for space and resources with diatoms could be much reduced, resulting in an increase of cyanobacteria. Competition between components of biofilms, however, has not been documented.

Vertical differences in physical stresses also appeared to affect biofilms on the shore, although to a much less extent and depending on season. Microbial communities did not differ significantly between the littoral fringe and the upper eulittoral zone investigated in the broad scale survey (Chapter 2) and in the local scale comparisons (Chapter 3). There was a trend, however, showing higher biomass in the upper eulittoral zone than in the littoral fringe in the spring, whilst in the summer differences were negligible. This result is in apparent contrast with several studies showing significant decreases in microalgal abundance (Underwood, 1984b; MacLulich, 1987) and species composition (Castenholz, 1963) towards the upper shore. The previous studies, however, compared microbial communities on tidal levels which were further apart than the two zones selected in my investigations. In my study these were both located on the upper shore, where the magnitude of physical stresses was at such a level that microbial communities could have been similarly affected. This probably also explains the slightly greater differences observed in the early spring, when physical stresses microbial communities were ameliorated in the upper eulittoral zone allowing proliferation of microalgae. Thompson (1996) also observed that differences between tidal levels were not consistent with seasons. High ephemeral macroalgal biomass preceded by an increase in the microbial components in a common feature of the littoral fringe of temperate shores (e.g. Hawkins, 1983).

Grazing is another factor limiting the abundance and development of intertidal biofilms (Cubit, 1984; Hill, 1990). In the experiment described in Chapter 5, the exclusion of limpets caused a significant increase in microbial films. The effect of grazing was evident on virgin substrata after 60 days of colonisation and on natural filmed surfaces in the surrounding area. Microalgal standing stock was not negatively correlated with densities of limpets in either seasons on the shore studied. It likely that on most moderately exposed rocky shores, grazing pressure is sufficient to prevent macroalgal growth and hence maintain similar low levels. Since the grazing effect is

overridden by a stronger seasonal pattern, this leads to elevated biomass in the winter and spring in the eulittoral. The grazing effect does not occur in the littoral fringe (except in the vicinity of refuges for littorinids) and seasonally varying physical features are probably paramount (Hawkins and Hartnoll, 1983; Hawkins, 1983). The influence of grazing is probably more important at small spatial scales (100 microns to metres), causing patchiness in the spatial distribution of diatoms and cyanobacteria. This was observed for limpets (Hill, 1990) and other grazers (Sommer, 1999a).

6.2.2 The importance of the rock substratum in relation to physical and biological factors.

The effect of the rock substratum was investigated for natural biofilm communities and on microbial colonisation processes in relation to physical factors such as season and tidal level using both a descriptive and an experimental approach. All investigations showed evidence of an effect of the rock type on intertidal biofilms. Significant differences in microalgal standing stock and microbial composition were observed among the 9 rock types considered in the studies.

On the basis of the patterns observed, two broad groups of rock types can be classified: carbonate rocks, characterised by high porosity, softness, and roughness, and quartz-based rocks, which are hard, little permeable to water and relatively smooth. Some rocks occupy an intermediate position, sharing features of both categories such as sandstone, which consists of both quartz and calcite grains, and has a very complex microtopography but low porosity. Dolomite, which calcium and magnesium carbonate, is not permeable to water but is a very hard rock.

Differences between rock types were not always apparent, and their magnitude varied with season and tidal level. Differences were more evident in winter-spring than in the summer. Seasonal patterns appeared to modulate the effect of the substratum on natural, mature microbial communities (Chapter 2) and on colonisation processes (Chapter 4). This suggests that during summer harsh physical stresses probably override the effects of the substratum on abundance of microbial communities. The importance of desiccation, insolation and thermal stresses in controlling microbial communities has been well documented in both tropical (Williams, 1994) and

temperate systems (Castenholz, 1963; Cubit, 1984; Thompson, 1996) and has been discussed in detail in chapters 2, 3 and 4. During summer physical stresses are the most limiting factors, leading to the disappearance of important components of the biofilms such as diatoms. Other limiting factors such as grazing become less important during summer and on the upper shore above the zone of peak abundance of limpets.

Seasonal variation also probably explains why the effects of the rock substratum varied with tidal level. The effect of rock type was more evident in the upper eulittoral than in the littoral fringe, but only during early spring. During summer there was no significant interaction between the factor rock type and tidal level, as levels of microalgal standing stock were generally very lower and more similar between the littoral and upper eulittoral zones. The microflora appeared to be similarly suppressed in both zones although in the upper eulittoral grazing could have confounded any effect of light or desiccation (Thompson, 1996).

Several properties of the rocks can have a different influence on microbial films. These include mineral composition, porosity, surface roughness, hardness and colour. Microalgal biomass estimated on rocky shores of differing geology was higher on porous, rough rocks such as chalk and limestone than more compact, smoother, less permeable rock such as granite and old red sandstone. A similar pattern was also observed in the local scale comparison between chalk and flint. These observations were experimentally tested and confirmed by comparing microbial colonisation on the soft, porous chalk and harder, smoother dolomite (see Chapter 4 and 5). Higher rates of colonisation were observed on chalk, which also showed greater microalgal abundance than dolomite in mature communities. Differences in the composition of microbial communities were also observed, but were less evident, generally limited to differences in the relative abundance of single components. For example, carbonate rocks generally supported a higher number of diatoms and low abundance of early stages of coralline algae.

Some properties such as chemical composition, microtopography and hardness have direct consequences on biofilms. For example, the chemical composition of chalk and limestone, which consist of calcium carbonate allows colonisation of endolithic cyanobacteria (Golubic *et al.*, 1975; Le Campion-Alsumard, 1989), thus increasing

microbial diversity and microalgal standing stock. Chemical composition and surface activity is only likely to be important during the very early phases of organic molecules adhesion and microbial colonisation. In particular, adhesion of bacteria to a new substratum is known to be influenced by surface tension which might vary depending on the type of substratum (Taylor *et al.*, 1997). Rock hardness determines the stability of the substratum and susceptibility to weathering effects. Epilithic organisms are more likely to be damaged or dislodged on friable rocks such as limestone and chalk than on flint or granite. Also grazing impact is likely to be higher on soft rocks, as grazers such as limpets are able to scrape and excavate soft substrata more efficiently (Hawkins *et al.*, 1989). In the exclusion experiment of chapter 5, differences between chalk and dolomite were much higher in plots where limpets were excluded. Also, chalk had significantly greater microalgal biomass in the exclusion plots than in the open areas, whilst similar values were observed for dolomite, suggesting that grazing pressure was higher on the softer rock. It might be possible however, that limpets grazed on chalk because of microalgae were more abundant.

The rock substratum appears to influence biofilms indirectly, by modulating the effect of other physical and biological variables. Porous rocks retain more water, keeping the surface damp during low tide. This is likely to reduce desiccation stresses experienced by intertidal microbial communities. Results from my work suggest that porosity can have strong positive effects on microbial communities. In the broad scale survey, differences in microalgal standing stock were proportional to the levels of porosity measured on each rock type. This was also confirmed by results obtained from the colonisation experiment (chapter 4), where the confounding effect of other physical features such as surface complexity was standardised. The effect of porosity on microbial films has not been documented elsewhere, some authors suggested that differences in the abundance and composition of biofilms between rock types were a consequence of different porosities (Blinn *et al.*, 1980; Edyvean *et al.*, 1985). Thermal stresses will be influenced by the colour of the rock substratum. For example, the surface temperatures recorded on white chalk were a few degrees lower than the dark dolomite in July. This small difference could be of major importance for diatoms, which are very sensitive to high insolation and thermal stresses occurring during summer (Blanchard *et al.*, 1997; Blanchard and Guarini, 1998).

Rocks characterised by a complex microtopography are likely to facilitate an increase in microbial biomass and composition, by providing more refuges from grazers, protection from desiccation and wave exposure. Microbial colonisation is facilitated by surface roughness. Hamilton and Duthie (1984) and Miller *et al.* (1987) observed that microbial colonisation first started in small pits and crevices and then extended to smoother areas of the substratum. Microtopography could have also a direct effect on species composition within the biofilms by providing a more diverse range of microhabitats (Thompson *et al.*, 1996). For example, adnate diatoms were observed mainly on rough substrata while stalked and filamentous colonies dominated smooth surfaces (Miller *et al.*, 1987; Snoeijs, 1991). In my study there were no apparent differences in the composition of main components of the microbial community between rough and smooth rocks. It is likely that microhabitat diversity affects microorganism more at species level, which was not considered here.

The biofilm is directly affected by season, tidal level, grazing and rock type. These factors, however, are interacting. For example, grazing pressure on biofilm communities can vary depending on season (Jenkins and Hartnoll, 2001; Jenkins *et al.*, 2001) and rock type. Conversely, biofilms can have an effect on these two factors. Endolithic cyanobacteria modify the microtopography of the rock (Naylor, 2001). Intensity of grazing depends also on the amount of microalgal biomass present on the rocks, as observed in chapter 5. Moreover, the growth of limpets appears to be dependent on microalgal food resources (Underwood, 1984a; Underwood, 1984c; Jenkins and Hartnoll, 2001).

6.3 Variability *versus* homogeneity of microbial films

6.3.1 Scales of spatial variability

Epilithic microbial communities investigated in my study were always located in the upper zones of moderately exposed shores. Therefore spatial patterns described here cannot be generalised to all rocky shores, as wave exposure and tidal level represent further sources of variation in the distribution and abundance of microbial films (Thompson, 1996; Jenkins and Hartnoll, 2001).

Natural epilithic biofilms showed a high level of spatial variarion at all scales, between shores (10's km), between plots (10's m), between replicate samples (10's cm) and within the same sample (100's μm). The degree of variability, however, was less evident at shore level, whilst increased at more localised scales. Differences between plots were observed independently of the season and on most of rock types examined. Greatest variability was observed within plots, among replicate samples. The level of spatial variability was greater when biolfilms were assessed in terms of microbial composition than microalgal standing stock. SEM analysis of microbial films detected a high degree of microheterogeneity within the same rock sample, at scale of microns.

Different factors are likely to cause spatial heterogeneity in microbial films. At large spatial scales, between shores, physical variables, such as local hydrodynamics, nutrients, shore morphology and microclimate are probably the most probable sources of variation. Different densities of grazers, which are known to significantly reduce microalgal abundance on the shores (Underwood, 1980; Hill, 1990; Roberts, 2002) might have also contributed to the variation in the overall microalgal abundance. In my work, however, there was no correlation between densities of limpets, which varied considerably within and among shores (see chapter 2) and total chlorophyll-a.
At smaller scales, biological factors, become more important. Macrobiota will influence the microbiota and represent a source of variation. In the shores examined in the broad and local scale comparisons (Chapter 2 and 3) macrobiota varied considerably. For example, on chalk shore barnacles were almost absent while a relatively high cover was observed on limestone shores. Distribution and colonisation of microbial films are likely to be affected by competition for space with macroalgae and sessile invertebrates. Within the macrobiota grazers, particularly limpets, can be considered as the main causes for variability in biofilms between and within plots. Limpets are sometimes characterised by a clumped distribution (Hartnoll and Hawkins, 1985; Hartnoll, 1986), and limpets generally forage within a radius of 0.5 m, therefore microbial communities are likely to be more grazed in areas near such clumps than in area where only few sparse limpets are present. The effect of grazing on microbial spatial variation, however, is more evident at microscopic scale, within a cm. Patterns of grazing have been recognised to play an important role in patchiness (Hawkins, 1989; Sommer 1999a). Different feeding systems in microphagous herbivorous also lead to various degrees of spatial microheterogeneity (Sommer,

1999b). In the current study, the analysis of grazed filmed rock chips collected during the spring-winter under scanning electron microscopy, often revealed a mosaic of micropatches of film and bare rock with only few sparse diatoms, reflecting the typical grazing marks of limpet radula.

The dispersion of the microflora varied with rock type. Greater variability and hence patchiness was generally observed in biofilms on carbonate rocks such as chalk and limestone than harder rock types. Spatial variability was particularly evident within plots. This could be explained by the softness and surface roughness which characterise these rock types. On soft rocks, limpet radula can excavate microalgae more efficiently, thus leaving the grazed rock completely bare. In contrast, the higher surface complexity on carbonate rocks provides more refuges from grazing. Therefore, on substrata with complex microtopography there will be heavily grazed, smooth areas and patches of biofilms restricted to small pits and microcrevices. As a result, patchiness caused by grazing can become exaggerated on carbonate rocks. Microtopography could, to a less extent, influence indirectly microheterogeneity by providing protection from physical stresses. During summer, microalgae settled in shaded microniches might survive better than on smooth areas, where desiccation and thermal stresses are stronger.

The influence of microtopography in increasing spatial variability was also evident when biofilms were investigated on machine cut, smooth chalk and flint rock tiles (Chapter 4and 5). Spatial variability within each rock type was considerably reduced when compared with the natural weathered surfaces (*cf* Chapter 2).

6.3.2 Homogeneity of biofilms

Despite considerable spatial variability, microbial films are surprisingly homogeneous when their biomass is compared at a broader spatial scale (Table 6.1). In Europe microalgal standing stock varies, on average between 1 $\mu g\ cm^{-2}$ and 20 $\mu g\ cm^{-2}$ chlorophyll-a, with lowest values corresponding to summer and the highest to spring-winter.

This implies that a similar amount of biomass is available for *in situ* consumption by grazers, although turnover could vary and lower biomass in lower latitudes could be more productive due to elevated grazing rates at higher temperatures.

The biofilm is also very homogeneous in composition. All studies describe intertidal epilithic biofilms as being dominated by microphototrophs, mainly represented by diatoms and cyanobacteria. In UK, composition of diatom assemblages is very similar, being dominated by few genera, including *Achnanthes, Fragilaria* and *Cocconeis.*

Table 6.1 – Range of average maximum (summer) and minimum (winter) values of microalgal standing stock (chlorophyll-a) for mid shore (eulittoral) communities in various European locations.

Location	Chlorophyll-a extraction methods	Range of values	References
UK:			
Isle of Man	Hot methanol 95 %	1.0 - 6.5 μg cm^{-2}	(Hill and Hawkins, 1991)
Isle of Man	Cold methanol 95 %	1.5 – 9.0 μg cm^{-2}	(Thompson, 1996)
Isle of Man	Cold methanol 95 %	2.0 – 7.0 μg cm^{-2}	(Jenkins *et al.*, 2001)
South west of England	Cold methanol 95 %	1.0 – 8.0 μg cm^{-2}	(Jenkins *et al.*, 2001)
South of England	Cold methanol 95 %	1.0 – 20.0 μg cm^{-2}	Present study
Europe:			
South west of Spain	Cold methanol 95 %	1.0 – 4.0 μg cm^{-2}	(Jenkins *et al.*, 2001)

6.3.3 Broader implications

The present study was aimed at investigating the importance of rock type on microbial communities. Some of the results obtained, however, can be considered in a broader context. In UK, there is great interest in coastal protection and conservation of shore habitats. The large variety of rock types characterising the English coast is a fundamental factor in determining the diversity of habitats for plants and animals (Covey, 2002). For example, soft cliffs are generally easily eroded and large lumps of rocks fall into the sea creating ledges of different slope, rock pools and deep crevices. This range of habitats facilitates colonisation of a wide range of organisms, including rarer species of shore birds. My work showed that intertidal microbial communities, including endolithic cyanobacteria, also can increase the ecological value of chalk

shores in terms of primary productivity, as microalgal abundance on this rock type was much higher than on harder non-carbonate rocks. In my study however, it was also shown that higher microalgal biomass has a positivie feedback on limpet grazing on chalk substrata. Grazing on soft rocks can have serious consequences for the erosion of the cliffs (Vidal, 2001). This highlights the importance of including microbial films in the assessment of the ecological value of the shores and for coastal management .

6.3.4 Conclusions and future work

This study showed that the nature of rock substratum influences the abundance and composition of microbial communities. Hardness, porosity and surface roughness are the properties of the rocks which most affected microbial films. In general, soft porous carbonate rocks with a complex microtopography support a higher microalgal biomass than hard, smooth siliceous rocks. These effects, however, are modified by physical and biological factors, such as seasonal patterns, tidal level and grazing.

More experimental work, however, is needed to identify the physical and chemical properties which affect biofilms. The examination of the endolithic component in the rocks would also help a more complete understanding of the ecology of microbial communities, particularly in relation to the rock substratum. There is a pressing need for studies of rates of production of the microflora and comparison with macroflora productivity. Isotope ratios (Bustamante *et al.*, 1995) would also be a valuable way of tracking the relative contribution of *in situ* microalgal production and advected macroalgal detritus in the energy budget of the shore.

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