UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS DO MAR E AMBIENTE

EXPERIMENTAL STUDY OF THE MACROBENTHIC COLONISATION AND SECONDARY PRODUCTION IN THE ARTIFICIAL REEFS OF ALGARVE COAST

(Tese para a obtenção do grau de doutor no ramo de Ciências do Mar,
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To those I love…….

Na luz do seu olhar tão lânguido, tão doce,
Havia o que quer que fosse
D'um íntimo desgosto:
Era um cão ordinário, um pobre cão vadio
Que não tinha coleira e não pagava imposto.
Acostumado ao vento e acostumado ao frio,
Percorria de noite os bairros da miséria
À busca dum jantar.
E ao ver surgir da lua a palidez etérea,
O velho cão uivava uma canção funérea,
Triste como a tristeza ossiânica do mar.
Quando a chuva era grande e o frio inclemente,
Ele ia-se abrigar às vezes nos portais;
E mandando-o partir, partia humildemente,
Com a resignação nos olhos virginais.
Era tranquilo e bom como as pombinhas mansas;
Nunca ladrou dum pobre à capa esfarrapada:
E, como não mordia as tímidas crianças,
As crianças então corriam-no a pedrada.

Uma vez casualmente, um mísero pintor
Um boémio, um sonhador,
Encontrara na rua o solitário cão;
O artista era uma alma heróica e desgraçada,
Vivendo numa escura e pobre água furtada,
Onde sobrava o génio e onde faltava o pão.
Era desses que têm o rubro amor da glória,
O grande amor fatal,
Que umas vezes conduz às pompas da vitória,
E que outras vezes leva ao quarto do hospital.

E ao ver por sobre o lodo o magro cão plebeu,
Disse-lhe: - "O teu destino é quase igual ao meu:
Eu sou como tu és, um proletário roto,
Sem família, sem mãe, sem casa, sem abrigo;

E quem sabe se em ti, ó velho cão de esgoto,
Eu não irei achar o meu primeiro amigo!..."

No céu azul brilhava a lua etérea e calma;
E do rafeiro vil no misterioso olhar
Via-se o desespero e ânsia d'uma alma,
Que está encarcerada, e sem poder falar.
O artista soube ler naquele olhar em brasa
A eloquente mudez dum grande coração;
E disse-lhe: - "Fiel, partamos para casa:
Tu és o meu amigo, e eu sou o teu irmão."

E viveram depois assim por longos anos,
Companheiros leais, heróicos puritanos,
Dividindo igualmente as privações e as dores.
Quando o artista infeliz, exausto e miserável,
Sentia esmorecer o génio inquebrantável
Dos fortes lutadores;
Quando até lhe acudiu às vezes a lembrança
Partir com uma bala a derradeira esp'rança,
Pôr um ponto final no seu destino atroz;
Nesse instante do cão os olhos bons, serenos,
Murmura-lhe: - Eu sofro, e a gente sofre menos,
Quando se vê sofrer também alguém por nós.

Mas um dia a Fortuna, a deusa milionária,
Entrou-lhe pelo quarto, e disse alegremente:
"Um génio como tu, vivendo como um pária,
Agrilhoado da fome à lúgubre corrente!
Eu devia fazer-te há muito esta surpresa,
Eu devia ter vindo aqui p'ra te buscar;
Mas moravas tão alto! E digo-o com franqueza
Custava-me subir até ao sexto andar.
Acompanha-me; a glória há de ajoelhar-te aos pés!..."
E foi; e ao outro dia as bocas das Frinés

Abriram para ele um riso encantador;
A glória deslumbrante iluminou-lhe a vida
Como bela alvorada esplêndida, nascida
A toques de clarim e a rufos de tambor!

Era feliz. O cão
Dormia na alcatifa à borda do seu leito,
E logo de manhã vinha beijar-lhe a mão,
Ganindo com um ar alegre e satisfeito.
Mas ai! O dono ingrato, o ingrato companheiro,
Mergulhado em paixões, em gozos, em delícias,
Já pouco tolerava as festivas carícias
Do seu leal rafeiro.

Passou-se mais um tempo; o cão, o desgraçado,
Já velho e no abandono,
Muitas vezes se viu batido e castigado
Pela simples razão de acompanhar seu dono.
Como andava nojento e lhe caíra o pelo,
Por fim o dono até sentia nojo ao vê-lo,
E mandava fechar-lhe a porta do salão.
Meteram-no depois num frio quarto escuro,
E davam-lhe a jantar um osso branco e duro,
Cuja carne servira aos dentes d'outro cão.
E ele era como um roto, ignóbil assassino,
Condenado à enxovia, aos ferros, às galés:
Se se punha a ganir, chorando o seu destino,
Os criados brutais davam-lhe pontapés.
Corroera-lhe o corpo a negra lepra infame.
Quando exibia ao sol as podridões obscenas,
Poisava-lhe no dorso o causticante enxame
Das moscas das gangrenas.

Até que um dia, enfim, sentindo-se morrer,
Disse "Não morrerei ainda sem o ver;

A seus pés quero dar meu último gemido..."
Meteu-se-lhe no quarto, assim como um bandido.
E o artista ao entrar viu o rafeiro imundo,
E bradou com violência:
"Ainda por aqui o sórdido animal!
É preciso acabar com tanta impertinência,
Que esta besta está podre, e vai cheirando mal!"
E, pousando-lhe a mão cariciosamente,
Disse-lhe com um ar de muito bom amigo:
"Ó meu pobre Fiel, tão velho e tão doente,
Ainda que te custe anda daí comigo."

E partiram os dois. Tudo estava deserto.
A noite era sombria; o cais ficava perto;
E o velho condenado, o pobre lazarento,
Cheio de imensas mágoas
Sentiu junto de si um pressentimento
O fundo soluçar monótono das águas.

Compreendeu enfim! Tinha chegado à beira
Da corrente. E o pintor,
Agarrando uma pedra atou-lh'a na coleira,
Friamente cantando uma canção d'amor.

E o rafeiro sublime, impassível, sereno,
Lançava o grande olhar às negras trevas mudas
Com aquela amargura ideal do Nazareno
Recebendo na face o ósculo de Judas.
Dizia para si: "È o mesmo, pouco importa.
Cumprir o seu desejo é esse o meu dever:
Foi ele que me abriu um dia a sua porta:
Morrerei, se lhe dou com isso algum prazer."

Depois, subitamente
O artista arremessou o cão na água fria.

E ao dar-lhe o pontapé caiu-lhe na corrente
O gorro que trazia
Era uma saudosa, adorada lembrança
Outrora concedida
Pela mais caprichosa e mais gentil criança,
Que amara, como se ama uma só vez na vida.

E ao recolher à casa ele exclamava irado:
"E por causa do cão perdi o meu tesouro!
Andava bem melhor se o tinha envenenado!
Maldito seja o cão! Dava montanhas d'oiro,
Dava a riqueza, a glória, a existência, o futuro,
Para tornar a ver o precioso objecto,
Doce recordação daquele amor tão puro."
E deitou-se nervoso, alucinado, inquieto.
Não podia dormir.
Até nascer da manhã o vívido clarão,
Sentiu bater à porta! Ergueu-se e foi abrir.
Recuou cheio de espanto: era o Fiel, o cão,
Que voltava arquejante, exânime, encharcado,
A tremer e a uivar no último estertor,
Caindo-lhe da boca, ao tombar fulminado,
O gorro do pintor!

Guerra Junqueiro : Fiel

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TÍTULO DA TESE: Estudo experimental da colonização e produção secundária macrobentónica em recifes artificiais da costa algarvia

RESUMO:

Os sistemas de recifes artificiais, implantados em todo o mundo, desempenham um papel importante no aumento da produção natural de recursos biológicos marinhos recursos. A implantação de recifes artificiais na costa Sul do Algarve teve como objectivo contribuir para a gestão e ordenamento das pescarias litorais, aumentar a produtividade natural das áreas circundantes, promover a biodiversidade e aumentar a protecção dos juvenis que migram para o litoral. Ao longo da costa sul do Algarve, sete sistemas artificiais foram implantados. No entanto, a investigação tem-se concentrado principalmente em populações de peixes, particularmente aquelas de importância económica. O presente estudo pretende contribuir para um melhor conhecimento das comunidades macrobentónicas associadas a estes sistemas de recifes artificiais. Estas comunidades que se encontram na base do sistema ecológico desempenham um importante papel ao nível da cadeia alimentar e da camuflagem das estruturas artificiais, tornando os recifes artificiais em locais mais atractivos para as populações piscícolas.

Várias experiências foram desenvolvidas com o intuito de recolher informação sobre as comunidades macrobentónicas, em particular em diversos grupos recifais no recife artificial de Faro/Ancão, e contribuir para o conhecimento sobre o funcionamento e ecologia dos recifes artificiais. Assim, um dos objectivos principais do presente trabalho foi analisar os diversos factores que poderão influenciar a colonização e sucessão das comunidades macrobentónicas, nomeadamente: **1)** caracterizar a evolução espaço-temporal da colonização de espécies macrobentónicas; **2)** estudar o efeito da orientação das superfícies na sucessão macrobentónica (horizontal *vs.* vertical); **3)** testar o efeito da profundidade (16 m *vs.* 20 m profundidade) e da estrutura recifal (camada superior *vs.* inferior do recife) na sucessão macrobentónica; **4)** estimar a produção secundária; **5)** analisar a estrutura trófica das comunidades macrobentónicas; **6)** comparar a sequência de colonização entre recifes artificiais em diferentes estados de "maturação"; **7)** o efeito do meio circundante para a colonização nos recifes artificiais; e **8)** testar se as comunidades dos recifes artificiais se assemelham às comunidades dos recifes naturais.

Como unidade de amostragem utilizou-se cubos de betão (15x15cm), de material idêntico ao da construção dos recifes artificiais. Estas unidades foram suspensas nos módulos recifais aquando da imersão destes. Foram recolhidas por mergulho autónomo, consoante o tempo delineado para cada experiência. Salienta-se que no estudo de comparação com o recife natural, estas unidades

de amostragem não foram utilizadas, mas foram raspado *quadrats* com 15cm de lado. No laboratório, como métodos não-destrutivo, foi utilizado o método do ponto de intersecção, onde os dados de percentagem de cobertura total foram recolhidos das faces dos cubos. De seguida, estas faces foram raspadas, para recolha e identificação dos organismos. Para este trabalho, analisaram-se quatro das seis faces amostradas, correspondendo à orientação horizontal: face superior *vs.* face inferior, e à orientação vertical: face interior *vs.* face exterior. Em termos estatísticos realizou-se a análise univariada (ANOVA) e/ou análise multivariada (ANOSIM, MDS, CLUSTER e SIMPER) com os diferentes tipos de dados, principalmente dados de percentagem de cobertura, abundância, biomassa e número de espécies.

De um modo geral, os valores de taxa de cobertura, número de *taxa*, de diversidade, de abundância e de biomassa aumentaram ao longo do tempo. A colonização e sucessão da comunidade epibêntica foi afectada pela orientação das superfícies, onde foi detectada diferenças na orientação horizontal, onde a superfície superior apresenta os valores mais elevados em relação ao número de espécies e de abundância média, excepto quando se utilizado o método não destrutivo de taxa de cobertura que apresenta valores inversos. Os dados de percentagem de cobertura dos grupos taxonómicos, foi observado que na superfície inferior teve uma colonização significativamente mais elevada, devido à dominância dos valores de cobertura por cirrípedes, favorecidos, provavelmente, pelos baixos níveis de sedimentação. Cirrípedes, briozoários e serpulídeos dominaram as amostras imediatamente após o início da experiência. A partir dos seis meses de colonização, a diversidade de espécies de invertebrados aumenta.

Organismos, consoante a sua mobilidade, parecem também ser afectados de modo diferentes. As espécies vágeis geralmente são negligenciadas, e apenas os organismos sésseis e espécies mais conspícuas são analisadas em estudos das comunidades de substrato rochoso. Portanto, o desenvolvimento dos componentes vágil e séssil da comunidade epibiótica foi analisado separadamente. As diferenças foram detectadas entre as amostras de superfícies horizontais, mas não em superfícies verticais. A análise multivariada detectou diferenças na estrutura da comunidade de macroinvertebrados bentónicos, quer considerando a componente séssil ou móvel. No entanto, só a fauna vágil apresentou diferenças significativas quando a análise de variância foi aplicada. Além disso, este estudo sugere que para as comunidades de substrato rochoso, a análise da fauna vágil é de extrema importância e deve ser tida em conta como uma característica importante no funcionamento do recife artificial.

A colonização da comunidade epibêntica foi também afectada pela profundidade e estrutura do RA, onde foi detectada que existem espécies que são afectadas com a profundidade, levando a concluir que mesmo pequenas diferenças na profundidade são importantes nestas comunidades; Em ambos os grupos recifais a diferentes profundidades foram caracterizados por *Balanus amphitrite*, *Gregariella subclavata*, *Musculus* cf. *subpictus*, *Paleanotus bellis* e *Syllidia armata*. Além disso, *Jassa marmorata* e *Bugula neritina* foram espécies típicas de 16 m, principalmente na camada superior do recife artificial, enquanto *Anomia ephippium* foi particularmente comum aos 20 m, especialmente na camada inferior. Em ambas as profundidades, a biomassa nas superfícies horizontais foi mais elevada na face superior do que na inferior. Nas superfícies verticais, os valores de biomassa obtidos foram semelhantes para ambas as camadas e profundidades.

A presença de uma comunidade preestabelecida, parece afectar a colonização, especialmente durante o primeiro ano; a inexistência de comunidade mais madura circunvizinha irá promover a colonização de novos substratos por organismos pioneiros (serpulídeos, cirrípedes, *Hiatella arctica*, *Polydora hoplura*), enquanto se existir uma comunidade já estabelecida, os novos

substratos serão colonizados por outras espécies mais competitivas (*Filograna implexa*, *Pomatoceros triqueter* e cnidários).

As capacidades de replicar um recife natural, mesmo para uma comunidade recifal com 16 anos, diferenças foram detectadas entre as duas comunidades, tanto em número de espécies, como na composição específica, estrutura trófica e da própria comunidades; no entanto, as duas comunidades em estudo apresentam espécies comuns, mas em proporções bastante diferentes. Na verdade, o recife natural apresentou valores mais elevados relativamente ao número de espécies de moluscos e biomassa de esponjas. Por outro lado, os substratos artificiais foram dominados por espécies de poliquetas (principalmente serpulídeos) e apresentaram os maiores valores de biomassa de antozoários. As estruturas tróficas de ambas as comunidades foram dominadas por organismos suspensívoros, principalmente nos recifes naturais (devido aos valores elevados de biomassa de esponjas), onde na comunidade do recife artificial, o domínio da suspensão foi compartilhada com organismos carnívoros (devido à elevada biomassa de antozoários).

Um dos aspectos menos estudos no domínio da investigação sobre o funcionamento dos recifes artificiais prende-se com a sua produção secundária. Nesse sentido, ao longo dos primeiros 12 meses após a implantação do recife artificial, estudou-se o efeito da orientação do substrato na produção secundária de epibentos. Verificou-se que a produção epibêntica foi mais elevada na superfície horizontal. No entanto, no final do período de estudo, a produção média apresentou valores semelhantes. As superfícies de orientação horizontal tiveram uma produção média entre 128 e 103 g m^2yr^1, enquanto as superfícies de orientação vertical apresentaram uma produção média entre 103 e 98 g m^2 yr^1. A partir destes valores extrapolou-se a produção média anual para o complexo recifal da costa algarvia, que após um ano de implantação geram cerca de 5 ton de fauna epibêntica.

De acordo com o presente estudo, e com a informação recolhida, pretendeu-se uma melhor compreensão dos padrões de colonização macrobentónica em recifes artificiais, de modo a melhor a gestão e funcionamento de recifes artificias para futuras implantações. Assim, a diferente exposição destas faces nomeadamente à luz, correntes e sedimentação poderá estar na origem dos resultados obtidos. Também outros factores, tais como profundidade, disponibilidade de larvas, ovos ou esporos no meio circundante, competição por uma comunidade pré estabelecida parecem afectar a colonização e sucessão destas comunidades. O tempo de imersão parece ser um dos factores determinantes nos processos de sucessão destas comunidades. Tornou-se evidente que, dos vários estudos abrangidos nesta tese, os recifes artificiais, de uma perspectiva ecológica, são um instrumento complementar de gestão e reabilitação de ecossistemas litorais.

Palavras-chave: Recifes artificiais, comunidade macrobentónica, padrões de colonização, Algarve

Experimental study of the macrobenthic colonisation and secondary production in the artificial reefs of Algarve coast

ABSTRACT

In the southern coast of the Algarve, the deployment of artificial reefs was undertaken to protect and enhance coastal fishery resources. It is extremely important to understand the factors, shaping macrobenthic communities, as they provide food and shelter for fish and other organisms economically important. These communities that if find in the base of the ecological system play an important role to the level of the alimentary chain and the camouflage of the artificial structures. This study provided the opportunity to understand the effectiveness and the biodiversity associated to these systems. Thus, another major goal was the analysis of the factors that will be able to affect the settling and succession of the macrobenthic communities in artificial reefs, namely, the effect of surfaces orientation, of depth and of the relative position inside reef, the effect of the surrounding habitats for the settling and if artificial reefs communities can imitate the natural reefs communities. This work will hopefully provide important data for understanding the patterns of benthic colonisation on artificial substrata for better management and functioning for future deployment.

Key-words:
Palavras-chave: Artificial reefs, macrobenthic comunity, colonisation patterns, Algarve

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CHAPTER I.

General introduction and objectives

The European Artificial Reef Research Network (EARRN; see Jensen, 1997) defines artificial reefs as "*submerged structures placed deliberately on the seabed to mimic some characteristics of natural reefs*". The use of the word submerged is deliberate; excluding structures such breakwaters, piers and jetties. Likewise, the requirement for reef deployment to be a deliberate act disassociates the true artificial reef from structures, which may claim that title in order to gain some ecological credibility. Structures that mimic natural reefs characteristics but have another primary purpose (such as piers and the steel jackets of oil/gas production platforms) may be described as secondary artificial reefs (Pickering *et al*., 1998).

Artificial reefs are employed for the purpose of promoting marine life in featureless bottom areas, primarily for fisheries enhancement. However, artificial reefs can fill a variety of roles beyond this, for example, as tools for: environmental mitigation, preservation and rehabilitation of natural habitats, aquaculture, tourism, control of coastal erosion, improvement of hydrodynamics for surfing and anti-trawling protection (Guillén *et al*., 1994; Antsulevich *et al*., 2000; Ramos-Esplá *et al*., 2000; Angel and Spanier, 2002; Ditton *et al*., 2002; Bacchiocchi and Airoldi, 2003). These structures have been deployed worldwide and, according to Seaman and Sprague (1991), the major areas presenting this kind of activity include the Mediterranean and Caribbean Seas, South-eastern Asia, Japan, North America, Australia and some islands in the South Pacific.

Regardless of the construction method, artificial reefs are generally designed to provide hard surfaces to which algae and invertebrates attach; the development of macrobenthic communities provides intricate structure and food for assemblages of fish (Pickering *et al*., 1998). For a better artificial reef management, a whole-ecosystem approach is necessary, incorporating studies of all aspects of hard substratum ecology, including both structural and functional variables, such as how quickly the substratum is colonised by epifauna and what factors influence rates of colonisation (Svane and Petersen, 2001). The capacity of food production is one of the potential parameters to evaluate artificial habitats as a feeding area (Itosu *et al*., 1999). Despite its importance, few productivity studies were undertaken (Steimle *et al*., 1999). The understanding of this aspect is of practical importance as epibiotic organisms are sources of food and shelter for many types of organisms (e.g. Connell and Andersen, 1999) and are an important component of subtidal systems. The recruitment onto artificial reefs will be structured by physical factors such as currents (which carry eggs, larvae and spores), water depth, distance to the shore or natural reefs, quality of the substratum material properties, orientation and its heterogeneity (Eckman, 1983; Roughgarden *et al*., 1988; Koehl, 2007). These in association with biological factors, like space competition and predation, may be important for ecology of these communities, influencing the subsequent recruitment, colonisation, succession and development (Svane and Petersen, 2001). The comprehension of artificial reefs as marine habitats and how to improve them as a replacement for natural reef, is an area of urgent need in coastal ecology. An immediate priority is the identification of the factors that affect species to differ between artificial and natural reefs. However, there is little understanding of the artificial reef structures on epibiota, and even less on how our current understanding of their ecology relates to natural reef. Composition of substratum appears as an obvious factor that may explain differences in *taxa* between artificial structures and rocky reef. Artificial reefs are made of materials naturally foreign to the marine environment (concrete) and are quite different from rocky reefs. Although differences in composition of substratum correlate with differences in epibiotic assemblages, this is also true for several other factors (e.g. age: introduced structures *vs.* seasoned reef) (Connell and Glasby, 1999). Artificial and natural reefs comparison is important in order to assess the performance of artificial reefs. Not only do artificial reefs provide surfaces of different compositions, but they may also provide surfaces of various orientations. Very different types of epibiotic assemblages have been shown to occur on surfaces of different orientations, and it is therefore likely, that the combined effects of surface composition and orientation may greatly influence the development of epibiotic assemblages (Glasby, 2000 and references there in; Glasby and Connell, 2001). The developing fouling assemblages are not necessary similar to epibioses on natural hard substrata. However, the effectiveness of artificial reefs deployment is to support assemblages and ecological processes similar to those of adjacent rocky shores. Promoting the establishment of assemblages, that are as similar as possible to those occurring on adjacent natural rocky substrata, could be a way to reduce the impacts of these structures (Bulleri, 2005). The artificial reefs will be colonised mainly by settling larvae and spores of a large number of epibenthic organisms as a consequence of the introduction of vacant hard substratum. Moreover, the effects of deploying artificial reefs clearly depend on the reef type and the surrounding environment. The neighbouring substrate may act as an important intermediate habitat that influences the spores and larvae availability (Svane and Petersen, 2001). As well, the presence of an established community in the artificial reef vicinity may increase both predation on larvae and space-competition, influencing the growth of species on a new substratum (Boaventura *et al*., 2002). The structure of the reef, offering several kinds of niches, in association with depth, will strongly influence the development rate of artificial reef epibioses. Relini *et al*. (1994) asserted that the epifauna density, biomass and cover decrease as a function depth. The highest rate is expected closer to the surface, primarily due to algal growth, with subsequent reduction with increasing depth (Svane and Petersen, 2001).

With so many known causes of variability in developing epifaunal assemblages, it is essential that any method used in studies of this nature provides a sensitive, accurate, and robust estimate of the assemblage structure. The study of sessile epibiotic communities of the subtidal hard substrate is complex as they cannot be studied easily by the normal quantitative methods applicable to infauna, due to the predominance of colonial organisms which are often of a complex topography (Hatcher, 1995, Steimle *et al*., 1999). A wide variety of techniques are routinely used in epifaunal studies to quantify the assemblage structure. These include percentage cover estimates, abundance counts and biomass determination (Beaumont *et al*., 2007 and references therein). Quantification using biomass measurements is considered to be more appropriately to examine such community (Hatcher, 1995). However, despite the variety of techniques that have been used to determine the extent of epifaunal fouling, there has been little comparative evaluation between the techniques used. The measurement of secondary production has assumed a fundamental role in the quantification of ecosystem dynamics, since production is one of the major pathways of energy flow (Möller *et al*., 1985; Morin *et al*., 1987; Ricciardi and Bourget, 1998). The estimation of secondary production is of basic importance to the rational management of natural resources, because it is the primary instrument for the evaluation of trophic potential of the components of each ecosystem (Tumbiolo and Downing, 1994).

The present study was conducted in the Algarve (Portugal) artificial reef complex. Currently, this artificial reef is composed of seven reef systems, spread over a total area of more than 43km^2. Each artificial reef system consists of a protection reef (PR) and an exploitation reef (ER). The PR consists of small concrete cubic units (2.7 m^3 each unit, weighting 3 tonnes), distributed by reef sets, each composed of 3 reef groups of 35 unit each, at depths ranging from 16 to 25 m. The ER consists of large concrete blocks (174 each unit, weighting over 40 tonnes), distributed in reef groups of 4 blocks each, at depths that range from 25 m to 40 m (for details see Monteiro and Santos, 2000; Santos and Monteiro, 2007). These multipurpose systems are used as a useful tool for management of local fisheries, both in terms of the protection of small fish and the enhancement of fishing ground. This coastal zone was selected for the deployment of the artificial structures due to the lack of high relief rocky bottom, lower fishing yield and the existence of natural features along the coast (e.g. Ria Formosa, Rio Arade, Ria de Alvor and Guadiana estuary), through the extension of their nursery potential offshore (Santos and Monteiro, 1997; Monteiro and Santos, 2000). In Portugal, research regarding these systems has been mainly focused on icthyological fauna, especially on commercial species (Santos, 1997; Santos *et al*., 1995; Santos and Monteiro, 1997, 1998 among others) and, until 2003, the ecology of mobile invertebrates, epifauna and epiflora, as integrant part of the food chain, had been disregarded (Wergikoski, 1999; Cancela da Fonseca *et al*., 2007).

Thesis rationale and aims

The present thesis attempts to investigate the artificial reef in relation to hard substratum ecology, with special emphasis on the role of fouling assemblages and epibioses. In 2002, the deployment of new reefs in the Algarve coast became an opportunity to understand the artificial reefs' epibioses. The first part of the present study corresponds to the results of a short-term colonisation (up to one year of study) (Chapter II and III). Various factors that influence the recruitment, such as surface orientation and depth of deployment of artificial reefs, were analysed. The evaluation of some techniques used in the assessment of subtidal epibiotic assemblage structure was studied. Also, to evaluate the Faro/Ancão artificial reefs communities function, the estimation of secondary production of epibenthic organisms was assessed (Chapter IV).

The second section of the thesis addresses the analysis of medium-term colonisation (between four and sixteen years of macrobenthic colonisation) (Chapter V and VI), mainly the influence of surrounding habitats in colonisation and potential mimic of the macrobenthic colonisation of artificial reefs as those on natural rocky area.

This thesis is developed in seven main sections. The present chapter (Chapter I) gives a general overview of the importance and background of the research theme, indicates the main objectives of the study and presents the structure of the thesis.

Chapter II presents several approaches to investigate the surface orientation effect in macrobenthic communities' colonisation, especially concerning the use of non-destructive and destructive methods to assess the percentage cover, abundance and biomass of macrobenthic organisms.

Chapter III illustrates the importance of depth on the distribution of the epibentic species. Depth alters light conditions and temperature, among others. In addition, morphological complexity of the artificial reefs will offer a great variety of conditions for the colonisation of the epibenthic fauna. Particularly, the effects on abundance and biomass production are discussed. It is critical to understand how the implantation of Faro/Ancão artificial reefs, will contributed to mitigate the natural reef-scarcity. Effects of substratum orientation on secondary production of epibenthos were evaluated over the following 12 months of deployment and the rough secondary production of the reef epifauna was estimated (Chapter IV).

Chapter V assesses the importance a nearby established community in the vicinity of the artificial reef on the recruitment, settlement and colonisation of macrobenthic communities. The effect of the presence/absence of pre-established nearby communities on the abundance, biomass and community structure of the epibenthic fauna are discussed.

Chapter VI focuses on the capacity of macrobenthic communities of the artificial reef structures to mimic those on natural rocky areas. It is important to evaluate the ecological value of the artificial reef surfaces as habitat for epibenthic fauna, in comparison to the natural rocky surfaces, which they intent to imitate. Therefore, species composition, community and trophic structure of both communities were compared.

Finally, Chapter VII presents the general conclusions of this work.

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CHAPTER II.

Macrobenthic community colonisation patterns on artificial reefs:

Analysis of the influence of surface orientation

Section 1.

Benthic succession on an artificial reef in the south of Portugal - Preliminary results

Moura, A.; Boaventura, D.; Cúrdia, J.; Carvalho, S.; Pereira, P.; Cancela da Fonseca, L.; Leitão, F.; Santos, M. N., and Monteiro, C. C. Benthic succession on an artificial reef in the south of Portugal - Preliminary results. *Revista Da Biologia*. 2004; 22:169-181.

Introduction

Artificial reefs are submerged structures deployed on the sea bottom to provide a substratum, minimizing the scarcity of natural reefs (Svane and Petersen, 2001). They have become an important resource enhancement technique to attract fish, to increase natural production of biological resources and also to improve fishing yields (Bohnsack and Sutherland, 1985). The deployment of artificial reefs has been associated not only with the exploitation of commercial fish resources, but also with the preservation and rehabilitation of marine habitats (Guillén *et al*., 1994; Allemand *et al*., 2000; Barnabé *et al*., 2000). Although the importance of these systems is recognised worldwide, little information is available regarding their biological and ecological features (Bohnsack and Sutherland, 1985). To date, most of the studies undertaken on artificial reefs have been focused on the enhancement of fishing success (Kevern *et al*., 1986; Polovina, 1991; Bombace, 1995; Lamadrid *et al*., 1995; Santaella and Revenga, 1995; Santos *et al*., 1995; Santos and Monteiro, 1998; Walker *et al*., 2000). Quantitative data regarding biological communities associated to these systems is scarce, particularly concerning the study of benthic communities (Badalamenti *et al*., 2000; D’Anna *et al*., 2000; Relini, 2000; Relini *et al*., 1994).

Since 1990, seven artificial reef systems have been deployed off the south coast of the Algarve by the INIAP/IPIMAR (Portuguese Institute for Agriculture and Fisheries Research) (Figure 1). These multipurpose systems were deployed to enhance local fisheries. The potential increase of food resources and shelter may extend the nursery effect provided by estuarine-lagoon systems along the coast (Monteiro and Santos, 2000). In Portugal, research regarding these systems has been mainly focused on icthyological fauna, especially on commercial species (Santos *et al*., 1995; Santos and Monteiro, 1997, 1998). The spatial and temporal patterns of macrobenthic communities within artificial reef systems are an important goal of a multidisciplinary project undertaken by the INIAP/IPIMAR. These communities are of particular interest as they are an important food resource for most of the commercial fish species associated with the artificial reef systems. Succession of the macrobenthic communities in the Faro/Ancão reef system will be analysed over the next several years, since artificial reef submersion. This paper presents results on early macrobenthic colonisation and succession within two artificial reef groups.

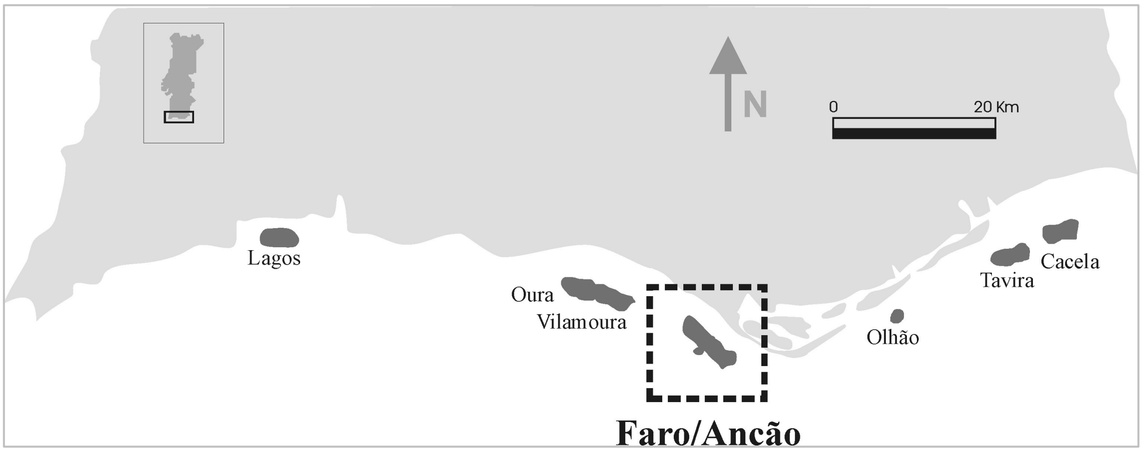

Figure 1. Study site. Artificial reefs on the southern coast of Portugal. The box indicates the Faro/Ancão reef where the study was conducted.

Material and methods

The Algarve artificial reef systems consist of both small (2.7m^3) and large concrete modules (174m^3) (Figure 2). Both structures were deployed along four distinct parallel lines to the coast, where small modules were placed at lower depths (18-25m) and the large ones on deeper waters (30-40m). The present work was carried out on the “Faro/Ancão” artificial reef, located off Faro (Algarve, southern Portugal) (cf. Figure 1), particularly on 2 adjacent small artificial reef groups (groups A10 and A11) of 35 modules each. These reef modules were deployed in August 2002, at approximately 20m depth.

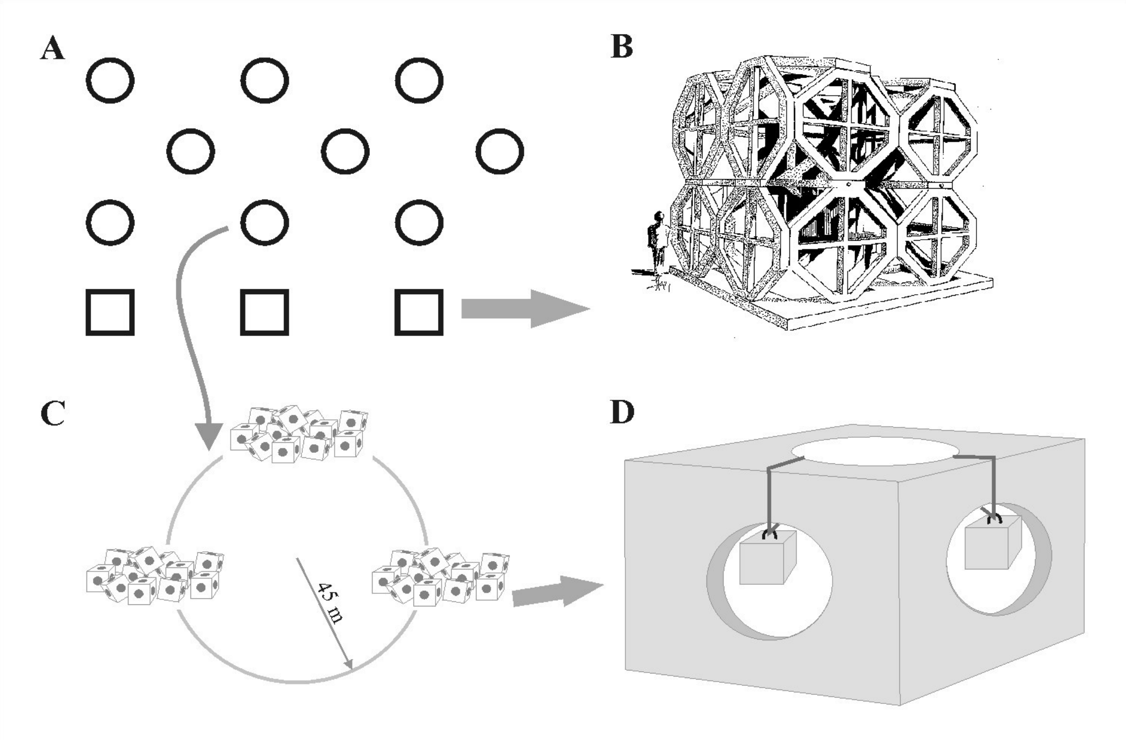

Figure 2. A -Faro/Ancão artificial reef system. B – Large module. C – Reef group with 3 groups of 35 small modules each. D – Small module detail with the cubic sample units used in this study.

The study of macrobenthic colonisation was performed using 15 x 15cm cubic sample units made of the same concrete material of reef modules. These units were suspended on the reef blocks at the time of the reef immersion to ensure that the colonisation patterns would be representative of those occurring throughout the reef modules (cf. Figure 2). Three replicate samples were retrieved by scuba diving 3 and 6 months after immersion (November 2002; February 2003). The different surfaces of the cubic samples were scraped to analyse macrobenthic colonisation and succession. Four out of the six cube surfaces (horizontal: top and bottom; vertical: inside and outside) were compared during the study to analyse surface orientation role on benthic colonisation. Horizontal and vertical surfaces are not compared together in the present study. This methodology ensures that only non-adjacent surfaces are compared, minimising contamination. Only a quarter of the material collected from each face was analysed in some of the samples. On those samples, extrapolated values for the total surface area are used. Bryozoans, hydroids, sponges, barnacles and ascidians were not quantified.

Data analysis

The community structure of the two artificial reef groups and on contrasting surfaces (horizontal and vertical) was evaluated throughout the sampling period using multivariate analyses. PRIMER v.5.0 software package was used for multivariate analyses (Clarke and Warwick, 1994). Similarity between faunal data was analysed by cluster and ordination techniques (non-metric multidimensional scaling - MDS) based on the Bray-Curtis similarity coefficient after fourth root transformation (Clarke, 1993). The cluster analysis used the unweighted pair group average algorithm (UPGMA) (Clarke and Warwick, 1994). To test for statistically significant differences between the macrobenthic colonisation on surface orientation and period of immersion, a two-way crossed ANOSIM test was performed using the same similarity matrix used for CLUSTER and MDS ordination. Species having the greatest contribution to dissimilarity among the pairs of surfaces, top *vs.* bottom and inside *vs.* outside, and period of immersion were determined using the similarity percentages routine (SIMPER) of the same statistical package.

Results

To date, 126 *taxa* have been identified in the “Faro/Ancão” artificial reef. 52 from class Polychaeta, 30 from phylum Mollusca and 35 Crustacea. Although barnacles and colonial *taxa* such as bryozoans, hydroids, sponges and ascidians were not analysed in this study, they were also found to be present in all samples. Data analysis showed that in both reef groups (A10 and A11), the mean abundance and the total number of species were higher on the top than on bottom surfaces (Figure 3A and 4A). These parameters tend to increase with submersion time, particularly on the top surface. The mean abundance of the outside and inside surfaces showed similar colonisation patterns for both reef groups (Figure 3B and 4B).

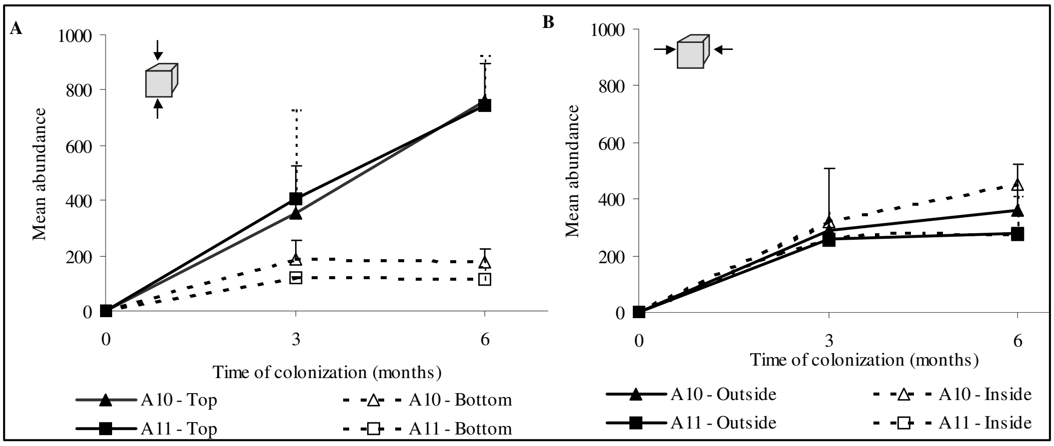

Figure 3. Mean abundance values (±SE) of the two studied reef groups (A10 and A11). A – Top *vs.* Bottom surfaces; B – Outside *vs.* Inside surfaces.

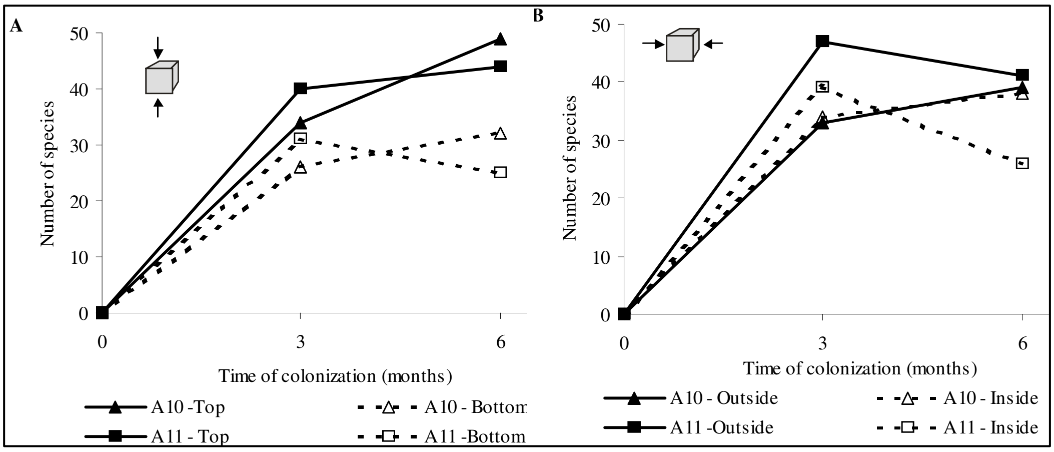

Figure 4. Number of species of the two studied reef groups (A10 and A11). A – Top vs. Bottom surfaces; B – Outside vs. Inside surfaces.

Diversity and evenness indices (Table I) increased with time in both groups studied and on all surfaces. The strongest increase in these indices occurred on the bottom surface. The A11 group generally presented higher diversity and evenness values than the A10 group.

Table I. Species diversity (H'log2 Shannon-Wiener index) and Pielou's evenness (J') values on the two studied reef groups (A10 and A11). Top *vs.* Bottom surfaces; Outside *vs.* Inside surfaces.

	H'(log2)				J'			
Time (month)	**A11 Top**	**A10 Top**	**A11 Bottom**	**A10 Bottom**	**A11 Top**	**A10 Top**	**A11 Bottom**	**A10 Bottom**
3	3.5	3.3	3.2	3.0	0.7	0.6	0.6	0.6
6	3.9	3.7	4.0	4.0	0.7	0.7	0.9	0.8
Time (month)	**A11 Outside**	**A10 Outside**	**A11 Inside**	**A10 Inside**	**A11 Outside**	**A10 Outside**	**A11 Inside**	**A10 Inside**
3	3.8	3.3	3.3	3.0	0.7	0.7	0.6	0.6
6	4.0	3.3	3.5	3.3	0.8	0.6	0.8	0.6

Throughout the sampling period, bivalves, amphipods and polychaetes, dominated the top surface, whereas on the bottom surface, polychaetes and bivalves were the more important taxonomic groups (Figure 5A and B). Amphipods were always present throughout the sampling period on both vertical surfaces. In general, polychaetes were the most abundant group on both inside and outside surfaces, even though there was a dominance shift from polychaetes to bivalves 6 months after submersion (Figure 5C and D).

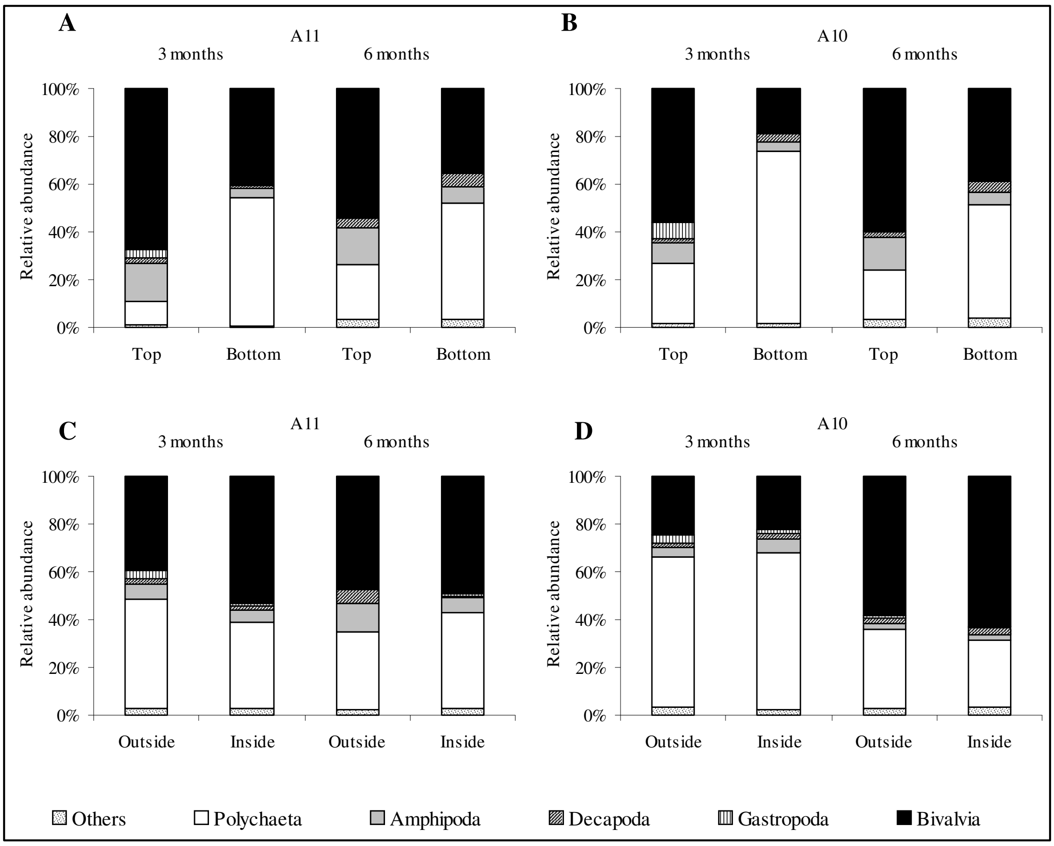

Figure 5. Relative abundance on the two studied reef groups (A10 and A11). A – Top *vs.* Bottom surfaces of the A11 reef group; B – Top *vs.* Bottom surfaces of the A10 reef group; C – Outside *vs.* Inside surfaces of the A11 reef group; D - Outside *vs.* Inside surfaces of the A10 reef group.

Cluster analysis (Figure 6A) individualised two main groups corresponding to the two sampling periods (3 and 6 months). Within both groups, the samples corresponding to each surface (top and bottom) cluster together, with no differentiation of the two reef groups. For vertical surfaces (inside and outside surfaces) only two groups were distinguished, corresponding to the different sampling periods (3 and 6 months) (Figure 6B). The configuration of the groups of samples obtained by MDS supported the results of the cluster analysis (Figures 7A and B). Thus, ordination and cluster diagrams for the macrofauna data suggests that differences on orientation and time of submersion were determinant for community composition. The results obtained with a two-way crossed ANOSIM showed that only the horizontal surfaces were significantly different (R = 0.74; p = 0.001), whereas for the vertical surfaces, the ANOSIM test showed no significant difference (R = 0.042; p = 0.265). The time of immersion was significant both for horizontal (R = 0.87; p = 0.001) and vertical surfaces (R = 0.89; p = 0.001).

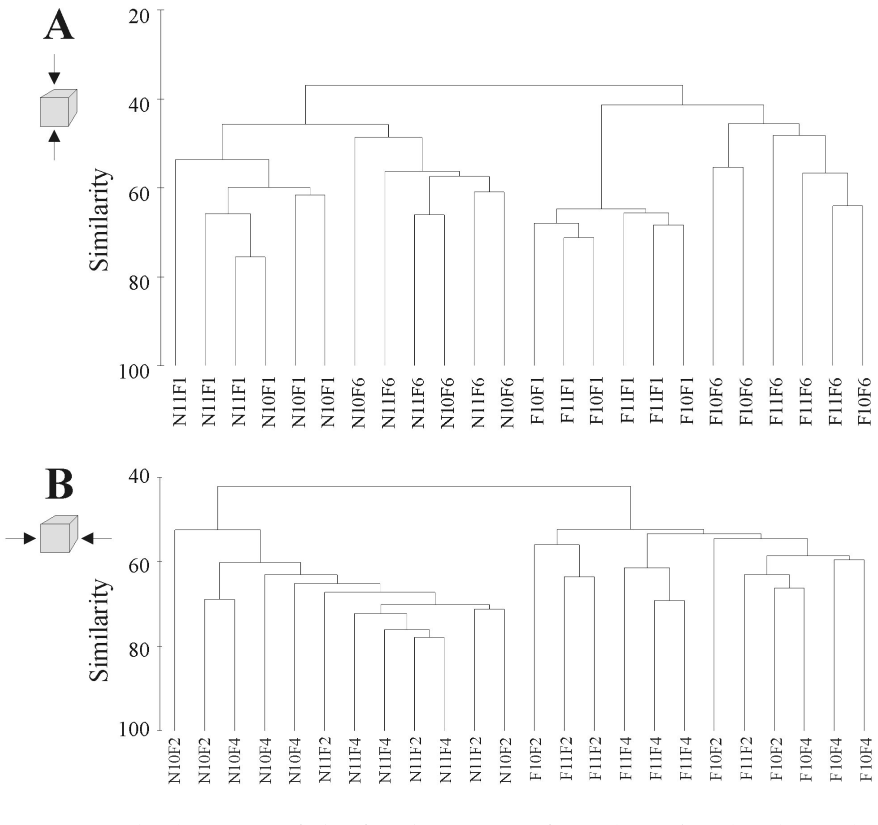

Figure 6. CLUSTER dendrograms of the fourth root transformed species-abundance data of the two studied reef groups (10 and 11). A – Top (F1) *vs.* Bottom (F6) surfaces, B – Outside (F2) *vs.* Inside (F4) surfaces, during sampling period (3 months – N; 6 months - F).

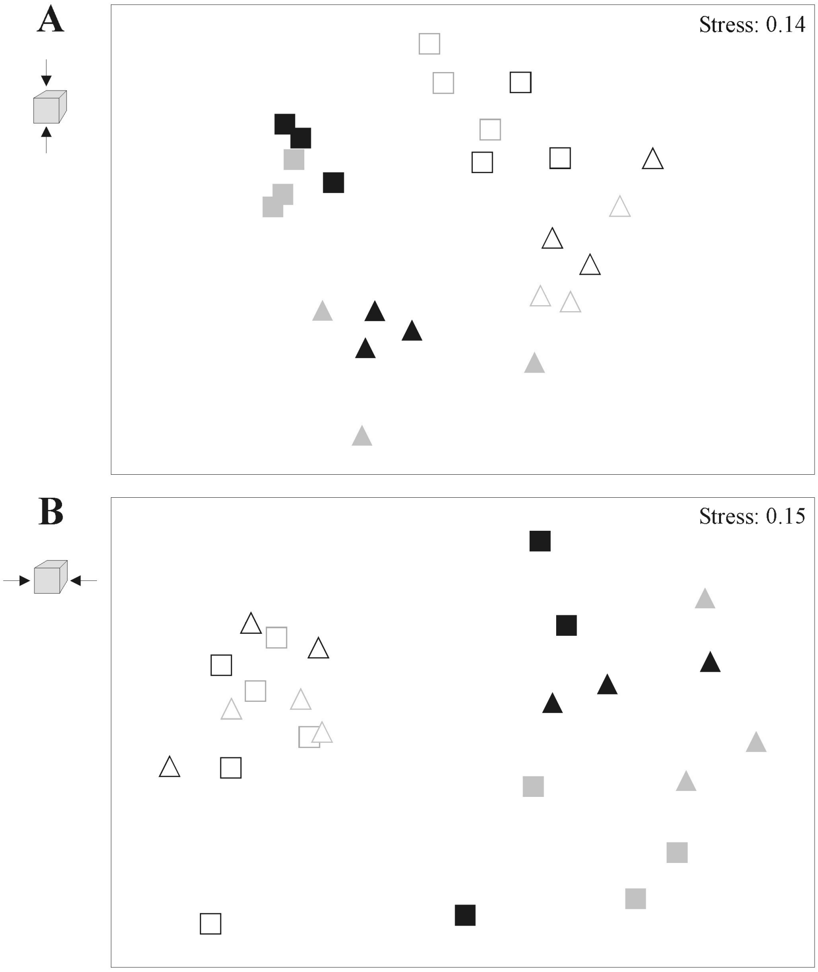

Figure 7. MDS ordination plots of the fourth root transformed species abundance data. Black symbols – A10 reef group; Grey symbols – A11 reef group; Non-filled symbols – 3 months of colonisation; Filled symbols – 6 months of colonisation. A – Top *vs.* Bottom surfaces; Square symbols – top surface; Triangles – bottom surface. B – Outside *vs.* Inside; Square symbols – outside surface; Triangles – inside surface.

Regarding horizontal surfaces, SIMPER analysis identified the bivalves *Hiatella arctica* (Linnaeus), *Musculus costulatus* (Risso), *Musculus* cf. *marmoratus* (Forbes) and undetermined Anomiidae, the amphipod *Microdeutopus versiculatus* (Bate) and the serpulids [und. Serpulidae, *Pomatocerus triqueter* (Linnaeus), *P. lamarckii* (Quatrefages), *Serpula concharum* Langerhans and *Serpula vermicularis* Linnaeus], as the species having the greatest contribution to the MDS ordination plot and ANOSIM results. Although most species were present on both horizontal surfaces, macrobenthic assemblages were significantly different especially due to the differences observed in the relative abundance. Top surfaces were characterised by *H. arctica*, *M. costulatus*, *M.* cf. *marmoratus*, und. Anomiidae and *Microdeutopus versiculatus*. In bottom surfaces, polychaete serpulids were the most characteristic *taxa*. Vertical surfaces presented a similar faunal composition, clearly dominated by serpulids and by *H. arctica*, especially on the inside surface. With submersion time increase, different species are present, such as the polychaetes *Capitella* spp.. Furthermore, the average abundance increases on all surfaces, except for serpulids and the amphipod *M. versiculatus* that after six months, were less abundant.

Discussion

Biodiversity on artificial reefs is related to different environmental and structural factors, such as morphological complexity of the reef, substratum composition, depth and distance from natural reef areas (Ardizzone *et al*., 1997). To evaluate the success of an artificial reef it is necessary to calculate its capacity as a feeding ground for demersal and rockfishes (Itosu *et al*., 1999). Thus, the efficiency of an artificial reef is related to biological colonisation, particularly biomass, species richness, number of ecological niches and food webs (Relini *et al*., 1994). The present study on the colonisation patterns of the "Faro/Ancão" artificial reef showed a general increase of the average abundance, number of species and diversity during the first six months of immersion. Three months after immersion the samples already presented 26-47 macrobenthic *taxa*, excluding colonial *taxa* and barnacles species. After sixth months of immersion 25-49 *taxa* were collected, whereas in the Loano reef (Ligurian sea, Italy), Relini *et al*. (1995) reported only 57 species after twelve months, including colonial *taxa* and barnacles. The top surface of the cubic samples presented higher number of species and mean abundance than the other surfaces in study. Similar patterns were also found by D'Anna *et al*. (2000) and by Glasby and Connell (2001). According to several other authors (Baynes *et al*., 1989; Ardizzone *et al*., 2000; Relini, 2000) the different exposure of each surface namely to light, currents and sedimentation strongly influences settlement and evolution patterns of benthic communities, arguing in favour of the present results. Time of submersion, however, appeared to be the main factor influencing the succession of these communities, with both artificial reef groups showing a similar pattern.

Vertical and bottom surfaces of the cubic samples have a similar faunal composition, with a clear dominance by serpulids after three months. D'Anna *et al*. (2000) observed that the early stages of the settlement are driven by encrusting forms like the serpulid worm *Pomatocerus triqueter*, a common species encroaching on clear surfaces. In that study the development of significant colonies of encrusting filter feeders, such as serpulids occurred inside the reef structure and in shaded niches. These organisms abundance decreased with time of submersion, and *Hiatella arctica* becomes the dominant species. These taxonomical groups are common colonizers of artificial reefs in many other artificial reef structures (e.g. Ardizzone *et al*., 2000; D'Anna *et al*., 2000). Contrasting with the results obtained for other European reefs, macroalgae were not found to colonize the reef during these first six months of study. Other studies in progress on the Algarve artificial reef system, addressing the comparison between different artificial reef groups, depth and temporal variation will hopefully provide important data for understanding the patterns of benthic colonisation on artificial substrata.

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Section 2.

Macrobenthic colonisation of artificial reefs on the southern coast of Portugal (Faro/Ancão, Algarve)

Boaventura, D.; Moura, A.; Leitão, F.; Carvalho, S.; Cúrdia, J.; Pereira, P.; Cancela da Fonseca, L.; Santos, M. N., and Monteiro, C. C. 2006. Macrobenthic colonisation of artificial reefs on the southern coast of Portugal (Ancão, Algarve). *Hydrobiologia*. 555(1):335-343.

Introduction

Artificial reefs are submerged structures placed on the seabed deliberately to imitate some characteristics of natural reefs, as defined by the European Artificial Reef Research Network (Jensen, 1997). Artificial reef systems are usually developed for fish exploitation, for protection of marine areas from illegal fisheries, and more recently, for preservation and rehabilitation of natural habitats (Pickering *et al*., 1998). These structures have been deployed worldwide and, according to Seaman and Sprague (1991), the major areas presenting this kind of activity include the Mediterranean and Caribbean Seas, South-eastern Asia, Japan, North America, Australia and some islands in the South Pacific. The expansion of this activity is related to the evolution of the structures and materials used in the reef's construction, as well as their purposes.

In Europe, the construction of artificial reefs started in the late 1960s. However, only in the 1980s and 1990s their use has increase significantly, especially in the Mediterranean (Allemand *et al*., 2000; Badalamenti *et al*., 2000; Barnabé *et al*., 2000; Moreno, 2000; Revenga *et al*., 2000).

In Portugal, seven artificial systems have been implanted along the southern coast of the Algarve over the last decade. The grounds for initiating this programme and for locating it on the southern coast of Portugal included the presence of several highly productive lagoon and estuarine systems in this region, the relative scarcity of natural reefs (especially on the south-eastern part of this coast), the high fishing intensity offshore, and the need to provide alternative means that would minimise the effect of fishing in order to yield a sustainable management of this coastal region (Monteiro and Santos, 2000). Research to date on these Portuguese artificial reefs has focussed mainly on fish populations, particularly those of economical importance (e.g. Santos, 1997; Santos and Monteiro, 1997, 1998). The present work aims to study the macrobenthic communities of the artificial reef structures, as these will enhance the food resources and shelter, making the reefs more attractive to fish. In particular, we aimed to experimentally analyse the sequence of colonisation of macrobenthic communities of the Faro/Ancão artificial reef system and to compare colonisation at two reef groups in surfaces with different orientation.

Material and methods

This study was carried out on the southern coast of Portugal (Algarve). Figure 1 shows the seven reef systems that were implanted on the southern coast since 1990. Two types of concrete modules were used in the Algarve reef systems: small modules (2.7 m^3) and large modules (174 m^3). These were deployed in different lines, the small modules being closer to the coast and at shallower depths than the large ones. The sequence of macrobenthic colonisation was experimentally analysed on the artificial reef system of Faro/Ancão (36º59’ N, 7 60’ W) (Figure 1). Several new reef groups were immersed at Faro/Ancão in the summer of 2002 (August) and the present work compared the patterns of colonisation between two small artificial reef groups (groups A10a and A11a) of 35 units each.

Figure 1. Study site. Location of artificial reefs on the southern coast of Portugal. The present study was conducted on the Faro/Ancão reef (indicated with number 4).

The study of colonisation of benthic communities was done using samples of concrete cubic units (15 x 15 cm) that were suspended at the small reef modules at a depth of approximately 20 m, at the time of the reef immersion. The cubic units were made from the same material used for the construction of the artificial reef modules. Four replicate samples were collected by scuba diving from two groups of the Faro/Ancão reef every 3 months from the starting date over a one-year period. Sampling was done using essentially non-destructive methods (point intersection) (see Hawkins and Jones, 1992 for a review) to assess the percentage cover of macrobenthic organisms in both vertical and horizontal surfaces. The percentage cover of sessile organisms was estimated with the intersection point method using a quadrat of 15 x 15 cm with 49 intersection points. The species present within the quadrat but which did not match any intersection point were recorded. In case of species overlapping sampling was stratified in different layers. The quadrats were photo-documented with a digital camera. Additionally, the different faces of the cubic samples were scrapped for *a posteriori* laboratory analysis. This manuscript is focused on the results from the non-destructive methods, i.e. percentage cover of sessile species and presence/absence data. The adopted methodology entailed sampling the species whose identification and quantification in quadrats was reliable. Very small organisms (e.g. amphipods) and/or highly motile species (e.g. crabs) were not quantified (see Hawkins and Jones, 1992).

Data analysis

The total percentage cover of replicate samples was analysed using anova. The factors tested were ‘reef groups’ (fixed, orthogonal, two levels) and ‘surfaces’ (fixed, orthogonal, two levels). Cochran’s C-test was used to check homogeneity of variance. Tests of homogeneity, anova and SNK (Student–Newman–Keuls) *a posteriori* comparison tests were done using GMAV5 for Windows Statistical Software (Institute of Marine Ecology, Sydney, Australia).

Results

Total percentage cover

Figure 2 shows the results of the total percentage cover of the top *vs.* bottom and outside *vs.* inside surfaces of the cubic samples, for the two studied reef groups. These results refer to the first 12 months after the reef immersion. The colonisation patterns for total percentage cover were similar at both studied reef groups (A10a and A11a).

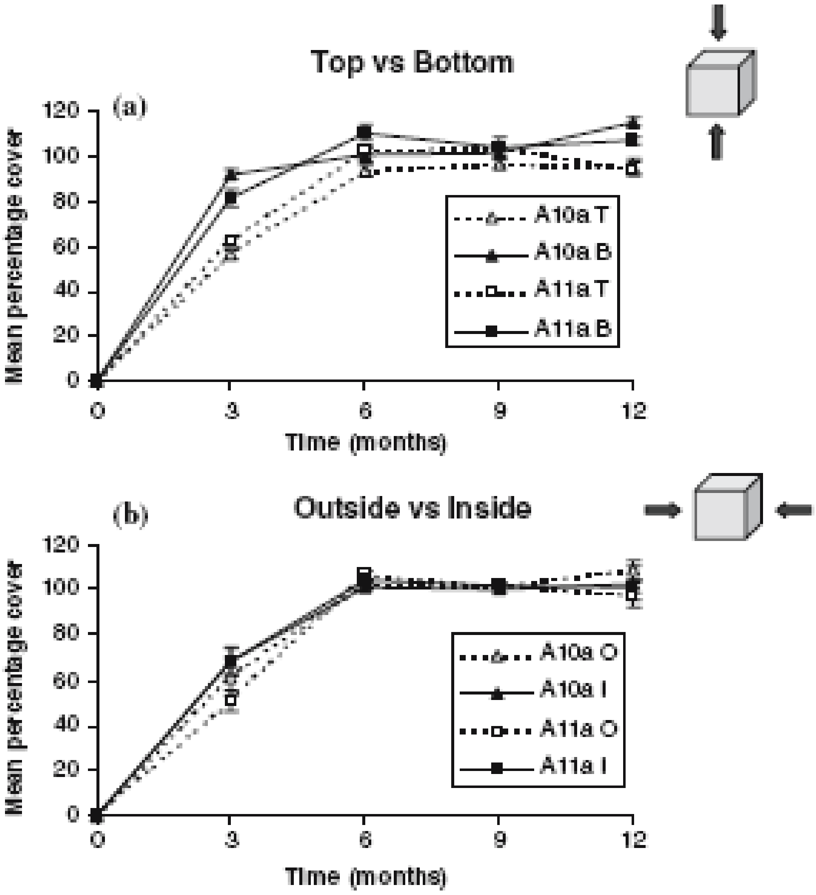
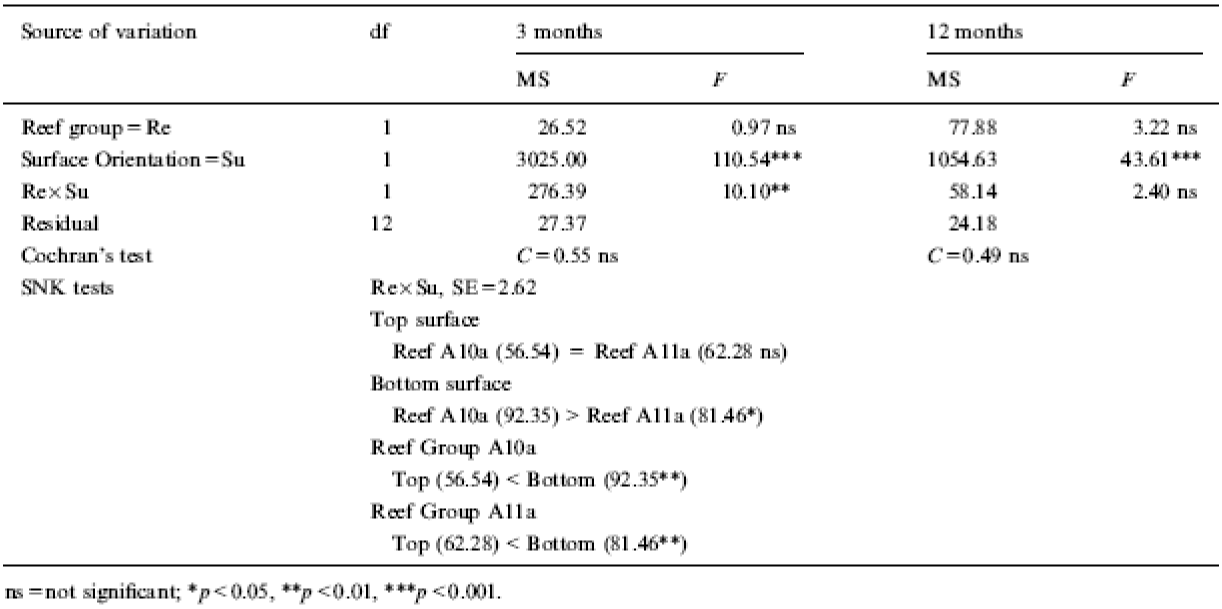


Figure 2. Mean percentage cover (±SE) on the two studied reef groups (A10a and A11a). (a) – Top (T) *vs.* Bottom (B) surfaces; (b) – Outside (O) *vs.* Inside (I) surfaces.

Three months after the beginning of the experiment the total cover exceeded 50% and after the sixth month the samples were totally colonised (values above 100% were obtained due to more than one layer of organisms). However, the comparison of the different cube surfaces revealed that the bottom surface had a higher colonisation than the top surface, particularly for the third and twelfth months, where these differences were significantly different (Table I). There was also a significantly higher total cover on the third month at the reef group A10a for the bottom surface (Table I, SNK test). No significant differences were observed on the total cover for the inside and outside surfaces (Table II).

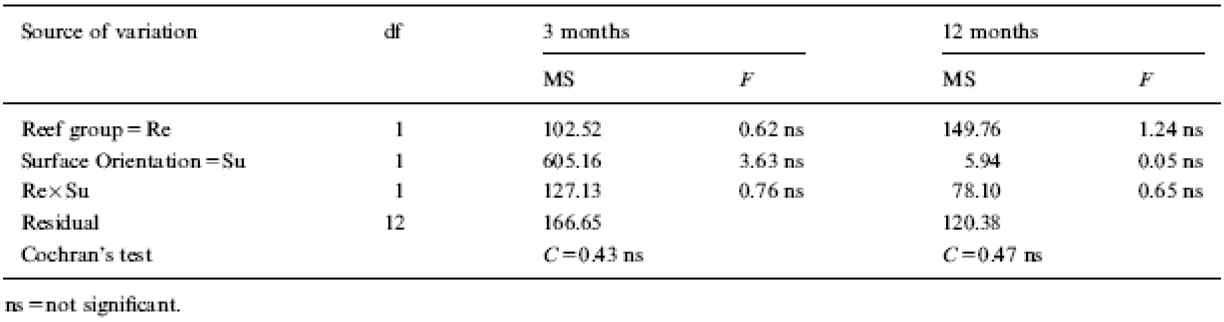
Table I. Results of ANOVA performed at months 3 and 12 for the top and bottom surfaces at the two studied reef groups.



Source of variation	df	3 months		12 months	
		MS	*F*	MS	*F*
Reef group = Re	1	26.52	0.97 ns	77.88	3.22 ns
Surface Orientation = Su	1	3025.00	110.54***	1054.63	43.61***
Re × Su	1	276.39	10.10**	58.14	2.40 ns
Residual	12	27.37		24.18	
Cochran's test		*C* = 0.55 ns		*C* = 0.49 ns	
SNK tests	Re × Su, SE = 2.62 Top surface Reef A10a (56.54) = Reef A11a (62.28 ns) Bottom surface Reef A10a (92.35) > Reef A11a (81.46*) Reef Group A10a Top (56.54) < Bottom (92.35**) Reef Group A11a Top (62.28) < Bottom (81.46**)				

ns = not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table II. Results of ANOVA performed at months 3 and 12 for the outside and inside surfaces at the two studied reef groups.



Source of variation	df	3 months		12 months	
		MS	*F*	MS	*F*
Reef group = Re	1	102.52	0.62 ns	149.76	1.24 ns
Surface Orientation = Su	1	605.16	3.63 ns	5.94	0.05 ns
Re × Su	1	127.13	0.76 ns	78.10	0.65 ns
Residual	12	166.65		120.38	
Cochran's test		*C* = 0.43 ns		*C* = 0.47 ns	

ns = not significant.

Major taxonomical groups

Cirripedia, Serpulidae, Bryozoa and Ascidiacea were the major sessile taxonomical groups to colonise the cubic samples (Figure 3 and 4) at both reef groups and for the different surfaces. Barnacles clearly dominated the samples throughout the year. Barnacle cover was already higher than that of other groups by the third month and continued to increase until the sixth month. There was a general decline in barnacle cover after the sixth month associated with an increase of other taxonomical groups such as bryozoans. This pattern was similar for both reef groups and for most of the surface orientations. The only exception was the higher mean percentage cover of barnacles found on the bottom surface on the third month with values of 76.6% and 68.1% for reef group A10a and A11a, respectively. These results conform to the significant differences obtained for the total percentage cover. Serpulids showed, in general, a small peak at the third month but mean percentage cover did not exceed 20%. The ascidians were more abundant on the bottom surface, which was less exposed to the light.

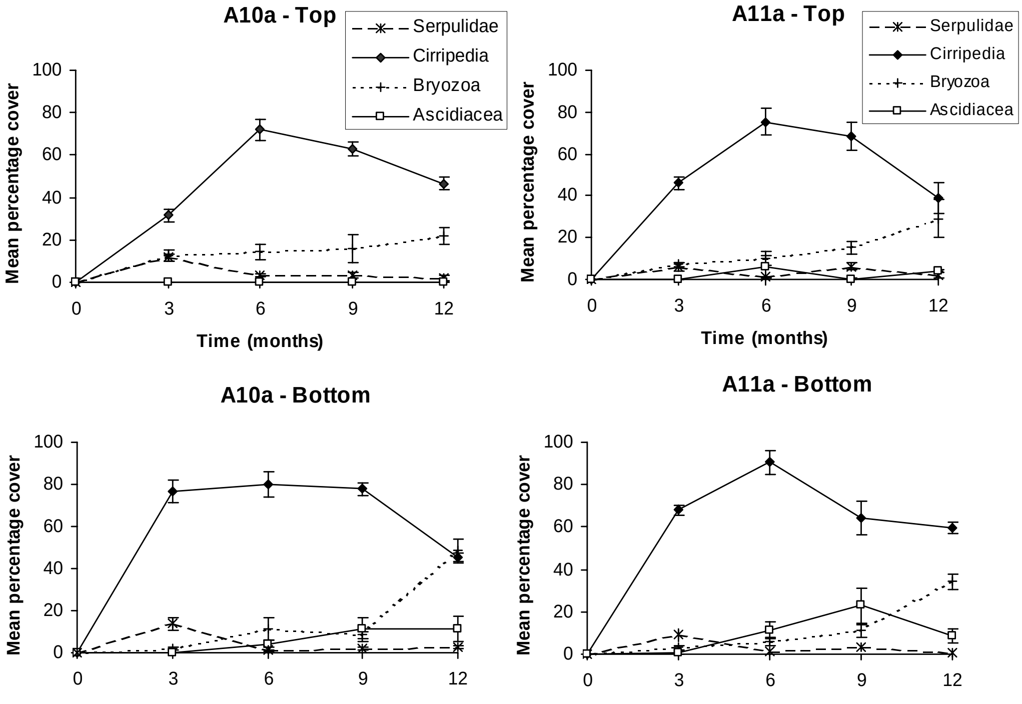


Figure 3. Major taxonomical groups at the two studied reef groups, Top *vs.* Bottom surfaces.

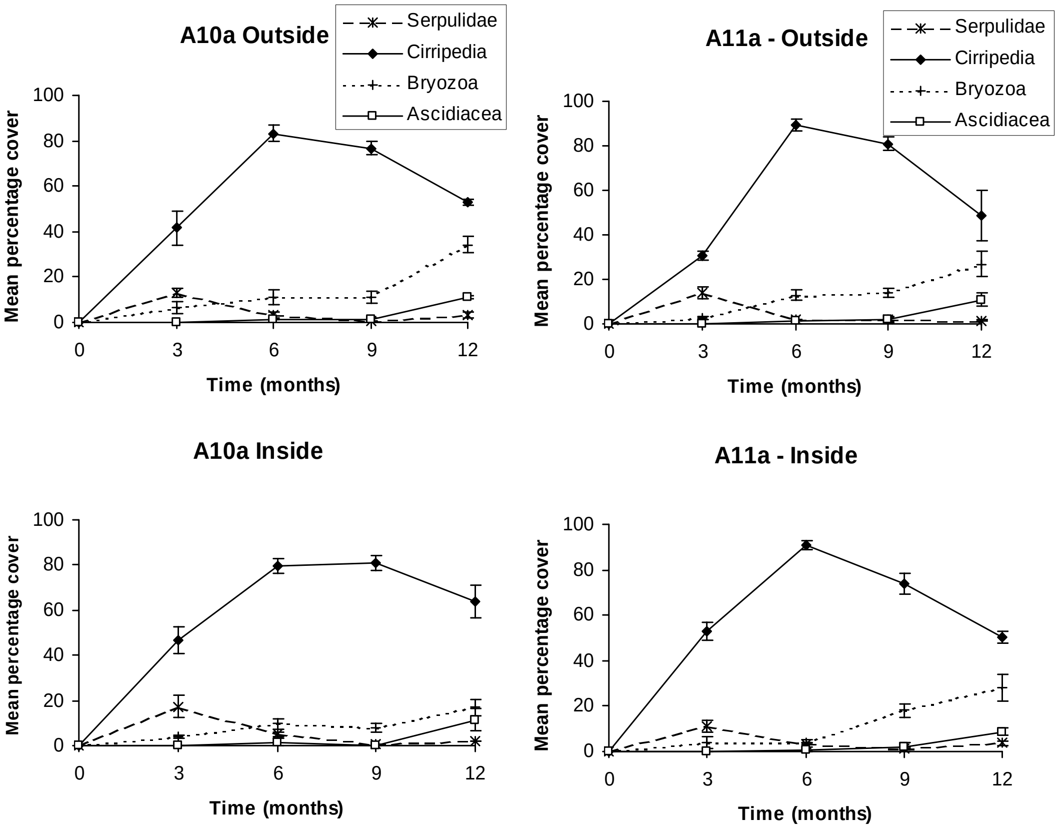


Figure 4. Major taxonomical groups at the two studied reef groups, Outside *vs.* Inside surfaces.

Other sessile groups such as Porifera, Hydrozoa, Anthozoa, and other sessile Polychaeta showed a generally low percentage cover which had increased after the sixth month of the experiment to 5–10%, particularly on the top surface where cover reached 20% (Figure 5). The list of identified species to date from the scrapped surfaces of the samples is given in the Appendix A. In general, non-sessile organisms (e.g. decapods, gastropods, bivalves) were also observed to increase after the sixth month but their densities have not been processed for this article.

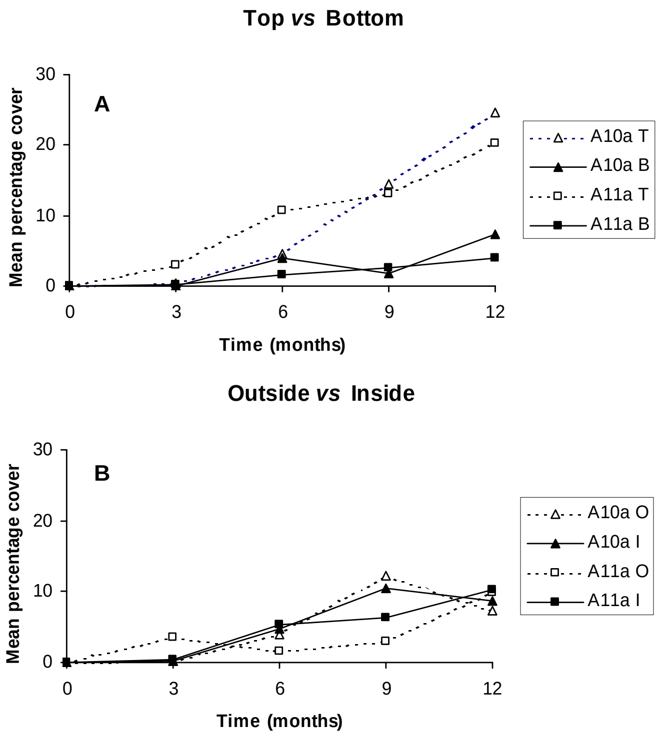


Figure 4. Major taxonomical groups at the two studied reef groups, Outside *vs.* Inside surfaces.

Discussion

Previous studies of artificial reefs have focussed mostly on aspects of fishery ecology; however, to manage and understand these artificial habitats it is essential to integrate all aspects of the hard substratum ecology (see Svane and Petersen, 2001 for a review). The present study has provided information on the patterns of macrobenthic colonisation of artificial reefs on the southern Portuguese coast. Two reef groups at the Faro/Ancão artificial reef system were analysed and colonisation was compared between surfaces with different orientation. The replicate cubic units used in this work were made with the same material of the reef structure (concrete) and were suspended at the reef blocks at the time of the reef immersion to ensure that the colonisation patterns would be representative of the reef structure. Three months after immersion more than half of the area of the samples was colonised by macrobenthic species and after the sixth month the entire surface was covered. Sessile encrusting organisms such as barnacles, bryozoans, serpulids and ascidians colonised the cubic samples with a clear dominance of barnacles. These taxonomical groups have been seen to colonise artificial reefs in many other artificial reef structures (e.g. Cummings, 1994; Relini *et al*., 1994; Badalamenti *et al*., 2000; D’Anna *et al*., 2000; Relini, 2000). Despite the general presence of these taxonomical groups in the first year of colonisation of artificial substrata, the sequence of macrobenthic colonisation varied with seasons or places in several studies conducted in the Mediterranean (e.g. Badalamenti *et al*., 2000; Relini, 2000). In the Faro/Ancão reef similar patterns of colonisation were obtained at the two studied reef groups. The heavy initial settlement of barnacles matches the observations of Cummings (1994) for the colonisation of an artificial reef in Florida. The barnacle cover declined at Faro/Ancão six months after immersion and a more heterogeneous community was established.

Surfaces with different orientation showed different colonisation patterns at both reef groups of the Faro/Ancão system. This result is consistent with previous work (e.g. D’Anna *et al*., 2000; Glasby and Connell, 2001), which showed that different assemblages developed on surfaces of different orientation. In this study the bottom surface of the cubic units revealed a significantly higher percentage of colonisation possibly related to lower sedimentation levels, particularly with respect to barnacle cover. Ascidians were also more abundant on this surface, which was less exposed to the light. Similar results were found by Hatcher (1998) in the Poole Bay Artificial Reef (UK), where the epibenthic biomass was seen to be greater on the bases of the samples than on the top of the samples, throughout the study.

In contrast with the results obtained for other European reefs (e.g. Hatcher, 1998; D’Anna *et al*., 2000; Relini, 2000), macroalgae were absent in our study during the first year of colonisation. Several explanations can be offered for this fact. Our study was done at 20 m depth and the turbidity of the water was generally high. Most of the studies where macroalgae were present during the first year were done at shallower depths and clearer waters (e.g. D'Anna *et al.*, 2000; Relini, 2000). Marked differences in colonisation stages were described by D'Anna *et al*. (2000) for clear and turbid waters, showing a major effect of light on algal cover. Ongoing studies on the comparison among different reefs on the southern coast, however, seem to indicate that both older artificial reef structures and artificial reefs which are closer to natural rock substrata have macroalgae (see also Monteiro and Santos, 2000). It is possible that macroalgae species will start to colonise the Faro/Ancão reef in later years. Other studies in progress at this region comparing different reefs, depths, and temporal variation will enable to clarify this and other points of colonisation patterns.

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Appendix A

Table A.1. List of identified *taxa* (Und.=Undetermined).

PORIFERA	
CNIDARIA	
Hydrozoa	Und. Hydrozoa
Anthozoa	Und. Anthozoa
PLATYHELMINTHES	
Turbellaria	Und. Turbellaria
NEMERTEA	
NEMATA	
ANNELIDA	
POLYCHAETA	
Polynoidae	*Malmgrenia ljungmani* (Malmgren, 1865)
	Und. Polynoidae
Pholoidae	*Pholoe synophtalmica* Claparède, 1868
Chrysopetalidae	Und. Chrysopetalidae
Phyllodocidae	Und. Phyllodocidae
Hesionidae	*Syllidia armata* Quatrefages, 1865
	Podarke sp.
	Und. Hesionidae
Syllidae	*Autolytus* spp.
	Autolytus cf. *alexandri* Malmgren, 1874
	Autolytus cf. *brachycephalus* (Marenzeller, 1874)
	Autolytus langerhansi Gidholm, 1967
	Proceraea sp.
	Pseudobrania limbata (Claparède, 1868)
	Sphaerosyllis cryptica Ben-Eliahu, 1977
	Sphaerosyllis hystrix Claparède, 1863
	Sphaerosyllis taylori Perkins, 1981
	Exogone naidina Oersted, 1845
	Exogone verugera Claparède, 1868
	Syllides articulocirratus (Gillandt, 1979)
	Pionosyllis sp.
	Syllis spp.
	Typosyllis hyalina (Grube, 1863)
	Typosyllis cf. *variegata* (Grube, 1860)
	Typosyllis spp.
	Und. Syllidae
Nereididae	*Websterinereis glauca* (Claparède, 1870)
Lumbrineridae	*Lumbrineris* sp.
Dorvilleidae	Und. Dorvilleidae

Table A.1. (Continued)

	Spionidae	*Polydora* spp.
	Chaetopteridae	*Chaetopterus variopedatus* (Renier, 1804)
	Ophellidae	*Aphelochaeta* sp.
	Capitellidae	*Capitella* spp.
	Ampharetidae	Und. Ampharetidae
	Terebellidae	Und. Amphitritinae
		Und. Terebellidae
	Sabellaridae	*Sabellaria* sp.
	Sabellidae	Und. Sabellidae
	Serpulidae	*Serpula concharum* Langerhans, 1880
		Serpula vermicularis Linnaeus, 1767
		Vermiliopsis cf. *monodiscus* Zibrowius, 1967
		Pomatocerus triqueter (Linnaeus, 1767)
		Pomatocerus lamarckii (Quatrefages, 1865)
		Hydroides cf. *norvegica* Gunnerus, 1768
		Hydroides cf. *nigra* Zibrowius, 1971
		Hydroides stoichadon Zibrowius, 1971
		Filograna sp.
		Protula sp.
		Filogranula cf. *stellata* (Southward, 1963)
		Und. Serpulidae
ARTHROPODA		
CRUSTACEA		
CIRRIPEDIA		
	Balanidae	*Balanus amphitrite* Darwin, 1854
		Megabalanus tulipiformis (Ellis, 1758)
OSTRACODA		
COPEPODA		
AMPHIPODA		
	Stenothoidae	*Stenothoe c*f. *valida* Dana, 1855
	Aoridae	*Aora gracilis* (Bate, 1857)
		Microdeutopus versiculatus (Bate, 1856)
		Microdeutopus armatus Chevreux, 1887
	Isaeidae	*Gammaropsis maculata* (Johnston, 1827)
	Corophiidae	*Corophium sextonae* Crawford, 1937
		Ericthonius brasiliensis (Dana, 1855)
	Ischyroceridae	*Jassa marmorata* Holmes, 1903
	Caprellidae	*Caprella* sp.
		Caprella acanthifera Leach, 1814
		Phtisica marina Slabber, 1769
TANAIDACEA		
	Apseudidae	*Apseudes talpa* (Montagu, 1808)
		Und. Tanaidacea
ISOPODA		
	Gnathiidae	Und. Gnathiidae
	Anthuridae	Und. Anthuridae
	Munnidae	Und. Munnidae
	Janiridae	*Janira maculosa* Leach, 1814
		Und. Isopoda
DECAPODA		
CARIDEA		
	Hippolytidae	*Hippolyte longirostris* (Czerniavsky, 1868)
		Hypolite varians Leach, 1814
		Thoralus cranchii (Leach, 1817)
		Eualus occultus (Lebour, 1936)

Table A.1. (Continued)

	Eualus sp.
	Und. Caridea
ANOMURA	
Galatheidae	*Galathea intermedia* Lilljeborg, 1851
Porcellanidae	*Pisidia* cf. *bluteli* (Risso, 1816)
BRACHYURA	
Majidae	*Inachus leptochirus* Leach, 1817
Xanthidae	*Pilumnus* cf. *hirtellus* (Linnaeus, 1761)
	Und. Decapoda
PYCNOGONIDA	Und. Pycnogonida
MOLLUSCA	
GASTROPODA	
Trochidae	*Jujubinus exasperatus* (Pennant, 1777)
Rissoidae	cf. *Pusillina sarsii* (Lovén, 1846)
	Alvania sp.
Cerithiidae	*Bittium* spp.
Muricidae	*Ocinebrina aciculata* (Lamarck, 1822)
	Muricidae sp.
Nassariidae	*Nassarius* cf. *pygmaeus* (Lamarck, 1822)
Pyramidellidae	*Chrysallida* spp.
	Odostomia spp.
Cylichnidae	cf. *Scaphander* sp.
Limapontiidae	cf. *Limapontia depressa* Alder & Hancock, 1862
	Und. Gastropoda
BIVALVIA	
Mytilidae	*Modiolus modiolus* (Linnaeus, 1758)
	Musculus costulatus (Risso, 1826)
	Musculus discors (Linnaeus, 1767)
Pinnidae	Und. Pinnidae
Pectinidae	Und. Pectinidae
Anomiidae	Und. Anomiidae
Ostreidae	Und. Ostreidae
Cardiidae	Und. Cardiidae
Tellinidae	*Tellina* sp.
Hiatellidae	*Hiatella arctica* (Linnaeus, 1767)
	Und. Bivalvia
BRYOZOA	
CYCLOSTOMATA	
Crisiidae	*Crisia ramosa* Harmer, 1891
CHEILOSTOMATA	
Scrupariidae	*Scruparia chelata* (Linnaeus, 1758)
Bugulidae	*Bugula neritina* (Linnaeus, 1758)
Umbonulidae	*Umbonula ovicellata* Hastings, 1944
Schizoporellidae	*Schizobrachiella sanguinea* (Norman, 1868)
ECHINODERMATA	
OPHIUROIDEA	
	Und. Ophiuroidea
ECHINOIDEA	
	Und. Echinoidea
CHORDATA	
UROCHORDATA	
Styelidae	*Botryllus schlosseri* (Pallas, 1766)
	Und. Urochordata

Section 3.

Is surface orientation determinant for colonisation patterns of vagile and sessile macrobenthos in artificial reefs?

Moura, A.; Cancela da Fonseca, L.; Cúrdia, J.; Carvalho, S.; Boaventura, D.; Cerqueira, M.; Leitão, F.; Santos, M. N., and Monteiro, C. C. 2008. Is surface orientation a determinant for colonisation patterns of vagile and sessile macrobenthos on artificial reefs? *Biofouling* 24(5):381-391.

Introduction

Over the years, artificial reefs (ARs) have been used for different purposes, including the prevention of trawling, increase of fishery yield and production, as well as for recreational diving, coastal protection and biodiversity conservation (Baine, 2001). The deployment of ARs provides a vacant hard substratum, which is colonised primarily by settling larvae and spores of a large number of epibenthic organisms. The ARs supply not only shelter for motile organisms, but also the hard surfaces required for the attachment of sessile invertebrates (Qiu *et al*., 2003). Therefore, ARs can supply a potential food resource via their associated fauna and also provide shelter for invertebrates and juvenile fish from natural predators. Nevertheless, the processes of colonisation and succession of these structures are not clearly understood and still poorly documented (Underwood and Chapman, 2006). Previous studies regarding the colonisation patterns of epibiota on ARs showed that the physical and biological environments strongly influence the subsequent recruitment, colonisation, succession and development (Eckman, 1983; Le Tourneux and Bourget, 1988; Roughgarden *et al*., 1988; Baynes and Szmant, 1989; Mullineaux and Butman, 1991). Surface characteristics such as spatial orientation, structural complexity, substratum composition and texture are known to affect the settlement of benthic invertebrates onto natural and artificial substrata (Jacobi and Langevin, 1996; Qiu *et al*., 2003; Brown, 2005; Bulleri, 2005a; Perkol-Finkel *et al*., 2006). In particular, surface orientation is described as a major determinant of habitat heterogeneity (Bourget *et al*., 1994; Glasby, 2000; Glasby and Connell, 2001; Bulleri and Chapman, 2004), greatly influencing the structure of epibiotic assemblages (Knott *et al*., 2004).

In the study of hard substratum communities, the main difficulty is to combine in a single scale, both solitary and colonial organisms (Qiu *et al*., 2003; Beaumont *et al*., 2007), and it is becoming necessary to use methods that provide a sensitive, accurate and robust estimate of the community structure (Beaumont *et al*., 2007). Despite the great variety of techniques used in the study of epifaunal communities, comparative evaluation of or between the techniques used is scarce (Beaumont *et al*., 2007). The abundance of epibiotic organisms is a precise variable but it is time-consuming. Furthermore, it can be applied only to solitary organisms and not to algae or colonial organisms (Pamintuan *et al*., 1994; Bulleri, 2005b). This limitation is crucial for the study of sessile benthos, as these organisms are dominant. One of the most common methods used for the estimation of abundance is the percentage cover sampling technique (Woodhead and Jacobson, 1985; Nelson *et al*., 1994; Hatcher, 1997, 1998; Glasby, 1999; Boaventura *et al*., 2006), which is an immediate method, although not suitable for mobile or less conspicuous fauna. The other common technique is biomass (Hatcher, 1995, 1997, 1998; Relini and Relini, 1997 and references therein; Qiu *et al*., 2003; Moura *et al*., 2006) which, although laborious, allows solitary and colonial organisms to be compared on the same scale.

The present study investigated how surface orientation (vertical orientation: outside *vs.* inside surfaces and horizontal orientation: top *vs.* bottom surfaces) affects the structure of epibenthos of Faro/Ancão AR during the first year of colonisation. Moreover, as both components (the sessile and vagile fauna) of hard substratum macrofaunal communities have different ecological patterns, this study also analyses whether those components are affected differently by surface orientation.

Material and methods

The present work was carried out on the ‘‘Faro/Ancão’’ AR system spread over an area of 12.2 km^2, located off Faro (Algarve, southern Portugal) on a sandy bottom (Figure

1A). The system is composed of small (2.7 m^3) and large (174 m^3) concrete modules. The small modules are used to build 21 AR groups (Figure 1B). Each AR group comprises three reef sets of 35 modules. Each reef group is arranged roughly as a triangle, with reef sets distanced by 70 m (Figure 1B). In each reef set, the modules were haphazardly arranged, comprising two layers (Figure 1C). This study was performed on two randomly selected AR groups submerged on August 2002 at 20 m depth.

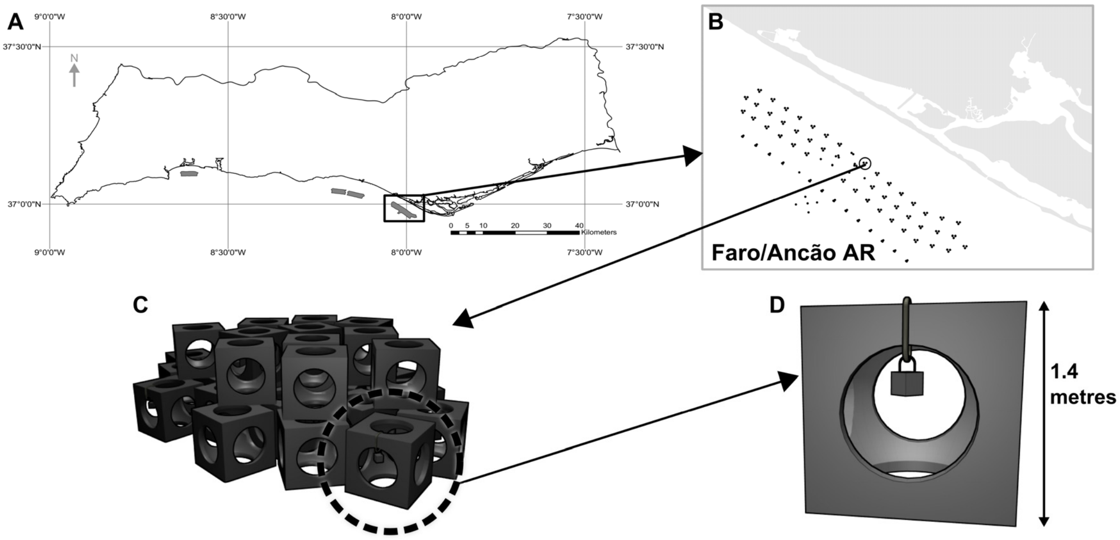

Figure 1. A – Map of the Algarve region with seven artificial reef systems (in grey); the artificial reef system of Faro/Ancão is shown within a box to individualise it. B – Faro/Ancão artificial reef system arrangement, each dot corresponds to a set of 35 concrete modules. C – Diagram showing the arrangement of the cubic modules that form a reef set; cubic sampling units are suspended randomly in the lower layer of modules. D – Reef module with suspended cubic sampling unit, used in the present work.

The study of macrobenthic colonisation was performed using cubic sample units (15 cm side length) made of the same concrete material as the reef modules. The cubic sample units were set randomly at the time of the reef immersion on the lower layer of the AR. As a consequence of their weight and their setting on the reef modules, the cubic units were firmly anchored to the reef modules, therefore ensuring an upright vertical position, even during rough weather. This vertical position results in different faces of the cube, namely two horizontal faces, (top and bottom of the cube) and four vertical faces (two pointing inside and two pointing outside the AR module) (see Figure 1D). During the first year of immersion, three replicate samples were retrieved from each reef group at 3 m intervals by scuba diving. However, only the 3- and 12-month immersion samples were analysed for this study. In the laboratory, the percentage cover of sessile organisms was estimated using point intersection methods (see Boaventura *et al*., 2006). Additionally, the different surfaces of the cubic samples were scraped to analyse macrobenthic colonisation and succession. Four out of the six cube surfaces (vertical: inside and outside; horizontal: top and bottom) were compared during the study to analyse the role of surface orientation on benthic colonisation. The samples were sieved through a 0.5 mm square mesh and the material retained was fixed in 4% buffered formalin. The material collected was sorted and identified to species level whenever possible. The biomass of each species was obtained for biological samples dried to a constant weight at 70ºC (usually for at least 24–48 h). The ash-free dry weight (AFDW) was determined by burning the animals at 450ºC for 4 h in a muffle furnace. The AFDW was calculated by subtracting the ash weight from the dry weight.

Data analysis

For the surface orientation tests, horizontal and vertical surfaces were not compared in a single analysis to ensure that only non-adjacent surfaces were compared. The design included a two-way ANOVA for the influence of surface orientation: ‘‘reef groups’’ (orthogonal, fixed with two levels: A10 and A11) and ‘‘reef surfaces’’ (orthogonal, fixed with two levels: top and bottom surfaces or outside and inside surfaces) for the number of *taxa*, the abundance and biomass data for the vagile fauna and the number of *taxa* and biomass data for the sessile fauna. The homogeneity of variance was checked with Cochran’s C-test and data were transformed when this ANOVA assumption failed

(Underwood, 1997). Student-Newman-Keuls (SNK) *a posteriori* comparison tests were used, when significant differences were detected.

In order to detect differences in macrobenthic structure on different surface orientations and reef group multivariate analyses were also used (PRIMER v.5.0 software package for multivariate analyses; Clarke and Warwick, 1994). The Bray–Curtis similarity index after fourth root transformation was applied for comparing the samples (Clarke, 1993). Similarities between faunal data were analysed by an ordination technique (non-metric multidimensional scaling - MDS) that was produced from the similarity matrices for the sessile and vagile fauna components, using biomass and abundance data, respectively. Two-way crossed ANOSIM (analysis of similarities) tests were applied to assess the significance of differences in macrobenthic colonisation patterns with respect to different surface orientation and reefs. The SIMPER (similarity percentages) routine of fourth-root transformed data was applied in order to obtain the contribution of each *taxon* to the dissimilarities between different reefs and surface orientation. When no differences were detected, the SIMPER routine was used to identify the characteristic *taxa* of each surface orientation or reef group.

Results

Invertebrate species from 16 phyla colonised the cubic units. The majority of the benthic *taxa* were polychaetes (108 *taxa*), followed by crustaceans (42 *taxa*) and molluscs (36 *taxa*).

Vertical surfaces

In general, the abundance, biomass and the number of *taxa* increased throughout the study period, with similar values between the outside and inside surfaces. Considering both ARs analysed for the sessile fauna, 3 months after AR deployment, between 16 and 26 *taxa* were identified on the outside surface, with biomass varying from 0.21–0.85 g × 0.0225 m^{-2}. On the other hand, the inside surface presented between 13 and 27 *taxa* with biomass of 0.23–1.0 g × 0.0225 m^{-2}. One year after the beginning of the study, the number of *taxa* on the outside samples ranged between 39 and 49 and biomass varied between 2.12 and 3.75 g × 0.0225 m^{-2}. The inside surface showed 33–49 *taxa* and biomass values varied between 1.52 and 3.33 g × 0.0225 m^{-2}. Nevertheless, the outside and the inside surfaces displayed no significant differences for biomass and number of *taxa* for the sessile fauna (Table I).

Table I. Results of the two-way ANOVA on biomass (B) and number of *taxa* (S) obtained on vertical surfaces (outside and inside) in two reef groups (A10 and A11) for sessile fauna assemblages during the study period (3 and 12 months).

Source of variation	df	B		S	
		MS	F	MS	F
3 months					
Reef group = Re	1	0.035153	0.58 ns	147.00	16.64 **
Surface Orientation = Su	1	0.007614	0.13 ns	5.33	0.60 ns
Re x Su	1	0.145718	2.41 ns	0.00	0.00 ns
Residual	8	0.060292		8.83	
Cochran's test		*C* = 0.45 ns		*C* = 0.41 ns	
SNK tests				Reef A11 > A10 **	
12 months					
Reef group = Re	1	0.55288	1.43 ns	4.08	0.12 ns
Surface Orientation = Su	1	0.55282	1.43 ns	36.75	1.09 ns
Re x Su	1	0.07094	0.18 ns	18.75	0.56 ns
Residual	8	0.38742		33.67	
Cochran's test		*C* = 0.53 ns		*C* = 0.54 ns	

ns = not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

Between 8 and 22 *taxa* were identified and 68–168 ind. × 0.0225 m^{-2} were counted in the vagile fauna in the first sampling period on the outside surfaces. The inside surfaces showed between 10 and 19 *taxa* with abundances of 55–168 ind. × 0.0225 m^{-2}. On both surfaces, biomass values reached a maximum of 0.01 × 0.0225 m^{-2}. After 12 months immersion, between 35 and 43 *taxa* were identified on the outside surface with abundance values of 1064 and 2228 ind. × 0.0225 m^{-2}. Biomass values varied between 0.09 and 0.25 g × 0.0225 m^{-2}. On the inside surfaces, between 30 to 40 *taxa* were identified, with abundance values of 536 and 1756 ind. × 0.0225 m^{-2}. The biomass values of these organisms varied between 0.05 and 0.19 g × 0.0225 m^{-2}. Nonetheless, no significant differences for abundance, biomass and number of *taxa* were observed (Table II). This pattern was observed for all sampling periods. In general, differences within reefs were also not significant with the exception of one period, after 3 months immersion, when reef A11 presented a higher number of sessile *taxa* than reef A10.

Table II. Results of the two-way ANOVA on abundance (Abd), biomass (B), and number of *taxa* (S) obtained on vertical surfaces (outside and inside) in two reef groups (A10 and A11) for vagile fauna assemblages during the sampling period (3 and 12 months).

Source of variation	df	Abd		B		S	
		MS	F	MS	F	MS	F
3 months							
Reef group = Re	1	5676.8	3.81 ns	0.000007	1.07 ns	102.08	4.77 ns
Surface Orientation = Su	1	520.1	0.35 ns	0.000003	0.44 ns	6.75	0.31 ns
Re x Su	1	184.1	0.12 ns	0.000018	2.61 ns	2.08	0.10 ns
Residual	8	1489.7		0.000007		21.42	
Cochran's test		*C* = 0.54 ns		*C* = 0.48 ns		*C* = 0.76 ns	
12 months							
Reef group = Re	1	340033	1.63 ns	0.000276	0.07 ns	18.75	1.87 ns
Surface Orientation = Su	1	712481	0.10 ns	0.004693	1.15 ns	18.75	1.87 ns
Re x Su	1	47628	0.64 ns	0.000238	0.06 ns	0.75	0.07 ns
Residual	8	208420		0.004078		10.00	
Cochran's test		*C* = 0.44 ns		*C* = 0.47 ns		*C* = 0.32 ns	

ns = not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

Additionally, the two-way crossed ANOSIM showed no significant differences between reefs or between the vertical surfaces for vagile and sessile fauna (Table III). The MDS plot on vagile and on sessile benthos confirmed the ANOSIM results; no differentiation was observed between the vertical surfaces and reef groups during the study year (Figure 2A and B).

Table III. Two way crossed ANOSIM results (R values) of epifaunal assemblage structure for vagile and sessile fauna obtained of vertical and horizontal surfaces in two reef groups (A10 and A11) during the sampling period (3 and 12 months).

	Vertical surfaces		Horizontal surfaces	
Fauna	Vagile Abundance	Sessile Biomass	Vagile Abundance	Sessile Biomass
3 months				
Reef group	- 0.056 (70)	0.278 (10)	- 0.037 (61)	0.185 (8)
Surface Orientation	- 0.185 (91)	0 (55)	0.537 (1)	0.37 (3)
12 months				
Reef group	0.185 (13)	0.13 (27)	0.5 (4)	0.074 (35)
Surface Orientation	- 0.056 (61)	- 0.074 (66)	0.963 (1)	0.796 (1)

Abundance and biomass data was used for vagile fauna and sessile fauna respectively. Significance of R values is presented in brackets (%).

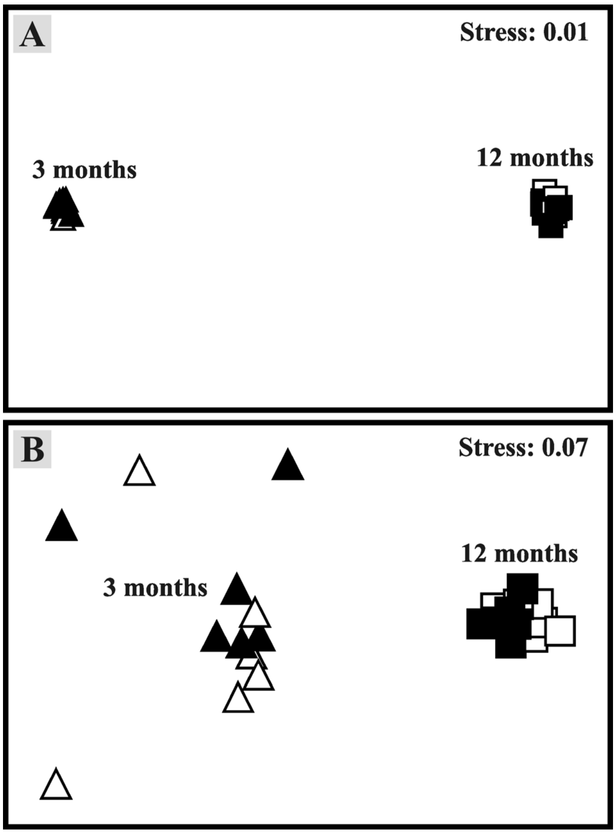

Figure 2. MDS ordination plots for vertical surfaces over one year after AR deployment. A – sessile fauna; B – vagile fauna. [△,▲] – 3 months; [☐,■] – 12 months; open symbols – outside surface; filled symbols – inside surface.

According to the SIMPER results (Table IV), after 3 months immersion, the sessile macrofaunal community was characterised by cirripeds, namely *Balanus amphitrite* Darwin, 1854, the bivalves *Hiatella arctica* (Linnaeus, 1767) and *Modiolarca* cf. *subpicta* (Cantraine, 1835), and by serpulids, mainly *Pomatoceros triqueter* (Linnaeus, 1767). The mobile benthic community was mainly characterised by the crustaceans *Microdeutopus gryllotalpa* Costa, 1853 and *Achelia longipes* Hodge, 1864, the Autolytinae nd. (not identified to species level) polychaetes and the gastropod *Pusillina inconspicua* (Alder, 1844).

Table IV. Species identified by SIMPER routine as having a high contribution to the average similarity within the outside and inside surfaces.

		Sessile benthos		Vagile benthos	
		Taxa	Contrib%	Taxa	Contrib%
3 months					
	Outside	Cirripedia	28.23	*Microdeutopus gryllotalpa*	16.23
		Pomatoceros triqueter	9.09	Autolytinae nd	12.29
		Modiolarca cf. *subpicta*	8.36	*Pusillina inconspicua*	8.7
				Achelia longipes	7.92
	Inside	Cirripedia	29.51	*Microdeutopus gryllotalpa*	16.48
		Hiatella arctica	8.9	*Pusillina inconspicua*	12.05
		Modiolarca cf. *subpicta*	8.64	*Achelia longipes*	10.12
				Autolytinae nd	9.69
12 months					
	Outside	Cirripedia	19.67	Nematoda	7.26
		Polydora hoplura	9.21	*Corophium* spp.	6.79
		Ascidiacea	9.11	*Paleanotus bellis*	6.16
		Anthozoa	8.3	*Syllidia armata*	5.4
				Pisidia cf. *bluteli*	4.95
				Achelia longipes	4.04
				Eumida sanguinea	4.04
				Autolytinae nd	3.82
				Nemertea	3.77
	Inside	Cirripedia	19.29	*Corophium* spp.	6.33
		Ascidiacea	9.13	Nematoda	6.27
		Bryozoa	9	*Syllidia armata*	5.74
		Polydora hoplura	8.93	*Paleanotus bellis*	5.57
				Pisidia cf. *bluteli*	4.89
				Syllis hyalina	4.25
				Achelia longipes	4.25
				Thalassema spp.	4.16
				Eumida sanguinea	4.08

Abundance and biomass data were used for vagile and sessile fauna, respectively. Data are presented for each sampling date

One year after AR deployment, cirripeds remained dominant but the polychaete *Polydora hoplura* Claparede, 1870 was also a characteristic species of the sessile fauna on this surface orientation. The increase in *P. hoplura* may be due to the increase in the number and size of *Megabalanus tulipiformis* (Ellis, 1758), whose walls are excavated by this polychaete (personal observation). For the sessile organisms, bryozoans and ascidians were also typical of the vertical surfaces. The nematodes, the polychaetes *Syllidia armata* Quatrefages, 1865 and *Paleanotus bellis* (Johnson, 1897), the decapod *Pisidia* cf. *bluteli* (Risso, 1816) and the amphipod *Corophium* spp. were important vagile macrofauna.

Horizontal surfaces

In general, the abundance, biomass and number of *taxa* increased throughout the study period, with top surfaces exhibiting higher values than bottom surfaces. In particular, after 3 months, the vagile fauna presented abundance values between 44 and 156 ind. × 0.0225 m^{-2} on the top surface, whilst the bottom surface showed 12 – 42 ind. × 0.0225 m^{-2}. After 12 months immersion, abundance on the top surfaces ranged between 1236 and 5088 ind. × 0.0225 m^{-2} and on the bottom surfaces between 364 and 1771 ind. × 0.0225 m^{-2}. The analysis of variances showed that this variable was significantly affected by surface orientation throughout the sampling period with higher values on the top surfaces (Table V). Regarding the number of *taxa*, at the beginning of this study, the top surface presented 6 to 23 *taxa*, while on the bottom surface between 6 and 14 *taxa* were identified. After 12 months immersion, the top surface exhibited between 35 and 52 *taxa*, whilst the bottom surface presented 26 to 37 *taxa*. The number of vagile *taxa* was also influenced by surface orientation as top surfaces presented significantly higher values than the bottom surfaces 1 year after the beginning of the study (Table V). On the other hand, significant differences were observed between reefs, reef A10 exhibiting higher abundance and number of *taxa* than reef A11 after 12 months immersion (Table

V). On both the horizontal surfaces after 3 months deployment, the vagile fauna biomass values reached a maximum of 0.01 g × 0.0225 m^{-2}. After 12 months, biomass values on the top surfaces varied between 0.20 and 1.27 g × 0.0225 m^{-2}, whilst on the bottom surfaces they ranged between 0.04 and 0.53 g × 0.0225 m^{-2}. The biomass of the vagile fauna showed significant differences after 12 months immersion, displaying higher biomass on the top compared to the bottom surfaces (Table V).

Table V. Results of the two-way ANOVAs performed on abundance (Abd), biomass (B), and number of *taxa* (S) obtained on horizontal surfaces (top – T and bottom – B) in two reef groups (A10 and A11) for vagile fauna assemblages during the study period (3 and 12 months).

Source of variation	df	Abd		B		S	
		MS	F	MS	F	MS	F
3 months							
Reef group = Re	1	396.75	0.58 ns	0.000001	0.29 ns	60.75	3.69 ns
Surface Orientation = Su	1	15768.75	23.21 **	0.000024	4.87 ns	70.08	4.25 ns
Re x Su	1	2268.75	3.34 ns	0.000004	0.77 ns	52.08	3.16 ns
Residual	8	679.50		0.000005		16.50	
Cochran's test		*C* = 0.54 ns		*C* = 0.40 ns		*C* = 0.31 ns	
SNK tests		Surface					
		T (98.5) > B (26.0) **					
12 months							
Reef group = Re	1	4219788	5.54 *	0.000094	0.00 ns	243.00	30.37 ***
Surface Orientation = Su	1	10149441	13.33 **	0.612033	6.39 *	560.33	70.04 ***
Re x Su	1	324065,00	0.43 ns	0.112387	1.17 ns	1.33	0.17 ns
Residual	8	761336		0.095841		8.00	
Cochran's test		*C* = 0.60 ns		*C* = 0.65 ns		*C* = 0.79 ns	
SNK tests		Reef		Surface		Reef	
		A10 (2450.7) > A11 (1264.7) *		T (0.63351) > B (0.18183) *		A10 (42.83) > A11 (33.83) ***	
		Surface				Surface	
		T (2777.3) > B (938.0) **				T (45.17) > B (31.50) ***	

ns = not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

In general, the assemblages of sessile organisms on the top surfaces were dominated by cirripeds (between 66 and 89%) and bivalves (between 4 and 11%). In contrast, on the bottom surfaces besides cirripeds (with 72 to 97%), colonial organisms with large dimensions such as ascidians, bryozoans and sponges (between 1 to 19%) dominated the biomass values. Between 13 and 33 *taxa* were identified with biomass values of 0.23– 0.55 g × 0.0225 m^{-2} on the top surface at the beginning of this study. On the other hand, the bottom surface presented between 14 and 26 *taxa* with biomass values of 0.17–2.50 g × 0.0225 m^{-2}. One year after the start of the investigation, the number of *taxa* on the top was between 34 and 45, and biomass values varied between 1.37 and

4.33 g × 0.0225 m^{-2}. The bottom surface showed 33 to 45 *taxa*, and biomass values varied between 1.85 and 3.57 g × 0.0225 m^{-2}. However, the biomass and the number of *taxa* for the sessile fauna appeared not to be influenced by the horizontal orientation, as no significant differences were detected (Table VI).

Table VI. Results of the two-way ANOVAs on biomass (B), and number of *taxa* (S) obtained on horizontal surfaces (top and bottom) in two reef groups (A10 and A11) for sessile fauna assemblages during the study period (3 and 12 months).

Source of variation	df	B		S	
		MS	F	MS	F
3 months					
Reef group = Re	1	0.126549	0.36 ns	48.00	1.37 ns
Surface Orientation = Su	1	1.391112	3.93 ns	8.33	0.24 ns
Re x Su	1	0.386694	1.09 ns	12.00	0.34 ns
Residual	8	0.354172		34.92	
Cochran's test		*C* = 0.97 ***		*C* = 0.74 ns	
12 months					
Source of variation	df	B		S	
		MS	F	MS	F
Reef group = Re	1	0.02072	0.03 ns	1.33	0.05 ns
Surface Orientation = Su	1	0.23705	0.34 ns	0.33	0.01 ns
Re x Su	1	2.93185	4.20 ns	12.00	0.47 ns
Residual	8	0.69857		25.75	
Cochran's test		*C* = 0.81 ns		*C* = 0.43 ns	

ns = not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

Moreover, multivariate analysis also showed that the structure of the vagile fauna community was affected by the horizontal orientation. The two-way crossed ANOSIM showed significant differences for the abundance of vagile organisms between surfaces during the sampling period (Table III). These differences were also observed in the MDS ordination concerning abundance data for vagile macrofauna, where two different groups were discernible, corresponding to the samples with different orientation (top and bottom surfaces) (Figure 3B). The biological structure of the vagile fauna on both horizontal surfaces, after 3 months immersion, were characterised by the presence of the amphipods *Ericthonius* spp. and *M. gryllotalpa*. Nevertheless, the high abundance of the gastropod *P. inconspicua*, and *M. gryllotalpa*, and the exclusive species *Microdeutopus* *armatus* Chevreux, 1887 on the top surface were responsible for the dissimilarity between the horizontal surfaces (Table VII). The bottom surfaces were distinguished by the exclusivity of the polychaete *P. bellis* (Table VII). After 12 months, the dissimilarity between surfaces was due to the higher abundance on the top than on the bottom surfaces; in particular of the *taxa Ericthonius* spp., *Thalassema* sp., the gastropod *Nassarius incrassatus* (Ström, 1768) and nematodes. *Caprella* spp. was also important in the dissimilarity between different orientations, as it was found in the top surfaces only.

Table VII. Species identified by SIMPER routine as having a high contribution to the average dissimilarity between the macrobenthic assemblages of top and bottom surfaces.

Vagile fauna	Top	Bottom	Cont%	Sessile fauna	Top	Bottom	Cont%
3 months							
Pusillina inconspicua	15.0	> 0.17	7.83				
Microdeutopus gryllotalpa	22.5	> 3.83	5.46				
Autolytinae nd	8.50	> 1.33	5.27				
Paleanotus bellis	0.0	< 3.17	4.45				
Microdeutopus armatus	2.5	> 0.0	4.33				
Pisidia cf. *bluteli*	4.33	> 1.0	3.96				
12 months							
Thalassema spp.	101.33	> 8.00	3.47	Ascidiacea	0.0	< 0.14	7.65
Nematoda	960.67	> 134.67	3.42	*Chaetopterus variopedatus*	0.12	> 0.0	6.18
Ericthonius spp.	42.67	> 1.33	3.21	Bryozoa	0.29	< 0.37	4.17
Jassa marmorata	99.33	> 9.33	2.9	*Leucandra aspera*	0.0	< 0.01	3.85
Stenothoe valida	67.33	> 3.33	2.87	*Serpula vermicularis*	0.0	< 0.02	3.44
Nassarius incrassatus	59.33	> 3.33	2.81	Anthozoa	0.06	< 0.08	3.23
Caprella spp.	14.0	> 0.0	2.68	Hydrozoa	0.03	> 0.01	3.12
Syllidae nd	12.0	< 20.0	2.03	Serpulidae nd	0.0	= 0.0	3.08
Chrysallida cf. *interstincta*	17.33	> 2.00	2.02	*Hiatella arctica*	0.17	> 0.08	3.01
Arthropoda nd	9.33	> 1.33	2.0	*Sphenia binghami*	0.0	= 0.0	2.97
Paromola cuvieri	7.33	> 0.67	2.0	*Pomatoceros triqueter*	0.01	> 0.0	2.62
Pusillina inconspicua	64.00	> 8.67	1.96	*Anomia ephippium*	0.01	> 0.0	2.61

Abundance and biomass data were used for vagile and sessile fauna respectively. Data are presented for each sampling date. Mean values and differences (< and >) are presented for horizontal surfaces.

Multivariate analysis showed no differences between surfaces and reefs after 3 months immersion for the biomass of sessile fauna, consequently dissimilarities between reefs were not analysed with the SIMPER routine (Table III). Except for the beginning of this study, the MDS ordinations of the sessile biomass data allowed two clear groups to be distinguished, corresponding to the samples with different orientation (top and bottom surfaces) (Figure 3A). The biological structure of the sessile macrofauna after 3 months immersion, was characterised by cirripeds and the bivalve *M.* cf. *subpicta*. At the last sampling period, the higher biomass values of ascidians, anthozoans and bryozoans on the bottom surfaces, and *Chaetopterus variopedatus* (Renier, 1804) and *H. arctica* on the top surfaces were important for the dissimilarity between orientations. In addition, the dissimilarity between surfaces was due to the exclusive presence of the polychaete *P. triqueter* on the top surface.

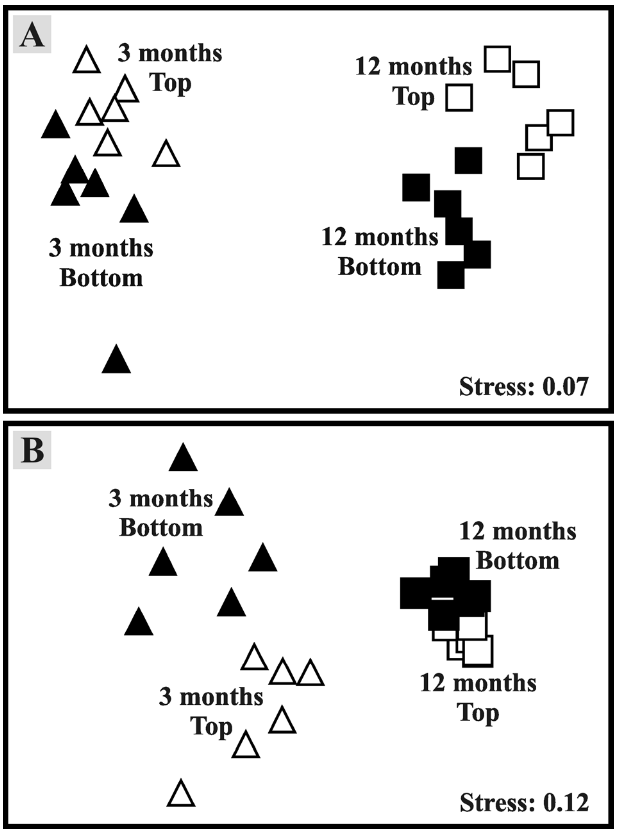

Figure 3. MDS ordination plots for horizontal surfaces over one year after AR deployment. A – sessile fauna; B – vagile fauna. [△,▲] – 3 months; [□,■] – 12 months; open symbols – top surface; filled symbols – bottom surface.

Discussion

The 1-year study of the epibenthic community of the Faro/Ancão AR showed that the colonisation process was affected by surface orientation. On the vertical surfaces, the macrobenthic colonisation appeared similar throughout the sampling period. No differences were observed for all the biological variables studied or for the community structure for either the sessile or motile fauna. On the other hand, the macrobenthic community structure of the horizontal surfaces analysed by multivariate analyses was different for both the vagile and sessile components. However, only the vagile fauna was significantly different when analysis of variance was applied. One year after AR deployment, the abundance, number of *taxa* and the biomass were significantly higher on the top compared to the bottom surfaces. These differences observed within horizontal surfaces may be due to a combination of both biological and environmental factors.

The sessile fauna was not significantly different although the top surfaces presented a higher number of *taxa* and more biomass compared to the bottom surfaces, which is in disagreement with the results of Boaventura *et al*. (2006) for the same sample units. These authors found that the bottom surfaces had significantly higher colonisation than the top surface, particularly after 3 and 12 months immersion. The differences found in both studies result from the use of different methods. Biomass was measured as AFDW in this study and percentage cover by the point intersection method (Boaventura *et al*., 2006). Therefore, the choice of the method used for estimating the contribution to the community of sessile *taxa* is of major importance as it can lead to different results and conclusions. Whenever possible, the use of more than one method is highly desirable in order to avoid misinterpretation of the data. Hatcher (1998) studied ARs with a pyramidal shape and observed that both the number of *taxa* and the total biomass were higher on the bases, suggesting that siltation, predation and the presence and growth of a thick, low-relief algal-hydroid turf, may have provided unfavourable conditions for barnacle and serpulid survival on the tops of the slabs. D'Anna *et al*. (2000) observed that a high rate of silt deposition led to a slowing down of the successional colonisation rate. Different exposure to light, currents and sedimentation were also pointed out by

Relini *et al*. (1994) as strongly influencing the settlement and development of benthic communities. Exposure to ocean currents increases a reef's exposure to larval recruits and may also increase the potential food supply (Bohnsack *et al*., 1991; Ginn *et al*., 2000). Baynes and Szmant (1989) observed that areas of high sessile benthic cover and species diversity corresponded to areas of strong circulation and low sedimentation. Within the Faro/Ancão AR system, Falcão *et al*. (2007) observed nutrient enrichment (both in the sediment and in the water), biogenic particles, microphytobenthos and the enhancement of organic sediment after AR deployment. These changes may increase the complexity of the biotic and abiotic habitat, ecological niches and food webs on a local scale. Although not quantified, top surfaces are more likely to retain sediment particles than bottom surfaces, and thus be subjected to higher sediment and/or organic stress. The top surfaces of the Algarve ARs normally present a layer of sediment that is re-suspended when disturbed (e.g. by scuba diver activity). However, the top surfaces were richer (in terms of abundance, biomass and species richness) than the bottom surfaces. This result seems to indicate that the sedimentation level in the Faro/Ancão AR is not sufficient to produce detrimental effects on the community. The frequent hydrodynamic disturbance observed within the Faro/Ancão AR system (due to waves and tidal currents) may remove sediment from the top surfaces, and may be key factors for the absence of thick sediment layers. On the other hand, it is known that intermediate levels of nutrient enrichment may lead to an increase in abundance and diversity of macrobenthic communities by providing extra resources to the populations either directly (detritivores) or indirectly (predators, by increasing the number of prey) (Pearson and Rosenberg, 1978; Magni, 2003). Therefore, a combination of low siltation and intermediate levels of nutrient enrichment may be the reason for the results obtained for the sessile component on the horizontal surfaces.

In this work, the quantitative sampling of small motile invertebrates may provide valuable data for evaluating the dynamics of these invertebrates within ARs. Vagile species were always more abundant on the top surfaces compared to the bottom surfaces. Intermediate abundances were observed for both inside and outside vertical surfaces. Therefore, although vagile species have the capacity to move throughout the cubic sampling unit (as in AR modules), it seems that they show a preference for some surfaces, particularly the top surface because of the high abundances observed. One of the factors that may be associated with the preference of the vagile fauna for some surfaces is the surface characteristics provided by the sessile fauna. It is known that the settlement of sessile organisms will promote higher spatial heterogeneity that will enhance the colonisation by other species (Bourget *et al*., 1994; Relini *et al*., 1994). A higher spatial heterogeneity within the top surfaces together with non-detrimental levels of siltation, the provision of food, and the protection from predators and currents promoted by sessile organisms could lead to the significantly higher abundance, number of *taxa* and greater biomass of motile invertebrates (Hatcher, 1998; Koehl, 2007). However, although some studies have documented the effect of surface orientation on epibenthic organisms (Glasby, 2000; Glasby and Connell, 2001; Bulleri and Chapman, 2004; Knott *et al*., 2004), the motile fauna has never been thoroughly examined. Therefore, the results regarding this fauna cannot be compared and fully discussed. Within horizontal surfaces, sessile epibenthic assemblages were dominated by cirripedes (mainly *Balanus amphitrite*). However, while bivalves (such as *Hiatella arctica* and *Modiolarca* cf. *subpicta*) were the second most abundant group on the top surfaces (and almost absent from the bottom surfaces), ascidians and bryozoans (mainly encrusting bryozoans) co-dominated on the bottom surfaces. During sample processing and sorting, it was observed that a lot of motile species (for example crustaceans) were present inside the shells of cirripedes and dead bivalves, but this was not the case for ascidians or encrusting bryozoans. Therefore, the differences in the sessile community structure could also be related to the increase in abundance, biomass, and diversity of motile organisms. Concerning the faunal composition of the vagile component, assemblages were dominated by polychaetes, namely Autolytinae nd, *Paleanotus bellis* and *Syllidia armata*, by pantopods such as *Achelia longipes* and by crustaceans like *Caprella* spp., *Corophium* spp., *Ericthonius* spp. and *Pisidia* cf. *bluteli*. This dominance pattern was similar for both the reef groups and for horizontal and vertical surfaces. However, 3 months after the beginning of the experiment the bivalve *M.* cf. *subpicta* and the amphipods *Microdeutopus armatus* and *Microdeutopus gryllotalpa* were also important colonisers, although *M. armatus* was unable to colonise the bottom surface. One year after deployment, the Faro/Ancão AR had not reached the stage of full maturity, as the *taxa* identified in this study were common pioneer *taxa*, encroaching on clear surfaces and therefore corresponding to a pioneer settlement period (Chalmer, 1982; Woodhead and Jacobson, 1985; Kocak and Zamboni, 1998; Moura *et al*., 2004, 2006, 2007). However, other authors have described similar community patterns, which show a clear period of dominance by mussels and oysters (Ardizzone *et al*., 1989, 2000), or macroalgae (Relini *et al*., 1994; Hatcher, 1998; Kocak and Zamboni, 1998). Although macroalgae are an important component of European AR communities, especially on top surfaces (Hatcher, 1998; D'Anna *et al*., 2000; Relini, 2000), in the Faro/Ancão AR system macroalgae were absent. Similar results were also reported by Relini and Relini (1997) for the benthic communities of Adriatic ARs. It should be noted nevertheless, that the present study was run for only 12 months and it is possible that different conclusions would have been drawn from a longer-term study, when this community reached a more stable and mature state.

The establishment of fouling assemblages is a complex process and understanding the relationship between the structural features of an AR and its developing benthic communities has great biological and ecological significance for reef rehabilitation and enhancement (Baine, 2001; Svane and Petersen, 2001 and references therein). The choice of the variables and methods used for community analysis when epifaunal recruitment or colonisation is studied can potentially generate different results of epifaunal assemblage structure. Moreover, the use of so many different methods and techniques in epifaunal studies undoubtedly confounds the problems of identifying important ecological processes and makes comparisons between different studies almost impossible (Relini and Relini, 1997; Knott *et al*., 2004; Beaumont *et al*., 2007). Another constraint in ecological quantitative studies of hard bottom communities is the use of sampling units that are expected to reproduce the conditions of the system being analysed. In the present study, cubic samples were suspended at a position within an opening of the AR module and it was expected that the overall patterns observed for these sampling units were representative of the AR modules. However, no clear differences were found in the colonisation/succession of the benthic communities between the cubic sampling units and the AR modules (unpublished data). The suspension of the cubic samples could not only alter the ‘‘normal’’ AR function but also receive different light and hydrodynamic (current velocity and direction) conditions, all of which can have a pronounced effect on larval settlement on the sample cube (Bohnsack *et al*., 1991; Glasby, 1999; Koehl, 2007). Moreover, patch size is an important regulating factor in recruitment, especially where the spatial distribution of the sessile biota on hard substrata is uneven, due to various ecological factors such as exposure to light, currents and sedimentation (Jensen and Collins, 1997, Svane and Petersen, 2001). However, when the arrangement of the modules forming the AR groups is considered, the random placement of the cubic sample units among the AR group is intended to represent the AR groups as a whole. Since there is a large variability of exposure within modules, the potential effects of the suspended cubic sampling units are therefore reduced.

Overall, in the present work it was evident that for the study of hard substratum communities, analysis of the vagile and the sessile components is of utmost importance. The exclusive use of the sessile fauna on hard substratum studies may result in biased conclusions when trying to assess the benthic community as a whole. The motile fauna component, which has been usually neglected in studies of epibiotic communities, should be taken into consideration as an important feature in the functioning of the AR. Finally, the study of the role of surface orientation on the epifaunal communities of ARs may provide important clues that may be used to adapt AR design in order to expand their purposes, namely the enhancement of biodiversity and food production to fish.

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CHAPTER III.

Macrobenthic community colonisation patterns on artificial reefs:

Analysis of the influence of depth and of the reef structure

Section 1.

Effect of depth and reef structure on early macrobenthic communities of the Algarve artificial reefs (southern Portugal)

Moura, A.; Boaventura, D.; Cúrdia, J.; Carvalho, S.; Cancela da Fonseca, L.; Leitão, F.; Santos, M. N. and Monteiro, C. C. 2007. Effect of depth and reef structure on early macrobenthic communities of the Algarve artificial reefs (southern Portugal). *Hydrobiologia* 580(1):173-180.

Introduction

Ecological processes concerning macrobenthic communities, such as recruitment, colonisation and succession, are strongly affected by the surrounding physical and biological environment (Svane and Petersen, 2001). Factors, such as temperature, salinity, light, depth, substratum and relative position to the seafloor play an important role in the settlement and recruitment of the organisms as well as in their growth (Kocak and Zamboni, 1998; Glasby, 1999a, b; Svane and Petersen, 2001). Intra- and inter-specific interactions in the community arise in the partition of resources such as space and food, contributing to the dominance or non-presence (local extinction) of species (Kocak and Zamboni, 1998).

Artificial reefs (ARs) have become an important resource of fisheries enhancement (Bohnsack and Sutherland, 1985). Most of the commercial fish species associated with the AR systems are attracted to food habits directly linked to the macrobenthos (Ardizzone *et al*., 1997; Itosu *et al*., 1999; Steimle *et al*., 2002). Due to the potential role of macrobenthic communities in providing food for commercial fish fauna, studies concerning macrobenthic colonisation assume importance to explain the patterns of fish associated with AR systems. Reefs with overlapping elements are particularly effective in maximizing the surface available for the colonisation of benthic organisms. They provide different conditions, namely in light, temperature and physical–chemical parameters (Relini *et al*., 1994).

In Portugal, AR systems made of concrete modules were deployed along the south coast of the Algarve. The coastal area where the reefs were deployed is characterised by a water temperature between 17.4ºC and 20.2ºC. Salinity of the upper layers of the water column is almost constant throughout the year in the south of coast of Algarve (ranging from 36.1 to 36.3 at the surface and from 36.1 to 36.4 at 50 m depth) (see Santos, 1997 for further details). The main objective of the artificial structures was to enhance local fisheries by extending the nursery effect of estuarine–lagoon systems present along the coast through the creation of potential food resources and shelter for juveniles of commercial fish fauna (see Santos, 1997; Santos and Monteiro, 1997, 1998 for further details). Although the importance of AR systems is recognised worldwide, research regarding Portuguese ARs systems has been focused mainly on ichthyofauna, especially commercial species (Santos and Monteiro, 1997, 1998). The Algarve AR systems are composed of small (2.7 m^3) and large concrete modules (174 m^3). This study was performed in the small modules, located at 16–20 m depth. This depth range for the deployment of AR modules was determined at its lower end because of bivalve dredge fishing (an important local fishery) and at its upper and due to the characteristics of these particular reef modules (in order to maximise the reef effect at the water column). So, the main objectives of the present study were (i) to detect the effect of depth on the macrobenthic species after 6 months of colonisation within two reef groups, and (ii) to test differences on the colonisation between two depth layers of reef modules (Upper and Lower).

Material and methods

The present work was carried out on the ‘‘Faro/Ancão’’ AR located off Faro, southern Portugal, particularly on two small AR groups, deployed in May 2003, at approximately 16 m (37º00.454’ N 8º02.171’ W) and 20 m depth (37º00.062’ N 8º02.482’ W) (groups A and C, respectively). Each one of these reef groups is composed of 35 concrete module units (2.7 m^3 each unit) jumbled up, comprising roughly two different depth layers.

Macrobenthic colonisation was investigated using sample units (15 × 15 × 15 cm) made of the same concrete material of reef modules. The cubic units were suspended on the upper and lower portions (Upper layer—UL and Lower layer—LL) of each reef groups at the time of reef immersion (four units in each layer of modules *per* AR). Three replicate samples were collected by scuba diving 6 months after immersion in November of 2003. In each cubic unit, to avoid the ‘‘border effect’’ on macrobenthic assemblages, the central area of the surface facing outwards was scraped for posterior analysis of macrobenthic communities.

The samples were sieved through a 0.5 mm square mesh, and the retained material fixed in 4% buffered formalin. All specimens were sorted and identified to the species level whenever possible. For the calculation of diversity and richness index, all *taxa* were included. Non-colonial organisms were counted, and barnacles and colonial species were assessed using biomass. The wet weight of each species was obtained after a 5-min drying period on blotting paper and was measured (to 0.001 g) with a digital weighing scale.

Data analysis

Macrobenthic community was evaluated using the PRIMER v.5.0 software. The analysis was carried out using fourth-root (√√)-transformed data, which is more sensitive to changes in less abundant species. Community patterns were assessed using non-metric multidimensional scaling ordination (MDS) based on a similarity matrix using Bray–Curtis coefficient. A two-way crossed analysis of similarity (ANOSIM) was performed to determine if there were any effects of depth and reef structure on community structure. The Similarities Percentages procedure (SIMPER) of fourth-root transformed data was used to determine the contributions from individual species to the Bray–Curtis dissimilarities between depths and layers of modules.

A two-way ANOVA was used to test for differences in mean density (D), mean biomass (B), Shannon–Wiener diversity (H'; $\log_2$) and mean number of species (S) of each group of samples. The design included two factors: ''Reef Depth'' (RD) orthogonal, fixed with two levels (A and C) and ''Reef Layer'' (RL) orthogonal, fixed with two levels (UL and LL). Cochran's C test was used to determine whether variances were heterogeneous and therefore if any data required an appropriate transformation (Underwood, 1997). Student Newman–Keuls (SNK) *a posteriori* comparison tests were used. ANOVA and SNK tests were carried out using GMAV5 for Windows Statistical Software (Institute of Marine Ecology, Sydney, Australia).

Results

Non-colonial organisms

A total of 78 non-colonial *taxa* were identified. The best represented taxonomical groups were the classes Polychaeta (31), Gastropoda (7), Bivalvia (8) and the orders Amphipoda (10), Isopoda (2) and Decapoda (7).

Data analysis for the non-colonial organisms showed similar values for D (20,681–25,066 individuals m^{-2}), for H' (21–27 species) and S (3.3– 3.8) between reef and reef structure (Table I). The two-way ANOVA showed no significant differences for the above-mentioned variables for both RD and RL factors.

Six species, *Gregariella subclavata* (Libassi), *Musculus* cf. *subpictus* (Cantraine), *Hiatella arctica* (L.), *Microdeutopus versiculatus* Chevreux, *Jassa marmorata* Holmes, and *Paleanotus* cf. *bellis* (Johnson), contributed to 67.1% of the total mean density values of the non-colonial organisms. The bivalves *G. subclavata* and *M.* cf. *subpictus* were the most abundant species. *G. subclavata* presented higher values in reef A than in reef C, and *J. marmorata* also showed a marked decrease with depth (Figure 1).

Table I. Mean and standard deviation of density (D, number of individuals m^{-2}), number of species (S), Shannon–Wiener diversity (H') and biomass (B, ww g m^{-2}) of macrofaunal assemblages calculated for each group and layer of modules.

	Reef Depth		Reef Layer			
	A	C	UL-A	UL-C	LL-A	LL-C
Non-colonial organisms						
D	23,585.2 ± 8,148.09	22,133.3 ± 5,572.99	25,066.7 ± 8,457.07	23,585.2 ± 3,393.34	22,103.7 ± 9,374.02	20,681.5 ± 7,733.50
S	25.3 ± 3.27	22.7 ± 5.35	27.7 ± 2.52	21.3 ± 7.37	23.0 ± 2.00	24.0 ± 3.46
H'	3.8 ± 0.15	3.4 ± 0.55	3.8 ± 0.21	3.3 ± 0.84	3.8 ± 0.10	3.5 ± 0.10
Barnacles and colonial organims						
B	1,309.8 ± 474.58	966.4 ± 519.45	1,716.3 ± 136.82	1,372.0 ± 374.05	903.3 ± 220.60	560.8 ± 202.81
S	7.2 ± 0.75	5.5 ± 1.38	7.3 ± 0.58	6.3 ± 1.53	7.0 ± 1.00	4.7 ± 0.58
H'	0.5 ± 0.26	0.2 ± 0.20	0.6 ± 0.26	0.2 ± 0.28	0.3 ± 0.18	0.2 ± 0.14

A = –16 m; C = –20 m; UL = Upper Layer; LL = Lower Layer

The two-way crossed ANOSIM showed a significant difference between A and C reefs, R = 0.53 (P = 0.02), but no dissimilarity between the Upper and the Lower layers of modules was observed (R = 0, P = 0.52). Furthermore, the MDS ordination presented an evident separation of the samples with differing depth (Figure 2a).

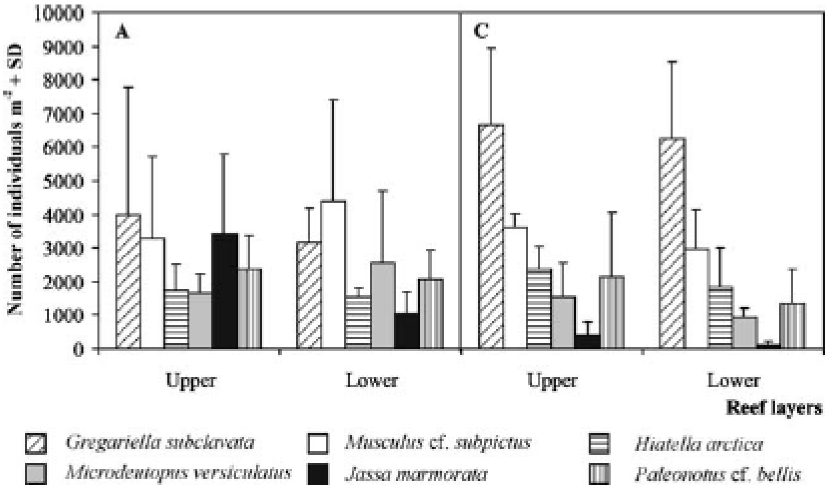


Figure 1. Mean density values and standard deviation (SD) of the most abundant non-colonial species in the two reef groups (A and C) and layers of modules (Upper and Lower).

As a RD effect was indicated by the ANOSIM test, a SIMPER analysis was carried out for A and C reef groups. The dominant *taxa* at A reef were *J. marmorata*, undetermined Turbellaria, *Syllidia armata* Quatrefages, and *Serpula vermicularis* L. (Table IIa). This reef presented exclusive *taxa*, such as undetermined Nudibranchia and *Nereis* cf. *zonata* Malmgren. On the other hand, *Corophium sextonae* Crawford, *Anomia* cf. *ephippium* L., *Pomatoceros triqueter* (L.) and *Pisidia* cf. *bluteli* (Risso) were particularly dominant at reef C.

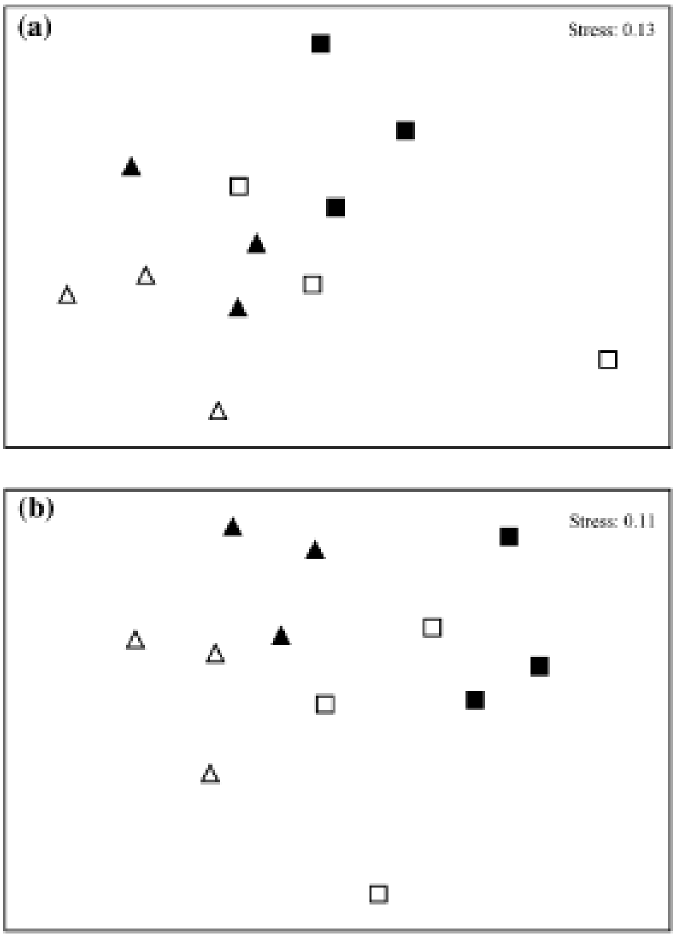
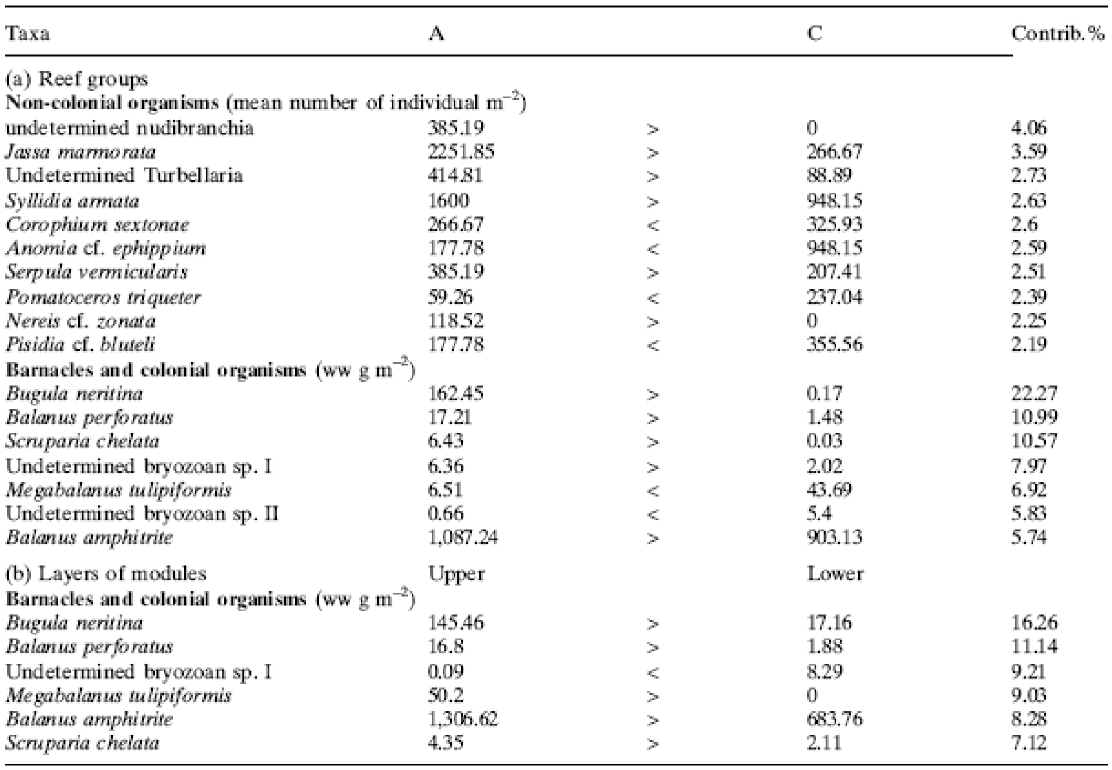


Figure 2. MDS ordination plots for reef groups and layers of modules of (a) non-colonial organisms abundance and (b) barnacles and colonial organisms biomass (A reef: Upper layer = △; Lower layer = ▲; C reef: Upper layer = □; Lower layer = ■).

Table II. Differences (< and >) in mean density (mean number of individuals m^{-2}) for non-colonial organisms and mean biomass values (ww g m^{-2}) for barnacles and colonial organisms. Contrib.% = *taxa* percentage contribution, from SIMPER analysis, to dissimilarities between (a) reef groups and (b) layers of modules.



Taxa	A		C	Contrib.%
(a) Reef groups				
Non-colonial organisms (mean number of individual m^{-2})				
undetermined nudibranchia	385.19	>	0	4.06
Jassa marmorata	2251.85	>	266.67	3.59
Undetermined Turbellaria	414.81	>	88.89	2.73
Syllidia armata	1600	>	948.15	2.63
Corophium sextonae	266.67	<	325.93	2.6
Anomia cf. *ephippium*	177.78	<	948.15	2.59
Serpula vermicularis	385.19	>	207.41	2.51
Pomatoceros triqueter	59.26	<	237.04	2.39
Nereis cf. *zonata*	118.52	>	0	2.25
Pisidia cf. *bluteli*	177.78	<	355.56	2.19
Barnacles and colonial organisms (ww g m^{-2})				
Bugula neritina	162.45	>	0.17	22.27
Balanus perforatus	17.21	>	1.48	10.99
Scruparia chelata	6.43	>	0.03	10.57
Undetermined bryozoan sp. I	6.36	>	2.02	7.97
Megabalanus tulipiformis	6.51	<	43.69	6.92
Undetermined bryozoan sp. II	0.66	<	5.4	5.83
Balanus amphitrite	1,087.24	>	903.13	5.74
(b) Layers of modules	Upper		Lower	
Barnacles and colonial organisms (ww g m^{-2})				
Bugula neritina	145.46	>	17.16	16.26
Balanus perforatus	16.8	>	1.88	11.14
Undetermined bryozoan sp. I	0.09	<	8.29	9.21
Megabalanus tulipiformis	50.2	>	0	9.03
Balanus amphitrite	1,306.62	>	683.76	8.28
Scruparia chelata	4.35	>	2.11	7.12

A = –16 m; C = –20 m

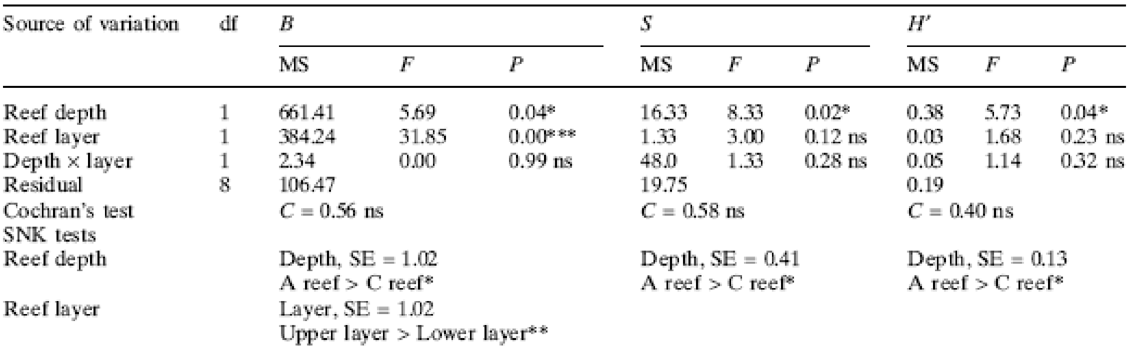
Barnacles and colonial organisms

Four species of the subclass Cirripedia were determined. Colonial organisms were mainly represented by Bryozoa (6 *taxa*) and Porifera (3 *taxa*).

Data analysis for the barnacles and colonial organisms showed higher B values in reef A (1,309 ww g m^{-2}) than in reef C (966 ww g m^{-2}), and in UL (1,372–1,716 ww g m^{-2}) to LL (903– 560 ww g m^{-2}). H' and S values were higher in lower depths, within layers of modules H' and S values were similar (Table I). The two-way ANOVA for B showed significant differences for both depth and layer factors (Table III). B values at reef A were significantly higher than at reef C. B was also higher in the UL than in the LL (see SNK in Table III). H' and S also displayed a depth effect as values were significantly higher at reef A than at reef C (Table III).

Balanus amphitrite Darwin comprised 87.4% of the total mean biomass of the barnacles and colonial organisms. This species presented higher values in the UL of both reefs, especially at reef A. The bryozoan *Bugula neritina* (L.), ranking second in biomass values accounted for 7% of the total, showing a decline with increasing depth (Figure 3).

Table III. Two-way ANOVA on mean biomass (B), number of species (S) and Shannon–Wiener diversity (H’) of barnacles and colonial organisms collected in the reef groups and layers of modules.



Source of variation	df	*B*			*S*			*H′*		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Reef depth	1	661.41	5.69	0.04*	16.33	8.33	0.02*	0.38	5.73	0.04*
Reef layer	1	384.24	31.85	0.00***	1.33	3.00	0.12 ns	0.03	1.68	0.23 ns
Depth × layer	1	2.34	0.00	0.99 ns	48.0	1.33	0.28 ns	0.05	1.14	0.32 ns
Residual	8	106.47			19.75			0.19		
Cochran’s test		*C* = 0.56 ns			*C* = 0.58 ns			*C* = 0.40 ns		
SNK tests										
Reef depth		Depth, SE = 1.02 A reef > C reef*			Depth, SE = 0.41 A reef > C reef*			Depth, SE = 0.13 A reef > C reef*		
Reef layer		Layer, SE = 1.02 Upper layer > Lower layer**								

(ns = not significant; *P < 0.05; **P < 0.01; ***P < 0.001). A = –16 m; C = –20 m

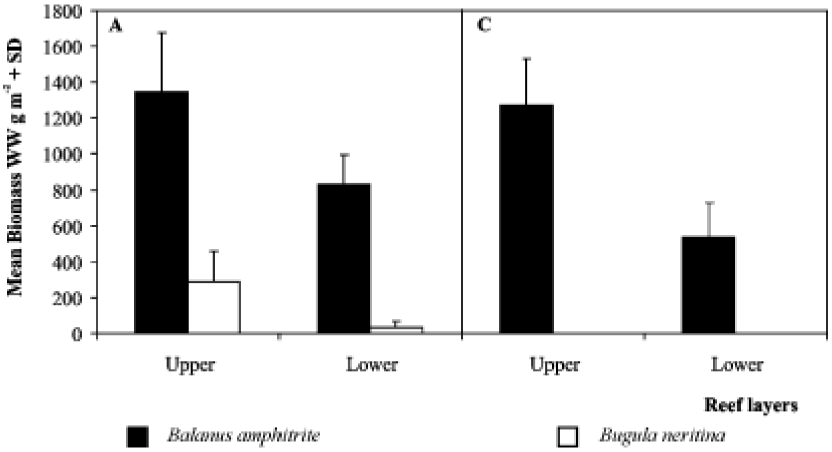


Figure 3. Mean biomass values and standard deviation (SD) of two the most dominant barnacles and colonial species in the two reef groups (A and C) and layers of modules (Upper and Lower).

Multivariate analyses using biomass values showed a clear effect of depth (R = 0.80, P = 0.01), i.e. assemblages at 16 m were significantly different from those at 20 m. There were also significant differences between assemblages in UL and LL (R = 0.54, P = 0.02). The MDS ordination corroborated the differences observed in ANOSIM analyses for depth and layer factors (Figure 2b).

SIMPER analysis showed that *Bugula neritina* and *Balanus perforatus* Brugiére have a strong contribution to the dissimilarity between depths (Table IIa), with higher B values in the reef A (Table IIa). *Scruparia chelata* (L.) and undetermined Bryozoa sp. I presented high B values in reef A, and *Megabalanus tulipiformis* (Ellis) presented highest values in reef C. Concerning the reef structure, the species *Bugula neritina* and *Balanus perforatus* presented higher values in the UL (Table IIb). Furthermore, the barnacle *M. tulipiformis* was exclusive of the UL and undetermined Bryozoa sp. I showed high B values on the LL. Despite being characteristic of every group of samples, *Balanus amphitrite* showed twice higher biomass values in UL compared to those observed in the LL.

Discussion

The biodiversity of ARs is related to different environmental and structural factors, such as morphological complexity of the reef, substratum composition, depth and distance from natural reef areas (Bohnsack *et al*., 1991). In the present work, the distributions of many species were depth-related, although this observation was more evident for colonial and barnacle species. The results were consistent with previous work (Relini *et al*., 1994; Kocak and Zamboni, 1998), with biomass and the number of sessile species decreasing with depth. Some faunal groups, like bryozoans, barnacles and molluscs occurred in different proportions according to depth. This correlation with depth was also observed in the Loano AR (Ligurian sea, Italy) (Kocak and Zamboni, 1998). Some species like *J. marmorata* and *B. neritina* were associated to lower depths, while *A.* cf. *ephippium*, *G. subclavata* and *M. tulipiformis* showed the reverse trend. Depth and reef structure alter light conditions and thus light is responsible for changes in community structure and composition (Bohnsack *et al*., 1991). Glasby (1999b) observed that differences in light intensity could influence (direct or indirectly) the settlement of various species, including bryozoans. The observed communities of the ‘‘Faro/ Ancão’’ AR, after 6 months of submersion, do not reflect a mature community. The time of colonisation needed to achieve mature macrofaunal communities for the Algarve reefs is still being studied. In the present study, the macrobenthic community of ‘‘Faro/Ancão’’ AR corresponds to a settlement period, with serpulids, barnacles, bryozoans and molluscs dominating the community. Nevertheless, this community typically composed of pioneer *taxa*, showed higher biodiversity (91 macrobenthic *taxa* after 6 months of immersion) than the Fregene AR, Tyrrhenian Sea, Italy (10 benthic invertebrate *taxa* after 6 months and 135 after 4 years of immersion) (Ardizzone *et al*., 1989) and than the Loano AR, Ligurian sea, Italy (57 species after 12 months) (Relini *et al*., 1995).

The bivalves (*G. subclavata* and *M.* cf. *subpictus*) and barnacles (*B. amphitrite*) dominated the macrobenthic communities in Algarve ARs, exhibiting a similar trend to that described for the modules at the Tyrrhenian Sea (Ardizzone *et al*., 1989). The opposite was observed in Loano AR, where these faunal groups were never dominant (Relini *et al*., 1994). Contrasting with the results obtained for other European reefs (Relini *et al*., 1994; Collins *et al*., 1995; Garrido *et al*., 1999), macroalgae were not found colonising the reefs during the 6-month period of this study. In Portugal, as in the Adriatic (Badalamenti *et al*., 1992) and Tyrrhenian Sea (Ardizzone *et al*., 1989), the benthic communities were characterised by the absence of macroalgae, being dominated by vagile and sessile macrofauna.

Regarding the layer effect, biomass and number of barnacle species and colonial species decreased from upper to lower layers of modules. In the Alcamo Marina reef, Tumbiolo *et al*. (1995) observed, however, the opposite pattern, with biomass increasing from the top to the lowest layer of modules of the reef. In the Alcamo Marina reef, the amount of sediment was positively related to biomass, affecting macrobenthic communities (Tumbiolo *et al*., 1995). Glasby (1999b) observed differences between assemblages close to and far from the seafloor, linking them to differences in sedimentation. In the present study, however, the assemblages at the upper layer of modules in the ‘‘Faro/Ancão’’ AR were probably less affected by darkness and other physical factors, such as sedimentation. As an overall conclusion, the small differences in depth (16–20 m) and the structure of the ARs influenced the development of subtidal epibiotic assemblages at the ‘‘Faro/Ancão’’ AR. This effect was more obvious for colonial organisms and for barnacles; and it seems that, for biomass values, the reef structure was important. The reasons for such differences are not clear, but may involve factors such as light, predation/grazing, larval behaviour and water flow at micro- or mesoscales.

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Section 2.

Biomass production of early macrobenthic communities at the Faro/Ancão artificial reef (Portugal): effect of depth and reef layer

Moura, A.; Boaventura, D.; Cúrdia, J.; Santos, M. N. and Monteiro, C. C. 2006. Biomass production of early macrobenthic communities at the Faro/Ancão artificial reef (Portugal): effect of depth and reef layer. *Bulletin of Marine Science* 78(1):83-92.

Introduction

One of the main goals in artificial reef construction programs is biomass enhancement, and thus the production of harvestable resources, particularly fish (Relini and Relini, 1997). Since most of the commercial fish species associated with artificial reefs have food habits directly associated with the macrobenthos (Ardizzone *et al*., 1997), studies concerning the macrobenthic colonisation assume a relevant importance (Itosu *et al*., 1999; Steimle *et al*., 2002). Measurements of benthic biomass living on artificial reefs may contribute to our understanding of the role that these structures play in enhancing fish production, whether an artificial reef produces new biomass or merely aggregates fish (Bohnsack and Sutherland, 1985).

Conservative methods, such as percent cover, may give rapid quantitative evaluations of the most conspicuous sessile biota, and rough estimates of biomass can be obtained from converting observed cover values into biomass (Harmelin and Bellan-Santini, 1997). The structure and function of subtidal, hard substrate, sessile epibiotic communities cannot be studied easily by the normal quantitative methods applicable to infauna, owing to the predominance of colonial organisms, which often present a complex topography. Quantification of such communities is more appropriately measured using biomass (Hatcher, 1995).

Reefs with overlapping elements are particularly effective in maximizing the surface available for the colonisation of benthic organisms and providing physical-chemical gradients (see Bortone and Kimmel, 1991; for more information; Glasby, 1999a). Macrobenthic recruitment, colonisation, and succession are in fact strongly affected by the surrounding physical and biological environment (Kocak and Zamboni, 1998; Glasby, 1999a; Svane and Petersen, 2001). But, in order to fully understand the processes that govern the development of artificial reefs, a considerable research effort is required (Svane and Petersen, 2001).

This study focused on the zoobenthic component of concrete two-layer artificial reef systems deployed off the south coast of the Algarve region in Portugal. The main objective of these structures was to enhance local fisheries by extending the nursery effect of some estuarine-lagoon systems along the coast (see Santos and Monteiro, 1997; 1998 for further details). To understand the potential enhancement value of the reef, especially as a source of food for fisheries resources, the biomass of the reef's epifauna was estimated. The aim of this study was to investigate the effect of depth and layer on early biomass production on vertical and horizontal surfaces.

Material and methods

The study was carried out on the Faro/Ancão artificial reef located off Faro, southern Portugal. The biomass of pioneer benthic invertebrates was estimated on the horizontal and vertical surfaces of two artificial reef groups, deployed in May 2003 at approximately 16 m (37° 00.454´ N 8° 02.171´ W) and 20 m depth (37° 00.062´ N 8° 02.482´ W). Each one of these reef groups is composed of 35 concrete module units (2.7 m^3 each unit) positioned in two layers (upper and lower layers; Figure 1). The study was performed using 15 × 15 × 15 cm cubic sample units made of the same concrete material of reef modules. These cubic units were suspended randomly on each layer from both reef groups at the time of reef immersion (four units in each layer per artificial reef; Figure 1). Each sample was suspended with two similar cables for precaution. No samples were lost during this experiment. Three replicate samples were collected by scuba divers 6 month after deployment (November 2003).

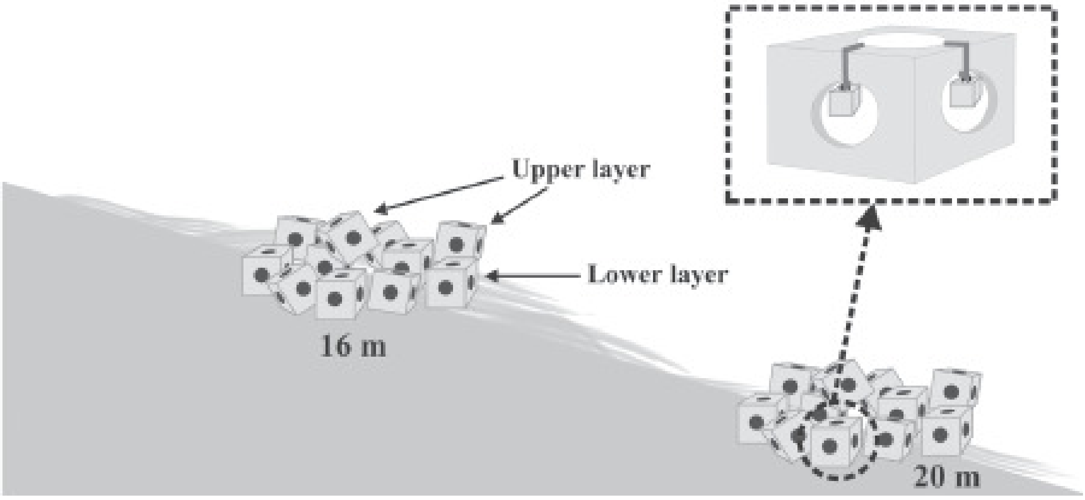


Figure 1. Artificial reefs deployed in two layers at two depths (16 and 20 m) on the southern coast of Portugal. Dashed box is detail of a concrete reef module with the cubic sample units used in this study.

Four of the six cube surfaces (horizontal: top *vs.* bottom; vertical: inside *vs.* outside) were scraped to analyze early macrobenthic total biomass. Biomass production was compared among these different surfaces for reef modules in different positions relative to the seafloor (upper, lower) between two reef groups at different depths. For the surface orientation tests, horizontal and vertical surfaces were not compared together in a single analysis to ensure that only non-adjacent surfaces are compared. The design included a three-way ANOVA for the influence of surface orientation: “reef depth” (orthogonal, fixed with two levels: 16 and 20 m), “reef layer” (orthogonal, fixed with two levels: upper and lower layer), and “reef surface” (orthogonal, fixed with two levels: top and bottom surfaces or outside and inside surfaces).

To examine the effect of depth and module layers on species biomass production, we analyzed a 7.5 × 7.5 cm sample from the outside surface. This size was selected after preliminary studies for determining the minimum sampling area and considering logistic constraints. To avoid the “border effect” we selected the central area of each outside surface. The collected material was sorted and identified to species level (whenever possible) and the biomass of each species determined. Total percent cover of major taxonomical groups was also recorded for the entire surface of the sample (15 ×

15 cm), using the point intersection method (49 intersection points; see D. Boaventura *et al*. in press; Hawkins and Jones, 1992 for a review).

In the laboratory, the sessile epibiota was carefully scraped from each cubic unit as soon as possible. The samples were sieved through a 0.5 mm square mesh, and the retained material was fixed in 4% buffered formalin. Wet weight (WW) was determined by blot drying each sample on paper towels for a few minutes before weighting. The dry weight was estimated by placing the samples in the oven at 70 ºC for at least 24–48 hours or until a constant weight was reached. The ash-free dry weight (AFDW) was determined by burning the animals at 450 ºC for 4 hours in a muffle furnace. The ash-free dry weight was calculated by subtracting the ashes weight from the dry weight.

Data analysis

Community structure was evaluated using the PRIMER v.5.0 software. The analysis was carried out using fourth-root (√√)-transformed data because this transformation is more sensitive to changes in less abundant species. Community patterns were assessed using non-metric multidimensional scaling ordination (MDS) based on a similarity matrix calculated using the Bray-Curtis coefficient. A two-way crossed analysis of similarity (ANOSIM) was performed to determine if there were any effects of depth and reef structure on community structure. The similarities percentages routine (SIMPER) of fourth-root transformed data was used to determine the contributions from individual species to the Bray-Curtis dissimilarities between different depths and layers.

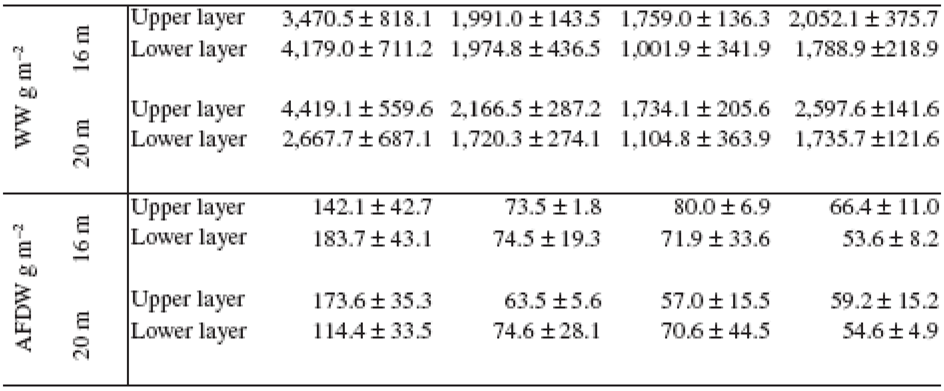
ANOVA was used to test for differences in biomass of each group of samples. Cochran's C - test was used to determine whether variances were heterogeneous and if any data therefore required transformation (Underwood, 1997). Student-Newman-Keuls (SNK) *a posteriori* comparison tests were used. ANOVA and SNK tests were carried out using GMAV5 for Windows Statistical Software (Institute of Marine Ecology, Sydney, Australia).

Results

Effect of surface orientation, depth, and layers on total biomass

Biomass values measured as WW and as AFDW were used to calculate artificial reef production (Table I). Data analysis showed that at both 16 and 20 m the total biomass (AFDW g m^{-2}) of the horizontal surfaces was higher on the top than on the bottom surfaces (Table I). The three-way ANOVA for total biomass showed a significant interaction between surfaces, depth, and layers, indicating that the biomass of the horizontal surfaces is not independent of depth and layer (Table IIA). The biomass on the top surface was significantly higher than on the bottom, except for the lower layer at 20 m depth, where the differences were not significant (SNK test, SE = 17.33; Table IIA). For the lower layer, biomass on the top surface was significantly higher at 16 m than at 20 m. Top surface at 20 m presented higher biomass on the upper layer than on the lower layer. The total biomass on the vertical surfaces showed no significant differences for both layers and depths (Table I, IIB).

Table I. Mean (± SD) of wet weight (WW) and ash-free dry weight (AFDW) for macrofaunal communities on the different surfaces, layers, and depths of cubic sampling units in Faro Ancão artificial reef, Portugal.



WW g m^{-2}	16 m	Upper layer	3,470.5 ± 818.1	1,991.0 ± 143.5	1,759.0 ± 136.3	2,052.1 ± 375.7
		Lower layer	4,179.0 ± 711.2	1,974.8 ± 436.5	1,001.9 ± 341.9	1,788.9 ±218.9
	20 m	Upper layer	4,419.1 ± 559.6	2,166.5 ± 287.2	1,734.1 ± 205.6	2,597.6 ±141.6
		Lower layer	2,667.7 ± 687.1	1,720.3 ± 274.1	1,104.8 ± 363.9	1,735.7 ±121.6
AFDW g m^{-2}	16 m	Upper layer	142.1 ± 42.7	73.5 ± 1.8	80.0 ± 6.9	66.4 ± 11.0
		Lower layer	183.7 ± 43.1	74.5 ± 19.3	71.9 ± 33.6	53.6 ± 8.2
	20 m	Upper layer	173.6 ± 35.3	63.5 ± 5.6	57.0 ± 15.5	59.2 ± 15.2
		Lower layer	114.4 ± 33.5	74.6 ± 28.1	70.6 ± 44.5	54.6 ± 4.9

Table II. Results from three-way ANOVA performed on total biomass obtained in reef and layer macrofaunal assemblages.

A) Horizontal surfaces				
Source of variation	df	MS	P	
Surface = Su	1	39,962.24	0.00	***
Depth = De	1	618.36	0.42	ns
Layer = La	1	2.79	0.96	ns
Su × De	1	533.26	0.45	ns
Su × La	1	613.68	0.42	ns
De × La	1	2,587.89	0.11	ns
Su × De × La	1	5,490.78	0.02	*
Res	16	901.30		
Cochran's test		C = 0.26 ns		
SNK tests	Su × De × La, SE = 17.33 16 m, upper, top > bottom* 16 m, lower, top > bottom** 20 m, upper, top > bottom** 20 m, lower, top = bottom ns Top, upper, 16 = 20 m ns Top, lower, 16 m > 20 m* Bottom, upper, 16 = 20 m ns Bottom, lower, 16 = 20 m ns Top, 16 m, upper = lower ns Top, 20 m, upper > lower * Bottom, 16 m, upper = lower ns Bottom, 20 m, upper = lower ns			
B) Vertical surfaces				
Source of variation	df	MS	P	
Surface = Su	1	786.65	0.22	ns
Depth = De	1	348.58	0.41	ns
Layer = La	1	53.38	0.74	ns
Su × De	1	121.41	0.62	ns
Su × La	1	197.56	0.53	ns
De × La	1	337.73	0.41	ns
Su × De × La	1	68.38	0.71	ns
Res	16	479.81		
Cochran's test		C = 0.51 ns		

ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Biomass for individual taxa (outside surface)

In total, 91 *taxa* were identified (for species checklist see D. Boaventura *et al*., in press). Those with higher biomass values were Cirripedia (four species, 506 g m^{-2} AFDW), Bivalvia (eight species, 168 g m^{-2} AFDW), and Bryozoa (six species, 109 g m^{-2} AFDW). Crustacea and Bryozoa dominated the percent cover at both layers and depths (Figure 2). Barnacle cover, especially *Balanus amphitrite* Darwin, 1854, clearly dominated, exceeding 45% cover. On the upper layer at 16 m, percent cover and biomass showed a similar trend with high values for barnacles (49.7%; especially *B. amphitrite* with 46.58 g m^{-2} AFDW) and the bryozoan *Bugula neritina* (Linnaeus, 1758) (41.5%, 21.62 g m^{-2} AFDW; Figure 2 and 3). Conversely, at 20 m on the lower layer, the bivalves *Anomia ephippium* Linnaeus, 1758 (33.94 g m^{-2} AFDW) and *Ostrea* cf. *edulis* Linnaeus, 1758 (6.45 g m^{-2} AFDW) had the highest biomass (Figure 3). A small increase in the percent cover of the bivalve *A. ephippium* (18.0%) and the bryozoan *Schizobrachiella sanguinea* (Norman, 1868) (6.6%) was observed at 20 m on the lower layer.

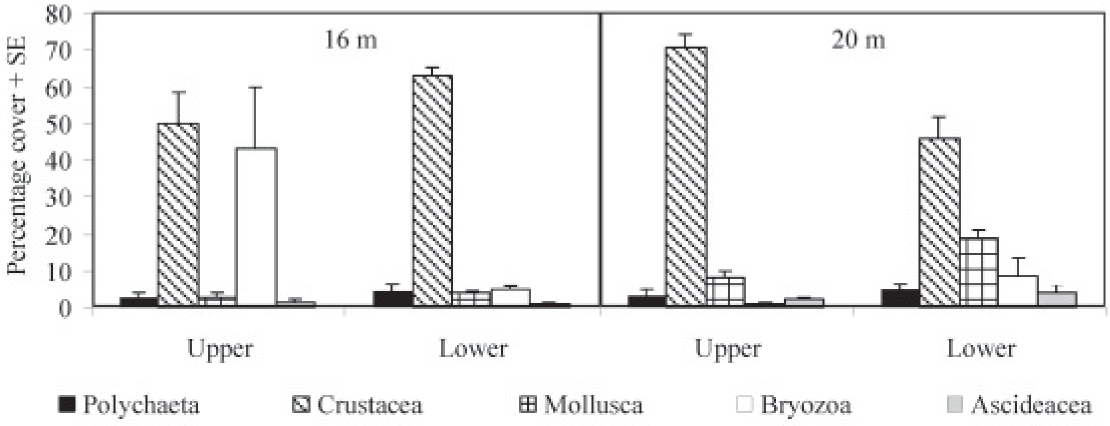


Figure 2. Mean (± SE) percent cover of the major macrofaunal taxonomical groups of the outside surface from the two reef layers (upper and lower) at two depths (16 and 20 m).

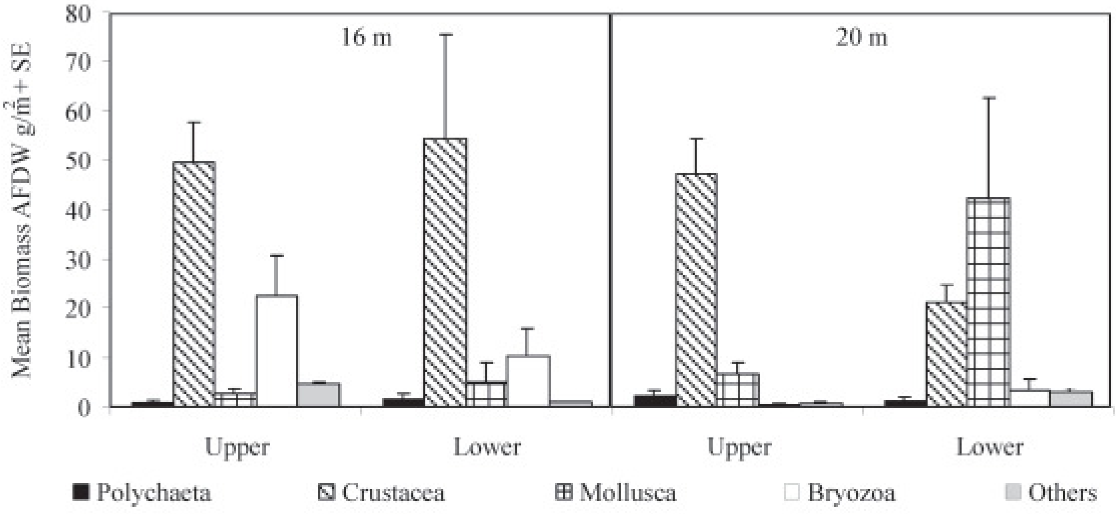


Figure 3. Mean (± SE) biomass values for major taxonomic groups in the two reef layers (upper and lower) at two depths (16 and 20 m).

The MDS ordination distinguished two main groups corresponding to the two different depths (Figure 4). Within both groups, the samples corresponding to each layer clustered together. The two-way crossed ANOSIM showed a significant difference between the 16 and 20 m reefs (R = 0.8, P = 0.01), but no dissimilarity between layers was observed (R = 0.3, P = 0.10). Additionally, SIMPER analyses indicated that the bryozoans *B. neritina* and *Scruparia chelata* (Linnaeus, 1758) had a strong contribution to the dissimilarity between depths, as they were exclusive to the 16 m reef (Table III). The *taxa* Ascideacea sp. I, *B. perforatus*, and Bryozoa sp. I had high biomass values at 16 m, but *A. ephippium* and the serpulids *Pomatoceros triqueter* (Linnaeus, 1767) and *Serpula vermicularis* (Linnaeus, 1767) had high values at 20 m.

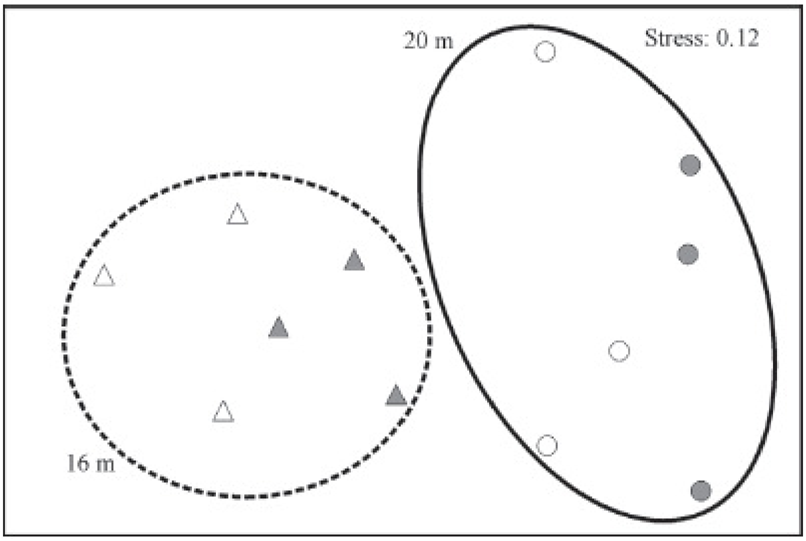
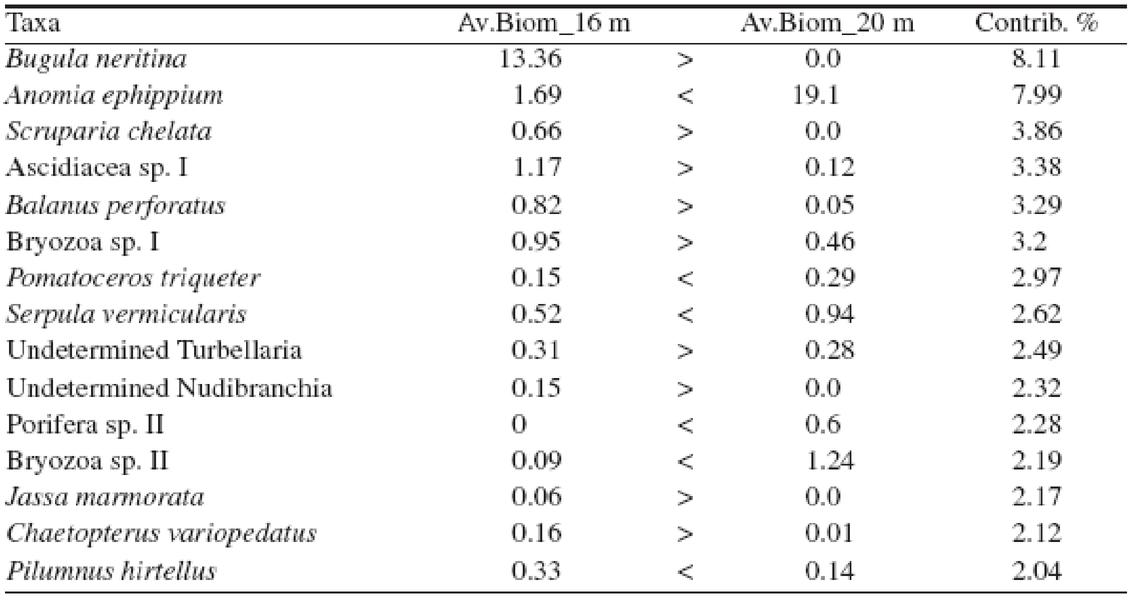


Figure 4. MDS ordination plots for reef groups and layers of the fourth-root transformed macrofaunal biomass data of the outside surface (△: reef at 16 m depth; ○: reef at 20 m depth; open symbols: upper layer; filled symbols: lower layer).

Table III. Summary of similarity terms (SIMPER) analysis (after fourth-root transformation). Differences (< and >) in mean biomass (AFDW g m^{-2}) of macrofaunal species contributing to dissimilarities between depths.



Taxa	Av.Biom_16 m		Av.Biom_20 m	Contrib. %
Bugula neritina	13.36	>	0.0	8.11
Anomia ephippium	1.69	<	19.1	7.99
Scruparia chelata	0.66	>	0.0	3.86
Ascidiacea sp. I	1.17	>	0.12	3.38
Balanus perforatus	0.82	>	0.05	3.29
Bryozoa sp. I	0.95	>	0.46	3.2
Pomatoceros triqueter	0.15	<	0.29	2.97
Serpula vermicularis	0.52	<	0.94	2.62
Undetermined Turbellaria	0.31	>	0.28	2.49
Undetermined Nudibranchia	0.15	>	0.0	2.32
Porifera sp. II	0	<	0.6	2.28
Bryozoa sp. II	0.09	<	1.24	2.19
Jassa marmorata	0.06	>	0.0	2.17
Chaetopterus variopedatus	0.16	>	0.01	2.12
Pilumnus hirtellus	0.33	<	0.14	2.04

Discussion

During 6 months of artificial reef submersion, serpulids, barnacles, bryozoans, and molluscs were able to settle and dominate the macrobenthic community of the Faro/Ancão artificial reef. Most of these groups are common pioneer *taxa* encroaching on clear surfaces (Chalmer, 1982; Kocak and Zamboni, 1998). Within this relatively short period, these communities achieved considerable abundances, biomass, and number of species, especially when compared to similar colonisation times from Mediterranean (Relini *et al.*, 1994; Kocak and Zamboni, 1998) and Florida (Cummings, 1994) artificial reefs, and from “early fouling” studies (Glasby, 2000; Glasby and Connell, 2001).

In the Faro/Ancão artificial reef, surfaces with different orientations had different epibiotic biomass patterns at 16 and 20 m. While no significant differences in biomass were found between vertical surfaces at the two depths, colonisation differed on the horizontal surfaces between the two depths. These results are consistent with previous works (e.g., D’Anna *et al.*, 2000; Glasby, 2000; Glasby and Connell, 2001), which showed that surfaces of different orientations develop different epibiotic assemblages. In this study, the top surface developed a significantly higher biomass, possibly related to higher sedimentation levels. Tumbiolo *et al*. (1995) observed the same trends for the Alcamo Marina reef. Such a decrease in the biomass and cover as a function of depth has also been highlighted in previous works (e.g., Relini *et al*., 1994; Kokac and Zamboni, 1998; Relini, 2000).

In the present work, the distribution of some species was depth-related, and influenced overall biomass and cover patterns. Previous studies have shown that some faunal groups, like bryozoans, barnacles, and serpulids occur in different proportions according to depth, with a clear dominance of barnacles (e.g., Kocak and Zamboni, 1998; Ardizzone *et al*., 2000; D'Anna *et al*., 2000; Glasby, 2000; Glasby and Connell, 2001). Barnacles, especially *B. amphitrite*, dominated the surface cover and the biomass of the macrobenthic communities in Faro/Ancão artificial reef. The opposite was observed in the Loano artificial reef, where this faunal group was never dominant (Relini *et al*., 1994). Other species such as the bryozoan *B. neritina* were also associated to the upper depth, while *A. ephippium* and serpulids showed a reverse trend. Depth and corresponding light levels influence (direct or indirectly) the community structure (Bohnsack *et al*., 1991) and the settlement of various species, including bryozoans (Glasby, 1999a). The general decrease in biomass and cover between the upper and lower reef layers was in contrast to biomass trends at the Alcamo Marina reef observed by Tumbiolo *et al*. (1995).

In this study, the central area of the cubic sample units was used to avoid the "border effect," allowing the comparison of opposite surfaces of the cubes. This procedure was applied to minimize contamination between adjacent cubic surfaces with different orientation. Therefore, it is possible that the biomass estimates of individual *taxa* were under- or overestimated, as some species occur in patchy distributions. Furthermore, direct comparisons between the cubic sampling units and the concrete reef modules have not yet been reported. Nevertheless, based on in situ visual surveys, we believe that the cubic units are representative of the artificial reef modules. Additionally, it should be noted that the present experiment was conducted for only 6 months and it is very likely that a longer-term study would produce different results. Early colonisers are good recruits to fresh substrata but poor competitors (Hatcher, 1997), thus the community structure at Faro/Ancão artificial reef will likely change with submersion time, with the total abundance and number of species increasing until reef maturity is reached. Species found on the Faro/Ancão artificial reef were not unique to this habitat, as they are found on natural rocky reefs (J. Cúrdia, IPIMAR - CRIPSul, pers. obs.), even though in very different proportions. The installation of artificial reefs to coastal sandy areas may increase local species diversity by allowing the settlement of new species that usually live on natural rocky reefs (Bacchiocchi and Airoldi, 2003). However, this must be balanced with the loss of biota on and in the seabed on which the reef has been placed (Jensen *et al.*, 2000). Those impacts are still unclear, but the introduction of new habitats could conceivably affect a variety of marine organisms (Glasby, 1999b; Bacchiocchi and Airoldi, 2003). Current Algarve artificial reef studies addressing the comparison between different artificial reef groups and temporal variation will hopefully provide important data for understanding the patterns of benthic colonisation on artificial substrata and the possible consequences of these changes on the structure and function of coastal assemblages in this region.

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CHAPTER IV.

Estimation of secondary production of the Faro/Ancão artificial reefs

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Moura, A.; Cancela da Fonseca, L.; Boaventura, D.; Santos, M. N., and Monteiro, C. C. Estimation of secondary production of the Faro/Ancão artificial reefs. Submitted to *Brazilian Journal of Oceanography*

Introduction

In spite of the growing use of reefs for restoration and mitigation, only few studies have provided substantive guidance on quantifying the necessary amount of structure to ensure adequate habitat replacement. Bohnsack and Sutherland (1985) referred the need of artificial reef productivity estimates to assess habitat restoration or mitigation success. Secondary production is a measurement of biological material created over a specific unit of time. This value is useful in comparing different habitats inhabited by a variety of species with different life histories and growth rates (Burton *et al*., 2002). Despite of their importance, few productivity studies have been undertaken and these have considered mostly fish (Johnson *et al*., 1994; Wilson *et al*., 2001). Traditional methods for calculating secondary production from the benthos have been applied to single animals or populations based on the change in body mass or growth over time. Generally, methods include those based on cohort analysis, size class based methods and the relationship between productivity and mortality (Morin *et al*., 1987). None of these methods are feasible when trying to quantify secondary production at the community level. In this case, biomass data was used to estimate secondary productivity of epifauna that colonised artificial reefs. Concrete reef units were deployed in south coast of Algarve to mitigate the scarcity of natural reefs and to enhance local fisheries. The development of the reef's epibenthic community, and its use as prey, has been reported (Leitão *et al*., 2007; Moura *et al*., 2008). In this study, the assessment of surface orientation in secondary production from the epifaunal benthos at two artificial reefs of the Faro/Ancão artificial reef system has been undertaken over a one year period.

Material and methods

This artificial reef system was spread over an area of 12.2 km^2 off Faro (Algarve, southern Portugal), on a sandy bottom. This study was performed on two randomly selected artificial reef groups submerged on August 2002 at 20 m depth. Each artificial reef group comprises three reef sets of 35 modules (each module with 2.7 m^3) (see Moura *et al*., 2008 for more information of these systems). The study of macrobenthic colonisation was performed using cubic sample units (15 cm side length) made of the same concrete material as the reef modules. The cubic sample units were set randomly at the time of the reef immersion. During the first year of immersion, three replicate samples were retrieved from each reef group at sixth and twelfth months by scuba diving. In laboratory, three out of the six cube surfaces (vertical: inside and outside; horizontal: top) were compared to investigate the role of surface orientation on benthic secondary production. Prior studies have concluded that no differences in abundance, biomass and number of *taxa* were observed between inside and outside surfaces (Moura *et al*., 2004, 2008), for the present study, both surfaces were analysed together as vertical surfaces. The samples were sieved through a 0.5 mm square mesh and the material retained was fixed in 4% buffered formalin. The biomass was obtained for biological samples dried to a constant weight at 70ºC (usually for at least 24–48 h). The ash-free dry weight (AFDW) was determined by burning the animals at 450ºC for 4 h in a muffle furnace. The AFDW was calculated by subtracting the ash weight from the dry weight.

Data analysis

The estimate production rates of the substrata with different orientation were calculated using the method of Boysen-Jensen (Yablonskaya *et al*., 1971). The growth increment is obtained from the sum of consumption and the biomass remaining, after subtracting the initial biomass:

$$P = B_e + B_2 - B_1;$$

the magnitude for consumption (B_e) was taken to be the difference between the initial (N_1) and final (N_2) numbers, multiplied by arithmetical mean of the initial (B_1/N_1) and the final (B_2/N_2) mean individual weights of the groups of animals under consideration. Thus B_e is given by the equation:

$$B_e = (N_1 - N_2) \times 1/2 \times (B_1/N_1 + B_2/N_2).$$

For some epifaunal species, biomass data was available, but abundance data was not. This occurred either because animals were colonial (thus it was not possible to count the number of individuals) or strongly bonded to the concrete surface (such as barnacles). In these cases it was not possible to account for production directly by applying Boysen-Jensen method. Secondary production was estimated by the P/B ratio (of 0.012) provided by Brey (2002) for miscellaneous benthic invertebrates.

Results

As expected, the estimated mean production increased after the artificial reef deployment (Figure 1). All surfaces showed a similar pattern, with a strong increase after six months. After six months, horizontal orientation surfaces exhibited a 10% higher production than vertical orientation surfaces. The horizontal orientation surfaces had a mean production of 128 g $m^{-2}yr^{-1}$, while vertical orientation surfaces showed a mean production of 103 g $m^{-2}yr^{-1}$. However, one year after deployment, the mean production of both orientations had a slow increment, showing similar values. Still horizontal orientation exhibited 10% more epibenthic production (103 g $m^{-2}yr^{-1}$) than vertical orientation (98 g $m^{-2}yr^{-1}$).

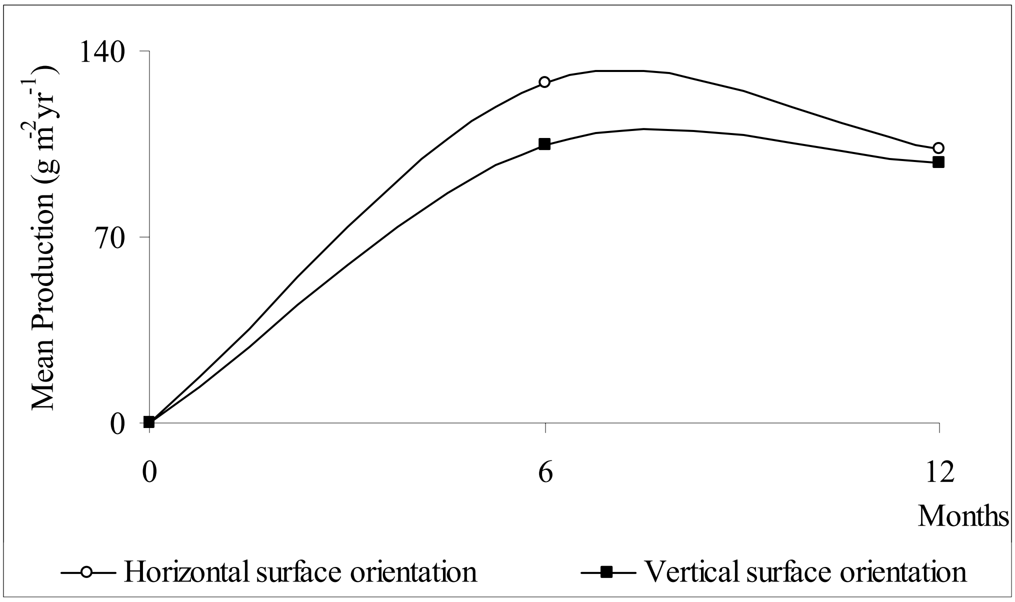

Figure 1. The production (g m^{-2} yr^{-1}) estimate for horizontal and vertical surfaces of the Faro/Ancão artificial reef system in the first year of deployment.

The mean annual production for the all Algarve AR complex was estimated by extrapolation of these data. This complex comprises approximately 81% of vertical and 19% of horizontal surfaces. After one year of deployment, the entire AR complex of the Algarve (with an area of 10ha of surface available for epibentic colonisation) will produce 10ton. On average, 8 ton will be produced on 8 ha of vertical surfaces and 2 ton in 2 ha of horizontal surfaces.

Discussion

Artificial reefs have the potential to fulfil many objectives for which they are promoted. However, their success will ultimately reflect the quality of prior planning and management (Baine, 2001). The Algarve artificial reef complex' construction required the development of a set of design parameters that should guarantee a persistent structural stability and population of fish to enhance local fisheries. Previously, for the AR fish assemblage, the rapid increase of macrobenthic community production in the months shortly after Faro/Ancão AR deployment, may have contributed to the fast rate of fish colonisation and stabilization referred by Leitão *et al*. (2008); as most of the resident fish species reefs are dependent on invertebrates for shelter purposes or for food (Leitão *et al*. 2007). While addressing the productivity issue, it is important to examine how epifauna colonising reef habitats enhance the availability of benthic invertebrate prey to fishery resource (Bohnsack *et al*., 1991). Thus, observations on both horizontal and vertical orientation of the artificial reef surfaces indicate that the initial epibenthic macrofauna colonisation on the horizontal surfaces is higher than on vertical surfaces, which contributed for substantially improved benthic secondary production. Nevertheless, the results indicate that, after one year, the surface orientation does not seem affect the production of reef surface area. Our primary objective was to quantify the effect of surface orientation on benthic secondary production. We recognize that secondary production is only one component of ecological benefit, but it is the only ecological metric at present that allows comparisons between habitat types with different species, life histories, and growth rates (Burton *et al*., 2002). Other components of ecosystem health, such as biodiversity, were not addressed here (but see Moura *et al*., 2004; 2008).

Furthermore, we intended to extrapolate the secondary production estimative for the Algarve artificial reef complex. These systems provide additional surface area of almost 50 000 m^2 for the development of encrusting communities (thus increasing species richness) and shelter that attracts different fish species; the attraction-production controversy (Svane and Petersen, 2001). After the first year of colonisation, A. Moura (unpublished data) reported that, for the Faro/Ancão artificial reefs, biomass was declining throughout the study of four years of colonisation. So, it’s also possible that secondary production could be diminishing throughout that period. Moreover, Moura *et* *al* (2006) observed that the biomass production was affected by depth and reef layer. Nevertheless, it can be argued that variability in the productivity of different *taxa* associated with structure s' age and environmental factors may be large. Artificial reefs apparently may enhance benthic secondary production per unit area in the Algarve southern coast, but further studies for a longer period of time are needed.

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CHAPTER V.

A four year study on the macrobenthic colonisation of artificial reefs: influence of surrounding habitats.

Moura, A.; Cúrdia, J.; Carvalho, S.; Cancela da Fonseca, L.; Boaventura, D.; Santos, M. N., and Monteiro, C. C. A four year study on the macrobenthic colonisation of artificial reefs: influence of surrounding habitats. Submitted to *Hydrobiologia.*

Introduction

Coastal environments throughout the world have experienced a proliferation of man-made artificial hard substrates such as artificial reefs (ARs), pier pilings, bulkheads, jetties and pontoons associated with coastal urbanization and boating/shipping activities (Atilla *et al*., 2003). Many ARs have been deployed to enhance the productivity of marine ecosystems in areas with paucity of natural rocky reefs (Monteiro and Santos, 2000; Svane and Petersen, 2001). They have the potential to provide not only shelter for mobile organisms (e.g. thigmotropism in fish), but also food, namely for commercial species (like cephalopods, crustaceans and finfish), as a consequence of the attachment of sessile invertebrates to the hard surfaces (Bohnsack and Sutherland, 1985; Ardizzone *et al*., 1997; Steimle *et al*., 2002). The presence of invertebrate and algal assemblages is essential for the effectiveness of ARs, contributing to the diversity of the community and to the variety of habitats. Furthermore, these species cement an AR, making the structure more stable and perhaps longer-lasting, and contribute to a higher resemblance to natural environments (Carter *et al*., 1985; Fitzhardinge and Bailey-Brock, 1989; Relini *et al*., 1994; Svane and Petersen, 2001).

The submersion of ARs on the seabed provides vacant hard substratum to be colonised by a large number of epibenthic organisms (Svane and Petersen, 2001). The establishment of fouling assemblages is a complex process, as many factors, both biological and physical, can influence the success, intensity and variability of recruitment of benthic marine invertebrates. The abundance of larvae in the water column is influenced by reproductive cycles but the success of recruitment is also associated with larval mortality during attachment. Physical factors such as currents, water depth, seasonality, substratum type and its heterogeneity is also of utmost importance for the establishment of fouling assemblages (Eckman, 1983; Roughgarden *et al*., 1988; Glasby, 1999; Chapman, 2002; Rule and Smith, 2005). In fact, the recruitment of benthic organisms to ARs occurs predominantly by migration from adjacent substrata or by settlement of spores and larvae (Cummings, 1994; Svane and Petersen, 2001). Consequently, the existence of already established communities in the vicinity will probably play an important role in recruitment of ARs as source of potential colonists.

Since 1990 seven AR systems were deployed along the southern coast of Portugal, over a total area of more than 43km^2, making it the largest project of this kind in Europe. The fish colonisation of Portuguese ARs has been relatively well studied (e.g. Santos and Monteiro, 1997, 1998, 2007; Monteiro and Santos, 2000). The knowledge of macrobenthic colonisation patterns is still in progress (Boaventura *et al*., 2006; Moura *et al*., 2004, 2006, 2007, 2008). However, the information regarding the role of nearby communities to the colonisation and succession of the ARs is still lacking. This information is important especially concerning AR systems with such dimension as it will contribute to a better knowledge of the possible consequences of these changes on the structure and function of coastal assemblages. Therefore, long-term spatial and temporal recruitment and succession patterns of hard bottom benthic assemblages were analysed over a period of four years in order to evaluate the role of nearby communities on epifaunal colonisation patterns on clear surfaces.

Material and methods

To study the effects of the surrounding environment on epifaunal colonisation patterns, clear sample units were immersed at one reef group with an established epibenthic communities (deployed in 1990) and at two newly deployed reef groups (both immersed at the beginning of the experience – August 2002). The three reefs groups were located in Faro/Ancão AR system (Algarve, southern Portugal) at approximately 20m depth (see Moura *et al*., 2008 for further details).

The experiment was carried out using 15 x 15 x 15cm cubic sample units, made of the same concrete material of reef modules. In August of 2002 the uncolonised cubic units were randomly hung on the reef blocks of three reef groups. Three replicate samples were retrieved by scuba diving after 6, 12, 24, 36 and 48 months of immersion. The outside vertical surfaces of the cubic samples were scraped to posterior analysis of macrobenthic colonisation and succession. The samples were sieved through a 0.5mm square mesh, and the retained material was fixed in 4% buffered formalin. The collected material was sorted and identified to species level (whenever possible) and the biomass of each species determined. Macrobenthic biomass was estimated as ash-free dry weight (AFDW) according to the technique used by Moura *et al*. (2006).

Data analysis

An asymmetrical analysis of variance (ANOVA) was used to test for differences on epifaunal abundance, number of *taxa* and biomass between areas with (old reef) and without (new reefs) nearby established communities. The method of construction of the asymmetrical analysis used in this study follows that described in Underwood (1997). The required sums of squares for the asymmetrical components of these analyses were also calculated following the latter author. The asymmetrical ANOVA was performed in three steps: (1) a 1-way ANOVA was done for all reefs to obtain a value of sum of squares (SS) for the comparison of all reefs; (2) a second 1-way ANOVA was done using only the values for the new reefs; (3) a value for the SS for the old reef was obtained by subtracting the SS for the new reefs from the SS for all reefs. Degrees of freedom and mean square (MS) values were similarly derived. An F-value for the comparison of the old reef and new reefs was obtained by dividing MS of the comparison of the old reef and new reefs by MS of the residual. The assumption of homogeneity of variances was tested by Cochran's C-test. However, when variances were heterogeneous but the analysis did not detect significant differences, according to Underwood (1997), the results were still considered valid because an excessive Type I error can't be made. Therefore, the untransformed data were analysed, because analysis of variance is relatively robust to heterogeneous variances (Underwood, 1997).

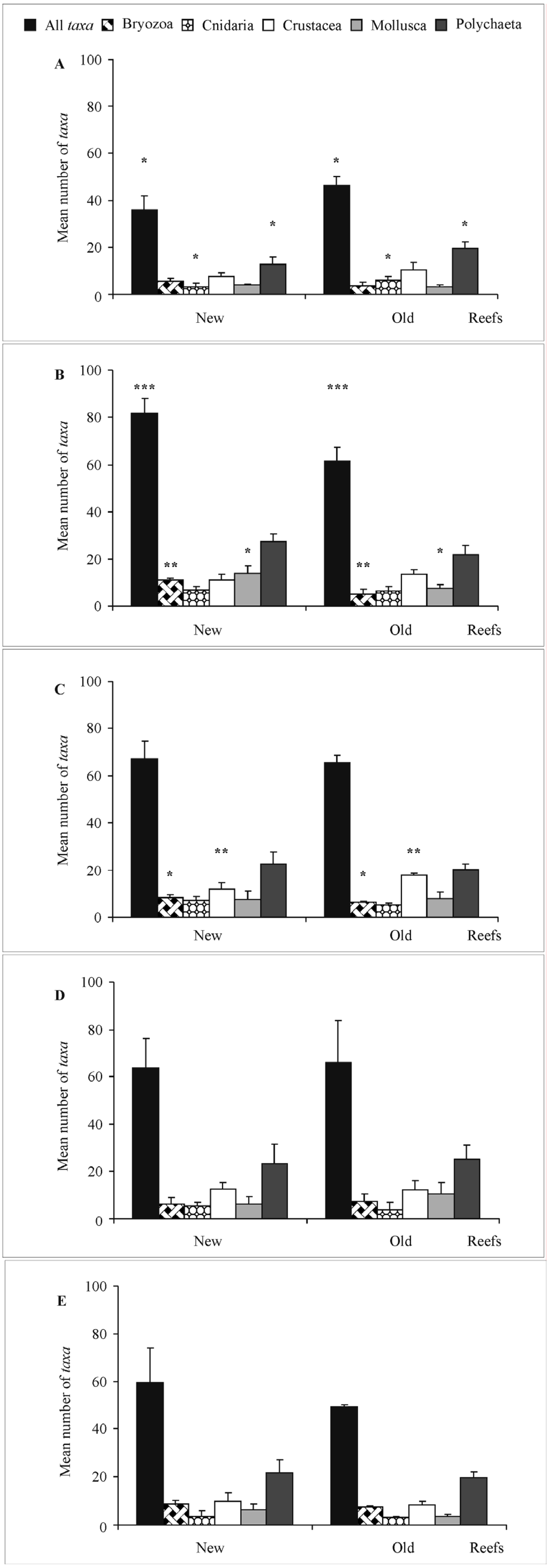
Multivariate statistical techniques were used to analyse the variation in species abundance and composition at each reef and at each sampling period using the PRIMER software package (Plymouth Marine Laboratories, UK). The Bray-Curtis index was used as faunal similarity coefficient calculated using species/abundance matrix after fourth-root (√√) transformation. The similarity matrix was used to generate 2-dimensional plots with the non-metric multi-dimensional scaling (nMDS) technique (Clarke, 1993). The SIMPER procedure (similarity percentage analysis, PRIMER v5.0) was used to identify the *taxa* that characterise each group identified by the non-metric multidimensional scaling (MDS) plots (Clarke, 1993).

Results

Species richness

A total of 246 *taxa* belonging to fifteen phyla were identified on the cubic units. Most benthic species were polychaetes (105 *taxa*), followed by crustaceans (44 *taxa*), molluscs (37 *taxa*), cnidarians (17 *taxa*) and bryozoans (19 *taxa*). After six months of deployment, the samples from the old AR exhibited significant higher number of *taxa* (46 *taxa*) than those of the new reefs (36 *taxa*) (ANOVA, $F = 6.8$; $p < 0.05$) (Figure 1 A). At this time a significant higher number of cnidarian (especially hydrozoans)

(ANOVA, F = 7.0; $p < 0.05$) and polychaete *taxa* (ANOVA, F = 9.2; $p < 0.05$) was observed on the assemblages of the older reef (Figure 1 A). Polychaetes were the group with the highest number of *taxa* at all reefs, and this pattern was consistent along time (Figure 1 A-E). After one year of immersion, the samples of the newly deployed ARs had a greater increase in the number of *taxa* (82 *taxa*), significantly overcoming the number of *taxa* in the old reef (61 *taxa*) (ANOVA, F = 19.6; $p < 0.001$) (Figure 1 B). This was probably due to the significant higher number of bryozoan species (ANOVA, F = 32.4; $p < 0.01$) and of molluscs (ANOVA, F = 7.9; $p < 0.05$) identified at the assemblages of the newly deployed reef (Figure 1 B). Significant differences between taxonomical groups were still observed after 24 months of deployment (Figure 1 C), with more bryozoan *taxa* (ANOVA, F = 7.6; $p < 0.05$) on newly deployed reefs, while at the older reef a higher number of crustacean *taxa* was observed (ANOVA, F = 30.4; $p < 0.01$) (Figure 1 C). Afterwards, there were no significant differences either for all *taxa* or the main taxonomic groups on the mean number of *taxa* between the new and old reefs (Figure 1 D-E).



* p < 0.05; ** p < 0.01; *** p < 0.001

Figure 1. Mean number of taxa for all taxa combined and for the main animal groups registered on areas with (Old) and without (New) nearby established communities during 6, 12, 24, 36 and 48 months of immersion (A, B, C, D and E, respectively). Significant results of the asymmetrical ANOVAs on mean number of taxa of the main animal groups are represented above columns.

Abundance

In general, the cubic samples placed on the old reef exhibited similar abundance values throughout the study period (Figure 2 A). After 6 months of deployment, the samples from the oldest reef showed higher abundance values (1123 ind. × $0.0225m^{-2}$) than the new reefs (369 ind. × $0.0225m^{-2}$) (Figure 2 A). However after 12 months of deployment, the new reef groups had a strong increase of abundance values with 2641 ind. × $0.0225m^{-2}$ (Figure 2 A). During the 24 to 36 months of immersion, new reefs assemblages' exhibit higher abundance values (between 2268 to 2035 ind. × $0.0225m^{-2}$, respectively) than old reef (1284 and 1239 ind. × $0.0225m^{-2}$, respectively) (Figure 2 A). After 48 months of deployment, the assemblages of all reefs showed a strong decrease in abundance. Nevertheless, new reefs' assemblages presented higher abundance values (451 ind. × $0.0225m^{-2}$) than assemblages of the old reef (250 ind. × $0.0225m^{-2}$) (Figure 2 A). Despite the observed trends, no significant differences were observed between ARs for abundance values (Table I), due to the high variability of the samples.

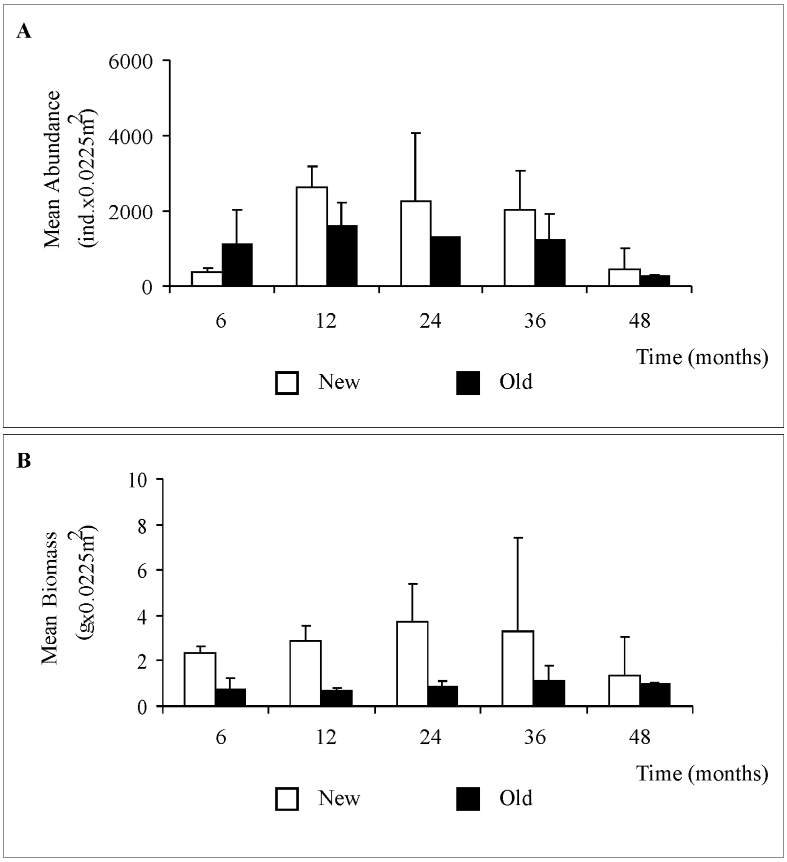


Figure 2. Mean abundance (ind. × $0.0225m^{-2}$) (A) and mean biomass (g × $0.0225m^{-2}$) (B) on areas with (Old) and without (New) nearby established communities during the study period (6, 12, 24, 36 and 48 months of immersion).

Table I. Asymmetrical ANOVA comparing the mean abundance on areas with (Old) and without (New) nearby established communities during the study period (6, 12, 24, 36 and 48 months of immersion).

		6 months		**12 months**		**24 months**		**36 months**		**48 months**	
Source of Variation	**df**	MS	F	MS	F	MS	F	MS	F	MS	F
Among all reefs	2	575021.8	2.06 ns	1140279.1	3.06 ns	3291456.1	1.70 ns	999717.3	1.05 ns	243855.4	1.21 ns
Within groups	6	279302.7		372044.4		1938165.3		949406.2		200719.7	
Total	8										
Cochran's test		*C* = 0.97 *		*C* = 0.40 ns		*C* = 1.00 ***		*C* = 0.54 ns		*C* = 0.93 *	
Among New	1	14016.7	1.00 ns	30530.7	0.08 ns	4646400.0	1.60 ns	732202.7	0.62 ns	406640.7	1.36 ns
Within groups	4	14055.3		359922.7		2906960.0		1187530.7		299570.8	
Total	5										
Cochran's test		*C* = 0.69 ns		*C* = 0.62 ns		*C* = 0.99 *		*C* = 0.65 ns		*C* = 0.94 ns	
Old vs New	**1**	1136026.9	4.07 ns	2250027.6	6.05 ns	1936512.0	1.00 ns	1267232.0	1.33 ns	81070.2	0.40 ns
Among New	**1**	14016.7	0.05 ns	30530.7	0.08 ns	4646400.0	2.40 ns	732202.7	0.77 ns	406640.7	2.03 ns
Residual	**6**	279302.7		372044.4		1938165.3		949406.2		200719.7	
Total	**8**										
Cochran's test		*C* = 0.98 **		*C* = 0.57 ns		*C* = 1.00 ***		*C* = 0.70 ns		*C* = 1.00 *	

ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

The MDS ordination showed a clear separation of two groups, corresponding to the ARs with different ages. Within the group of samples corresponding to the ARs newly deployed, the samples were clustered together, with no differentiation of the samples from the two reef groups. This pattern was observed for all sampling periods (Figure 3 A-E).

After 6 months, the SIMPER routine distinguished the old reef assemblages from the new reefs assemblages' by the higher abundance of *Filograna implexa* Berkeley, 1827, *Polydora caeca* Webster, 1879 and *Serpula vermicularis* Linnaeus, 1767, and exclusive presence of *Vermiliopsis striaticeps* (Grube, 1862) and *Protula* spp. (Table II). Conversely, the bivalve *Hiatella arctica* (Linnaeus, 1767) showed higher values in the samples deployed at the new reefs. After 12 months, the dissimilarity between old and new reefs was due to the presence of *V. striaticeps* (exclusive) and *Pomatoceros triqueter* (Linnaeus, 1758) at the older reef, and to the higher abundance of copepods, the polychaetes *Polydora hoplura* Claparède, 1870 and *P. caeca* and the bivalve *Kellia suborbicularis* (Montagu, 1803) (exclusive) in the new reefs (Table II).

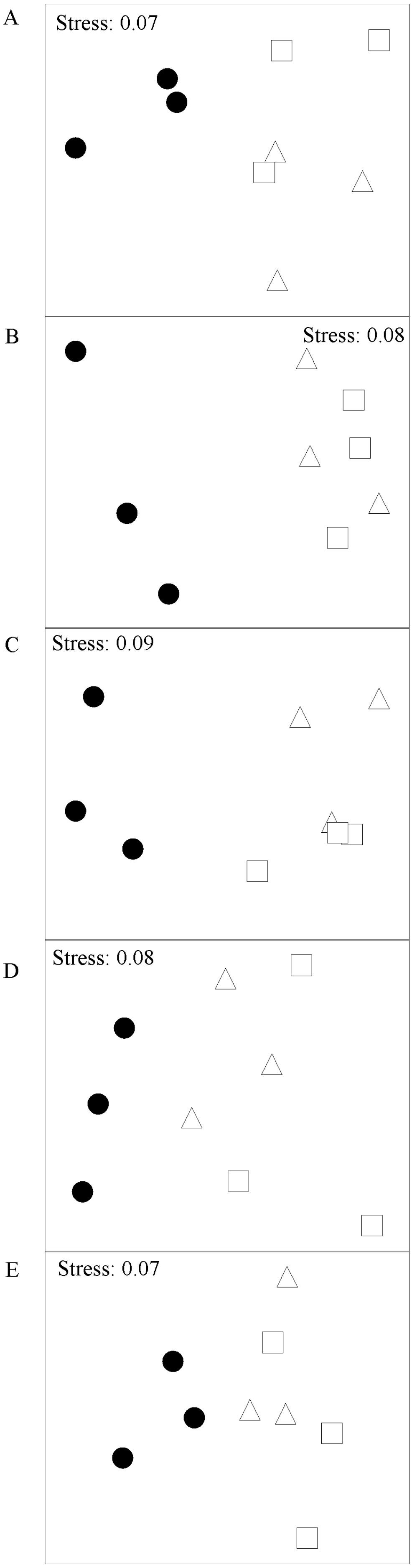


Figure 3. MDS ordination comparing abundances data from assemblages on areas with (black filled ball symbols) and without (new reef 1 – open square symbols; new reef 2 – open triangle symbols) nearby established communities during 6, 12, 24, 36 and 48 months of immersion (A, B, C, D and E, respectively).

Table II. Species identified by SIMPER routine as having a high contribution to the average dissimilarity between the abundance of epibenthic assemblages on areas with (Old) and without (New) nearby established communities during the study period (6, 12, 24, 36 and 48 months of immersion). Mean values and differences (< and >) are presented.

Taxa	New		Old	Contrib%
6 months				
Filograna implexa	5.33	<	698	7
Vermiliopsis striaticeps	0	<	40	4.7
Hiatella arctica	134.5	>	12	3.72
Polydora caeca	2.83	<	59.33	3.51
Serpula vermicularis	1.5	<	40.33	3.5
Protula sp.	0	<	10.67	3.34
12 months				
Copepoda	218	>	25.33	2.24
Polydora hoplura	533.33	>	58.67	2.21
Kellia suborbicularis	18	>	0	2.12
Polydora caeca	30	>	1.33	2.01
Vermiliopsis striaticeps	0	<	12	1.96
Pomatoceros triqueter	10.67	<	101.33	1.93
24 months				
Polydora hoplura	96.67	>	2.67	2.3
Anthozoa	1.33	<	42.67	2.26
Vermiliopsis striaticeps	0	<	16	2.2
Lembos cf. *websteri*	27.33	>	0	2.12
Elasmopus rapax	0	<	42.67	2
Copepoda	1.33	<	32	1.74
36 months				
Corophium spp.	1.33	<	34.67	2.22
Tanaidacea sp. I	54.67	>	2.67	2.16
Alvania cf. *semistriata*	0	<	24	2.09
undetermined Cirratulidae	28	>	0	2.05
Lembos cf. *websteri*	38.67	>	0	1.9
Gnathia sp.	11.33	>	0	1.72
48 months				
Chone spp.	1	<	59.33	4.44
Amphipoda sp. I	0	<	24.33	3.99
Anthozoa	4.17	<	22.33	2.69
Lembos cf. *websteri*	21.83	>	0.67	2.53
Phascolosoma granulatum	7.5	>	1	2.06
Eumida sanguinea	2.83	>	0	1.83

After two years of immersion, differences between assemblages were mainly due to higher abundance of *P. hoplura* and the exclusive presence of the amphipod *Lembos* cf.

websteri Bate, 1857 at the recent reef groups. Additionally, at the older reef, anthozoans, the amphipod *Elasmopus rapax* Costa, 1853 and the exclusive presence of *V. striaticeps* was important to the dissimilarity between assemblages (Table II). After three years of immersion, the old reef assemblages were distinguished from the new ones by exhibiting higher abundance of the amphipod *Corophium* spp., and also by the exclusive presence of the gastropod *Alvania* cf. *semistriata* (Montagu, 1808) (Table II). What is more, the new reef assemblages were characterised by higher abundance of Tanaidacea sp. I and the exclusive presence of *L.* cf. *websteri*, undetermined Cirratulidae and *Gnathia* sp. (Table II). The higher abundance of the polychaete *Chone* spp., anthozoans and Amphipoda sp. I (exclusive) on the assemblages of the older reef, contributed to the dissimilarity between old and recent reefs group after four years of immersion. On the other hand, *L.* cf. *websteri* persisted as a characteristic species on the new reefs group assemblages, along with the sipunculid *Phascolosoma granulatum* Leuckart, 1828 and the polychaete *Eumida sanguinea* (Örsted, 1843) (Table II).

Biomass

The assemblages of the newly deployed reefs showed higher biomass values than the assemblages of the older reef throughout the sampling time (Figure 2 B). Generally, the assemblages of the new reef groups had 3 to 4 times more biomass than on the assemblages of the old reef group, with the exception of the samples with 4 years of immersion where lower differences were detected (Figure 2 B). However, the biomass of epibiota was only significantly higher on samples from the newly deployed reefs at 6, 12 and 24 months after deployment (Table III; Figure 2 B). After 36 months, no significant differences were observed (Table III; Figure 2 B). On the assemblages of the new reefs there was a pattern of biomass increase until the second year (maximum of

3.7 g × 0.0225m^{-2}), followed by a decline, reaching a minimum of 1.3 g × 0.0225m^{-2} after 4 years; whereas on the older reef, biomass was rather constant throughout the sampling period (between 0.7 and 1.1 g × 0.0225m^{-2}) (Figure 2 B). Biomass dominance was different between reefs at all sampling periods (Figure 4 A–E). Along time, the biomass of the new reefs was always dominated by crustaceans, namely the barnacles *Balanus amphitrite* Darwin, 1854 and *Megabalanus tulipiformis* (Ellis, 1758), and after two years bryozoans were also important for the biomass. On the old reef, biomass dominance changed within time periods; after 6 months, crustaceans (65%) and polychaetes (29%) were the taxonomical groups that contributed the most to the biomass after 6 months of immersion (Figure 4 A). After one year crustaceans (53%), polychaetes (18%), also cnidarians (particularly anthozoans) (17%) were relevant to the total biomass (Figure 4 B). Two years after deployment, bryozoans presented an important contribute to total biomass in old reefs (10%) (Figure 4 C). After three and four years of immersion, no differences were observed in the taxonomical groups that contributed to the higher biomass values (Figure 4 D-E). Macroalgae were only recorded 36 months after immersion at the new reefs, but were never observed at the old throughout the study period.

Table 3. Asymmetrical ANOVA comparing the mean biomass on areas with (Old) and without (New) nearby established communities during the study period (6, 12, 24, 36 and 48 months of immersion).

		6 months		**12 months**		**24 months**		**36 months**		**48 months**	
Source of Variation	**df**	MS	F	MS	F	MS	F	MS	F	MS	F
Among all reefs	2	2.8	27.97 ***	5.3	21.33 **	8.3	3.66 ns	9.9	0.78 ns	1.9	1.09 ns
Within groups	6	0.1		0.3		2.3		12.6		1.8	
Total	8										
Cochran's test		C = 0.83 ns		C = 0.74 ns		C = 0.70 ns		C = 0.96 *		C = 0.98 **	
SNK tests		Old < New_1 = New_2		Old < New_1 = New_2							
Among New	1	0.4	14.63 *	0.5	1.31 ns	0.1	0.03 ns	9.9	0.53 ns	3.6	1.33 ns
Within groups	4	0.0		0.4		3.4		18.6		2.7	
Total	5										
Cochran's test		C = 0.85 ns		C = 0.76 ns		C = 0.71 ns		C = 0.97 ns		C = 0.98 *	
Old vs New	**1**	5.1	52.20 ***	10.2	40.75 ***	16.4	7.27 *	9.8	0.78 ns	0.3	0.18 ns
Among New	**1**	0.4	3.73 ns	0.5	1.91 ns	0.1	0.04 ns	9.9	0.79 ns	3.6	2.00 ns
Residual	**6**	0.1		0.3		2.3		12.6		1.8	
Total	**8**										
Cochran's test		C = 0.72 ns		C = 0.95 ns		C = 0.97 ns		C = 0.97 *		C = 1.0 **	
SNK tests		Old reef < New reef		Old reef < New reef		Old reef < New reef					

ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

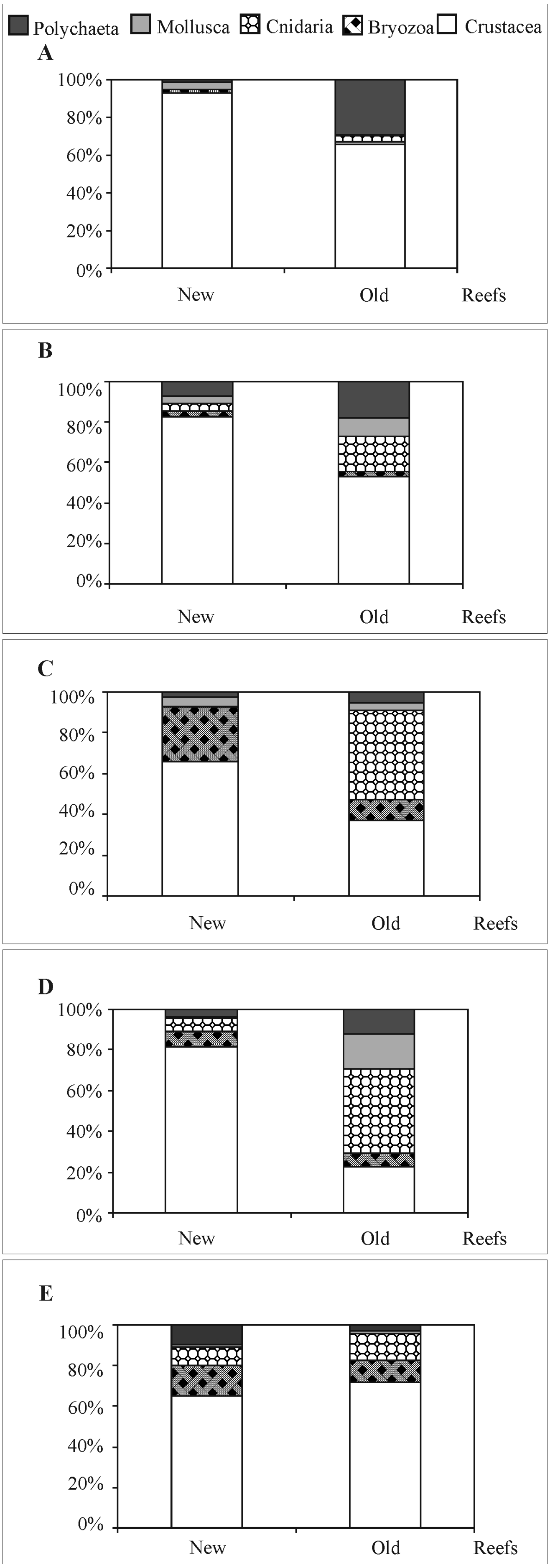


Figure 4. Percentage biomass of the main animal groups on areas with (Old) and without (New) nearby established communities during the study period 6, 12, 24, 36 and 48 months of immersion (A, B, C, D and E, respectively).

Discussion

Community structure and development on hard substrata are determined by complex abiotic and biotic interactions (Mullineaux and Butman, 1991; Kocak and Zamboni, 1998; Koehl, 2007). The colonisation process is determined by several factors, one of them being the source of recruits. In the present work, differences were detected concerning abundance, biomass and community structure between samples deployed in areas with nearby established hard bottom communities (old reef, an AR deployed more than a decade ago) and samples deployed in areas without any hard-bottom communities in the vicinity (new reefs, ARs deployed in sandy bottoms at the same time as the samples).

Since, the old reef group at the time of the present study was strongly colonised, it was expected that the new available substratum submerged on this reef would be more easily colonised (Roughgarden *et al*., 1988; Cummmings, 1994; Petersen and Svane, 1995; Svane and Petersen, 2001). However, the assemblages of the old reef only exhibit higher abundance and number of *taxa* after six months of immersion. After that, the development of species richness, abundance and biomass in older AR assemblages were overcome by those in new reefs. ARs are important for adjacent fish as food resource (Lindquist *et al*., 1994; Relini *et al*. 2002; Fabi *et al*., 2006; Leitão *et al*., 2007), so the differences observed may be related with a higher predation pressure on the sample units' placed on the colonised AR, maintaining the low values of old reef assemblages. On the other hand, the recent deployed ARs, have lower fish densities and biomass (IPIMAR, unpublished data), and the predation on macrofauna is consequently lower. Additionally, the presence of surrounding anthozoan species in the old AR may also affect the epibiotic colonisation on the sample units, by predation on larvae and space-competition (Osman and Whitlatch, 1998, 2004) contributing to a depressed number of larvae available for colonisation. The established macrofauna community may suppress the growth of other resident species and inhibit the invasion of subsequent new species (Connell and Slatyer, 1977; Greene *et al*., 1983; Carter *et al*., 1985; Boaventura *et al*., 2002a). Accordingly, intra and inter-specific relationships for space and food in the community can lead to some species dominance or absence (Moyse and Hui, 1981; Mullineaux and Butman, 1991; Rodríguez *et al*., 1993; Boaventura *et al*., 2002b, 2003). Many research projects have been undertaken that describe benthic succession using different experimental approaches involving artificial reefs or defaunated natural substrata (Bailey-Brock, 1989; Relini *et al*., 1994; Brown and Swearingen, 1998; Kocak and Zamboni, 1998; Glasby and Connell, 2001). However, few studies concluded that true ecological succession occurs in marine habitats (Scheer, 1945), since the episode of a true succession is difficult to identify (Greene *et al*., 1983; Carter *et al*., 1985; Nandakumar, 1996). In the Faro/Ancão ARs, the dominant *taxa* of the new reefs assemblages on the first and second year of immersion were typical pioneer *taxa* of vacant substrata (barnacles, serpulids, *Hiatella arctica* and *Polydora hoplura*). Those *taxa* have been pointed by several authors as encroaching on clear surfaces (Chalmer, 1982; Kocak and Zamboni, 1998). While after 3 and 4 years of deployment, the assemblages of the new reefs were characterised by the dominance of several crustaceans (like Tanaidacea sp. I and *Lembos* cf. *websteri*) and bryozoans. On the old reef assemblages, the succession of species occurred differently. One and two years after deployment, the characteristic species were polychaetes (such as *Filograna implexa* and *Pomatoceros triqueter*) and cnidarians (anthozoans and hydrozoans). After three and four years of immersion, anthozoans, crustaceans (like *Corophium* spp.) and polychaetes (like *Chone* spp.) were the species typical of old reef assemblages. It seems that settlement/colonisation of the vacant substrata is different, namely due to the presence or absence of a mature community in the surrounding areas. On the new reefs the assemblages were dominated by pioneer organisms, typical of the colonisation of clean substrata; whereas in old reefs, these organisms were less representative and the assemblages were dominated by other species.

Previous studies on the Algarve ARs described that older AR structures at lower depth have important macroalgae colonisation (Monteiro and Santos, 2000). However, macroalgae appeared only on the new reefs assemblages, at the 36 and 48 months sampling dates and their biomass values were very low, with insignificant contribution for the total biomass. The factors that are influencing the algae development are still unclear. Fortunately, other studies in progress at this region comparing artificial reefs with natural reefs and over a longer time frame will contribute to a better knowledge on the colonisation and succession processes occurring in the artificial reefs' system of the Algarve. Nevertheless, the present study showed that future studies on colonisation and succession of subtidal epibiotic assemblages, must take into consideration the existence or not of nearby communities as well the faunal composition of those communities as they influence the colonisation patterns.

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CHAPTER VI.

Are artificial reefs macrobenthic communities' alike to those of natural reefs?

Moura, A.; Cúrdia, J.; Carvalho, S.; Cancela da Fonseca, L.; Boaventura, D.; Santos, M. N. and Monteiro, C. C. Are artificial reefs macrobenthic communities' alike to those of natural reefs? Submitted to *Estuarine, Coastal and Shelf Science*.

Introduction

Artificial reefs (ARs) have been regarded as potentially useful tools for the management of fishing activities, enhancing the productivity of commercial species and mitigating habitat losses (Svane and Petersen, 2001). These man-made structures have also been deployed to provide hard substrates, where natural rocky reefs (NRs) are scarce (Bohnsack and Sutherland, 1985). The installation of ARs within coastal sandy areas will provide suitable substrata for benthic invertebrates and fish (Santos *et al.*, 1995), contributing to the increase of local diversity (Bohnsack and Sutherland 1985; Perkol-Finkel *et al.*, 2006).

In general, NRs have a wide range of slopes, resulting in several ecological niches (i.e. crevices, pools and overhangs), whereas the orientation of the substratum on ARs is mainly vertical with even surfaces. So, the more complex and heterogeneous reef structure is, the greater array of niches is offered, and that will affect the establishment and development of the communities (Glasby and Connell, 2001; Brown, 2005; Perkol-Finkel *et al.*, 2006). Therefore, it is important to understand if the structural characteristics of ARs will promote a similar establishment of benthic communities to those occurring within NRs (Svane and Petersen, 2001). Comparisons with NRs are of upmost importance not only to evaluate the performance of ARs but also to assess the potential ecological effects of introducing artificial structures into the marine environment (Knott *et al.*, 2004).

Despite the proliferation of works supporting the introduction of artificial structures on coastal areas, the full impact of this action is still unclear, but early indications suggest that it may be profound (Connell and Glasby, 1999; Glasby, 1999a; Bacchiocchi and Airoldi, 2003; Moschella *et al.*, 2005). Moreover, the settlement on the ARs of new species must be balanced with the loss of biota on and in the seabed on which the reef has been placed (Jensen *et al.*, 2000).

Since 1990, seven AR systems were deployed along the southern coast of Portugal, over a total area of more than 43km^2, making it the largest project of this kind in Europe. The ARs were deployed to enhance local fisheries and to provide artificial hard substrates to minimize the scarcity of natural reefs, but also as a management tool to preserve and promote the rehabilitation of marine habitats. They were deployed in areas next to estuaries and near-shore habitats. As they have the potential to increase food resources and shelter, they contributed to extend the nursery effect of estuarine-lagoon systems along the coast (Monteiro and Santos, 2000). However, it is important to evaluate the ecological value of these AR surfaces as habitat for epibenthic assemblages, in comparison to the NR surfaces they intent to mimic. So, in the present study we aimed to evaluate if macrobenthic community structure of ARs is similar to the one of nearby NRs. For the present study, the NRs benthic communities were compared to those of ARs deployed in 1990 (the oldest ARs in the southern coast of Portugal).

Material and methods

In order to accomplish the objectives of the present study, four locations were sampled: two artificial reef sets (AR#1 and AR#2) deployed in 1990 and two natural reefs (NR#1 and NR#2), at approximately 20m depth. Data collection was performed in August 2006. At each sampling station, three quadrats (15 x 15cm) of vertical surface orientation were scraped, and the macrobenthic community was collected by suction sampling. The samples were sieved through a 0.5 mm square mesh, and the retained material was fixed in 4% buffered formalin. The collected material was sorted and identified to species level (whenever possible) and the biomass of each species per area determined. Macrobenthic biomass (ash free dry weight) was determined according to the technique applied in Moura *et al.* (2006).

Data analysis

As many species of hard substrata are colonial organisms, their quantification is only possible using percentage cover methods and/or biomass data. Due to the sampling method, only biomass measures were used for colonial organisms (such as Hidrozoa, Bryozoa, Tunicata and Porifera) and barnacles. Therefore, the abundance data presented in this study is only related to organisms that can be counted. Differences in community structure between the two reef types were evaluated using both univariate and multivariate statistical analyses. One-way ANOVA was used to test for differences between natural and artificial reefs concerning the number of *taxa*, abundance, biomass and taxonomic distinctness. The assumption of homogeneity of variances was tested by Cochran's C-test. However, when variances were heterogeneous but the analysis did not detect significant differences, according to Underwood (1997) the results were still considered valid because an excessive Type I error couldn't be made. Therefore, the untransformed data were analysed, because analysis of variance is relatively robust to heterogeneous variances (Underwood, 1997). To assess the taxonomic distinctness of each sample, three measures were calculated: the average taxonomic distinctness (Δ^+), the total taxonomic distinctness ($s\Delta^+$) and the variation in taxonomic distinctness (Λ^+). These measures were based on a presence/absence (P/A) matrix and were calculated using the DIVERSE procedure in the PRIMER computer software package. According to Warwick and Clarke (1995), these indices have the potential to be more sensitive to assess community structure than species diversity.

The sampled communities have both colonial (not counted) and non-colonial (counted) *taxa*, and in order to assess community structure holistically a P/A matrix was used for the multivariate analysis with the Bray-Curtis index. The SIMPER procedure (similarity percentage analysis) was used to identify those *taxa* that characterise each group identified by the non-metric multidimensional scaling (MDS) plots. A one-way analysis of similarity (ANOSIM) was also performed to compare the two reef types.

Additionally, biomass production estimates were related to the feeding types of the *taxa*. Based on published data, the feeding structure analysis was performed by assigning each *taxon* to one or more trophic groups. Four feeding types were established: suspension feeding, deposit feeding, herbivory and carnivory. Quite often, the degree of speculation involved in this calculation is fairly high. Many species can exhibit different modes of feeding in particular situations. Therefore, biomass values regarding each *taxon* with more than one feeding types were evenly distributed among the feeding groups assigned to that particular *taxon* (Boaventura *et al.*, 1999). Taking into account that trophic groups were assigned according to organism's life traits described in the literature, these should be regarded as functional groups instead of taxonomical categories.

Results

In the present study, 162 *taxa* were identified on the ARs and 218 on the NRs (Table I). The measures of biodiversity indicated significant differences between the reef types (Table II). The NRs supported a more diverse community, with significant higher values of Δ^+ (ANOVA; $F = 18.0$, $p < 0.01$) and $s\Delta^+$ (ANOVA; $F = 5.5$, $p < 0.05$), while samples from the ARs displayed lower diversity and higher evenness with values of Λ^+ significant higher than NRs (ANOVA; $F = 26.0$, $p < 0.001$) (Table II). The most represented phyla in terms of number of *taxa* for ARs were Annelida (66 *taxa*) and Arthropoda (37 *taxa*), while for NRs were Mollusca (63 *taxa*) and Annelida (58 *taxa*). Considering these phyla, less than 50% of the *taxa* were common to both reef types (Table I). No significant differences were observed for the number of *taxa* between the NRs (84-110 *taxa*) and the ARs (59-109 *taxa*) (ANOVA; $F = 4.0$, $p > 0.05$).

Table I. Shared species based on taxonomic groups by the two reef types.

Phylum		Number of taxa	
	% Share	AR	NR
Chordata	0	2	5
Mollusca	36	27	63
Echinodermata	40	2	5
Rhodophyta	40	2	5
Cnidaria	42	7	10
Annelida	48	66	58
Arthropoda	48	37	49
Bryozoa	50	10	14
Echiura	100	1	1
Granuloreticulosa	100	1	1
Nematoda	100	1	1
Nemertina	100	1	1
Phoronida	100	1	1
Platyheminthes	100	1	1
Porifera	100	2	2
Sipuncula	100	1	1
		162	218

Table II. Mean values of average (Δ+), total (sΔ+) and variance (Λ+) in taxonomic distinctness referred to the artificial (AR) and natural (NR) reefs samples and calculated with equal weighting between taxonomic levels. Mean is expressed with it standard error. Summary of one-way ANOVA exploring differences between the three different diversity indices for the two types of reefs used in the study.

	AR		NR	
Δ^+	89.4 ± 1.1	<	91.5 ± 0.7	**
sΔ^+	7649.7 ± 1449.6	<	9277.9 ± 877.3	*
Λ^+	411.7 ± 30.3	>	323.0 ± 29.9	***

*** $P<0.001$, ** $P<0.01$, * $P<0.05$

Multivariate analyses indicated a strong effect of the reef type on macrobenthic community structure (ANOSIM; R = 0.981, p = 0.002). The MDS analysis of the *taxa* data clearly presented distinct community structures between both artificial and natural reefs. The samples from the same type of substrate were grouped together, indicating their high similarity (Figure 1).

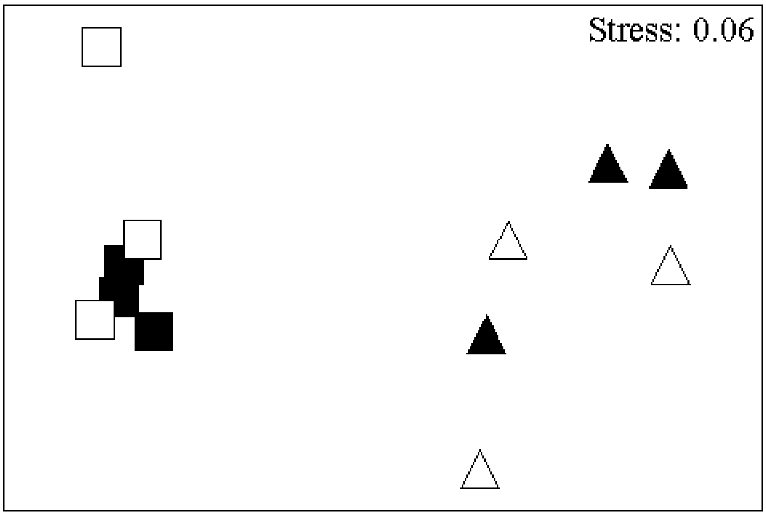

Figure 1. MDS ordination plots on presence/absence of *taxa* for the artificial (■ - AR#1, □ - AR#2) and natural (▲ - NR#1, △ - NR#2) reefs

According to the SIMPER analysis (Table III), dissimilarity between the artificial and natural reefs was due to higher frequency of several polychaete species on the ARs [*Trypanosyllis zebra* (Grube, 1840), *Lysidice ninetta* Audouin and Milne-Edwards, 1833 and several serpulids, such as undetermined Serpulidae, *Vermiliopsis striaticeps* (Grube, 1862) and *Pomatoceros triqueter* (Linnaeus, 1767)], whereas molluscs (such as the bivalve *Cardita calyculata* (Linnaeus, 1758), and several gastropods *Chauvetia* sp., *Chrysallida* cf. *emaciata* (Brusina, 1866), *Capulus ungaricus* (Linnaeus, 1758), *Bittium* cf. *latreilleii* (Payraudeau, 1826) and *Bittium* spp.) were more frequent on the NRs.

Table III. Comparison between artificial reef (AR) and natural reef (NR), based on presence/absence data. Contrib% is the percentage contribution of each *taxa* to the average Bray–Curtis dissimilarity between the groups of samples, also expressed as a cumulative percentage Cum%. *Taxa* are listed in decreasing order of importance.

Taxa	AR	NR	Contrib%	Cum.%
Average dissimilarity between AR and NR = 54.8				
Trypanosyllis zebra	1.00	0.00	1.00	1.00
Tubuliporidae	0.00	1.00	1.00	2.00
Vermiliopsis striaticeps	1.00	0.00	1.00	3.00
Lumbrineris gracilis	0.00	1.00	1.00	4.00
Chauvetia sp.	0.00	1.00	1.00	5.00
Corophium spp.	1.00	0.00	1.00	6.00
Cystodytes dellechiajei	0.00	1.00	1.00	7.00
Escharoides coccinea	0.17	1.00	0.85	7.86
Maera cf. *hirondellei*	0.00	0.83	0.84	8.70
Lysidice ninetta	1.00	0.17	0.84	9.54
Cardita calyculata	0.00	0.83	0.84	10.38
Chrysallida cf. *emaciata*	0.00	0.83	0.83	11.21
Cumacea	0.00	0.83	0.83	12.04
Capulus ungaricus	0.00	0.83	0.82	12.86
Bittium cf. *latreilleii*	0.00	0.83	0.82	13.67
Algae	0.00	0.83	0.82	14.49
Chartella papyracea	0.83	0.00	0.81	15.30
Serpulidae indeterminate	0.83	0.17	0.74	16.04
Cirratulidae indeterminate	0.83	0.17	0.73	16.77
Bittium spp.	0.17	0.83	0.72	17.49
Pomatoceros triqueter	0.83	0.17	0.70	18.19

Cut-off at 18% of Contrib% to the dissimilarity.

Concerning biomass, ARs exhibited values between 0.4 and 0.9 g × $0.0225m^{-2}$, however for the NRs, the biomass' values showed a higher variation among samples (0.6 to 5.9 g × $0.0225m^{-2}$). This large variation in biomass was due to a single species, the sponge Porifera sp. I, as its biomass ranged from 0.01 to 5.4 g × $0.0225m^{-2}$. Nevertheless, no significant differences for biomass data were observed (ANOVA; $F = 2.3$, $p > 0.05$), due to the high variability of the samples.

The high percentage of suspension feeding on the NRs was once more due to the dominance of Porifera sp. I, which accounted for 80% of the biomass of this feeding mode (Figure 2; Table IV). However, even when this species was removed, the proportion of suspension feeding remained high (62%) on the NRs, especially due to the bryozoan *Pentapora fascialis* (Pallas, 1766). On the ARs, the proportion of the biomass linked to carnivory was also important (28%). That proportion was due to the biomass dominance of certain *taxa* such as anthozoans (63%) and *Pilumnus hirtellu*s (Linnaeus, 1761) (13%) (Table IV). The proportion of the biomass associated to detritivores (those of specimens presenting suspension and deposit feeding modes) was comparatively higher on the NRs.

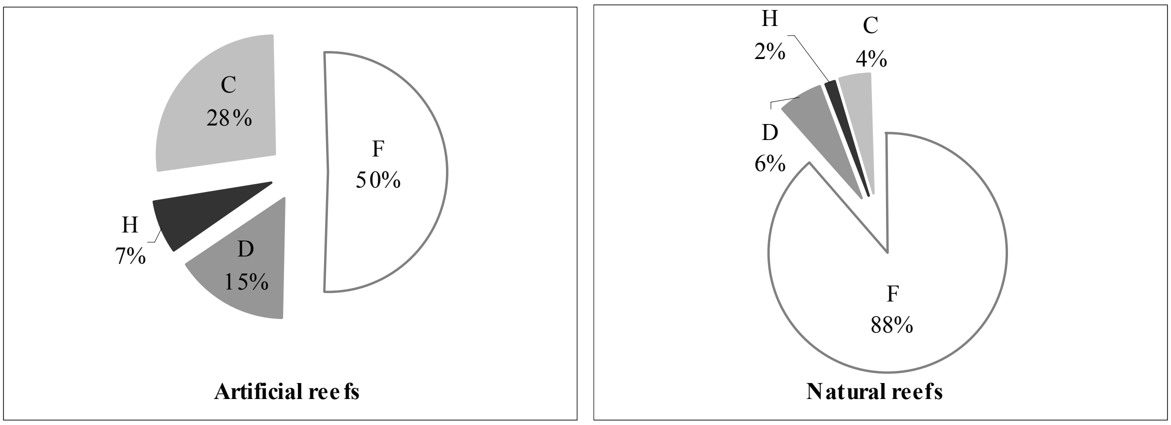

Figure 2. Estimated contribution (%) of different feeding types to macrobenthic biomass on artificial and natural reefs (F – suspension feeder; D – deposit feeder; H - herbivore; C- carnivore).

Table IV. The dominance and cumulative dominance percentage of the five dominant *taxa* in terms of biomass.

	Taxa	Dominance (%)	Cumulative dominance (%)
Artificial reef			
	Anthozoa	30.3	30.3
	Algae	14.4	44.7
	Phascolosoma granulatum	7.8	52.5
	Pilumnus hirtellus	6.0	58.4
	Celleporina caliciformis	5.9	64.3
Natural reef			
	Porifera sp. A	58.4	58.4
	Algae	9.0	74.7
	Pentapora fascialis	7.2	81.9
	Retusa truncatula	7.2	83.6
	Escharoides coccinea	1.7	85.2

The NRs presented abundance values between 369 and 577 ind. × $0.0225m^{-2}$, whilst the ARs showed broader ranges (385-1191 ind. × $0.0225m^{-2}$) and no significant differences were observed in abundance between both reef types (ANOVA; $F = 3.8$, $p > 0.05$), owing to the high variability of the samples. Abundance was dominated by nematodes and foraminiferans within both types of reefs (Table V). Anthozoans, the polychaetes *Chone* spp. and the isopod *Janira maculosa* Leach, 1814 also presented high abundance within ARs. On the other hand, on the NRs, high abundance was found for the sipunculid *Phascolosoma granulatum* Leuckaert, 1828 and for the crustaceans *Lembos websteri* Bate, 1857 and *Thoralus cranchii* (Leach, 1817).

Table V. The dominance and cumulative dominance percentage of the five dominant *taxa* in terms of abundance.

	Taxa	Dominance (%)	Cumulative dominance (%)
Artificial reef			
	Nematoda	20.9	20.9
	Foraminifera	7.7	28.6
	Chone spp.	6.5	35.0
	Anthozoa	5.6	40.7
	Janira maculosa	5.2	45.9
Natural reef			
	Foraminifera	21.1	21.1
	Nematoda	4.5	25.6
	Phascolosoma granulatum	3.7	29.3
	Lembos websteri	3.4	32.7
	Thoralus cranchii	2.9	35.6

It is worth noting that, as previously referred, colonial organisms and barnacles were not quantified in terms of abundance. Therefore, the abundance values reported in this study are underestimated.

Discussion

ARs are increasingly being used as alternatives for hard substratum, in order to overcome NRs scarcity. Thus, it is important to understand if the ARs can efficiently provide similar habitats to NRs areas. The degree to which the biological communities on these materials become similar to those on NRs have not been well demonstrated yet (Thanner, 2006). Regarding the present study, the results suggested that substrata type plays an important role in determining community structure. Differences were found between artificial and natural reef communities, including higher number of *taxa* and diversity (Δ^+ and sΔ^+) and lower evenness (Λ^+) within NRs. The taxonomic distinctness indices applied to the P/A data highlighted some situations of environmental stress, in particular within ARs. It is known that these variables can be strongly influenced by the environment characteristics (Warwick and Clarke, 1998). Disturbed biotic assemblages have been shown to comprise species that are closely related to one another (low Δ^+) and at the same time high Λ^+, as some *taxa* become over-represented and others under-represented (Clarke and Warwick, 1998; Ceschia *et al.*, 2007). These indices, based on P/A data, treated all species as equal and do not take into account the value and the ecological significance of each species. However, the taxonomic distinctness indices combined with the number of *taxa* may help in the interpretation of the information, not only indicating differences in the number of *taxa* between locations, but also differences in the taxonomic spread, which are equally important (Ceschia *et al.*, 2007). At ARs, the highest Λ^+ values might be the result of a lower spatial heterogeneity. Similar findings were observed by Perkol-Finkel and Benayahu (2005), indicating that the benthic community of the former may still be undergoing changes and reshaping its features even 10 years after deployment. This is an interesting result because previous studies have found that subtidal assemblages on artificial structures were more diverse than on

NRs (Connell and Glasby, 1999). Therefore, different macrobenthic patterns can be observed depending, for example on depth, ARs' design and surrounding environment. Changes in macrobenthic structure within both reef types were also verified by multivariate analyses. The results supported that after 16 years after deployment, ARs and nearby NRs present different community structures. These results emphasize that time may represent a key factor in the development of benthic communities of ARs. With such differences observed after 16 years, one might suggests that it will take a considerable longer period for these ARs to eventually clone the NRs macrobenthic communities. This suggests that specific characteristics of ARs should be taken into account very carefully, when choosing man-made structures to suppress NRs scarcity. If the goals are largely dependent on the benthic communities that will establish in the deployed structures (hypothetically the same as in nearby NRs), the results in the short/medium term may be substantially different. Although, macrobenthic community structure within the two reef types was different, both substrates shared a considerable number of *taxa*. These common *taxa* were, however, represented differentially. For example, in terms of number of *taxa*, several serpulid species were mainly dominant on ARs, while algae and molluscs dominated within NRs. Several authors (such as Knott *et al.* 2004; Perkol-Finkel *et al.* 2005, 2006) also observed that the biota found on artificial substrata was not unique, but common species, however found in very different proportions when compared to natural rocky reefs. The same phyla dominance in similar studies was also reported by Knott *et al.* (2004).

Regarding biomass production, the presence of sponges was important within NRs. The differences between assemblages on ARs and NRs were mainly due to higher biomass values of sponges on NRs, while anthozoan species were an important supplier to the biomass of ARs. This was clearly illustrated in the trophic structure of the macrobenthic community, since the higher biomass of a particular sponge (Porifera sp. I) contributed to the clear dominance of suspension feeders within NRs. On the other hand, there was a co-dominance of suspension feeders and carnivores within ARs, as anthozoans can share both feeding modes. The trophic distribution may be influenced by factors such as turbulence, which is important in distributing particulate food, keeping particles in suspension, thus favouring suspension feeders (Probert, 1984; Boaventura *et al.*, 1999). A greater number of sponge *taxa* on NRs than on ARs were also observed by Knott *et al.* (2004). They suggested that the differences detected may have derived from the different abilities of artificial and natural substrata to attract or sustain certain benthic invertebrates. The differences between the niches provided by NRs and by artificial structures, such as a greater surface heterogeneity of NRs, may affect the recruitment and growth of invertebrates resulting in the observed differences in the biomass and richness of *taxa*. Additionally, the recruitment of some species (namely sponges) is so slow that it takes very long periods of time before assemblages on artificial structures become like those on NRs (Perkol-Finkel *et al.*, 2005, 2006). Despite being in the water for 16 years, the assemblages of sponges that have developed on ARs surfaces are quite scarce compared to those on NRs.

The different complexity and heterogeneity offered by the surfaces on NRs can play an important role in determining the structure of epibenthic assemblages, by affecting a variety of ecological processes (Bourget *et al.* 1994; Archambault and Bourget, 1996; Menconi *et al.*, 1999). On the other hand, concrete has certain physical and chemical properties which may affect the settlement, growth or survival of organisms (Connel and Glasby, 1999; Knott *et al.*, 2004; Bulleri, 2005b). Consequently, artificial structures may act by changing the patterns of distribution of locally abundant species rather than increasing species diversity (Glasby, 1999a; Bacchiocchi and Airoldi, 2003). Artificial substrata may be replacing natural habitats for some species, but Connell and Glasby (1999) suggested that as habitat for assemblages of epibiota, NRs may not be adequately replaced by urban structures. Nevertheless, when NRs are inexistent or scarce, the addition of artificial substrata will create additional patches of hard substrata, and will contribute to the increase of biodiversity, especially within sandy clean areas. These areas are known to have low diversity values when compared to fine sand or muddy areas (Snelgrove and Butman, 1994). The deployment of artificial structures had been also associated with the dispersal of species that are poor dispersers to cover greater distances by using urban structures as "stepping-stones" (Reed *et al.*, 2000; Glasby and Connell, 2001; Bacchiocchi and Airoldi, 2003; Kinlan and Gaines, 2003; Zintzen *et al.*, 2006). In the present study, most of the species identified within ARs had been already reported either on hard and/or soft substrata along the Portuguese coast. Moreover, studies focussed on commercial fish species undertaken since 1990, showed that ARs are a useful management tool, enhancing and diversifying the catches, thus contributing to the enhancement of local artisanal fisheries, extremely relevant for this region (Leitão *et al.*, 2007; Santos and Monteiro, 2007).

In marine ecosystems, fisheries play a major role in biodiversity and productivity decline. These factors, together with environmental degradation and climate changes are increasing dramatically and threatening many habitats throughout the world (Bulleri, 2005b). As a consequence, the increase of artificial structures submersion (e.g. artificial reefs, seawalls, breakwaters) is predicted in the next few decades (Bulleri, 2005b and references therein). Understanding the mechanisms which cause differences between assemblages on artificial structures from those occurring on natural habitats, is important for improvement of the artificial structures design. Designing a heterogeneous AR will increase diversity and abundance of species and, depending on the level of similarity to the overall composition of the natural surroundings, may also elevate diversity and species abundance in the entire area (Perkol-Finkel *et al.*, 2006). Future research should include experiments over longer time periods, in order to fulfil knowledge gaps concerning the late stages of AR communities' development. It would also be interesting to test the influence of different artificial structures on macrobenthic colonisation patterns, such as the increasing of surfaces heterogeneity.

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CHAPTER VII.

General conclusions

Community structure and development on hard substrata are determined by complex abiotic and biotic interactions. The colonisation process is influenced by a number of factors, and the present study has provided information that has allowed several conclusions to be drawn concerning the macrobenthic community colonisation patterns of artificial reefs on the southern Portuguese coast:

Chapter II reported a short-term colonisation study (1-year), where the role of surface orientation on the epifaunal communities of ARs was analysed. The evaluation of some techniques used in the assessment of subtidal epibiotic assemblage structure was also assessed. As expected, the mean abundance, number of species, diversity, biomass and percentage of cover of the epibenthic community, on the "Faro/Ancão" artificial reef system, tended to increase with time of immersion. Nevertheless, the epibenthic community showed to be affected by surface orientation: on the vertical surfaces, the macrobenthic colonisation appeared similar throughout the study period, while, differences on horizontal surfaces were detected. The abundance, number of *taxa* and the biomass were significantly higher on the top compared to the bottom surfaces. On the contrary, the bottom surfaces had significantly higher colonisation than the top surfaces, when only percentage cover was assess. Also, the macrobenthic community structure of the horizontal surfaces seems to affect in a different way for both vagile and sessile components. Additionally, the obtained information suggests that, for hard substratum communities, the choice of the method used for estimating the contribution to the community is of major importance, as it can lead to complementary results and conclusions. Whenever possible, the use of more than one method is highly desirable in order to avoid misinterpretation.

Also, in a short-term colonisation study (6 months), the influence of depth and of the reef structure (Chapter III) on the macrobenthic community colonisation patterns was analysed. In this chapter, the distribution of some species was depth-related, and influenced overall biomass, abundance and cover patterns. The small differences in depth (16–20 m) and the structure of the ARs influenced the development of subtidal epibiotic assemblages at the ‘‘Faro/Ancão’’ AR. This effect was more obvious for colonial organisms and for barnacles; and it seems that, for biomass values, the reef structure was important, decreasing from upper to lower layers of modules.

The estimation of secondary production of epibenthic organisms was assessed (Chapter IV) over the 12 months after deployment, to evaluate the Faro/Ancão artificial reefs communities function. Also, the effect of substratum orientation on secondary production of epibenthos was evaluated. Epibenthic production was greater on the horizontal than on the vertical orientation surfaces, during the initial macrofauna colonisation. Nevertheless, the results indicate that after one year of colonisation, the surface orientation does not seem to affect the production of reef surface area.

The second section of the thesis analysed long-term colonisation processes. Community structure differences among old and new reefs were examined during a four year study (Chapter V). The settlement/colonisation of the vacant substrata appears to be different according to the presence or absence of a mature community in the surrounding areas. On the new reefs the assemblages were dominated by pioneer organisms (e.g. barnacles, the bivalve *Hiatella arctica* and polychaetes such as serpulids and *Polydora hoplura*), typical of the colonisation of clean substrata; whereas in old reefs, these organisms were less representative and the assemblages were dominated by other species (e.g. polychaetes, such as *Filograna implexa* and *Pomatoceros triqueter*, and cnidarians, like anthozoans and hydrozoans).

The epifaunal invertebrates associated with natural and artificial habitats (after 16 years after deployment) were investigated (Chapter VI) and differences were detected between the two distinct reefs habitats on species richness, species composition, community and trophic structure. The biota found on artificial substrata was not unique, however, common species were found in very different proportions when compared to natural rocky reefs. The trophic structure for both communities was dominated by suspension-feeding organisms.

This manuscript hopefully highlights some important issues for the macrobenthic communities' colonisation in artificial reef, which may improve futures artificial reefs deployment and better management:

1. The surface orientation effect in macrobenthic communities' colonisation;
2. The importance of depth on the distribution of the epibentic species;
3. The importance a nearby established community in the vicinity of the artificial reef on the recruitment, settlement and colonisation of macrobenthic communities;
4. The capacity of macrobenthic communities of the artificial reef structures to mimic those on natural rocky areas.

As it was evident, from the various case studies covered in the thesis, artificial reefs, from an ecological perspective, exhibit significant potential as a tool in the rehabilitation of coastal ecosystems. Future research should include experiments over longer time periods, in order to fulfil knowledge gaps concerning the late stages of artificial reef communities' development. It would also be interesting to test the influence of different artificial structures on macrobenthic colonisation patterns, such as the increasing of surfaces heterogeneity. Moreover, these studies should be extended to all of the Algarve recifal system, as those macrobenthic communities are subject to different physic-chemical and biological characteristics.