UNIVERSIDADE DE LISBOA
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“However fragmented the world, however intense the national rivalries, it is an inexorable fact that we become more interdependent every day. I believe that national sovereignties will shrink in the face of universal interdependence. The sea, the great unifier, is man's only hope. Now, as never before, the old phrase has a literal meaning: We are all in the same boat.”

Jacques-Yves Cousteau

in National Geographic Magazine, 1981

Table of Contents

Resumo

O nível crescente de pressão humana sobre os ecossistemas marinhos levou a uma maior sensibilização para a necessidade de compreender a interconexão de efeitos directos e indirectos sobre comunidades e populações. Assim, na avaliação de associações de peixes marinhos, assiste-se a uma transição da monitorização do efeito da pesca em populações exploradas para a análise do efeito de várias fontes de impacto sobre comunidades.

Esta tese contribui para as novas exigências metodológicas da avaliação de integridade funcional em associações de peixes marinhos, utilizando a costa portuguesa como área de estudo. O primeiro estudo pretende analisar a distribuição e caracterização das associações de peixes em recifes rochosos e áreas de substrato móvel, baseando-se apenas em dados publicados. Embora seja possível uma caracterização geral, vários aspectos negativos dessas abordagens foram identificados e demonstrados, como as lacunas de informação, a questionável comparabilidade dos dados e a falta de detalhe sobre variação a pequena escala. Os dois estudos que se seguem destinam-se a compreender a variabilidade em recifes temperados. O primeiro centra-se na quantificação da complexidade topográfica, através da proposta de um novo índice que reflecte a resposta das comunidades de peixes a características topográficas; o segundo analisa a influência dessa complexidade no poder estatístico para detectar alterações em métricas de estrutura e função, com algumas orientações sobre o esforço amostral. Os dois estudos finais propõem um quadro metodológico para a definição de tipos de associações de peixes funcionalmente homogéneos, tendo em conta a variabilidade externa. No primeiro estudo, associações de peixes de recife são classificadas em tipos que persistem face à variabilidade entre transectos, enquanto que no segundo estudo, associações de substrato móvel da plataforma continental são classificadas em áreas que conservam a sua homogeneidade face à variação inter-anual contida em 5 anos de dados de monitorização.

Palavras-chave: peixes marinhos, gestão ambiental, monitorização ambiental, metodologia, Portugal.

Summary

The increasing level of human pressure on marine ecosystems has led to higher awareness towards the need to understasnd the inter-connection of direct and indirect effects on communities and populations. Therefore, in the assessment of marine fish assemblages, there is an ongoing shift from monitoring the effect of fishing in exploited populations to analysing the effect of several impact sources on communities.

This thesis contributes to the new methodological requirements of functional integrity assessments in marine fish assemblages, using the Portuguese coast as a study area. The first study attempts to analyse the distribution and characterisation of fish assemblages in rocky reefs and soft substrate areas by relying only on published data. Although a rough characterisation is achieved, several negative aspects of these approaches were pinpointed and demostrated, namely information gaps, questionable data comparability and the lack of detail on fine-scale variation. The two studies that follow are aimed at understanding variability in temperate reefs. The first one focuses on quantifying topographic complexity, through the proposal of a new index that reflects the response of fish communities to topographic features, and the second one analyses the influence of this complexity on the power to detect changes in structural and functional fish-based metrics, with some guidelines regarding sampling efforts. The two final studies propose a framework for the definition of functionally homogeneous fish assemblage types, while taking into account background variability. In the first study, reef fish assemblages are classified into types that persist in the face of fine-scale among-transect variability, while in the second study, soft substrate fish assemblages of the continental shelf are classified into areas that retain their homogeneity in the face of inter-annual variation, as described by 5 years of monitoring data.

Keywords: marine fish, environmental management, environmental monitoring, methodology, Portugal.

Resumo alargado

Sabe-se que o ambiente marinho suporta cerca de 25% das espécies da Terra, existindo provavelmente ainda um número muito elevado de espécies marinhas por descrever. Esta enorme biodiversidade fornece bens e serviços inestimáveis ao ecossistema, do qual as populações humanas são uma parte muito importante. No entanto, o uso desses recursos pelo Homem tornou-se insustentável, e a pressão sobre o ambiente marinho disparou nas últimas décadas, atingindo níveis alarmantes em algumas regiões do globo. Isto levou a uma maior sensibilização dos decisores, e vários instrumentos políticos internacionais impõem agora a necessidade de evitar a deterioração e assegurar o uso sustentável dos recursos. Na verdade, os princípios da abordagem ecossistémica, propostos pela Convenção sobre a Diversidade Biológica no ano 2000, tiveram uma grande contribuição na forma como a política internacional lida com o equilíbrio entre conservação e uso de recursos no ambiente marinho. Em geral, esses princípios afirmam não só a necessidade de avaliar e antecipar possíveis impactos noutras áreas do ecossistema que não estão diretamente sob pressão, mas também a necessidade de incorporar a dimensão humana na equação, incluindo todas as partes interessadas e as áreas da sociedade que dependem directa ou indirectamente dos recursos.

Em comunidades de peixes, estes novos requisitos levaram a uma mudança de abordagem, de uma gestão apenas centrada nos efeitos da pesca em populações comercialmente exploradas para uma gestão mais integrada e transversal, onde várias fontes de impacto são tidas em conta, bem como o seu efeito em toda a comunidade. Esta abordagem tem sido aplicada em rios e estuários europeus no âmbito da Directiva Quadro da Água, onde as ferramentas com maior sucesso se centram em aspectos funcionais das comunidades, em vez de se focarem na identidade taxonómica. Isto ocorre geralmente com a divisão das espécies em grupos que possuem uma característica funcional comum (*e.g.* carnívoros, sedentários, resiliência elevada). Este aspecto tem sido fundamental para ajudar na distinção entre variabilidade natural e antropogénica, dado que grupos funcionais não só respondem de modo mais previsível aos impactos, como permitem maior resistência à variabilidade natural (quando espécies são substituídas por outras do mesmo grupo ao longo de um gradiente).

Esta tese consiste numa série de estudos que visam contribuir para as novas exigências metodológicas de abordagens funcionais baseadas em comunidades de peixes marinhos, tentando preencher algumas das lacunas relacionadas com a variabilidade e

heterogeneidade ambiental. De facto, apenas lidando com a incerteza e a heterogeneidade podemos efetivamente detectar impactos antropogénicos, compreender as limitações dos métodos e adaptar as necessidades de gestão aos recursos disponíveis.

O primeiro estudo foi um exercício de caracterização de comunidades de peixes marinhos com base apenas em dados publicados sobre a costa de Portugal continental, abrangendo áreas de substrato móvel até aos 200m de profundidade e recifes rochosos perto da costa. Independentemente da própria caracterização, este exercício teve por objetivo abordar criticamente estes procedimentos, através da identificação de problemas e capacidades. Foram definidos critérios para a inclusão de estudos publicados e os conjuntos de dados foram padronizados através do uso de proporções de abundância de espécies e da classificação em secções espaciais e temporais comparáveis. As espécies foram classificadas e incluídas em diversos grupos funcionais e todas as análises foram realizadas separadamente para uma abordagem taxonómica clássica e uma abordagem com grupos funcionais. A influência da estação do ano, profundidade e latitude foram avaliadas através de análise de variância multivariada usando permutações (PERMANOVA) e modelos lineares com base em distância (DISTLM) e as associações de peixes foram caracterizados com base em métodos de ordenação multivariada e classificação hierárquica. Foram encontradas diferenças significativas entre estudos que empregam diferentes métodos em recifes rochosos e os *outliers* não foram utilizados na caracterização. Devido a limitações no detalhe espacial e temporal em dados publicados, apenas foi realizada uma caracterização de tipos de associações de peixes pouco detalhada e a larga escala, com conjuntos de dados divididos em recifes rochosos, substrato móvel pouco profundo (0−20 m), substrato móvel intermédio (20−100 m) e substrato móvel profundo (100−200 m). Não foram detectadas diferenças sazonais, mas uma relação não linear com a latitude foi encontrada em associações de substrato móvel profundo. Durante o exercício, as escalas temporais e espaciais foram forçadas pela qualidade dos dados e algumas análises não detectaram padrões conhecidos. Diferenças entre os métodos e tipos de dados levaram à necessidade de adoptar um grande número de pressupostos questionáveis para conseguir alguma comparabilidade. No geral, o exercício proporcionou uma visão prática sobre o uso de dados publicados para planeamento num contexto de gestão costeira, apontando a comparabilidade dos dados, a escala e as lacunas de informação como severas limitações destas abordagens.

No segundo estudo foi proposta uma nova forma de quantificar complexidade topográfica em recifes rochosos usando o método “*chain and tape*”. Este método consiste em calcular o rácio entre a distância linear entre os pontos de início e fim de uma corrente ou cabo chumbado (*chain*) moldada à superfície do substrato e o seu comprimento total estendido, uma medida conhecida como o índice de rugosidade do substrato (SR). Esta medida tem várias vantagens no campo quando comparada com outros métodos, mas têm sido apontados alguns pontos fracos. No entanto, ainda é uma das medidas de topografia mais utilizadas em ecologia de peixes de recife. O estudo realizado propôs um índice de topografia combinado (CTI), que utiliza o método "*chain and tape*" no campo, com resultados que se aproximam de métodos mais complexos, superando o índice SR tradicional. O CTI é estruturado como uma soma ponderada de três características topográficas: índice SR, o número de ondulações (NC) e o relevo máximo vertical (MVR), sendo que a NC e MVR são aplicados coeficientes de peso que variam entre 0 e 1. Para estabelecer os coeficientes de peso, foram amostradas associações de peixes em seis locais de calibração, representando um gradiente de complexidade topográfica. Foram calculadas combinações de coeficientes de modo a que o CTI ficasse optimamente correlacionado com vários parâmetros estruturais de comunidades de peixes. A lista de possíveis soluções foi então aplicada a nove perfis esquemáticos publicados anteriormente e comparada com outros métodos. O índice final foi estabelecido como: $CTI = (1 - SR) + NC/25 + MVR/25$. No final, o desempenho preditivo do CTI e do tradicional SR foi testado, aplicando-os a 11 novos locais como variáveis preditoras de abundâncias de espécies em modelos lineares com base em distância (DISTLM). O CTI superou o SR quando adicionado a três variáveis previamente inseridas no modelo (profundidade, percentagem de cobertura com areia, percentagem de cobertura com calhau rolado), explicando 5,6% de variação adicional ao utilizar todas as espécies e 8,1% utilizando apenas as espécies criptobênticas, enquanto o SR não mostrou efeitos adicionais significativos.

O terceiro estudo teve como objetivo compreender a relação entre a complexidade topográfica em recifes ao longo da costa portuguesa e o poder estatístico para detectar alterações em métricas baseadas em associações de peixes. Em 14 locais ao longo da costa foram realizados seis transectos de censos visuais em mergulho para cada local e recorreu-se a simulações de Monte Carlo para gerar um grande número de replicados para representar a hipótese nula (ausência de alteração) e simular três hipóteses alternativas que representam diferentes magnitudes de alteração. O poder estatístico para detectar diferenças entre as hipóteses nula e alternativa foi estimado através de 10 mil testes de Mann-Whitney para números de replicados entre 2 e 15. O poder estatístico mostrou tendência para variar de acordo com a complexidade topográfica, particularmente no caso de alterações pequenas e médias nos valores das métricas e quando se utiliza um número reduzido de replicados. Enquanto o poder aumenta com o aumento da complexidade para a maioria dos indicadores, alguns mostraram tendências decrescentes. Para uma alteração de grande magnitude foi necessário um número de replicados entre 5 e 15, dependendo da métrica, para estabilizar o poder estatístico acima de 0,80 independentemente das características do habitat. Um poder estatístico de 0,95, contudo, não foi atingido para a maioria das métricas em todos os locais, mesmo quando se utilizam 15 replicados. A observação de que a capacidade para detectar a degradação e recuperação de associações de peixes de recife parece variar de acordo com características do habitat significa que um programa de monitorização que está correctamente calculado para uma determinada área pode não ser directamente aplicável num recife nas proximidades. Para além da necessidade de maximizar o poder estatístico, este estudo enfatizou a necessidade de ter em conta a heterogeneidade do habitat nestes cálculos, estimando um esforço amostral que seja independente do habitat e apropriado para a escala e locais de interesse.

No quarto estudo propôs-se uma estrutura inovadora para definir tipos de associações de peixes de recifes rochosos através de um método aglomerativo denominado "*metric pairwise constrained k-means*" (MPCK-means). Foram amostradas associações de peixes em 14 locais ao longo da costa portuguesa e foram agrupados os locais com base em várias categorias grupos funcionais, numa abordagem que tem em conta a variabilidade que resulta do método de amostragem e de causas naturais. Este procedimento foi seguido de um segundo processo aglomerativo baseado na identidade dos grupos formados com cada uma das categorias funcionais, e que encontra padrões que minimizem a perda de informação ao integrar os resultados desses agrupamentos individuais. A classificação final foi obtida por comparação de três algoritmos de geração de consenso, tendo o melhor resultado mantido um índice médio de informação mútua normalizada de 0,605. Através de análises PERMANOVA hierárquicas foram encontradas diferenças significativas entre os tipos de associações e identificadas as métricas menos resistentes à variabilidade natural. Como complemento, foi proposto um método baseado num algoritmo classificador (*k-nearest neighbours*) para inserir novos locais na tipologia definida, tendo como base apenas variáveis ambientais que não são diretamente afectadas pela presença de impactos antrópogénicos. Os parâmetros do modelo de classificação foram optimizados e o melhor desempenho foi alcançado com votação ponderada por distância inversa dos quatro vizinhos mais próximos, com uma precisão média de classificação de 96,08%.

No último estudo propõe-se a aplicação do método utilizado anteriormente para identificar tipos de associações de peixes de substrato móvel ao longo da costa portuguesa. Usando o mesmo algoritmo *MPCK-means*, as estações de amostragem foram agrupadas com base em categorias de grupos funcionais, tendo em conta a variabilidade contida em 5 anos de monitorização com arrasto de fundo. Neste processo, foi comparada a utilização de dados em biomassa e abundância. Todos os agrupamentos feitos com base em categorias individuais foram combinados num conjunto final de sete tipos de associação de peixes. O efeito da profundidade, tipo de substrato e da variação latitudinal nos padrões de agrupamento foi também avaliado. Os dados de biomassa revelaram padrões mais homogéneos que facilitaram a distinção de áreas, mas a combinação de métricas com base em biomassa e abundância obteve os melhores resultados na definição de uma solução final.

A aplicação das classificações obtidas deve estar ligada a uma base de dados sólida, dado que os métodos utilizados são limitados pela qualidade e tamanho da base de dados que os suporta. Quanto maior for a base de dados, melhores serão as capacidades de previsão, um aspecto crucial num contexto de monitorização, uma vez que é necessário identificar o potencial de um determinado local, mesmo que o presente estado das comunidades esteja longe do ideal. No entanto, modelar o resultado da monitorização de uma comunidade de peixes inteira é imensamente complexo, a um nível que ainda está fora do alcance à luz do conhecimento actual. As razões pelas quais um peixe é encontrado num determinado local num determinado momento são uma combinação muito complexa de factores ambientais, interações entre indivíduos dentro das populações e comunidades e processos fisiológicos e preferências individuais.

Por estas razões, é muito importante realizar análises de poder estatístico, compreender as probabilidades de erro, limitar a variabilidade ambiental e avançar com o melhor plano de monitorização possível para o orçamento disponível. Um indicador incapaz de oferecer segurança suficiente limitará inevitavelmente a confiança dos responsáveis pelas fontes de impacto e a sua cooperação. A simplificação da complexidade dos sistemas através de formas eficientes e responsáveis é essencial para garantir que as ferramentas desenvolvidas são de confiança e aplicáveis num contexto de monitorização.

List of papers

This thesis consists of a series of papers, each one corresponding to a chapter, from 2 to 6. The author wrote all papers and was responsible for experimental design, field surveys and data analysis. Remaining authors collaborated in some or several of these procedures.

Chapter 2

Pais, M.P., Henriques, S., Costa, M.J., Cabral, H.N., 2012. A critical approach to the use of published data for baseline characterisation of marine fish assemblages: An exercise on Portuguese coastal waters. Ocean & Coastal Management 69, 173−184.

Chapter 3

Pais, M.P., Henriques, S., Costa, M.J., Cabral, H.N., 2013. Improving the “chain and tape” method: A combined topography index for marine fish ecology studies. Ecological Indicators 25, 250−255.

Chapter 4

Pais, M.P., Henriques, S., Costa, M.J., Cabral, H.N. Topographic complexity and the power to detect structural and functional changes in temperate reef fish assemblages: the need for habitat-independent sample sizes. Submitted to Journal of Applied Ecology.

Chapter 5

Pais, M.P., Henriques, S., Batista, M.I., Costa, M.J., Cabral, H.N., 2013. Seeking functional homogeneity: A framework for definition and classification of fish assemblage types to support assessment tools on temperate reefs. Ecological Indicators 34, 231−245.

Chapter 6

Pais, M.P., Henriques, S., Murta, A.G., Azevedo, M., Costa, M.J., Cabral., H.N. Identifying functional homogeneity in a dynamic environment: Aplication to soft-substrate fish assemblages off the Portuguese coast. Submitted to Journal of Sea Research.

For every paper, a QR code like the one presented here is provided at the start of the corresponding chapter. Scanning this code with any smartphone or tablet will link to the published version. Alternatively, scanning this example code will lead to an index containing all papers.

Chapter 1

General introduction

The marine environment is known to support about 25% of the Earth's species, and there is reason to believe there may be yet a very large number of marine species awaiting description (Mora *et al.*, 2011). This huge biodiversity provides invaluable goods and services to the ecosystem, of which human populations are a very important part (Worm *et al.*, 2008). However, the use of these resources by humankind has become far from sustainable, and the anthropogenic pressure on the marine environment has escalated in the last decades, reaching alarming levels in some regions of the globe (Halpern *et al.*, 2008). This has led to increasing awareness among decision-makers, and several international policy tools now enforce the need to prevent deterioration and ensure the sustainable use of resources. In fact, the 12 principles of the ecosystem approach proposed by the Convention of Biological Diversity (CBD, 2000) have had a great contribution to the way international policy deals with the balance among conservation and use of resources in the marine environment. In general, these principles state not only the need to assess and anticipate possible impacts in other areas of the ecosystem that are not directly under pressure but also the need to incorporate the human dimension into the equation, including all stakeholders and areas of the society that directly and indirectly depend on the resources. The need for an ecosystem approach to fisheries (Garcia and Cochrane, 2005; Jennings, 2005) has led to the establishment of ecosystem-based fisheries management practices (Link, 2002; Nicholson and Jennings, 2004) and ultimately to the concept of ecosystem-based management of the marine environment (Browman and Stergiou, 2004; McLeod *et al.*, 2005). This concept is now introduced into marine policy worldwide (Forst, 2009) and plays a strong part in European marine policy tools such as the Marine Strategy Framework Directive (MSFD; EC, 2008) and the Common Fisheries Policy (CFP; EC, 2011).

In a healthy ecosystem, communities are only limited by environmental factors and the result of their own interaction (predation, competition, reproduction) (Diaz *et al.*, 2004; Johnson *et al.*, 2012). In the marine environment, one of the main factors that can determine the potential community composition of a given habitat is the type of substrate, which can roughly be divided into hard substrate (reefs) and soft substrate (sand, mud). In soft substrate communities, sediment properties can have a direct effect on benthic organisms (Rhoads, 1974) but the main driving force for both benthic and demersal organisms is usually depth, and changes in community composition tend to occur along a depth gradient (Lampitt *et al.*, 1986; Sousa *et al.*, 2005, 2006; Johnson *et al.*, 2012). In rocky reefs, communities are highly dependent on the complexity of the substrate, as a rough surface provides conditions for the fixation of algae and sessile invertebrates, which in turn will provide food and shelter for adult and juvenile fish and mobile invertebrates. The occurrence of holes and crevices of several sizes, boulders, cobble and large blocks also provides shelter from hydrodynamic stress and increases the number of niches available for species to thrive (Ferreira *et al.*, 2001; García-Charton and Pérez-Ruzafa, 2001; Fraschetti *et al.*, 2005). In addition to spatial variability due to environmental factors, there is also significant temporal variability at several scales, from movement of organisms across the day (*e.g.* Gibson *et al.*, 1998), to weekly, monthly or seasonal patterns (*e.g.* Cadée, 1986; Holbrook *et al.*, 1994; Beyst *et al.*, 2001), decadal climatic oscillations (Henriques *et al.*, 2007; Kröncke *et al.*, 2013), as well as large scale climate change (Roessig *et al.*, 2005; Gamito *et al.*, 2012).

As a system becomes impacted by human-induced pressures such as fishing, sewage discharges and habitat loss, changes occur that lead to a shift in the overall state of the communities, with drastic reductions in abundance or disappearance of sensitive species and increases in abundance of stress-tolerant opportunistic species (*e.g.* Islam and Tanaka, 2004; Arévalo *et al.*, 2007; McKinley and Johnston, 2010). The challenge that stands before environmental managers and the scientific community in general is to be able to search within this immense pool of variability and detect which part of it is directly or indirectly related to human action. Not only must we accomplish this but it must be done in a timely manner, so that management decisions can effectively reverse degradation.

The way scientists and managers are dealing with natural variability can be roughly divided into two main approaches: the first one is to incorporate or model the behaviour of environmental variables and the second one is to minimise their effects by partitioning the area of interest into homogeneous sections. Incorporating environmental variability can be accomplished either by using a BACI (before-after control-impact) approach or its variations (*e.g.* Underwood, 1994), where changes in the control sites before and after an impacted situation are assumed to be due to natural causes, or by identifying environmental variables responsible for changes, quantifying and excluding their effects (*e.g.* García-Charton *et al.*, 2004). These approaches, however, are mostly suited for relatively small-scale studies or direct assessments of a known impact source. In national and international large scale monitoring programmes, however, modelling every possible combination of environmental conditions and their interactions is an extremely difficult task, and thus the most frequently used methods aim to reduce environmental variability by defining relatively homogeneous units within which the state of the communities is assessed (Maxwell and Buddemeier, 2002). This can be achieved either through a top-down approach, by delimiting areas with similar habitat features at an appropriate scale

(Valesini *et al.*, 2003; Ramos *et al.*, 2012), or through a bottom-up approach, by grouping areas with similar community composition for the organisms of interest (Araújo and Azevedo, 2001; Bremner *et al.*, 2003). A top-down approach is advantageous for standardising assessment scales, since environmental homogeneity can be organised hierarchically according to scale (Costello, 2009). On the other hand, such an approach is not guaranteed to lead to units supporting homogeneous communities, particularly of free-swimming organisms (Stoner, 2003). This reason supports the need for bottom-up definition of units using the communities of interest, followed by inference about the range of environmental features where those communities occur. With such an approach, spatial and temporal scales will likely vary according to the type of organism considered but the resulting units will more accurately reflect the achievable level of homogeneity within the variables that will in fact be monitored, such as species abundances.

Dealing with uncertainty in ecology and environmental management is undoubtedly a challenging task. In addition to natural variability, there are also sampling errors that contribute to background noise. These include systematic errors due to the selectivity of the methods, observer bias and other constraints, as well as random errors, generated by the movement of organisms relative to the sampling units (Vézina, 1988; Irigoyen *et al.*, 2013; McClanahan *et al.*, 2007). While an adequate monitoring plan can minimise the influence of random errors and small-scale natural variability, marine ecosystems present an additional barrier, as all processes occur under large masses of saltwater, in an environment that is still harsh for humans. For this reason, marine monitoring usually requires complex and/or expensive resources and techniques, and budget constraints tend to be very limiting in every monitoring programme (Borja and Elliott, 2013). This leads to institutions and managers often resorting to less demanding solutions that are, more often than not, inadequate and insufficient (de Jonge *et al.*, 2006; Borja and Elliott, 2013; Pais, 2013). Using available published data or scattered databases to design large scale monitoring plans is not an uncommon alternative to more expensive approaches but this can lead to huge data gaps and a general disregard for the actual methods that will be applied in the field. Another approach is to apply a monitoring programme that fulfils the (often lax) requirements of national or international policy tools but does not necessarily provide sufficient certainty in estimates and can therefore mislead managers, with ecological and socioeconomic consequences. One of the clearest examples of this is fisheries stock assessments and predictive models, which are the basis for the establishment of regulatory measures that can be costly if unnecessarily applied and catastrophic if a decline passes undetected (Maxwell and Jennings, 2005). In fact, it has been suggested that catch data fails to reflect the real state of populations (Pauly *et al.*, 2013), and even some of the most complete stock survey programmes have been shown to often require a decade to provide, with enough certainty, indication that a worrying decline is occurring (Maxwell and Jennings, 2005).

While uncertainty is still a complex challenge at the population level, it can easily escalate in complexity when assessing marine communities or, ultimately, the whole ecosystem (Pais, 2013). Lessons learned from community-based assessments in transitional waters for the European Water Framework Directive (WFD; EC, 2000) have shown that the choice of sampling method and protocol can have huge impacts on the perceived abundance of some species (Pasquaud *et al.*, 2012), and that some of the monitoring plans already established are leading to much more uncertainty than would be desirable for decision support purposes (Gamito *et al.*, 2012). For these reasons, there is evidence to support the need to prioritise scientific background knowledge regarding environmental features and sampling procedures in the planning phase of community-based assessments in the marine environment, in order to minimise problems that may arise once monitoring programmes are established.

Among all organisms in the marine environment, fish have been given a minor role in marine community-based assessments required by European policy tools, and their management has been mostly focused on the impact of fishing in a few commercially important populations. However, due to their great socioeconomic importance, as well as their valuable contribution to the stability of the ecosystem (Holmlund and Hammer, 1999), monitoring the state of fish communities is now a requirement of more recent tools like the MSFD, as well as part of monitoring and management plans in Marine Protected Areas (MPA) worldwide (García-Charton *et al.*, 2000; Côté *et al.*, 2001; Denny and Babcock, 2004; EC, 2008). In fact, the diversity of niches occupied by fish species, from bottom-dwelling invertebrate feeders to pelagic plankton filterers, has been pointed out as one of the main advantages of using fish communities as indicators of ecosystem instability and degradation (Harrison and Whitfield, 2004). This, allied to their relative easiness of identification, can provide the bases for short-term indicators of anthropogenic impacts on the marine environment. Nevertheless, high mobility, schooling behaviour and cryptic habits are some of the behavioural aspects of fish that can greatly affect the variability of abundance estimates, and thus our ability to discern human-driven from natural changes (Samoilys and Carlos, 2000; Maxwell and Jennings, 2005; McClanahan *et al.*, 2007; Irigoyen *et al.*, 2013). In order to minimise these issues, promising results have been obtained by classifying species according to functional guilds that respond more predictably to stress (Elliott *et al.*, 2007; Rochet and Trenkel, 2003). These functional approaches have been thoroughly used in streams and estuaries, where several measurable aspects of fish communities, such as species richness, total abundance and functional guilds are combined into multimetric indices that are able to detect deviation from a functionally healthy community (Pérez-Dominguez *et al.*, 2012). This approach has recently been brought into the marine environment, both in a similar form (*e.g.* Henriques *et al.*, 2008) and also as a set of tools to support ecosystem-based fisheries management (Rochet and Trenkel, 2003; Nicholson and Jennings, 2004) but its application is still at a very early stage. While many studies have stressed the need for functional approaches, there are still large methodological gaps regarding their practical application, particularly the response of functional guilds to specific pressure sources and their relationship with uncertainty and environmental heterogeneity. It is therefore urgent, given the urgency of detecting and acting upon human impacts on the marine environment, that these gaps are filled before the functional integrity of fish communities can be properly assessed.

This thesis consists of a series of works that aim to contribute to the new methodological requirements of community-based, functional approaches to marine fish assemblage monitoring, by attempting to fill in some of the gaps related to background variability and environmental heterogeneity. In fact, only by dealing with uncertainty and heterogeneity can we effectively detect anthropogenic impacts, understand the limitations of the methods and adapt management needs to the available resources. In chapter 2, a classification of fish assemblage types on the Portuguese coast is attempted using only published data as a basis. The problems and achievements of such a procedure are analysed and demonstrated, and some alternatives and good practices are suggested. The problems of using published data set the basis for approaching some of the practical aspects that can only be solved when using field data that incorporate fine scale variability with standardised and directly comparable sampling methods. Chapters 3 and 4 approach the issues of uncertainty and heterogeneity in temperate reefs, focusing on two different perspectives. In chapter 3, a method is proposed to quantify topographic complexity as perceived by fish communities, a solution to incorporate the fact that different organisms have different responses to habitat heterogeneity. This is followed, in chapter 4, by an assessment of the relationship between topographic complexity and the effort needed to detect changes in metrics of structural and functional integrity with enough certainty. Finally, chapters 5 and 6 describe and apply a framework for the definition of fish assemblage types that retain the best achievable level of structural and functional homogeneity, considering natural and sampling-related variability. In chapter 5 the method is applied to temperate reefs, taking into account the variability associated with underwater visual census and, in chapter 6, the same method is applied to soft-substrate areas along the coast, taking into account inter-annual variability along 5 years of bottom-trawl surveys.

Overall, this thesis proposes new approaches and methodologies for community-based assessment of marine fish, integrated in an ecosystem approach. In this way, it attempts to strengthen the bases that are needed for a reliable and scientifically supported use of fish communities as short-term indicators of human impacts on the functional integrity of marine ecosystems.

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Chapter 2

A critical approach to the use of published data for baseline characterisation of marine fish assemblages: An exercise on Portuguese coastal waters.

Miguel Pessanha Pais, Sofia Henriques, Maria José Costa, Henrique N. Cabral.

Abstract

Policy-makers are becoming increasingly aware of the importance of coastal fish communities for the integrity of marine habitats. However, initial assessments are often done prior to any major funding and available published data is sometimes seen as a low-cost approach to this problem. The present work constituted an exercise on marine fish assemblage characterisation, by relying only on published data from mainland Portugal, covering soft substrate areas and nearshore rocky reefs. Regardless of the characterisation itself, this exercise aimed to critically approach these procedures, by pinpointing problems and highlighting achievements. Criteria were defined for the inclusion of published studies and included datasets were standardised in terms of species proportions, spatial and temporal scales. Species were assigned to ecological guilds and all analyses were performed separately for species and guild data. The influence of season, depth and latitude were analysed through multivariate analysis of variance using permutations and distance-based linear models and assemblages were characterised based on multivariate ordination and hierarchical classification methods.

Significant differences were found between studies employing different methods in rocky reefs and outliers were not used in assemblage characterisation. Due to limitations in spatial and temporal detail in published data, only a rough, large-scale characterisation of assemblage types was accomplished, with datasets divided in rocky reefs, shallow soft-substrate (0−20 m), intermediate soft-substrate (20−100 m) and deep soft substrate (100−200 m). No seasonal differences were detected but a non-linear relationship with latitude was found in deep soft-substrate assemblages. During the exercise, spatial and temporal scales were forced by the quality of the data and some analyses failed to detect known patterns. Differences between methods, designs and types of data required assumptions to be made regarding data comparability. Overall, the exercise provided a hands-on insight into the use of published data for planning in a coastal management context, pinpointing data comparability, scale and information gaps as severe limitations of these approaches. In order to improve planning and design, some proposed measures were highlighted, such as the establishment of national databases and the use of pilot studies.

2.1. Introduction

Due to the consequences of increasing anthropogenic pressure on the marine environment, several national and international policies are addressing the need to maintain the integrity of marine ecosystems (Ricketts and Harrison, 2007; Borja *et al.*, 2008; Katsanevakis *et al.*, 2011). In fact, in Europe, the importance of coastal fish communities for the stability of the ecosystem and their role in supporting stocks is being increasingly addressed by both international policies and local management requirements (Borja, 2006; EC, 2008; Stål *et al.*, 2008). Moreover, the high socio-economic value of fish, allied to their relative easiness of identification, diversity of ecological guilds, longevity, among others, are important advantages of using them as quality indicators for water bodies (Whitfield and Elliott, 2002; Harrison and Whitfield, 2004).

On the marine environment, most of the work has been centred on the impact of fishing on exploited fish species (e.g. Rice, 2000; Sainsbury *et al.*, 2000) or, more recently, on an ecosystem approach to fisheries management (e.g. Browman and Stergiou, 2004; Jennings, 2005) that constitutes a holistic approach to fisheries but leaves a minor role to other anthropogenic impacts (Dethlefsen and Tiews, 1985). Adding the human dimension to the ecosystem is now deeply embedded in European policy, with the inter-connection of all species and habitats being a keystone principle in Integrated Coastal Zone Management. In fact, there is increased awareness that the effects of humans on exploited resources are reciprocal (Forst, 2009). With this in mind, the implementation of the Marine Strategy Framework Directive in 2008 (EC, 2008) has set the goals for improving the state of European marine waters but the scientific community is yet struggling to understand some of the underlying fundamental differences between marine habitats that define the "best" potential community a given area can support.

So far, the most common fish-based tools created in response to policy requirements in streams and estuaries (Roset *et al.*, 2007; Cabral *et al.*, 2012) and, more recently, in coastal waters (Henriques *et al.*, 2008a,b) are multimetric indices, which rely mainly on functional guilds, by comparing measurable aspects of a community with "reference" values that correspond to a certain "status" in a given "type" of habitat. However, these (and likely other) tools have requirements that need to be fulfilled before they can be successfully applied in a monitoring context, namely the definition of habitat "types" and the understanding of how the relative importance of each functional unit in a community varies between habitats (Henriques *et al.*, 2008b).

The downside is that initial assessments are often done prior to any major funding and, more often than desirable, have no choice but to rely on available published data compiled in a database of previous studies for a given area (Aiken *et al.*, 1999; Elliott *et al.*, 1999). Because published data have served different purposes, often there is valuable information missing regarding habitat characteristics that would prove useful in defining habitat types (Olenin and Daunys, 2004). However, since what is ultimately measured is the supported community, some information can be obtained by looking at the assemblages themselves. Assuming that in a baseline characterisation of coastal areas, data should represent the natural state of a system, surveys performed in heavily impacted sites should be avoided, as they are likely to support "unnatural" communities (Smith *et al.*, 1999; Guidetti *et al.*, 2003). Another important aspect when characterising assemblages in a monitoring context is that species lists are insufficient, as important variations in abundance would pass unnoticed (Hewitt *et al.*, 2005; de Jonge *et al.*, 2006). On the other hand, it is known that different sampling designs and methods can lead to different abundance estimates for a given area (Willis *et al.*, 2000; Pasquaud *et al.*, 2012), which, coupled with the fact that raw abundances are not frequently published (e.g. Olenin and Daunys, 2004), can prevent the use of abundance values for analysis.

The present work constitutes an exercise of marine fish assemblage characterisation using only published data as a basis. As a case study, we will focus on the coastal waters of mainland Portugal down to 200 m deep, covering soft substrate areas and nearshore rocky reefs, in an attempt to understand the main gradients and factors delimiting fish assemblages, not only in terms of species composition but also using ecological guild data. The study ultimately aims to critically approach such procedures, by pinpointing problems and highlighting achievements.

2.2. Materials and methods

2.2.1. Study area

The Portuguese coast is located in western Iberian Peninsula and extends from the Minho river mouth southwards along the 9º W meridian, and then eastwards at cape São Vicente (figure 2.1). The continental shelf is relatively narrow and its most conspicuous irregularity is the Nazaré Canyon. Reaching depths of around 5000 m, this depression divides the western shelf in a northern, flatter section up to 70 km wide, and a southern, steeper section up to 20 km wide until cape São Vicente, then reaching a width of about 30 km in the south coast (Gomes *et al.*, 2001). Over the shelf, upwelling occurs during warm months (April-September) due to predominant northern winds. In winter, only intermittent and weaker upwelling periods are observed. Average sea surface temperature usually stands in the 14−21 ºC range (Lemos and Pires, 2004).

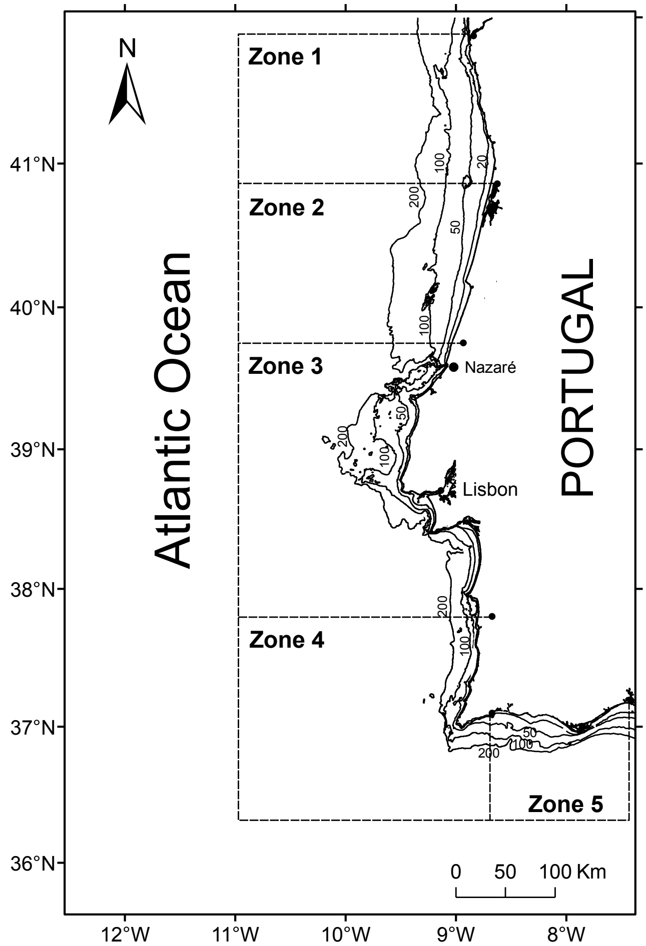

Figure 2.1. Map of the study area with coastal zones delimited by IPIMAR (1980, 1981a,b, 1982, 1984) based on hydro-morphological criteria. The 20 m, 50 m, 100 m and 200 m isobaths are represented.

2.2.2. Data collection and assumptions

For the purpose of this study, no privileged access to databases was used and only publicly available data was considered. In general, it was found that data on composition and abundance of fish assemblages in the study area is not easily accessible, being mostly scattered across several sources in research papers, academic theses and technical reports. Nevertheless, an effort was made to compile data from various locations, depth ranges, seasons and substrates.

The first step in data collection was to define the basic criteria that any published dataset should fulfil to be included in the exercise, by defining a simple decision tree illustrated in figure 2.2. This step was crucial in order to ensure an acceptable degree of comparability between studies that are likely to differ in their design and goals.

In order to minimise the introduction of samples from highly impacted assemblages, nearshore sites were given priority when located near or inside protected areas. Moreover, given the unavoidable differences in abundance values between sampling methods and designs and to maximise the available data, relative abundances were calculated for every dataset to conserve the proportion of the assemblages represented by each species or guild. Another important criterion was the possibility to organise data into seasons in order to introduce seasonal variability into the analysis.

For each site and season, data were aggregated into a single data point and were assumed representative of the proportion of species and guilds on that site and season. As a result, by the end of the data collection process, each entry in the database represented the relative proportion of species in an area of the coast in a particular season, delimited by depth ranges. In order to avoid confusion regarding the concept of sample, data points are referred in the text as "datasets", since they are the result of aggregated samples in a dataset.

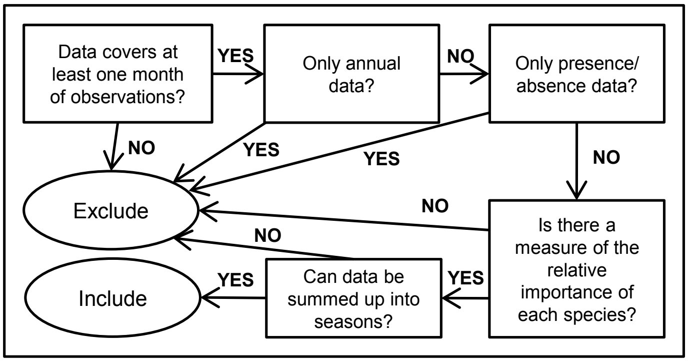

Figure 2.2. Decision-tree illustrating the selection criteria for data to include in the exercise.

Most of the data used for soft substrate assemblages along the coast were gathered from technical reports of the trawl surveys performed by the Portuguese Institute for Fisheries and Sea Research (IPIMAR) that are grouped according to pre-defined depth ranges and coastal sections (zones 1 to 5 in figure 2.1). This zonation was indivisible in the published data, however, it is based on hydro-morphological criteria and zones can be roughly corresponded to homogenous fish communities, considering the large spatial scale (Gomes *et al.*, 2001). Therefore, in order to overcome this constraint and standardise latitudinal location across the database, all collected datasets were assigned to these zones.

Concerning sampling procedures on the collected datasets (table 2.1), otter trawl was the most frequent method used on deeper soft substrate, complemented with beam trawl data to characterise some areas approximately 10−30 m deep (Abreu, 2005) and beach seine fisheries data for some areas shallower than 10 m (Cabral *et al.*, 2003). Beach seine, despite not being intentionally performed with the purpose of surveying fish assemblages, provides rather complete data due to the low selectivity of the fishing gear (Cabral *et al.*, 2003). All rocky reefs were sampled using underwater (SCUBA) visual census methods that varied from timed random paths, to fixed distance transects and stationary observations.

Compiled studies were organised into 69 datasets (table 2.1) and the taxonomy was updated and corrected according to FishBase online database (Froese and Pauly, 2011).

Table 2.1. Summary of the references from which the data were collected. See figure 2.1 for coastal zone limits.

Reference	Sampling years	Substrate	Latitude zones	Seasons sampled	Depth range (m)	Sampling method/gear	Nr. of datasets
IPIMAR,1980	1979	S	1, 2, 3, 5	Su	20 - 200	Otter trawl	8
IPIMAR, 1981a	1979	S	1, 2, 3, 4, 5	W	20 - 200	Otter trawl	9
IPIMAR,1981b	1980	S	3, 4, 5	Sp	20 - 200	Otter trawl	6
IPIMAR, 1982	1980	S	1, 2, 3, 4, 5	Su	20 - 200	Otter trawl	10
IPIMAR, 1984	1980	S	1, 2, 3, 4, 5	W	20 - 200	Otter trawl	10
Henriques, 1993	1993	R	3	Sp/Su/A/W	1 - 15	VC random	4
Rodrigues, 1993	1993	R	3	Sp/Su/A/W	10 - 25	VC transect	4
Almeida, 1997	1997	R	5	Su	17 - 25	VC stationary	1
Almada *et al.*, 2002	1999	R	3	Sp/Su/A/W	1 - 20	VC transect	4
Cabral *et al.*, 2003	1999	S	3	Sp/Su/A	0 - 20	Beach seine	3
Prista *et al.*, 2003	2001	S	3	Sp/Su/A/W	10 - 30	Otter trawl	4
Almada *et al.*, 2004	2004	R	3	A	1 - 20	VC transect	1
Gonçalves, 2004	2002	R	4	Su	19 - 23	VC random	2
Abreu, 2005	2005	S	5	Sp	10 - 30	Beam trawl	1
Maranhão *et al.*, 2006	2004-05	R	3	Su	1 - 15	VC random	2

VC- visual census, S- soft, R- rock, Sp- spring, Su- summer, A- autumn, W- winter.

2.2.3. Guild classification

One of the advantages of using fish as ecological indicators is the large variety of ecological guilds, which can respond more predictably to alterations on the ecosystem (Elliott *et al.*, 2007; Halpern and Floeter, 2008). Therefore, not only is there a need for guilds to play an important role in monitoring and assessment tools (de Jonge *et al.*, 2006; Henriques *et al.*, 2008b) but also the baseline characterisation of habitats and assemblages should take into account the distribution of these guilds regardless of individual species. For this reason, all species were incorporated into a total of 37 ecological guilds from seven categories (table 2.2), based on previous work by Henriques *et al.* (2008a,b).

Table 2.2. List, by category, of the ecological guilds used in the analysis.

Category	Guild	code	Category	Guild	code
Substrate dependence	S resident	Sres	Migration	non-migratory	nomig
	O resident	Ores		oceanadromous	oce
	R resident	Rres		catadromous	cat
	I resident	Ires		anadromous	ana
	S dependent	Sdep		anfidromous	anf
	O dependent	Odep	Trophic	Invertebrate feeders	inv
	R dependent	Rdep		omnivore	om
	I dependent	Idep		macrocarnivore	mac
Mobility	high	himob		zooplanktivore	zoo
	medium	medmob		piscivore	pi
	territorial	terr		herbivore	he
	sedentary	sed	Resilience	very low	VLresi
Habitat	demersal	dem		low	Lresi
	pelagic	pel		medium	Mresi
	reef-associated	reef		high	Hresi
	bathydemersal	batd	Spawning season	spring	SPspn
	bathypelagic	batp		summer	SUspn
	benthopelagic	bentp		autumn	AUspn
				winter	WIspn

I- rocky intertidal, S- soft substrate, R- rocky substrate, O- offshore. See section 2.2.3 for a detailed description and references.

On substrate dependence guilds, species were considered “resident” when a particular substrate is needed for settlement, life and reproduction to occur and “dependent” when a particular substrate is needed to partially fulfil the requirements of the species life-cycle (*e.g.* food, reproduction, protection). The term “offshore” was used when species inhabit or depend on deeper waters, mostly indifferent to the type of substrate beneath.

Migration and trophic guilds were based on the review on estuarine fish guilds by Elliott *et al.* (2007), with some adaptations to marine communities. Species were considered “invertebrate feeders” when they feed mostly on non-planktonic invertebrates, otherwise being considered “zooplanktivore”, along with other zooplankton feeders (*e.g.* species that feed on hydroids and fish eggs/larvae). “Herbivore” species feed mostly on benthic and planktonic macro and microalgae and macrophytes. Detritus and opportunistic feeders were included along with other “omnivore” species. “Macrocarnivores” feed both on macroinvertebrates and fish and species that feed almost exclusively on fish were included on the “piscivore” guild.

Habitat guilds were adapted from Holthus and Maragos (1995) and resilience guilds were based on the estimated minimum population doubling time (Musick, 1999) and classified as “high” (up to 1.4 years), “medium” (1.4−4.4 years), “low” (4.5−14 years) and “very low” (more than 14 years) according to FishBase data (Froese and Pauly, 2011). The proportion of individuals that fit each guild by category (hereafter designated “guild data”) was calculated for each dataset, constituting a separate data matrix.

2.2.4. Data analysis

In order to study the differences between groups in terms of guilds or species composition, both types of data were used in the analyses. On all initial analyses, soft and hard substrates were separated, since otherwise the expected differences between them would dominate multivariate analyses.

2.2.4.1. Minimising the effect of sampling methods

Prior to characterising the assemblages, the multivariate methods described below were used to analyse potential confounding variation due to differences in sampling methods, by isolating zone 3 (figure 2.1), which presented the highest variability in terms of methods for rocky reefs and shallow soft substrate areas (table 2.1). Methods in zone 3 that were significantly dissimilar and considered less representative of the assemblage (lower number of species counted in average) were marked for exclusion. Unconstrained ordination and classification methods were then used to compare these deviant datasets with all data available and exclude similar datasets from other coastal zones.

2.2.4.2. Assemblage characterisation

All multivariate analyses were based on Bray-Curtis dissimilarities (Bray and Curtis, 1957) and no further transformations were performed besides the standardisation per total in each dataset, since variations in the proportions of the most common species were important, considering the relatively short coastal area and depth range covered.

In order to test the effects of categorical factors in the assemblage structure using Bray-Curtis dissimilarities and not having to comply with normality assumptions, multivariate analysis of variance using permutations (PERMANOVA) was applied. This method partitions the total sum of squares based on the distance measure of choice and calculates a pseudo-F statistic, analogous to the F statistic in traditional ANOVA (Anderson, 2001; McArdle and Anderson, 2001). Unlike in a typical assessment, where experiments are designed *a priori*, the use of published data often leads to heavily unbalanced data (unequal number of samples within each level of a factor), and complex designs are sometimes impossible to test due to lack of replication and coverage of all possible combinations. However, using one-way PERMANOVA will perform the correct tests with minor concerns, under the assumption that individual data points are permutable under the null hypothesis (Anderson *et al.*, 2008). Therefore, for the purpose of this exercise, all factors were analysed separately, with possible interactions being merely observed in multivariate space or minimised by selectively removing confounding datasets for each analysis. In the case of depth ranges and latitude zones on soft substrate datasets, PERMANOVA was used to test their effect as qualitative (categorical) predictors and distance-based linear models (DISTLM; Legendre and Anderson, 1999, McArdle and Anderson, 2001) to test their effects as quantitative (gradient) predictors (using the average depth per dataset).

Another important factor when dealing with unbalanced data from various sources is the differences in multivariate dispersion between groups, to which PERMANOVA is sensitive (Anderson *et al.*, 2008). Therefore, a test of homogeneity of dispersions (PERMDISP; Anderson, 2006) based on distances from centroids was used to complement the analysis.

In order to observe data in unconstrained multivariate space based on the Bray-Curtis dissimilarities, Principal Coordinates Analysis (PCO; Torgerson, 1958; Gower, 1966) was used.

However, since in many cases the differences between groups are not evident when visualising axes that maximise total variance, a canonical analysis of principal coordinates (CAP; Anderson and Robinson, 2003; Anderson and Willis, 2003) was used to find axes in multivariate space that best discriminate between groups of interest.

Some observations were made concerning the species and guilds responsible for the observed patterns by representing Spearman's rank correlations of the original variables with PCO and CAP axes and group cohesion was analysed by calculating classification success rates through a "leave-one-out" procedure (Lachenbruch and Mickey, 1968).

P values were obtained using 9999 permutations. In PERMANOVA analyses, whenever the number of unique permutations available did not reach 100 due to lack of replicates, *P*-values were based on the Monte Carlo method proposed by Anderson and Robinson (2003). All analyses were performed using PRIMER v6 with PERMANOVA+ package and the level of statistical significance adopted was 0.05.

2.3. Results

A total of 201 species from classes Chondrichthyes and Actinopterygii were counted on all 69 datasets included in the exercise.

2.3.1. Minimising the effect of sampling methods

Despite the criteria adopted when selecting published data, some unwanted variation due to sampling methods was only visible when comparing datasets. Due to the diversity of sampling methods in shallow areas of coastal zone 3 (figure 2.1, table 2.1), comparisons were only made within this zone.

PERMANOVA found significant differences between methods using species and guild data for both substrates, yet leaving a single method per substrate in this case would lead to the loss of a large number of datasets and prevent further characterisation of the assemblages. Therefore, the criteria adopted were the average number of species counted by each method (per dataset) for this coastal zone and the within and between-group similarities (table 2.3).

Table 2.3. Within-substrate comparison between different sampling methods used in shallow areas (less than 50 m deep) of coastal zone 3 (see figure 2.1) with the average number of species (and standard deviation) calculated from n available datasets. Average Bray-Curtis similarities are shown for both species (in bold) and guild data. All PERMANOVA pairwise comparisons showed significant differences between methods.

	Method	n	Nr. of species	Average Bray-Curtis similarity				
				OT	BS	VCR	VCT	VCTb
Soft substrate	Otter trawl (OT)	4	21 ± 7	**51.21** 74.50	-	-	-	-
	Beach seine (BS)	3	38 ± 8	**18.32** 48.33	**39.77** 69.63	-	-	-
Rocky reefs	Random path (VCR)	6	32 ± 11	-	-	**65.92** 91.10	-	-
	Transect (VCT)	5	49 ± 7	-	-	**25.63** 78.24	**52.12** 81.72	-
	Benthic transect (VCTb)	4	13 ± 2	-	-	**2.31** 52.60	**8.09** 60.81	**59.33** 86.37

For soft substrates, otter trawl had less species per dataset, in average, than beach seine, which is probably related to the fact that seine nets sample a wider depth amplitude and have a smaller mesh size (Cabral *et al.*, 2003). However, otter trawl in this case can be seen as complementary in terms of depth and habitat, as it can sample areas that are deeper and adjacent to rocky coastal areas (Prista *et al.*, 2003), therefore supporting the decision to maintain both methods for this depth range. When including the remaining dataset from Abreu (2005), located in the south coast (zone 5) and sampled with beam trawl, in a cluster analysis by group average, all datasets were clustered at 8.4% similarity using species data and at 46.7% using guild data. Upon removal of the latter dataset, clustering level improved to 17.6% similarity for species data but maintained at 46.7% for guild data, which led to the decision of not discarding any datasets for functional characterisation. However, all comparative analyses relying on soft substrate data for this depth range were limited to zone 3 (see section 2.3.2).

On rocky reef datasets, however, a study by Rodrigues (1993) (see table 2.1) applied a visual census method with a clear focus on benthic species, which led to an overall lower number of species counted and low average similarities with other methods (table 2.3), some of them performed on the exact same site (table 2.1). This led to the decision of excluding these datasets and cluster analysis, along with unconstrained ordination (figure 2.3) were used to decide on the exclusion of data from other coastal zones. All rocky reef datasets clustered at 4.7% similarity with species data and 54.05% with guild data. Since data from zone 5 published by Gonçalves (2004) (see table 2.1) clustered with the datasets to be removed, when using both species and guilds (figure 2.3), these datasets were also excluded. Upon removal of all datasets with benthic-oriented methods, all data clustered at 15.4% using species data and 67.4% using guild data.

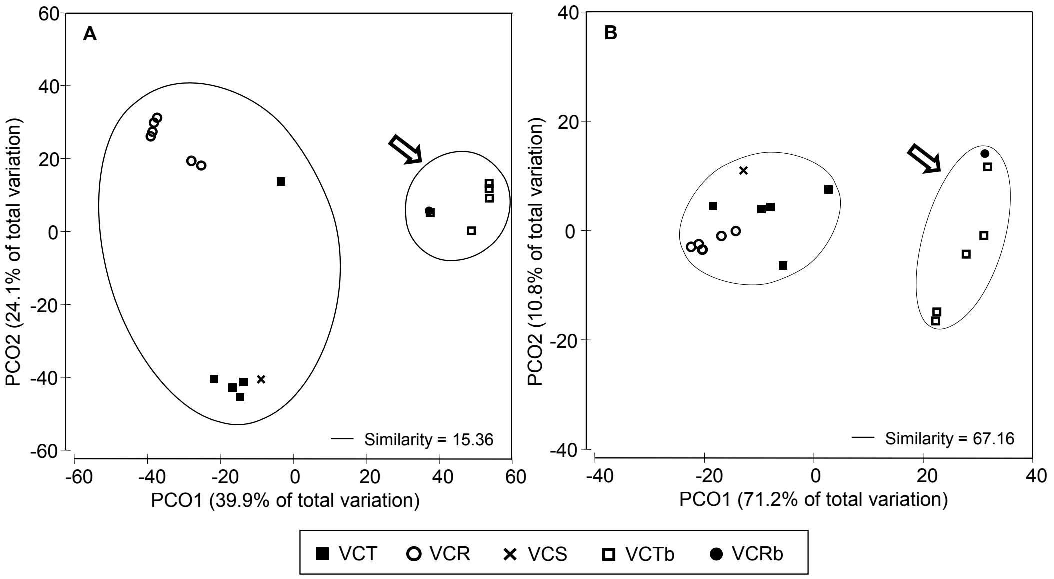

Figure 2.3. Principal coordinates analysis (PCO) using species (A) and guild (B) data. The group signalled by an arrow was excluded from further analyses due to underestimation of assemblage composition (see table 2.3). Cumulative explained variation was A) 87.80% and B) 77.68%. Similarity contours represent the level at which the accepted datasets were clustered by group average. For guild code correspondence see table 2.2, for species codes see appendix 2A.

2.3.2. Assemblage characterisation

After excluding rocky reef datasets with deviant sampling methods, the resulting data were mostly from coastal zone 3 (see figure 2.1) and depths were not evenly distributed across sites (see table 2.1), thus not allowing any tests regarding latitude or depth. Only seasonal variation was tested, showing no significant differences when using species (pseudo-F=0.317, *P*=0.9763) and guild data (pseudo-F=0.283, *P*=0.9974).

For soft substrate assemblages, latitude and depth were tested as gradient and categorical predictors, while excluding confounding datasets in each case, which would bring additional, unequally distributed variance across levels of the factor under test (table 2.4). Depth showed significant results both as a quantitative and a categorical predictor for coastal zone 3, while latitude showed significant differences only when treated as categorical. These observations support the hypotheses that functional and compositional changes in fish assemblages on the study area may occur along a depth gradient but do not seem to have a linear relationship with latitude (Hewitt *et al.*, 2005). In fact, pairwise tests overall tend to show a difference between the central zones 3 and 4 with northern and southern areas (zones 1 and 5, respectively) (table 2.4). Multivariate dispersion was significantly different between depth ranges in zone 3 using species (F=49.542, *P*=0.0003) and guild (F=27.490, *P*=0.0002) data, with deep (100−200 m) assemblages being significantly less variable in multivariate space. Overall, no significant differences were found between the available seasons (spring, summer and winter).

Table 2.4. Influence of latitude, depth and season on soft substrate assemblages using species and guild data. For depth as a quantitative gradient, the average depth per dataset was used, whereas in the categorical test, depth ranges corresponded to shallow (S; 0−20m), intermediate (I;20−100m) and deep (D;100−200m) areas. *P*-values were bolded when considered significant and underlined if significantly different multivariate dispersions were found in PERMDISP analyses. For pairwise tests, significantly different pairs are shown in brackets.

		Quantitative (DISTLM)		Categorical (PERMANOVA)		
	Data type	pseudo-F	*P*	pseudo-F	*P*	Pairwise
Latitude (20-200 m)	Species	2.0637	0.0949	2.7401	**0.0039**	(1,3)(1,4)(5,3)(5,4)
	Guilds	2.8159	0.0728	3.2628	**0.0073**	(1,3)(1,4)(1,5)
Depth (zone 3)	Species	9.5534	**0.0002**	5.9709	**0.0002**	(S,I)(I,D)(S,D)
	Guilds	10.474	**0.0013**	5.3630	**0.0050**	(S,D)(I,D)
Season	Species	-	-	1.1079	0.3248	-
	Guilds	-	-	0.8998	0.4991	-

Given that depth ranges were indivisible in some datasets (IPIMAR, 1980, 1981a, 1981b, 1982, 1984), depth was treated as categorical and the effects of season and latitude were tested separately within each depth range (table 2.5). PERMDISP found differences in multivariate dispersions between seasons in intermediate depths (20−100 m) but

PERMANOVA did not find enough evidence to reject the null hypothesis. Latitudinal location (coastal zones), however, showed significant differences on the guild and species composition of soft substrate assemblages sampled 100−200 m deep.

Table 2.5. Influence of latitude zones and seasons within depth ranges on soft substrate assemblages. Data for areas shallower than 20 m were not available for all coastal zones. PERMANOVA tests using species and guild data. *P*-values were bolded when considered significant and underlined if significantly different multivariate dispersions were found in PERMDISP analyses.

		Depth range					
		0−20 m		20−100 m		100−200 m	
	Data type	pseudo-F	*P*	pseudo-F	*P*	pseudo-F	*P*
Latitude	Species	-	-	1.3225	0.2068	4.6681	**0.0002**
	Guilds	-	-	1.5845	0.1675	6.2984	**0.0004**
Season	Species	0.4537	0.9640	1.8793	0.0701	0.3470	0.9372
	Guilds	0.2813	0.9512	2.5696	0.0568	0.3041	0.8966

The pattern responsible for this difference can be seen on the CAP plot (figure 2.4) for the 100−200 m depth range, where the canonical axes are drawn to best discriminate between latitude zones as categorical predictors. By looking at the plot, it is evident that datasets located to the right of the plot are mainly from central locations on the coast (zones 2, 3 and 4) and dominated by two species of snipefish (*Macroramphosus gracilis and Macroramphosus scolopax*), whereas datasets to the left are mainly constituted by hake (*Merluccius merluccius*) and other species. When plotting guild data (not shown), the results were similar, since datasets to the right of the plot were dominated by all the guilds represented by snipefish.

After analysing substrates independently, datasets were separated into four basic assemblage "types", according to the results obtained: rocky subtidal (R; permanently submerged rocky reefs down to a depth of 20 m), shallow soft-substrate (SS; soft substrate down to 20 m deep), intermediate soft-substrate (IS; soft substrate 20 to 100 m deep) and deep soft-substrate (DS; soft substrate 100 to 200 m deep). The distribution of all datasets was then analysed using species and guild data in unconstrained multivariate space using PCO (figure 2.5a,c), where the distinction between depth ranges in soft substrate areas was once again overruled by the presence of datasets dominated by a few species of the genus *Macroramphosus* (figure 2.5a). However, when looking at the variation along the vertical axis, assemblage types are not separated, being dispersed along a gradient, with a clear overlap between depth ranges that becomes even clearer when looking at functional guild composition (figure 2.5c).

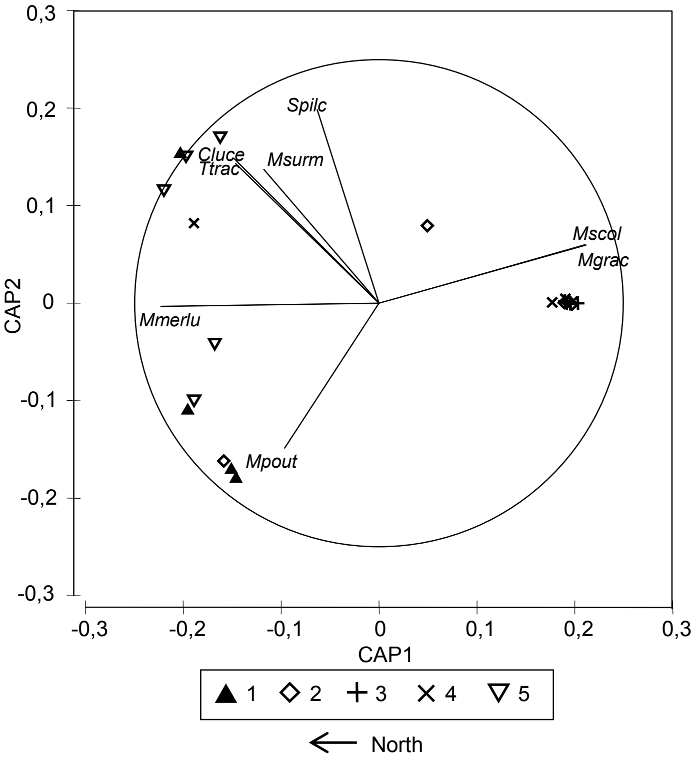

Figure 2.4. Canonical Analysis of Principal Coordinates (CAP) of species data using datasets from the 100−200m depth range. Axes are drawn to maximise the distinction between latitude zones 1 (north) to 5 (south). 83.34% variation was explained by m=2 PCO axes. Correlations with canonical axes are only shown when ρ > |0.6|. For species code correspondence see appendix 2A.

In order to look at the multivariate distribution of the established types without the strong influence of atypical datasets, a CAP analysis was used to find the axes that best discriminate between the defined assemblage types (figure 2.5b,d). In figure 2.5b, a distinction between types became evident, with the “leave-one-out” allocation procedure correctly classifying 69.8% of the datasets. The most distinct type were rocky reefs, with 100% correct classifications, and IS assemblages, with 87.5%. The only clear overlap when using species data was between IS and DS assemblages, where 9 out of 21 IS datasets were allocated to the DS group and 5 out of 22 DS datasets were misclassified as IS.

When using guild data (figure 2.5d), groups were more distinct, leading to 79.9% of correct allocations. Classification success for R assemblages was 91.7%, with one dataset misclassified as SS. DS assemblages had 81.8% correct classifications, with 4 datasets classified as IS, and SS had 62.5% success, overlapping with both R and IS assemblages.

The largest functional overlap was observed in IS assemblages (47.6% success), with 8 datasets classified as IS, and 3 as SS.

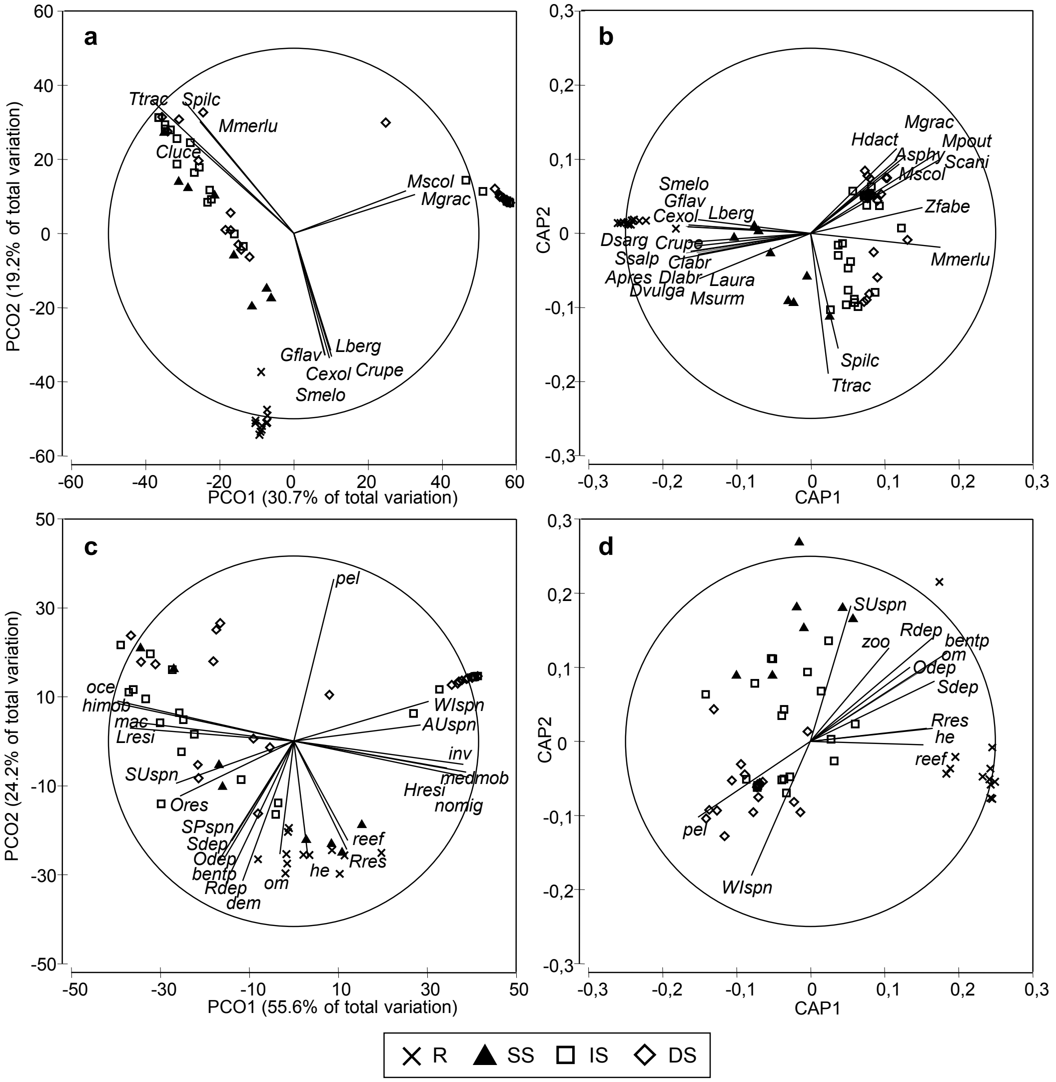

Figure 2.5. Unconstrained (PCO) and constrained (CAP) ordination plots to discriminate among the defined assemblage types, using species (a,b) and guild (c,d) data. Cumulative explained variation was a) 49.89% c) 79.84% b) 99.48% by m=6 PCO axes and d) 49.89% by m=3 PCO axes. Correlations with canonical axes are only shown when ρ > |0.6|. For guild code correspondence see table 2.2, for species codes see appendix 2A.

The objective of this exercise is to attempt a functional characterisation from published data that mostly focus on species, so individual species will deliberately not be given much attention, serving occasionally as a justification for functional aspects of the assemblages. However, by comparing Spearman's correlations of species proportions with the PCO and CAP axes when using species data (figure 2.5a,b), it is evident that the influence of highly dominant gregarious species like *Macroramphosus* spp. was minimised, with a gradient of species that characterise each assemblage type standing out in multivariate space, from seabream (*Diplodus* spp.) in rocky reefs and shallow sandy areas to pilchard (*Sardina pilchardus)* and horse mackerel (*Trachurus trachurus)* in shallow and intermediate soft substrates and hake (*M. merluccius*) and blue whiting (*Micromesistius poutassou*) in intermediate and deep soft-substrate assemblages.

For the same reasons, when using guild data, Spearman's correlations of guild proportions with the canonical axes of CAP (figure 2.5d) provide a clearer functional characterisation of the assemblage types. Winter spawners are mostly associated with deeper assemblages, while species from shallow soft substrates and rocky reefs are mostly summer spawners. Pelagic species characterise soft substrates and get more abundant in deeper areas while benthopelagic species are characteristic of shallow soft substrates. On rocky reefs most species are reef-associated. On the trophic category, omnivores and herbivores are more associated with reefs and zooplanktivores with shallow soft substrates. The average number of species per dataset (standard deviation in brackets) was 38 (14) for rocky reefs, 33 (16) for shallow soft substrates, 24 (8) for intermediate soft substrates and 24 (9) for deep soft substrates.

2.4. Discussion

2.4.1. Comparability of methods and designs

This study was an exercise that aimed to characterise marine fish assemblages according to species composition and functional guilds, by relying exclusively on published data, an approach intended as a hands-on confrontation with the problems arising from such procedures.

The first issue when compiling existing data is the comparability of different sampling methods. In fact, the published data gathered comes from samples collected with pure ecological studies in mind, which most likely differ in design and purpose (Elliott *et al.*, 1999; Olenin and Daunys, 2004; Hewitt *et al.*, 2005). On fish assemblage studies, it has been shown that sampling gear type and selectivity (Willis *et al.*, 1993; Pasquaud *et al.*, 2012), observational focus in visual census techniques (De Girolamo and Mazzoldi, 2001) and even the observer's experience (Kulbicki and Sarramégna, 1999) can significantly influence species composition estimates at a local scale. Likewise, aspects such as the number of replicates (Samoylis and Carlos, 2000), sampling periodicity (Pasquaud *et al.*, 2012), variability of habitats sampled (Jones, 1988) and the spatial scale considered

(Jones, 1988; Anderson and Millar, 2004; García-Charton *et al.*, 2004) can affect sample variability and hence the power to detect trends and patterns.

In the present study, finding a common ground in terms of spatial scale and sampling-related issues was no easy task, with datasets having to be standardised by the smallest indivisible spatial (coastal zones) and temporal (seasons) scales found in published literature. Another important aspect is the type of compositional data that best fits the objective of each study, with fisheries-related data often being reported in biomass (Cabral *et al.*, 2003), random path visual census in frequency of occurrence (Henriques, 1993, Maranhão *et al.*, 2006) and even sometimes using a categorical scale of abundance (Almada *et al.*, 2004). These cases were the exception, rather than the rule but the bias due to the overestimation of large fish in biomass data and to the underestimation of rarer gregarious species in frequencies of occurrence had to be assumed, despite the fact that these datasets did fit the overall pattern in multivariate analyses. Given this, in order to make datasets comparable, species data were standardised by total and all measures were assumed to represent the relative “importance” of each species for that particular study. All the above mentioned adjustments led to a very limiting starting point, forcing conclusions to be drawn from large coastal areas and masking within-season variation into single entries in the database.

By looking at the comparison of methods for areas shallower than 20 m (see section 2.3.1), it is evident that achieving unquestionable comparability between datasets was an impossible task, and too much strictness in the exclusion criteria would have prohibited any characterisation. However, datasets from rocky reefs that came from studies with a clear focus on benthic fish (Rodrigues, 1993, Gonçalves, 2004) counted significantly less species, making them unsuited for assemblage characterisation, even at a large scale. The problem of the observer’s focus in visual census techniques has been pointed out by many authors (Harmelin-Vivien *et al.*, 1985; Willis, 2001), since focusing on demersal fish underestimates cryptobenthic fish and vice-versa. The results observed in the present study show that the lack of a consensual practice regarding visual census methods can be a strong argument against the use of published data for rocky reef characterisation purposes, and an approach that covers substrate, crevices and water column (*e.g.* De Girolamo and Mazzoldi, 2001) should always be the choice when characterising assemblages in a monitoring context.

Given the overall differences found between methods within the same coastal area, the decision to exclude datasets based on the average number of species, coupled with multivariate classification and ordination methods assured a certain level of functional and compositional similarity between datasets to be included in the exercise.

2.4.2. Characterisation of assemblage types: problems and achievements

Even considering all limitations, the compiled datasets allowed a rough functional characterisation to be achieved, by using multivariate analysis to test how different substrates, depth ranges and seasons affected both species and guild data.

Overall, substrate and depth were identified as the main factors responsible for differences in assemblage distribution at this spatial scale. The difference between soft substrates and rocky reefs is known and was expected prior to data analysis (*e.g.* Pihl and Wennhage, 2002) but it is also known that different structural characteristics within the same substrate, like different types of sediment (Demestre *et al.*, 2000) or rocky reef areas of different complexity (Jones, 1988; García-Charton and Pérez-Ruzafa, 2001) can greatly affect the supported fish assemblage. These factors could not be tested from the published data analysed, even though it can be argued that a balance has to be achieved between the assessment scale and the applicability of the tools and monitoring procedures, in order to optimise costs (Caughlan and Oakley, 2001). Indeed, the coverage of a wide range of small-scale habitat variability with sufficient compositional homogeneity to compare relatively distant locations has been found in many studies (Jones, 1988; García-Charton *et al.*, 2004) but the main issue here is the context and purpose of the management objectives. In fact, if a monitoring programme aims to detect anthropogenic impacts on coastal fish communities, the scale must be chosen so that the natural variability inherent to habitat types is smaller than the expected variation due to impacts (Osenberg *et al.*, 1994).

Depth could not be tested for rocky reef data due to lack of detailed information but incorporating this variability into an overall habitat unit at this spatial scale was acceptable. Within soft substrates, the range covered was much larger, and significant functional and compositional differences were found when treating depth as a quantitative gradient and a categorical factor. However, the overlap between depth ranges was evident in multivariate ordination, so the establishment of strict depth boundaries in a gradient can be an arbitrary decision with little ecological meaning. As depth increases, changes occur in water temperature, salinity, pressure, light intensity and other factors that affect fish distribution according to ecological needs and physiological tolerances (Rice, 2005). Demestre *et al.* (2000) and Catalán *et al.* (2006) observed that depth was the main limiting factor for species distribution on soft substrate of the north-western Mediterranean continental shelf and the studies on demersal assemblages by Gomes *et al.* (2001) and Sousa *et al.* (2005) also identified depth as one of the main factors influencing the distribution of fish, crustaceans and cephalopods on the Portuguese shelf and upper slope.

Based on the ordination plots, Bray-Curtis similarity values and the “leave-one-out” allocation success, it is evident that the most pronounced differences between assemblages occur when species data is used. This is due to the fact that species are directly affected by small-scale habitat characteristics (Rice, 2005), while guilds tend to suffer smaller variations in abundance as some species are replaced by others of the same guild. The fact that proportions of individual species are very sensitive to environmental variation makes the distinction between natural and pressure-induced changes very difficult. In addition, individual species, except in the case of indicator species, provide little information about the state or “health” of an assemblage, compared to ecological guilds (Elliott *et al.*, 2007). However, as observed on within-group similarity values, though guild data can distinguish different substrates and depths at a relatively large biogeographic scale, smaller variations are probably more difficult to detect, and thus a careful selection of the guilds that are affected by each source of anthropogenic pressure is key for successfully managing coastal fish assemblages (Henriques *et al.*, 2008a).

2.4.2.1. Rocky reefs (0−20 m deep)

Rocky reefs identified in the present study display typical warm-temperate characteristics (Almada *et al.*, 1999; Henriques *et al.*, 1999). In these areas, the increase in turbulence and the decrease in water temperature, photoperiod, prey availability, among other factors, in autumn and winter, favour spring and summer spawning (Almada *et al.*, 1999). Due to the high productivity and complexity of rocky reefs, most species are very linked to the substrate throughout their whole lifecycle (Almada *et al.*, 1999; Henriques *et al.*, 1999; García-Charton and Pérez-Ruzafa, 2001; Pihl and Wennhage, 2002), hence the abundance of rock residents is characteristic of this substrate, making it vulnerable to impacts that negatively affect habitat integrity (Guidetti *et al.*, 2002).

The herbivores trophic guild was also characteristic of rocky reef assemblages. However, this is mainly due to *Sarpa salpa*, the only species, among the most common, whose adults are almost exclusively herbivore. The occurrence of few herbivore species on temperate rocky reefs might be related to the seasonal variability of algal biomass not favouring exclusive herbivory (Horn and Ojeda, 1999), however, as Bellwood *et al.* (2003) have pointed out in a much more diverse system, a single species can dominate a key function in the community, thus reinforcing the need to use abundance data rather than species lists and to always assess functional integrity.

Due to the a higher exposure to dominant winds and wave action (Sousa *et al.*, 2005), rocky reefs in the north coast of Portugal (zones 1 and 2) are difficult to sample using underwater visual census (Henriques *et al.*, 1999) and very few data were found for these areas, except for occasional species lists. This limitation led to year-round rocky reef data being mostly available in the centre and south, in areas that are sheltered from the predominant north-western winds (Lemos and Pires, 2004). Effects of latitude in rocky reefs were therefore not addressed in this exercise but variation is known to exist due to the location of the Portuguese coast in the gradient between Mediterranean and Northern-Atlantic waters, and even climatic oscillations have been shown to affect the predominance of species with cold-temperate or warm-temperate affinities (Henriques *et al.*, 2007).

2.4.2.2. Shallow soft substrate (0−20 m deep)

Multivariate analyses revealed a certain degree of similarity between shallow soft substrates and rocky reefs, probably due to factors associated with coastal productivity and to the frequent occurrence of shallow sandy areas near rocky reefs, with species known to occur on both substrates (Demestre *et al.*, 2000; Prista *et al.*, 2003). However, shallow soft-substrate assemblages were characterised by the co-occurrence of resident benthic species like *Mullus surmuletus* with highly mobile pelagic species like *Trachurus trachurus* and *Sardina pilchardus*, as observed by Catalán *et al.* (2006) near the Guadalquivir river mouth on the Gulf of Cadiz. The fact that pelagic zooplanktivores like *S. pilchardus* exhibit gregarious behaviour explains the abundance of the zooplanktivore guild on these assemblages.

2.4.2.3. Deep and intermediate soft substrate (20−200 m deep)

The demersal soft-substrate surveys conducted by the IPIMAR were published in technical reports with detailed abundance data (although along fixed coastal zones and depth ranges) only for the period used in this exercise (1979−1980), being subsequently included in integrative studies and published in journal articles by Gomes *et al.* (2001) (1985−1988) and Sousa *et al.* (2005) (1989-1999), where a division of assemblage types was accomplished based on species composition. Since the present work was intended as an exercise, using twenty-year-old data was not a major concern, as it were the only usable data to cover the whole continental shelf, with winter, summer and spring surveys and different depth ranges.

The most abundant species belonging to deeper assemblages are more independent from the substrate and exhibit gregarious behaviour. The occurrence of gregarious species dominated assemblages 20−200 m deep, and the use of proportions emphasised these differences. Although the use of proportions was the only way of standardising data in the present study, standardising by area or using raw abundances should always be favoured. Another solution is to exclude pelagic species from datasets (Gomes *et al.*, 2001, Sousa *et al.*, 2005), thus revealing the underlying patterns of less abundant species but losing information regarding functional guild composition.

Besides the pelagic guild, winter spawners dominated intermediate and deep soft-substrate assemblages, as pelagic species on upwelling systems tend to spawn when offshore transport is minimal, with planktivore juveniles feeding during the summer upwelling period (Santos *et al.*, 2001).

Assemblages from intermediate depths (20−100 m) were dominated by the pelagic species *T. trachurus* and *S. pilchardus* and deeper assemblages (100−200 m) were characterised by *M. merluccius*, a species occupying higher trophic levels. This increase in trophic level in offshore waters is typical of coastal upwelling systems, since offshore transport of primary productivity leads to a distribution pattern where species that feed on primary producers (*e.g. S. pilchardus*) are closer to the coastline and higher trophic levels place further away (Vinogradov and Sushkina, 1978; Gomes *et al.*, 2001).

Although deep soft-substrate assemblages are known to be more stable (Sousa *et al.*, 2005), there is a known influence of seasonality on rocky reefs (Almada *et al.*, 1999), shallow soft-substrates (Cabral *et al.*, 2003) and intermediate soft-substrates (Gomes *et al.*, 2001). However, no significant differences between seasons were found on the species and guild composition of these assemblages. This is likely due to the fact that each sample in the analysis represented a whole season, with within-season variation being represented by different studies, with different methods, in different locations. This likely led to too much variation and not enough replicates to achieve sufficient power to detect differences. Another possible explanation, as found by Pihl and Wennhage (2002), is that seasonal differences can affect the total number of individuals, thus the use of abundance proportions may mask those effects.

A non-linear relationship with latitude zones was found in deep soft-substrate assemblages, with snipefish (*Macroramphosus* spp.) dominating zone 3. The explanation for this fact was attributed by Marques *et al.* (2005) to the presence of the Setúbal Canyon but also the Cascais and Nazaré Canyons might have an important role in extending the distribution of these species into areas closer to the coast. Moreover, *T. trachurus* and *M.*

merluccius were less abundant on central coastal zones at this depth range, which has been attributed by Marques *et al.* (2005) and Sousa *et al.* (2005) to a predator-prey relationship, since the main prey of these species, *M. poutassou*, occurs mainly deeper than 200 m in the region off Lisbon.

An important aspect when using old datasets to characterise assemblages has to do with highly dominant species, which can change the functional characteristics of a community when a particular year is less favourable. In fact, the data used in the present study (1979−1980) correspond to a period of very high abundance of snipefish compared to the present state, and Marques *et al.* (2005) have pointed out a significant population decline due to unsuccessful recruitment in the year 2000 which, according to recent surveys, was maintained until present.

While the abundance of snipefish in some datasets dominated unconstrained ordination, the use of CAP attenuated those effects, thus allowing the characterisation of each habitat type without having to remove species or use data transformations, as the relative proportion of each species was deliberately conserved. Moreover, the inclusion of guild data on multivariate analysis provided important information on marine fish assemblages and established a link between the definition of management units and the development of monitoring tools.

2.5. Conclusions

Collecting published data can be useful in the case of social or economic indicators and statistics, or even ecological data that can be assumed somewhat stationary. In some cases, a few summarised values may be enough to support the design of a management plan. However, when dealing with data that can be highly variable in space and time, with very complex interactions, our study has demonstrated that summarising data masks a great deal of complexity. In this exercise, although assemblage types could be characterised to a certain degree using published data, it was not without a significant amount of doubtful assumptions and important information gaps.

Although we considered an extreme case, where no access to raw data is available, it served as a case study to highlight several problems that can be faced by coordinating entities, regardless of the type of monitoring objectives and ecosystem parameters to be measured. The main issues found can be summarised in the following topics:

1. Spatial and temporal scales were forced by the quality of the data, as important information regarding habitat complexity and environmental gradients are lost when

data is summarised for publication. Some analyses failed to detect patterns that have been observed at regional and local scales.

2. The stricter the requirements for including datasets in the analyses, the more comparable they become but the less the information available to support conclusions.
3. Different methods, designs and data types prevented the comparability of some studies and forced questionable assumptions (sections 2.2.2 and 2.3.1).
4. Missing data for some areas and time periods limited the analyses of spatial and temporal patterns.
5. Some distribution patterns could only be related to complex interactions between hydrology, habitat structure and biotic variables (such as predator-prey relationships).
6. Some older datasets were found to have different species compositions when compared to more recent observations of the same sites.

In order to avoid or minimise some of the problems demonstrated in this study, a number of guidelines that have been referred by other authors are highlighted:

1. The quantity and quality of available data should be exhaustively assessed before deciding to use them to support management decisions. The creation and maintenance of national databases of publicly available data can greatly improve this process (Hiscock *et al.*, 2003).
2. Species lists are not useful for most monitoring and assessment programmes (de Jonge *et al.*, 2006, Halpern and Floeter, 2008) and information on species abundances should always be favoured.
3. Besides species composition, functional aspects of the communities must be assessed when analysing patterns, defining management units and developing monitoring tools (de Jonge *et al.*, 2006)
4. Spatial and temporal scales adopted have to depend on monitoring objectives and environmental and habitat variability must be quantified in order to permit the distinction between natural and anthropogenic variation (García-Charton and Pérez-Ruzafa, 2001; Anderson and Millar, 2004).
5. Pilot studies should always be a part of every planning phase, since sampling methods and designs can greatly influence the power to detect changes (Osenberg *et al.*, 1994). All aspects, from reference values to warning thresholds should take into account a standard sampling design and should be able to adapt to changes in objectives or funding constraints.

6. Policy requirements should be viewed as an opportunity to acquire solid scientific knowledge on ecosystem patterns and responses (Elliott *et al.*, 1999; de Jonge *et al.*, 2006).

In the present study, even in a relatively small area, with all the effort employed in finding and filtering available data, an extensive list of issues was found. This can be seen as a wakeup call, stressing the importance of following the proposed guidelines and ultimately the need for funding in the planning phase of new management challenges, so that problems in monitoring designs can be anticipated, rather than corrected at a greater expense. Managing an area based on an inappropriate scale or using insufficient sampling can lead to assessment errors that ultimately carry environmental and socioeconomic consequences. It is on the best interest of regions and countries to actually ensure environmental quality and sustainability, rather than fulfil requirements with the sole purpose of fulfilling them.

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Appendix 2A. Correspondence between codes in the ordination plots (figures 2.4 and 2.5a,b) and species names.

Code	Species name
Apres	*Atherina presbyter* Cuvier, 1829
Asphy	*Argentina sphyraena* Linnaeus, 1758
Cexol	*Centrolabrus exoletus* (Linnaeus, 1758)
Cluce	*Chelidonichthys lucernus* (Linnaeus, 1758)
Crupe	*Ctenolabrus rupestris* (Linnaeus, 1758)
Dlabr	*Dicentrarchus labrax* (Linnaeus, 1758)
Dvulga	*Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817)
Dsarg	*Diplodus sargus* (Linnaeus, 1758)
Gflav	*Gobiusculus flavescens* (Fabricius, 1779)
Hdact	*Helicolenus dactylopterus* (Delaroche, 1809)
Laura	*Liza aurata* (Risso, 1810)
Lberg	*Labrus bergylta* (Ascanius, 1767)
Mgrac	*Macroramphosus gracilis* (Lowe, 1839)
Mmerlu	*Merluccius merluccius* (Linnaeus, 1758)
Mpout	*Micromesistius poutassou* (Risso, 1827)
Mscol	*Macroramphosus scolopax* (Linnaeus, 1758)
Msurm	*Mullus surmuletus* Linnaeus, 1758
Scani	*Scyliorhinus canicula* (Linnaeus, 1758)
Smelo	*Symphodus melops* (Linnaeus, 1758)
Spilc	*Sardina pilchardus* (Walbaum, 1792)
Ssalp	*Sarpa salpa* (Linnaeus, 1758)
Ttrac	*Trachurus trachurus* (Linnaeus, 1758)
Zfabe	*Zeus faber* Linnaeus, 1758

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Chapter 3

Improving the “chain and tape” method: A combined topography index for marine fish ecology studies.

Miguel Pessanha Pais, Sofia Henriques, Maria José Costa, Henrique N. Cabral.

Abstract

The "chain and tape" method is used to quantify topographic complexity in reef ecology studies, consisting of the ratio of the linear distance between the start and end points of a chain moulded to the surface of the substrate to its stretched length, a measure known as the substrate rugosity (SR) index. This measure has several advantages in the field when compared to other methods but some weaknesses have been pointed out. However, it is still one of the most frequently used topography measures in reef fish ecology. The present study proposes a combined topography index (CTI) that uses the "chain and tape" method in the field, with results that can match more complex methods, outperforming the traditional SR index. The CTI is structured as a weighted sum of 3 topographic features: SR index, number of corrugations (NC) and maximum vertical relief (MVR), where NC and MVR are given weight coefficients ranging from 0 to 1. In order to establish weight coefficients, fish assemblages were sampled at 6 training sites, representing a topographic complexity gradient. A series of candidate weight combinations were then selected so that CTI was optimally correlated with each one of several fish assemblage parameters. The list of possible solutions was then applied to nine previously published schematic profiles and compared with other methods. The final index was established as: CTI = (1−SR) + NC/25 + MVR/25. Ultimately, the predictive performance of CTI and SR was tested by applying them to 11 new sites as predictors of species abundances in distance-based linear models. The CTI outperformed SR when added to 3 previously fitted variables (depth, percent cover of sand, percent cover of cobble), explaining 5.6% additional variation when using all species and 8.1% when using only cryptobenthic species, whereas SR showed no significant additional effects.

3.1. Introduction

The quantification of environmental parameters that drive the abundance and distribution of species in an ecosystem is a major part of both fundamental ecological studies and environmental monitoring (Costello, 2009). In marine reef ecology, one of the most important parameters is the structural complexity of the underlying substrate, which can ultimately determine the number of niches available when considering substrate alone.

To this day, many measurements of structural complexity have been applied in reef ecology, such as the diversity of shapes or “growth forms” (*e.g.* Luckhurst and Luckhurst, 1978), the diversity of substrate types and boulder sizes (*e.g.* García-Charton and Pérez-Ruzafa, 2001) or the calculation of complexity indices that are known *a priori* for a number of substrates (*e.g.* Roberts and Ormond, 1987; Brokovich *et al.*, 2006). These measures are often used to complement a measure of surface topography that quantifies the complexity of the underlying substrate (Underwood and Chapman, 1989; McCormick, 1994), and the most common choice is the application of cost-effective small-scale field methods by SCUBA divers, who can accomplish enough detail with minimum cost, when compared to more expensive technology (Costello, 2009; Johnson *et al.*, 2012).

In a study published in 1994, a series of performance tests were applied to a number of these methods using both real and schematic reef profiles (McCormick, 1994). Most of the methods tested required the use of a field profile gauge, an apparatus with eleven 1m long graduated needles placed 10cm apart, which are able to move vertically on a frame when placed over the substrate. The only method tested that did not require a field profile gauge was an adaptation of Risk’s “chain and tape” method (Risk, 1972; Luckhurst and Luckhurst, 1978), where a chain is moulded to the surface of the substrate, and its length is compared to the horizontal distance covered. This method usually leads to the calculation of a substrate rugosity index (SR) that has two variations. In Risk’s index, a variable chain length is used to cover a fixed horizontal distance (“tape”), leading to the calculation of a chain:tape ratio, which is mainly used for smaller scales (1m^2). On larger scales (100m^2), due to practical field constraints, many researchers rely on a fixed chain length and invert the index to a tape:chain ratio, so that the index maintains a linear response, decreasing as the horizontal distance covered decreases (with increasing rugosity) (*e.g.* Grigg, 1994; Öhman and Rajasuriya, 1998).

McCormick (1994) pointed out several weaknesses of the SR index, demonstrating that it could not distinguish among substrata with very different profiles, especially between a single large corrugation and a series of small corrugations. Nevertheless, the “chain and tape” method is still one of the most frequently applied in marine fish ecology studies (*e.g.*

Öhman and Rajasuriya, 1998; Ferreira *et al.*, 2001; García-Charton and Pérez-Ruzafa, 2001; Brokovich *et al.*, 2006), most likely due to its advantages in the field: 1) there is no need to invest in or build a calibrated apparatus that is difficult to carry underwater; 2) the "chain" can be replaced by a thin leaded rope and either of them can be carried on a reel, along with a measuring tape for the linear distance, while performing other tasks; 3) the length of the "chain" can be adjusted to cover smaller (*e.g.* quadrats) or larger areas (*e.g.* transects).

The common practice of introducing other variables to complement the weaknesses of SR in modelling approaches can lead to variable correlation problems. Moreover, having two or more variables to describe topography can be seen as an unnecessary increase in dimensionality that adds to model complexity (Raudys and Jain, 1991). With this in mind, and taking into account that the main drawbacks of the SR index are related to the number and height of corrugations, the present study aims to incorporate these parameters into a combined topography index (CTI) that uses the "chain and tape" method in the field, with results that outperform the traditional SR index.

3.2. Materials and methods

3.2.1. Study area

In order to study the relationship between topographic features and fish communities, 6 sites were chosen along a 7km stretch of coast located off Cascais, Portugal, an area sheltered from the prevailing north winds and representing a topographic complexity gradient (henceforth referred to as "training sites"). In order to test the index, an additional 11 test sites along a 250km stretch of the Portuguese coast were sampled (table 3.1).

3.2.2. Fish sampling method

Fish were sampled during daytime hours using visual censuses obtained by SCUBA-diving along 50m strip transects (Harmelin-Vivien *et al.*, 1985). In order to sample both demersal and cryptobenthic species, each transect was travelled twice for each replicate (De Girolamo and Mazzoldi, 2001), with a first pass for demersal species (50m x 2m) and a second for cryptobenthic species (50m x 1m). In order to minimise the disturbance on fish behaviour, transects were deployed while performing the first pass, with cryptobenthic fish sampled while reeling the transect, by searching in crevices and under cobbles ≤20cm in diameter.

A total of 3 transects per site, per season were performed, starting each time at a random point and allocated to pre-determined depth intervals (3−6m and 8−11m), according to each site’s characteristics. On cryptobenthic transects, only the families Blenniidae, Bothidae, Batrachoididae, Callionymidae, Congridae, Gadidae (subfamilies Lotinae and Phycinae), Gobiesocidae, Gobiidae, Muraenidae, Scorpaenidae, Scophthalmidae, Soleidae, Syngnathidae and Tripterygiidae and the species *Ctenolabrus rupestris* (L.) and *Labrus mixtus* L. were counted. Due to ontogenic variations in behaviour, fish smaller than 5cm TL from the genus *Symphodus* were also counted on cryptobenthic transects (but not considered cryptobenthic species). All others were counted on demersal transects.

Table 3.1. Location and description of the training and test sites according to average values for the environmental variables measured. Depth in metres.

	Site	Lat (N)	Long (W)	1−SR	NC	MVR	depth	%S	%CS
Training sites	Cascais 1	38°41.3'	9°21.9'	0.05	1	1.5	5.2	5.0	0.0
	Cascais 2	38°41.5'	9°22.5'	0.15	4	1.6	7.9	15.3	0.0
	Cascais 3	38°42.0'	9°24.8'	0.20	3	1.6	3.5	12.5	0.0
	Cascais 4	38°42.1'	9°24.0'	0.27	1	1.4	4.6	11.7	0.0
	Cascais 5	38°41.3'	9°25.5'	0.33	5	2.5	9.2	13.0	0.0
	Cascais 6	38°41.6'	9°26.7'	0.34	4	4.6	8.1	19.0	0.0
Test sites	Sesimbra 1	38°27.0'	9°01.2'	0.28	5	2.3	4.9	0.0	4.0
	Sesimbra 2	38°27.0'	9°01.2'	0.32	5	2.2	9.1	1.5	13.0
	Sesimbra 3	38°26.1'	9°03.8'	0.36	3	2.3	5.3	0.0	33.6
	Sesimbra 4	38°26.1'	9°03.8'	0.30	3	3.1	9.2	0.0	18.7
	Sines 1	37°58.0'	8°52.8'	0.31	3	2.1	10.1	11.1	0.0
	Sines 2	37°55.3'	8°48.6'	0.20	4	1.4	8.4	32.0	0.0
	Porto Covo 1	37°52.4'	8°48.3'	0.36	3	2.6	11.7	0.0	0.0
	Porto Covo 2	37°50.9'	8°48.0'	0.27	4	2.1	9.5	0.0	0.0
	Algarve 1	37°5.4'	8°40.1'	0.05	0	1.5	3.3	31.6	2.1
	Algarve 2	37°4.5'	8°18.7'	0.25	3	1.4	3.1	10.2	11.1
	Algarve 3	37°5.7'	8°23.2'	0.23	3	0.8	4.5	5.1	0.0

Lat- Latitude, Long- Longitude, SR- Substrate rugosity index, NC- Number of corrugations, MVR- Maximum vertical relief (in metres), %S- Percentage cover of sand, %CS- Percentage cover of cobble. See section 2.3 for a detailed description of the variables.

Since topographic complexity remains similar yearlong, fish at the training sites were sampled in winter, spring and summer, in order to account for seasonal variation (autumn was not sampled due to turbulent sea conditions). For the test sites, an effort was made to cover a wide array of conditions (*i.e.* depth, exposure, latitude), while maximising the potential of each site by sampling fish assemblages during summer, which is close to the spawning season for many species (Almada *et al.*, 1999).

3.2.3. Topography sampling method

In the proposed method, the aim is to sample an area in the same period of time it takes to apply the usual “chain and tape” method, while recording additional features to improve the final index value. Due to the relatively large scale covered by fish transects (50m x 2m), a fixed chain length of 25m was adopted and the substrate rugosity (SR) index was calculated as the ratio of the horizontal distance covered by the contoured chain to its stretched length (Grigg, 1994). For this purpose, a reel with a 25m long leaded rope was used, along with a 25m reeled measuring tape. Both the leaded rope and the measuring tape are anchored at the starting point, randomly placed for each replicate. Then, one diver carrying a depth gauge (*e.g.* in a dive computer) unreels the leaded rope, while making sure it closely follows the contours of the substrate in a fixed direction. A second diver follows behind, unreeling the measuring tape while maintaining linear tension.

The diver carrying the measuring tape must count the number of significant height variations (≥ 0.5 metres was adopted in this study) as they appear, recording the total number of upwards (N_u) and downwards (N_d) elevation changes along the profile. This is used to calculate an approximation of the number of corrugations (NC), defined as

$$\mathrm{NC} = \frac{N_u + N_d}{2} \tag{1}$$

The linear distance (Ld) given by the measuring tape from the anchor point to the end of the leaded rope is measured and the substrate rugosity index (SR) calculated as

$$\mathrm{SR} = \frac{Ld}{Lc} \tag{2}$$

where Lc is the stretched length of the “chain”, or leaded rope (25m). The diver with the leaded rope then records the depth (in metres) at the deepest (D_d) and shallowest (D_s) points on the profile to permit calculation of the maximum vertical relief (MVR) for that replicate:

$$\mathrm{MVR} = D_d - D_s \tag{3}$$

Additionally, the diver with the measuring tape records the total distance travelled over several types of substrate (rock, sand, cobble). The percentage of tape length covered by each substrate provides an approximate measure of substrate heterogeneity without much additional effort. Three replicates were performed per site, placed 3 metres apart and following the direction of highest complexity within the defined depth ranges. However, due to the random placement of transects and the mobility of fish assemblages, no direct comparison between topography and fish replicates can be made. Thus, results were averaged across the fish transects and values were assumed representative of each site (table 3.1).

3.2.4. Building and testing the index

A linear combination was chosen in order to merge the rugosity index (SR), the number of corrugations (NC) and the maximum vertical relief (MVR) into a single final index. This was accomplished through a weighted sum of these components.

Since SR decreases linearly from a maximum value of 1 as rugosity increases (Grigg, 1994; McCormick, 1994), while NC and MVR tend to increase with complexity, the variation of the rugosity term was inverted by changing it to $1-SR$, which in practice represents the proportion of measuring tape that is left after reaching the end of the “chain” (Öhman and Rajasuriya, 1998).

The final form of the combined topography index (CTI) is therefore

$$\mathrm{CTI} = (1 - \mathrm{SR}) + W_{\mathrm{NC}} \cdot \mathrm{NC} + W_{\mathrm{MVR}} \cdot \mathrm{MVR} \tag{4}$$

where SR is the rugosity index (eq. (2)), NC is the number of corrugations (eq. (1)), MVR is the maximum vertical relief in metres (eq. (3)) and W_{NC} and W_{MVR} are the weight coefficients for NC and MVR, respectively.

The calculation of weight coefficients for the CTI was approached as an optimisation problem, with McCormick’s tests as performance goals. As a starting point, all 9 fish transects per training site (3 per season) were used to establish an initial list of 4 possible solutions, by setting W_{NC} and W_{MVR} to values ranging from 0 to 1, so that the final index value had an optimal Pearson’s correlation with each one of 4 fish assemblage parameters (table 3.2). The list of parameters with expected positive correlations with topographic complexity was adapted from McCormick (1994) and the solution for each parameter was found using the optimisation algorithm in Microsoft Excel Solver (Fylstra *et al.*, 1998).

In order to allow direct performance comparisons with McCormick’s approach, the list of possible weight combinations was tested by calculating the CTI for the exact same schematic profiles (figure 3.1). These theoretical profiles, although originally based on coral reefs, cover a wide range of shapes, slopes and heights that are transversal to tropical and temperate reefs, even if we consider a larger scale. Profile 1 can represent any transition from a large flat block to sand on a lower level, or to bedrock; profile 2 represents a tall hill; profile 3 is found on temperate reefs when a large rock is eroded at the base due to wave action, sand and boulders, while the top is unaffected; profiles 4 and 5 represent any surface with medium hills or blocks; profile 6 can be found when a large block is detached from a cliff and some smaller blocks fall near it; profiles 7, 8 and 9 can represent several different shapes of wave-eroded tilted rock layers.

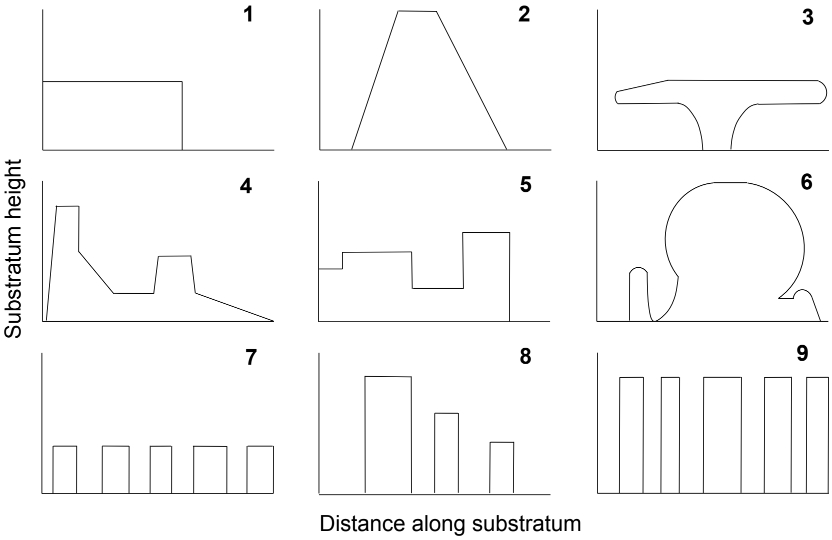

Figure 3.1. Schematic profiles from McCormick (1994).

Contour length was measured using image analysis software, by laying a “chain” of fixed length (equal to the horizontal distance of the profiles) 3 times over each profile. Once the chain ended, the horizontal distance covered was measured and no further corrugations were counted. In order to cover the whole profile, one chain was laid from the leftmost point in the profiles, one at a random point in the centre and another from the rightmost point. Both the SR and all versions of the CTI were then calculated for each of the three measurements and the final index values were averaged to represent each profile.

Candidate weight combinations and the SR index were compared according to the order of complexity among the 9 profiles, by analysing Kendall’s rank correlations with the three best performing profile gauge methods, namely the sum of consecutive needle height differences (CHD), vectors standard deviation (VSD) and substratum angle standard deviation (SASD) (McCormick, 1994). Finally, the individual performance of indices on the profiles was graphically analysed.

After selecting the weight coefficients based on training sites and schematic profiles, the predictive performances of the proposed CTI and the original SR index were compared, by using them separately as predictor variables to model Bray-Curtis dissimilarity matrices of square-root transformed species densities at the test sites. This was accomplished by using distance-based linear models (DISTLM), a routine that attempts to model a multivariate data cloud described by any distance measure of choice, by partitioning variation according to a regression model (Legendre and Anderson, 1999). *P*-values for the pseudo-F ratios were calculated through 9999 permutations and considered significant at $P < 0.05$.

3.3. Results and discussion

The present study proposes a combined topography index that merges three topographic features to improve the performance of the traditional SR index. Of the four candidate weight combinations in table 3.2, the one that maximised the correlation with the total number of species, CTI_{S1}, is redundant with 1−SR, as it gives zero weight to both NC and MVR, and was therefore discarded.

Table 3.2. Performance summary of the three separate terms in the CTI and final index values, calculated using four optimised combinations for W_{NC} and W_{MVR}. Pearson correlation coefficients that were maximised for each solution (S1-S4) are bolded, underlined values were not significant at α=0.05. The number of species whose abundances were correlated with the CTI is also shown.

			CTI weight solutions				Separate terms		
			CTI_{S1}	CTI_{S2}	CTI_{S3}	CTI_{S4}			
	Fish assemblage parameters	W_{NC}	0.00	0.04	0.05	0.00	1−SR	NC	MVR
		W_{MVR}	0.00	0.04	0.06	0.19			
1	Total number of species		**0.094**	-0.006	-0.029	-0.011	0.094	-0.151	-0.060
2	Total density (fish.m^{-2})		0.388	**0.414**	0.412	0.384	0.388	0.337	0.338
3	Density of cryptic individuals (fish.m^{-2})		0.357	0.393	**0.395**	0.371	0.357	0.331	0.336
4	Density of rock residents (fish.m^{-2})		0.366	0.379	0.381	**0.436**	0.366	0.203	0.421
	Mean (Standard deviation)[a]		0.371	0.395	0.396	0.397	0.371	0.290	0.365
			(0.016)	(0.040)	(0.049)	(0.106)	(0.016)	(0.062)	(0.141)
	No. of correlated species[b]		7	8	8	10	7	9	8
	No. of correlated cryptic species[b]		3	3	3	3	3	1	3

CTI- Combined topography index, SR- Substrate rugosity index, NC- Number of corrugations, MVR- Maximum vertical relief, W_{NC}- Weight coefficient for the number of corrugations, W_{MVR}- Weight coefficient for maximum vertical relief.

[a] Mean correlations did not take into account parameter 1, due to non-significant results.

[b] Number of significant correlations out of a total of 48 species,14 of which are cryptobenthic.

Unlike the results found by McCormick (1994) in the Great Barrier Reef, no solution was able to find a significant correlation with the total number of species. In fact, conclusions regarding the influence of topography on fish species richness are highly variable (*e.g.* Luckhurst and Luckhurst, 1978; Gratwicke and Speight, 2005), even on different substrates in the same region (Öhman and Rajasuriya, 1998). This is probably related to the erratic behaviour of this parameter, as counting rarer species can be unrelated to habitat features (Poos and Jackson, 2012). The remaining parameters showed significant correlations with substrate complexity, with the three CTI configurations performing better than the individual terms. The effect of vertical relief in the training sites is evident, with MVR dominating CTI_{S4} and showing significant correlations with a larger number of species.

Despite having significant correlations with assemblage parameters, the three candidate solutions are likely to respond differently to changes in the shape of substrate profiles. This is best observed when applied to the schematic profiles proposed by McCormick (1994), which vary in complexity and shape (figure 3.1). In fact, the results of Kendall's correlation in table 3.3 lead to the decision of discarding CTI_{S4}, since the order of complexity of the nine profiles according to this index was the least correlated with the order achieved using the best profile gauge methods. The fact that NC was not taken into account in this configuration and that excessive weight was given to MVR result in higher values for profiles with higher peaks, regardless of their shape.

Table 3.3. Kendall rank correlations between several index values obtained for 9 schematic profiles adapted from McCormick (1994). All values were significant at α=0.05.

	CHD	VSD	SASD
1−SR	0.719	0.764	0.689
CTI_{S2}	0.764	0.719	0.733
CTI_{S3}	0.764	0.719	0.733
CTI_{S4}	0.719	0.629	0.733

CTI S2-S4- Combined topography index according to the configurations proposed in table 3.2, SR- Substrate rugosity index, CHD- Consecutive height differences, VSD- Vectors standard deviation, SASD- Substratum angle standard deviation.

The two remaining configurations, CTI_{S2} and CTI_{S3}, had similar results and the traditional SR index also showed equivalent performance in terms of the order of complexity, although it was different from the substratum angles standard deviation (SASD). This index varies with the angular standard deviation of the angles formed by lines joining two consecutive needles in a profile gauge and vertical, while vectors standard deviation (VSD) depends on the angles formed by vectors perpendicular to these lines, making SASD much more sensitive to variations in height, to which SR is not very sensitive (McCormick, 1994). However, these results refer to ranks only, and thus do not take into account how different indices distinguish among different topographic features. Therefore, the final decision can only be made when graphically comparing the relative values obtained when using CTI_{S2}, CTI_{S3}, the SR index and McCormick's CHD (figure 3.2).

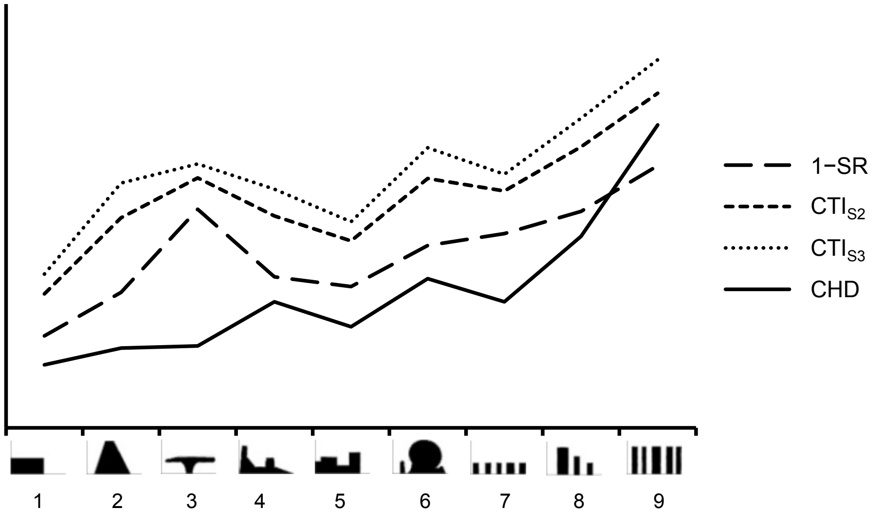

Figure 3.2. Comparison of relative index values for 9 schematic profiles. Vertical axis represents increasing complexity according to the indices but units vary and are scaled for comparison purposes and thus not shown. Schematic profiles and CHD values from McCormick (1994). *Legend*: SR- Substrate rugosity index, CTI- Combined topography index, CHD- Consecutive height differences.

The weaknesses of the SR index applied to these profiles have already been pointed out by McCormick (1994) and were the motivation for this study but figure 3.2 clearly shows its lack of sensitivity to tall and highly corrugated structures, since it only focuses on the perimeter of the shapes regardless of their number or height, giving too much importance to the tabulate outcrop in profile 3. What is accomplished by introducing sensitivity to height and the number of corrugations in the CTI is a small increase in the response to the number of corrugations and a clear valorisation of higher structures, which is evident in the increased values of profiles 2, 4 and 6 and the reduced importance given to the perimeter of the tabulate outcrop when compared to the SR index. Moreover, it is clear that the weight given to vertical relief in CTI_{S3} is too high, since the complexity of higher profiles is always overestimated regardless of shape, so the distinction of the more complex tabulate outcrop from its neighbours becomes less pronounced. Considering this, CTI_{S2} was considered the best configuration for the index, achieving a better balance between corrugations and vertical relief. Therefore, with $W_{NC} = W_{MVR} = 0.04 = 1/25$, the final configuration of the combined topography index according to eq. (4) becomes

$$\mathrm{CTI} = (1 - \mathrm{SR}) + \frac{\mathrm{NC}}{25} + \frac{\mathrm{MVR}}{25} \quad (\mathrm{MVR\ in\ metres}^{1})$$

It is also evident that no single index has an ideal response regarding the perceived complexity by itself, with the CHD method failing to quantify the complexity of the tabulate outcrop and the CTI approximating profiles 3 and 6, which have different configurations. This behaviour of the CTI, however, is because the relative weights of rugosity and vertical relief have been tuned to maximise total abundance (CTI_{S2} in table 3.2). In fact, the CTI gives a high value to profile 3, since larger caves and indentations can provide shelter to more fish and cave-dwelling species, and the large corrugation in profile 6 can also provide shelter for more juveniles and adults and therefore increase the carrying capacity of a habitat in terms of total abundance, while protecting the fish from predators and wave action (Henriques and Almada, 1998; García-Charton and Pérez-Ruzafa, 2001).

Table 3.4. Performance of the substrate rugosity index and the combined topography index in distance-based linear models (DISTLM), when modelling Bray-Curtis dissimilarity matrices of square-root transformed species densities. Indices were used alone in marginal tests and then introduced in a model with 3 previously fitted variables (depth, percentage cover of sand and percentage cover of cobble). Both the additional proportion of variation explained by fitting the indices and the cumulative variation explained by the whole model are shown. *P*-values are underlined when non-siginificant.

			Pseudo-F	P	Proportion	Prop. Model
Marginal tests	All species	SR	5.6916	0.0001	15.5%	–
		CTI	6.9317	0.0001	18.3%	–
	Cryptobenthic species	SR	7.9882	0.0001	20.5%	–
		CTI	10.351	0.0001	25.0%	–
Whole model	All species	SR	1.3268	<u>0.2111</u>	3.2%	33.1%
		CTI	2.4328	0.0144	5.6%	35.5%
	Cryptobenthic species	SR	2.163	<u>0.0639</u>	4.9%	36.3%
		CTI	3.7421	0.0074	8.1%	39.4%

SR- Substrate rugosity index, CTI- Combined topography index.

Although the linear correlation with assemblage parameters has been optimised, and taking into account oscillations due to seasonal patterns, this does not mean causality or good performance in a modelling context. For this purpose, distance-based linear models (DISTLM) were used to test the CTI and the SR index (table 3.4) in new, independent test sites covering a wide variety of conditions (table 3.1). This approach resulted in 2.8%

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[1] For units in feet, use CTI = (1−SR) + NC/25 + MVR/82.

(whole assemblage) and 4.5% (cryptobenthic only) more variation explained with the CTI, when compared to the SR in marginal tests. However, to better understand the explanatory capabilities of the topography indices in a context of intricate effects and interactions, they were fitted into a model after fitting three potentially confounding variables (depth and percent cover of sand and cobble). This way, by using type I (sequential) sums of squares, the effects of a new variable are calculated over and above the effects of the previously fitted variables (Anderson *et al.*, 2008). Fitting the CTI to this model achieved a significant gain in 5.6% more variation explained for the whole model using the complete assemblage and 8.1% using only cryptobenthic species, whereas the SR index had no significant additional effects. The main advantage is that this was achieved only through index configuration and not by adding dimensions to the model, something that is often undesirable, due to added complexity (Raudys and Jain, 1991).

This behaviour optimisation of the index was not intended as a tailor-made approach to fit our data, and care was taken to use independent data to test its performance. Instead, it was a way of looking at topography “through the eyes of fish”, since habitat classifications are highly dependent upon the organisms of interest and the existence of a universal measure is unlikely (Costello, 2009). Overall, the CTI showed promising results, at a time where there is a need to detect and act upon anthropogenic impacts to marine ecosystems, by isolating them from natural variation (Henriques *et al.*, 2008). Further developments should go into applying the index in other areas, especially in highly diverse tropical reefs, as well as optimising weight coefficients to other biological elements, such as sessile macroinvertebrates.

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Chapter 4

Topographic complexity and the power to detect structural and functional changes in temperate reef fish assemblages: The need for habitat-independent sample sizes.

Miguel Pessanha Pais, Sofia Henriques, Maria José Costa, Henrique N. Cabral.

Abstract

Functional approaches have shown promising results to detect degradation in marine fish assemblages. However, background variability significantly affects the amplitude of change that is detectable by a monitoring plan, and failing to detect such changes can have devastating consequences and carry aggravated recovery costs due to unnoticed degradation. The present study aimed to understand the relationship between topographic complexity in temperate reefs and the power to detect variations in fish-based metrics. Underwater visual census of fish assemblages was performed using strip transects and a Monte Carlo simulation approach was used to generate a large number of replicates and simulate three alternative hypotheses representing different magnitudes of change. Statistical power to detect differences between null and alternative hypotheses was estimated through 10,000 Mann-Whitney tests for numbers of replicates ranging from 2 to 15. Power tended to vary with topographic complexity particularly with small and medium changes in metric values and when using small sample sizes. While power increased with complexity for most metrics, some showed decreasing trends. With a large effect, 5−15 transects were needed, depending on the metric, to stabilise power above 0.80 independently of habitat features. A power of 0.95, however, could not be achieved for most metrics in all sites, even when using 15 transects. The observation that the power to detect degradation and recovery in temperate reef fish seems to vary with habitat features means that a monitoring programme that is correctly planned for a particular area may not be directly applicable in a nearby reef. Adding to the need to maximise power in monitoring, this study highlights the need to take into account habitat variability in these calculations and estimate habitat-independent sample sizes that are appropriate for the scale and location of interest.

4.1. Introduction

The increasing anthropogenic pressure on marine habitats has stressed the need to develop tools and methodologies to monitor and detect degradation in time to take appropriate measures. This is now a requirement of several international policies, as well as local management plans (*e.g.* Rogers and Greenaway, 2005; Borja *et al.*, 2010). In temperate climates, rocky reefs are known to support a great diversity of fish species by providing shelter and ideal conditions for feeding and reproduction (Almada *et al.*, 1999). However, fish assemblages in nearshore reefs are particularly vulnerable to anthropogenic pressure sources such as fishing, chemical and organic pollution and habitat loss, among others (*e.g.* McKinley and Johnston, 2010; Henriques *et al.*, 2013a). Due to the role of fish in the ecosystem, as well as their socioeconomic importance (Holmlund and Hammer, 1999), failure to detect and act upon anthropogenic impacts can have devastating consequences. This has led to the development of tools and indicators that aim to constitute short-term warnings of community degradation (Pérez-Dominguez *et al.*, 2012), and a move from single-species approaches to community-based assessments (Nicholson and Jennings, 2004). In addition, fish species can be assigned to functional guilds that have been shown to respond more predictably to stress, aiding in the distinction between natural and human-induced change (Elliott *et al.*, 2007). However, these tools and measures are often not linked to standardised sampling methods and the adequacy of the applied methodology to the location to be monitored or the issue to tackle is rarely assessed (de Jonge *et al.*, 2006).

When establishing a monitoring programme, or even when designing a field experiment to detect a particular effect, the main goal is to be able to detect a signal in a pool of background noise. In the case of fish assemblages, a signal can be a change in abundance of a particular species, an increase or decrease in species richness, or a change in abundance of a functional guild (*e.g.* García-Charton *et al.*, 2000; Guidetti *et al.*, 2002, 2003; Maxwell and Jennings, 2005; McKinley and Johnston, 2010; Henriques *et al.*, 2013a). Besides natural variation, sampling error also plays an important part in background noise. In underwater visual census, the most common sampling method for reef fish, this includes systematic errors due to rarity or low detectability, and random errors, mainly due to the mobility of fish around the sampling units (Samoilys and Carlos, 2000; Irigoyen *et al.*, 2013).

When detecting an impact on fish communities, the same principles of any statistical test must be applied, and the concepts of type I and type II errors must be brought into context, as well as their practical consequences (Peterman, 1990; Fairweather, 1991). In a monitoring context, the probability of committing a type I error (α) translates as the probability of detecting an impact where there is none, while the probability of a type II error (β) is the probability of failing to detect an impact that is occurring (Fairweather, 1991; Osenberg *et al.* 1994; Mapstone, 1995). This is an important aspect, since ecologists are often more concerned with α, while β is frequently neglected (Peterman, 1990). In a monitoring context, a type I error will lead to managers taking action where none is needed, with consequences for industries that constitute the main sources of impact. On the other hand, a type II error can be devastating for marine communities and the costs of recovery will be aggravated as a result of long periods of unnoticed degradation (Maxwell and Jennings, 2005). Therefore, failing to detect an impact carries a cost that is too high to ignore and, in the context of environmental monitoring, usually higher than the cost of wrongly detecting an impact (Fairweather, 1991; Mapstone, 1995).

Statistical significance, however, is not a synonym of biological relevance (Mapstone, 1995; Steidl and Thomas, 2001), and there is a need to define the magnitude of change that is relevant in a monitoring context. This magnitude can be defined as the minimum impact threshold that would require management actions and must therefore be detected by a monitoring plan (Mapstone, 1995; Munkittrick *et al.*, 2009). The link between statistical significance and biological relevance is in practice established by the complement of β, or the probability of detecting a true (biologically relevant) impact (Thomas and Juanes, 1996). In statistical terms, this is known as power (1−β), a probability that can be increased by increasing the number of replicates, reducing among-sample variability (*e.g.* increasing the size of each transect), increasing the minimum range of variation to be detected (effect size) or increasing the probability (α) of committing a type I error (Steidl and Thomas, 2001). Establishing a balance among all the variables that interact with power is the goal of statistical power analysis (Cohen, 1988), a procedure that has been highly recommended for fish community monitoring and management but also one that is still rarely used (Maxwell and Jennings, 2005).

In the assessment of reef fish assemblages through underwater visual census there is no agreement regarding the number of replicates or the size of each transect (*e.g.* Mosquera *et al.*, 2000). In fact, calculations of sampling effort are known to be not only survey-specific but also location-specific (Maxwell and Jennings, 2005). Several environmental features are known to affect fish assemblage characteristics in temperate reefs, particularly reef complexity, a feature whose effect has been thoroughly studied (*e.g.* García-Charton and Pérez-Ruzafa, 2001; Gratwicke and Speight, 2005; Pais *et al.*, 2013). However, the effect of habitat complexity on the variability of estimates has only been scarcely addressed in the literature (*e.g.* Mouillot *et al.* 1999; Cote *et al.* 2013) and, to the authors' knowledge, there is no study that directly relates habitat complexity with statistical power, particularly in light of the recent adoption of functional approaches, which are somewhat intuitively expected to reduce the sampling effort necessary to detect change (McClanahan *et al.* 2007).

The present study analysed the relationship between topographic complexity and the power to detect structural and functional changes in temperate reef fish assemblages along the Portuguese coast. The main goals were (1) to identify trends in the relationship of individual fish community metrics with topographic complexity and (2) to calculate the number of replicates needed to achieve enough power to detect meaningful changes. Fish assemblages were sampled through underwater visual census in 14 sites located along the Portuguese coast and power was estimated for several magnitudes of change and sample sizes. Different metrics behaved differently in relation to reef complexity, some with positive and some with negative trends. Increasing the number of replicates was shown to gradually flatten trendlines and stabilise power across habitats but the sampling effort required was usually large and only able to detect large changes in community structure. The results suggest that an increase in α is likely the best short term solution for more cost-effective monitoring and stress the need for pilot studies that allow calculation of habitat-independent sample sizes in the planning phase of monitoring programmes and ecological experiments.

4.2. Materials and methods

4.2.1. Study area

Rocky reefs located on the Portuguese coast (SW Europe) are typical of warm-temperate regions and are affected by summer upwelling (Almada *et al.*, 1999; Henriques *et al.*, 2007). With both Northern-Atlantic and Mediterranean influence, the area provides conditions for a relatively large number of fish species to occur, and thus it can be used to study the influence of local environmental factors on settlement and survival, ultimately determining the best potential assemblage supported by a given habitat. For this study, 14 sites were sampled along approximately 300 km in the centre and south coasts (figure 4.1), located at depths between 3 and 11 m and covering a wide range of environmental conditions and topographic complexity, from flat layers of rock to large blocks with caves and crevices. In order to minimise the effect of anthropogenic disturbance on the results, care was taken to avoid sites under direct influence of impact sources.

4.2.2. Fish assemblage surveys and topographic complexity

Sampling was performed during summer in order to optimise the potential of each site to support fish assemblages (Henriques *et al.*, 2013b). Data were collected through SCUBA-diving visual census during daytime and replicates within each site covered several tidal states in order to reflect local variability. In order to minimise underestimation of fish abundances, each replicate consisted of two passes, one directed to demersal species (50 m x 2 m) and another to cryptobenthic species (50 m x 1 m) (Cheal and Thompson, 1997; De Girolamo and Mazzoldi, 2001). The first pass was performed while unreeling a 50 m thin rope, with an average swimming speed of 8 m per minute, with cryptobenthic fish sampled in the opposite direction, while reeling the transect, by searching in crevices and under cobbles ≤20 cm in diameter (average speed of 3 m per minute). On cryptobenthic transects, the families Blenniidae, Bothidae, Batrachoididae, Callionymidae, Congridae, Gadidae (subfamilies Lotinae and Phycinae), Gobiesocidae, Gobiidae, Muraenidae, Scorpaenidae, Scophthalmidae, Soleidae, Syngnathidae and Tripterygiidae and the species *Ctenolabrus rupestris* and *Labrus mixtus* were counted. Due to ontogenic variations in behaviour, fish smaller than 5 cm (total length) from the genus *Symphodus* were also counted on cryptobenthic transects. All others were counted on demersal transects. For each replicate, both passes were merged by standardising abundance per square metre of transect.

A total of six transects were performed per site by two experienced observers (three by each observer), starting each time at a random point and allocated to pre-determined depth intervals (3−6m and 8−11m), according to each site’s characteristics. The adopted method aimed to minimise sampling error and was the result of a short pilot study where among-observer estimates were calibrated, transect length and orientation was chosen and species were assigned to demersal and cryptobenthic transects (Henriques *et al.*, 2013a). In order to quantify topographic complexity, the “chain and tape” method was applied using a 25 m long leaded rope and the combined topography index (CTI) was calculated (see Pais *et al.*, 2013 for details). Three replicates of the method were performed and CTI values were averaged to represent topographic complexity at each site (table 4.1).

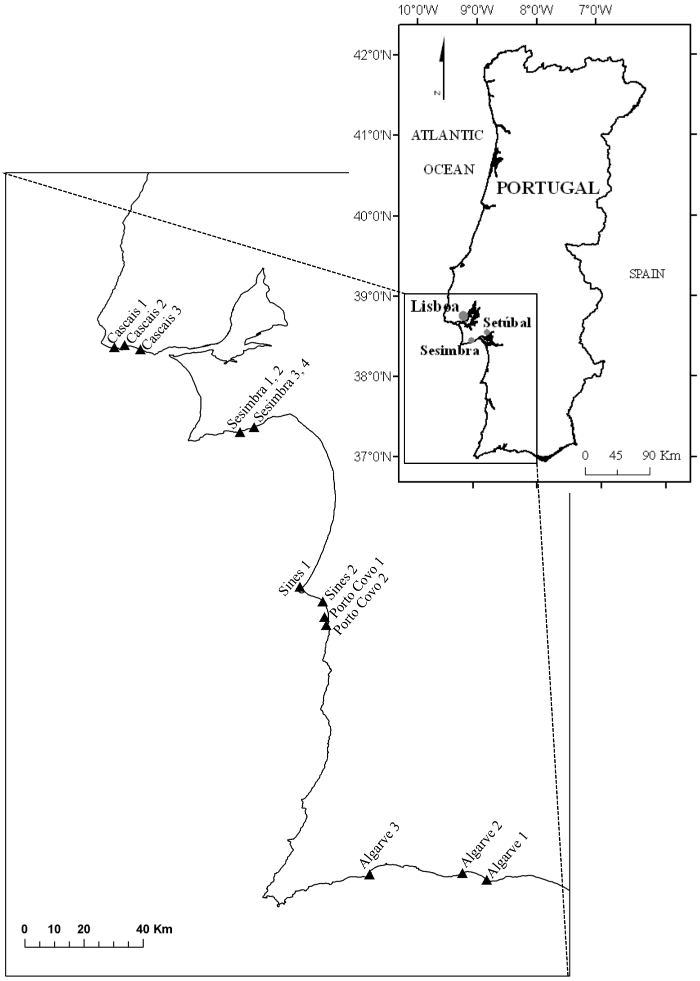

Figure 4.1. Study sites, located in nearshore rocky reefs along the Portuguese coast.

4.2.3. Fish-based metrics

Apart from the usual measures of species richness and total density, eight community metrics, which have been shown to vary in the face of anthropogenic and environmentally-induced disturbances in the study area, were selected (Henriques *et al.*, 2013a): Species with tropical affinity have known distributions from West Africa to southern Iberian Peninsula (Henriques *et al.*, 2007); Density of opportunists represents a single species, *Coris julis* (Linnaeus, 1758), known to occur in almost every site, and to thrive even in the presence of moderate contamination (Henriques *et al.*, 2013a,b); Density of territorial individuals represents species that actively defend territories (usually holes or crevices) at least during a period in their lifecycle; Target species are the ones that have high commercial value or represent an important part of the local fishing industry. The density of trophic guilds was also assessed, namely invertebrate feeders (feed mostly on non-planktonic invertebrates), omnivores (opportunistic feeders and detritivores), herbivores (feed on benthic and planktonic micro and macroalgae and macrophytes), and macrocarnivores (feed mostly on macroinvertebrates and fish). Species were classified into guilds based on previous studies (Henriques *et al.*, 2007; Halpern and Floeter, 2008; Henriques *et al.*, 2008, 2013a), FishBase online database (Froese and Pauly, 2012) and the authors' field observations.

Table 4.1. Mean values for the combined topography index (CTI), depth and all fish-based metrics for the studied sites. Sites are ordered according to topographic complexity. See figure 4.1 for site locations.

Site	Average CTI	Average depth (m)	Species richness	Total density	Opportunists	Territorial	Target species	Tropical affinity	Invertebrate feeders	Omnivores	Herbivores	Macrocarnivores
Algarve 3	0.120	3.3	10	1.000	0.038	0.667	0.142	0.685	0.157	0.755	0.083	0.005
Cascais 3	0.259	6.6	13	1.035	0.103	0.260	0.180	0.140	0.602	0.428	–	0.005
Cascais 2	0.370	4.0	16	1.960	0.100	0.357	0.395	0.133	1.205	0.687	–	0.068
Algarve 2	0.390	4.5	12	1.073	–	0.343	0.285	0.375	0.113	0.953	–	0.007
Sines 2	0.402	8.4	12	0.900	0.040	0.393	0.065	0.263	0.478	0.393	0.012	0.003
Algarve 1	0.404	3.1	11	0.640	0.007	0.307	0.108	0.415	0.085	0.552	–	0.003
Porto Covo 2	0.498	9.5	11	0.817	0.043	0.270	0.127	0.182	0.317	0.367	0.010	–
Sines 1	0.518	10.1	12	1.380	0.317	0.313	0.227	0.287	0.638	0.577	–	0.015
Sesimbra 2	0.524	9.3	18	2.442	0.227	0.847	0.578	0.318	1.042	1.185	0.018	0.193
Sesimbra 3	0.560	4.9	18	4.543	0.173	1.880	0.623	1.150	1.907	1.978	0.638	0.020
Sesimbra 1	0.566	5.3	18	2.668	0.217	0.810	0.703	0.675	1.198	1.140	0.283	0.047
Porto Covo 1	0.586	11.7	12	1.782	0.117	0.180	0.300	0.233	0.542	0.552	0.002	0.003
Sesimbra 4	0.594	9.1	14	7.177	0.182	1.883	0.253	0.317	5.450	1.707	0.002	0.018
Cascais 1	0.670	8.6	13	2.830	0.150	0.323	0.208	0.275	1.993	0.742	–	0.020

4.2.4. Calculation of statistical power

Since fish abundance estimates are not guaranteed to comply with assumptions for parametric tests and non-parametric approaches are now widely used in fish ecology studies (*e.g.* Anderson and Millar, 2004), a non-parametric approach to power analysis was followed. In order to simulate variability and generate a probability distribution for every metric, Monte Carlo simulations were used based on the abundance of individual species, using the histogram obtained from field data as a probability distribution. This procedure assumes that species can be independently shuffled among replicates within the same site to simulate among-transect variability. In some particular cases, however, specific habitat differences can have an effect on some species and thus their occurrence cannot be considered independent within the same transect. This was the case for *Callionymus lyra*, *Callionymus reticulatus*, *Ctenolabrus rupestris*, *Gobius xanthocephalus* and *Tripterygion delaisi*. Some pairs of these species had correlated abundances across all sites (r>0.6), and are known to be linked with particular habitat features: *Callionymus* spp. are only found in sand deposits, *G. xanthocephalus* thrive in rock/sand boundary areas and *C. rupestris* and *T. delaisi* are associated with large blocks and crevices (M.P. Pais, pers. obs.). For this reason, these species were simulated separately using the Iman-Conover method, which retains the correlation among abundance values in the simulations (Iman and Conover, 1982), and were then merged with the remaining species. A total of 100,000 transects were simulated per site, and all fish-based metrics were calculated for each individual transect.

In order to calculate power for every number of transects between 2 and 15, an adaptation of the method proposed by Thomas and Juanes (1996) for non-parametric tests was applied. For every metric in every site, three alternative hypotheses (H_A) were simulated: $H_{S,}$ H_M and H_L (small, medium and large effect sizes, respectively). The distribution of values for these hypotheses was generated from the 100,000 values simulated from field data (representing H_0), by subtracting a percentage of the mean of H_0 to all values. This percentage is known as the relative effect size (Steidl and Thomas, 2001). For example, in the case of a small effect size in metric *m*, on site *i*, $H_S = H_0 – \mu H_0 \times ES_S$, where H_S are the 100,000 transects representing the alternative hypothesis for a small effect size, H_0 are the 100,000 transects simulated from field data for site *i*, μH_0 is the mean value for metric *m* calculated from all 100,000 transects in H_0 and ES_S is the relative effect size representing a small variation in metric *m*. Values for the relative effect sizes in table 4.2 were established by looking at average differences between impacted and control sites in the study area (Henriques *et al.*, 2013a), as well as differences found in other studies covering a variety of metrics and impact sources (*e.g.* Harmelin *et al.*, 1995; Wantiez *et al.*, 1997; García-Charton *et al.*, 2000; Guidetti *et al.*, 2002, 2003; McKinley and Johnston, 2010).

For every number of transects from 2 to 15, power was calculated by taking *n* transects, without replacement, from the pool of 100,000 transects representing H_0, repeating the same for the pool of transects representing H_A, and performing a two-tailed Mann-Whitney U test. This was done 10,000 times for each number of transects and the proportion of tests (out of 10,000) rejecting the null hypothesis (for α=0.05) translate as the statistical power of the test (Thomas and Juanes, 1996). The relationship between topographic complexity and power was then visually analysed through scatterplots and trendlines. Additionally, the minimum and maximum number of transects, among all sites, that are necessary to detect a given effect with powers of 0.95 ($\beta=\alpha$) and 0.8 ($\beta=0.2$) were calculated. All simulations were performed using Statistica 11 software (www.statsoft.com). Sampling and Mann-Whitney tests were performed in R version 2.13.0 (R Development Core Team, 2011).

Table 4.2. Relative effect sizes adopted for each fish-based metric to represent three alternative hypotheses, corresponding to small, medium and large changes in fish assemblages.

	Relative effect size (%)		
Metric	Small	Medium	Large
Species richness	10	25	50
Total density	20	50	90
Opportunists	20	50	90
Territorial	20	50	90
High commercial value	30	60	100
Tropical affinity	10	50	100
Invertebrate feeders	20	40	80
Omnivores	20	40	80
Herbivores	20	40	80
Macrocarnivores	20	40	80

In order to assess if sites with larger mean values for the null hypothesis were artificially increasing power due to the calculation of relative effect sizes, Pearson correlation coefficients were calculated among absolute effect sizes at each site and the achieved power for 10 transects with a medium effect size. Correlations were weak and non-significant for all metrics, except for species richness, where power slightly increased with absolute effect size ($r=0.68$; $p<0.05$).

4.3. Results

Scatterplots in figure 4.2 show the relationship between topographic complexity (measured by the CTI) and the power to detect small, medium and large variations in metric values with an increasing number of replicates. Even when using 15 transects, the power to detect a small effect was low for almost every metric. However, in areas of higher topographic complexity, the power to detect changes was higher for 4 out of 10 metrics, namely species richness, opportunists, target species and invertebrate feeders. On the other hand, a decreasing trend was found for the density of macrocarnivores.

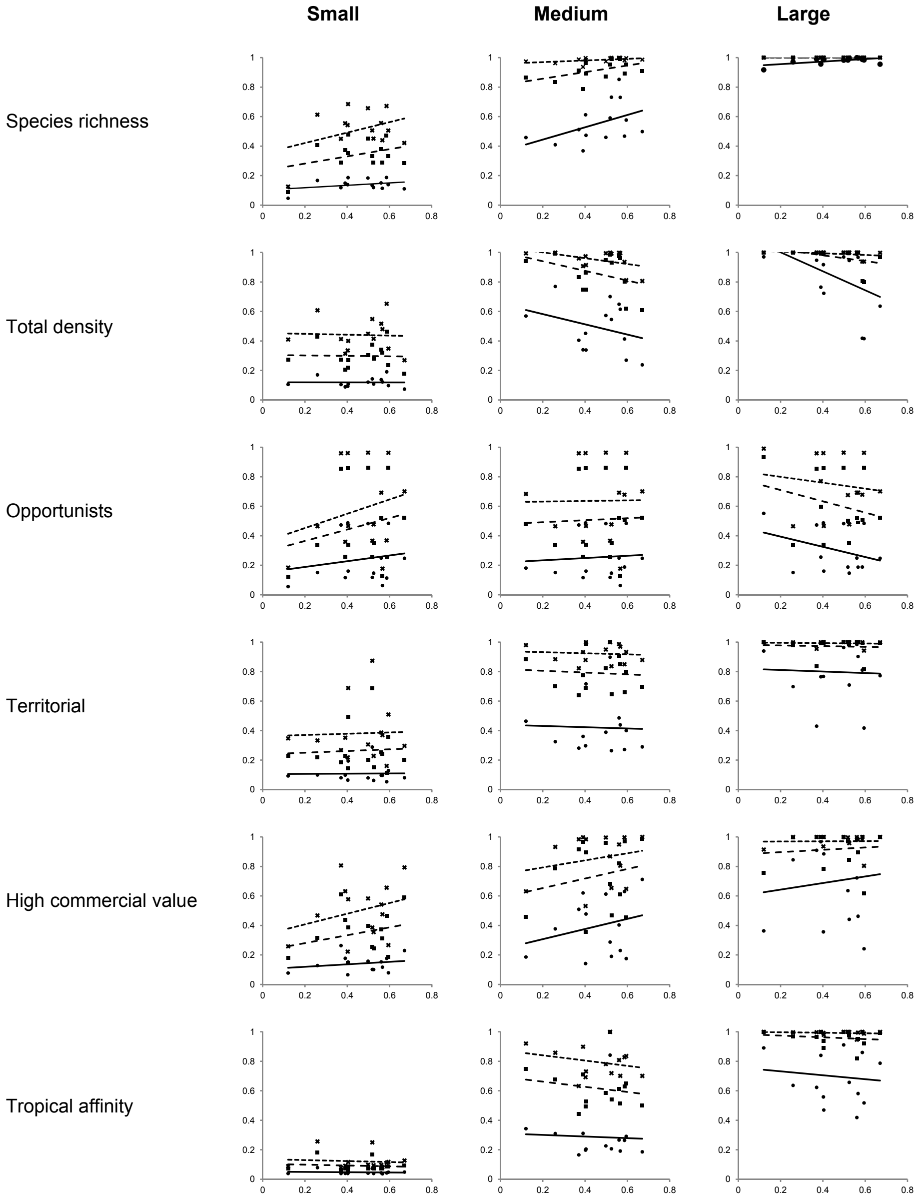

Figure 4.2. Scatterplots and trendlines showing the correlation between topographic complexity (horizontal axis) and power (vertical axis) to detect small, medium and large effect sizes with three levels of sampling effort. Circles and solid lines correspond to 5 replicates, squares and long-dashed lines correspond to 10 replicates and crosses and short-dashed lines correspond to 15 replicates.

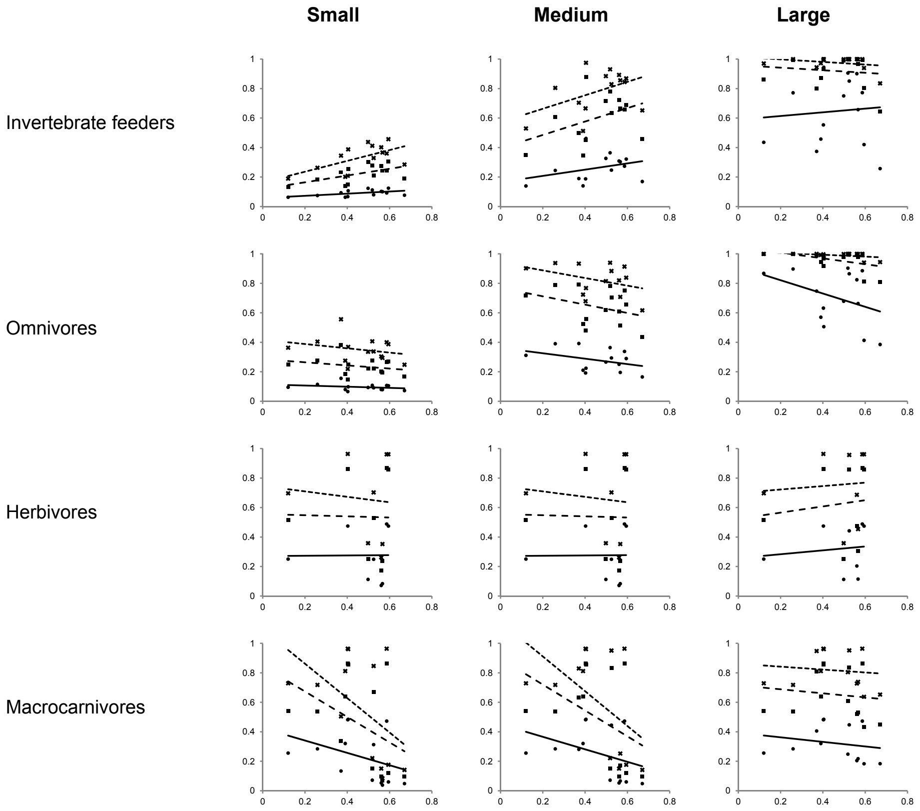

Figure 4.2 (cont.).

With a medium effect size, variations in some metrics were easier to detect but only when using 15 replicates. Only with species richness were 10 transects enough to achieve high power. For a change of this magnitude, power tended to increase with topography when measuring species richness, target species and invertebrate feeders, while total density, omnivores and macrocarnivores showed a decreasing trend (figure 4.2). Finally, with a large effect size, 15 transects were enough to stabilise trendlines at high power in most metrics. The only exceptions were the three metrics that have shown the poorest results overall, namely the density of opportunists, herbivores and macrocarnivores, which showed highly variable results, irrespective of effect size and topography. With 5 transects, however, while the achieved power can be acceptable in some cases, variation with topography is still visible, particularly an increase in power for the density of target species and invertebrate feeders and a decrease in power for total density and the density of omnivores. For species richness, however, 5 transects were enough to stabilise power at high levels for all sites.

Tables 4.3 and 4.4 show the percentage of sites where a given level of power was achieved and the minimum and maximum number of replicates required to achieve it. These results show that for a power of 0.8 (β = 0.2), achieving a habitat-independent sample size (i.e. number of replicates that achieve the 0.8 power threshold across all sites) is possible for almost all metrics (5−15 range) but only when dealing with large effect sizes (table 4.3).

Table 4.3. Minimum (min) and maximum (max) number of replicates, across all sites, needed to achieve a power of 0.8 for each effect size (ES). The maximum number of replicates corresponds to a habitat-independent sample size whenever this level of power was achievable for 100% of sites.

	Small ES			Medium ES			Large ES		
	% sites	min	max	% sites	min	max	% sites	min	max
Species richness	0	−	−	100	5	11	100	4	5
Total density	0	−	−	100	6	15	100	4	10
Opportunists*	31	8	8	31	8	8	38	7	8
Territorial	7	13	13	100	5	14	100	4	10
High commercial value	7	15	15	71	6	15	100	4	15
Tropical affinity	0	−	−	50	5	15	100	4	10
Invertebrate feeders	0	−	−	64	9	15	100	4	14
Omnivores	0	−	−	64	11	15	100	5	10
Herbivores†	38	8	8	38	8	8	50	8	9
Macrocarnivores*	38	8	15	46	8	15	54	8	15

* Calculated for a total of 13 sites.
† Calculated for a total of 8 sites.

Table 4.4. Minimum (min) and maximum (max) number of replicates, across all sites, needed to achieve a power of 0.95 for each effect size (ES). The maximum number of replicates corresponds to a habitat-independent sample size whenever this level of power was achievable for 100% of sites.

	Small ES			Medium ES			Large ES		
	% sites	min	max	% sites	min	max	% sites	min	max
Species richness	0	−	−	93	6	14	100	4	6
Total density	7	13	13	64	8	14	86	4	13
Opportunists*	31	13	14	31	13	14	38	11	14
Territorial	0	−	−	43	6	14	86	4	15
High commercial value	0	−	−	50	8	15	79	4	15
Tropical affinity	0	−	−	7	7	7	100	4	15
Invertebrate feeders	0	−	−	7	13	13	79	5	13
Omnivores	0	−	−	0	−	−	86	6	11
Herbivores†	38	13	14	38	13	14	50	13	14
Macrocarnivores*	23	13	13	31	13	14	38	13	15

* Calculated for a total of 13 sites.
† Calculated for a total of 8 sites.

With medium effect sizes, habitat-independent sample sizes were found only for species richness, total density and the density of territorial species (11−15 range). For a power of 0.95 (β = α), results were generally poor (table 4.4), with habitat-independent sample sizes being achievable only with large changes in species richness (6) and the density of species with tropical affinity (15). However, 15 replicates were sufficient to achieve a power of 0.95 in 86% of sites when using total density and the density of territorial and omnivore species. As seen in figure 4.2, the density of opportunists, herbivores and macrocarnivores had the lowest values for power, and thus a power of 0.8 was not attainable on more than 50% of sites even with large effect sizes. For these metrics, although a power of 0.95 could be achieved for a small effect size in a small percentage of sites (table 4.4), this did not improve with increased effect sizes (although the number of replicates needed to achieve the same power decreased).

4.4. Discussion

The present study analysed the statistical power to detect changes in fish-based metrics across different reefs on the Portuguese coast, focusing on the correlation between power and topographic complexity. Building on the recommendations of previous studies (*e.g.* Peterman, 1990; Fairweather, 1991; García-Charton *et al.*, 2000; Maxwell and Jennings, 2005), our results reiterate the need for power analysis on the planning phase of any monitoring programme or ecological experiment. For parametric tests, an estimation of power is relatively straightforward to obtain based on values for α, variance and expected effect size (Cohen, 1988). Thus, even a simple exercise based on previous studies can help establish a more suited sampling effort. However, ecological data are known to often fail to fulfil assumptions for parametric tests (Olden *et al.*, 2008), and fish ecology studies often resort to non-parametric or semi-parametric alternatives that do not rely on assumptions regarding probability distributions (Anderson and Millar, 2004). For non-parametric approaches, there is considerably less information available on the estimation of power, and not even Cohen (1988) mentions power calculations for such tests, which may be part of the reason why so many experiments and monitoring programmes are ignoring β. The method used on the present study follows the suggestions of more recent studies that mention non-parametric power (Thomas and Juanes, 1996; Steidl and Thomas, 2001; Mumby, 2002) and shows that it is possible to calculate power for non-parametric tests with relative ease by relying on fast computer processors available nowadays. Nonetheless, these simulation methods carry a few disadvantages in relation to parametric approaches. The first is related with the need for probability distributions in Monte Carlo methods (Metropolis and Ulam, 1949). While other methods of estimating variability can be used that do not split original sampling units (*e.g.* bootstrap), the simulation methods used can generate more realistic outputs, as they allow species to freely shuffle among replicates. However, each individual species must follow a particular probability distribution to reflect the original data, and the lack of evidence to go with a particular theoretical distribution may be the reason one chooses to go non-parametric in the first place. In this study, the histograms generated from the original six replicates were used as a basis for simulations. This, however, is assuming that fish abundance is a discrete variable and thus, what is referred to as “density” is in fact abundance (discrete) relative to a fixed sampling area. With other methods, such as timed transects or random paths (Harvey *et al.*, 2004), and whenever area has to be considered a variable, density must be simulated based on continuous probability distributions and hence some parameterisation will be needed. The second issue of such approaches is the need for field data to be taken on the same conditions that will be found during the intended experiments or monitoring surveys. This is ideally achieved through a pilot study, although previous data may be usable in case of monitoring programmes already underway (Mumby, 2002). These data are crucial to simulate the expected shape of the probability distribution of each variable, in cases where it is difficult to fit a known theoretical distribution to the data.

While this study focused on the power to detect changes in structural and functional fish assemblage metrics, it is very important that simulations are done based on species abundances, and not directly on metric values calculated from the original replicates. This estimates variability more accurately by incorporating the effect of functional redundancy on the final metric values, one of the reasons functional guilds are more stable in the face of natural variation, as the absence of one species is balanced by another species of the same guild (Nicholson and Jennings, 2004; Pais *et al.*, 2012).

The underwater visual census method applied aimed to be the best possible method to minimise sampling error at the studied sites. While among transect variability could in theory be reduced by increasing the area covered by each transect (Irigoyen *et al.*, 2013), a length of 50 m was found, based on the authors’ previous experience, to be the maximum achievable in at least some of the reefs studied. Significantly increasing this distance would lead to transects crossing the reef borders into sandy substrate or increase the probability of two replicates falling on the exact same area. The width of demersal transects (2 m) was established based on the available underwater visibility at the most turbid locations (often 3−4 m), particularly those under the influence of estuarine plumes, so that the effects of differences in visibility on abundance estimates were minimised. For cryptobenthic transects, however, a width of 1 m was used, since actively searching inside crevices and under cobbles significantly increased sampling time and required observers to focus on a narrower strip (Cheal and Thompson, 1997), in order to count cryptic fish that would occasionally flee from their shelters. This thorough search, as described by Beldade and Gonçalves (2007), significantly reduces the underestimation of cryptic species and, consequently, minimises the associated sampling errors. When both passes (demersal and cryptobenthic) were merged into one replicate and abundance was standardised per unit area, this implies that each fish counted on cryptobenthic passes (50 m^2) has twice the weight of a fish counted on demersal passes (100 m^2), although each species is assigned to only one of them. Nevertheless, the eventual errors induced by this linear conversion most likely outweigh the advantages of thoroughly searching a narrower area (Cheal and Thompson, 1997).

The effect sizes considered were relative to the estimated mean for each metric on each site, which means that a “large” effect size is different in any two sites, in absolute terms. However, since different sites have different potential assemblages they can support, setting an absolute value for the expected magnitude of change would generate unrealistic outcomes, and thus a relative effect size will more accurately reflect the amount of change required, standardised by the site’s potential. In addition, the inexistence of correlations among absolute effect sizes and the achieved power leads to the perception that this was not the main driving force for the obtained results. Relative effect sizes have the additional advantage of being easier to obtain in published literature. For species richness, changes smaller than 10% were found in response to sewage and nutrient run-offs, (McKinley and Johnston, 2010), decreases of 25−30% were found for sewage discharges and industrial effluents (Guidetti *et al.*, 2002; McKinley and Johnston, 2010) and 50−60% changes were found in response to nutrient run-offs, fish farms, industrial effluents (McKinley and Johnston, 2010) and marine protected areas (MPA; Wantiez *et al.*, 1997). Changes in total abundance of about 25% were found in MPA (Harmelin *et al.*, 1995), while sewage discharges have led to changes of 40% (McKinley and Johnston, 2010). Fish farms led to changes of around 100% in total abundance (McKinley and Johnston, 2010) and much larger changes, up to 6-fold, have been observed in response to sewage and MPA (García-Charton *et al.*, 2000; Guidetti *et al.*, 2002, 2003).

Our results have shown that, overall, power tends to vary with topographic complexity (and likely habitat features in general) whenever sampling effort is relatively low or effect sizes are small. In fact, when sample sizes and effect sizes are fixed, power will be mainly affected by the inherent variability of abundance estimates (Maxwell and Jennings, 2005) which, in turn, are known to be affected by habitat features (Irigoyen *et al.*, 2013). It is important to stress, however, that the relationship among power and topography is not being treated as causal and many factors may be influencing power. Nevertheless, reef complexity is known to be one of the main features responsible for changes in reef communities, as well as being relatively easy to measure prior to monitoring (García-Charton *et al.*, 2000).

The power to detect changes in species richness was seen to increase with topography. Although some correlation between absolute effect sizes and power were found for this metric, the main cause for such results is probably the fact that that the number of species tends to be more stable across replicates on more complex reefs, given that increasing sample size quickly flattened trendlines. This is probably due to the fact that these habitats provide ideal conditions for foraging, shelter and reproduction, thus favouring the permanence of species (Almada *et al.*, 1999; Gratwicke and Speight, 2005). In flatter reefs, the occasional occurrence of mobile species is more frequent, and thus the number of species counted within a sampling unit is prone to vary (McClanahan *et al.*, 2007; Cote et al, 2013).

Species that are more abundant tend to generate more stable estimates than rarer species, mainly due to higher probability of capture or detection (Mouillot *et al.*, 1999; Maxwell and Jennings, 2005). This was found for most functional guilds but for total density, however, the larger numbers found in more complex reefs (Pais *et al.*, 2013) also tended to be more variable, leading to a reduction in power with complexity for small sample sizes. In fact, some of the main species responsible for increased abundances in more complex reefs are gregarious species such as Sparids, who find shelter under large crevices and near blocks, as well as juveniles from other families, such as Gobiids that gather in small schools prior to settlement (Beldade *et al.*, 2006). This increases the variability of estimates, as the difference between counting or missing a school can be in the order of tens of individuals and small sample sizes can lead to increased error. This, however, was easily minimised by increasing the number of replicates. On the other hand, increasing the sample size was not enough to achieve acceptable power, even with large effects, for opportunist species, herbivores and macrocarnivores. Opportunists and herbivores are represented by a single species each, namely *Coris julis* and *Sarpa salpa* (Linnaeus, 1758), respectively. Therefore, their variation is not sustained by functional redundancy. While *C. julis* was consistently abundant at a few sites, it was much less abundant at other sites and highly variable in terms of occurrence. The fact that some distributional patchiness is observed in this species contributes to low power to detect changes in most sites. *Sarpa salpa* was not observed in 6 out of 14 sites, and was relatively rare in all but two sites. However, even when abundant its gregarious behaviour and wide home range can lead to great variability among replicates (Henriques *et al.*, 2013b). This is also the case for macrocarnivores, a guild that includes several species which are relatively rare, cryptic and solitary (*e.g.* moray eels, scorpionfishes). These three metrics share the same issue of high noise, from which it is difficult to extract a signal, particularly one that will be often small in amplitude. While they have shown a tendency to respond to impacts in the study area (Henriques *et al.*, 2013a), the lack of rejection of a null hypothesis must not be taken as a guarantee that these guilds are not being impacted. Guidetti *et al.* (2002), for example, have not been able to find significant differences in the abundance of *S. salpa* at a sewage outfall, although they were seemingly far less abundant than at control sites.

This study considered the conventional case where α=0.05 as a way of understanding the main issues posed by such an assumption. However, by establishing a β higher than 0.05 (power < 0.95), we are implying that the cost of a type II error is lower than the cost of a type I error, which is known to be untrue (Mapstone, 1995). Even a power of 0.8, considered acceptable for social sciences by Cohen (1988) and attainable for almost every metric in the present study, assumes that a type I error is four times more costly than a type II error. This can be a problem, considering that 15 transects per site, the maximum considered in the present study, is already a very large effort for the usual budget in any monitoring study. In fact, many studies addressing the capacity of existing monitoring programmes to detect changes have found them to be insufficient (*e.g.* Nicholson and Jennings, 2004; Maxwell and Jennings, 2005; Gamito *et al.*, 2012).

Given these issues, it has been suggested that the most immediate and low-cost solution available to increase power is simply to increase α (Peterman, 1990; Osenberg *et al.*, 1994; Mapstone, 1995; Maxwell and Jennings, 2005). This will act as a buffer to prevent the required sample sizes from escalating, while increasing the probability α of detecting an impact or recovery that did not occur. If setting this probability as high as 20% can be accepted by stakeholders, achieving a power of 80% (β=α) or even 90% (β<α) will be easier and more adequate in a monitoring context (Mapstone, 1995).

As a conclusion, this study stresses the need to consider habitat variability in the planning phase of every monitoring programme or ecological experiment. If different reefs are to be monitored, they all should be surveyed with a standardised method, using habitat-independent sample sizes. This concept is easy to understand but can be difficult (and costly) to calculate. Therefore, we suggest that, in the absence of initial funding for a pilot study, the required sample sizes should be roughly estimated based on parametric approaches, with parameters taken from available literature, preferably from sites with low complexity. After a first period of monitoring, the data gathered can be used to estimate variability through simulations and re-calculate the minimum effort needed to achieve pre-determined values for α and β for all sites and metrics. This can restrict future comparisons with the first period of monitoring but the advantages will likely compensate for the loss.

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Chapter 5

Seeking functional homogeneity: A framework for definition and classification of fish assemblage types to support assessment tools on temperate reefs.

Miguel Pessanha Pais, Sofia Henriques, Marisa I. Batista, Maria José Costa, Henrique N. Cabral

Abstract

Due to their important role in the ecosystem and high economic value, there is a need to assess the effect of anthropogenic impacts on marine fish assemblages. However, this can only be achieved if variations due to natural causes are known. Moreover, while most assessment tools rely on functional traits, bottom-up habitat classification frameworks tend to use species composition. The present study proposes an innovative framework to define fish assemblage types through metric pairwise constrained k-means (MPCK-means) clustering of sites based on functional guild categories and univariate metrics, an approach that takes into account within-site variability due to the sampling method and natural causes. This was followed by a label-based ensemble clustering approach, which finds patterns that minimise information loss when integrating clustering results from individual metrics. In order to test the method, fish assemblages on 14 nearshore rocky reefs along the Portuguese coast were sampled. The final typology configuration achieved through ensemble clustering consisted of three assemblage types and maintained average normalised mutual information of 0.605 with the individual clustering results. Nested PERMANOVA found differences among types and the most variable metrics in the face of natural variation were identified. Ultimately, a k-nearest neighbours classifier was optimised to label new sites, based only on environmental variables that are unlikely to be directly affected by the presence of anthropogenic impacts. Optimal performance for the classification model was achieved with inverse distance-weighted voting of the 4 nearest neighbours with an average classification accuracy of 96.08%.

5.1. Introduction

Increasing pressure on the marine environment has led to several policies stressing the need to improve the state of marine ecosystems in the near future and ensure the sustainable use of resources, such as the European Marine Strategy Framework Directive (EC, 2008). Besides the alarming pressure of the fishing industry (Worm *et al.*, 2006), marine fish assemblages, particularly in nearshore rocky reefs, are affected by many other pressure sources (Henriques *et al.*, 2013a). Being in many aspects a highly valued resource (Holmlund and Hammer, 1999), it has become urgent to develop and apply methods that can enable scientists and managers to detect and act upon the sources of pressure affecting fish assemblages.

However, locations with long-term monitoring programmes that enable the comparison of conditions before and after the presence of a particular pressure source are the exception rather than the rule (Borja *et al.*, 2012), and there is a need to develop tools that can signal managers when a fish assemblage has been or is being affected by human activities, without knowledge of the previous state of the system. For this purpose, functional guild approaches have been successfully used in streams and estuaries (Roset *et al.*, 2007; Pérez-Dominguez *et al.*, 2012), not only because they have a broader geographical application but also because the response of functional guilds to pressure sources can be more predictable and easy to interpret than that of individual species (Elliott *et al.*, 2007). However, changes due to anthropogenic pressures can only be detected when the range of variation due to natural causes is known (Osenberg et al., 1994; García-Charton and Pérez-Ruzafa, 2001).

There are several ways to minimise the effects of habitat in environmental monitoring, which usually involve the establishment of either type-specific or site-specific reference values that represent an ideal situation in the absence of impact or a goal for a given management programme (Borja *et al.*, 2012). In site-specific approaches, each site has its own reference, which may be theoretical (*e.g.* modelled or historical data) or a direct comparison with one or more control sites with similar characteristics. On the other hand, type-specific approaches begin by establishing habitat types that share certain environmental characteristics and are assumed to support the same potential communities in the absence of impact. The delimitation of habitat types is usually achieved by either a bottom-up approach, where the communities themselves are divided into clusters with similar species composition, or a top-down approach, where sites with similar environmental features are grouped based on quantitative or qualitative criteria (Maxwell and Buddemeier, 2002).

While site-specific approaches allow for greater detail and precision, they are highly impractical at larger scales, thus the definition of habitat types is the most frequent method to support national monitoring programmes and international policy requirements (Borja *et al.*, 2012). For this purpose, many national and international habitat classification frameworks have been established (Costello, 2009). However, the concept of "habitat" varies not only according to scale but also according to the organisms in question, so top-down approaches may be useful for administrative purposes but are not guaranteed to delimit homogeneous communities for all organisms at the scale needed for a particular management objective (Costello, 2009). Moreover, habitat classification frameworks that use variables such as algal cover and the diversity of sessile fauna to classify sites at smaller scales are of little use in a monitoring context because these variables are also affected by impact sources (*e.g.* Arévalo *et al.*, 2007) and thus site classification would be biased due to an already altered system.

There is still a discrepancy between species-based classification tools and guild-based assessment tools (Pais *et al.*, 2012; Henriques *et al.*, 2013a). This is an important issue, since functional guilds are more resilient to natural variation, as species are replaced by others from the same guild. This leads to areas with homogeneous guild abundance values tending to be larger than areas with homogeneous species composition (Pais *et al.*, 2012), which is a desirable characteristic of a management-oriented habitat typology, as a large number of types can be impractical or even impossible for medium to large scale monitoring (Johnson *et al.*, 2012). Moreover, unlike species that are either present or absent, the choice of functional guilds is arbitrary and can ultimately depend on management objectives, the expected response to impact sources, among other criteria (Elliott *et al.*, 2007; Henriques *et al.*, 2008, 2013a,b).

In the study of fish-habitat relationships, there is a long history of application of statistical methods that rely on assumptions regarding independence, linearity of responses or probability distributions (Knudby *et al.*, 2010). In fact, ecological data is known to rarely satisfy such conditions (Olden *et al.*, 2008) and fish species and guilds have been shown to have complex, non-linear responses to habitat variables (Friedlander and Parrish, 1998). All these constraints call for non-parametric methods that can deal with complex interactions, non-linearity and unusual distributions. Complex statistical tools that can find patterns and perform predictions based on empirical data have been developed in the field of artificial intelligence and experienced a huge progress in the last decade (Olden *et al.*, 2008). These tools are known as machine learning (ML) techniques and rely on algorithms that are designed to deal with classic statistical problems, such as regression, clustering and classification, by interpreting complex (and often large) databases without having to comply with assumptions and yet outperforming classic procedures (Crisci *et al.*, 2012).

Due to the potential of ML algorithms for interpreting patterns in ecological data, their use is steadily increasing. Nevertheless, when compared to other fields, ML applications in ecology are still at an embryonic stage, probably due to a language barrier between ecologists and computer experts (Olden *et al.*, 2008), aggravated by the fact that some complex models may need very large datasets (Raudys and Jain, 1991) that are often nonexistent in ecology. Despite this, some techniques have shown promising results with ecological data (*e.g.* Crisci *et al.*, 2012).

In the present study, machine learning algorithms are combined with permutation-based statistical tests to propose a bottom-up approach for the delimitation of reef fish assemblage types based on structural and functional metrics. Additionally, a quantitative model for the classification of new sites according to the established types is tested, by relying on a set of environmental variables that are not affected by most impact sources. During the process, the behaviour of several fish-based metrics in the face of natural variation is also assessed.

5.2. Materials and methods

5.2.1. Study area

In order to delimit assemblage types that reflect the potential assemblage characteristics associated with environmental variables, an effort was made to select sites without direct influence of impact sources in order to minimise their influence. A total of 14 sites covering a wide array of environmental conditions on nearshore temperate reefs were selected along a 300 km stretch of the Portuguese coast (figure 5.1). In order to optimise their potential to support fish assemblages, surveys were performed during summer, near the spawning season for many species (Henriques *et al.*, 2013b).

5.2.2. Fish sampling method

Fish assemblages were sampled during daytime using SCUBA diving underwater visual census along 50 m strip transects. Each transect was travelled twice, with a first pass for demersal species (50 m x 2 m) and a second for cryptobenthic species (50 m x 1 m). A 50 m long thin rope was deployed while sampling demersal species, with cryptobenthic fish sampled while reeling the rope, by searching in crevices and under cobbles ≤ 20 cm in diameter (Henriques *et al.*, 2013a; Pais *et al.*, 2013).

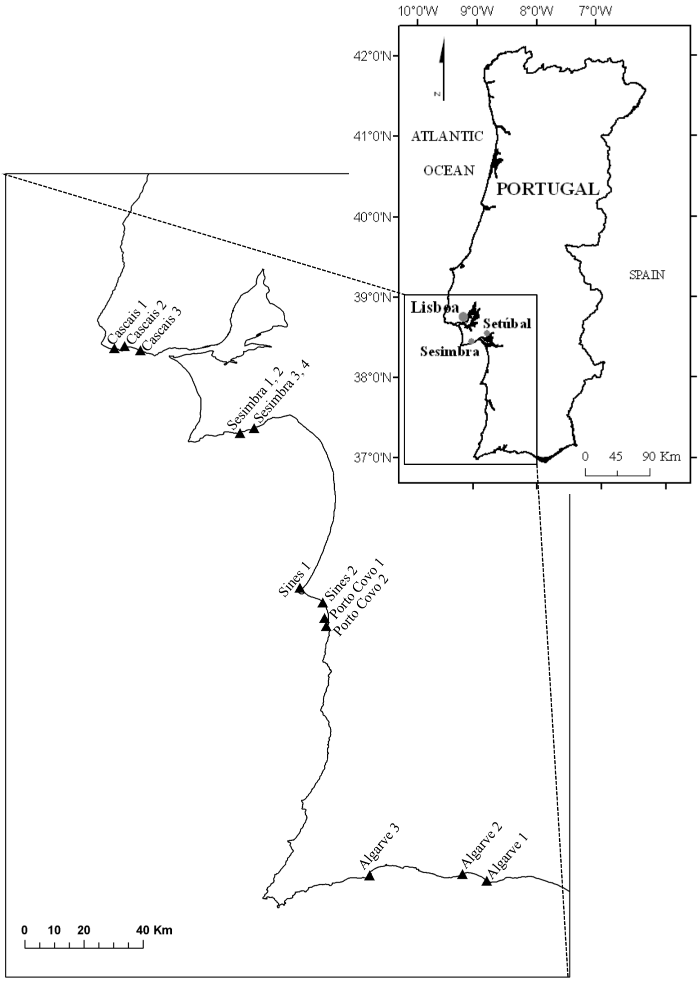


Figure 5.1. Study sites, located in nearshore rocky reefs along the Portuguese coast.

Based on a pilot study to establish a representative number of replicates and calibrate size and abundance estimates between observers (see Henriques *et al.* 2013a), a total of 6 transects per site were performed, half by each observer (M.P. Pais and S. Henriques), starting each time at a random point allocated to one of two pre-determined depth intervals (0−5 m and 5−10 m), according to each site's characteristics.

5.2.3. Fish-based metrics and guild classification

Metrics were selected based on previous compilations that took into account their use in monitoring and assessment tools and programmes (Henriques *et al.*, 2008; Pais *et al.*, 2012, Henriques *et al.*, 2013a,b;). A total of 47 metrics were calculated for each transect, representing a range of structural and functional fish assemblage characteristics including diversity, composition, abundance, trophic structure, habitat association, nursery function, mobility, resilience, spawning season and biogeographic affinity (table 5.1).

Table 5.1. Description of the metrics that integrate the univariate and multivariate functional categories used.

Category	**Metric**	**Description**
Univariate categories[a]	Total density	Total density of fish.
	Total density of juveniles	Total density of fish under the size at first maturity.
	Total density of adults	Total density of fish with or over the size at first maturity.
	Total density (cryptobenthic)	Total density of crypobenthic fish.
	Rock residents	Total density of fish that spend their whole life cycle on rocky substrate.
	Dominance	Number of species that make up 90% of the total abundance.
	Species richness	Total number of species.
	Average length	Average total length of all fish.
	Average length (adults)	Average total length of fish with or over the size at first maturity.
	Average trophic level	Average number of energy-transfer steps required to get to the position that each fish occupies in the food chain.
	Pelagic/demersal ratio	Ratio of the density of pelagic fish to the density of demersal fish.
	Commercial/non-commecial ratio	Ratio of the density of fish with "medium" and "high" commercial value to the density of fish with "low" commercial value.
Symphodus and *Labrus*	Density of *Symphodus* spp.	Density of two genera with nesting behaviour that are highly dependent on algae covered rocky substrates.
	Density of *Labrus* spp.	
Trophic guilds	Invertebrate feeders	Density of fish that feed mostly on non-planktonic invertebrates.
	Omnivores	Density of oportunistic feeders and detritivores.
	Macrocarnivores	Density of fish that feed mostly on macroinvertebrates and fish.
	Zooplanktivores	Density of fish that feed mostly on planktonic invertebrates and fish eggs/larvae.
	Herbivores	Density of fish that feed mostly on benthic and planktonic micro and macroalgae and macrophytes.
Mobility	High mobility	Density of highly mobile and migratory fish.
	Medium mobility	Density of fish with movement patterns on the order of tens of meters.
	Territorial	Density of fish with limited movement and territorial behaviour.
	Sedentary	Density of benthic fish with limited movement and well defined home ranges.
Resilience	Low resilience	Density of fish with minimum population doubling time of 4.5−14 years.
	Medium resilience	Density of fish with minimum population doubling time of 1.4−4.4 years.
	High resilience	Density of fish with minimum population doubling time of up to 1.4 years.
Spawning season	Spring spawners	Density of fish with spawning season in March−May.
	Summer spawners	Density of fish with spawning season in June−August.
	Autumn spawners	Density of fish with spawning season in September−November.
	Winter spawners	Density of fish with spawning season in December−February.
GBGT	Gobiidae	Density of four families of benthic fish that are highly dependent on microhabitat features, such as holes and crevices, for shelter and reproduction.
	Blenniidae	
	Gobiesocidae	
	Tripterygiidae	
Biogeography	Temperate	Density of fish that occur in cold and warm-temperate areas.
	Warm-temperate	Density of fish that occur from the the western entrance of the English Channel to the Mediterranean and north-western coasts of Africa.
	Cold-temperate	Density of fish that occur from the North Sea to the Atlantic coast of the Iberian Peninsula.
	Tropical	Density of fish that occur from tropical west Africa to the entrance of the Mediterranean and southern Iberian Peninsula.
	Eurythermic	Density of fish that occur in a wide latitudinal range.
Habitat	Generalists	Density of fish that use all or most habitat categories, especially rocky substrates and less frequently water column and sand.
	Rock and cave	Density of fish that inhabit cavities in the rocks.
	Rock specialists	Density of fish that inhabit rock bumps and flats, mainly those covered by algae but also bare.
	Sand specialists	Density of fish that inhabit sandy substrates at the bottom of slopes or on flats among rocky outcrops.
	Water column	Density of fish that inhabit the water column, irrespective of the substrate below.
	Water column and algae	Density of fish that inhabit the water column over algae covered substrates.
	Water and cave	Density of fish that inhabit the water column, usually inside large caves.

[a] These metrics were used individually in the analyses.

All metrics in the “univariate” group in table 5.1 were treated individually in the analyses, while all other metrics were treated as part of their multivariate categories (for simplicity, the term “functional categories” will henceforth refer to both univariate metrics and multivariate categories). Species were classified into guilds based on previous studies (Henriques *et al.*, 2008, Tsikliras *et al.*, 2010; Henriques *et al.*, 2013a;), FishBase online database (Froese and Pauly, 2012) and the authors’ field observations.

Migration and trophic guilds were based on Elliot *et al.* (2007) with the adapted classification by Henriques *et al.* (2008), habitat guilds were adapted from Fasola *et al.* (1997), resilience guilds were based on the estimated minimum population doubling time (Musick, 1999) and trophic levels were calculated according to Pauly and Christensen (1998). Classification according to trophic level, resilience and length at first maturity data were gathered from FishBase (Froese and Pauly, 2012).

5.2.4. Environmental variables

In the absence of a long monitoring history on a particular site, and given that the best potential assemblage it can support is limited by environmental features, the classification of new sites must ideally take into account environmental variables that are known to affect rocky reef communities but stay unaffected in the presence of impact sources.

Exposure to wave action can greatly affect both the physical structure of a reef and the supported communities (Burrows *et al.*, 2008), thus affecting the characteristics of fish assemblages both directly and indirectly (*e.g.* Fulton and Bellwood, 2004). In order to calculate measures of exposure per site, the map-based method of Burrows *et al.* (2008) was applied to the study area using Geographical Information Systems (GIS) software. A detailed vector map of the Portuguese and Northern African coasts was taken from the Global Self-consistent, Hierarchical, High-resolution Shoreline (GSHHS) dataset (available at: www.ngdc.noaa.gov/mgg/shorelines/gshhs.html). Since the model requires a grid-based map, ArcGIS 9.3 software was used to project the vector coastline and convert the vector image to a gridded dataset with a cell size of 200 m and different codes for land and sea cells (Burrows *et al.*, 2008). The resulting gridded map was then imported into specialised software (Wave Fetch Model available at http://www.sams.ac.uk/michael-burrows) that was used to identify coastal cells and calculate wave fetch for each coastal cell as the distance to the closest land in each of 16 angular sectors of 22.5°. Maximum fetch distance was set to 200 km and map size was set to be larger than this radius for every sampled site to avoid miscalculations of wave fetch due to map borders. In order to minimise the effect of having a coastline converted to square-shaped cells and to reduce the number of variables, only 8 directions were considered (N, NE, E, SE, S, SW, W, NW) with each included sector represented by the average between its fetch value and the two adjacent excluded sectors.

While wave fetch values at a grid size of 200 m can predict rocky shore communities to a certain degree (Burrows *et al.*, 2008), they do not capture fine-scale differences between sites. At a smaller scale, depth, surface topography and heterogeneity are the main variables that are known to affect fish assemblage structure (Johnson *et al.*, 2012) and are not likely to change due to most impact sources. Surface topography was quantified using the combined topography index (CTI; Pais *et al.*, 2013), that employs the "chain and tape" method using a fixed chain length (Grigg, 1994). For each replicate, a 25 m long leaded rope was deployed along the contour of the substrate and the linear distance travelled (Ld) was measured with a measuring tape, then the substrate rugosity (SR) index was calculated as SR = $Ld/25$. The number of upwards (N_u) and downwards (N_d) elevation changes with more than 0.5 m were counted and used to calculate an approximate measure of the number of corrugations as NC = $(N_u + N_d)/2$ and depth at the shallowest (D_s) and deepest (D_d) points along the profile was measured with a depth gauge to calculate the maximum vertical relief as MVR = $D_d – D_s$. The CTI was then calculated as

$$\mathrm{CTI} = (1 - SR) + \frac{NC}{25} + \frac{MVR}{25}$$

In addition, average depth was estimated per replicate as $(D_s + D_d)/2$ and the percentage of sand, rock and cobbles covered by the measuring tape were used as a measure of substrate heterogeneity (Pais *et al.*, 2013).

5.2.5. Typology definition

5.2.5.1. Clustering sites according to categories of fish-based metrics

Because fish-based metrics can be used individually and respond differently to environmental conditions (Öhman and Rajasuriya, 1998), the first step in the approach is to look at how similar different assemblages are when using different metrics to describe them. For this purpose, Euclidean distance matrices were calculated separately for each functional category. Because units within categories are the same and the magnitude of values is meaningful, no data transformations were applied.

In order to cluster the surveyed assemblages using each category individually, a method was needed that could test all possible combinations to find clusters that maximise the distinction between an unknown number of groups of unknown size heterogeneity at an unknown scale.

In order to achieve such a classification, metric pairwise constrained k-means (MPCK-means), a semi-supervised clustering algorithm, was applied (Bilenko *et al.*, 2004). This algorithm has the same underlying philosophy of traditional unsupervised k-means clustering, where data points are iteratively relocated so that the dataset is partitioned into K clusters that minimize the total squared Euclidean distance of the points to their cluster centroids. What makes MPCK-means powerful is that it combines two methods of semi-supervision: metric learning and pairwise constraints. In the first step of the process, must-link and cannot-link constraints are defined between pairs of data points, based on known information (Wagstaff *et al.*, 2001). Then, these pairwise constraints are used to adapt the underlying distance measure, so that the distance between must-link pairs is minimised and the distance between cannot-link pairs is maximised in multidimensional space. This method allows the algorithm to learn a distance measure that more accurately reflects the concept of similarity contained in the supervision. Moreover, separating two must-link data points or joining two cannot-link data points in MPCK-means comes with a cost, which can be weighted relative to the cost of increasing within-cluster distance if constraints are fulfilled (Bilenko *et al.*, 2004).

In the present study, the goal was to cluster sites along the coast based on their inherent variability, represented by six replicate transects of 100 m^2. For this purpose, individual transects were used as data points and must-link constraints were established between all pairs of transects within a site. In this way, each site was represented by a cloud of points, whose dissociation carried a cost in the objective function to be optimised by the iterative process.

In order to observe if any replicates were clearly deviant from within-site multivariate species composition, a principal coordinates analysis (Gower, 1966; not shown) was used to represent all transects in unconstrained multivariate space based on Bray-Curtis dissimilarities using untransformed species densities. This led to the exclusion of one replicate from Sesimbra 4 (figure 5.1) from further analyses, since the abnormally large number of individuals from a few species in this replicate would affect the clustering process. After removal of this transect, a total of 83 data points representing 14 sites and 288 must-link pairwise constraints were used to run the algorithm for each functional category.

Given a set of data points X, a set of must-link constraints $\mathcal{M}$ and in the absence of cannot-link constraints, the objective function $\mathcal{J}_{\text{mpckm}}$ to be minimised by the MPCK-means algorithm is

$$\mathcal{J}_{mpckm} = \sum_{x_i \in X} \left(\left\| x_i - \mu_{l_i} \right\|^2_{A_{l_i}} - log\left(det\left(A_{l_i}\right) \right) \right)$$
$$+ \sum_{(x_i, x_j) \in \mathcal{M}} w_{ij} \left(\frac{1}{2} \left\| x_i - x_j \right\|^2_{A_{l_i}} + \frac{1}{2} \left\| x_i - x_j \right\|^2_{A_{l_j}} \right) \mathbb{1}[l_i \neq l_j]$$

The first summation refers to the distance of all data points to their respective cluster centroids, where x_i is a data point assigned to cluster $l_i \in \{1, \dots, K\}$ with centroid μ_{l_i}. In order to allow clusters to have different shapes, the metric learning process generates a separate positive-definite diagonal matrix $A_{l_i}$for each cluster, which corresponds to giving different weights to input variables to minimise the distance between must-link pairs. These K diagonal matrices are then used to parameterise Euclidean distances within each cluster and are re-adjusted in each iteration (see Bilenko *et al.*, 2004 for details). The second summation refers to the penalties for must-link constraint violation, where two points x_i and x_j are a must-link pair of data points. The term w_{ij} has to be defined *a priori*, and represents the cost of violating a constraint, relative to the cost of increasing distances to centroids. This cost is then multiplied by the second term, which increases the penalty for points that are further apart according to the learned distance metrics for both involved clusters, l_i. and l_j. The third term is an indicator function $\mathbb{1}$, which assumes the value 0 if false (no penalty if x_i and x_j were assigned to the same cluster) or the value 1 if true (must-link points were assigned to different clusters, so the cost is counted).

Prior to the clustering process, both the number of clusters K and the relative cost of constraint violation w_{ij} were unknown for each functional category. Therefore, a method was followed to optimise both parameters so that within-cluster distances were minimised and no constraints were violated. Starting at K=2 and w_{ij}=1, K was gradually increased until a constraint was violated. If a constraint was violated, w_{ij} was increased by an integer, followed by another increase in K. If after reaching K=n the algorithm returned one or more empty clusters, a value of K=n−1 was adopted. This method relies simply on the self-limitation of the algorithm, since an empty cluster means that it would likely be a “boundary” cluster composed from a mixture of other clusters, thus setting w_{ij} to a value that is high enough will make other clusters claim their members, leaving an empty cluster. For each individual functional category, a maximum of 10 initialisations using a “weighted farthest-first” algorithm (Bilenko *et al.*, 2004) were used to optimise the initial cluster seeds, with 100 iterations to minimise the objective function.

5.2.5.2. Cluster ensembles and definition of assemblage types

Given that fish-based metrics can be used individually or in different combinations depending on the context, sites should belong to the same assemblage type if their response pattern when using different functional categories is somewhat similar.

For this purpose, it is necessary to incorporate the results of the MPCK-means clustering algorithm for the different functional categories and reach a final combined result. However, due to the metric learning process involved and the different nature, units and number of variables in each case, combining results while taking into account the original distances between sites in each case would be an extremely complex approach. Instead, Strehl and Ghosh (2002) proposed label-based ensemble clustering algorithms that provide the tools to combine multiple clustering results for a set of objects (sites) into a combined solution, by relying only on cluster membership, assuming that sites that tend to be labelled with the same cluster name using different descriptors should belong in similar clusters in the final assembled solution.

This cluster ensemble process attempts to combine an input set of r clustering results, denoted by Λ, into an integrated clustering solution λ. Since all individual clustering results have equal importance in the ensemble, the goal of the process is to find a clustering solution that shares the most information with all the original results. This is accomplished through pairwise mutual information (Cover and Thomas, 2006), which quantifies the information shared between a pair of clustering results. In order to have a measure ranging from 0 to 1, the normalised mutual information (NMI) between a pair of clusterings $\lambda^{(a)}$ and $\lambda^{(b)}$, with $k^{(a)}$ and $k^{(b)}$ clusters respectively, is

$$NMI(\lambda^{(a)}, \lambda^{(b)}) = \frac{\sum_{h=1}^{k^{(a)}} \sum_{l=1}^{k^{(b)}} n_{h,l} \log\left(\frac{n \cdot n_{h,l}}{n_h^{(a)} n_l^{(b)}}\right)}{\sqrt{\left(\sum_{h=1}^{k^{(a)}} n_h^{(a)} \log \frac{n_h^{(a)}}{n}\right)\left(\sum_{l=1}^{k^{(b)}} n_l^{(b)} \log \frac{n_l^{(b)}}{n}\right)}}$$

where n is the total number of sites, $n_h^{(a)}$ is the number of sites in cluster h according to clustering results $\lambda^{(a)}$, $n_l^{(b)}$ is the number of sites in cluster l according to $\lambda^{(b)}$ and $n_{h,l}$ is the number of sites that are in cluster h according to $\lambda^{(a)}$ as well as in cluster l according to $\lambda^{(b)}$. All r individual clustering results $\lambda^{(q)}$ belonging to the input set Λ are ultimately compared one by one with the integrated clustering solution λ and lead to the calculation of the average normalised mutual information (ANMI), the objective function to be maximised by the ensemble clustering algorithm:

$$ANMI(\Lambda, \lambda) = \frac{1}{r}\sum_{q=1}^{r} NMI\left(\lambda, \lambda^{(q)}\right)$$

Strehl and Ghosh (2002) propose three algorithms to solve the cluster ensemble problem, each with its own strengths and weaknesses: the cluster-based similarity partitioning algorithm (CSPA), the hypergraph-partitioning algorithm (HGPA) and the meta-clustering algorithm (MCLA) (see Strehl and Ghosh, 2002 for details). Because each algorithm tends to perform better with some types of data and context, the approach followed was to use all three of them and adopt the one that returns the highest ANMI (a step known as the 'supra-consensus' function).

Because simply using all functional categories in the ensemble clustering algorithm would produce too much noise and generate a solution with very low ANMI values (Strehl and Ghosh, 2002), functional categories were divided into two subsets based on second stage Spearman rank correlation matrices (Somerfield and Clarke, 1995) and individual clustering results.

Second stage correlations measure the relationship among all the Euclidean distance matrices for every functional category, and thus a high value means that categories tend to bring together the same assemblages, thus potentially leading to similar clustering results. Due to this, since there was a relatively large group of highly correlated matrices ($r_s > 0.9$), they were excluded and a single category was kept as a proxy on the ensemble clustering process. When generating a combined clustering result, it was unclear whether introducing categories with different patterns would improve results. Therefore, two different approaches were compared by using two different subsets of categories. Subset 1 consisted of a group of categories with highly correlated distance matrices ($0.8 \leq r_s \leq 0.9$), meaning that they lead to similar relative among-site distances, while subset 2 used all functional categories with second stage $r_s \leq 0.9$, in order to represent all patterns present in the data. Cases where the MPCK-means algorithm was unable to find partitions in the dataset (K=1) were excluded from the subsets.

Because within-type cohesion can only be evaluated by looking at the variability at several scales (type, site and transect), the values for all functional categories in the typologies resulting from the two subsets were then compared through nested multivariate (or univariate) analysis of variance using permutations (PERMANOVA), a method that is analogous to traditional ANOVA or MANOVA if based on an Euclidean distance matrix but calculates the significance of the pseudo-F statistic by permutation of residuals under the null hypothesis, thus avoiding the need to comply with distributional assumptions (Anderson, 2001; McArdle and Anderson, 2001). Due to the fact that bottom-up classifications can lead to unbalanced group sizes, an additional concern is the different multivariate dispersions among groups, to which PERMANOVA is sensitive, thus a test of homogeneity of dispersions (PERMDISP; Anderson, 2006) based on distances to centroids was used to complement the interpretation of results. For both PERMANOVA and PERMDISP, 9999 permutations were used and results were considered significant at $P < 0.05$.

Effects of assemblage types (fixed), sites (random, nested in types) and residuals (transects) were calculated through sequential (type I) sums of squares and the estimated components of variation for these three hierarchical levels were compared for all functional categories. PERMANOVA results for the two ensemble approaches (resulting from the two category subsets) were compared, and the one showing significant differences among assemblage types for the most categories and with the highest percent variation explained by this highest hierarchical level was chosen as the final configuration. Average values for individual metrics were calculated to characterise each final assemblage type and, in order to assess the detectability of individual metrics in the face of natural variation, an average signal-to-noise ratio (STN) was calculated for every metric as the ratio of the mean to the standard deviation within each assemblage type, averaged across all types. This can be seen as a particular case of Cohen's d (Cohen, 1988), where the mean value is compared to zero.

Finally, average values for environmental variables were calculated for each assemblage type and species that characterise each type were identified through a Canonical Analysis of Principal Coordinates (CAP; Anderson and Robinson, 2003; Anderson and Willis, 2003), by finding axes in multivariate space that best distinguish between types and overlaying vectors representing the species with highest Spearman correlations with canonical axes (Anderson *et al.*, 2008). CAP used individual transects as data points and was based on a

Bray-Curtis dissimilarity matrix of square-root transformed species densities. Type cohesion according to the CAP model was assessed using a leave-one-out allocation procedure (Lachenbruch and Mickey, 1968).

5.2.5.3. Classification model

After the definition of assemblage types, there is a need for a classification tool that can assign new sites into the defined types based on environmental attributes that are unlikely to change due to the presence of an impact source. In order to achieve this, a *k*-nearest neighbours classifier (kNN; Cover and Hart, 1967) was applied.

Unlike other learning algorithms where labelled data is used initially to train the model and then discarded, kNN is a lazy classifier, since it does not involve an initial training phase. Instead, labelled data are stored and used for comparison every time a new object is to be classified. With all data described by the same n attributes, the k neighbours with the shortest Euclidean distance to the new object in n-dimensional space determine its classification.

In the simplest form of the kNN method, the label attributed to the new object is simply the most frequent among its k nearest neighbours, which is known as the “majority voting” rule (Cover and Hart, 1967). However, if class representation is heavily unbalanced, classes with more objects in the training data are more likely to be classified as nearest neighbours and thus lead to biased classifications (Coomans and Massart, 1982). One way of overcoming this problem is by changing the voting rule, so that the vote from each neighbour consists of the inverse of its distance to the object to be classified. This inverse-distance weighted (IDW) voting rule gives more weight to nearer neighbours in the final decision (Dudani, 1976). Amongst the several variations of kNN algorithms available, the WEKA implementation of the IBk algorithm was chosen (Aha *et al.*, 1991; available at http://weka.sourceforge.net/doc/weka/classifiers/lazy/IBk.html), which is identical to the simplest form of kNN but includes a step to normalise all input variables by their ranges and incorporates some rules to deal with missing data.

Pearson correlation coefficients were calculated among environmental variables and no redundancies ($r \geq |0.95|$) were found. Therefore, a total of 12 environmental variables (see section 5.2.4 and table 5.4) were used in the classification algorithm, with each CTI replicate (and associated substrate cover percentages and average depth) representing the smallest unit of variation for each object in the training set, since wave fetch values were constant for every replicate within a site. Depending on the area and topographical homogeneity of each reef, 2 to 4 CTI replicates were used to characterise each site, as a way of balancing representation and redundancy. This led to a total of 49 objects available to train and test the model.

The definition of the number of neighbours k and the choice of voting rule are of crucial importance to the behaviour of the classification tool (Coomans and Massart, 1982; Raudys and Jain, 1991; Liu *et al.*, 2003). For this purpose, the algorithm was run for every combination of k ϵ {2,3,4,5} and the two voting rules (majority rule and inverse distance-weighting). A 3-fold cross-validation with stratified sampling was used to calculate classification accuracy for each combination, which ensures a realistic scenario where all assemblage types are represented in the training data in the correct proportions, while using all data points as test data, 1/3 at a time, to estimate performance variability. The combination with the highest classification accuracy was established as the final configuration for the algorithm.

In order to further attempt to fine-tune the model by removing any irrelevant features, a backward elimination method was applied, by iteratively removing one variable at a time and re-running the kNN algorithm with a 3-fold cross-validation for performance estimation. Only variables whose removal resulted in improved classification accuracy were excluded by the backward elimination process.

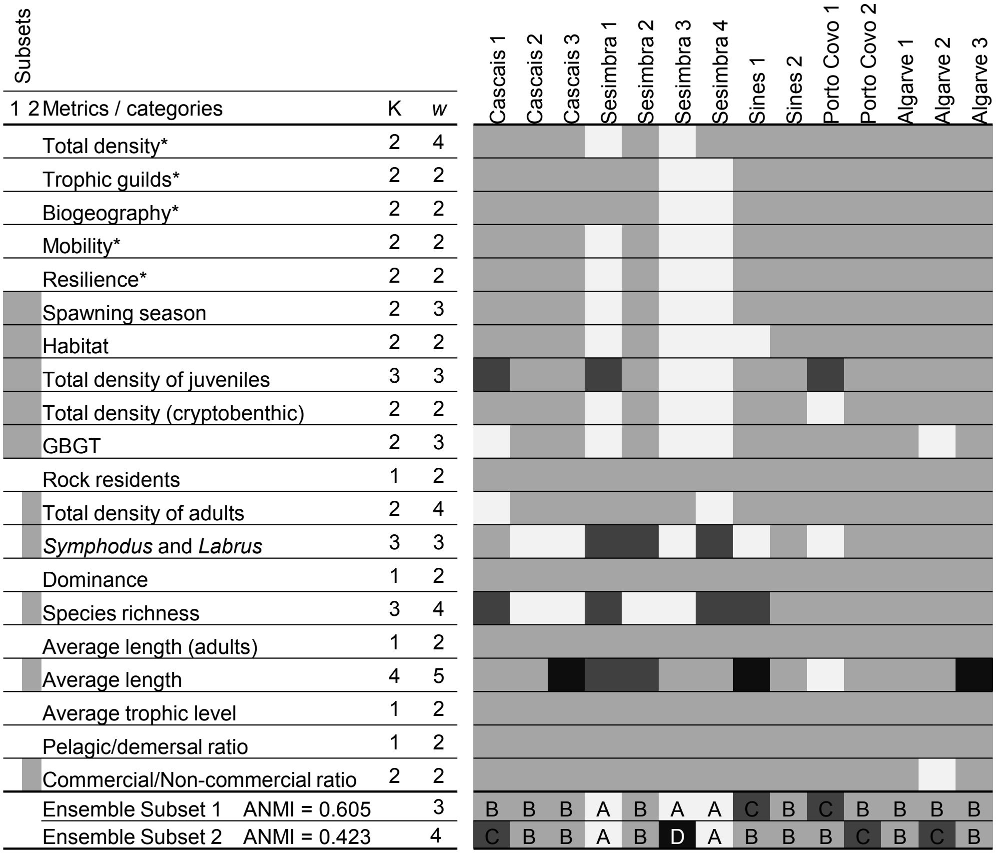
5.2.5.4. Algorithms and software

Distance and correlation matrices, second stage Spearman rank correlation matrices, CAP, PERMANOVA and PERMDISP were calculated using PRIMER 6 with PERMANOVA+ package, the MPCK-means algorithm was applied, optimised and evaluated using RapidMiner 4.2 and the ensemble cluster algorithms were run using a specialised MATLAB function (available at http://www.lans.ece.utexas.edu/~strehl/soft.html). The kNN classifier was applied, optimised and evaluated in RapidMiner 5 by importing the W-IBk algorithm via the WEKA (Hall *et al.*, 2009) extension to RapidMiner.

5.3. Results

5.3.1. Individual and ensemble clustering of sites

Results for the MPCK-means clustering of sites based on each functional category are presented in figure 5.2. For the majority of functional categories, only 2 partitions were separated by the algorithm, with sites in the region of Sesimbra tending to form a separate cluster. When describing sites according to the “total density of juveniles”, “species richness” and the density of “*Symphodus* and *Labrus*”, a division in K=3 groups was achieved, while K=4 was only achieved with “average length”. For “rock residents”, "dominance", "average length of adults", "average trophic level" and "pelagic/demersal ratio", the algorithm was not able to partition the dataset, so these categories were not included in any subsets for ensemble clustering.



Subsets 1	Subsets 2	Metrics / categories	K	*w*	Cascais 1	Cascais 2	Cascais 3	Sesimbra 1	Sesimbra 2	Sesimbra 3	Sesimbra 4	Sines 1	Sines 2	Porto Covo 1	Porto Covo 2	Algarve 1	Algarve 2	Algarve 3
		Total density*	2	4	▒	▒	▒	□	▒	□	▒	▒	▒	▒	▒	▒	▒	▒
		Trophic guilds*	2	2	▒	▒	▒	▒	▒	□	□	▒	▒	▒	▒	▒	▒	▒
		Biogeography*	2	2	▒	▒	▒	▒	▒	□	□	▒	▒	▒	▒	▒	▒	▒
		Mobility*	2	2	▒	▒	▒	□	▒	□	□	▒	▒	▒	▒	▒	▒	▒
		Resilience*	2	2	▒	▒	▒	□	▒	□	□	▒	▒	▒	▒	▒	▒	▒
▒	▒	Spawning season	2	3	▒	▒	▒	□	▒	□	□	▒	▒	▒	▒	▒	▒	▒
▒	▒	Habitat	2	2	▒	▒	▒	□	▒	□	□	□	▒	▒	▒	▒	▒	▒
▒	▒	Total density of juveniles	3	3	▓	▒	▒	▓	▒	□	□	▒	▒	▓	▒	▒	▒	▒
▒	▒	Total density (cryptobenthic)	2	2	▒	▒	▒	□	▒	□	□	▒	▒	□	▒	▒	▒	▒
▒	▒	GBGT	2	3	□	▒	▒	□	▒	□	□	▒	▒	▒	▒	▒	□	▒
		Rock residents	1	2	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
	▒	Total density of adults	2	4	□	▒	▒	▒	▒	▒	□	▒	▒	▒	▒	▒	▒	▒
	▒	*Symphodus* and *Labrus*	3	3	▒	□	□	▓	▓	□	▓	□	▒	□	▒	▒	▒	▒
		Dominance	1	2	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
	▒	Species richness	3	4	▓	□	□	▓	□	□	▓	▓	▒	▒	▒	▒	▒	▒
		Average length (adults)	1	2	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
	▒	Average length	4	5	▒	▒	█	▓	▓	▒	▒	█	▒	□	▒	▒	▒	█
		Average trophic level	1	2	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
		Pelagic/demersal ratio	1	2	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
	▒	Commercial/Non-commercial ratio	2	2	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	□	▒
		Ensemble Subset 1 ANMI = 0.605		3	B	B	B	A	B	A	A	C	B	C	B	B	B	B
		Ensemble Subset 2 ANMI = 0.423		4	C	B	B	A	B	D	A	B	B	B	C	B	C	B

* excluded due to high second stage correlation with "spawning season".

Figure 5.2. Partitioning of the data into clusters according to each individual functional category through MPCK-means. Sites with similar shaded cells along horizontal lines were clustered together according to that category. For each category, the optimised number of clusters (K) and the weight given to pairwise constraints (w) is shown. Members of both subsets are identified by shaded cells on the first two columns. Ensemble clustering results and average normalised mutual information (ANMI) values according to the meta-clustering algorithm (MCLA) are represented in the bottom rows.

Analysis of the second stage Spearman correlation coefficients showed that "spawning season" was highly correlated ($r_s > 0.90$) with "total density", "trophic guilds", "biogeography", "mobility" and "resilience", so this category was kept as a proxy and the others were not included on any subsets. Based on the defined criteria (see section 5.2.5.2), functional categories were included in subsets 1 and 2 (figure 5.2) to integrate the cluster ensemble phase.

When running the three ensemble clustering algorithms with both subsets to optimise the ANMI at an optimal number of clusters K, the meta-clustering algorithm (MCLA) had the best performance, with ANMI values of 0.605 and 0.423 for subset 1 and 2, respectively, followed by the cluster-based similarity partitioning algorithm (CSPA), with an ANMI of 0.492 and 0.401 and the hypergraph partitioning algorithm (HGPA) with values of 0.437 and 0.332. Therefore, the two ensemble solutions found by the MCLA were kept for comparison and are represented in figure 5.2. For subset 1, three clusters were established, with cluster A integrating three sites, cluster C with two sites and cluster B with 9 sites. For subset 2, four clusters were found, again featuring a large cluster B with 8 sites, a cluster A with 2 sites, a cluster C with 3 sites and a cluster D with a single site.

5.3.2. Variability of fish-based metrics and characterisation of assemblage types

Comparison of the estimated components of variation in nested PERMANOVA analyses for the assemblage typologies achieved with both subsets is presented in table 5.2. Given that variation among transects is the same for both typologies, the balance between the components of variation due to sites (nested) and assemblage types is of crucial importance. Because the sum of squared effects was calculated sequentially, there are no overlapping components and the variation due to a hierarchically superior factor is calculated over and above the variation due to the factor below.

In general, considering that heterogeneous cluster sizes led to significant differences in dispersions for both cluster configurations, care should be taken when analysing significant results, however, the frequency of non-significant results reveals that subset 1 performed better in terms of the relative importance of assemblage types in explaining total variation. In the case of “habitat”, “total density (cryptobenthic)” and “average length of adults”, no significant differences were found among the assemblage types established with subset 2, whereas for subset 1 PERMANOVA found a significant effect. For “GBGT”, “dominance”, “average trophic level”, “pelagic/demersal ratio” and “commercial/non-commercial ratio”, no significant differences were found among types with any of the ensemble solutions, with most variation occurring among individual transects. In the case of “species richness” and the density of “*Symphodus* and *Labrus*”, however, among-site variation was predominant. Analysis of table 5.2 led to the decision of adopting the typology configuration achieved with subset 1 (figure 5.2), with sites divided into three assemblage types.

Table 5.2. Results of the nested PERMANOVA for each individual functional category, with factor “sites” (random) nested within “types” (fixed). Bottom values for each row represent the variation partitioning according to the three hierarchical levels using sequential sum of squares. In order to visualise components of variation in the original units (see Table 3), top values represent the square rooted component of variation due to each factor. Values are bolded when no significant differences were found in PERMANOVA and underlined if clusters were found to have significantly different multivariate dispersions in PERMDISP.

		Subset 1 (K=3)			Subset 2 (K=4)		
Categories	NV	Types	Sites	Resid	Types	Sites	Resid
Total density	1	1.247	0.704	0.950	1.179	0.682	0.950
		52.7%	16.8%	30.6%	50.4%	16.9%	32.8%
Total density of juveniles	1	1.087	0.581	0.827	1.042	0.543	0.827
		53.7%	15.3%	31.0%	52.6%	14.3%	33.1%
Total density of adults	1	0.211	0.166	0.299	0.199	0.164	0.299
		27.6%	17.1%	55.2%	25.4%	17.3%	57.3%
Total density (cryptobenthic)	1	0.867	0.616	0.813	**0.774**	0.642	0.813
		42.0%	21.2%	36.8%	35.8%	24.6%	39.5%
Rock residents	1	0.937	0.450	0.407	0.968	0.295	0.407
		70.5%	16.2%	13.3%	78.8%	7.3%	13.9%
Dominance	1	**0.000**	0.741	1.320	**0.000**	0.794	1.320
		0.0%	23.9%	76.1%	0.0%	26.6%	73.4%
Species richness	1	**1.571**	2.336	0.951	**1.250**	2.412	0.951
		28.0%	61.8%	10.3%	18.9%	70.2%	10.9%
Average length	1	**1.533**	2.203	1.975	**0.000**	2.505	1.975
		21.2%	43.7%	35.1%	0.0%	61.7%	38.3%
Average length (adults)	1	3.615	2.256	3.302	**0.768**	3.456	3.302
		45.0%	17.5%	37.5%	2.5%	51.0%	46.5%
Average trophic level	1	**0.000**	0.080	0.208	**0.026**	0.067	0.208
		0.0%	12.8%	87.2%	1.4%	9.4%	89.2%
Pelagic/demersal ratio	1	**0.000**	0.137	0.223	**0.000**	0.146	0.223
		0.0%	27.4%	72.6%	0.0%	30.0%	70.0%
Commercial/Non-commercial ratio	1	**0.000**	0.394	0.511	**0.000**	0.389	0.511
		0.0%	37.2%	62.8%	0.0%	36.6%	63.4%
Symphodus and *Labrus*	2	**0.000**	0.046	0.035	**0.000**	0.045	0.035
		0.0%	63.6%	36.4%	0.0%	62.7%	37.3%
Trophic guilds	5	0.825	0.687	0.881	0.760	0.692	0.881
		35.3%	24.5%	40.2%	31.5%	26.1%	42.4%
Mobility	4	0.706	0.570	0.846	0.652	0.573	0.846
		32.4%	21.1%	46.5%	29.0%	22.3%	48.7%
Resilience	3	0.926	0.533	0.912	0.876	0.517	0.912
		43.5%	14.4%	42.1%	41.1%	14.3%	44.6%
Spawning season	4	1.410	1.083	1.380	1.267	1.115	1.380
		39.3%	23.2%	37.6%	33.8%	26.2%	40.0%
GBGT	4	**0.416**	0.453	0.750	**0.352**	0.471	0.750
		18.4%	21.8%	59.8%	13.7%	24.4%	61.9%
Biogeography	5	0.765	0.714	0.874	0.724	0.705	0.874
		31.5%	27.4%	41.1%	29.4%	27.8%	42.8%
Habitat	7	0.627	0.572	0.876	**0.543**	0.597	0.876
		26.4%	22.0%	51.6%	20.8%	25.1%	54.1%

NV- number of variables.

The calculation of average values and standard deviations (table 5.3) was an important complement to table 5.2, since it distinguished between metrics that were not significantly different due to high variance from metrics that were stable but similar across types (the case of ”dominance” and “average trophic level”). In addition, several metrics had average signal-to-noise (STN) ratios smaller than 1, which can be seen as an indicator (considering that standard deviations are not guaranteed to be symmetrical from the mean) that they are excessively variable to be considered different from zero, and therefore their use in a monitoring context should be discouraged.

Table 5.3. Mean values (standard deviation in brackets) for every fish-based metric within each of the three assemblage types. Average signal-to-noise ratios (STN; see section 5.2.5.2) lower than 1 are signalled with “−“, otherwise are signalled with “+”. Legend: N- number of transects.

			Type			
Category	Metric	Units	A (N=17)	B (N=54)	C (N=12)	STN
Univariate categories	Total density	fish.m^{-2}	3.776 (1.440)	1.411 (1.009)	1.581 (1.261)	+
	Total density of juveniles	fish.m^{-2}	2.960 (1.250)	1.025 (0.811)	1.120 (1.084)	+
	Total density of adults	fish.m^{-2}	0.816 (0.414)	0.386 (0.321)	0.461 (0.258)	+
	Total density (cryptobenthic)	fish.m^{-2}	2.526 (1.377)	0.850 (0.790)	0.865 (1.137)	+
	Rock residents	fish.m^{-2}	2.493 (0.985)	0.763 (0.440)	0.803 (0.246)	+
	Dominance*	nr. species	7 (1)	7 (2)	7 (1)	+
	Species richness*	nr. species	16 (3)	13 (3)	12 (3)	+
	Average length	cm	10 (3)	9 (3)	13 (4)	+
	Average length (adults)	cm	16 (4)	12 (4)	20 (4)	+
	Average trophic level*	-	3.11 (0.15)	3.14 (0.26)	3.20 (0.03)	+
	Pelagic/demersal ratio*	-	0.321 (0.323)	0.260 (0.253)	0.189 (0.114)	+
	Commercial/non-commecial ratio*	-	0.459 (0.380)	0.546 (0.720)	0.464 (0.336)	+
Symphodus and *Labrus**	Density of *Symphodus* spp.	fish.m^{-2}	0.065 (0.048)	0.065 (0.054)	0.068 (0.044)	+
	Density of *Labrus* spp.	fish.m^{-2}	0.028 (0.016)	0.010 (0.016)	0.027 (0.018)	+
Trophic guilds	Invertebrate feeders	fish.m^{-2}	2.026 (1.120)	0.666 (0.772)	0.590 (0.195)	+
	Omnivores	fish.m^{-2}	1.396 (0.782)	0.674 (0.393)	0.564 (0.213)	+
	Macrocarnivores	fish.m^{-2}	0.029 (0.028)	0.034 (0.149)	0.009 (0.015)	−
	Zooplanktivores	fish.m^{-2}	0.000 (0.000)	0.024 (0.085)	0.417 (1.173)	−
	Herbivores	fish.m^{-2}	0.325 (0.583)	0.014 (0.051)	0.001 (0.003)	−
Mobility	High mobility	fish.m^{-2}	0.582 (0.458)	0.373 (0.365)	0.351 (0.186)	+
	Medium mobility	fish.m^{-2}	1.184 (0.609)	0.380 (0.299)	0.977 (1.192)	+
	Territorial	fish.m^{-2}	1.321 (0.594)	0.419 (0.281)	0.247 (0.109)	+
	Sedentary	fish.m^{-2}	0.689 (0.913)	0.239 (0.583)	0.007 (0.013)	−
Resilience	Low resilience	fish.m^{-2}	0.028 (0.016)	0.012 (0.016)	0.028 (0.018)	+
	Medium resilience	fish.m^{-2}	1.406 (0.641)	0.674 (0.446)	0.688 (0.189)	+
	High resilience	fish.m^{-2}	2.341 (1.194)	0.724 (0.740)	0.866 (1.194)	+
Spawning season	Spring spawners	fish.m^{-2}	2.846 (1.180)	1.140 (0.945)	1.378 (1.235)	+
	Summer spawners	fish.m^{-2}	2.828 (1.375)	1.003 (0.833)	1.218 (1.144)	+
	Autumn spawners	fish.m^{-2}	0.726 (0.715)	0.195 (0.187)	0.189 (0.088)	+
	Winter spawners	fish.m^{-2}	1.784 (1.075)	0.705 (0.776)	0.517 (0.229)	+
GBGT*	Gobiidae	fish.m^{-2}	1.224 (1.165)	0.437 (0.595)	0.433 (1.181)	−
	Blenniidae	fish.m^{-2}	0.269 (0.111)	0.184 (0.119)	0.188 (0.076)	+
	Gobiesocidae	fish.m^{-2}	0.027 (0.035)	0.001 (0.005)	0.008 (0.018)	−
	Tripterygiidae	fish.m^{-2}	0.485 (0.230)	0.066 (0.115)	0.033 (0.034)	+
Biogeography	Temperate	fish.m^{-2}	1.448 (1.115)	0.546 (0.792)	0.438 (0.166)	+
	Warm-temperate	fish.m^{-2}	1.461 (0.663)	0.384 (0.306)	0.268 (0.114)	+
	Cold-temperate	fish.m^{-2}	0.074 (0.059)	0.081 (0.105)	0.523 (1.201)	−
	Tropical	fish.m^{-2}	0.724 (0.620)	0.310 (0.223)	0.260 (0.088)	+
	Eurythermic	fish.m^{-2}	0.071 (0.169)	0.090 (0.230)	0.093 (0.127)	−
Habitat	Generalists	fish.m^{-2}	0.186 (0.101)	0.101 (0.173)	0.217 (0.178)	+
	Rock and cave	fish.m^{-2}	0.035 (0.037)	0.005 (0.010)	0.015 (0.019)	−
	Rock specialists	fish.m^{-2}	0.842 (0.303)	0.377 (0.189)	0.294 (0.113)	+
	Sand specialists	fish.m^{-2}	1.201 (1.172)	0.378 (0.577)	0.014 (0.020)	−
	Water column	fish.m^{-2}	0.029 (0.057)	0.002 (0.009)	0.003 (0.009)	−
	Water column and algae	fish.m^{-2}	0.919 (0.771)	0.367 (0.333)	0.800 (1.294)	−
	Water and cave	fish.m^{-2}	0.564 (0.270)	0.179 (0.208)	0.238 (0.140)	+

* No significant effects of "type" in PERMANOVA (see Table 5.2).

By analysing table 5.3, type A assemblages stand out as having higher density for most metrics and tending to have higher species richness. When looking at proportions relative to total density, type A also had the highest proportion of juveniles (78% when compared to 73% and 71% of types B and C, respectively) and the highest proportion of cryptobenthic individuals (67% against 60% for B and 55% for C). In terms of trophic guild composition, all types are dominated by invertebrate feeders and omnivores. However, type A assemblages are characterised by occasionally having higher densities of herbivores. Most species are territorial or with medium mobility, spawning in spring and summer, and the families Tripterygiidae (triplefin blennies) and Gobiesocidae (clingfish) are characteristic of this type.

Type B is the most common assemblage type in the surveyed areas. It can be seen as very similar to type A in terms of proportions of functional guilds but the overall lower densities make them very different. Almost all individuals are invertebrate feeders or omnivores, with occasional occurrences of all other guilds. Territorial and medium and high mobility species are present in nearly equal proportions.

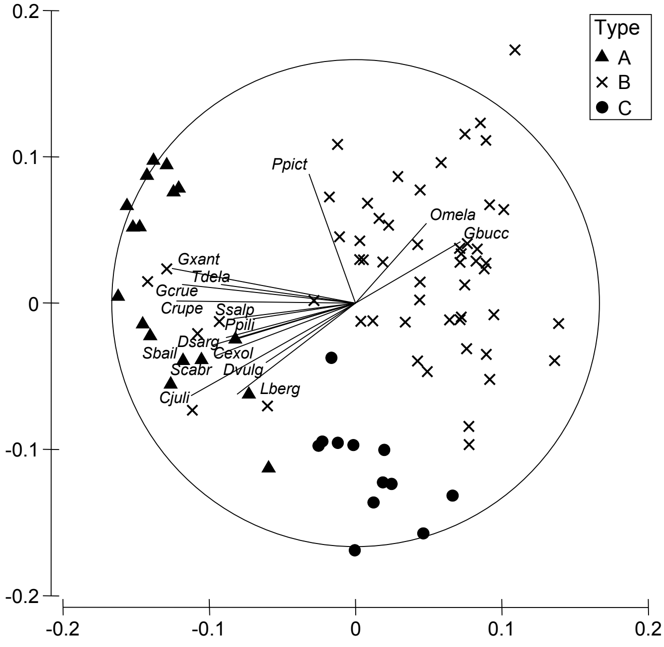


Figure 5.3. Canonical analysis of principal coordinates (CAP) based on Bray-Curtis dissimilarity matrices of square-root transformed species densities, with axes drawn to maximize discrimination among assemblage types. An average of 86.75% classification success was achieved with 72.12% variation explained by m=6 principal coordinates axes. Spearman correlations of species densities with canonical axes are shown if $r_s > |0.4|$.See section 5.3.2 for details on the leave-one-out allocation procedure. For species code correspondence see appendix 5A.

Finally, type C assemblages are characterised by a higher density of larger individuals, with an average of 13 cm TL against 10 cm and 9 cm for A and B, respectively. Moreover, the occasional occurrence of a great number of zooplanktivores was also found on both sites representing this type. This type has the lowest proportion of territorial species and the highest proportion of winter spawners and cold-temperate species.

In the CAP model (figure 5.3), the leave-one-out procedure led to a classification accuracy of 86.75%. Type C had the highest percentage of correct classifications according to species composition (91.67%), with one transect misclassified as type B, type A had 88.24% correct classifications, with two misclassifications as type C and type B had 85.19% correct classifications, with 5 transects classified as type A and 3 as type C. All transects of type B assemblages misclassified as type A were from Sesimbra 2.

In terms of Spearman correlations of species densities with both canonical axes, type A stands out as having higher densities of several species, especially from the families Blenniidae, Gobiidae, Labridae and Sparidae. The painted goby *Pomatoschistus pictus* (Malm, 1865) is less abundant in type C and the species *Oblada melanura* (Linnaeus, 1758) and *Gobius bucchichi* Steindachner, 1870 are mostly found on type B assemblages.

Finally, table 5.4 shows the average values per type of the environmental variables that were used as input in the classification model. These data show that type A assemblages are located in areas completely sheltered from the prevailing north winds, with relatively high topographic complexity and occasional occurrence of cobble.

Table 5.4. Mean values (standard deviation in brackets) for all environmental variables within each assemblage type. N = 12, 33 and 4 for type A, B and C, respectively. See section 5.2.4 for a detailed description.

	Type		
Variable	A	B	C
Topography	0.573 (0.078)	0.421 (0.213)	0.552 (0.075)
Sand (%)	0.5 (1.7)	12.1 (16.3)	5.6 (11.1)
Cobble (%)	16.9 (20.6)	3.1 (9.7)	0.0 (0.0)
Average depth (m)	6.4 (2.2)	6.5 (2.6)	10.9 (0.9)
Fetch N (km)	0.0 (0.0)	0.3 (0.9)	26.3 (27.5)
Fetch NE (km)	0.0 (0.0)	0.4 (1.4)	1.4 (1.7)
Fetch E (km)	13.9 (5.2)	8.2 (18.2)	0.1 (0.1)
Fetch SE (km)	46.0 (8.7)	48.5 (40.5)	0.0 (0.0)
Fetch S (km)	89.4 (48.3)	110.7 (63.9)	33.3 (38.5)
Fetch SW (km)	44.8 (33.1)	111.2 (90.8)	100.0 (115.5)
Fetch W (km)	0.1 (0.1)	42.9 (57.6)	166.7 (38.5)
Fetch NW (km)	0.0 (0.0)	8.5 (33.1)	113.3 (47.5)

Type C assemblages are associated with sites that are exposed to the west and northwest, mainly covered by rock, reaching higher depths and with relatively high topographic complexity. Type B assemblages, which represent the majority, can be found in a larger array of conditions. However, they are mostly found in sites exposed to the south, with occasionally high sand cover, including all sites with lower topographic complexity.

5.3.3. *k*-nearest neighbours classification model

Results of the parameter optimisation process for the kNN algorithm using a 3-fold cross-validation are shown in table 5.5. Overall, inverse-distance weighted (IDW) voting showed better results when compared to traditional majority rule (MR) voting, with overall higher classification accuracy and lower variability. When using IDW voting, classification accuracy peaked at 96.08% (5.55% standard deviation) for k=4, thus this was the chosen configuration for the kNN classification model.

Table 5.5. Classification accuracy (standard deviation in brackets) results for the 3-fold cross-validation in each combination of kNN parameters. The number of nearest neighbours k was optimised for each of the two voting rules: majority (MR) and inverse distance-weighted (IDW).

Voting rule	*k*	Accuracy (%)
IDW	2	89.83 (2.79)
	3	89.83 (2.79)
	4	96.08 (5.55)
	5	85.78 (5.65)
MR	2	79.66 (2.25)
	3	77.70 (7.16)
	4	75-86 (14.27)
	5	81.86 (7.97)

A backward elimination algorithm was applied to the kNN classifier with optimised parameters in order to eliminate input variables that negatively affect classification accuracy. The procedure suggested the removal of “topography” and “Fetch SW”, leading to an average increase of 1.84% (3.93% s.d.) accuracy, which was not considered a significant improvement, and thus all variables were kept in the final classification model. In the confusion matrix for the 3-fold cross-validation procedure with IDW voting and k=4 (table 5.6), only two samples from a type B site were misclassified as type A when using all environmental input variables.

Table 5.6. Confusion matrix of the 3-fold cross-validation procedure for the final classification model using k=4 neighbours and inverse distance-weighted voting. Mean recall (standard deviation in brackets) is 97.98% (2.86%) and mean precision is 96.30% (5.24%), for a total classification accuracy of 97.08% (5.55%).

	True A	True B	True C	Precision
Pred. A	12	2	0	85.71%
Pred. B	0	31	0	100.00%
Pred. C	0	0	4	100.00%
Recall	100.00%	93.94%	100.00%	

5.4. Discussion

The present study proposed an innovative framework to define fish assemblage types through non-hierarchical clustering of sites based on functional guild categories, while taking into account within-site variability. This is followed by a meta-clustering approach, which finds patterns that minimise information loss when integrating individual clustering results. Ultimately, a k-nearest neighbours (kNN) classifier was optimised and applied, to test its potential as a classification tool for new sites, based only on environmental variables that are unlikely to be directly affected by the presence of anthropogenic impacts.

In top-down definition of habitat units, using hierarchical classification methods is useful, as they allow the selection of an appropriate scale by simply altering the hierarchical level at which sites are clustered (Costello, 2009). In bottom-up approaches, however, the scale of the resulting types should reflect the maximum homogeneity that can be detected by species abundance estimates (Schoch and Dethier, 1996), since these are the values that will be monitored in order to detect changes.

While k-means is the most frequently used non-hierarchical method (Jackson *et al.*, 2010), the MPCK-means algorithm used in the present study permitted the inclusion of a whole new dimension into the definition of types, which is the variation that occurs within each unit. This seems to be a promising way of incorporating not only natural variability but also the variability that is inherent to the sampling method, since each site is represented by a neighbourhood of 6 linked replicates. In fact, in the context of environmental monitoring, two sites can only belong to different types if they can be distinguished even when considering within-type variability due to sampling and natural causes (Underwood and Chapman, 1998).

The metric learning process, which distinguishes MPCK-means from other constraint-based algorithms such as COP-Kmeans (Wagstaff *et al.*, 2001), is another crucial aspect, since in ecology it is often difficult to define the most appropriate distance measure to describe similarities among data points, as different measures tend to highlight different features of the data (Clarke *et al.*, 2006). In the case of univariate categories such as “species richness” and “total density” that do not benefit from the relative weighting of input variables, it is still an advantage because the algorithm still adjusts the distance measure differently for each cluster, thus allowing clusters with different shapes and sizes in multidimensional space (Bilenko *et al.*, 2004). While it can be argued that Euclidean distance is not a good starting point for dealing with species abundance data, the abundance of functional guilds has a completely different meaning, since they represent values that indicate a linear numerical deviation from a particular assemblage state or “health”. Moreover, while having two assemblages without a particular species can be meaningless in terms of similarity, having two assemblages without macrocarnivores or even without representatives of the family Gobiesocidae carries a meaning that approximates both assemblages under the assumption that they are missing those guilds for similar reasons.

The use of label-based ensemble clustering algorithms to combine clustering results from individual MPCK-means results proved an efficient method, considering that incorporating each individual learning and optimisation process that led to the final labels for each functional category would be an extremely complex process. These methods can also be useful in a monitoring context to combine data from different studies with different clustering methods (hierarchical, non-hierarchical, expert-judgement), given that the resulting labels are meaningful (Strehl and Ghosh, 2002).

For both subsets, the meta-clustering algorithm (MCLA) outperformed both the hypergraph-partitioning algorithm (HGPA) and the cluster-based similarity partitioning algorithm (CSPA) in terms of ANMI values. The poor performance of the HGPA can be attributed to the fact that this algorithm will always try to force clusters of approximately similar size (*i.e.* similar number of objects), thus yielding low resemblance with the original clustering results that showed highly unbalanced cluster sizes. The CSPA is the simplest approach, where all occurrences of a given pair of sites on the same cluster are summed up, representing a level of similarity among them. However, the presence of several sites in the Sesimbra region that frequently clustered together probably dominated this procedure, masking other less frequent patterns (see Strehl and Ghosh, 2002 for details). Therefore, running all three algorithms (supra-consensus) is an important step in the process of optimising the ANMI in the proposed classification framework, since the outcome and characteristics of individual clustering results for each functional category are unknown *a priori*. The poor results obtained with subset 2 stress the importance of narrowing the input data according to their second stage correlation coefficients. By trying to extract a consensus from functional categories that lead to very different clustering patterns, not only do the resulting types retain little mutual information with the original clusters but also the patterns within the original data are lost in the process.

Differences found among types in the final typology showed promising results, with most functional categories showing significant differences. However, some metrics were shown to be highly variable, such as the density of the families Gobiidae and Gobiesocidae, which had low signal-to-noise ratios. These (and the other two families in the “GBGT” category) represent the most abundant, substrate-dependent cryptobenthic species on the study area (Beldade *et al.*, 2006), hence it is very important to take them into account in typology definition. However, the family Gobiidae has ontogenic changes in behaviour, from small free-swimming juveniles that gather in schools near the reef (Beldade *et al.*, 2006) to adults that become benthic and solitary, thus generating a large variability in abundance estimates. The family Gobiesocidae, in its turn, has very strict habitat requirements, with species strongly associated with cobble (*e.g.* Henriques *et al.*, 2002), thus their occurrence is more likely in transects that happen to cross cobble deposits. This inherent variability of some cryptobenthic families leads to low power to detect changes; however, more effort should be put into understanding the maximum potential of a given site to support them, in a way that power can be slightly improved by limiting within-type variability.

Variability issues and lack of power also apply to other metrics such as “pelagic/demersal ratio” and “commercial/non-commercial ratio”, which make sense conceptually but require pelagic, commercially important species that are associated with soft substrates at greater depths (Pihl and Wennhage, 2002; Pais *et al.*, 2012) to be counted through visual censuses on rocky reefs, which results on a very low signal-to-noise ratio. Another metric that is intuitively introduced in multimetric assessment tools is species richness (Pérez-Domínguez *et al.*, 2012) but results suggested that it may also be prone to excessive variation, mainly due to the probability of counting rarer species.

Overall, several metrics that have been shown to respond to nearshore anthropogenic impacts, such as the density of juveniles, the density of habitat generalists and territorial species and trophic guild composition (Henriques *et al.*, 2013a), have also shown significant differences among assemblage types, which seems like a promising result in terms of controlling natural variability. However, care should be taken when analysing metrics with low signal-to-noise ratios (such as the density of macrocarnivores and herbivores).

When clustering sites using each functional category individually, the Sesimbra region, namely the sites coded Sesimbra 1, 3 and 4, clustered separately from most of the other sites, leading to the formation of assemblage type A. In fact, all these sites are within the Arrábida Marine Park, a relatively recent protected area with unique characteristics in terms of shelter and habitat complexity which support high diversity and abundance (Gonçalves *et al.*, 2002). However, Sesimbra 2, a site only tens of meters away from Sesimbra 1 and located in deeper waters, consistently clustered with type B assemblages when using functional categories, while the discriminant analysis with species abundances was unable to separate them from type A assemblages. This is an important aspect to highlight, and seems to be related with the fact that species composition is more prone to manifest spatial autocorrelation, with nearby sites tending to share common species (Legendre, 1993), to which the Bray-Curtis coefficient is sensitive (Clarke *et al.*, 2006). However, as results in the present study seem to show, using assemblage metrics attenuates these effects, since in a functional point of view, there is no reason for two nearby sites to have the same carrying capacity and therefore the same guild abundances, total density or even species richness. Overall, as Sesimbra 2 tended to show lower density values for several metrics when compared to Sesimbra 3 and 4 and the nearby shallow Sesimbra 1, it came near the lower expected potential of type B assemblages.

Type B assemblages had an overall lower density of several guilds, which in turn can be associated with several environmental drivers, such as lower complexity (García-Charton and Pérez-Ruzafa, 2001) or higher exposure (Fulton and Bellwood, 2004). The fact that environmental conditions supporting type B assemblages were more variable than in the case of the other two assemblage types leads to the hypothesis that a lower density can also mean that frequencies of occurrence are more erratic and therefore the power to detect changes decreases. With less power, variations due to small scale habitat changes become more difficult to distinguish and therefore increasing the spatial scale (*i.e.* to include more environmental variability) is an acceptable compromise, which was accomplished automatically by the MPCK-means algorithm. The only species that characterised type B assemblages in CAP were *Gobius bucchichi* and *Oblada melanura*, due to the fact that they are more abundant in south coast assemblages, all classified as type B. This, however, did not significantly affect guild composition, with *Gobius xanthocephalus* and *Diplodus* spp. gradually outnumbering G. bucchichi and *O. melanura*, respectively, in sites located further north.

Type C assemblages, located in sites exposed to the predominant wind and wave direction, were characterised by the presence of larger fish, zooplanktivores, cold-temperate species and winter-spawners. In fact, higher exposure to wave action can encumber a site's capability to support large numbers of resident species, with highly mobile pelagic species (often zooplanktivore) approaching these sites in search of food and shelter (García-Charton and Pérez-Ruzafa, 2001), while taking advantage of their better swimming performance to resist hydrodynamic stress (Fulton and Bellwood, 2004). Moreover, these highly mobile offshore opportunistic species from upwelling-driven coasts characteristically spawn in winter, when offshore larval transport is minimised (Santos *et al.*, 2001).

Although a pilot study was performed to maximise the total number of species counted and the choice of six 50 m long replicates seems like a costly and time-consuming approach for most available monitoring budgets, results from metric variability, variation partitioning and spatial pattern discrimination found nonetheless a great amount of variation that is characteristic of highly dynamic systems with complex interactions. This points towards the need for pilot studies and power analysis prior to monitoring, since power seems to depend on what is being measured, as well as where it is being measured. The variability that is inherent to each metric, and hence its impact-detection capability, is at least type-specific, so the sample size needed to detect a fixed impact will probably be different for each assemblage type. These aspects should be a part of sampling design, so that the limitations of a monitoring programme are known (Osenberg *et al.*, 1994; Mapstone, 1995).

A typology is useless if there is no way of classifying new sites without prior knowledge on the potential state of their fish assemblages. Therefore, a simple kNN classification model was applied, where new sites are classified by comparing environmental features with a database of sites with known assemblages and negligible impact sources, in order to achieve a degree of functional homogeneity that is type-specific. This does not replace, and is not to be confused with, national and international habitat classification frameworks, such as the UK Joint Nature Conservation Committee's marine habitat classification (Connor *et al.*, 2004), which is being integrated into European decision-support tools through the European Nature Information System (http://eunis.eea.europa.eu/). These frameworks are of crucial importance to clarify and standardise terms and scales for habitat classification across the world, thus facilitating communication between countries and inter-calibration processes. The approach proposed is a fish-based classification model, specifically aimed at supporting functional guild approaches and multimetric assessment tools.

The extensive history of fish-habitat relationship studies has shown that the reasons why a particular fish is located in a particular area when an assemblage is surveyed are many, and can range from tolerance to abiotic variables such as temperature and salinity to complex biotic interactions, such as reproduction, predation and competition (Hayes *et al.*, 1996). Moreover, the presence of anthropogenic impacts can directly affect species composition and abundance but it can also affect the environment, thus indirectly affecting fish assemblages (Johnson *et al.*, 2012). These facts pose a problem in a management context, since on one hand there is a need for extremely complex models to accurately predict the exact assemblage that would be found in a site at a given time but on the other hand these models require information about variables such as temperature, salinity, nutrient concentrations, algae cover, abundance of other organisms, among others, that will probably be altered when a site to be classified is already under stress.

“Data mining” approaches such as the one used in the present study have been criticised for using indirect or coincidental relationships as if they were direct causes (Guisan *et al.*, 2002), and some authors stress the need to understand the complexity rather that simplify the problem (Diaz *et al.*, 2004). However, no assumptions regarding causality were made in the proposed framework. Environmental variables were selected by compiling a list of variables that are known to affect fish assemblages on temperate reefs and excluding the ones that are known to be directly affected by most impact sources. Therefore, given the overwhelming complexity of fish-habitat relationships, the only way to reduce the number of variables that are used to classify a new site is to reduce the complexity of the problem itself (Raudys and Jain, 1991). In this study, complexity was ultimately reduced to a simple classification problem, where the output variable is the probability of belonging to one of three assemblage types, decided through an inverse-distance weighted vote of the four nearest neighbours.

While no assumptions are made regarding the direct effect of each environmental variable on each individual fish-based metric, there is an implicit assumption that a new site with similar environmental features is expected to have metric values that fall within the expected type-specific range. It was due to this fact that variable selection through backward elimination simply focused on maximising the performance at solving this problem, and not at predicting each individual metric value, since that would greatly increase the complexity and assume that metric values were predictable by (possibly linear) combinations of the available set of environmental variables (Knudby *et al.*, 2010). The method used for variable selection will tend to remove variables that are similar across all data points or whose behaviour is more erratic, contributing to increased noise.

Therefore, the suggested removal of topography (measured through the combined topography index) was expected, since it had the smallest scale of variation available in the model, with different values for each replicate, while fetch values and depth were stable on each site. “Fetch SW”, however, was variable among sites within each type. Nevertheless, improvements made by removing such variables were not significant and they were therefore kept in the model, as the gain in performance did not justify losing their eventual contribution for the classification of new sites. This is particularly true in the case of topography, which has been shown to significantly influence reef fish assemblages in the study area (Pais *et al.*, 2013).

Other similar classification frameworks exist that are based on previous data to classify a new site. An example is the nearest-replicate classification scheme proposed by Valesini *et al.* (2003) to classify new sites in Australia into one of 6 habitat types defined by expert knowledge by using 7 environmental variables. This method bases the classification on a 2-dimensional representation of the replicates with known labels, by reducing the original 7 dimensions to 2 principal components. Although the same method can be applied in full 7-dimensional space (Valesini *et al.*, 2003), the 2-D configuration was preferred due to the possibility of graphical representation and the reduced dimensionality. This is a very useful method for a top-down approach, and principal components can be used if linearity is assumed, allowing the establishment of a boundary limit of application, outside which a new site is not classified. However, in a bottom-up definition of types, the resulting relationship among environmental variables that explain the expected functional structure are not guaranteed to be linear, and the different types will probably not be clearly distinguished by two orthogonal principal component axes. In addition, even if the classification is done in multidimensional space, using a single neighbour to classify a new site would generate too much variability, with the possibility of larger, more variable classes like type B assemblages claiming a member simply by chance.

Due to this, using a simple nearest neighbour classifier (k=1) was not considered an option, as it would not reflect the measures of uncertainty that motivated the proposed approach. Therefore, having k=4 neighbours casting a vote that is proportional to their distance to the new site in 12-dimensional space is a simple, fuzzy approach that is robust to outliers and makes no assumptions regarding relationships among input variables. In fact, kNN is the simplest supervised learning algorithm (Crisci *et al.*, 2012), which can achieve good results with relatively small training datasets (Raudys and Jain, 1991) and a performance comparable to more complex and data-demanding methods such as Artificial Neural Networks, if parameters are optimised (Liu *et al.*, 2003).

Using only 14 sites along the coast is of course not enough to build a complete classification framework upon. However, this work aimed solely to propose an alternative methodological approach to the problem of fish community assessments based on type-specific goals or reference values. The high correct classification rates obtained with the model can be a consequence of the relatively small number of sites, adding to the fact that the 8 average fetch values were necessarily similar within sites. This leads to a small database that is easy to learn and probably difficult to extrapolate from. As with other lazy classifiers, kNN should not be used to predict assemblage types outside previous experience. However, it should ideally be used in parallel with a constantly growing database and be continuously improved with new sites supporting known assemblage types and even changes in the number of types. While one must be careful when adding new dimensions to the model, as the number of labelled replicates needed is a direct consequence of this (Raudys and Jain, 1991), adding a new environmental variable to the model can be done by simply filling in its value for every entry in the database, since data normalisation is integrated into the IBk algorithm (Aha *et al.*, 1991).

Given that pristine conditions are almost nonexistent and probably unattainable in an industrialised world (Roset *et al.*, 2007), having a quality-controlled database of the least impacted sites (preferably located in marine protected areas) to represent the potential assemblages that a given set of conditions can support has been proposed as a solution for the establishment of reference values (Mee *et al.*, 2008). The typology definition and classification framework proposed in the present study is a flexible tool that can be used in parallel with such a database, while taking into account natural and sampling-related variability into the definition of assemblage types and progressively improving classification performance as more data is introduced into the database covering different conditions.

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Appendix 5A. Correspondence between codes in the CAP plot (figure 5.3) and species names.

Code	Species	Family
Cexol	*Centrolabrus exoletus* (Linnaeus, 1758)	Labridae
Cjuli	*Coris julis* (Linnaeus, 1758)	Labridae
Crupe	*Ctenolabrus rupestris* (Linnaeus, 1758)	Labridae
Dsarg	*Diplodus sargus* (Linnaeus, 1758)	Sparidae
Dvulg	*Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817)	Sparidae
Gbucc	*Gobius bucchichi* Steindachner, 1870	Gobiidae
Gcrue	*Gobius cruentatus* Gmelin, 1789	Gobiidae
Gxant	*Gobius xanthocephalus* Heymer and Zander, 1992	Gobiidae
Lberg	*Labrus bergylta* Ascanius, 1767	Labridae
Omela	*Oblada melanura* (Linnaeus, 1758)	Sparidae
Ppict	*Pomatoschistus pictus* (Malm, 1865)	Gobiidae
Ppili	*Parablennius pilicornis* (Cuvier, 1829)	Blenniidae
Sbail	*Symphodus bailloni* (Valenciennes 1839)	Labridae
Scabr	*Serranus cabrilla* (Linnaeus 1758)	Serranidae
Ssalp	*Sarpa salpa* (Linnaeus 1758)	Sparidae
Tdela	*Tripterygion delaisi* Cadenat & Blache 1970	Tripterygiidae

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Chapter 6

Identifying functional homogeneity in a dynamic environment: Application to soft-substrate fish assemblages off the Portuguese coast.

Miguel Pessanha Pais, Sofia Henriques, Alberto G. Murta, Manuela Azevedo, Maria José Costa, Henrique N. Cabral.

Abstract

In fish community assessment, measures of functional integrity have shown to be the most suited to detect anthropogenic impacts in a background of natural variation. However, minimising background noise involves the establishment of homogeneous areas, which is still mostly done based on taxonomic approaches or by relying on environmental features. The present study proposes a framework to identify functional homogeneity in demersal soft-substrate fish assemblages along the Portuguese coast, by relying on fish-based metrics describing a series of functional traits. Using metric pairwise constrained k-means (MPCK-means), sampling stations were clustered based on individual categories of functional traits, while taking into account the minimum achievable homogeneity contained in 5 years of bottom trawl surveys. All individual clustering solutions resulting from different functional categories were ultimately combined into a final set of seven assemblage types using an ensemble clustering algorithm. The effect of depth, substrate type and latitudinal variation in clustering patterns was also assessed. Biomass-based traits revealed more homogeneous patterns and facilitated the distinction of areas but the combination of biomass and abundance-based metrics achieved the best results in the definition of a final solution.

6.1. Introduction

Pelagic and demersal fish assemblages in soft-substrates have mostly been assessed and managed through single species approaches, mainly with the purpose of assuring the sustainable exploitation of a few commercially important species. However, the increasing anthropogenic pressures and the threat of climate change on the marine environment led to an increased awareness towards the existence of indivisible linkages in an ecosystem, where disrupting the balance of a fish community can lead to indirect impacts on species that have important roles or constitute valuable resources (Vasas *et al.*, 2007). In this context, the inclusion of the “Ecosystem Approach” concept into international policy was an important step but brought new challenges to the scientific community, by stressing the need to identify and fill in knowledge gaps and detect methodological misfits (Borja, 2006).

From the experience gathered in the assessment of fish assemblages in streams and estuaries, there is a general consensus regarding the need to assess functional integrity, rather than the presence or absence of particular species (Elliott *et al.*, 2007). In fact, along spatial and temporal gradients, some species are often replaced by others who occupy similar niches and are therefore functionally redundant, thus making functional guilds more resilient to natural variation (Bremner *et al.*, 2003; Nicholson and Jennings, 2004; Fulton *et al.*, 2005; Pais *et al.*, 2012; Henriques *et al.*, 2013). In addition, the response of functional guilds to stress is more predictable, as members of the same guild tend to be similarly affected (Micheli and Halpern, 2005; Mouillot *et al.*, 2012). Besides functional guilds, other metrics can give important information regarding the overall structure of an assemblage, such as species richness, total abundance and information on the average size or weight per individual (Nicholson and Jennings, 2004; Fulton *et al.*, 2005; Sousa *et al.*, 2006).

In a monitoring context, the goal is to distinguish signals from background noise. Signals constitute actual anthropogenic impacts on the ecosystem, and noise is mostly represented by natural variation and the uncertainty associated with monitoring design (Nicholson and Jennings, 2004). In short, the wider the noise range, the larger the minimum signal that can be detected, and failure to detect an impact can have ecological and socioeconomic consequences (Osenberg *et al.*, 1994). The amount of noise is usually minimised by reducing the scale to more homogeneous areas, which is frequently done either through a top-down definition of habitat types based on environmental parameters (*e.g.* Valesini *et al.*, 2003), or through a bottom-up delimitation of areas with homogeneous species composition, often inferring about environmental predictors (*e.g.* Gomes *et al.*, 2001; Sousa *et al.*, 2005). Both these approaches have some disadvantages in a community assessment context, since it is known that, particularly at larger scales, having areas that are environmentally identical is no guarantee that they will support similar assemblages (Costello, 2009), and defining areas based uniquely on the abundance of taxa leads to greater sensitivity to spatial and temporal variability (Bremner *et al.*, 2003; Pais *et al.*, 2012). While habitat types are usually defined based on some criteria of spatial homogeneity, assemblage types, in a functional sense, should represent assemblages that show a similar pattern when described by different functional categories alone, or combined into multi-trait assessment tools.

In addition, spatial homogeneity in assemblage composition is known to vary across time, through the response of biotic factors (*i.e.* tolerance, adaptation, recruitment, competition) to environmental variation (Johnson *et al.*, 2012). Indeed, inter-annual climatic oscillations have been shown to lead to short-term changes in fish assemblage composition in soft-substrate habitats (Allen, 2008; Paiva *et al.*, 2013) and nearshore reefs (Henriques *et al.*, 2007).

In this context, the present study proposes a framework to delimit functionally homogeneous assemblage types through a bottom-up approach, while ensuring that assemblages can only belong to different types if they prevail in the face of inter-annual variability. This is accomplished by adapting a method proposed in a recent work by Pais *et al.* (2013), which uses metric pairwise constrained k-means clustering (MPCK-means; Bilenko *et al.*, 2004), a semi-supervised machine learning algorithm, to group sites according to several univariate (*e.g.* species richness, total abundance) and multivariate (*e.g.* trophic, mobility, resilience) functional categories. Ultimately, clustering results for individual categories are combined using an ensemble clustering algorithm (Strehl and Ghosh, 2002), in order to find common patterns and delimit assemblage types.

The main goals of the approach are: 1) to understand how different areas of the Portuguese coast cluster when described by different categories of functional traits, while taking into account inter-annual variability, 2) to find common patterns among each individual clustering result and delimit assemblage types and 3) to analyse possible linkages between assemblage types and environmental features.

6.2. Material and methods

6.2.1. Study area

The Portuguese coast (SW Europe) extends from 41° 50’ N down along the 9° W meridian, then eastwards at around 37° N (figure 6.1). In the north-western coast, the continental shelf is wider (70 km) and coarse sand and gravel deposits are more frequent. In the centre there are three submarine canyons, with the Nazaré canyon being the deepest and northernmost (39º 36’ N), followed by the Lisbon (38º 27’ N) and Setúbal (38º 17’ N) canyons. In the south-western coast, the shelf is narrower and steeper (20−30 km), with a lower energy environment promoting the deposit of fine sand near the coast. In the south coast (Algarve), mud deposits are frequent. In deep areas along the coast, mud and fine sand are predominant (Gomes *et al.*, 2001; Martins *et al.*, 2012).

Near the coast, upwelling occurs mainly from April to September, due to prevailing northerly winds. Winter upwelling occurs mainly in years of positive NAO, when northerly winds are more frequent (see Henriques *et al.*, 2007 for further details). The south coast is located near the Mediterranean and north-western Africa and is influenced by unique oceanographic processes, like the westward and northward drift of dense Mediterranean waters at higher depths, contrasting with the eastward drift of Atlantic waters near the surface (Mason *et al.*, 2005).

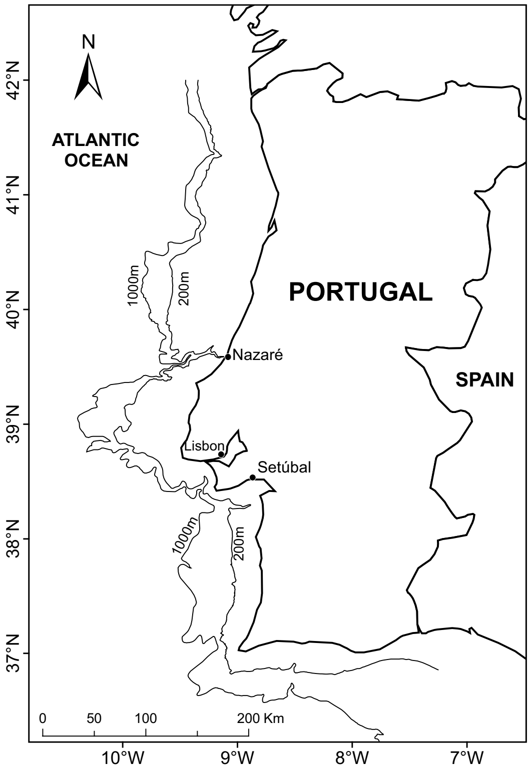

Figure 6.1. Map of the Portuguese coast with lines representing the 200 m and 1000 m isobaths. Survey data only covered depths down to 500 m but a deeper isobath is displayed for a clearer visualisation of submarine canyons.

6.2.2. Sampling method and fish-based metrics

Fish assemblage data was gathered for five consecutive years, from 2006 to 2010, in bottom trawl surveys of the Instituto Português do Mar e da Atmosfera (IPMA; Portuguese Institute for the Ocean and Atmosphere), during September−October. This period marks an important climatic oscillation, since 2006 to 2008 had positive winter NAO index, while the 2009/2010 and 2010/2011 winters had strongly negative NAO index (NOAA data based on monthly averages, available at www.cpc.ncep.noaa.gov). Sampling stations were sampled every year with a 30-minute tow at a constant speed of approximately 3.5 knots during daytime, using a bottom trawl with a 14 m headline, 20 mm cod-end mesh size and ground rope with rollers. All hauls were imported into geographical information system (GIS) software and represented by their start and end points, so that each sampling station was identified by a cloud of neighbouring points. All points were then converted to Voronoi cells that were merged into a polygon per station, representing all points that are closer to that station's samples than to any other point (Aurenhammer, 1991).

Species whose lifecycle is mostly pelagic were removed, since bottom trawl highly underestimates their abundance. These species were *Alosa falax*, *Auxis rochei*, *Atherina presbyter*, *Belone belone*, *Engraulis encrasicolus*, *Gadiculus argenteus*, *Liza* spp., *Macroramphosus* spp., *Mola mola*, *Sardina pilchardus*, *Scomber* spp., *Spicara maena* and *Vinciguerria poweriae*. Additionally, the species *Micromesistius poutassou*, *Trachurus trachurus* and *Trachurus picturatus*, although they constitute an important part of the catch in Portuguese bottom trawl fisheries, were removed from the analysis as they would lead to increased variability due to gear selectivity and distributional patchiness and would therefore mask underlying patterns and dominate clustering solutions. After removal of these species, 120 species were left on the database and used in the analyses. Fish were weighted and both abundance (fish per hour) and biomass (kg per hour) data were used in the analyses.

Fish-based metrics and functional traits were selected taking into account previously studied patterns and responses to disturbance. For each tow, species richness, average trophic level per fish (Pauly and Christensen, 1998) and average weight (kg) per fish were calculated, followed by 23 metrics measured in both biomass and abundance (see table 6.1 for a detailed description). A total of 49 metrics were used, some individually (univariate categories) and some organised into multivariate functional categories, namely trophic guilds, mobility, commercial value, resilience and biogeography. Species were classified into guilds based on previous studies (Henriques *et al.*, 2008; Pais *et al.*, 2012) and FishBase online database (Froese and Pauly, 2012). Trophic guilds were based on the classification adopted by Henriques *et al.* (2008) and species were allocated according to food items and known diet data available on FishBase. Resilience guilds are based on the estimated minimum population doubling time (Musick, 1999). Metrics under the "univariate" group in table 6.1 were used individually in the analyses, while all other metrics were used as part of their multivariate categories.

Table 6.1. Description of the metrics that integrate the univariate and multivariate functional categories used.

Category	Metric	Description
Univariate	Species richness	Total number of species.
	Average trophic level	Average number of energy-transfer steps required to get to the position that each fish occupies in the food chain.
	Average weight	Average kilograms per fish
	Total abundance	Total number of individuals.
	Total biomass	Total kilograms of catch.
	Dominance	Number of species that make up 90% of the total abundance/biomass.
	Rock dependent	Total abundance/biomass of species that depend on rocky substrates for a part of their life cycle.
	Soft-substrate residents	Total abundance/biomass of species that live in close relationship with soft-substrate areas throughout their life cycle.
	Offshore residents	Total abundance/biomass of species that spend most of their life cycle in deep, open waters far from the coast.
	Flatfish	Total abundance/biomass of fish of the order Pleuronectiformes.
	Chondrichthyes	Total abundance/biomass of sharks, rays, skates and chimaeras.
Trophic guilds	Invertebrate feeders	Density of fish that feed mostly on non-planktonic invertebrates.
	Omnivores	Density of opportunistic feeders and detritivores.
	Zooplanktivores	Density of fish that feed mostly on planktonic invertebrates and fish eggs/larvae.
	Macrocarnivores	Density of fish that feed mostly on macroinvertebrates and fish (includes piscivores).
Mobility	High mobility	Density of highly mobile and migratory fish.
	Medium mobility	Density of fish with movement patterns on the order of tens of meters.
	Sedentary	Density of benthic fish with limited movement and well defined home ranges.
Commercial value	Low value	Total abundance/biomass of species with low or no commercial value in the fisheries industry.
	Medium value	Total abundance/biomass of species that are frequently caught but do not have high value in the market.
	High value	Total abundance/biomass of species with high commercial value.
Resilience	Low/very low	Density of fish with minimum population doubling time of > 4.5 years.
	Medium	Density of fish with minimum population doubling time of 1.4−4.4 years.
	High	Density of fish with minimum population doubling time of up to 1.4 years.
Biogeography	Temperate	Density of fish that occur in cold and warm-temperate areas.
	Warm-temperate	Density of fish that occur from the western entrance of the English Channel to the Mediterranean and north-western coasts of Africa.
	Eurythermic	Density of fish that occur in a wide latitudinal range.

6.2.3. Environmental variables and trawling impact

In order to detect possible links between the achieved assemblage types and the environment, data on some of the most frequent environmental factors affecting soft-substrate fish assemblages were collected. Depth per trawl was measured as the average between its start and end points. Substrate types were taken from official sediment charts of the Portuguese Hydrographic Institute (scale 1:150,000) and classified as gravel (more than 15% of particles with size >2 mm), coarse sand (predominantly ≥0.25 mm), fine sand (predominantly <0.25 mm and less than 25% <0.062 mm) and mud (more than 25% <0.062 mm). Each tow was assigned to the substrate type upon which it travelled the most distance. Additionally, since many environmental patterns occur along a latitudinal gradient (*e.g.* climate, average sea surface temperature, influence of Mediterranean waters, shelf morphology, proximity to biogeographic regions), the average latitude for each tow (in decimal degrees) was used as a proxy for all these patterns. No redundant ($r > |0.95|$) environmental variables (including substrate types with binary coding) were found in Pearson correlation matrices.

In order to delimit areas with some degree of functional homogeneity in the face of spatial and inter-annual variability, there is a need to minimise the confounding effect of fishing intensity (*e.g.* bottom trawl), the main activity responsible for both direct and indirect impacts on soft-substrate fish assemblages (Broadhurst *et al.*, 2006). In a recent study by Henriques *et al.* (unpublished results), trawling pressure on the study area was quantified using Vessel Monitoring System (VMS) data from 2006 to 2007 (as it can be assumed representative of the whole 2006−2010 period). Points were filtered by speed (2−5 knots) and areas closer than 6 nautical miles from the coastline were discarded (due to port activity and trawl prohibition). Route lines were drawn per day and vessel and the density of route lines per square nautical mile was calculated. The resulting levels of intensity were then divided into five relative classes and every sample was labelled with its corresponding class (Henriques *et al.*, unpublished results). The balance between information loss and impact minimisation was then visually assessed, by successively removing a class of fishing intensity at a time, from highest to lowest, and seeing the amount of information loss in a spatial representation of all sampling units. This has led to the decision of discarding only the level 5 intensity class, since discarding also level 4 would lead to a loss of spatial cover in some sections of the coast. After the removal of 27 hauls located in areas with higher fishing pressure, a total of 423 hauls (20−460 m deep) performed in a period of 5 years across 86 sampling stations were used in the analyses.

6.2.4. Definition of assemblage types

6.2.4.1. Clustering sampling stations according to categories of fish-based metrics

In order to cluster sampling stations while taking into account inter-annual variability, a metric pairwise constrained k-means (MPCK-means; Bilenko *et al.*, 2004) algorithm was used. While its goal is similar to traditional unsupervised k-means clustering, MPCK-means adds the possibility of defining pairwise must-link and cannot-link constraints and a metric learning procedure (Bilenko *et al.*, 2004). On the supervised phase of the process, must-link constraints were created among each pair of hauls within a sampling station, so that every station was represented by a cloud of must-linked points that the algorithm tries not to dissociate, while at the same time minimising distances to centroids within each cluster. The metric learning process, in its turn, adjusts the original Euclidean distance matrices by weighting input variables so that the distance between must-link pairs is minimised. This is done separately for each cluster and re-adjusted on each iteration, allowing clusters to have different shapes (Bilenko *et al.*, 2004). For each individual functional category, a maximum of 10 initialisations using a "weighted farthest-first" criterion (Bilenko *et al.*, 2004) were used to optimise the initial cluster seeds, with a maximum of 200 iterations to reach an optimal solution.

Although constrains are violable, there is a need to define a parameter *w*, which represents the cost of violating a constraint, relative to the cost of increasing within-cluster distance. Moreover, as in traditional k-means, the number of clusters k must be defined *a priori* (Bilenko *et al.*, 2004). Since there is no interest in violating constraints and k and *w* are unknown for every category and univariate metric, a method was applied to optimise both parameters so that within-cluster distances were minimised and no constraints were violated. This method involves setting a value for k and gradually increasing *w* by integer values until no constraints are violated, then k is increased and the same step is repeated until the algorithm returns one or more empty clusters at k = n, which leads to a choice of k = n – 1 for that category. This assumes that empty clusters are a collection of boundary objects, which are claimed by their must-link pairs, thus impeding the algorithm from converging into a solution for that number of clusters.

Gregarious species and distributional patchiness can lead to outlying samples, which in turn would affect the MPCK-means results. This problem was minimised by eliminating local outliers for each functional category, through a density-based multivariate outlier detection algorithm. In this method, the relationship between the density of points around a sample and the density of points surrounding its *x* nearest neighbours is used to calculate a local outlier factor (LOF; Breunig *et al.*, 2000) for each sample, meaning in general terms that a LOF higher than 1 may be considered relatively deviant from the neighbouring pattern. This is accomplished by calculating a LOF for every sample using the nearest *x* neighbours, for every *x* within a specified range (in this study, values between 10 and 20 were adopted), and returning the highest achieved LOF as the final value for that sample. In the end, samples with a LOF above a defined threshold are considered outliers and discarded (see Breunig *et al.*, 2000 for details). In the present study, establishing a threshold value of 2 was a more conservative approach that led to discarding significantly fewer samples, while retaining a larger number of relatively cohesive samples (LOF < 2) as input for the MPCK-means algorithm. Some advantages of this approach are the fact that the number of outliers to discard is not pre-defined but is instead dictated by the data, and the fact that the concept of “isolation” in space is relative to the neighbouring density (meaning that a sample must have a much greater distance from a naturally dispersed neighbourhood, when compared to a more cohesive one, to be considered an outlier) (Breunig *et al.*, 2000).

In order to analyse the combined role of environmental variables (depth, latitude and substrate) in explaining MPCK-means clustering results, a model distance matrix was built for every functional category, so that hauls in the same cluster have distance 0 and hauls in different clusters have distance 1. Distance based linear models (DISTLM; McArdle and Anderson, 2001), where then used to find the best combination of environmental variables to model each distance matrix using the ‘BEST’ selection procedure to minimise a distance-based analogue to the Akaike Information Criterion (AIC; Akaike, 1973). All substrate categories were coded as binary variables and merged into a single set in the DISTLM procedure (Anderson et al, 2008).

6.2.4.2. Cluster ensembles and characterisation

In order to combine MPCK-means clustering results for individual functional categories into a final set of assemblage types, the cluster ensemble method described by Strehl and Ghosh (2002) was applied. This method is based simply on cluster membership, and achieves a final assembled result based on cluster labels (*i.e.* cluster name or number) assigned to each sampling station. In this way, it assumes that sampling stations with similar patterns across a set of functional categories should belong in the same type.

Strehl and Ghosh (2002) propose three algorithms to reach an ensemble clustering solution, namely the cluster-based similarity partitioning algorithm (CSPA), the hypergraph-partitioning algorithm (HGPA) and the meta-clustering algorithm (MCLA), each with a different approach and different performances according to the characteristics of the dataset. The method followed, therefore, was to run all three algorithms and choose the one with the best overall result (known as the ‘supra-consensus’ function). The performance of these algorithms is measured by calculating pairwise mutual information among the solution and each input set of cluster labels, and normalising the final result into an index that varies from 0 (low) to 1 (high), known as the average normalised mutual information (ANMI; Strehl and Ghosh, 2002).

Second stage Spearman rank correlations (Somerfield and Clarke, 1995) were used to compare Euclidean distance matrices among samples for every functional category, using untransformed data (units within categories are similar). Correlations were then represented graphically by using the complete linkage method in agglomerative hierarchical clustering (figure 6.2), so that each node in the dendrogram represents the minimum Spearman correlation among members of each group. There were several subgroups of functional categories that led to similar distance matrices but there was not a clear separation of a few outlying categories that could be discarded without losing important information. It was therefore decided to include all functional categories as input for the ensemble clustering phase, so that all subgroups of patterns are taken into account and weighted by the number of functional categories they contain (*i.e.* if more categories lead to one clustering pattern, that pattern is more likely to be reflected in the final solution).

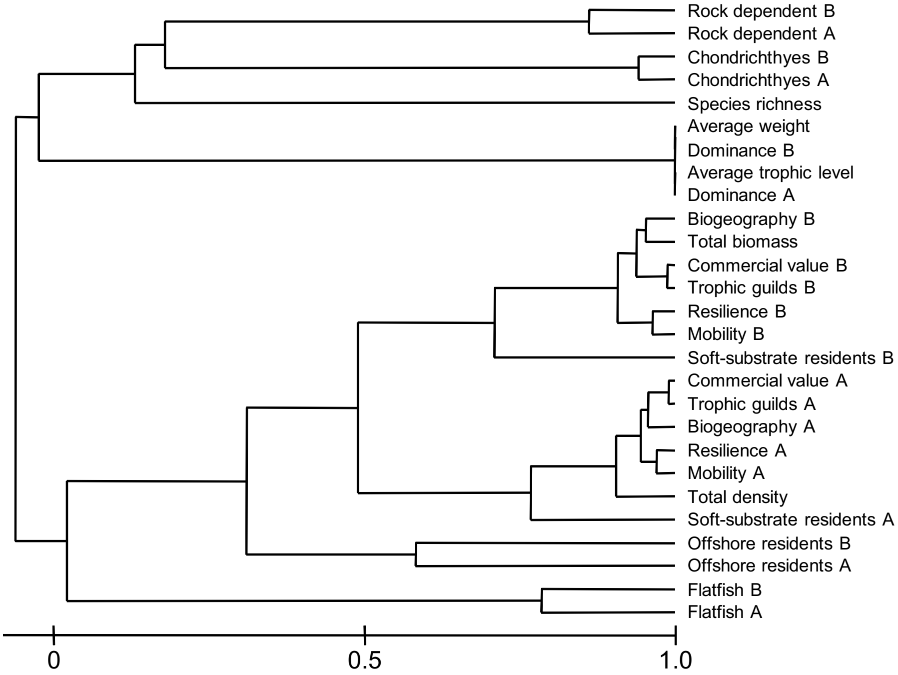

Figure 6.2. Complete linkage clustering dendrogram based on second stage Spearman rank correlation (horizontal axis) of Euclidean distance matrices for individual functional categories. *Legend:* A- abundance, B- biomass.

In order to test which type of data (abundance or biomass) can lead to the best results, or whether a combination of the two is more advantageous, three subsets of functional categories were used as input for the ensemble clustering algorithms: one with all categories, one with all abundance-based categories and one with all biomass-based categories, which led to the establishment of 3 candidate sets of assemblage types.

The final sets of assemblage types were then compared according to within-type cohesion and among-type differences, by looking at the variability occurring at several assessment scales (type, station and individual hauls) for each functional category in each of the three sets. This was accomplished through permutation-based (multivariate or univariate) nested analysis of variance (PERMANOVA) based on Euclidean distance matrices. In this way the analysis is analogous to traditional ANOVA or MANOVA but the significance of the statistic is calculated not from a table of a known probability distribution but through 9999 permutations of residuals to represent the null hypothesis (Anderson, 2001; McArdle and Anderson, 2001). The effect of assemblage types (fixed), sites (random, nested in types) and residuals (transects) was calculated through sequential (type I) sum of squares and results were considered significant at $P < 0.05$.

The estimated components of variation for the three hierarchical levels (types, sites and residuals) were compared for all functional categories in all three sets. The set showing a significant effect of factor “types” for the most categories, with the highest percentage of variation associated with higher level factors (*i.e.* types and stations) was chosen as the final adopted configuration. The final set of assemblage types was then characterised, by calculating average values and standard deviations for every metric, so that both the magnitude of values for each assemblage type and the variation of each metric in the face of inter-annual variability could be analysed.

6.2.5. Algorithms and software

PRIMER 6 software with PERMANOVA+ package (www.primer-e.com) was used to compute second stage Spearman rank correlation matrices, hierarchical clustering, CAP and PERMANOVA. Calculation of local outlier factors, outlier elimination and MPCK-means clustering were performed using RapidMiner 4.2 (www.rapid-i.com). Ensemble cluster algorithms were run in a specialised MATLAB package (available at http://www.lans.ece.utexas.edu/~strehl/soft.html).

6.3. Results

6.3.1. Clustering of sampling stations according to individual categories

In the dendrogram based on second stage Spearman correlations among Euclidean distance matrices for each functional category (figure 6.2), a great heterogeneity of responses was evident, and even some abundance/biomass pairs of the same category showed different patterns. This was particularly noticeable on the subgroup formed by biogeography, total abundance/biomass, trophic guilds, resilience, mobility and sand residents, where the pattern is similar within abundance or biomass data but both types of data only cluster near a minimum coefficient of 0.5. On the other hand, rock dependent, Chondrichthyes and Flatfish tend to show similar patterns when calculated in terms of abundance and biomass, while Dominance (in abundance and biomass), average weight and average trophic level lead to exactly the same pattern in terms of ranked distances among samples (second stage $r_s = 1$).

The number of partitions k achieved with the MPCK-means algorithm varied between 2 and 7, for abundance data (figure 6.3) and 3 and 7, for biomass data (figure 6.4). Most categories led to a partitioning of the dataset that contains one large cluster, covering most of the study area, interspersed by smaller clusters restricted to a few sampling stations, which differ among categories. In general, clusters with higher species richness (figure 6.3a), as well as total biomass (figure 6.4b), tend to be located in the centre and southwest coasts, while higher average trophic levels (figure 6.3) can be found in a single cluster covering the northwest and centre. In terms of biogeographic affinity (figures 3i and 4i), a latitudinal gradient is also evident, with a large number of small clusters forming in the south and southwest coasts featuring a larger average abundance and biomass of warm-temperate species.

As expected, areas near the coast stand out in terms of the abundance of rock dependent species (figure 6.3e), while the algorithm was only able to separate areas in the southwest when using biomass data (figure 6.4e).

Results of the DISTLM procedure revealed the cases where some of the three most commonly used environmental variables (depth, latitude-related patterns and substrate) were not informative to model cluster membership for each functional category (table 6.2). R^2 values were generally low, with the percentage of variation explained by models ranging between 3.2% (flatfish abundance) and 32.8% (species richness). Depth was included in almost all models, being only excluded for species richness, dominance (in abundance) and the abundance of trophic guilds. Latitude (*i.e.* latitudinal patterns) did not contribute with additional information regarding total abundance, abundance of offshore residents, abundance of flatfish and biomass of sand residents. Substrate type was included in all models except for commercial value (in abundance) and biogeography (in biomass).

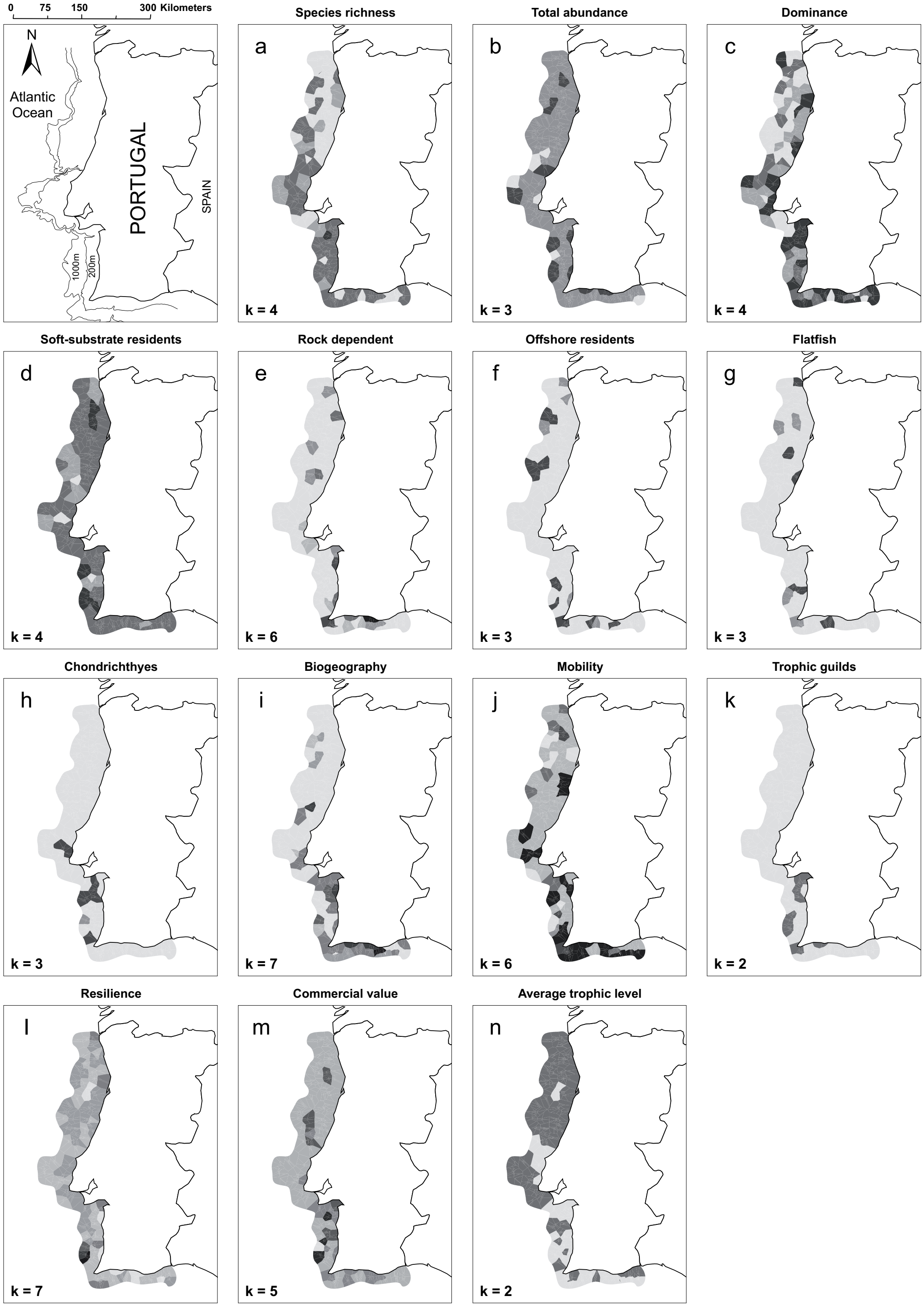

Figure 6.3. MPCK-means clustering results for individual abundance-based functional categories (including average trophic level). The number of clusters *k* for each solution is represented, and darker shades denote higher average values for fish-based metrics per cluster. In the case of multivariate categories, a single metric was chosen to be represented the category in the shaded scale: Biogeography- warm-temperate species, Mobility- highly mobile species, Trophic guilds- macrocarnivores, Resilience- high, Commercial value- high value.

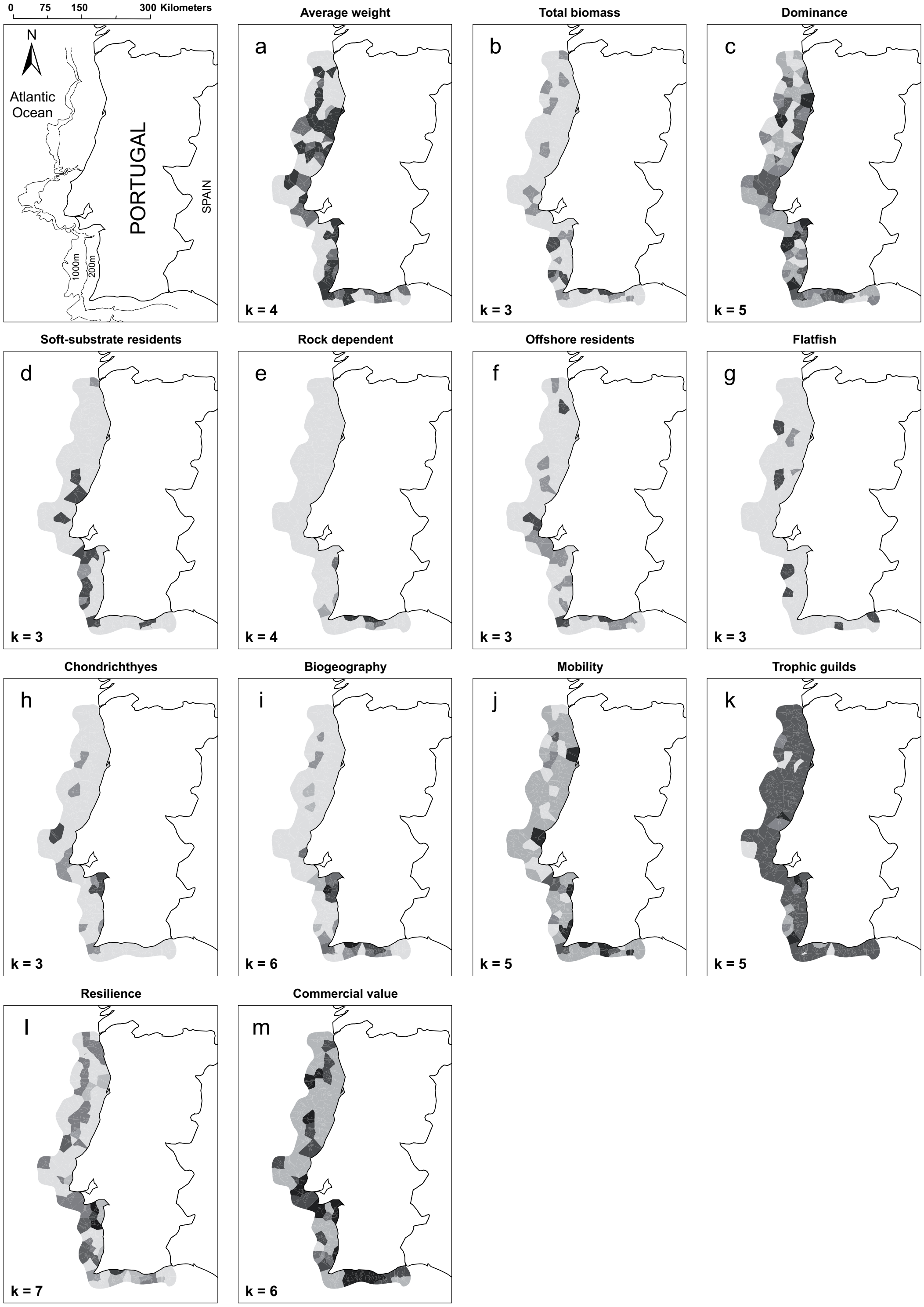

Figure 6.4. MPCK-means clustering results for individual biomass-based functional categories. The number of clusters *k* for each solution is represented, and darker shades denote higher average values for fish-based metrics per cluster. In the case of multivariate categories, a single metric was chosen to be represented the category in the shaded scale: Biogeography- warm-temperate species, Mobility- highly mobile species, Trophic guilds- macrocarnivores, Resilience- high, Commercial value- high value.

Table 6.2. Results of the distance-based linear models for individual functional categories, using a brute-force selection procedure to model cluster memberships resulting from MPCK-means clustering. Environmental variables selected in the best model for each case are marked with an x, and individual R^2 and Akaike Information Criterion (AIC) values are stated.

	Functional categories	Depth	Latitude	Substrate	AIC	R^2
General	Species richness[a]		x	x	-800.3	0.328
	Average trophic level[a]	x	x	x	-576.4	0.173
	Average weight[b]	x	x	x	-548.5	0.124
Abundance	Total abundance	x		x	-778.4	0.137
	Dominance		x	x	-458.7	0.111
	Rock dependent	x	x	x	-1242.7	0.155
	Soft-substrate residents	x	x	x	-607.7	0.105
	Offshore residents	x		x	-776.5	0.061
	Flatfish	x		x	-847.9	0.032
	Chondrichthyes	x	x	x	-1067.3	0.100
	Trophic guilds		x	x	-1060.2	0.128
	Mobility	x	x	x	-546.4	0.141
	Commercial value	x	x		-689.6	0.111
	Resilience	x	x	x	-503.5	0.067
	Biogeography	x	x	x	-659.4	0.231
Biomass	Total biomass	x	x	x	-838.7	0.171
	Dominance	x	x	x	-425.4	0.076
	Rock dependent	x	x	x	-801.9	0.132
	Soft-substrate residents	x		x	-734.6	0.128
	Offshore residents	x	x	x	-736.7	0.126
	Flatfish	x	x	x	-917.7	0.073
	Chondrichthyes	x	x	x	-893.1	0.079
	Trophic guilds	x	x	x	-804.8	0.067
	Mobility	x	x	x	-570.8	0.071
	Commercial value	x	x	x	-552.8	0.113
	Resilience	x	x	x	-510.6	0.126
	Biogeography	x	x		-736.8	0.123
Ensemble results	Abundance only	x	x	x	-402.9	0.123
	Biomass only	x	x	x	-398.0	0.111
	All	x	x	x	-422.1	0.160

[a] Metric included in the abundance subset for ensemble clustering.
[b] Metric included in the biomass subset for ensemble clustering.

6.3.2. Ensemble clustering and definition of assemblage types

All ensemble clustering results for the three subsets used (all categories, biomass only and abundance only) achieved their highest ANMI values with the CSPA algorithm and k=7 clusters (figure 6.5). However, the achieved ANMI values were not very high (0.225 for CSPA abundance, 0.240 for CSPA biomass and 0.211 for CSPA all). Depth, latitude and substrate were all important in explaining variation among clusters in the three ensemble subsets (table 6.2), with CSPA all achieving the highest R^2, followed by CSPA abundance and CSPA biomass.

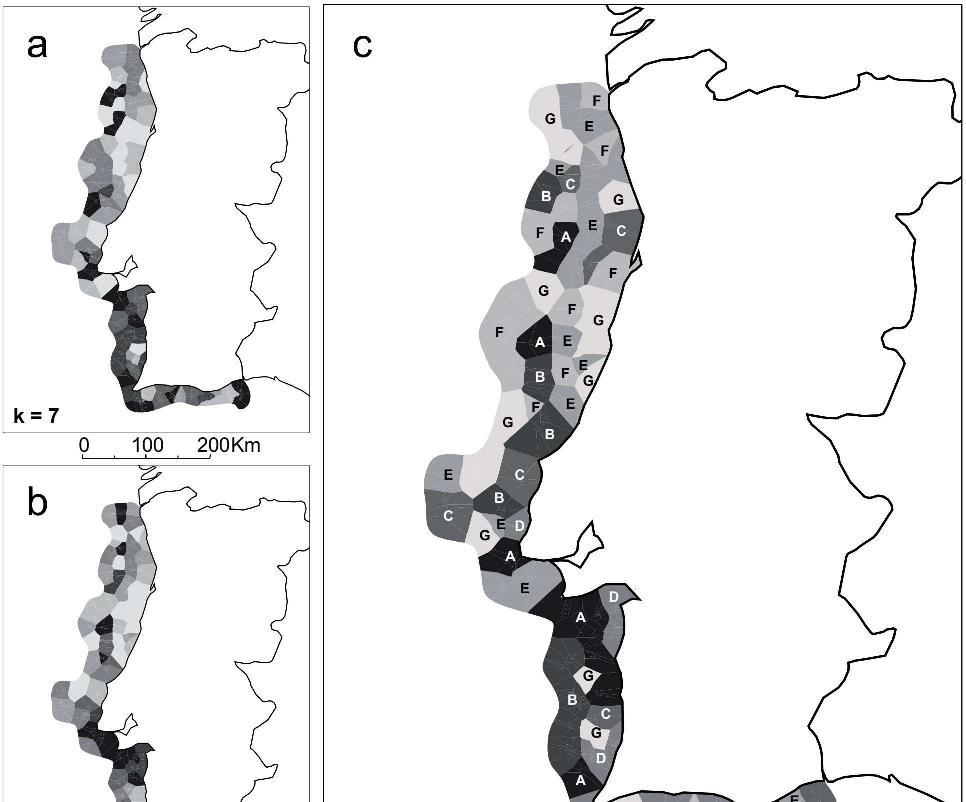

Figure 6.5. Results of the ensemble clustering process using cluster-based similarity partitioning algorithm (CPSA) with three different subsets of functional categories: a) only abundance-based categories, b) only biomass-based categories, c) all categories. The number of clusters k for each solution is represented, and darker shades represent higher average total abundance (a, c) or total biomass (b) per cluster. Final ANMI values for the three subsets were (a) 0.225, (b) 0.240 and (c) 0.211.

The 7 clusters formed by each of the three ensemble results represent three candidate sets of assemblage types, one maximising within-type homogeneity in terms of abundance (CSPA abundance), another maximising homogeneity in terms of biomass (CSPA biomass), and, finally, one seeking a pattern that integrates both types of data into a single solution (CSPA all). In order to choose the best candidate for a final set of assemblage types, nested PERMANOVA (table 6.3) was used to quantify the components of variation attributed to each of three different scales (hauls, sampling stations and assemblage types). It is evident that the subset using only abundance data had the poorest performance, with only 4 functional categories, besides species richness and average trophic level, showing significant differences among types (namely dominance, rock dependent, offshore residents and Chondrichthyes). When using biomass-based categories to define assemblage types, abundance-based metrics did not fit the overall pattern, with no significant differences among types in most cases. However, results for biomass-based categories were considerably better, with all categories (except for the biomass of flatfish) showing significant differences among types with a higher percentage of variation attributed to assemblage types.

Remarkably, when incorporating all categories (including abundance/biomass pairs of the same metrics) to reach an ensemble clustering solution (CSPA all), all functional categories showed significant differences among types (except for the abundance and biomass of flatfish). This was accompanied by a higher percentage of variation attributed to assemblage types and a lower percentage attributed to among-station variability within types.

Since CSPA all optimised the relationship with environmental variables and the differences among assemblage types for abundance and biomass data (tables 2 and 3), this was adopted as the final set of assemblage types (figure 6.5c).

6.3.3. Characterisation of assemblage types

The final seven types were labelled A to G, from highest to lowest average total abundance. Each assemblage type was then characterised according to average values for environmental variables (table 6.4), average values for each individual metric (table 6.5) and the average proportion of guilds within functional categories (table 6.6).

Table 6.4. Average values and standard deviations (in brackets) for environmental variables, based on the location of hauls assigned to each of the seven assemblage types. For sediment types, percentages represent the proportion of hauls that fall within each substrate category.

			Sediment type			
Type	Latitude (ºN)	Depth (m)	M	FS	CS	G
A	38.463 (1.242)	157.5 (65.1)	28.0%	70.0%	2.0%	0.0%
B	38.723 (1.241)	259.7 (119.1)	50.8%	40.7%	0.0%	8.5%
C	38.633 (1.61)	174.1 (105.3)	28.0%	28.0%	18.0%	26.0%
D	37.273 (0.566)	97.9 (62.1)	52.5%	24.6%	3.3%	19.7%
E	40.201 (1.382)	104.5 (58.8)	32.1%	49.4%	1.2%	17.3%
F	40.399 (0.94)	146.6 (75.5)	23.5%	57.4%	7.4%	11.8%
G	39.419 (1.48)	116.6 (49)	14.8%	49.2%	24.6%	11.5%

M- mud, FS- fine sand, CS- coarse sand, G- gravel.

Type A assemblages were mainly located in fine sand deposits (occasionally with mud) at intermediate to deep locations, located in several areas from north to south, including the area surrounding the Lisbon and Setúbal canyons (figure 6.5, table 6.4). These assemblages have the highest average total abundance, total biomass and species richness (table 6.5), with invertebrate feeders representing 92.2% of the total abundance (table 6.6). They also have the highest average biomass of Chondrichthyes, the highest average weight per fish (table 6.5) and highest proportion of species with low commercial value (table 6.6).

Some of the deepest areas (mostly > 200m), including sampling stations around the Nazaré canyon, tended to support type B assemblages. These assemblages were mostly located over areas of mud and fine sand, in a wide latitudinal range along exposed, west-facing areas of the coast (figure 6.5c, table 6.4). Type B also has relatively high average species richness, total abundance and total biomass, the highest average abundance of offshore residents, the lowest abundance of rock dependent species and the lowest average weight per fish. The highest biomass of flatfish and the highest abundance of Chondrichthyes also belong to type B assemblages (table 6.5). In terms of guild proportions, invertebrate feeders and macrocarnivores are equally present, with the first being more abundant. This is also reflected in the patterns observed for the commercial value and resilience categories (table 6.6).

Table 6.5. Average values and standard deviations (in brackets) for every fish-based metric within each of the seven assemblage types.

	Category	Metric	Units	A	B	C	D	E	F	G
General		Species richness	nr. species	11 (7)	10 (3)	8 (3)	10 (3)	6 (3)	6 (3)	7 (3)
		Average trophic level	–	3.85 (0.43)	3.85 (0.44)	3.82 (0.49)	3.62 (0.31)	4.20 (0.32)	4.19 (0.36)	4.03 (0.27)
		Average weight	kg	0.131 (0.084)	0.084 (0.058)	0.102 (0.068)	0.126 (0.052)	0.107 (0.046)	0.092 (0.053)	0.121 (0.071)
Abundance	Univariate	Total abundance	fish.h^{-1}	7328.8 (20811.8)	2319.3 (3149.8)	1292.5 (5863.3)	1163.3 (1101.6)	602.087 (632.64)	568.668 (552.128)	363.767 (450.641)
		Dominance	nr. species	3 (2)	2 (2)	3 (2)	4 (2)	2 (1)	2 (1)	3 (2)
		Rock dependent	fish.h^{-1}	96.2 (91.2)	41.8 (77.5)	63.1 (175.2)	603.9 (747.8)	73.3 (257.3)	31.7 (57.9)	51.0 (83.9)
		Soft-substrate residents	fish.h^{-1}	7106.7 (20815.7)	2211.5 (3144.0)	1113.8 (5842.0)	412.1 (932.9)	519.0 (599.5)	496.9 (537.7)	301.5 (439.8)
		Offshore residents	fish.h^{-1}	409.0 (579.0)	558.3 (839.6)	192.4 (189.5)	500.8 (498.8)	461.6 (564.2)	496.2 (532.0)	215.5 (291.0)
		Flatfish	fish.h^{-1}	3.2 (4.4)	10.0 (25.4)	4.2 (10.2)	10.9 (27.1)	2.8 (4.5)	7.5 (12.2)	5.3 (21.8)
		Chondrichthyes	fish.h^{-1}	3.9 (6.0)	5.3 (17.1)	0.6 (1.1)	1.5 (2.9)	0.1 (0.628)	0.0 (0.3)	1.0 (3.1)
	Trophic	Invertebrate feeders	fish.h^{-1}	6745.9 (20822.9)	1687.7 (3059.1)	966.2 (5795.6)	219.6 (832.0)	63.6 (244.4)	15.1 (24.4)	101.4 (316.8)
		Omnivores	fish.h^{-1}	37.1 (56.5)	5.6 (18.7)	18.1 (60.8)	328.5 (475.1)	7.9 (31.1)	5.5 (37.4)	21.2 (57.2)
		Zooplanktivores	fish.h^{-1}	34.9 (123.6)	0.5 (1.6)	0.7 (2.2)	7.8 (36.7)	0.2 (0.9)	0.3 (1.1)	0.1 (0.4)
		Macrocarnivores	fish.h^{-1}	498.5 (583.0)	619.0 (857.5)	259.3 (223.5)	601.0 (578.3)	524.5 (606.8)	520.9 (536.6)	241.0 (304.6)
	Mobility	High	fish.h^{-1}	127.8 (148.2)	45.0 (70.1)	98.5 (188.9)	698.0 (790.7)	67.2 (249.7)	26.6 (57.5)	51.5 (84.7)
		Medium	fish.h^{-1}	7166.4 (20805.5)	2248.6 (3143.1)	1121.7 (5841.3)	440.8 (948.2)	522.8 (599.8)	505.4 (538.4)	304.6 (441.2)
		Sedentary	fish.h^{-1}	22.0 (40.0)	18.8 (31.8)	23.3 (62.7)	12.6 (36.9)	6.1 (27.1)	9.7 (21.6)	7.6 (17.5)
	Commercial value	Low value	fish.h^{-1}	6795.6 (20816.0)	1700.3 (3056.8)	972.7 (5793.5)	245.3 (829.3)	68.3 (245.3)	18.1 (55.9)	102.1 (316.3)
		Medium value	fish.h^{-1}	52.7 (73.2)	33.9 (57.6)	12.7 (22.1)	241.8 (330.9)	10.7 (31.9)	10.7 (36.9)	24.9 (58.0)
		High value	fish.h^{-1}	468.0 (589.3)	578.5 (857.1)	258.9 (239.2)	670.1 (635.0)	517.3 (607.5)	513.0 (535.0)	236.8 (306.6)
	Resilience	Low / very low	fish.h^{-1}	403.7 (591.9)	578.1 (835.0)	175.5 (189.0)	204.1 (232.5)	462.3 (566.0)	500.8 (534.5)	212.1 (293.5)
		Medium	fish.h^{-1}	174.4 (231.0)	57.6 (121.8)	101.2 (160.9)	650.5 (698.8)	72.1 (254.7)	31.4 (70.0)	55.5 (88.4)
		High	fish.h^{-1}	6738.2 (20824.9)	1676.4 (3058.6)	967.642 (5794.2)	302.3 (842.1)	61.8 (244.6)	9.6 (23.1)	96.0 (317.3)
	Biogeography	Temperate	fish.h^{-1}	7153.5 (20816.4)	2254.4 (3139.4)	1154.6 (5839.2)	717.6 (1016.2)	568.3 (629.3)	516.1 (539.2)	324.4 (444.3)
		Warm-temperate	fish.h^{-1}	57.7 (166.4)	8.6 (18.3)	15.2 (54.4)	145.4 (244.9)	7.6 (33.0)	4.2 (9.4)	3.2 (5.4)
		Eurythermic	fish.h^{-1}	104.0 (145.0)	38.7 (53.3)	71.3 (104.1)	286.3 (478.9)	19.0 (43.0)	20.4 (31.4)	33.6 (60.4)

Table 6.5 (cont.). Average values and standard deviations (in brackets) for every fish-based metric within each of the seven assemblage types.

	Category	Metric	Units	A	B	C	D	E	F	G
Biomass	Univariate	Total biomass	kg.h^{-1}	311.405 (687.450)	110.078 (98.716)	53.129 (142.672)	134.315 (117.838)	51.313 (45.875)	43.465 (50.348)	29.131 (21.152)
		Dominance	kg.h^{-1}	3.320 (1.889)	2.593 (1.261)	3.060 (1.449)	3.803 (1.526)	1.926 (1.01)	2.059 (1.006)	2.836 (1.474)
		Rock dependent	kg.h^{-1}	24.330 (24.937)	7.803 (14.844)	8.112 (24.560)	90.911 (115.572)	7.569 (17.908)	5.744 (10.454)	7.079 (10.269)
		Soft-substrate residents	kg.h^{-1}	281.546 (684.983)	99.662 (96.570)	38.585 (138.526)	35.509 (37.778)	43.400 (42.751)	38.285 (48.143)	21.623 (16.963)
		Offshore residents	kg.h^{-1}	60.966 (38.494)	46.518 (43.011)	21.453 (22.619)	76.779 (93.655)	42.023 (42.969)	37.398 (47.229)	20.277 (14.658)
		Flatfish	kg.h^{-1}	0.180 (0.316)	0.542 (1.267)	0.159 (0.400)	0.310 (0.736)	0.278 (0.869)	0.318 (0.623)	0.250 (0.620)
		Chondrichthyes	kg.h^{-1}	4.007 (8.479)	1.699 (6.403)	0.849 (1.988)	2.474 (5.459)	0.083 (0.404)	0.029 (0.239)	0.79 (2.545)
	Trophic	Invertebrate feeders	kg.h^{-1}	229.641 (678.457)	53.940 (95.322)	23.296 (136.401)	6.678 (21.877)	1.951 (6.961)	0.327 (0.383)	2.694 (8.454)
		Omnivores	kg.h^{-1}	4.018 (6.153)	0.726 (2.272)	2.654 (11.337)	40.590 (61.936)	1.127 (3.868)	0.648 (4.426)	2.305 (5.509)
		Zooplanktivores	kg.h^{-1}	1.489 (5.099)	0.008 (0.034)	0.017 (0.053)	0.313 (1.658)	0.023 (0.100)	0.029 (0.128)	0.008 (0.037)
		Macrocarnivores	kg.h^{-1}	76.237 (40.328)	55.396 (47.066)	27.028 (24.064)	86.464 (92.449)	48.209 (45.285)	42.446 (50.286)	24.124 (18.086)
	Mobility	High	kg.h^{-1}	24.419 (23.698)	5.748 (10.112)	10.566 (24.819)	97.153 (114.447)	6.962 (17.352)	4.163 (8.169)	6.761 (9.906)
		Medium	kg.h^{-1}	282.893 (686.059)	101.971 (96.860)	38.743 (138.535)	36.25 (39.071)	43.404 (42.909)	38.555 (48.165)	21.481 (16.883)
		Sedentary	kg.h^{-1}	4.071 (8.026)	2.342 (5.741)	3.675 (10.234)	0.807 (1.179)	0.943 (4.220)	0.732 (1.805)	0.888 (2.313)
	Commercial value	Low value	kg.h^{-1}	230.965 (679.181)	54.468 (95.168)	23.301 (136.366)	6.163 (21.872)	2.053 (6.967)	0.449 (1.084)	2.729 (8.414)
		Medium value	kg.h^{-1}	7.385 (9.225)	5.065 (10.568)	1.576 (2.220)	26.807 (34.781)	1.876 (4.348)	2.143 (7.809)	2.857 (5.730)
		High value	kg.h^{-1}	73.036 (38.180)	50.540 (45.872)	28.117 (30.095)	101.336 (104.943)	47.381 (44.955)	40.858 (49.597)	23.545 (17.793)
	Resilience	Low / very low	kg.h^{-1}	62.734 (37.959)	51.393 (43.136)	19.609 (21.001)	30.897 (28.731)	43.629 (43.197)	40.706 (49.355)	20.264 (15.076)
		Medium	kg.h^{-1}	20.462 (23.986)	5.218 (10.917)	8.996 (15.332)	82.946 (98.406)	5.904 (17.141)	2.558 (6.676)	6.526 (10.36)
		High	kg.h^{-1}	228.189 (678.585)	53.45 (95.021)	24.389 (136.591)	20.454 (42.450)	1.765 (6.965)	0.187 (0.681)	2.339 (8.477)
	Biogeography	Temperate	kg.h^{-1}	291.671 (686.412)	104.194 (97.007)	43.279 (138.73)	90.976 (99.739)	47.78 (44.685)	40.099 (48.690)	25.212 (19.166)
		Warm-temperate	kg.h^{-1}	3.874 (11.343)	0.393 (0.645)	1.871 (10.754)	17.896 (37.980)	0.422 (1.394)	0.216 (0.791)	0.256 (0.542)
		Eurythermic	kg.h^{-1}	15.795 (15.486)	4.877 (6.989)	7.684 (12.115)	24.85 (39.947)	3.016 (5.802)	3.093 (5.454)	3.5 (5.774)

Type C assemblages occur in a great variety of conditions, from north to south, in deeper and shallower areas and in every type of substrate (figure 6.5c, table 6.4). This type has the lowest abundance of offshore residents and intermediate values for average species richness (table 6.5). In terms of guild proportions (table 6.6), type C is very similar to type B, both in abundance and biomass, except in the biogeography category, where the average abundance of eurythermic species is higher when compared to temperate species (table 6.6).

Type D assemblages are found mostly in nearshore shallow areas with mud and fine sand deposits, located in the south and southwest coasts (figure 6.5c, table 6.4). These assemblages have the highest value for dominance, meaning that more species share the top 90% of the total abundance/biomass. The highest abundance of flatfish is also found in type D assemblages, and the abundance and biomass of rock dependent species is the highest of all types. Type D also has the lowest average trophic level and a high average weight (table 6.5). While macrocarnivores are the most represented trophic guild, the omnivores guild has the highest proportion in abundance and biomass of all types. Highly mobile and eurythermic species also occur in larger proportions in type D, when compared to other types (table 6.6).

While type D assemblages are mostly found in the south and southwest, type E assemblages are found in shallow and intermediate areas in the northwest (figure 6.5c). These assemblages contrast with type D assemblages and have much lower species richness and total abundance. Type E assemblages have the lowest abundance of flatfish, the lowest values for dominance (fewer species tend to dominate the assemblages) and the highest trophic level and commercial value (table 6.5). Species with low resilience dominate in proportion, with macrocarnivores being the most represented trophic guild (table 6.6).

Type F assemblages are mostly found to the north of the Nazaré canyon (figure 6.5c), in areas usually deeper than type E assemblages but similar in terms of substrate types (table 6.4). When compared to type E, these assemblages are equally poor in abundance and species richness, with high average trophic level and low average weight. However, rock dependent species and flatfish are less abundant in average (table 6.5). In terms of proportions, both types are very similar, apart from a higher average proportion of species with low or very low resilience and a lower proportion of species with low commercial value in type F (table 6.6).

Finally, type G assemblages occur from north to south, at different depth ranges and mostly over fine and coarse sand deposits (figure 6.5c, table 6.4). This type has the lowest total abundance and low species richness. It also has the lowest abundance of soft-substrate residents and a high average weight (table 6.5). Macrocarnivores and invertebrate feeders are the most represented trophic guilds in terms of abundance, with macrocarnivores representing a much higher proportion of total biomass (table 6.6).

Table 6.6. Proportion represented by each guild in multivariate categories within each of the seven assemblage types.

			A	B	C	D	E	F	G
Abundance	Trophic	Invertebrate feeders	92.2%	73.0%	77.7%	19.0%	10.7%	2.8%	27.9%
		Omnivores	0.5%	0.2%	1.5%	28.4%	1.3%	1.0%	5.8%
		Zooplanktivores	0.5%	0.0%	0.1%	0.7%	0.0%	0.1%	0.0%
		Macrocarnivores	6.8%	26.8%	20.8%	52.0%	88.0%	96.1%	66.3%
	Mobility	High	1.7%	1.9%	7.9%	60.6%	11.3%	4.9%	14.2%
		Medium	98.0%	97.2%	90.2%	38.3%	87.7%	93.3%	83.8%
		Sedentary	0.3%	0.8%	1.9%	1.1%	1.0%	1.8%	2.1%
	Commercial value	Low value	92.9%	73.5%	78.2%	21.2%	11.5%	3.3%	28.1%
		Medium value	0.7%	1.5%	1.0%	20.9%	1.8%	2.0%	6.8%
		High value	6.4%	25.0%	20.8%	57.9%	86.8%	94.7%	65.1%
	Resilience	Low/very low	5.5%	25.0%	14.1%	17.6%	77.5%	92.4%	58.3%
		Medium	2.4%	2.5%	8.1%	56.2%	12.1%	5.8%	15.3%
		High	92.1%	72.5%	77.8%	26.1%	10.4%	1.8%	26.4%
	Biogeography	Temperate	97.8%	97.9%	93.0%	62.4%	95.5%	95.5%	89.8%
		Warm-temperate	0.8%	0.4%	1.2%	12.7%	1.3%	0.8%	0.9%
		Eurythermic	1.4%	1.7%	5.7%	24.9%	3.2%	3.8%	9.3%
Biomass	Trophic	Invertebrate feeders	73.7%	49.0%	44.0%	5.0%	3.8%	0.8%	9.2%
		Omnivores	1.3%	0.7%	5.0%	30.3%	2.2%	1.5%	7.9%
		Zooplanktivores	0.5%	0.0%	0.0%	0.2%	0.0%	0.1%	0.0%
		Macrocarnivores	24.5%	50.3%	51.0%	64.5%	94.0%	97.7%	82.8%
	Mobility	High	7.8%	5.2%	19.9%	72.4%	13.6%	9.6%	23.2%
		Medium	90.9%	92.6%	73.1%	27.0%	84.6%	88.7%	73.7%
		Sedentary	1.3%	2.1%	6.9%	0.6%	1.8%	1.7%	3.0%
	Commercial value	Low value	74.2%	49.5%	44.0%	4.6%	4.0%	1.0%	9.4%
		Medium value	2.4%	4.6%	3.0%	20.0%	3.7%	4.9%	9.8%
		High value	23.5%	45.9%	53.1%	75.5%	92.3%	94.0%	80.8%
	Resilience	Low/very low	20.1%	46.7%	37.0%	23.0%	85.1%	93.7%	69.6%
		Medium	6.6%	4.7%	17.0%	61.8%	11.5%	5.9%	22.4%
		High	73.3%	48.6%	46.0%	15.2%	3.4%	0.4%	8.0%
	Biogeography	Temperate	93.7%	95.2%	81.9%	68.0%	93.3%	92.4%	87.0%
		Warm-temperate	1.2%	0.4%	3.5%	13.4%	0.8%	0.5%	0.9%
		Eurythermic	5.1%	4.5%	14.5%	18.6%	5.9%	7.1%	12.1%

6.4. Discussion

The present study proposed a framework to identify areas of functional homogeneity using soft-substrate demersal fish assemblages on the Portuguese coast. This method allows for large-scale inter-annual variability to be incorporated into the clustering process, so that only areas that can be distinguished even in the face of inter-annual variation are clustered separately. This way, the degree of homogeneity achieved within clusters is a function of inherent background variability. This is based on the principle that we can only be certain that an impact is occurring if it has a magnitude larger than the background variability we face with the current method (Hurlbert, 1984). All changes occurring within the range of natural variation, even if they are due to anthropogenic impacts, will certainly not be detected by the monitoring programme (Nicholson and Jennings, 2004). In the case of the annual surveys used in the present study, the five 30-minute hauls per station, one per year, performed in autumn along five consecutive years, represent the range of variation that is expected mainly due to natural causes at this scale, such as climatic oscillations reflected in the NAO index. In fact, a study performed in the area by Paiva *et al.* (2013) analysed data on fish species for the same period used in the present study (2006−2010) and noticed dramatic changes in 2009−2010 due to an abrupt change from a positive NAO index to a historically low index in the winter of 2010. However, only pelagic species, occupying lower trophic levels, were observed but this had measurable indirect impacts on top predators, such as the shearwater *Calonectris diomedea borealis* (Paiva *et al.*, 2013). In the same way, climatic oscillations and, at a larger time scale, climate change, may cause a rapid response in lower trophic levels with repercussions in whole communities (Hobday, 2011; Rombouts *et al.*, 2013). Therefore, a method that can incorporate inter-annual variability into the definition of homogeneous units will be more resilient to the dynamic nature of baseline conditions.

While there was an effort to minimise the effect of trawling impact on results, there was a need to establish a balance between the minimum acceptable level of impact and the magnitude of information loss. This led to the decision of excluding only the level of highest intensity, thus the results may still incorporate some patterns which are due to variation in trawling impact. However, in most countries with a long history of human impacts on the marine ecosystem, it is very unlikely or even impossible to find pristine conditions or areas with neglectable impacts, and even if usable pre-impact data were available, they would probably represent unattainable patterns under the present conditions (Roset *et al.*, 2007). Therefore, considering that classes of trawling impact are relative, and not absolute measures of what constitutes a “high” impact (Henriques *et al.*, unpublished results), and assuming that spatial patterns that persist over a five year period are mostly due to natural factors (Sousa *et al.*, 2005), it can be seen as an acceptable compromise.

Due to the fact that fish abundance data often presents unusual distributions, and that relationships among variables is often non-linear and with very complex interactions, the use of machine learning algorithms in the study of fish-habitat relationships is steadily increasing (Knudby *et al.*, 2010). In fact, such methods rely on very little assumptions and usually use the high processing power of modern computers to find patterns. In the present study, using MPCK-means to cluster sampling stations has two main advantages when compared to other non-hierarchical clustering algorithms. The first one is the fact that each sampling station can be represented by a cloud of points in multidimensional space that represent the variability occurring within each station. The second one is the metric learning process, which can weight input variables to better describe the proximity of points within a station. In the case of univariate measures, such as species richness, the metric learning process is useful as it allows for different clusters to have different shapes (Bilenko *et al.*, 2004), an important aspect since the magnitude of variation dictated by inter-annual variability tends to be cluster-specific (Pais *et al.*, 2013).

When clustering stations according to individual functional categories, apart from the expected pattern in biogeographic affinities, other traits revealed a latitudinal pattern. Species richness tended to be lower in the northwest coast, and trophic level was higher. The increase in species richness to the south was also observed by Sousa *et al.* (2006) and attributed to differences in shelf morphology and the influence of the Mediterranean current. In terms of trophic level, the reason for the observed patterns is probably a combination of complex interactions. However, since summer upwelling is higher in the southwest coast, thus favouring an increase in primary productivity, the predominance of lower trophic levels in the south and southwest is expected (Gomes *et al.*, 2001). In fact, assemblage types E and F, located mainly at intermediate depths in the north and centre, had higher proportions of macrocarnivores but this was clearly due to a significant decrease in the abundance and biomass of the remaining trophic guilds. In addition to topography and primary productivity, the south and southwest coasts are also characterised for having a more irregular coastline, interspersed with capes that provide shelter from prevailing north-westerly winds and waves. This combination of factors promotes the establishment of more substrate-dependent species, thus soft-substrate residents and rock dependent species are more abundant in these areas. Rock dependent species, in particular, are strongly associated with areas near rocky reefs, as they depend on them for feeding, shelter or reproduction (Pais *et al.*, 2012). In addition, the higher diversity of skates and rays in the southwest (Figueiredo *et al.*, 2007), allied to a greater availability of prey, may be related to a greater abundance and biomass of Chondrichthyes.

The abundance and biomass of flatfish were the only metrics that did not show significant differences among types in all proposed solutions. Individual clustering patterns revealed scattered areas of higher biomass or abundance that persisted but this pattern seemed to be unrelated to global trends. This is probably related to the inefficiency of the gear in capturing flatfish, as the trawl used in the surveys had rollers in the ground rope. This fact, allied to their spatial and temporal variability and distributional patchiness (Teixeira and Cabral, 2009), makes flatfish very difficult to manage with these surveys. However, due to their high economic value and strong linkages to sediment quality, it is very important that at least population-based assessments are done, and that their distributional patterns are well understood to implement appropriate management strategies (Teixeira and Cabral, 2009).

The results obtained with individual functional categories revealed a great diversity of patterns, with sampling stations clustering differently according to the functional categories used. This can be related to the results obtained by Bremner *et al.* (2003) in a study comparing different approaches to the characterisation of benthic invertebrate fauna in the English Channel, where different sampling stations grouped differently if described by taxonomic approaches, trophic guild composition or a combination of functional traits. In fact, this stresses the importance of choosing the right combination of functional traits in a monitoring context, since it is known that different traits respond differently to both natural and anthropogenic stress (Micheli and Halpern, 2005; Mouillot *et al.*, 2012). The alternative proposed in the present study is to cluster each functional category individually, allowing patterns attributable to each category to be visually analysed and incorporated into the method. This intends to minimise subjectivity when using functional traits to delimit assemblage types, since the number of traits selected would affect clustering results (Micheli and Halpern, 2005). Of course, in the ensemble clustering phase, the initial choice of categories to include is still subjective, and analysing the second stage correlation of all categories helps remove some of the subjectivity involved. For example, in the study by Pais *et al.* (2013) using rocky reef fish assemblages, a large group of functional categories were shown to have high second stage correlation, with a few categories standing out as outliers from the most common pattern. In this case, it is wiser to exclude these outliers from the ensemble phase, since they will not have enough weight to make their individual patterns represented in the final solution. Pais *et al.* (2013) noticed that using only categories with very different patterns led to a compromise that did not retain individual identities. In the present study, however, the pattern of the second stage correlation was different, with several cohesive groups that differed considerably among them. Since every category has the same weight in the cluster ensemble algorithms (Strehl and Ghosh, 2002), having several categories representing similar patterns ensured their presence in the final solution, and removing categories would weaken their representation. This is probably the main reason why combining abundance and biomass data into the final solution achieved the best results. By combining functional traits measured in abundance and biomass, similar patterns that occur irrespective of the type of data were strengthened, and the final solution was able to minimise information loss and achieve significant differences among types for almost all metrics.

Although abundance data is commonly used in the assessment and characterisation of demersal fish assemblages (*e.g.* Rogers and Ellis, 2000; Gaertner *et al.*, 2005; Sousa *et al.*, 2005), biomass-related metrics are known to be more useful in detecting the impacts of fishing (Nicholson and Jennings, 2004; Fulton *et al.*, 2005), as large-bodied species are usually targeted by fisheries, while small-sized fish in the lower levels of the food chain tend to be more affected by natural variation (Rochet *et al.*, 2010). In the present study, biomass data had an overall better performance, translated into a better connection with environmental variables and significant results among assemblage types when using biomass-based metrics alone. This reveals that biomass was more stable in the face of inter-annual variation, as the component of variation attributed to individual hauls and stations was smaller.

While most categories led to a partitioning of sampling stations into 3 or 4 clusters, the diversity of patterns found led to a final set of 7 assemblage types. This is the maximum number of clusters found in individual solutions, and all ensemble clustering algorithms were used to find a value for k with the highest ANMI value within the original range of k values (Strehl and Ghosh, 2002). Moreover, using the ‘supra-consensus’ approach, where all three ensemble clustering algorithms are used, proved to be an essential step in every assessment that applies this method. In fact, while the meta-clustering algorithm (MCLA) performed best in rocky reefs, after the removal of outliers (Pais *et al.*, 2013), the cluster-based similarity partitioning algorithm (CSPA) outperformed the others in the present study. This is due to the fact that each algorithm is better suited to a particular type of data, thus naturally returning higher ANMI values. The hypergraph partitioning algorithm (HGPA) performed poorly in both studies, as it tends to look for evenly sized clusters, not being able to deal with the unevenness found in this type of data. The MCLA, on the other hand, can deal with unbalanced clusters but assumes correspondence among the original clusters, thus it tends to perform poorly when there is a great diversity of individual clustering patterns, as was the case with this dataset (Strehl and Ghosh, 2002). The CSPA is the simplest of all approaches and the most tolerant to noise and pattern diversity. It assumes that a pair of objects that are clustered together often in individual clustering results should be considered similar. The number of times every pair of objects is clustered together is converted into a new similarity matrix, which is in turn partitioned again for the final solution. The ‘supra-consensus’ function is therefore an objective way of automatically choosing the best algorithm (Strehl and Ghosh, 2002).

In the past, other authors have characterised demersal assemblages on the study area. Using 4 years of survey data on fish, crustaceans and cephalopods, Gomes *et al.* (2001) identified 5 assemblage types, distributed according to depth and latitude. This classification was further optimised by Sousa *et al.* (2005) using more data (11 years), leading to the delimitation of two types (shallow and intermediate) to the north of the Nazaré canyon, two types (shallow and intermediate) to the south of Lisbon, and a single deep assemblage type. The approach followed in the present study does not intend to replace these classifications, which are very important decision-support tools for multi-species fisheries management. In fact, the main patterns found in those studies were due to commercially important species, usually with pelagic life cycles and gregarious behaviour, which were not included in this study. This may have contributed to the fact the tendency of richness to decrease onto the shelf break was not very marked, since most of the excluded species occurred preferentially in shallow or intermediate areas (Sousa *et al.*, 2005). However, the higher species richness observed in assemblage types located beyond the shelf break (deeper than 200 m) is a pattern that is consistent with observations by Sousa *et al.* (2006) using data from 1989-1999.

In general, taxonomic approaches tend to be affected by the tendency of nearby areas to have similar species composition (Legendre, 1993), and thus focusing on species identities will often lead to clearer biogeographic patterns. Using strictly functional approaches leads to higher variation at a smaller scale, as different functional patterns in neighbouring areas are detected, whereas they would be masked by putting emphasis on species identities (Bremner *et al.*, 2003). In the study area, this can be observed to a certain degree in a study by Pais *et al.* (2012), who found marked differences in fish species abundances among depth and latitude intervals to be less pronounced when using functional guild data. As well, in a different approach that focused on skates, rays and associated captures, Figueiredo *et al.* (2007) delimited 6 clusters along the Portuguese coast, which also showed some degree of inter-annual variation but were more independent from geographic proximity.

As Bremner *et al.* (2003) noticed with benthic communities, some patterns were found with functional traits that were not clear with a taxonomic approach. Although this was evident in the present study, some similarities with previous characterisations were retained, namely the delimitation of depth strata and the role of the Nazaré region as the most conspicuous division in latitudinal gradients. In fact, type D assemblages described in the present study are related to the “shallow southern” assemblage of Sousa *et al.* (2005), characterised by the predominance of rock dependent sparids. Types E and F are somewhat related to “intermediate northern” assemblages, along with types C and G that cover some of the regions along the coast at intermediate depth ranges. Finally, “deep” assemblages described by Sousa *et al.* (2005) include sampling stations from this study’s type B assemblages. However, this is likely not due to a direct link between species and functional traits but due to similar broad-scale patterns in the response of species and traits to predominant environmental features.

In addition to multi-species stock management, where species identities are important, the proposed framework does not replace habitat classification frameworks, such as the UK Joint Nature Conservation Committee’s marine habitat classification (Connor *et al.*, 2004), integrated into European management programmes through the European Nature Information System (http://eunis.eea.europa.eu/). These hierarchical classifications are usually based on top-down approaches that go from larger to small scale habitat variability, and are very useful as way of establishing common concepts in international policies (Costello, 2009).

The proposed framework can be seen as a new approach to an old problem, a method that intends to understand how different areas of the same coastal region tend to organise into different clusters when using different metrics describing structural and functional traits, and ultimately attempts to identify functionally similar assemblages that retain their characteristics in the face of temporal variation. It is therefore a useful tool in support for the application of tools that assess functional integrity, such as ecosystem approaches to fisheries management (Fulton *et al.*, 2005) or multimetric indices (Henriques *et al.*, 2008). Nonetheless, this is not a method that is useful by itself, and it must be accompanied by ways of quantifying reference values or management goals and further efforts to optimise the power to detect environmental degradation in a timely manner.

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Chapter 7

General discussion and final remarks

The present thesis approached some of the challenges posed by uncertainty in estimates and environmental heterogeneity when monitoring marine fish communities, and some of the consequences of ignoring them. The pressure to solve these issues is enormous, as deadlines established by international policies are often set unrealistically and do not take into account the amount of information gaps and the path that is yet to be travelled before tools can be effectively applied. Nevertheless, these requirements and deadlines are undoubtedly catalytic and usually lead to huge responses by the scientific community (Elliott *et al.*, 1999; de Jonge *et al.*, 2006).

Overall, the work presented constitutes a contribution to the ongoing discussion regarding fish community-based assessments in marine monitoring and management. In chapter 2, it became evident that relying uniquely on published data is not an option in most cases. Although such an approach can be a good starting point, it is certainly not a solution, as a great amount of essential information is often missing, such as the exact composition of every field sample or information on environmental data at the time of collection. This can be improved if national databases of raw field data are built and quality-controlled (Hiscock *et al.*, 2003) but differences in sampling methods, gear selectivity, observer experience, among others, may still encumber data comparability and thus their use in supporting fine-scale monitoring issues. The best way to achieve enough control over background noise is to understand it and quantify it for a specific, standard methodology that is previously tested in pilot studies (Osenberg *et al.*, 1994). Even though chapters 3 to 6 approached issues that were not solvable by relying on published data, the results and patterns found are only valid for the particular sampling methods applied. If longer transects are used, if a different visual census method is applied or if a bottom trawl with a different width or mesh size is used, then all steps, from the calculation of sample sizes to the definition of homogeneous units, are no longer fully applicable and should be calibrated for the new method.

In ecological experiments, environmental variability and heterogeneity can mask results by generating highly variable responses, and controlling confounding variables is a top priority (García-Charton *et al.*, 2000). This can be done by using control locations, comparing different sites with negligible environmental differences or modelling the direct relationship among environmental factors and the variables of interest (García-Charton and Pérez-Ruzafa, 2001; Pardal *et al.*, 2004; Aguado-Giménez *et al.*, 2012; Bustamante *et al.*, 2012; Henriques *et al.*, 2013). In a monitoring context, however, areas to be sampled are usually much larger, and the expected impacts and their effects are often unknown and likely differ from one area to another (de Jonge *et al.*, 2006). In addition, knowledge on the previous state of the systems is scarce, and many times managers are presented with sites that are already at an altered state due to prolonged exposure to anthropogenic stress. Therefore, the challenge that stands before scientists and environmental managers is to be able to monitor a large number of sites and design tools that are able to detect changes and identify an already impacted site in a relatively short period of time, all this with enough certainty to support decisions with minimal resource waste (Caughlan and Oakley, 2001).

The characterisation achieved with published data in chapter 2, despite all the problems encountered, was very important to pinpoint issues that served as a basis to support decisions in the chapters that followed. However, due to the fact that a different approach was followed in chapters 5 and 6, the patterns observed are not directly comparable. One of the main issues found in chapter 2 was the need to use proportions in order to standardise the data. While proportions can more accurately represent the relative importance of each guild in an assemblage, they don’t incorporate the different magnitudes of abundance in which changes occur. As an example, if in the absence of impact, location A is estimated to have 50 omnivores in a total of 100 fish and location B has 500 omnivores in a total of 1000 fish, they both are 50% composed of omnivores but the overall potential of each location is extremely different. Another issue is the dependency among different groups, since a 10% increase in the proportion of omnivores may be the result of a 10% decrease in all the remaining trophic groups, while the absolute number of omnivores in the system stays unaltered. Using absolute abundances instead of proportions, however, leads to more difficult interpretations when comparing sites with different total abundance or biomass, and sites with a higher overall abundance will tend to have higher values for most metrics, even if all proportions are conserved. Nevertheless, in order to establish functionally homogeneous units and understand the range of variation of estimates, using absolute values leads to more meaningful patterns.

A greater degree of cohesion within types was found in chapter 2 when using guild data but the overall multivariate pattern was very similar for guilds and species proportions, which was mainly attributed to gregarious species in soft-substrate areas. This led to patterns in guild composition reflecting the guilds represented by dominant gregarious species, a pattern that was enhanced due to the use of proportions. In chapter 6, the removal of such species, even if they represent an important part of the catches (as seen in Sousa *et al.*, 2005), revealed underlying functional patterns that were not previously evident, particularly a more scattered mesh of different types instead of contiguous, large latitudinal sections and depth intervals that are more influenced by spatial autocorrelation (Bremner *et al.*, 2003).

Of all the studies presented, chapter 3 stands out as being the only one not directly addressing marine fish assemblages but instead focusing on the quantification of habitat complexity in temperate reefs. Indeed, if one needs to understand the influence of “topographic complexity” on fish community measures, there is a need to define and quantify this concept, something that the substrate rugosity index by itself falls short of accomplishing (McCormick, 1994). This of course does not mean that the combined topography index (CTI) is the ultimate answer, and time will tell if the method is able to consistently perform across a variety of areas and organisms. What may be key for such a measure to work is its intended plasticity, given by the weight coefficients for the different components. The CTI may have performed well at quantifying topographic complexity in chapters 4 and 5 because it was originally built for fish assemblages on these sites but if it ends up not performing so well in coral reefs, or with sessile invertebrates, it is adaptable, it can be changed to reflect the way a particular organism perceives its habitat and may work better with slight changes on the weight given to each component. In addition, there are of course several other measures of habitat complexity, such as algae cover and structure, the diversity of cobble sizes and the size and number of holes and crevices (*e.g.* García-Charton and Pérez-Ruzafa, 2001; Horta e Costa *et al.*, 2013) that can complement the CTI, possibly with improved results at predicting fine scale variation on species composition and abundance.

The findings in chapter 4 point to an astonishingly high number of replicates needed to provide an acceptable probability of false negatives, a number that was of course not applied in chapter 5, since the original 6 replicates per site were used in that study. This is not problematic *per se*, since the conclusions regarding metrics that did vary significantly among assemblage types are still valid, with the same probability of error of 5% (Fairweather, 1991; Mapstone, 1995). What happens is that we cannot conclude that the metrics that failed to show significant differences among sites or types are actually similar across sites, due to high β. This led to more difficulty in interpreting patterns, something that could only be achieved through detailed tables and signal-to-noise ratios. However, in a monitoring context, having a low β is crucial and cannot be overlooked, for the reasons pointed out in chapter 4.

The practical application of the classifications achieved in chapters 5 and 6 must be linked to a solid database. The use of machine learning methods may aid in the complex process of finding patterns but it is only as good as the data that supports the learning phase (Knudby *et al.*, 2010; Crisci *et al.*, 2012). The larger the database, the better the predictive capabilities, a crucial aspect in a monitoring context, since there is a need to identify the potential of a given location, even if the present condition of the communities is far from ideal. Regarding predictive models, suggest that we must understand the complexity of the processes, instead of simplifying them (*e.g.* Diaz *et al.*, 2004), and it can even be argued that even the outcome of a coin flip can be modelled and predicted, given the right set of variables. That may indeed be true, however, modelling the outcome of a survey of a whole ecological community is immensely complex, to a level that is yet beyond our reach. The reasons why a fish is found on a particular place at a given time are a very complex combination of environmental factors, interactions among individuals within populations and communities and individual physiological processes and motivations (Johnson *et al.*, 2012). Nonetheless, not being certain that the fish should be there is a problem when trying to understand why it is not, which is why prediction, whether simple or complex, is certainly a very important part of environmental monitoring.

The approach followed in chapters 5 and 6 was indeed a simplification of the problem, done by incorporating the result of environmental and sampling variability into the definition of assemblage types, so that predictive models only have to assign each area to a particular type, which then has a range of expected values for a given metric. In practice these types are “homogeneous” units that are as cohesive as background variability allows. At a local scale, such as in environmental impact assessment of a known pressure source, this approach can be too simplistic, since it may happen that “types” are too large to discern small-scale variations. In these cases, a classic experimental design is probably the best choice, and modelling the direct effect of environmental factors on species abundances is probably easier (García-Charton and Pérez-Ruzafa, 2001; Aguado-Giménez *et al.*, 2012).

The methods proposed in the present thesis aim to support fish assemblage-based tools that seek to measure structural and functional integrity. These tools must function as an early warning for managers, so they must provide relatively short-term responses to anthropogenic stress. In fact, most of the tools developed for fish assemblages in streams and estuaries are simply combinations of metrics (Roset *et al.*, 2007; Pérez-Dominguez *et al.*, 2012). Every metric is scored taking into account the potential of each site through the comparison with reference value thresholds that represent an acceptable level of impact. These scores are then summed up into a final index value that is then translated into a qualitative classification scale, such as “good” or “bad” status (Hering *et al.*, 2006). Although simplistic in their results, these tools must be developed with the same rigour that defines every ecological experiment. These indicators provide the warning signs that support the decision to take action regarding a particular location, and therefore assessment errors may lead to unnecessary costs or catastrophic ecological consequences (Mapstone, 1995; Maxwell and Jennings, 2005). For these reasons, it is very important to perform power analyses, understand the probabilities of error, limit environmental variability and come up with the best possible monitoring design for the available budget (Caughlan and Oakley, 2001). If an indicator is unable to provide enough certainty that an impact is occurring, then the ones responsible for the sources of impact are even less likely to trust them and be willing to cooperate to solve the problem. It is the responsibility of those that understand the complexity to be able to simplify it in an efficient, responsible way, in order to ensure that these tools can be trusted and applied in a monitoring context (Willby, 2011).

From where we stand, the goal established by the European Marine Strategy Framework Directive in 2008 of achieving “good environmental status” by 2020 (EC, 2008) is very near. While some progress has been made by the scientific community and dedicated working groups, there are still many questions to be answered and even the concept of “good” status is still a matter for discussion. In a period of economic crisis, not every country will be able to establish new monitoring plans with ease. Therefore, more than ever, there is a need for careful planning, re-design of the established plans and profound scientific understanding of the measures that are best at detecting degradation, the magnitude of background variability and the amount of effort needed to support decisions with enough certainty. Performing a poorly structured monitoring plan with insufficient sampling may prove to be not much more than a waste of valuable resources. While not acting at all is certainly cheaper, it is not and should never be an option, given the alarming levels of resource overexploitation we face today (Worm *et al.*, 2006). Monitoring must occur and it must be cost-effective, even if that means reducing the number of sampling stations and increasing within-station replication, so that spatial extension is sacrificed to the benefit of statistical power. More than fulfilling the requirements of national and international policy tools, governmental institutions, the scientific community and resource users must work towards stopping the degradation of marine ecosystems, even if not a single policy tool demands it, or the day will come when there will be nothing left to govern, nothing left to study and nothing left to live from. Initial funds must always be directed into solving basic questions, such as the ones addressed in this thesis, in order to allow the establishment of solid foundations for the development of effective assessment tools and methods. We cannot aspire to reach our destination without first building the road.

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Annex I

Species list and guild classification

Species	Family	Troph level	Lm (cm)	Habitat	Migration	Mobility	Residency				Dependence				Troph. guild	Spawning				Commercial value	Resil	Beogeographic affinity	Reef habitat subgroup
							S	O	R	I	S	O	R	I		Sp	Su	Au	Wi				
Acantholabrus palloni	Labridae	3.5	15.7	reef-associated	n	mm	0	1	1	0	0	0	0	0	inv	1	0	0	0	€	M	Temperate	rockspe
Alosa alosa	Clupeidae	3.6	33.5	pelagic	ana	hm	0	0	0	0	0	0	0	0	zoo	1	0	0	0	€€€	M	Temperate	
Alosa fallax	Clupeidae	3.6	28	pelagic	ana	hm	0	0	0	0	0	0	0	0	zoo	1	0	0	0	€€	M	Temperate	
Amblyraja radiata	Rajidae	4	61.5	demersal	oce	hm	1	1	0	0	0	0	0	0	ma	1	0	1	0	€€€	L	Cold-temperate	
Ammodytes tobianus	Ammodytidae	3.2	12.6	demersal	n	te	1	0	0	0	0	0	0	0	zoo	1	0	1	0	€	H	Cold-temperate	
Anguilla anguilla	Anguillidae	3.5	54.6	demersal	cat	hm	0	0	0	0	1	0	1	0	ma	1	1	0	0	€€€	VL	Temperate	
Anthias anthias	Serranidae	3.8	16.9	reef-associated	n	mm	0	0	1	0	0	0	0	0	zoo	1	1	0	0	€	M	Warm-temperate	rockspe
Aphia minuta	Gobiidae	3.1	5.7	demersal	n	te	1	0	0	0	0	0	0	0	ma	0	1	0	0	€	M	Temperate	wat
Apletodon dentatus	Gobiesocidae	3.1	3.1	demersal	n	te	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	rockcave
Apletodon incognitus	Gobiesocidae	3.1	2.2	demersal	n	te	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	rockcave
Apogon imberbis	Apogonidae	3.9	9.5	reef-associated	n	mm	0	0	0	0	1	0	1	0	zoo	0	1	0	0	€	H	Tropical	rockcave
Argentina sphyraena	Argentinidae	3.6	21.2	bathydemersal	n	mm	0	0	0	0	0	0	0	0	ma	1	1	0	0	€	M	Temperate	
Argyrosomus regius	Sciaenidae	4.3	101.7	benthopelagic	oce	hm	0	0	0	0	1	0	0	0	ma	1	1	0	0	€€€	L	Warm-temperate	
Arnoglossus imperialis	Bothidae	3.8	15.7	demersal	n	mm	1	0	0	0	0	0	0	0	ma	1	0	0	0	€	H	Tropical	
Arnoglossus laterna	Bothidae	3.6	9.6	demersal	n	mm	1	0	0	0	0	0	0	0	ma	1	1	0	0	€	M	Warm-temperate	
Arnoglossus thori	Bothidae	3.3	11.3	demersal	n	mm	1	0	0	0	0	0	0	0	ma	1	1	0	0	€	M	Warm-temperate	
Atherina boyeri	Atherinidae	3	5.7	demersal	anf	hm	0	0	0	0	0	0	0	0	ma	1	1	0	0	€	H	Temperate	wat
Atherina presbyter	Atherinidae	3.7	9.7	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	0	1	0	0	€	H	Temperate	wat
Balistes capriscus	Balistidae	3.5	29.5	reef-associated	n	hm	0	0	0	0	1	0	1	0	inv	0	1	0	0	€€	H	Eurythermic	watalgae
Belone belone	Belonidae	4.2	30.2	pelagic	oce	hm	0	0	0	0	0	0	0	0	pi	1	1	0	0	€	M	Temperate	wat
Beryx decadactylus	Berycidae	4.3	29.9	bathydemersal	n	mm	0	1	0	0	0	0	0	0	ma	0	1	1	1	€€	L	Oceanic	
Blennius ocellaris	Blenniidae	3.5	12.9	demersal	n	te	0	0	1	0	0	0	0	0	om	1	1	0	0	€	M	Warm-temperate	
Boops boops	Sparidae	3	19.8	demersal	oce	hm	0	0	0	0	1	1	1	0	om	1	0	0	0	€€	M	Eurythermic	watalgae
Bothus podas	Bothidae	3.4	26.5	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	0	0	€€	H	Tropical	
Brama brama	Bramidae	4.1	53.5	bathypelagic	oce	hm	0	0	0	0	0	0	0	0	ma	0	1	0	0	€€€	L	Oceanic	
Buglossidium luteum	Soleidae	3.3	7.6	demersal	n	mm	1	0	0	0	0	0	0	0	inv	1	0	0	1	€€	M	Warm-temperate	
Callanthias ruber	Callanthiidae	3.8	34.1	demersal	n	mm	0	0	0	0	0	1	0	0	ma	0	0	0	1	€	M	Warm-temperate	
Callionymus lyra	Callionymidae	3.3	15	demersal	n	mm	1	0	0	0	0	1	1	0	inv	1	1	0	1	€	M	Temperate	sandspe
Callionymus maculatus	Callionymidae	3.3	10.6	demersal	n	mm	0	1	0	0	0	0	0	0	inv	1	1	0	1	€	H	Temperate	sandspe
Callionymus reticulatus	Callionymidae	3.3	7.6	demersal	n	mm	1	0	0	0	0	1	1	0	inv	1	0	0	0	€	H	Temperate	sandspe
Callionymus risso	Callionymidae	3	7.6	demersal	n	mm	1	0	0	0	0	1	1	0	inv	1	1	1	0	€	H	Warm-temperate	sandspe
Capros aper	Caproidae	3.1	18.5	demersal	n	mm	1	0	0	0	0	0	0	0	inv	1	1	0	0	€	H	Temperate	
Carcharhinus plumbeus	Carcharhinidae	4.5	125.7	reef-associated	oce	hm	0	0	0	0	1	1	1	0	ma	0	1	0	0	€€	VL	Eurythermic	wat
Centrolabrus exoletus	Labridae	3.5	8.5	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Cold-temperate	watcave
Cepola macrophthalma	Cepolidae	3.1	42	demersal	n	se	0	1	0	0	0	0	0	0	zoo	1	1	1	0	€	M	Temperate	
Chelidonichthys cuculus	Triglidae	3.8	14.6	demersal	n	mm	0	0	0	0	1	1	1	0	ma	1	1	0	0	€	M	Temperate	
Chelidonichthys lucernus	Triglidae	3.7	25.8	demersal	n	mm	1	0	0	0	0	0	1	0	ma	1	1	0	0	€€€	M	Temperate	
Chelidonichthys obscurus	Triglidae	3.4	20.7	demersal	n	mm	1	0	0	0	0	0	1	0	ma	1	0	0	1	€€	M	Warm-temperate	
Chelon labrosus	Mugilidae	2.4	38.1	demersal	anf	mm	0	0	0	0	0	0	0	0	om	0	0	0	1	€€	M	Temperate	wat
Chromis chromis	Pomacentridae	3	9.2	reef-associated	n	mm	0	0	1	0	0	0	0	0	zoo	0	1	0	0	€	M	Tropical	rockcave
Ciliata mustela	Lotidae	3.5	15.7	demersal	oce	hm	0	0	0	0	1	0	1	1	inv	1	0	0	1	€	H	Cold-temperate	rockcave
Citharus linguatula	Citharidae	4	15.2	demersal	n	mm	1	0	0	0	0	0	0	0	ma	1	0	0	1	€€	M	Temperate	
Conger conger	Congridae	4.3	125.3	demersal	oce	hm	0	0	0	0	0	0	1	0	ma	0	1	0	0	€€€	VL	Temperate	rockcave
Coris julis	Labridae	3.2	16.2	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	M	Temperate	gen
Coryphoblennius galerita	Blenniidae	2.2	5.5	demersal	n	te	0	0	1	1	0	0	0	0	om	1	1	0	0	€	H	Warm-temperate	rockspe

Lm- length at first maturity, **S**- soft substrate, **O**- offshore, **R**- rocky subtidal, **I**-rocky intertidal, **Troph**- trophic, **Sp**- Spring, **Su**- summer, **Au**- autumn, **Wi**- winter, **Resil**- resilience, **n**- non-migratory, **ana**- anadromous, **cat**- catadromous, **anf**- anfidromous, **oce**- oceanadromous, **hm**- high mobility, **mm**- medium mobility, **se**- sedentary, **te**- territorial, **he**- herbivore, **zoo**- zooplanktivore, **om**- omnivore, **inv**- invertebrate feeder, **ma**- macrocarnivore, **pi**- piscivore, **€**- low value, **€€**- medium value, **€€€**- high value, **VL**- very low, **L**- low, **M**- medium, **H**- high, **wat**- water column, **watcave**- water column/caves, **watalgae**- water column/algae, **sandspe**- sand specialist, **rockspe**- rock specialist, **rockcave**- rock/caves, **gen**- generalist

Species	Family	Troph level	Lm (cm)	Habitat	Migration	Mobility	Residency				Dependence				Troph. guild	Spawning				Commercial value	Resil	Beogeographic affinity	Reef habitat subgroup
							S	O	R	I	S	O	R	I		Sp	Su	Au	Wi				
Ctenolabrus rupestris	Labridae	3.3	10.1	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	1	€	M	Temperate	watcave
Cubiceps gracilis	Nomeidae	3.6	56.8	pelagic	oce	hm	0	0	0	0	0	0	0	0	zoo					€	M	Eurythermic	
Dasyatis pastinaca	Dasyatidae	4.1	43	demersal	n	mm	1	1	0	0	0	0	0	0	ma	0	1	0	0	€€	VL	Temperate	
Deania calcea	Centrophoridae	4.2	49.2	bathydemersal	n	mm	0	1	0	0	0	0	0	0	ma	0	0	0	1	€€	VL	Eurythermic	
Deltentosteus quadrimaculatus	Gobiidae	3.1	5.8	demersal	n	te	1	0	0	0	0	0	0	0	inv	1	0	0	0	€	H	Warm-temperate	
Dentex dentex	Sparidae	4.5	45.4	benthopelagic	n	mm	0	0	0	0	0	1	1	0	om	1	0	0	0	€€€	M	Warm-temperate	gen
Dentex macrophthalmus	Sparidae	3.4	32.4	benthopelagic	oce	hm	0	0	0	0	0	1	1	0	om	1	0	0	0	€€€	M	Tropical	gen
Dentex maroccanus	Sparidae	3.8	20	benthopelagic	n	mm	0	0	0	0	0	1	1	0	om	1	1	0	0	€€€	M	Warm-temperate	gen
Dicentrarchus labrax	Moronidae	3.8	44.4	demersal	oce	hm	0	0	0	0	1	0	1	0	ma	1	0	0	1	€€€	M	Temperate	gen
Dicentrarchus punctatus	Moronidae	3.9	39.1	pelagic	n	mm	0	0	0	0	0	0	0	0	ma	1	0	0	1	€€€	M	Temperate	gen
Dicologlossa cuneata	Soleidae	3.3	14.9	demersal	n	mm	1	0	0	0	0	0	0	0	inv	1	1	0	0	€€€	H	Temperate	
Dicologlossa hexophthalma	Soleidae	3.4	12.9	demersal	n	mm	1	0	0	0	0	0	0	0	inv					€	H	Tropical	
Diplecogaster bimaculata	Gobiesocidae	3.3	4.5	demersal	n	te	0	0	0	0	0	0	1	0	inv	1	1	0	0	€	M	Temperate	
Diplodus annularis	Sparidae	3.4	16	benthopelagic	n	mm	0	0	0	0	0	0	1	0	om	1	0	0	0	€€€	M	Warm-temperate	watalgae
Diplodus bellottii	Sparidae	3.5	15.6	benthopelagic	n	mm	0	0	0	0	0	0	1	0	om	1	0	0	0	€€€	M	Warm-temperate	watalgae
Diplodus cervinus	Sparidae	3	31.6	reef-associated	oce	hm	0	0	0	0	0	0	1	0	om	1	0	0	1	€€€	L	Warm-temperate	watalgae
Diplodus puntazzo	Sparidae	2.9	34.1	benthopelagic	oce	hm	0	0	0	0	0	0	1	0	om	0	1	1	0	€€€	M	Tropical	watalgae
Diplodus sargus	Sparidae	3	21	demersal	oce	hm	0	0	0	0	0	0	1	0	om	1	0	0	1	€€€	M	Tropical	watalgae
Diplodus vulgaris	Sparidae	3.2	16.5	benthopelagic	oce	hm	0	0	0	0	0	0	1	0	om	0	0	1	1	€€€	H	Warm-temperate	watalgae
Echiichthys vipera	Trachinidae	4.4	10	demersal	n	se	1	0	0	0	0	0	0	0	ma	1	1	0	0	€	H	Temperate	
Engraulis encrasicolus	Engraulidae	3.1	10.4	pelagic	oce	hm	0	0	0	0	0	0	0	0	zoo	1	1	0	0	€€	H	Temperate	
Entelurus aequoreus	Syngnathidae	3.5	23.8	demersal	n	mm	0	0	1	0	0	0	0	0	inv	0	1	0	0	€	M	Cold-temperate	
Eutrigla gurnardus	Triglidae	3.6	26	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	0	1	€€	M	Temperate	
Gadiculus argenteus	Gadidae	3.5	10.2	pelagic	n	mm	0	0	0	0	0	0	0	0	inv	1	0	0	1	€	H	Temperate	
Gaidropsarus guttatus	Lotidae	2.8	16.3	demersal	n	mm	0	0	0	0	1	0	1	0	om	1	1	0	0	€	L	Tropical	rockcave
Gaidropsarus mediterraneus	Lotidae	3.4	29	demersal	oce	hm	0	0	0	0	0	1	1	1	om	1	1	0	0	€	L	Temperate	rockcave
Gaidropsarus vulgaris	Lotidae	3.3	34.1	demersal	n	mm	0	0	0	0	1	0	1	0	ma	1	0	0	1	€	L	Cold-temperate	rockcave
Galeus melastomus	Scyliorhinidae	4.2	41.5	bathydemersal	n	mm	0	0	0	0	0	1	0	0	ma	1	1	1	1	€€	L	Temperate	
Gobius auratus	Gobiidae	3	7	demersal	n	te	1	0	0	0	0	0	0	0	om	1	0	0	0	€	H	Temperate	sandspe
Gobius bucchichi	Gobiidae	3.1	7	demersal	n	te	0	0	1	0	0	0	0	0	om	0	1	0	0	€	M	Tropical	sandspe
Gobius cobitis	Gobiidae	3	15.2	demersal	n	te	0	0	1	1	0	0	0	0	om	1	0	0	0	€	M	Warm-temperate	rockspe
Gobius cruentatus	Gobiidae	3.1	11.8	demersal	n	te	0	0	1	0	0	0	0	0	om	0	0	0	1	€	M	Warm-temperate	sandspe
Gobius gasteveni	Gobiidae	3.2	8.2	demersal	n	te	1	1	0	0	0	0	0	0	om	1	1	0	0	€	M	Warm-temperate	sandspe
Gobius niger	Gobiidae	3.2	11.5	demersal	n	te	0	0	1	1	0	0	0	0	om	1	0	0	1	€	M	Temperate	rockspe
Gobius paganellus	Gobiidae	3.3	8.8	demersal	n	te	0	0	1	1	0	0	0	0	om	1	0	0	1	€	M	Warm-temperate	rockspe
Gobius xanthocephalus	Gobiidae	3.1	7	demersal	n	te	0	0	1	0	0	0	0	0	om	0	1	0	0	€	H	Warm-temperate	sandspe
Gobiusculus flavescens	Gobiidae	3.2	4.5	demersal	n	mm	0	0	0	0	1	0	1	0	zoo	1	1	0	0	€	H	Cold-temperate	watalgae
Gymnammodytes cicerelus	Ammodytidae	3.4	11.2	demersal	n	mm	1	0	0	0	0	0	0	0	zoo	0	0	0	1	€	H	Warm-temperate	
Gymnammodytes semisquamatus	Ammodytidae	2.7	18.5	demersal	n	mm	0	0	0	0	1	0	1	0	zoo	0	1	0	0	€	M	Cold-temperate	
Halobatrachus didactylus	Batrachoididae	3.9	29	demersal	n	se	1	0	0	0	0	0	0	0	ma	1	1	0	0	€€	L	Warm-temperate	gen
Helicolenus dactylopterus	Sebestidae	3.8	19	bathydemersal	n	se	0	1	0	0	0	0	0	0	ma	1	1	0	0	€€€	VL	Eurythermic	
Hippocampus guttulatus	Syngnathidae	3.5	10.6	demersal	n	se	0	0	1	0	0	0	0	0	zoo	1	1	0	0	€	M	Warm-temperate	rockspe
Hippocampus hippocampus	Syngnathidae	3.2	10	demersal	n	se	0	0	1	0	0	0	0	0	zoo	1	1	1	0	€	H	Warm-temperate	rockspe
Hyperoplus lanceolatus	Ammodytidae	4.2	17.6	demersal	oce	hm	0	1	0	0	0	0	0	0	ma	0	1	0	0	€	M	Cold-temperate	
Isurus oxyrinchus	Lamnidae	4.5	170.5	reef-associated	oce	hm	0	0	0	0	0	0	0	0	ma	1	1	0	0	€€€	VL	Eurythermic	

Lm- length at first maturity, **S**- soft substrate, **O**- offshore, **R**- rocky subtidal, **I**-rocky intertidal, **Troph**- trophic, **Sp**- Spring, **Su**- summer, **Au**- autumn, **Wi**- winter, **Resil**- resilience, **n**- non-migratory, **ana**- anadromous, **cat**- catadromous, **anf**- anfidromous, **oce**- oceanadromous, **hm**- high mobility, **mm**- medium mobility, **se**- sedentary, **te**- territorial, **he**- herbivore, **zoo**- zooplanktivore, **om**- omnivore, **inv**- invertebrate feeder, **ma**- macrocarnivore, **pi**- piscivore, **€**- low value, **€€**- medium value, **€€€**- high value, **VL**- very low, **L**- low, **M**- medium, **H**- high, **wat**- water column, **watcave**- water column/caves, **watalgae**- water column/algae, **sandspe**- sand specialist, **rockspe**- rock specialist, **rockcave**- rock/caves, **gen**- generalist

Species	Family	Troph level	Lm (cm)	Habitat	Migration	Mobility	Residency				Dependence				Troph. guild	Spawning				Commercial value	Resil	Beogeographic affinity	Reef habitat subgroup
							S	O	R	I	S	O	R	I		Sp	Su	Au	Wi				
Labrus bergylta	Labridae	3.1	31.9	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€€	L	Cold-temperate	watalgae
Labrus merula	Labridae	3.2	25	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	0	0	1	€€	M	Warm-temperate	watalgae
Labrus mixtus	Labridae	3.9	19.9	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€€	L	Temperate	watcave
Labrus viridis	Labridae	3.8	27.5	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	0	0	1	€€	L	Warm-temperate	watalgae
Lebetus guilleti	Gobiidae	3.1	2	reef-associated	n	te	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Temperate	
Lepadogaster candolii	Gobiesocidae	2.8	5.4	demersal	n	te	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	M	Warm-temperate	rockcave
Lepadogaster lepadogaster	Gobiesocidae	3.3	4.8	demersal	n	te	0	0	1	1	0	0	0	0	inv	1	1	0	0	€	M	Warm-temperate	rockcave
Lepadogaster purpurea	Gobiesocidae	3.3	5.4	demersal	n	te	0	0	1	1	0	0	0	0	inv	1	0	0	1	€	M	Temperate	rockcave
Lepidopus caudatus	Trichiuridae	3.8	89.6	bathydemersal	oce	hm	0	0	0	0	0	1	0	0	ma	1	0	0	1	€€€	M	Eurythermic	
Lepidorhombus boscii	Scophthalmidae	3.7	25	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	0	0	0	€€€	M	Temperate	
Lepidorhombus whiffiagonis	Scophthalmidae	4.2	32.7	bathydemersal	n	mm	1	1	0	0	0	0	0	0	ma	1	0	0	0	€€€	L	Temperate	
Lepidotrigla cavillone	Triglidae	3.2	7.1	demersal	n	mm	1	1	0	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	sandspe
Lepidotrigla dieuzeidei	Triglidae	3.3	12.9	demersal	n	mm	1	1	0	0	0	0	0	0	inv	0	1	0	0	€	H	Warm-temperate	sandspe
Lesueurigobius sanzi	Gobiidae	3.6	7.6	demersal	n	te	0	1	0	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	sandspe
Leucoraja naevus	Rajidae	3.9	39.6	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	1	1	€€€	L	Temperate	
Lichia amia	Carangidae	4.5	98.8	pelagic	oce	hm	0	0	0	0	0	0	0	0	pi	0	1	0	0	€€	M	Warm-temperate	
Lipophrys canevae	Blenniidae	2.1	5.4	demersal	n	te	0	0	1	1	0	0	0	0	om	1	1	0	0	€	H	Warm-temperate	rockspe
Lipophrys pholis	Blenniidae	3.1	18.5	demersal	n	te	0	0	1	1	0	0	0	0	om	1	1	0	0	€	M	Temperate	rockspe
Lithognathus mormyrus	Sparidae	3.4	22.9	demersal	n	mm	1	0	0	0	0	0	0	0	om	1	1	0	0	€€€	M	Warm-temperate	sandspe
Liza aurata	Mugilidae	3.1	37.3	pelagic	cat	hm	0	0	0	0	0	0	0	0	om	0	1	1	0	€€	M	Temperate	wat
Lophius piscatorius	Lophiidae	4.4	82.8	bathydemersal	n	se	1	1	0	0	0	0	0	0	ma	1	1	0	1	€€€	L	Temperate	
Macroramphosus gracilis	Centriscidae	3.4	8.6	pelagic	n	mm	0	0	0	0	0	0	0	0	inv	0	0	0	1	€	H	Eurythermic	
Macroramphosus scolopax	Centriscidae	3.5	12.3	pelagic	n	mm	0	0	0	0	0	0	0	0	inv	0	0	1	1	€	M	Eurythermic	
Maurolicus muelleri	Sternoptychidae	3	5.8	bathypelagic	n	mm	0	0	0	0	0	0	0	0	inv	1	1	0	0	€	M	Eurythermic	
Merlangius merlangus	Gadidae	4.4	31.3	benthopelagic	oce	hm	0	0	0	0	1	1	1	0	ma	1	1	1	0	€€€	M	Cold-temperate	
Merluccius merluccius	Merlucciidae	4.4	60.1	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	0	0	1	€€€	L	Temperate	
Microchirus azevia	Soleidae	3.2	20.2	demersal	n	mm	1	1	0	0	0	0	0	0	inv	1	0	0	1	€€€	H	Temperate	
Microchirus boscanion	Soleidae	3.2	12.9	demersal	n	mm	1	1	0	0	0	0	0	0	inv	1	0	0	1	€	H	Tropical	
Microchirus ocellatus	Soleidae	3.2	12.9	demersal	n	mm	1	1	0	0	0	0	0	0	inv	0	0	0	1	€	H	Warm-temperate	
Microchirus variegatus	Soleidae	3.3	21.2	demersal	n	mm	1	1	0	0	0	0	0	0	inv	1	0	0	1	€€€	M	Temperate	
Micromesistius poutassou	Gadidae	4	21.4	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	1	0	0	1	€€	M	Temperate	
Molva molva	Lotidae	4.3	89.9	demersal	oce	hm	0	1	0	0	0	0	0	0	ma	1	1	0	0	€€	L	Cold-temperate	
Monochirus hispidus	Soleidae	3.5	12.9	demersal	n	mm	1	1	0	0	0	0	0	0	inv	1	1	0	0	€€	H	Tropical	
Mugil cephalus	Mugilidae	2.1	54.6	benthopelagic	cat	hm	0	0	0	0	1	1	1	0	ma	0	1	1	0	€€	M	Tropical	wat
Mullus barbatus	Mullidae	3.2	15.5	demersal	n	mm	1	1	0	0	0	0	0	0	inv	1	1	0	0	€€€	M	Temperate	sandspe
Mullus surmuletus	Mullidae	3.4	26.2	demersal	oce	hm	1	1	0	0	0	0	0	0	inv	1	1	0	0	€€€	M	Temperate	sandspe
Muraena helena	Muraenidae	4.2	76.7	reef-associated	n	se	0	0	1	0	0	0	0	0	ma	1	1	0	0	€€€	M	Warm-temperate	rockcave
Mustelus mustelus	Triakidae	3.8	72.9	demersal	n	hm	0	0	0	0	0	0	0	0	ma	0	1	0	0	€€€	VL	Temperate	
Myliobatis aquila	Myliobatidae	3.6	91.4	benthopelagic	n	mm	1	1	0	0	0	0	0	0	ma	0	1	0	0	€€	VL	Tropical	
Oblada melanura	Sparidae	3	20.6	benthopelagic	oce	hm	0	0	0	0	0	0	1	0	om	1	1	0	0	€€	M	Tropical	watalgae
Ophisurus serpens	Ophichthidae	4.1	120.4	reef-associated	n	se	1	1	0	0	0	0	0	0	inv	1	1	0	0	€	VL	Tropical	
Pagellus acarne	Sparidae	3.5	17.7	benthopelagic	oce	hm	0	1	0	0	0	0	1	0	ma	1	0	1	0	€€€	M	Temperate	gen
Pagellus bogaraveo	Sparidae	3.5	33.6	benthopelagic	n	mm	0	1	0	0	0	0	1	0	ma	1	0	0	1	€€€	L	Temperate	gen
Pagellus erythrinus	Sparidae	3.4	28.9	benthopelagic	n	hm	0	1	0	0	0	0	1	0	ma	1	0	1	0	€€€	M	Temperate	gen
Pagrus auriga	Sparidae	3.4	44	benthopelagic	oce	hm	0	1	0	0	0	0	1	0	inv	0	0	0	1	€€€	VL	Tropical	gen

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Species	Family	Troph level	Lm (cm)	Habitat	Migration	Mobility	Residency				Dependence				Troph. guild	Spawning				Commercial value	Resil	Beogeographic affinity	Reef habitat subgroup
							S	O	R	I	S	O	R	I		Sp	Su	Au	Wi				
Pagrus caeruleostictus	Sparidae	3.8	48.8	benthopelagic	oce	hm	0	1	0	0	0	0	1	0	ma	1	1	1	0	€€€	M	Warm-temperate	gen
Pagrus pagrus	Sparidae	3.7	35.2	benthopelagic	oce	hm	0	1	0	0	0	0	1	0	ma	1	1	0	0	€€€	M	Warm-temperate	gen
Parablennius gattorugine	Blenniidae	2.9	18.5	demersal	n	te	0	0	1	1	0	0	0	0	om	1	1	0	0	€	H	Warm-temperate	rockspe
Parablennius incognitus	Blenniidae	2.4	4.3	demersal	n	te	0	0	1	0	0	0	0	0	om	1	1	0	0	€	H	Warm-temperate	rockspe
Parablennius pilicornis	Blenniidae	3.2	8.6	demersal	n	te	0	0	1	1	0	0	0	0	om	1	1	0	0	€	H	Tropical	rockspe
Parablennius rouxi	Blenniidae	2.6	5.8	demersal	n	te	1	0	1	0	0	0	0	0	om	1	1	0	0	€	H	Warm-temperate	rockspe
Parablennius ruber	Blenniidae	2.9	8.5	demersal	n	te	0	0	1	0	0	0	0	0	om	0	0	0	1	€	H	Temperate	rockspe
Parablennius sanguinolentus	Blenniidae	2.1	12.9	demersal	n	te	0	0	1	1	0	0	0	0	om	1	1	0	0	€	M	Warm-temperate	rockspe
Parablennius tentacularis	Blenniidae	3.1	10	demersal	n	te	0	0	1	1	0	0	0	0	om	1	0	0	0	€	H	Warm-temperate	rockspe
Lipophrys trigloides	Blenniidae	3.5	8.8	demersal	n	te	0	0	1	1	0	0	0	0	om	1	0	0	1	€	H	Warm-temperate	rockspe
Solea lascaris	Soleidae	3.2	17	demersal	n	mm	1	1	0	0	0	0	0	0	inv	1	0	0	1	€€€	M	Temperate	
Phycis phycis	Phycidae	4.3	35.6	benthopelagic	n	mm	0	0	0	0	1	1	1	0	inv	1	0	0	0	€€€	M	Warm-temperate	rockcave
Platichthys flesus	Pleuronectidae	3.2	23.6	demersal	cat	hm	1	1	0	0	0	0	0	0	ma	0	0	0	1	€€€	M	Temperate	
Plectorhinchus mediterraneus	Haemulidae	4	37	demersal	n	mm	1	1	0	0	0	0	0	0	inv					€€€	M	Warm-temperate	
Pleuronectes platessa	Pleuronectidae	3.3	43.5	demersal	oce	hm	1	1	0	0	0	0	0	0	inv	1	0	0	1	€€€	L	Temperate	
Pollachius pollachius	Gadidae	4.2	67.5	benthopelagic	oce	hm	0	1	0	0	0	0	0	0	inv	1	0	0	1	€€€	M	Cold-temperate	gen
Pomadasys incisus	Haemulidae	3.8	29	demersal	n	mm	0	0	0	0	1	1	1	0	inv	0	1	0	0	€€€	M	Warm-temperate	
Pomatomus saltatrix	Pomatomidae	4.5	64.6	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	1	1	0	0	€	M	Eurythermic	
Pomatoschistus lozanoi	Gobiidae	3.1	5.8	demersal	n	se	0	0	0	0	1	0	1	0	inv	1	1	0	0	€	H	Cold-temperate	sandspe
Pomatoschistus marmoratus	Gobiidae	3.3	5.8	demersal	n	se	1	0	0	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	sandspe
Pomatoschistus microps	Gobiidae	3.3	7.3	demersal	anf	hm	0	0	0	0	1	0	0	0	inv	1	1	0	0	€	H	Temperate	sandspe
Pomatoschistus minutus	Gobiidae	3.2	6.1	demersal	oce	hm	0	0	0	0	1	0	0	0	inv	1	0	0	1	€	H	Temperate	sandspe
Pomatoschistus pictus	Gobiidae	3.1	4.5	demersal	n	se	1	0	0	0	0	0	0	0	inv	1	1	0	1	€	H	Temperate	sandspe
Scophthalmus maximus	Scophthalmidae	4	25.5	demersal	oce	hm	1	0	0	0	0	0	0	0	ma	1	1	0	0	€€€	M	Temperate	
Raja brachyura	Rajidae	4	60.6	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	0	1	€€€	L	Temperate	
Raja clavata	Rajidae	3.8	45.4	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	0	0	0	€€€	L	Temperate	
Raja microocellata	Rajidae	3.9	44	demersal	n	mm	1	1	0	0	0	0	0	0	pi	0	1	0	0	€€€	L	Cold-temperate	
Raja miraletus	Rajidae	3.8	35.6	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	0	0	€€€	L	Warm-temperate	
Raja montagui	Rajidae	3.7	39.3	demersal	n	mm	1	1	0	0	0	0	0	0	inv	0	1	0	0	€€€	L	Temperate	
Raja undulata	Rajidae	3.5	56.4	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	0	0	€€€	L	Warm-temperate	
Raniceps raninus	Gadidae	3.8	17.1	demersal	oce	hm	0	0	0	0	0	0	1	0	ma	0	1	0	0	€	M	Cold-temperate	
Rostroraja alba	Rajidae	4.4	120	demersal	n	mm	1	1	0	0	0	0	0	0	ma					€€€	L	Temperate	
Sarda sarda	Scombridae	4.5	35	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	1	1	0	0	€€€	M	Eurythermic	wat
Sardina pilchardus	Clupeidae	2.8	14.6	pelagic	oce	hm	0	0	0	0	0	0	0	0	zoo	0	0	0	1	€€€	M	Temperate	
Sarpa salpa	Sparidae	2	28	benthopelagic	n	mm	0	0	1	0	0	0	0	0	he	1	0	1	0	€€	M	Tropical	watalgae
Scomber colias	Scombridae	3.9	27.8	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	0	0	0	1	€€	H	Tropical	
Scomber scombrus	Scombridae	3.7	25.2	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	1	1	0	0	€€	M	Cold-temperate	
Scophthalmus rhombus	Scophthalmidae	3.8	41.5	demersal	oce	hm	1	0	0	0	0	0	0	0	ma	1	1	0	0	€€€	M	Warm-temperate	
Scorpaena notata	Scorpaenidae	3.5	15.2	demersal	n	se	0	0	1	0	0	0	0	0	ma	1	1	0	0	€	M	Warm-temperate	rockcave
Scorpaena porcus	Scorpaenidae	3.9	16.4	demersal	n	se	0	0	1	0	0	0	0	0	ma	1	1	0	0	€€	M	Warm-temperate	rockcave
Scorpaena scrofa	Scorpaenidae	4.3	29	demersal	n	se	0	0	0	0	1	1	1	0	ma	0	1	0	0	€	H	Warm-temperate	gen
Scyliorhinus canicula	Scyliorhinidae	3.7	53.5	demersal	n	mm	1	0	0	0	0	0	1	0	ma	1	1	1	1	€€	L	Temperate	
Scyliorhinus stellaris	Scyliorhinidae	4	85.6	reef-associated	n	mm	1	0	0	0	0	0	1	0	ma	1	1	0	0	€€	L	Temperate	
Seriola dumerili	Carangidae	4.5	70.2	reef-associated	oce	hm	0	0	0	0	0	0	0	0	ma	1	1	0	0	€€€	M	Warm-temperate	
Serranus atricauda	Serranidae	4.3	27.8	demersal	n	mm	0	1	1	0	0	0	0	0	ma	1	1	0	0	€€€	L	Warm-temperate	watcave

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Species	Family	Troph level	Lm (cm)	Habitat	Migration	Mobility	Residency				Dependence				Troph. guild	Spawning				Commercial value	Resil	Beogeographic affinity	Reef habitat subgroup
							S	O	R	I	S	O	R	I		Sp	Su	Au	Wi				
Serranus cabrilla	Serranidae	3.3	13.6	demersal	n	mm	0	1	1	0	0	0	0	0	ma	1	1	0	0	€	M	Tropical	watcave
Serranus hepatus	Serranidae	3.5	15.7	demersal	n	mm	0	1	1	0	0	0	0	0	ma	1	1	0	0	€	M	Warm-temperate	watcave
Serranus scriba	Serranidae	3.8	17.9	demersal	n	se	0	1	1	0	0	0	0	0	ma	1	1	0	0	€	M	Temperate	rockcave
Solea senegalensis	Soleidae	3.1	34.1	demersal	n	mm	1	0	0	0	0	0	0	0	inv	1	0	0	1	€€€	L	Warm-temperate	
Solea solea	Soleidae	3.1	29.9	demersal	oce	hm	1	1	0	0	0	0	0	0	inv	0	0	0	1	€€€	M	Temperate	
Sparus aurata	Sparidae	3.4	31.9	demersal	n	mm	0	1	1	0	0	0	0	0	om	0	0	1	0	€€€	M	Warm-temperate	gen
Sphoeroides pachygaster	Tetraodontidae	4.2	24.1	demersal	n	mm	0	1	0	0	0	0	0	0	inv	1	1	1	1	€	M	Eurythermic	
Spicara maena	Centracanthidae	4.2	15.7	pelagic	n	mm	0	0	0	0	0	0	0	0	zoo	0	1	1	0	€	M	Warm-temperate	
Spondyliosoma cantharus	Sparidae	3.3	31.1	benthopelagic	oce	hm	0	0	0	0	1	1	1	0	om	1	1	0	0	€€	M	Temperate	watalgae
Sprattus sprattus	Clupeidae	3	8.5	pelagic	oce	hm	0	0	0	0	0	0	0	0	zoo	1	1	0	0	€€	H	Temperate	wat
Squalus blainville	Squalidae	4	60.6	demersal	n	mm	0	0	0	0	0	0	0	0	ma	1	1	1	1	€€€	VL	Eurythermic	
Symphodus bailloni	Labridae	3.3	12.9	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	M	Temperate	rockspe
Symphodus cinereus	Labridae	3.3	9.8	demersal	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	M	Warm-temperate	rockspe
Symphodus mediterraneus	Labridae	3.1	10.6	demersal	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	rockspe
Symphodus melops	Labridae	3.3	13.1	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	1	€	M	Cold-temperate	rockspe
Symphodus ocellatus	Labridae	3.3	7.1	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	rockspe
Symphodus roissali	Labridae	3.5	10.4	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	M	Warm-temperate	rockspe
Symphodus rostratus	Labridae	3.4	7	reef-associated	n	mm	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	rockspe
Synaptura lusitanica	Soleidae	3.8	23.8	demersal	n	mm	1	0	0	0	0	0	0	0	inv	1	1	0	0	€€€	M	Tropical	
Syngnathus abaster	Syngnathidae	3.2	13.5	demersal	anf	se	0	0	1	0	0	0	0	0	inv	1	1	1	0	€	H	Temperate	rockspe
Syngnathus acus	Syngnathidae	3.4	29	demersal	n	se	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	M	Temperate	rockspe
Syngnathus rostellatus	Syngnathidae	3.7	12.3	demersal	n	se	0	0	1	0	0	0	0	0	inv	0	1	0	0	€	H	Cold-temperate	rockspe
Syngnathus typhle	Syngnathidae	4.3	15.7	demersal	n	se	0	0	1	0	0	0	0	0	inv	1	1	1	0	€	M	Temperate	rockspe
Taurulus bubalis	Cottidae	3.6	11.6	demersal	n	mm	0	0	1	0	0	0	0	0	ma	1	0	0	1	€	M	Cold-temperate	rockcave
Thorogobius ephippiatus	Gobiidae	3	8.8	demersal	n	te	0	0	1	0	0	0	0	0	om	1	1	0	0	€	M	Temperate	sandspe
Torpedo marmorata	Torpedinidae	4.5	53.5	reef-associated	n	mm	1	1	0	0	0	0	0	0	ma	0	0	1	0	€€	L	Temperate	sandspe
Torpedo nobiliana	Torpedinidae	4.5	90	benthopelagic	oce	hm	1	1	0	0	0	0	0	0	pi	0	0	1	1	€€	L	Temperate	sandspe
Torpedo torpedo	Torpedinidae	4.5	24.5	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	0	0	€€	L	Warm-temperate	sandspe
Trachinotus ovatus	Carangidae	3.7	39.1	pelagic	n	mm	0	0	0	0	0	0	0	0	inv	0	1	0	0	€€	M	Warm-temperate	wat
Trachinus draco	Trachinidae	4.2	30.6	demersal	n	se	1	1	0	0	0	0	0	0	ma	1	1	0	0	€	M	Temperate	
Trachinus radiatus	Trachinidae	3.97	29	demersal	n	se	1	1	0	0	0	0	0	0	ma	1	1	0	0	€	M	Warm-temperate	
Trachurus mediterraneus	Carangidae	3.6	12.3	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	0	1	0	0	€€	M	Warm-temperate	
Trachurus picturatus	Carangidae	3.3	34.1	benthopelagic	oce	hm	0	0	0	0	0	0	0	0	ma	0	0	0	1	€€	M	Warm-temperate	
Trachurus trachurus	Carangidae	3.6	22.5	pelagic	oce	hm	0	0	0	0	0	0	0	0	ma	0	0	0	1	€€€	M	Temperate	
Trigla lyra	Triglidae	3.5	40.3	bathydemersal	n	mm	1	1	0	0	0	0	0	0	inv	1	0	1	1	€€€	M	Warm-temperate	sandspe
Trigloporus lastoviza	Triglidae	3.4	21.3	demersal	n	mm	1	0	0	0	0	0	1	0	inv	0	1	0	0	€€	M	Eurythermic	sandspe
Tripterygion delaisi	Tripterygiidae	3.4	6.3	demersal	n	te	0	0	1	0	0	0	0	0	inv	1	1	0	0	€	H	Warm-temperate	rockspe
Trisopterus luscus	Gadidae	3.7	26.4	benthopelagic	oce	hm	0	0	0	0	1	1	1	0	ma	1	0	0	0	€€€	M	Temperate	watcave
Trisopterus minutus	Gadidae	3.8	19.6	benthopelagic	n	mm	1	0	0	0	1	1	1	0	ma	1	0	0	0	€€€	M	Temperate	watcave
Uranoscopus scaber	Uranoscopidae	4.4	23.8	demersal	n	se	1	1	0	0	0	0	0	0	pi	0	1	0	0	€	M	Warm-temperate	
Zenopsis conchifer	Zeidae	4.5	44	benthopelagic	n	mm	1	0	0	0	0	1	1	0	pi	0	1	0	0	€	L	Eurythermic	
Zeugopterus punctatus	Scophthalmidae	4	15.7	demersal	n	mm	1	0	0	0	0	0	0	0	ma	1	1	0	0	€€	M	Cold-temperate	rockcave
Zeugopterus regius	Scophthalmidae	3.4	12.9	demersal	n	mm	1	1	0	0	0	0	0	0	ma	1	1	0	0	€€	H	Cold-temperate	rockcave
Zeus faber	Zeidae	4.5	37.6	benthopelagic	oce	hm	0	0	0	0	0	1	1	0	ma	1	0	0	1	€€€	L	Eurythermic	gen

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