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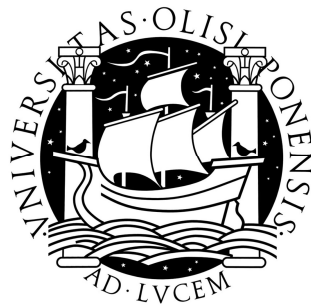## FACULDADE DE CIÊNCIAS

DEPARTAMENTO DE BIOLOGIA ANIMAL

MARINE FISH ASSEMBLAGE TYPOLOGIES FOR THE PORTUGUESE COAST IN THE CONTEXT OF THE EUROPEAN MARINE STRATEGY DIRECTIVE

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Resumo

O meio marinho engloba ecossistemas de elevada complexidade que suportam uma enorme biodiversidade, fornecendo inúmeros bens e serviços. No entanto, está actualmente sujeito a pressões crescentes como a pesca comercial, a contaminação com substâncias nocivas e nutrientes, a introdução de espécies exóticas, a perda de habitat, entre outras, que têm vindo a contribuir para a degradação da biodiversidade, com graves consequências ecológicas e socio-económicas.

Face a este problema, têm surgido várias iniciativas a nível nacional e internacional tendo em vista a protecção e conservação do meio marinho. A Convenção das Nações Unidas sobre a Lei do Mar (UNCLOS) é o quadro legal básico internacional que governa os usos do mar, delimitando acções para a preservação dos ecossistemas marinhos, juntamente com a Convenção sobre Diversidade Biológica. Na Europa, várias políticas comunitárias incidem sobre a temática do meio marinho, tais como as Directivas Habitats (92/43/EEC) e Aves (79/409/EEC), a Directiva Quadro da Água (DQA; 2000/60/EC), a Política Comum das Pescas, o ICES e convenções regionais como a Convenção OSPAR (Atlântico Nordeste), a Convenção de Helsínquia (Mar Báltico), a Convenção de Barcelona (Mar Mediterrâneo) e a Convenção de Bucareste (Mar Negro). No entanto, nenhuma constitui uma abordagem integrativa da necessidade de protecção e conservação dos ecossistemas marinhos da Europa e a falta de articulação entre as várias estratégias e convenções é responsável pela inadequação do quadro institucional da União Europeia (UE) para a gestão do meio marinho.

Por esta razão, o Sexto Programa de Acção para o Ambiente da UE (Decisão 1600/2002/EC) comprometeu-se a desenvolver uma Estratégia Temática para a protecção e conservação do ambiente marinho, levando à apresentação de uma proposta de uma Directiva "Estratégia para o Meio Marinho" (DEMM), que tem como principal objectivo atingir o 'bom estado ambiental' das águas marinhas sob jurisdição dos Estados Membros da UE até 2021, delimitando acções para prevenir futura deterioração.

Na DEMM estão delimitadas quatro regiões marinhas: o Mar Báltico, o Atlântico Nordeste, o Mar Mediterrâneo e o Mar Negro. Na região do Atlântico Nordeste estão definidas quatro sub-regiões, estando Portugal inserido, juntamente com França e Espanha, na sub-região que se estende desde a Baía da Biscaia para sul ao longo da costa ibérica até ao estreito de Gibraltar e também na sub-região constituída pelos arquipélagos dos Açores, Madeira e Ilhas Canárias.

No âmbito da Directiva, é requerido a cada Estado Membro o delineamento de uma Estratégia para a Protecção do Ambiente Marinho, consitente com as estratégias da região em que se insere e seguindo um Plano de Acção pre-definido. Em primeiro lugar neste Plano é necessária uma avaliação inicial integral do estado ambiental e do impacto das actividades humanas nas águas marinhas, delimitando tipologias e indicando valores de referência que definam o conceito de 'bom estado ambiental'.

Na DQA, apenas 19,8% das águas marinhas europeias estão incluídas e, ao contrário de elementos biológicos como o fitoplâncton, as macroalgas e os macroinvertebrados bentónicos, cuja avaliação é tida em conta nas zonas costeiras, os peixes estão apenas incluídos na análise da qualidade das águas interiores e de transição, constituíndo assim um novo requisito para a avaliação da qualidade ecológica do meio marinho.

O elevado valor socio-económico dos peixes, aliado à sua fácil identificação, diferenças no grau de mobilidade com muitos casos de dependência do substrato, longevidade e possibilidade de inclusão das espécies em grupos ecológicos que respondem de forma mais previsível a impactos são algumas das vantagens da sua utilização como indicadores de qualidade ecológica.

As ferramentas de gestão ambiental que usam peixes marinhos têm até agora sido centradas na gestão das pescas, focando-se em populações de espécies exploradas. No entanto existem algumas propostas mais recentes centradas numa Abordagem Ecossistémica da gestão das pescas, mais enquadradas no âmbito da DEMM, mas deixando um papel menor para os restantes impactos, existindo assim uma lacuna metodológica no que respeita à avaliação da qualidade de associações de peixes marinhos englobando todo o ecossistema.

No âmbito da DQA têm surgido várias propostas metodológicas e ferramentas para a avaliação da qualidade ecológica de associações de peixes em rios e estuários, que poderão servir de base à construção de ferramentas para a avaliação de associações de peixes marinhos, dado que ambas as Directivas têm abordagens e objectivos semelhantes, devendo assim ser implementadas tendo como base ferramentas e métodos comparáveis.

A maioria destas ferramentas é apresentada sob a forma de um índice de qualidade ecológica composto por vários componentes mensuráveis (métricas) de uma associação de peixes. Tal como para a DQA, os índices multimétricos são uma abordagem adequada para a avaliação ecológica a realizar no âmbito da DEMM, sendo assim urgente a delimitação de tipologias de associações de peixes, por forma caracterizá-las quanto à composição e abundância de espécies e compreender a representatividade dos grupos ecológicos em cada uma delas.

O presente trabalho teve como objectivo a delimitação e caracterização de tipologias de associações de peixes da plataforma continental portuguesa, desde a zona intertidal até à batimétrica dos 200 metros, através de pesquisa bibliográfica e compilação de dados de abundância e composição de espécies, cobrindo um grande espectro de variabilidade ambiental e diversidade de habitats, por forma a compreender não só os principais factores e gradientes responsáveis pela delimitação de diferentes associações, mas também a forma como as espécies e os grupos ecológicos diferenciam e caracterizam cada tipologia definida, estabelecendo assim as bases necessárias para a futura definição de valores de referência e escolha das métricas que irão integrar um índice multimétrico para a avaliação do estado ambiental das associações de peixes no âmbito da DEMM.

Após a recolha bibliográfica, apenas os conjuntos de dados que apresentavam valores de abundância (absoluta ou relativa) foram seleccionados para a análise, sendo a possibilidade de divisão desses conjuntos em estações do ano outro critério importante na selecção, por forma a permitir a avaliação do efeito da sazonalidade. De forma a maximizar o número de conjuntos de dados utilizáveis as abundâncias foram re-calculadas como proporções do total de cada conjunto.

As espécies presentes num total de 86 conjuntos de dados compilados foram agrupadas em 37 grupos ecológicos divididos em sete categorias (dependência do substrato, mobilidade, habitat, migração, grupos tróficos, resiliência e época de reprodução) e foram construídas três matrizes de dados: uma com as abundâncias relativas das espécies, outra com as proporções relativas dos grupos ecológicos por categoria e outra com o número de espécies por grupo ecológico. Estas matrizes foram utilizadas durante todo o processo de definição de tipologias e analisadas em paralelo.

Por forma a identificar o principal gradiente de distribuição das espécies e grupos ecológicos e delimitar tipologias de acordo com o agrupamento das amostras com base nos três tipos de dados, foi utilizada uma análise de correspondências com extracção de tendências por segmentos (DCA; Detrended Correspondence Analysis), com introdução posterior de valores de latitude e profundidade para análise da correlação destes factores com o gradiente principal de distribuição de espécies (indirect gradient analysis).

Com base na DCA, verificou-se uma forte influência da profundidade e do tipo de substrato na definição do gradiente principal e foram estabelecidas seis tipologias distintas: intertidal rochoso (IR; peixes que se encontram em poças de maré durante a baixa-mar), subtidal rochoso natural (NR; recifes rochosos submersos até à profundidade de 30 m e zonas intertidais durante a preia-mar), subtidal rochoso

artificial (AR; recifes artificiais submersos colocados sobre substrato móvel até 25 m de profundidade), substrato móvel pouco profundo (SS; substrato arenoso ou vasoso até aos 20 m de profundidade), substrato móvel de profundidade intermédia (IS; substrato arenoso ou vasoso dos 20 aos 100 m de profundidade) e substrato móvel profundo (DS; substrato arenoso ou vasoso dos 100 aos 200 m de profundidade).

Para verificar a robustez das tipologias definidas, calculou-se a similaridade média de Bray-Curtis entre os conjuntos de dados de cada tipologia e a dissimilaridade média de Bray-Curtis entre tipologias, juntamente com uma análise de similaridades (ANOSIM) entre tipologias, por forma a testar a significância das diferenças encontradas. As espécies e grupos ecológicos que contribuem em maior percentagem para estes valores de similaridade e dissimilaridade foram identificadas através de uma análise SIMPER (similarity percententage analysis).

Verificou-se que as diferenças verificadas entre amostras e entre tipologias são mais acentuadas quando se usam dados de abundância e composição de espécies do que quando se usam grupos ecológicos, dado que ao longo do gradiente ambiental as espécies vão sendo substituídas por outras dos mesmos grupos ecológicos, fazendo com que estes sejam mais estáveis face à variabilidade ambiental natural do sistema. Este facto, aliado à maior facilidade de identificação dos impactos proporcionada pelos grupos ecológicos sugere que este tipo de dados é mais adequado para a avaliação da qualidade ecológica de um sistema.

Na zona intertidal verificou-se que as espécies residentes territoriais caracterizam as associações de peixes do tipo IR, sendo sobretudo omnívoras, devido à elevada competitividade destes habitats. Nas associações de tipo NR observou-se que a maioria das espécies são residentes, sem comportamentos migratórios e muito dependentes do substrato, são invertívoras e reproduzem-se sobretudo na primavera e no verão. As de tipo AR caracterizam-se pela presença constante de espécies que se encontram na zona arenosa circundante, mas que dependem de formações rochosas para alimento, abrigo ou reprodução, exibindo comportamentos migratórios. Nas associações de tipo SS predominam os invertívoros, macrocarnívoros e zooplanctívoros, muito associados ao substrato, sendo que espécies residentes coexistem com outras de maior mobilidade, que tiram partido da disponibilidade de alimento e abrigo associadas às zonas costeiras e estuarinas. As de tipo SI distinguem-se por possuírem espécies menos dependentes do substrato, existindo uma predominância de espécies pelágicas e oceanádromas, de elevada mobilidade, que se reproduzem sobretudo no inverno. Por fim, nas áreas mais profundas, as espécies encontradas em associações de peixes de tipo DS ocupam níveis tróficos

superiores, havendo uma predominância de invertívoros e macrocarnívoros que se reproduzem também maioritariamente no inverno.

Para testar os efeitos da latitude, os conjuntos de dados foram divididos em cinco zonas latitudinais, coincidentes com as adoptadas pelo Instituto Português de Investigação das Pescas e do Mar (IPIMAR) nos cruzeiros demersais. Em seguida, foram utilizadas ANOSIM's para testar diferenças entre zonas latitudinais e entre estações do ano dentro de cada tipologia. Quando as diferenças encontradas foram estatisticamente significativas foi realizada uma análise SIMPER para identificar as espécies e grupos ecológicos que mais contribuem para a estas diferenças.

Nas associações de tipo DS verificou-se uma forte influência da latitude em todos os tipos de dados, que se deve sobretudo à elevada abundância de *Macroramphosus* spp. e *Capros aper* na zona central da costa portuguesa. Estas observações podem estar relacionadas com a topografia dos fundos marinhos, devido à presença dos canhões da Nazaré, Cascais e Setúbal neste local.

Quanto às diferenças sazonais, apenas nas associações de tipo IS se verificaram diferenças a larga escala, possivelmente relacionadas com o regime de afloramento costeiro, que aumenta a sua intensidade nos meses de verão, contribuindo para a predominância de *Sardina pilchardus*, uma espécie zooplanctonívora, sendo que no inverno a espécie macrocarnívora *Trachurus trachurus* é mais abundante.

Este trabalho permitiu verificar que a utilização de dados de composição de espécies juntamente com dados de grupos ecológicos em análise multivariada é um método eficaz para o estabelecimento de tipologias de associações de peixes marinhos. Contrariamente ao verificado quando apenas espécies individuais são utilizadas na definição de tipologias, com o método utilizado no presente trabalho é possível fazer a ligação entre a delimitação de unidades de gestão e as ferramentas utilizadas na avaliação do estado ambiental, que recorrem sobretudo a métricas relacionadas com grupos funcionais.

Com as tipologias de associações de peixes para o meio marinho definidas no presente trabalho ficaram assim estabelecidas as bases para a quantificação das proporções típicas de espécies e grupos ecológicos, por forma a permitir um cálculo adequado dos valores de referência a adoptar para a avaliação do estado ambiental requerida no âmbito da DEMM.

Palavras-chave: Directiva "Estratégia para o Meio Marinho"; ecologia marinha; grupos ecológicos; associações de peixes; plataforma continental; Portugal.

Summary

The proposed European Marine Strategy Directive (MSD) enforces the need for protection and conservation of the marine environment, having as the main objective the achievement of 'good environmental status' of the marine waters under jurisdiction of the Member States by 2021. In the MSD, fish are included as a biological element, thus constituting a new requirement for the assessment of marine waters that needs to be evaluated on the initial assessment to be presented by the fourth year after entry into force. These requirements urge the definition of marine fish assemblage typologies in order to permit the establishment of type-specific reference values that characterise a 'good' marine fish assemblage.

With the aim of establishing and characterising marine fish assemblages for the Portuguese continental shelf, from intertidal areas down to the 200 m isobath, a large variety of available data from studies conducted in Portuguese waters was collected and species were assigned into ecological guilds of several categories. Using guild and species data independently, a detrended correspondence analysis identified depth and bottom type as the factors underlying the main distribution gradient and led to the establishment of six assemblage typologies.

A non-metric analysis of similarities (ANOSIM) tested the consistency of the defined typologies and a similarity percentage analysis (SIMPER) routine identified the species and guilds that characterise each typology. Furthermore, the effects of latitude and seasonality were tested using ANOSIM and SIMPER within each typology, revealing that the first mainly affects soft substrate assemblages 20 to 100 m deep and the latter is noticed only deeper assemblages, within the same substrate.

The established typologies revealed distinct structural and functional characteristics, thus requiring the establishment of different reference values for quality assessment.

Keywords: Marine Strategy Directive; marine ecology; ecological guilds; fish assemblages; continental shelf; Portugal.

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CHAPTER 3

Chapter 1

General Introduction

Covering approximately 71% of the Earth surface and containing 90% of the biosphere, the marine environment includes complex and highly productive ecosystems that support huge biodiversity, supplying numerous resources and services (EU, 2005a). However, increasing anthropogenic pressure due to commercial fishing, chemical contamination, eutrophication, introduction of invasive species and habitat loss, allied to the effects of climate change, have significantly contributed to biodiversity loss and degradation of marine communities (EU, 2002, 2005a, 2006, 2007b; Borja, 2006; Mee *et al.*, in press).

In an effort to conserve and protect the marine environment, several national and international initiatives have surged. The United Nations Convention on the Law of the Seas (UNCLOS, 1982) is the basic international legal framework governing the uses of the sea and delimiting actions for the preservation of marine ecosystems, together with the 1992 Convention on Biological Diversity (CBD). In Europe, several community policies and regional conventions refer to the marine environment, such as the Habitats (92/43/EEC) and Birds (79/409/EEC) Directives, the Water Framework Directive (WFD; 2000/60/EC; EU, 2000), the Common Fisheries Policy, the International Council for the Exploration of the Sea (ICES) and regional seas conventions like the OSPAR Convention (North-East Atlantic), the Helsinki Convention (Baltic Sea), the Barcelona Convention (Mediterranean Sea) and the Bucharest Convention (Black Sea), but none constitute an strong and integrative approach that enforces the need for protection of the marine waters under jurisdiction of the Member States of the European Union (EU) (Borja, 2006).

Accounting for this lack of articulation between the various European strategies and conventions, the sixth action programme for the environment of the European Union (EU) (Decision 1600/2002/EC) has committed to develop a Thematic Strategy for the protection and conservation of the marine environment (EU, 2002), leading to its proposal in 2005, along with the proposal of an European Marine Strategy Directive (MSD; EU, 2005a,b,c). Later, the "Green Paper" on the European Maritime Policy (EU, 2006) was adopted, leading to the proposal of an Integrated Maritime Policy for the EU after the results of a one-year stakeholder consultation process, in a package named "The Blue Book" (EU, 2007b). The latter, together with the MSD, constitute a two pillar approach to the marine policy of the EU (Mee *et al.*, in press), the "Blue Book" referring

to the sustainable use of goods and services of marine waters and the MSD assuring the integrity of the ecosystems.

On the definition of "coastal waters" included in the WFD (EU, 2000) only approximately 19.8% of the European marine waters are covered (Borja, 2005), thus not fulfilling the need for an assessment of the status of the marine environment as a whole. However, the range of application of the MSD extends to the outermost reach of the area under sovereignty or jurisdiction of Member States, requiring the achievement of 'good environmental status' of the marine environment by 2021 and the design of monitoring and conservation programmes in order to prevent future deterioration (EU, 2005b).

In the proposed Directive, three marine regions were originally delimited: the Baltic Sea, the North-East Atlantic and the Mediterranean Sea (EU, 2005b), with the Black Sea being added as a fourth region in the most recent common position adopted by the Council due to Bulgaria and Romania joining the EU in 2007 (EU, 2007a). In the North-East Atlantic, four sub-regions are defined, with Portugal being included in the third sub-region, extending from the Bay of Biscay southwards along the Iberian coast until the Straight of Gibraltar (also including marine waters under jurisdiction of France and Spain) and in the fourth sub-region, constituted by the Azores, Madeira and the Canary Islands (EU, 2005b).

Each Member State is required to design a Strategy for the Protection of the Marine Environment, consistent with the marine region concerned, by following a pre-determined Action Plan. The first task of the Action Plan, to be achieved by the fourth year after entry into force of the MSD, consists of an initial assessment and identification of the anthropogenic impacts affecting marine waters, by defining typologies and reference values that correspond to 'good environmental status', which is defined as "*the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for uses and activities by current and future generations*" (EU, 2007a).

Furthermore, the MSD states that the ecological assessment should follow an "Ecosystem Approach", as presented by the CBD, by integrating scientific knowledge about the ecosystems with the management of human activities, in order to achieve a

sustainable use of marine resources and the maintenance of ecosystem integrity (CBD, 1998, 2000).

In this context there is an urgent need to understand and quantify the concept of 'good status', by characterising marine habitats with different "intrinsic conditions" in order to establish criteria that define a "healthy" system.

Despite the ecological and socio-economic importance of marine fish, these are not included in the quality assessment of coastal waters required by the WFD (EU, 2000). However, table 1 of the Annex III of the proposed MSD requires the inclusion of *"information on the structure of fish populations, including the abundance, distribution and age/size structure of the populations"* (EU, 2005b, 2007a), constituting a new requirement for the quality assessment of marine waters and hence requiring the development of new tools and methodologies.

Despite the problems related to the selective nature of sampling gears, the high sampling effort needed to characterise assemblages, the high mobility that permits the avoidance of impact sources and the relatively high tolerance of some species to stress, the advantages of using fish as ecological quality indicators clearly outrun these aspects. Fish are normally present in all aquatic systems, there is available information on how species respond to stress, identification of species is relatively easy, there are both mobile and sedentary species, thus permitting the assessment of local and broader impacts, their relative longevity permits a record of the impacts of stress for long periods of time and their social and economic value facilitates the communication with stakeholders and the general public (Karr, 1981; Karr *et al.*, 1986; Whitfield and Elliott, 2002; Harrison and Whitfield, 2004).

Additionally, one of the most useful advantages of fish is the fact that species can be easily combined into functional groups, or "guilds", that respond to stress in a more predictable way (Whitfield and Elliott, 2002; Harrison and Whitfield, 2004; Elliott *et al.*, 2007), which also makes assessment tools that use a guild approach more broadly applicable than others that refer to species, which are highly variable between regions.

The assessment of the structural and functional integrity of fish communities, as stated in the definition of 'good environmental status' (EU, 2007a), in a way that alterations in these communities due to anthropogenic impacts are understood by managers and decision-makers can be more efficiently achieved by adopting a multimetric index approach (de Jonge *et al.*, 2006), consisting of several measurable aspects (metrics) of

the structure (e.g. abundance, diversity) and function (e.g. guilds, trophic levels) of the community assembled in a single index that outputs the quality of the system.

Based on the multimetric Index of Biotic Integrity (IBI), described originally by Karr (1981) and further explained by Karr *et al.* (1986), there are many examples of multimetric indices developed to assess the quality of fish assemblages of rivers and transitional waters in the context of the WFD (e.g. Shiemer, 2000; Oberdorf *et al.*, 2002; Breine *et al.*, 2004, 2007; Harrison and Whitfield, 2004; Coates *et al.*, 2007; see Roset *et al.*, 2007 for a review), which provide the basis for the development of tools that evaluate the quality of marine fish communities in the context of the MSD, as the similar objectives of both directives should be faced with similar and comparable tools.

Although site-specific reference values based on local environmental conditions can be delimited (Roset *et al.*, 2007), these are usually best suited for local management purposes and would make the intercalibration process within marine regions very difficult. A type-specific approach is therefore the most appropriate and broadly used method, consisting of the definition of groups of faunal homogeneity by means of the application of clustering methods (Roset *et al.*, 2007). Clustering can be based either on a set of environmental properties (e.g. physico-chemical) or on the abundance and composition of species. The first approach defines potential habitat units with similar conditions but the latter distinguishes more realistic units, since, due to the very dynamic nature of the environment, it is very difficult to gather a set of environmental variables that completely explains species distribution (de Jonge *et al.*, 2006).

After the definition of habitat units or typologies, the thresholds for community metrics to be classified as high quality can be defined using various methods: (1) adopting minimally impacted sites as a reference, (2) using historical data, (3) calculating theoretical values based on models of species distribution or (4) directly assigning values by expert opinion based on background experience and personal observations (Vincent *et al.*, 2002; Borja, 2005; Roset *et al.*, 2007).

Although data from a historical period with minimal or inexistent anthropogenic impacts is sometimes available (e.g. Andersen *et al.*, 2004), it is very difficult or even impossible to distinguish the natural evolution of a site from the alterations that are due to deterioration, thus past conditions may not be recoverable in the present environment (Roset *et al.*, 2007; Mee *et al.*, in press). Moreover, sites with pristine, impact-free conditions are probably inexistent or rare in European marine waters due to industrialisation and sea currents (Andersen *et al.*, 2004) and thus reference values

should come from the least impacted sites within each typology, or even the best scoring site for each metric, rather than an ideal condition, since unrealistic recovery objectives could be unattainable (Roset *et al.*, 2007). This has led to the idea of "naturalness" (Hiscock *et al.*, 2003; Derous *et al.*, 2007), that describes how unaffected by anthropogenic impacts are the natural rates of change of a particular site, a concept that may lead to more realistic objectives, though being also difficult to define (Mee *et al.*, in press).

Regardless of the concept adopted, the definition of habitat units and the understanding and quantification of the "typical" structural and functional characteristics of fish assemblages as well as their temporal and spatial variation are urgent tasks to be fulfilled by all Member States as a basis for the establishment of reference values to be incorporated into the development of tools for ecological status assessment.

The present dissertation aims to define and characterise marine fish assemblage typologies for the Portuguese continental shelf based on composition and abundance of species and ecological guilds, as well as to analyse their seasonal and spatial variability in order to build a solid basis for the ecological status assessment and monitoring tools required by the MSD.

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Typology definition for marine fish assemblages in the context of the European Marine Strategy Directive: the Portuguese continental shelf

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Abstract

The requirements of the European Marine Strategy Directive urge the establishment of solid reference values for marine populations, which can only be achieved by first delimiting assemblage typologies for the marine waters under jurisdiction of each Member State. In order to establish typologies for marine fish assemblages, a large variety of available data from Portuguese waters was collected. A detrended correspondence analysis identified depth and bottom type as the factors responsible for the main gradient underlying the distribution of species and ecological guilds and permitted the establishment of six assemblage typologies. A non-metric analysis of similarities (ANOSIM) characterised the consistency of the typologies and a similarity percentage analysis (SIMPER) routine pointed out the species and guilds that characterised each typology. Using the same analysis within each typology, seasonality and latitude showed negligible effects in general, the first having an effect only on soft substrates 20 to 100 m deep and the latter on deeper soft substrate assemblages.

Keywords: Marine Strategy Directive; marine ecology; ecosystem management; fish assemblages; ecological guilds; continental shelves; Portugal.

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1. Introduction

Due to the consequences of an increasing anthropogenic pressure on the marine environment and accounting for the lack of articulation between the various strategies and conventions at both international and European levels (Borja, 2006), the sixth action programme for the environment of the European Union (EU) (Decision 1600/2002/EC) has committed to develop a Thematic Strategy for the protection and conservation of the marine environment, leading to the proposal of the European Marine Strategy Directive (MSD) that aims to achieve 'good status' of the marine waters under jurisdiction of the Member States by 2021 (EU, 2005a, b, c).

By the fourth year after entry into force of the MSD, Member States are required to present a complete evaluation of the ecological state and anthropogenic pressures of the marine waters under their jurisdiction, delimiting typologies and type-specific reference values in order to establish ecological quality standards (EU 2005b). This requirement urges the discussion and establishment of the concept of 'good status' of marine populations as well the definition of ecologically meaningful management units for assessment and monitoring of ecological status.

As opposed to other biological elements like phytoplankton, algae and benthic macroinvertebrates, whose monitoring is required by the Water Framework Directive (WFD) on the marine environment (EU, 2000), fish are deliberately excluded from the assessment of this area, therefore being a new requirement for ecological quality assessment of marine waters on the range of application of the MSD (EU, 2005b). Moreover, the high socio-economical value of fish, allied to their relative easiness of identification, diversity of ecological and trophic guilds, longevity, among others, are important advantages of using them as ecological quality indicators for water bodies (Whitfield and Elliott, 2002).

In this context, the political requirements so far have led to a number of papers focusing on fish as ecological indicators for streams (e.g. Schiemer, 2000; Oberdorff *et al.*, 2002; Breine *et al.*, 2004) and estuaries (e.g. Cabral *et al.*, 2001; Harrison and Whitfield, 2004; Breine *et al.*, 2007; Coates *et al.*, 2007) and a notorious methodological gap regarding the assessment of ecological status of marine waters using fish.

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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, including an integrated approach of the whole ecosystem supporting the stocks (e.g. Browman and Stergiou, 2004; Jennings, 2005) that fits the approach proposed by the MSD, but leaves a minor role to other anthropogenic impacts (Guidetti *et al.*, 2002).

For the reasons mentioned above, it is urgent to define reference values that characterize a 'good' marine fish assemblage, but not without first understanding what are the natural factors affecting the distribution of marine fish in order to establish types of assemblages from which to extract reference values.

There are many examples of authors that have studied how biotic and abiotic factors affect the abundance and distribution of fish populations and communities in Europe (e.g. Demestre *et al.,* 2000; García-Charton and Pérez-Ruzafa, 2001; Catalán *et al.*, 2006), however, most of the work so far has focused on a particular family or species or on a specific type of habitat, but the establishment of typologies of fish assemblages requires a wider approach.

For the Portuguese coast, a few examples of published work that constitute an important background for the establishment of marine fish community typologies are the studies performed by Gomes *et al.* (2001) and Sousa *et al.* (2005) for demersal soft-substrate fish species of the continental shelf and upper slope (20-710m deep), using data from bottom trawl surveys of the Portuguese Institute for Fisheries and Sea Research (IPIMAR), the work by Henriques *et al.* (1999) describing the composition and abundance of rocky reef fish species prior to the establishment of the Arrábida marine protected area, the characterization of the fish communities inhabiting the soft-substrate coastal area adjacent to the Tagus estuary by Prista *et al.* (2003) and the data on fish assemblages inhabiting rocky intertidal areas during low tide (Faria and Almada 1999, 2001) and high tide (Faria and Almada, 2006). In addition, the establishment, in 1990, of artificial reefs in soft bottom sediment near Ria Formosa, southern Portugal (Monteiro *et al.*, 1994; Santos *et al.,* 2005), creates another important habitat that should be taken into account when establishing typologies for the continental shelf of this area, since there is evidence that these reefs differ in some aspects of fish assemblage structure from the nearby natural rocky reefs (Santos *et al.,* 1995; Almeida, 1997).

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With a broad variety of habitats, from rocky intertidal and subtidal areas to shallow and deep plains of sandy or muddy substrate, continental shelves are a very important source of primary production, providing nursery areas for juvenile fish and supporting commercially exploited fish stocks (Gomes *et al.* 2001; Sousa *et al.*, 2005). For this reason, the establishment of typologies for marine fish in these areas is particularly important to support policy-defined management units.

The present study aims to establish marine fish assemblage typologies for Portuguese coastal waters, ranging from the upper limit of the intertidal areas down to approximately 200 meters deep, by compiling and for the first time approaching as a whole a broad collection of available data on composition and abundance of marine fish, covering a wide range of environmental variability and habitat diversity in order to understand not only the main gradients and factors delimiting fish assemblages, but also to study variations in individual species and ecological guilds within and between typologies.

2. Materials and Methods

2.1. Study area

The Portuguese continental shelf waters are included in ICES region IXa and in the Northeastern Atlantic eco-region of the MSD, sharing sub-region responsibilities with France, in the Bay of Biscay, and Spain, from the northern coast southwards to the straight of Gibraltar (EU, 2005b). The portuguese coast extends from the Minho river mouth southwards along the 9ºW meridian, then eastwards at cape São Vicente (approximately 37ºN). The continental shelf is relatively narrow and its most conspicuous irregularity is the Nazaré Canyon. Situated on the west coast, at about 39º30'N, and 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 20km wide until cape São Vicente, then reaching a width of about 30km in the south coast (Gomes *et al.*, 2001).

Over the shelf, the upper layers of water are under the influence of upwelling during the summer months (July-September) due to predominant northern winds. In winter, the

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wind regime becomes more variable and only intermittent and weaker upwelling periods are observed (Fiúza *et al.*, 1982).

In the present study, a depth limit was established at the 200 m isobath, adopted as a rough approach to the continental shelf border, as the variable depth of the border itself along the coast would affect the analysis.

2.2. Data sources and collection

Most of the data on composition and abundance of fish assemblages from Portugal is not easily available, consisting mainly of unpublished theses and technical reports, but an effort was made during the present study to compile the maximum possible data from various locations, depths, seasons, sampling methods and sediment types.

Since the present study aims to establish basic typologies for future management and assessment of ecological status, only abundance data, rather than presence-absence, were considered, as important variations in abundance would pass unnoticed until total disappearance of taxa (Hewitt *et al.*, 2005).

Mainly due to bottom morphology, different sampling methods are best suited for different substrate types. On the collected datasets (table 1), bottom trawl was the most frequent method used on soft substrate, underwater (SCUBA) visual census was the only method used on natural and artificial reefs and intertidal rocky platforms were sampled with tide pool census.

In spite of being the most suited methods available to assess fish diversity within each type of substrate, the number of individuals counted by each method is very different, thus making absolute frequency comparisons between substrates unfeasible. With the purpose of minimising the effects of sampling methods on the establishment of typologies, relative frequencies were calculated in order to allow the comparison between datasets, though maintaining the proportion represented by each species or guild. Apart from this, all ordinations were run on untransformed data, since data transformations usually reduce the effect of variations in the proportion of the most abundant species or guilds, which is not desired when establishing the bases for ecological status assessment of marine communities (Hewitt *et al.*, 2005).

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Another important selection criterion was the possibility to divide datasets into seasons whenever possible in order to analyse seasonal variability.

A total of 86 datasets were compiled (table 1) and the taxonomic nomenclature was updated and corrected according to FishBase online database (Froese and Pauly, 2007).

Table 1: Summary of the references from which the data were collected. The type of substrate and the number of datasets extracted for the present study are specified.
Legend: I – rocky intertidal, S – soft, R – rock, AR – artificial rock.

Reference	Substrate	Nr. of datasets
Arruda (1979)	I	2
IPIMAR (1980)	S	8
IPIMAR (1981a)	S	9
IPIMAR (1981b)	S	6
IPIMAR (1982)	S	10
IPIMAR (1984)	S	10
Henriques (1993)	R	4
Rodrigues (1993)	R	4
Souto (1993)	AR	2
Almeida (1996)	R	2
Almeida (1997)	R / AR	2
Faria (2000)	I	4
Almada *et al.* (2002)	R	4
Paiva (2002)	I	4
Cabral *et al.* (2003)	S	3
Prista *et al.* (2003)	S	4
Almada *et al.* (2004)	R	1
Gonçalves (2004)	R	2
Abreu (2005)	S	1
Batista (2005)	S	1
Faria and Almada (2006)	R	1
Maranhão *et al.* (2006)	R	2
	TOTAL	86

2.3. Guild classification

Previewing the future use of fish guilds in ecological quality indices for marine waters (Henriques *et al.*, submitted), the definition of typologies must take into account the distribution of these guilds regardless of individual species. For this reason all the species were included in a total of 37 ecological guilds from seven categories (table 2), based on available data from FishBase online database (Froese and Pauly, 2007), personal observations of the authors and expert consultation (Appendix I).

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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, etc.). The term "offshore" was used when species inhabit or depend on deeper waters, not considering the type of substrate beneath (e.g. pelagic species).

Table 2: List, by category, of the ecological guilds used in the analysis. Legend: I – rocky intertidal, S – soft substrate, R – rocky substrate. See section 2.3 for a detailed description.

Category	Guild	Category	Guild
Substrate dependence	S resident offshore resident R resident I resident S dependent offshore dependent R dependent I dependent	Migration	non-migratory oceanadromous catadromous anadromous anfidromous
Mobility	high medium territorial sedentary	Trophic	invertivore omnivore macrocarnivore zooplanktivore piscivore herbivore
Habitat	demersal pelagic reef-associated bathydemersal bathypelagic benthopelagic	Resilience	very low low medium high
		Spawning season	spring summer autumn winter

Migration and trophic guilds were adapted from the review on estuarine fish guilds by Elliott *et al.* (2007), with some alterations. In the latter, species were considered "invertivore" 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 and classified as "high" (up to 1.4 years), "medium" (1.4 to 4.4 years), "low" (4.5 to 14 years) and "very

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low" (more than 14 years) (Froese and Pauly, 2007). Using guild classification, two separate data matrices were built, one with the relative frequency of individuals that fit each guild by category (hereafter designated "guild frequencies") and another with the number of species per guild.

2.4. Data analysis

One of the advantages of using fish as ecological indicators is the large variety of ecological guilds that respond very typically to alterations on the ecosystem (Elliott *et al.*, 2007). For this reason, all the analyses were performed on the species, the guild frequencies and the number of species per guild matrices in parallel. In all permutation tests, a maximum of 999 permutations were performed and the level of statistical significance adopted was 0.05 for all analyses.

2.4.1. Main gradients and typology definition

Multivariate ordination was used to identify the main gradients and habitat types affecting the distribution of fish. To account for the marked arch effect produced by correspondence analysis (CA), and considering that the variability associated with the main ecological gradient is retrieved mainly by the first axis, a detrended correspondence analysis (DCA; Hill and Gauch, 1980) was performed using Canoco for Windows 4.5 software (ter Braak and Šmilauer, 2002). Since no covariables or environmental variables were included for direct analysis, detrending by segments was the method chosen (Lepš and Šmilauer, 2003). In order to interpret the influence of latitude and depth on species and guild variability along the main gradient, the correlation of these variables with the first axis was analysed via indirect gradient analysis.

The resulting typologies were characterised using the PRIMER v.5 (Plymouth Routines in Multivariate Ecological Research) software package (Clarke and Warwick, 2001). The average within-group Bray-Curtis similarity and between-group dissimilarities were calculated and a non-parametric one-way analysis of similarity (ANOSIM) was performed in order to evaluate the distinction between the defined typologies. The species and guilds with the highest contribution to the average similarity within

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typologies and to the average dissimilarity between typologies were identified using the similarity percentage analysis (SIMPER) routine.

2.4.2. Latitude and Seasonality

All the datasets were grouped into five latitude intervals that corresponded to the zones adopted in the sampling surveys conducted by the Portuguese Institute for Fisheries and Sea Research (IPIMAR), former National Institute for Fisheries Research (INIP). Zone 1 extends from Caminha (41°52'N) to Ovar (40°51'N), zone 2 from Ovar to S. Pedro de Moel (39°45'N), zone 3 from S. Pedro de Mo el to Cercal (37°48'N), zone 4 from Cercal to Lagos (37°6'N, 8°40'W), on the sout h coast, and zone 5 from Lagos to Vila Real de Santo António (37°11'N, 7°24'W). The a nnual average sea surface temperature (SST) was calculated for each sample using data from ICOADS (2002) and a strong negative correlation was found between latitude and SST (r=-0.82, p<0.05) on the compiled datasets, indicating that latitude zones can be used as an indirect measure of the influence of SST.

In order to evaluate the effect of latitude and seasonality within each of the resulting typologies, differences in the fish assemblage structure between different latitude zones and seasons were tested through one-way ANOSIM routines applied to Bray-Curtis similarity matrices. Whenever significant differences were found, a SIMPER analysis routine was used to understand the main species and guilds characterising each season and latitude zone.

3. Results

A total of 212 species were found on the compiled surveys belonging to 67 families of the classes Chondrichthyes and Actinopterygii (Appendix I). The most represented families on the database were Sparidae (21 species), Gobiidae (18 species), Labridae (13 species), Soleidae (11 species) and Blenniidae (10 species).

3.1. Main gradients and typology definition

The main gradients retrieved by DCA using species (figure 1A), guild frequencies (figure 1B) and number of species per guild (figure 1C) were coincident, revealing a strong negative correlation of depth with the first axis (-0.775 for species data, -0.664 for guild frequencies and -0.708 for the number of species per guild) as well as a strong

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influence of substrate type on the distinction between fish assemblages. Latitude was not correlated with the main gradient using the three data sets (0.049 for species data, -0.051 for guild frequencies and -0.038 for the number of species per guild).
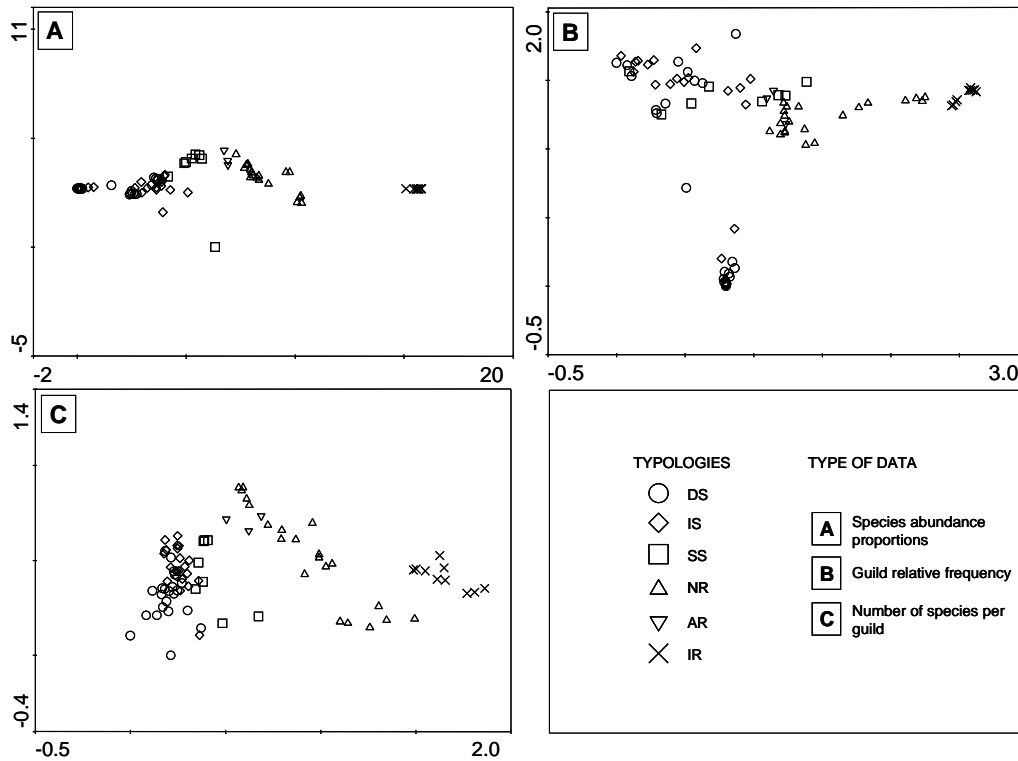
Figure 1: Detrended Correspondence Analysis plots of samples using three types of data as variables. Axes values are in standard deviation units of species turnover. See section 3.1 for details. Legend: IR – rocky intertidal, NR – natural rocky subtidal, AR – artificial rocky subtidal, SS – shallow soft-bottom, IS – intermediate soft-bottom, DS – deep soft-bottom.

The DCA plot of samples using species data (figure 1A) had a gradient length of 15.81 standard deviation (SD) units on the first axis, with no species shared between both ends of the gradient, total inertia was 12.738 and the first two axes represented 12.9% of the variance of the species data. With guild frequencies data (figure 1B), the gradient represented by the first axis was 2.623 SD units long and the total inertia was 1.191, with the first two axes explaining 43.8% of the total variance. The analysis relative to the number of species per guild (figure 1C) had 63.9% of the variance explained by the first two axes, with the shortest gradient length (1.862 SD units) and a total inertia of 0.324.

According to the ordination analyses, six basic assemblage typologies were defined: rocky intertidal (IR; fish inhabiting intertidal pools at low tide), natural rocky subtidal (NR; permanently submerged rocky reefs down to a depth of 30 m and intertidal areas

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sampled during high tide), artificial rocky subtidal (AR; artificial reefs over soft-bottom flats down to 25 m deep), shallow soft-bottom (SS; sandy or muddy substrate down to 20 m deep), intermediate soft-bottom (IS; sandy or muddy substrate 20 to 100 m deep) and deep soft-bottom (DS; sandy or muddy substrate 100 to 200 m deep). Significant differences were found between the defined typologies using the ANOSIM routine on the species (R=0.638; p<0.001), the guild frequencies (R=0.414; p<0.001) and the number of species per guild (R=0.408; p<0.001) data.

The highest average similarities within typologies were obtained using the number of species per guild (table 3C), with the species frequencies providing the lowest values (table 3A) and the average dissimilarities between typologies were higher when using species frequencies (table 3A) and lower when using guild data (table 3B,C).

Table 3: Average percent Bray-Curtis dissimilarity matrices between the defined typologies using three types of data. (A) species abundance, (B) guild frequency, (C) number of species per guild. Values within brackets represent the average within-group similarity. Cases where the dissimilarity was not significant on ANOSIM pairwise tests are marked *. Legend: IR – rocky intertidal, NR – natural rocky subtidal, AR – artificial rocky subtidal, SS – shallow soft-bottom, IS – intermediate soft-bottom, DS – deep soft-bottom.

A	IR	NR	AR	SS	IS	DS
	(57.25)	(22.35)	(46.05)	(25.17)	(23.29)	(31.62)
IR	0.00	98.65	99.95	99.99	100.00	100.00
NR	98.65	0.00	83.43*	96.92	98.94	99.57
AR	99.95	83.43*	0.00	88.54	93.51	97.49
SS	99.99	96.92	88.54	0.00	87.21	93.53
IS	100.00	98.94	93.51	87.21	0.00	76.63
DS	100.00	99.57	97.49	93.53	76.63	0.00

B	IR	NR	AR	SS	IS	DS
	(94.18)	(67.22)	(76.77)	(57.99)	(55.38)	(57.38)
IR	0.00	45.75	57.28	60.54	70.97	73.07
NR	45.75	0.00	35.27*	47.00	54.66	58.24
AR	57.28	35.27*	0.00	42.57*	52.50*	57.10
SS	60.54	47.00	42.57*	0.00	45.78*	52.00
IS	70.97	54.66	52.50*	45.78*	0.00	46.49*
DS	73.07	58.24	57.10	52.00	46.49*	0.00

C	IR	NR	AR	SS	IS	DS
	(80.27)	(64.00)	(77.17)	(69.89)	(75.45)	(73.01)
IR	0.00	51.18	54.02	63.06	62.15	61.98
NR	51.18	0.00	33.42*	38.13*	39.67	41.88
AR	54.02	33.42*	0.00	31.44*	29.34	32.65*
SS	63.06	38.13*	31.44*	0.00	28.53*	31.32*
IS	62.15	39.67	29.34	28.53*	0.00	26.41*
DS	61.98	41.88	32.65*	31.32*	26.41*	0.00

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The most distinct typology (with the highest average within-group similarities and between-group dissimilarities) was IR (table 3) and the most similar typologies (lowest average dissimilarity values that did not reject the null hypothesis in ANOSIM pairwise tests) were NR and AR when using both species (table 3A; R=0.086; p>0.05) and guild (table 3B; R=0.099; p>0.05) frequencies. When using the number of species per guild (table 3C), although the comparison between NR and AR did not reject the null hypothesis (R=-0.108; p>0.05), IS and DS assemblages had the lowest dissimilarity percentage (26.41%; R=0.041; p>0.05).

The SIMPER analysis routine revealed that the species with the highest percent contribution for the similarity between DS datasets were *Macroramphosus gracilis* and *Macroramphosus scolopax* (67.46%), *Micromesistius poutassou* (11.18%), *Merluccius merluccius* (9.59%) and *Trachurus trachurus* (9.25%), on IS were *T. trachurus* (34.20%), *Sardina pilchardus* (16.42%), *M. merluccius* (13.03%), *M. scolopax* (12.28%), *M. gracilis* (12.27%) and *Trisopterus luscus* (3.07%) and on SS were *T. trachurus* (33.37%), *Callionymus lyra* (23.09%), *Arnoglossus laterna* (14.31%) and *Diplodus bellottii* (10.30%). The main species associated with NR were *Diplodus vulgaris* (15.62%), *Coris julis* (9.48%), *Boops boops* (6.56%), *Sarpa salpa* (6.17%), *Parablennius pilicornis* (6.13%), *Gobiusculus flavescens* (5.52%), *Tripterygion delaisi* (5.15%), *Diplodus sargus* (4.97%), *Symphodus melops* (4.03%) and *Labrus bergylta* (3.53%), while those characteristic of AR datasets were *D. bellottii* (14.21%), *C. julis* (14.21%), *Scorpaena notata* (11.72%), *Diplodus annularis* (9.55%), *T. luscus* (8.86%), *D. vulgaris* (7.33%), *Pagellus acarne* (4.69%), *T. trachurus* (4.41%), *B. boops* (3.65%) and *Diplodus puntazzo* (3.44%). On IR datasets *Lipophrys pholis* (52.17%), *Coryphoblennius galerita* (27.88%), *Lepadogaster lepadogaster* (9.44%) and *Paralipophrys trigloides* (4.72%) were the most typical species.

On soft substrate, the guild frequency metrics with the highest percent contribution for the dissimilarity between the SS and IS typologies were the frequency of pelagic (6.52%), high mobility (6.11%) and oceanadromous (6.09%) individuals, more abundant on datasets from intermediate depths, and the frequency of spring spawning (6.16%), non-migratory (6.10%) and medium mobility (5.88%) individuals, more abundant on shallow datasets. Between IS and DS datasets, the dissimilarity was mainly due to the frequency of high mobility (6.79%) and oceanadromous (6.78%) individuals, more abundant in the first, and the frequency of macrocarnivore (7.09%), invertivore (7.47%) and non-migratory (6.78%) individuals, more abundant in the latter. The total number of species showed a decreasing trend with depth on soft substrate,

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with average values of 33 ± 16 in SS datasets, 27 ± 15 in IS datasets and 24 ± 9 in DS datasets. In addition, the number of spring spawning and medium resilience species also tended to decrease with depth and showed a high percent contribution to the dissimilarity between shallow and intermediate datasets (7.77% and 7.38% respectively) as well as between intermediate and deep datasets (7.77% and 7.23% respectively).

The similarity between NR assemblages was mainly due to the contribution of the frequency of spring (12.20%) and summer (9.85%) spawning, non-migratory (9.94%), demersal (6.87%), invertivore (6.74%) and rock resident (6.71%) individuals, as well as to the number of spring (9.81%) and summer (8.23%) spawning, non-migratory (8.58%) and demersal (7.13%) species. AR assemblages were characterised by the high percent contribution of the frequency of spring spawning (12.53%), medium resilience (12.49%), rock dependent (9.10%) and non-migratory (8.18%) individuals and by the number of spring (9.54%) and summer (6.69%) spawning, medium resilience (8.64%) and non-migratory (6.69%) species. Finally, the contribution of the frequency of demersal (11.86%), non-migratory (11.83%), rock resident (11.83%), spring spawning (11.75%) and territorial (11.71%) individuals, as well as the number of demersal (11.65%), spring spawning (11.10%), non-migratory (10.53%) and territorial (9.69%) species to the similarity between datasets characterised IR fish assemblages.

3.2. Latitude

Although latitude did not show a significant influence on the main gradient (see section 3.1), differences between latitude zones were found significant within DS assemblages using the ANOSIM routine on species (R=0.477; p<0.001), guild frequency (R=0.454; p<0.001) and number of species per guild (R=0.260; p<0.05) data.

On DS assemblages, the percent contribution of *M. poutassou* (85.46%) and *M. merluccius* (10.50%) characterised the datasets from zone 1, *M. scolopax* and *M. gracilis* had the highest contribution on zones 2 (88.88%), 3 (98.34%) and 4 (91.81%) and *T. trachurus* (43.75%), *M. merluccius* (42.36%) and *M. poutassou* (7.13%) on zone 5 (see section 2.4.2 for zone limits). Despite the dominance of *M. gracilis* and *M. scolopax* on the central zones 2, 3 and 4, the species that best distinguished zone 2 from zone 3 (with the highest contribution for the dissimilarity between zones) were *M.*

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poutassou (31.72%) and *T. trachurus* (10.17%) and zone 4 was characterised by the presence of *P. acarne* (16.89%), *M. merluccius* (13.92%) and *T. trachurus* (10.45%), all of these species being absent in zone 3, which showed a greater abundance of *M. gracilis*, *M. scolopax* and *Capros aper*. The DS datasets from zone 1 were characterised by the percent contribution of macrocarnivore (18.79%), high mobility (14.21%), pelagic (14.05%) and oceanadromous (13.91%) individuals, zone 2 by pelagic (22.82%), winter spawning (19.53%), medium resilience (11.18%) and non-migratory (9.67%), zone 3 by non-migratory (15.47%), medium mobility (15.47%), invertivore (15.42%) and pelagic (14.89%), zone 4 by non-migratory (15.08%), medium mobility (15.07%), winter spawning (14.63%) and pelagic (13.84%) and zone 5 by macrocarnivore (16.81%), low resilience (10.24%), oceanadromous (8.90%) and high mobility (8.90%) individuals.

The average number of species per sample was lower on the north (15 ± 4 on zone 1) and south (19 ± 11 on zone 5) zones and higher on the central zones (30 ± 10 on zone 2, 31 ± 3 on zone 3 and 22 ± 6 on zone 4), which was evident on the analysis performed with the number of species per guild, where the number of spring spawning, macrocarnivore, non-migratory and medium mobility species contributed cumulatively to more than 30% of the within-zone similarity in all zones.

IS datasets showed no differences between latitude zones in general, except for zones 1 and 4, which only revealed significant dissimilarity using species data (R=0.556; p<0.05), mainly due to the percent contributions of *S. pilchardus* (20.31%) and *T. trachurus* (19.33%), both more abundant in the north. On SS, only zones 3 and 5 were represented, with no significant differences on all data types. On NR, using datasets from zones 3, 4 and 5, only the first two zones showed significant differences using species (R=0.568; p<0.01) and guild frequency (R=0.594; p<0.01) data, but not with the number of species per guild (R=0.169; p>0.05). IR showed no influence of latitude and AR assemblages were not included in the analysis, as they are located exclusively on the south coast.

3.3. Seasonality

The effect of seasonality on the species and guild composition within the typologies was generally low, except for IS assemblages, where winter was significantly dissimilar

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from summer and spring concerning species composition (80.85%; R=0.201; p<0.05 and 85.81%; R=0.431; p<0.05, respectively), guild frequencies (47.30%; R=0.206; p<0.05 and 48.90%; R=0.388; p<0.05, respectively) and number of species per guild (23.22%; R=0.296; p<0.05 and 30.33%; R=0.228; p<0.05 respectively). The SIMPER analysis routine attributed the highest percent contributions for the dissimilarity between winter and summer/spring datasets to the species *T. trachurus* and to the frequency of macrocarnivores, spring spawners and high mobility individuals, more abundant in winter, and to the species *S. pilchardus*, *M. scolopax* and *M. gracilis* and the frequency of invertivores, medium mobility and non-migratory individuals, more abundant in summer and spring. The highest contributions concerning the number of species were due to spring and winter spawning, medium resilience, macrocarnivore, high mobility and oceanadromous species, all more numerous in summer and spring.

No significant influence of seasonality was detected on DS, SS and NR assemblages for all types of data used. On artificial rocky reefs and rocky intertidal platforms the analysis was not performed due to lack of sufficient data in order to calculate the significance of the R statistic.

4. Discussion

Six assemblage typologies were successfully delimited on the Portuguese continental shelf, taking into account not only species composition and relative abundance but also the relative frequency and composition of ecological guilds. Substrate type and depth were identified as the main factors underlying differences in assemblage distribution.

Substrate is known to be a very important habitat structuring factor, since it provides different shelter, types and quantities of food and other important conditions that influence survival rates and habitat selection on species with different ecological needs (Rice, 2005). Several authors have demonstrated that differences in fish assemblages can occur not only between very different bottom types, like soft and hard substrates (Pihl and Wennhage, 2002), but also between different structural characteristics within the same substrate, like different types of sediment (Demestre *et al.*, 2000) or rocky reef areas of different complexity (García-Charton and Pérez-Ruzafa, 2001). However, in the present study, subtle differences were incorporated into habitat characteristics at

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a larger scale, in order to establish typologies that cover a wide range of natural variability.

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 delimiting the distribution of fish, crustaceans and cephalopods on the Portuguese shelf and upper slope.

On the DCA plots of samples the scale of the second axis is an artifact of the detrending process and has no ecological meaning (Lepš and Šmilauer, 2003), thus the distribution of samples was analysed only along the first axis. Using all types of data, a group of six datasets that were sampled using underwater visual census in rocky subtidal areas, four in the Berlengas islands (Rodrigues, 1993) and two in Sagres (Gonçalves, 2004), were persistently plotted isolated and closer to the IR assemblages than other NR datasets. This group illustrates the importance of an adequate sampling plan on the assessment of assemblage composition, as these six datasets were sampled with a focus on cryptic species, thus containing a larger proportion of rocky substrate residents of the families Blenniidae and Gobiidae, some of them, like *Gobius paganellus* and *Parablennius gattorugine*, also present in tide pools (Faria and Almada, 2006). Although these datasets were included in the present study and classified as NR assemblages, similar surveys should not be used to assess ecological status. Instead, multiple visual census surveys focused on different niches should be performed in order to assess assemblage composition more accurately (De Girolamo and Mazzoldi, 2001).

Based on the results of DCA and Bray-Curtis similarity and dissimilarity indices, 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 frequency as some species are replaced by others of the same guild. An example is the replacement of the invertivore species *M. scolopax* and *M. gracilis*, abundant in DS assemblages by *L. lepadogaster* and *G. paganellus*, also invertivore and abundant in IR assemblages, two typologies that occupy opposite ends of the gradient.

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When using guild data, as relative frequencies are more easily affected than alterations in species composition, the number of species per guild is more resistant to variation and consequently the shortest length of gradient and the lowest dissimilarities between typologies correspond to this type of data. Thus, with very low within-group similarities, the proportions of individual species are very sensitive to environmental variation, hence making 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 ecological status of an assemblage, compared to ecological guilds (Elliott *et al.*, 2007). However, as observed on within-group similarity values, though guild data can distinguish typologies at a relatively large biogeographic scale, smaller variations are more difficult to detect, therefore, a careful selection of the community metrics that best detect impacts associated with the most important pressures affecting each typology is required (Henriques *et al.*, submitted).

The NR typology identified in the present study displays typical characteristics of warm-temperate rocky reefs (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, are responsible for the observed predominance of summer and spring spawners (Almada *et al.*, 1999). Due to the high productivity and complexity of rocky reefs, most species are very substrate-dependent (Almada *et al.*, 1999; Henriques *et al.*, 1999; García-Charton and Pérez-Ruzafa, 2001; Pihl and Wennhage, 2002), hence the abundance of non-migratory, demersal and rocky substrate residents being characteristic of this typology, which makes the NR assemblages vulnerable to impacts that negatively affect habitat characteristics (Guidetti *et al.*, 2002).

Invertivore species constitute the main trophic guild in NR assemblages, as zoobenthos are the most reliable prey in an environment where the biomass of algae and plankton has significant seasonal variability (Fiúza *et al.*, 1982; Almada *et al.*, 1999). The occurrence of few herbivore species on temperate rocky reefs verified by many authors (e.g. Almada *et al.*, 1999; Horn and Ojeda, 1999) has also been noticed in the present study, with *S. salpa* being the only species, among the most common, whose adults are almost exclusively herbivore. This fact is in part related to the seasonality of algal biomass, which decreases in winter (Horn and Ojeda, 1999).

Due to the cold temperatures in winter and a higher exposure to dominant winds and wave action (Sousa *et al.*, 2005), rocky reefs in the north coast of Portugal (zones 1

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and 2) are very difficult to sample using underwater visual census (Henriques *et al.*, 1999) and therefore no data was found for these areas. Nonetheless, the available datasets suggested no significant influence of latitude on the south coast, as zone 3 was similar to zone 5. The observed differences between zones 3 and 4 in ANOSIM were due to differences in sampling methods, as samples in Sagres (zone 4), as referred previously, were focused on cryptic species (Gonçalves, 2004). Between zones 4 and 5 only three permutations were possible and, despite the acceptance of the null hypothesis in ANOSIM, the significance of the result is not clear.

Despite the known seasonal variations in the environment, no significant differences between seasons were found on the species and guild composition of NR assemblages of the centre and south coast. This is supported by the observations in Beja (1995) concluding that winter stress does not have a very marked effect on rocky reef fish of the southwest coast of Portugal, compared to other temperate reefs. In addition, Pihl and Wennhage (2002) observed that seasonal differences affect mainly the number of individuals, thus the use of abundance proportions in the present study attenuates those effects.

The formation of a separate group of AR datasets on DCA plots when using species data led to the inclusion of these datasets in a different typology. Although differences between NR and AR assemblages were not significant according to ANOSIM, few permutations were possible due to the reduced number of AR datasets available, since there are only a few, relatively recent artificial reefs in Portugal (Monteiro *et al.*, 1994; Santos *et al.*, 2005). The significance of these results must therefore be viewed with some reservations.

When compared to nearby natural reefs, artificial reefs are known to support different fish assemblages (Santos *et al.*, 1995; Almeida, 1997; Perkol-Finkel *et al.*, 2006) that are mainly due to isolation and structural differences (Santos *et al.*, 2005; Perkol-Finkel *et al.*, 2006). Additionally, artificial reefs of the south coast of Portugal were built over sandy substrate with the aim of supporting fish stocks (Monteiro *et al.*, 1994), therefore having pressures and management objectives that are different from those of natural reefs.

In contrast with NR assemblages, where demersal residents were typical, benthopelagic rock dependent species like *T. luscus*, *D. vulgaris*, *D. annularis* and *P. acarne* were more characteristic of AR assemblages. This is probably due to the

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location of artificial reefs over soft substrate, thus attracting mobile species that depend on hard substrate for feeding, shelter and/or reproduction, performing migrations from the nearby sandy areas and form the Ria Formosa lagoon. This "oasis" effect reported by Santos *et al.* (2005) depends on the level of isolation from nearby natural reefs and is mainly due to the increase in primary productivity that leads to the enrichment of the benthic community of the surrounding substrate (Falcão *et al.*, 2007), hence the larger proportion of invertivores and macrocarnivores observed in the present study.

Due to the scarcity of AR datasets, it was not possible to test the effect of seasonality in the present study. However, Santos *et al.* (2005) observed that, on these reefs, fish density decreased in winter, which would not necessarily affect abundance proportions, and that the reefs closer to Ria Formosa are affected by the migration of juveniles from the lagoon in autumn, which was not verified in other reefs, therefore being an occurrence related to the particularities of the surrounding environment and not inherent to artificial reefs.

These results highlight the particularities of these assemblages and support the need for a specific AR typology for ecological status assessment and environmental monitoring.

Although not included on the requirements of the MSD (EU, 2005b), intertidal rocky platforms are known to be very important as nursery areas for some commercially important species (Faria and Almada, 2006). Moreover, considering their vulnerability to human intervention, monitoring and management of these habitats are extremely relevant, hence the inclusion of this typology in the present study.

IR assemblages of the Portuguese coast are characterised by the presence of cryptic species of the families Blenniidae, Gobiidae and Gobiesocidae that are highly dependent on this habitat for food, shelter and reproduction (Faria and Almada, 2006). This was observed in the present study, as the non-migratory, demersal and intertidal resident species constituted the most characteristic guilds of these assemblages. The high proportion of territorial individuals clearly distinguishes this typology, as the limited availability of suitable shelters and nests in a pool leads to competition and individuals that are unable to establish a territory are forced to leave (Faria and Almada 1999, 2001). Another consequence of competition and unstable characteristics of this typology is the predominance of omnivore species, as specialisation in food types is disadvantageous in a highly competitive environment (Faria and Almada, 2001).

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Although resident species were characteristic, as they persisted between datasets, juveniles of mobile species typical of soft substrates (e.g. *Ciliata mustela* and *Gaidropsarus mediterraneus*) and nearby rocky subtidal areas (e.g. *S. melops* and *D. sargus*) were frequently found on the collected datasets, thus emphasising the importance of these habitats as nursery areas.

For the same reasons previously mentioned for NR assemblages, spring spawning species were typical of IR datasets, some starting their breeding period in winter, like *L. pholis* and others extending it to the summer months, like *C. galerita* (Faria and Almada, 2001). Apart from this fact, the significance of the effect of seasonality was unclear due to the fact that some of the datasets could not be separated into seasons, however, the predominant sizes of individuals are known to vary seasonally according to the recruitment period of each species (Faria and Almada, 2001) and a decrease in abundance of benthic species of intertidal areas during winter has been observed by Faria and Almada (2006), who suggested that the inactivity of species that stay sheltered in holes and crevices for longer periods of time makes them more difficult to detect when sampling tide pools.

Although the scarcity of available data on fish assemblages from tide pools in zones 1, 2 and 4 discourages general conclusions on this matter, the observations of the present study did not suggest a significant influence of latitude on this typology. Similar observations were made by Arruda (1979) and Faria and Almada (2001) which suggest that differences between IR assemblages to the north and south of Lisbon affecting the most common species are probably due to specific habitat complexity and wave exposure characteristics rather than a direct consequence of latitude. This fact is very important for this typology and stresses the importance of incorporating environmental and microhabitat characteristics into the assessment of these areas, in order to be able to isolate the variability that is due to anthropogenic pressures (García-Charton and Pérez-Ruzafa, 2001).

The demersal soft-bottom surveys conducted by the IPIMAR were planned for the estimation of stocks of a few commercially important species, and thus are not ideal for use in the establishment of typologies based on distribution patterns (Gomes *et al.*, 2001). Nevertheless, the collected data cover the whole continental shelf, with winter, summer and spring surveys, therefore allowing for the effect of latitude and seasonality to be more accurately tested, as well as the limits between assemblages, which on this substrate are not established by marked morphological boundaries and hence very

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difficult to define. Due to this fact, previous works by Gomes *et al.* (2001) and Sousa *et al.* (2005) using fish, cephalopods and crustaceans, have been successful in identifying patterns and delimiting assemblages at an acceptable scale.

The study performed by Gomes *et al.* (2001) using species biomass data from 1985 to 1988 delimited four to five assemblages based on depth (20 to 500 m) and latitude and Santos *et al.* (2005), using 11 years of survey data (1989-1999) and a similar method, established five assemblage types partially similar to the previous ones, but covering a wider depth range (20 to 700 m). These studies, however, did not include data on shallower soft-bottom assemblages, which were included in the present study due to their importance for juvenile fish and to the particularities associated with the proximity of estuaries (Cabral *et al.*, 2003; Prista *et al.*, 2003).

Unlike rocky reefs, where depth was limited due to the sampling method, soft-bottom datasets covered a wide depth range (0 to 200 m), thus depth was the main structuring factor within this substrate. The decreasing trend observed in the average number of species as depth increased was due to the fact that these habitats gradually loose complexity and conditions become more stable in deeper areas, thus providing a smaller number of niches for demersal species (Demestre *et al.*, 2000). This occurrence affected the number of species attributed to each guild, which also showed decreasing values from SS to DS assemblages.

Another noticeable effect was the gradual homogenisation of soft-bottom typologies verified as the dissimilarity between them decreased from species abundance data to guild data. However, since these assemblage limits were clearly defined when using species data and verified by other authors (Gomes *et al.*, 2001; Sousa *et al.*, 2005), three typologies were adopted instead of a single soft-bottom typology, thus a careful selection of the guilds that best characterise and detect typology-specific impacts is necessary.

In order to cover the shallowest soft-bottom area, otter trawl data was used to characterise areas approximately 10 to 30 m deep (Prista *et al.*, 2003; Abreu, 2005) and beach seine fisheries data for the area shallower than 10 m (Cabral *et al.*, 2003). The latter, despite not being intentionally performed with the purpose of characterising fish assemblages, provides rather complete data, due to the low selectivity of the fishing gear (Cabral *et al.*, 2003).

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SS assemblages were characterised by the presence of non-migratory species of medium mobility like *C. lyra*, *A. laterna* and *D. bellottii*, but some highly mobile species like *T. trachurus* and *Scomber japonicus* were also frequent. This was also observed by Catalán *et al.* (2006) on soft-bottoms near the Guadalquivir river mouth on the Gulf of Cadiz, where resident species coexist with others that take advantage of these highly productive areas.

The most represented trophic guilds on this typology were the macrocarnivores (*T. trachurus*, *S. japonicus*), the invertivores (*C. lyra*, *D. bellottii*) and the zooplanktivores (*S. pilchardus*), which confirms the observed by Prista *et al.* (2003), who additionally referred the occurrence of the zooplanktivore juveniles of *T. trachurus* in shallow areas near the Tagus estuary.

As the abundance of spring spawning, non-migratory and invertivore species verified in SS assemblages was also characteristic of NR assemblages, these typologies were closely related in terms of guild composition both in ordination plots and dissimilarity values which is 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 in both substrates (Demestre *et al.*, 2000; Prista *et al.*, 2003).

Although the small number of samples allowed few permutations, the results showed no significant influence of seasonality. However, Cabral *et al.* (2003) detected seasonal variations at a local scale, with *S. pilchardus* and *S. japonicus* being more abundant in spring and summer and *T. trachurus* and *D. bellottii* in autumn. These observations suggest that the acceptance of the null hypothesis in ANOSIM routines either is an artifact due to the small number of possible permutations or a consequence of the expansion of the area and thus the inclusion of additional environmental variability into the data.

Latitude did not show a significant effect on SS assemblages, since no differences were found between zone 3 and zone 5, however, data covering a wider latitudinal range would be necessary to conclude if these assemblages differ from the northern coast, where river runoff is higher (Santos *et al.*, 2005).

Although useful as a source of information on SS assemblages, beach seine fisheries data should not be included for monitoring purposes in the context of the MSD, as it

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would encourage an activity that inflicts considerable damage on benthic communities and juvenile fish (Cabral *et al.*, 2003).

The most abundant fish belonging to deeper assemblages showed a higher level of independence from substrates and gregarious behaviour as a defence strategy due to the lack of physical shelter in the water column. The occurrence of gregarious species had a strong influence in abundance proportions of IS and DS assemblages due to the high density of these species, with 90% of the total abundance being made up by 12 species in IS assemblages and only by 6 species in DS assemblages.

Winter spawners constituted a characteristic guild of IS and DS 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).

IS assemblages were dominated by the highly mobile pelagic species *T. trachurus* and *S. pilchardus*, which made up more than 37% of the total abundance. These species strongly influenced the abundance of the oceanadromous, high mobility and winter spawning guilds verified in the present study.

The latitudinal variation in species abundance verified in IS assemblages due to *S. pilchardus* and *T. trachurus* being more abundant in the north has a possible explanation in the more persistent upwelling verified to the north of the Nazaré canyon due to constant northern wind stress during the upwelling season and higher river runoff (Santos *et al.*, 2005), which favours feeding conditions for juveniles and zooplanktivore adults (Gomes *et al.*, 2001; Santos *et al.*, 2001). A similar zonation was observed by Gomes *et al.* (2001), who outlined that *S. pilchardus* plays an important role on the trophic web as a link between plankton and larger macrocarnivore fish, especially to the north of the Nazaré canyon.

Upwelling regime was also the main factor responsible for the seasonal differences found between IS assemblages, with the zooplanktivore *S. pilchardus* being more abundant during the upwelling season and the macrocarnivore adults of *T. trachurus* during winter.

The analysis of the most characteristic guilds revealed that DS assemblages were characterised by species occupying higher trophic levels, with macrocarnivore species like *T. trachurus* and *M. merluccius* persisting between datasets.

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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 (i.e. in SS and IS assemblages) and higher trophic levels (e.g. *M. merluccius*) place further away (i.e. in IS and DS assemblages) (Gomes *et al.*, 2001).

In contrast with the studies by Gomes *et al.* (2001) and Santos *et al.* (2005), where most pelagic species were excluded from the analysis, *Macroramphosus* spp. constituted more than 46% of the total abundance of DS assemblages, since this depth interval covers the typical distributional range of these gregarious species (Marques *et al.*, 2005). The data used in the present study (1979-1980) correspond to a period of very high abundance (Marques *et al.*, 2005) compared to the present state, since the abundance of *Macroramphosus* spp. has suffered a significant decline due to unsuccessful recruitment in the year 2000 which, according to recent surveys, was maintained until present (Marques *et al.*, 2005). However, these species continue to be characteristic of these assemblages and significant alterations in assemblage limits are not likely to have occurred, as Santos *et al.* (2005) verified with demersal assemblage limits during an 11-year period.

In the present study, seasonal variations in species and guilds were not significant, however, latitude was an important structuring factor. The abundance of *Macroramphosus* spp. and *C. aper* in the centre of the west coast 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.

The low proportion of *T. trachurus* and *M. merluccius* verified in DS assemblages near zone 3, as well as being related to the high proportion of *Macroramphosus* spp., is also due to the fact that *M. poutassou*, which constitutes one of the main preys of these species, occurs mainly in areas deeper than 200 m in the region off Lisbon (Marques *et al.*, 2005; Sousa *et al.*, 2005). These aspects strongly influenced the guild composition of these assemblages and so further assessment is necessary in order to clarify if the division of the DS typology in latitudinal zones is necessary or if the depth limit must be increased in some areas according to the steepness of the shelf.

Although pelagic species that exhibit demersal behaviour are captured by bottom trawls, sampling design should be corrected and data from pelagic trawl surveys

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should be used in order to adapt these surveys to the requirements of the MSD, correctly assess assemblage composition, adjust assemblage limits and minimise the probability of unwanted variations in ecological status due to inadequate sampling.

5. Conclusion

Assemblage typologies were successfully defined in the present study, constituting an important step towards the establishment of reference values for the assessment of ecological status of marine fish assemblages in the context of the MSD.

Marine fish assemblage typologies are usually delimited using species data (e.g. Demestre *et al.*, 2000; Gomes *et al.*, 2001; Sousa *et al.*, 2005; Catalán *et al.*, 2006), but the establishment of fish-based indices for ecological quality assessment usually involves grouping species in ecological guilds that facilitate the identification of pressure sources affecting the assemblages (Elliott *et al.*, 2007). The inclusion of guild data on multivariate analysis of assemblage distribution proved to be an important method for the definition of marine fish assemblage typologies, which permits the analysis of the persistence of typologies when the type of data is changed, thus establishing a link between the design of management units and the development of monitoring tools that support management.

The results obtained led to the conclusion that guild data should be used in ecological status assessment of marine fish assemblages, since they are more resistant than species data to minor environmental variations and facilitate the identification of pressures. Moreover, the characteristics of the established typologies stress the need for a definition of type-specific reference conditions, so that these values take into account the guild proportions that characterise each typology, with a careful selection of the metrics that are most affected by typology-specific pressures being a key factor for a successful detection and consequent intervention on the sources.

As the use of a single sampling method for all typologies is impossible, these should be defined and standardised for the monitoring of fish assemblages required by the MSD. Additionally, the importance of seasonality should be taken into account in the design of management tools and possible alterations due to the incorporation of this variability

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into yearly datasets or the establishment of a standard sampling season should be carefully assessed.

Because ecologically-defined marine fish assemblage frontiers are highly variable, policy-defined management units have an important role in balancing ecological homogeneity and management procedures and responsibilities. Only this way, and not the opposite, can the ecological status be successfully assessed and the impacts predicted.

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Chapter 3

General Discussion and Final Remarks

So far, marine environmental policies have focused on a sectorial approach to the activities responsible for pollution or resource exploitation (Hiscock *et al.*, 2003) and regional conventions that lack the articulation needed in order to achieve the common objective of conservation and sustainable use of marine ecosystems and resources of the European Union (EU) (Borja, 2006). Therefore, the objectives outlined by the EU Maritime Policy (EU, 2007b) and the European Marine Strategy Directive (MSD; EU, 2007a) require a new approach to the management of marine ecosystems (Borja, 2006).

The assessment of 'environmental quality' required by the MSD, being based on an "Ecosystem Approach" (CBD, 2000), gives a central role to habitat characteristics and community ecology (Browman and Stergiou, 2004; Rice, 2005), rather than focusing merely on exploited populations, and integrates anthropogenic disturbances as part of a dynamic system that needs to be understood in order to define and quantify the concept of 'good environmental status'.

Portugal, in this context, faces the challenge of possessing one of the largest Exclusive Economic Zones in the EU, thus having an urgent need and the responsibility to stand as an example in the definition of management units that are both ecologically and politically meaningful, as a basis for the development of management tools for assessment, monitoring and identification of the sources of impact as required by the MSD.

In the present study, data on fish assemblages from a broad variety of marine habitats of the Portuguese continental shelf were collected from the available literature and multivariate analysis techniques were performed in order to delimit assemblage typologies.

Unlike the majority of studies, which describe fish assemblages using species composition only (e.g. Demestre *et al.,* 2000; García-Charton and Pérez-Ruzafa, 2001; Gomes *et al.*, 2001; Sousa *et al.*, 2005; Catalán *et al.*, 2006), the present study adopted a methodology that incorporates not only species data, but also abundance and diversity of ecological guilds, comparing results independently obtained with each type of data in order to understand how they affect the grouping of datasets and the robustness of assemblage typologies. This way, the data are analysed in order to reach a consensus between structural and functional aspects of fish assemblages, thus

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establishing a link between typology definition and the design of quality assessment tools based on type-specific reference conditions, since most community metrics adopted in fish-based multimetric indices, as observed in the context of the European Water Framework Directive (WFD; EU, 2000) include guild data as a measure of the functional integrity of a community (e.g. Harrison and Whitfield, 2004; Breine *et al.*, 2007; Coates *et al.*, 2007).

In the marine environment, the limits between habitat units are often very variable and differences between assemblages are sometimes subtle and gradual, particularly in substrates where habitat structure and complexity are less important than other factors like depth and temperature (Gomes *et al.*, 2001; Sousa *et al.*, 2005). The use of ecological guild data in typology definition thus allows a more accurate judgement of the need to define different reference thresholds, hence attributing different typologies, in cases where species composition is clearly different while guild proportions might be similar.

In the present study, considering that the different sampling plans and methods could create large amounts of unexplained variability, the use of unconstrained ordination proved to be an efficient method for the establishment of typologies, since plotting datasets on a multidimensional space allows for a better judgement and correction of misclassifications than in the case of groups being delimited automatically by clustering algorithms.

In addition, as the graphical interpretation of a large amount of datasets, species and guilds would be very difficult, the similarity percentage analysis (SIMPER) routine performed in the present study was a successful method for the identification of the species and guilds that characterise previously delimited typologies. Moreover, this method has the advantage of assigning a single species or guild into various groups, thus taking into account ubiquitous species like *Boops boops*, *Trachurus trachurus* or *Macroramphosus* spp. that were relatively abundant in more than one group. This is also a characteristic of the non-hierarchical k-means clustering, which calculates the mean abundance of each species in a k number of groups (Lepš and Šmilauer, 2003), however, since in this method the groups are defined automatically by a clustering algorithm they would present similar problems to the ones described above, hence this method was not used.

Despite the lack of available data to cover all possible combinations of seasonal and latitudinal variability, an effort was made in order to cover the gaps with observations

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from local studies performed in the same locations. Except for intermediate soft-bottom (IS) assemblages, where the influence of the upwelling regime was most noticed, the results suggested an apparent negligibility of seasonal variability at a larger scale. However, local seasonal variations in marine fish assemblages should be taken into account, such as the variations in species abundances verified by Santos *et al.* (2005) in an artificial rocky reef (AR) closer to Ria Formosa due to migrations from the lagoon and the seasonal variations of some species in a shallow soft-bottom (SS) assemblage observed by Cabral *et al.* (2003). These variations may influence guild composition and thus affect the assessment of environmental status, and so there is a need to establish a monitoring plan in the context of the MSD that takes into account this local seasonal variability.

In order to solve the issue of seasonality, a standard monitoring period or season can be adopted, based on the stability of the system (e.g. Deegan *et al.*, 1997) or other seasonally variable factors with unpredictable effects that are not related to anthropogenic disturbance (e.g. upwelling, migrations, hydrology and climate). Another possible approach is the incorporation of data from all seasons (e.g. Henriques *et al.* submitted), thus merging all seasonal variability into a single dataset. However, the effects of these approaches need further analyses in order to achieve the best balance between cost and representativeness of the sampling plan.

Except for deep soft-bottom (DS) assemblages, different latitudes showed no significant differences, particularly with guild data, which suggest that no distinction is necessary concerning reference values for community metrics. However, there is still a need to overcome the practical difficulties associated with the sampling of natural rocky reefs (NR) and SS assemblages from the northwest coast in order to fully understand the influence of latitude in this typology, as the differences in temperature, wave exposure and wind regime are likely to have an influence on assemblage composition (Henriques *et al.*, 1999; Sousa *et al.*, 2005).

The latitudinal differences observed in DS assemblages were mainly attributed to the bathymetric characteristics of the shelf off Lisbon, which could indicate that latitude by itself has possibly a minor role in the differences observed between zones. However, a solution is yet to be found concerning the establishment of reference values, since this central area of the west coast showed differences in ecological guild composition when compared to the north and south portions of the coast.

As verified in the present study, species composition and guild proportions vary significantly between typologies, which emphasises the need for an adaptation of quality assessment tools to the various typologies, by choosing the community metrics that best detect typology-specific impacts and delimiting different reference thresholds for similar metrics, also known as type-specific reference conditions (Roset *et al.*, 2007). In this context, the threshold values above which an assemblage is to be considered in 'excellent' quality have to be based on the typical proportions of species and ecological guilds that characterise each assemblage typology, as well as their variability, in order to predict and establish a realistic environmental quality scale that accounts for the natural response of the assemblages when facing anthropogenic disturbances. This study has contributed significantly to a general understanding of how and why different guilds or species are dominant in different typologies, and work is in progress for the quantification of these variations.

Considering the abovementioned, the choice of community metrics for a marine fish-based multimetric index has to be based not only on structural and functional aspects of the assemblages but also on the type of impacts that are related to the anthropogenic pressures affecting each assemblage type. For this purpose, the most adequate and commonly used method is the DPSIR (Drivers-Pressures-Status-Impact-Response) approach (Elliott, 2002; Borja *et al.*, 2006), which can be applied in order to guarantee that all pressure sources can be identified by a quality assessment tool, therefore allowing managers and decision-makers to take appropriate measures to fulfil the requirements of the MSD of improving the environmental status and preventing future deterioration (EU, 2007a).

The next step in typology definition is the classification and characterisation of marine fish assemblage typologies for areas deeper than 200 m under jurisdiction of Portugal in order to cover the whole range of application of the MSD, though a greater homogeneity is expected at these depths (Gomes *et al.*, 2001; Sousa *et al.*, 2005). Furthermore, the methodology used in the present study should also be applied to marine waters of the Azores and Madeira islands, being imperative that all phases of the implementation of the MSD in Portugal are accompanied by a national intercalibration process between sub-regions, in a way that both the concept of 'good environmental status' and the tools used in the quality assessment are equivalent and comparable.

Knowing that hard-bottom areas located deeper than 40 m cannot be sampled by visual census using standard diving equipment nor bottom trawls, there is still a knowledge gap regarding the assemblage composition of these areas off the Portuguese coast, being often mapped and identified as "untrawlable areas" in groundfish surveys (e.g. Gomes *et al.*, 2001; Sousa *et al.*, 2005). Therefore, various solutions are possible considering that these areas are to be included in the range of application of the MSD: either these areas are included in the monitoring plan and fully sampled with pelagic trawls, baited fishing gear and remotely operated image recording equipment (Sedberry and Van Dolah, 1984), which would be the most realistic approach but would hugely increase monitoring costs, or a partial sampling survey is performed using only pelagic trawls, which would lack information on the species exhibiting demersal behaviour, or the environmental quality of the assemblages is inferred from the nearby trawlable areas, assuming that there are no significant differences in the degree of anthropogenic disturbance.

The main difficulties encountered on the present study were due to the fact that data on fish assemblages from Portugal are not easily available and that there is still a large amount of dispersed unpublished academic dissertations and internal institutional reports. This fact not only emphasises the need for a database of publicly funded data on the marine environment (Elliott and de Jonge, 1996), making information widely available and thus permitting a more cost-effective implementation and monitoring (de Jonge *et al.*, 2006), but also the urgent need for an extensive pilot-study using standardised sampling plans for all the biological elements whose assessment is required by the MSD, in order to test or define typologies, correctly establish reference values and optimise the monitoring procedures to be adopted.

The present study represents a very important step towards the implementation of the MSD, as it successfully delimited and characterised marine fish assemblage typologies for the Portuguese continental shelf from intertidal areas down to the 200 m isobath. Moreover, it also constituted an integrated review on published data for this region, thus contributing to a better understanding of marine fish ecology and distribution on a broad variety of habitats and establishing a starting point for the forthcoming challenges of the European Maritime Policy.

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Appendix

Appendix I: Database of the species identified in all the studies conducted on the Portuguese continental shelf, down to the 200 m isobath, analysed in the present study (in alphabetical order), with the ecological guild assigned to each species by category. Legend: **S**- soft substrate, **R**- rocky substrate, **I**- rocky intertidal, **resid.**- resident, **dep.**- dependent, **he**- herbivore, **inv**- invertivore, **ma**- macrocarnivore, **om**- omnivore, **pi**- piscivore, **zoo**- zooplanktivore, **VL**- very low, **L**- low, **M**- medium, **H**- high, **n**- non-migratory, **ana**- anadromous, **anf**- anfidromous, **cat**- catadromous, **oce**- oceanadromous, **te**- territorial, **se**- sedentary, **mm**- medium mobility, **hm**- high mobility.

	Family	Habitat	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Trophic	Resilience	Migration	Mobility
Acantholabrus palloni (**Risso, 1810**)	Labridae	reef-associated	0	1	0	0	0	0	inv	M	n	mm
Alosa alosa (**Linnaeus, 1758**)	Clupeidae	pelagic	0	0	0	0	0	0	zoo	M	ana	hm
Alosa fallax (**Lacépède, 1803**)	Clupeidae	pelagic	0	0	0	0	0	0	zoo	M	ana	hm
Amblyraja radiata **Donovan, 1808**	Rajidae	demersal	1	0	0	0	0	0	ma	L	oce	hm
Ammodytes tobianus **Linnaeus, 1758**	Ammodytidae	demersal	1	0	0	0	0	0	zoo	H	n	te
Anthias anthias (**Linnaeus, 1758**)	Serranidae	reef-associated	0	1	0	0	0	0	ma	M	n	mm
Aphia minuta (**Risso, 1810**)	Gobiidae	demersal	1	0	0	0	0	0	ma	M	n	te
Apletodon dentatus (**Facciolà, 1887**)	Gobiesocidae	demersal	0	1	0	0	0	0	zoo	H	n	te
Apletodon incognitus (**Hofrichter & Patzner, 1997**)	Gobiesocidae	demersal	0	1	0	0	0	0	zoo	H	n	te
Argentina sphyraena **Linnaeus, 1758**	Argentinidae	bathydemersal	0	0	0	0	0	0	ma	M	n	mm
Argyrosomus regius (**Asso, 1801**)	Sciaenidae	benthopelagic	0	0	0	1	0	0	ma	L	oce	hm
Arnoglossus imperialis (**Rafinesque, 1810**)	Bothidae	demersal	1	0	0	0	0	0	ma	H	n	mm
Arnoglossus laterna (**Walbaum, 1792**)	Bothidae	demersal	1	0	0	0	0	0	ma	M	n	mm
Arnoglossus thori **Kyle, 1913**	Bothidae	demersal	1	0	0	0	0	0	ma	M	n	mm
Aspitrigla cuculus (**Linnaeus, 1758**)	Triglidae	demersal	0	0	0	1	1	0	ma	M	n	mm
Atherina boyeri **Risso, 1810**	Atherinidae	demersal	0	0	0	0	0	0	ma	H	anf	hm
Atherina presbyter **Cuvier, 1829**	Atherinidae	pelagic	0	0	0	0	0	0	ma	H	oce	hm
Balistes capriscus **Gmelin, 1789**	Balistidae	reef-associated	0	0	0	1	1	0	inv	H	n	hm
Belone belone (**Linnaeus, 1761**)	Belonidae	pelagic	0	0	0	0	0	0	pi	M	oce	hm
Beryx decadactylus **Cuvier, 1829**	Berycidae	bathydemersal	0	0	0	0	0	0	ma	L	n	mm
Boops boops (**Linnaeus, 1758**)	Sparidae	demersal	0	0	0	1	1	0	om	M	oce	hm
Bothus podas (**Delaroche, 1809**)	Bothidae	demersal	1	0	0	0	0	0	ma	H	n	mm
Brama brama (**Bonnaterre, 1788**)	Bramidae	bathypelagic	0	0	0	0	0	0	ma	L	oce	hm
Buenia jeffreysii (**Günther, 1867**)	Gobiidae	reef-associated	0	0	0	1	1	0	inv	H	n	te
Buglossidium luteum (**Risso, 1810**)	Soleidae	demersal	1	0	0	0	0	0	inv	M	n	mm
Callanthias ruber (**Rafinesque, 1810**)	Callanthiidae	demersal	0	0	0	0	0	0	ma	M	n	mm

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Appendix I (cont.)

	Family	Functional guild	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Feeding guild	Resilience	Migration	Mobility
Callionymus lyra **Linnaeus, 1758**	Callionymidae	demersal	1	0	0	0	1	0	inv	M	n	mm
Callionymus maculatus **Rafinesque, 1810**	Callionymidae	demersal	0	0	0	0	0	0	inv	H	n	mm
Callionymus reticulatus **Valenciennes, 1837**	Callionymidae	demersal	1	0	0	0	1	0	inv	H	n	mm
Callionymus risso **Lesueur, 1814**	Callionymidae	demersal	1	0	0	0	1	0	inv	H	n	mm
Capros aper **(Linnaeus, 1758)**	Caproidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Centrolabrus exoletus **(Linnaeus, 1758)**	Labridae	reef-associated	0	1	0	0	0	0	inv	H	n	mm
Cepola macrophtalma **(Linnaeus, 1758)**	Cepolidae	demersal	0	0	0	0	0	0	zoo	M	n	se
Chelidonichthys lastoviza **(Bonnaterre, 1788)**	Triglidae	demersal	1	0	0	0	1	0	inv	M	n	mm
Chelidonichthys lucernus **(Linnaeus, 1758)**	Triglidae	demersal	1	0	0	0	1	0	ma	L	n	mm
Chelidonichthys obscurus **(Bloch & Schneider, 1801)**	Triglidae	demersal	1	0	0	0	1	0	ma	M	n	mm
Chelon labrosus **(Risso, 1827)**	Mugilidae	demersal	0	0	0	0	0	0	om	M	anf	mm
Chromis chromis **(Linnaeus, 1758)**	Pomacentridae	reef-associated	0	1	0	0	0	0	inv	M	n	mm
Ciliata mustela **(Linnaeus, 1758)**	Lotidae	demersal	0	0	0	1	1	1	inv	H	oce	hm
Citharus linguatula **(Linnaeus, 1758)**	Citharidae	demersal	1	0	0	0	0	0	ma	M	n	mm
Clinitrachus argentatus **(Risso, 1810)**	Clinidae	demersal	0	1	0	0	0	0	inv	M	n	se
Conger conger **(Linnaeus, 1758)**	Congridae	demersal	0	0	0	0	1	0	ma	VL	oce	hm
Coris julis **(Linnaeus, 1758)**	Labridae	reef-associated	0	1	0	0	0	0	inv	M	n	mm
Coryphoblennius galerita **(Linnaeus, 1758)**	Blenniidae	demersal	0	1	1	0	0	0	om	H	n	te
Ctenolabrus rupestris **(Linnaeus, 1758)**	Labridae	reef-associated	0	1	0	0	0	0	ma	M	n	mm
Dasyatis pastinaca **(Linnaeus, 1758)**	Dasyatidae	demersal	1	0	0	0	0	0	ma	VL	n	mm
Deania calcea **(Lowe, 1839)**	Centrophoridae	bathydemersal	0	0	0	0	0	0	ma	VL	n	mm
Deltentosteus quadrimaculatus **(Valenciennes, 1837)**	Gobiidae	demersal	1	0	0	0	0	0	inv	H	n	te
Dentex dentex **(Linnaeus, 1758)**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	M	n	mm
Dentex macrophthalmus **(Bloch, 1791)**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	M	oce	hm
Dentex maroccanus **(Valenciennes, 1830)**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	M	n	mm
Dicentrarchus labrax **(Linnaeus, 1758)**	Moronidae	demersal	0	0	0	1	1	0	ma	M	oce	hm
Dicentrarchus punctatus **(Bloch, 1792)**	Moronidae	pelagic	0	0	0	0	0	0	ma	M	n	mm
Dicologlossa cuneata **(Moreau, 1881)**	Soleidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Diplecogaster bimaculata **(Bonnaterre, 1788)**	Gobiesocidae	demersal	0	0	0	0	1	0	om	M	n	te
Diplodus annularis **(Linnaeus, 1758)**	Sparidae	benthopelagic	0	0	0	0	1	0	inv	M	n	mm

	Family	Functional guild	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Feeding guild	Resilience	Migration	Mobility
Diplodus bellottii (Steindachner, 1882)	Sparidae	benthopelagic	0	0	0	0	1	0	inv	M	n	mm
Diplodus cervinus (Lowe, 1838)	Sparidae	reef-associated	0	0	0	0	1	0	om	L	oce	hm
Diplodus puntazzo (Cetti, 1777)	Sparidae	benthopelagic	0	0	0	0	1	0	om	M	oce	hm
Diplodus sargus (Linnaeus, 1758)	Sparidae	demersal	0	0	0	0	1	0	om	M	oce	hm
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)	Sparidae	benthopelagic	0	0	0	0	1	0	inv	H	oce	hm
Echiichthys vipera (Cuvier, 1829)	Trachinidae	demersal	1	0	0	0	0	0	ma	H	n	se
Engraulis encrasicolus (Linnaeus, 1758)	Engraulidae	pelagic	0	0	0	0	0	0	zoo	H	oce	hm
Entelurus aequoreus (Linnaeus, 1758)	Syngnathidae	demersal	0	1	0	0	0	0	ma	M	n	mm
Eutrigla gurnardus (Linnaeus, 1758)	Triglidae	demersal	1	0	0	0	0	0	ma	M	n	mm
Gadiculus argenteus Guichenot, 1850	Gadidae	pelagic	0	0	0	0	0	0	inv	H	n	mm
Gaidropsarus guttatus (Collett, 1890)	Lotidae	demersal	0	0	0	1	1	0	om	M	n	mm
Gaidropsarus mediterraneus (Linnaeus, 1758)	Lotidae	demersal	0	0	0	0	1	1	om	L	oce	hm
Galeus melastomus Rafinesque, 1810	Scyliorhinidae	bathydemersal	0	0	0	0	0	0	ma	L	n	mm
Gobius auratus Risso, 1810	Gobiidae	demersal	1	0	0	0	0	0	om	H	n	te
Gobius bucchichi Stendachner, 1870	Gobiidae	demersal	0	1	0	0	0	0	om	H	n	te
Gobius cobitis Pallas, 1814	Gobiidae	demersal	0	1	1	0	0	0	om	M	n	te
Gobius cruentatus Gmelin, 1789	Gobiidae	demersal	0	1	0	0	0	0	om	M	n	te
Gobius gasteveni (Miller, 1974)	Gobiidae	demersal	1	0	0	0	0	0	om	H	n	te
Gobius niger Linnaeus, 1758	Gobiidae	demersal	0	1	1	0	0	0	ma	M	n	te
Gobius paganellus Linnaeus, 1758	Gobiidae	demersal	0	1	1	0	0	0	inv	M	n	te
Gobius roulei de Buen, 1928	Gobiidae	bathydemersal	0	0	0	0	0	0	inv	H	n	te
Gobius xanthocephalus Heymer & Zander, 1992	Gobiidae	demersal	0	1	0	0	0	0	inv	H	n	te
Gobiusculus flavescens (Fabricius, 1779)	Gobiidae	demersal	0	0	0	1	1	0	zoo	H	n	mm
Gymnammodytes cicerelus (Rafinesque, 1810)	Ammodytidae	demersal	1	0	0	0	0	0	zoo	H	n	mm
Gymnammodytes semisquamatus (Jourdain, 1879)	Ammodytidae	demersal	0	0	0	1	1	0	zoo	M	n	mm
Halobatrachus didactylus (Bloch & Schneider, 1801)	Batrachoididae	demersal	1	0	0	0	0	0	ma	L	n	se
Helicolenus dactylopterus (Delaroche, 1809)	Sebestidae	bathydemersal	0	0	0	0	0	0	ma	VL	n	se
Hippocampus guttulatus Cuvier, 1829	Syngnathidae	demersal	0	1	0	0	0	0	zoo	M	n	se
Hippocampus hippocampus (Linnaeus, 1758)	Syngnathidae	demersal	0	1	0	0	0	0	zoo	H	n	se

Appendix I (cont.)

	Family	Functional guild	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Feeding guild	Resilience	Migration	Mobility
Hyperoplus lanceolatus (Le sauvage, 1824)	Ammodytidae	demersal	0	0	0	0	0	0	ma	M	oce	hm
Labrus bergylta (Ascanius, 1767)	Labridae	reef-associated	0	1	0	0	0	0	om	L	n	mm
Labrus merula Linnaeus, 1758	Labridae	reef-associated	0	1	0	0	0	0	inv	M	n	mm
Labrus mixtus Linnaeus, 1758	Labridae	reef-associated	0	1	0	0	0	0	ma	L	n	mm
Labrus viridis Linnaeus, 1758	Labridae	reef-associated	0	1	0	0	0	0	ma	L	n	mm
Lepadogaster candollei Risso, 1810	Gobiesocidae	demersal	0	1	0	0	0	0	inv	M	n	te
Lepadogaster lepadogaster (Bonnaterre, 1788)	Gobiesocidae	demersal	0	1	1	0	0	0	inv	M	n	te
Lepadogaster purpurea (Bonnaterre, 1788)	Gobiesocidae	demersal	0	1	1	0	0	0	inv	M	n	te
Lepidopus caudatus (Euphrasen, 1788)	Trichiuridae	bathydemersal	0	0	0	0	0	0	ma	M	oce	hm
Lepidorhombus boscii (Risso, 1810)	Scophthalmidae	demersal	1	0	0	0	0	0	ma	M	n	mm
Lepidorhombus whiffiagonis (Walbaum, 1792)	Scophthalmidae	bathydemersal	1	0	0	0	0	0	ma	L	n	mm
Lepidotrigla cavillone (Lacepède, 1801)	Triglidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Lepidotrigla dieuzeidei Blanc & Hureau, 1973	Triglidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Lesueurigobius sanzi (de Buen, 1918)	Gobiidae	demersal	0	0	0	0	0	0	inv	H	n	te
Leucoraja fullonica (Linnaeus, 1758)	Rajidae	bathydemersal	1	0	0	0	0	0	ma	L	n	mm
Leucoraja naevus (Müller & Henle, 1841)	Rajidae	demersal	1	0	0	0	0	0	ma	L	n	mm
Lichia amia (Linnaeus, 1758)	Carangidae	pelagic	0	0	0	0	0	0	ma	M	oce	hm
Lipophrys canevae (Vinciguerra, 1880)	Blenniidae	demersal	0	1	1	0	0	0	om	H	n	te
Lipophrys pholis (Linnaeus, 1758)	Blenniidae	demersal	0	1	1	0	0	0	om	M	n	te
Lithognathus mormyrus (Linnaeus, 1758)	Sparidae	demersal	1	0	0	0	0	0	inv	M	n	mm
Liza aurata (Risso, 1810)	Mugilidae	pelagic	0	0	0	0	0	0	om	M	cat	hm
Liza ramada (Risso, 1810)	Mugilidae	pelagic	0	0	0	0	0	0	om	L	cat	hm
Lophius piscatorius Linnaeus, 1758	Lophiidae	bathydemersal	1	0	0	0	0	0	ma	L	n	se
Macroramphosus gracilis (Lowe, 1839)	Centriscidae	pelagic	0	0	0	0	0	0	inv	H	n	mm
Macroramphosus scolopax (Linnaeus, 1758)	Centriscidae	pelagic	0	0	0	0	0	0	inv	M	n	mm
Malacocephalus laevis (Lowe, 1843)	Macrouridae	bathydemersal	0	0	0	0	0	0	ma	L	n	mm
Maurolicus muelleri (Gmelin, 1789)	Sternoptychidae	bathypelagic	0	0	0	0	0	0	inv	M	n	mm
Merlangius merlangus (Linnaeus, 1758)	Gadidae	benthopelagic	0	0	0	1	1	0	ma	M	oce	hm
Merluccius merluccius (Linnaeus, 1758)	Merlucciidae	demersal	1	0	0	0	0	0	ma	L	n	mm

	Family	Functional guild	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Feeding guild	Resilience	Migration	Mobility
Microchirus azevia **(Brito Capello, 1867)**	Soleidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Microchirus boscanion **(Chabanaud, 1926)**	Soleidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Microchirus ocellatus **(Linnaeus, 1758)**	Soleidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Microchirus variegatus **(Donovan, 1808)**	Soleidae	demersal	1	0	0	0	0	0	inv	M	n	mm
Micromesistius poutassou **(Risso, 1827)**	Gadidae	pelagic	0	0	0	0	0	0	ma	M	oce	hm
Mola mola **(Linnaeus, 1758)**	Molidae	pelagic	0	0	0	0	0	0	om	L	oce	hm
Molva molva **(Linnaeus, 1758)**	Lotidae	demersal	0	0	0	0	0	0	ma	L	oce	hm
Monochirus hispidus **Rafinesque, 1814**	Soleidae	demersal	1	0	0	0	0	0	inv	H	n	mm
Mugil cephalus **Linnaeus, 1758**	Mugilidae	benthopelagic	0	0	0	1	1	0	ma	M	cat	hm
Mullus barbatus **Linnaeus, 1758**	Mullidae	demersal	1	0	0	0	0	0	inv	M	n	mm
Mullus surmuletus **Linnaeus, 1758**	Mullidae	demersal	1	0	0	0	0	0	ma	M	oce	hm
Muraena helena **(Linnaeus, 1758)**	Muraenidae	reef-associated	0	1	0	0	0	0	ma	M	n	se
Mustelus mustelus **(Linnaeus, 1758)**	Triakidae	demersal	0	0	0	0	0	0	ma	VL	n	hm
Myliobatis aquila **(Linnaeus, 1758)**	Myliobatidae	benthopelagic	1	0	0	0	0	0	ma	VL	n	mm
Nerophis lumbriciformis **(Jenyns, 1835)**	Syngnathidae	demersal	0	1	0	0	0	1	ma	M	n	se
Oblada melanura **(Linnaeus, 1758)**	Sparidae	benthopelagic	0	0	0	0	1	0	om	M	oce	hm
Oxynotus centrina **(Linnaeus, 1758)**	Dalatiidae	bathydemersal	0	0	0	0	0	0	inv	VL	n	hm
Pagellus acarne **(Risso, 1827)**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	M	oce	hm
Pagellus bellottii **Steinsachner, 1882**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	M	n	mm
Pagellus bogaraveo **(Brünnich, 1768)**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	L	n	mm
Pagellus erythrinus **(Linnaeus, 1758)**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	M	n	hm
Pagrus auriga **Valenciennes, 1843**	Sparidae	benthopelagic	0	0	0	0	1	0	inv	VL	oce	hm
Pagrus pagrus **(Linnaeus, 1758)**	Sparidae	benthopelagic	0	0	0	0	1	0	ma	M	oce	hm
Parablennius gattorugine **(Linnaeus, 1758)**	Blenniidae	demersal	0	1	1	0	0	0	om	H	n	te
Parablennius incognitus **(Bath, 1968)**	Blenniidae	demersal	0	1	0	0	0	0	om	H	n	te
Parablennius pilicornis **(Cuvier, 1829)**	Blenniidae	demersal	0	1	1	0	0	0	he	H	n	te
Parablennius rouxi **(Cocco, 1833)**	Blenniidae	demersal	1	1	0	0	0	0	om	H	n	te
Parablennius ruber **(Valenciennes, 1836)**	Blenniidae	demersal	0	1	0	0	0	0	om	H	n	te
Parablennius sanguinolentus **(Pallas, 1814)**	Blenniidae	demersal	0	1	1	0	0	0	he	M	n	te
Paralipophrys trigloides **(Valenciennes, 1836)**	Blenniidae	demersal	0	1	1	0	0	0	om	H	n	te

Appendix I (cont.)

	Family	Functional guild	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Feeding guild	Resilience	Migration	Mobility
Phycis phycis **(Linnaeus, 1766)**	Phycidae	benthopelagic	0	0	0	1	1	0	inv	M	n	mm
Platichthys flesus **(Linnaeus, 1758)**	Pleuronectidae	demersal	1	0	0	0	0	0	ma	M	cat	hm
Plectorhinchus mediterraneus **(Guichenot, 1850)**	Haemulidae	demersal	1	0	0	0	0	0	inv	M	n	mm
Pleuronectes platessa **Linnaeus, 1758**	Pleuronectidae	demersal	1	0	0	0	0	0	inv	L	oce	hm
Pollachius pollachius **(Linnaeus, 1758)**	Gadidae	benthopelagic	0	0	0	0	0	0	inv	M	oce	hm
Pomadasys incisus **(Bowdich, 1825)**	Haemulidae	demersal	0	0	0	1	1	0	inv	M	n	mm
Pomatomus saltatrix **(Linnaeus, 1766)**	Pomatomidae	pelagic	0	0	0	0	0	0	ma	M	oce	am
Pomatoschistus marmoratus **(Risso, 1810)**	Gobiidae	demersal	1	0	0	0	0	0	inv	H	n	se
Pomatoschistus minutus **(Pallas, 1770)**	Gobiidae	demersal	0	0	0	1	0	0	inv	H	oce	hm
Pomatoschistus pictus **(Malm, 1865)**	Gobiidae	demersal	1	0	0	0	0	0	inv	H	n	se
Pseudocaranx dentex **(Bloch & Schneider, 1801)**	Carangidae	reef-associated	0	0	0	1	1	0	inv	M	n	mm
Raja brachyura **Lafont, 1873**	Rajidae	demersal	1	0	0	0	0	0	ma	L	n	mm
Raja clavata **Linnaeus, 1758**	Rajidae	demersal	1	0	0	0	0	0	ma	L	n	mm
Raja microocellata **Montagu, 1818**	Rajidae	demersal	1	0	0	0	0	0	pi	L	n	mm
Raja miraletus **Linnaeus, 1758**	Rajidae	demersal	1	0	0	0	0	0	ma	L	n	mm
Raja montagui **Fowler, 1910**	Rajidae	demersal	1	0	0	0	0	0	inv	L	n	mm
Raja undulata **Lacepède, 1802**	Rajidae	demersal	1	0	0	0	0	0	ma	L	n	mm
Sarda sarda **(Bloch, 1793)**	Scombridae	pelagic	0	0	0	0	0	0	ma	M	oce	hm
Sardina pilchardus **(Walbaum, 1792)**	Clupeidae	pelagic	0	0	0	0	0	0	zoo	M	oce	hm
Sardinella aurita **Valenciennes, 1847**	Clupeidae	reef-associated	0	0	0	0	0	0	zoo	H	oce	hm
Sarpa salpa **(Linnaeus, 1758)**	Sparidae	benthopelagic	0	1	0	0	0	0	he	M	n	mm
Scomber japonicus **Houttuyn, 1782**	Scombridae	pelagic	0	0	0	0	0	0	ma	M	oce	hm
Scomber scombrus **Linnaeus, 1758**	Scombridae	pelagic	0	0	0	0	0	0	ma	M	oce	hm
Scophthalmus maximus **(Linnaeus, 1758)**	Scophthalmidae	demersal	1	0	0	0	0	0	ma	M	oce	hm
Scophthalmus rhombus **(Linnaeus, 1758)**	Scophthalmidae	demersal	1	0	0	0	0	0	ma	M	oce	hm
Scorpaena notata **Rafinesque, 1810**	Scorpaenidae	demersal	0	1	0	0	0	0	ma	M	n	se
Scorpaena porcus **Linnaeus, 1758**	Scorpaenidae	demersal	0	1	0	0	0	0	ma	M	n	se
Scorpaena scrofa **Linnaeus, 1758**	Scorpaenidae	demersal	0	0	0	1	1	0	ma	H	n	se
Scyliorhinus canicula **(Linnaeus, 1758)**	Scyliorhinidae	demersal	1	0	0	0	1	0	ma	L	n	mm

	Family	Functional guild	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Feeding guild	Resilience	Migration	Mobility
Scyliorhinus stellaris (**Linnaeus, 1758**)	Scyliorhinidae	reef-associated	1	0	0	0	1	0	ma	L	n	mm
Seriola dumerili (**Risso, 1810**)	Carangidae	reef-associated	0	0	0	0	0	0	ma	M	oce	hm
Serranus atricauda (**Günther, 1874**)	Serranidae	demersal	0	1	0	0	0	0	ma	L	n	mm
Serranus cabrilla (**Linnaeus, 1758**)	Serranidae	demersal	0	1	0	0	0	0	ma	M	n	mm
Serranus hepatus (**Linnaeus, 1758**)	Serranidae	demersal	0	1	0	0	0	0	ma	M	n	mm
Serranus scriba (**Linnaeus, 1758**)	Serranidae	demersal	0	1	0	0	0	0	ma	M	n	se
Solea lascaris (**Risso, 1810**)	Soleidae	demersal	1	0	0	0	0	0	inv	M	n	mm
Solea senegalensis **Kaup, 1858**	Soleidae	demersal	1	0	0	0	0	0	inv	L	n	mm
Solea solea (**Linnaeus, 1758**)	Soleidae	demersal	1	0	0	0	0	0	inv	M	oce	hm
Sparus aurata **Linnaeus, 1758**	Sparidae	demersal	0	1	0	0	0	0	om	M	n	mm
Sphoeroides pachygaster (**Müller & Troschel, 1848**)	Tetraodontidae	demersal	0	0	0	0	0	0	inv	M	n	mm
Spicara maena (**Linnaeus, 1758**)	Centracanthidae	pelagic	0	0	0	0	0	0	zoo	M	n	mm
Spondyliosoma cantharus (**Linnaeus, 1758**)	Sparidae	benthopelagic	0	0	0	1	1	0	om	M	oce	hm
Sprattus sprattus (**Linnaeus, 1758**)	Clupeidae	pelagic	0	0	0	0	0	0	zoo	H	oce	hm
Squalus blainville (**Risso, 1827**)	Squalidae	demersal	0	0	0	0	0	0	ma	VL	n	mm
Symphodus bailloni (**Valenciennes, 1839**)	Labridae	reef-associated	0	1	0	0	0	0	om	M	n	mm
Symphodus cinereus (**Bonnaterre, 1788**)	Labridae	demersal	0	1	0	0	0	0	inv	M	n	mm
Symphodus melops (**Linnaeus, 1758**)	Labridae	reef-associated	0	1	0	0	0	0	inv	M	n	mm
Symphodus ocellatus **Forsskål, 1775**	Labridae	reef-associated	0	1	0	0	0	0	inv	H	n	mm
Symphodus roissali (**Risso, 1810**)	Labridae	reef-associated	0	1	0	0	0	0	inv	M	n	mm
Symphodus rostratus (**Bloch, 1791**)	Labridae	reef-associated	0	1	0	0	0	0	inv	H	n	mm
Synaptura lusitanica **Capello, 1868**	Soleidae	demersal	1	0	0	0	0	0	inv	M	n	mm
Synchiropus phaeton (**Günther, 1861**)	Callionymidae	demersal	0	1	0	0	0	0	inv	H	n	mm
Syngnathus acus **Linnaeus, 1758**	Syngnathidae	demersal	0	1	0	0	0	0	zoo	M	n	se
Taurulus bubalis (**Euphrasen, 1786**)	Cottidae	demersal	0	1	0	0	0	0	ma	M	n	mm
Thorogobius ephippiatus (**Lowe, 1839**)	Gobiidae	demersal	0	1	0	0	0	0	om	M	n	te
Torpedo marmorata **Risso, 1810**	Torpedinidae	reef-associated	1	0	0	0	0	0	ma	L	n	mm
Torpedo nobiliana **Bonaparte, 1835**	Torpedinidae	benthopelagic	1	0	0	0	0	0	pi	L	oce	am
Torpedo torpedo (**Linnaeus, 1758**)	Torpedinidae	demersal	1	0	0	0	0	0	ma	L	n	mm
Trachinotus ovatus (**Linnaeus, 1758**)	Carangidae	pelagic	0	0	0	0	0	0	ma	M	n	mm

Appendix I (cont.)

	Family	Functional guild	S-resid.	R-resid.	I-resid.	S-dep.	R-dep.	I-dep.	Feeding guild	Resilience	Migration	Mobility
Trachinus draco (**Linnaeus, 1758**)	Trachinidae	demersal	1	0	0	0	0	0	ma	M	n	se
Trachinus radiatus **Cuvier, 1829**	Trachinidae	demersal	1	0	0	0	0	0	ma	M	n	se
Trachurus picturatus (**Bowdich, 1825**)	Carangidae	benthopelagic	0	0	0	0	0	0	ma	M	oce	am
Trachurus trachurus (**Linnaeus, 1758**)	Carangidae	pelagic	0	0	0	0	0	0	ma	L	oce	hm
Trigla lyra (**Linnaeus, 1758**)	Triglidae	bathydemersal	1	0	0	0	0	0	inv	M	n	mm
Tripterygion delaisi **Cadenat & Blache, 1970**	Tripterygiidae	demersal	0	1	0	0	0	0	inv	H	n	te
Trisopterus luscus (**Linnaeus, 1758**)	Gadidae	benthopelagic	0	0	0	1	1	0	ma	M	oce	hm
Trisopterus minutus (**Linnaeus, 1758**)	Gadidae	benthopelagic	1	0	0	1	1	0	ma	M	n	mm
Uranoscopus scaber **Linnaeus, 1758**	Uranoscopidae	demersal	1	0	0	0	0	0	ma	M	n	se
Zeugopterus punctatus (**Bloch, 1787**)	Scophthalmidae	demersal	1	0	0	0	0	0	ma	M	n	mm
Zeugopterus regius (**Bonnaterre, 1788**)	Scophthalmidae	demersal	1	0	0	0	0	0	ma	H	n	mm
Zeus faber **Linnaeus, 1758**	Zeidae	benthopelagic	0	0	0	0	1	0	ma	L	oce	hm