Universidade de Lisboa Faculdade de Ciências Departamento de Biologia Animal



Stock assessment and management of multi-species fisheries: the case study of flatfish fisheries in the Portuguese coast

Célia Maria Mascarenhas dos Santos Teixeira

Doutoramento em Biologia Especialidade de Biologia Marinha e Aquacultura 2009

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Tese orientada por Professor Doutor Henrique Nogueira Cabral

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To the memory of my Daddy, and to my Mummy

À memória de meu Pai, e à minha Mãe

$\mathsf{T}_{\mathsf{ABLE}} \text{ of } \mathsf{C}_{\mathsf{ONTENTS}}$

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Resumo

Nas últimas décadas têm sido intensificados os sinais de sobre-exploração de muitos mananciais de recursos vivos marinhos. Neste contexto, uma gestão sustentável dos ecossistemas marinhos reveste-se da maior importância e urgência. Tradicionalmente, os modelos de gestão incidiam sobre uma única espécie-alvo ou pescaria, tendo-se revelado pouco eficazes. Mais recentemente, tem-se assistido a uma mudança de paradigma de gestão, segundo a qual os modelos integradores e holísticos tem sido indicados como aqueles que poderão garantir a desejada sustentabilidade das pescarias. As pescarias multi-específicas são um caso particularmente complexo em termos de gestão, uma vez que capturam uma grande diversidade de espécies e utilizam um conjunto alargado de artes e técnicas de pesca. A pesca de Pleuronectiformes na costa portuguesa constitui um desses exemplos de pescarias marcadamente multi-específicas. Esta pesca é uma actividade tradicional com grande importância socio-económica nalgumas regiões da costa de Portugal. Embora os quantitativos desembarcados das espécies de peixe-chatos representem apenas 4% do total de peixes desembarcados, a sua importância a nível económico é bastante superior, representando 11% do valor total de peixes.

No decurso do presente trabalho foram estudados alguns aspectos da biologia e ecologia destes recursos, caracterizadas as suas pescarias e avaliado o estado de exploração dos mananciais das espécies de Pleuronectiformes com interesse comercial na costa portuguesa.

Este trabalho é constituído por nove capítulos organizados em quatro partes. Na introdução geral (Capítulo 1) são abordadas a importância da avaliação e gestão dos stocks de pesca, destacando as pescarias multi-específicas pela sua complexidade, atribuindo particular destaque à pesca de Pleuronectiformes, que constitui uma importante actividade socio-económica para algumas comunidades da costa portuguesa para a qual não existem estudos de avaliação, sendo as medidas de gestão incipientes.

No Capítulo 2 foi efectuado um estudo comparativo da dieta, crescimento e reprodução do linguado legítimo, *Solea solea* (Linnaeus, 1758), e do linguado do

Senegal, *Solea senegalensis* Kaup, 1858, que ocorrem em simpatria na costa portuguesa. Ambas as espécies alimentaram-se principalmente de crustáceos, poliquetas e bivalves. A dieta variou consoante a época do ano, com o tamanho e o sexo dos indivíduos. O linguado legítimo consumiu uma maior diversidade de presas comparativamente ao linguado do Senegal e durante o Outono-Inverno verificou-se uma sobreposição trófica elevada entre as fêmeas das duas espécies. A actividade alimentar foi mais elevada durante a Primavera-Verão, particularmente no caso das fêmeas e dos indivíduos de menores dimensões. A idade e crescimento foi determinada com base na leitura de otólitos, e os parâmetros de crescimento da equação de von Bertalanffy permitiram verificar que fêmeas e machos apresentam crescimento diferenciado, atingindo as fêmeas maiores dimensões. A análise macroscópica das gónadas permitiu determinar que a época de postura ocorre principalmente no Outono-Inverno, para ambas as espécies.

O estudo da ecologia alimentar, crescimento e ciclo sexual do linguado da areia, *Solea lascaris* (Risso, 1810) (Capítulo 3), revelou que a dieta desta espécie é constituída, principalmente, por misidáceos, anfípodes e poliquetas. A diversidade de presas ingeridas apresentou variações sazonais e ontogénicas. O crescimento foi diferente para fêmeas e machos, atingindo as fêmeas dimensões superiores às dos machos. A época de postura estendeu-se do Inverno à Primavera.

No Capítulo 4 foi estudada a ecologia alimentar, o crescimento e a reprodução da carta, Citharus linguatula (Linnaeus, 1758), do areeiro-de-quatro-pintas, Lepidorhombus boscii (Risso, 1810), da solha, Platichthys flesus (Linnaeus, 1758), e da azevia, Microchirus azevia (de Brito Capello, 1867). A carta e o areeiro-de-quatropintas consumiram principalmente peixes, decápodes e misidáceos, e a solha alimentou-se preferencialmente de equinodermes, crustáceos e bivalves. A azevia alimentou-se principalmente de presas com pouca mobilidade, sendo os itens mais importantes na dieta os poliguetas e alguns decápodes. A composição da dieta variou com a época do ano, com o tamanho e com o sexo dos indivíduos. A actividade alimentar foi mais intensa no caso das fêmeas e dos indivíduos de menores dimensões, durante a Primavera-Verão. A azevia foi a espécie que apresentou um maior espectro alimentar, particularmente os machos e os indivíduos de maiores dimensões, durante o Outono-Inverno. Para as várias espécies estudadas, o crescimento foi diferente consoante o sexo dos indivíduos, atingindo as fêmeas maiores dimensões que os machos (excepto para C. linguatula). A época de postura do areeiro-de-quatro-pintas e da solha ocorreu no Outono-Inverno, enquanto que para a carta e a azevia verificou-se a existência de indivíduos maduros durante praticamente todo o ano.

A análise da diversidade genética e da estruturação populacional de *S. solea* e *S. senegalensis* foi abordada no Capítulo 5. Este estudo foi baseado na análise da sequência completa do citócromo b do ADN mitocondrial de amostras obtidas ao longo da área de distribuição das espécies consideradas. Ambas as espécies apresentaram baixa diversidade haplotípica e moderada a elevada diversidade nucleotídica. *S. solea* apresentou estruturação populacional, ocorrendo divergência genética entre as populações do Atlântico e do Mediterrâneo, e dentro do Mediterrâneo, entre as populações do ocidente e do oriente. *S. senegalensis* apresentou um padrão de heterogeneidade genética entre populações separadas geograficamente.

No Capítulo 6 foram analisados os desembarques mensais da pesca comercial de várias espécies de Pleuronectiformes, efectuados ao longo da costa portuguesa, entre os anos de 1992 e 2005. As espécies mais importantes em termos de desembarques foram os linguados, a azevia, a solha, a carta, a língua (Dicologlossa cuneata (Moreau, 1881)), o pregado (Scophthalmus maximus (Linnaeus, 1758)), o rodovalho (Scophthalmus rhombus (Linnaeus, 1758)) e os areeiros (Lepidorhombus boscii (Risso, 1810) e Lepidorhombus whiffiagonis (Walbaum, 1792)). Com o objectivo de identificar quais os factores que melhor explicavam as tendências verificadas nos desembarques, foram aplicados modelos lineares generalizados aos desembarques mensais das espécies consideradas sendo as variáveis explicativas o índice de oscilação do Atlântico Norte, a temperatura da água à superficie, a componente escalar do vento e a precipitação. Na série temporal considerada, os desembarques não apresentaram uma tendência comum para os diferentes grupos de espécies consideradas. Para a maioria dos grupos de espécies, foram encontradas relações significativas entre o mês e a temperatura da água à superfície e os desembarques por unidade de esforço. Para algumas espécies, o padrão de variação sazonal dos desembarques por unidade de esforço reflectiu as migrações efectuadas aquando da reprodução, período no qual se verifica a concentração de indivíduos nalgumas zonas da plataforma continental, o que aumenta a sua vulnerabilidade à pesca.

No Capítulo 7 foi caracterizada a pesca de peixes-chatos na costa portuguesa. A frota que captura Pleuronectiformes, apresentou grande heterogeneidade, sendo a grande maioria constituída por pequenas embarcações, que utilizam várias artes de pesca. Com recurso a uma análise canónica de correspondências foi possível identificar vários segmentos de frota, que utilizam diferentes artes e/ou técnicas de pesca. A maioria das espécies de peixes-chatos foi capturada conjuntamente com choco, polvos e raias, principalmente por pequenas embarcações que operam junto à costa. Os areeiros e a carta foram capturados conjuntamente com a pescada, pequenos peixes pelágicos e tamboris, por embarcações que operam com rede de arrasto. Para investigar a variação dos desembarques na série temporal considerada (1992-2005), foram

utilizados modelos lineares generalizados, sendo os preditores o ano, o mês, o porto de desembarque e o comprimento da embarcação. Os preditores que melhor explicaram a variação dos desembarques foram o porto de desembarque, o comprimento da embarcação e o mês. Estes resultados sugerem que existe elevada variabilidade espacio-temporal, mas que as características técnicas das embarcações também têm um importante contributo na variação dos desembarques.

O Capítulo 8 teve como objectivo determinar quais as espécies-alvo e as capturas acessórias da pesca com redes de emalhar na costa centro de Portugal, e assim estimar a percentagem de rejeições associada a esta pescaria. Este estudo foi baseado em campanhas de amostragem com observadores a bordo das embarcações da pesca comercial na zona de Setúbal e Sesimbra. As espécies-alvo destas embarcações foram o linguado legítimo, o linguado do Senegal e o choco. As capturas acessórias corresponderam a cerca de 60% do total capturado, sendo rejeitadas cerca de 40% das espécies acessórias. Foram várias as razões pelas quais as espécies foram rejeitadas, nomeadamente por não terem valor comercial, por se encontrarem em mau estado de conservação e por isso não poderem ser vendidas, ou porque os indivíduos capturados se encontram abaixo do tamanho mínimo autorizado. Estimouse que as rejeições desta pescaria, cuja frota é constituída por cerca de 200 embarcações, totalizem 174 toneladas ano⁻¹.

Finalmente, no Capítulo 9, é feita a síntese das principais conclusões e indicados alguns tópicos que necessitariam de investigação futura.

Palavras-chave: Pleuronectiformes, pequena pesca, pesca multi-específica, avaliação de stocks, gestão

SUMMARY

Multi-species and multi-gear fisheries present several problems to management. Traditionally, marine resources stock assessment and fisheries management was based in a single-species approach, which clearly proved to be ineffective. The present work analysed the flatfish fisheries along the Portuguese coast as a case study of a multi-species and multi-gear small-scale fisheries. Biological and ecological data regarding the main flatfish species with commercial importance for these fisheries was obtained. The diet, age and growth and spawning period of the common sole (Solea solea), Senegalese sole (Solea senegalensis), sand sole (Solea lascaris), bastard sole (Microchirus azevia), flounder (Platichthys flesus), spotted flounder (Citharus linguatula) and four-spotted megrim (Lepidorhombus boscii) were characterized. Common sole, Senegalese sole, sand sole and bastard sole fed mainly on crustaceans (amphipods, decapods and mysids), polychaetes and molluscs, while the spotted flounder and four-spotted megrim consumed mainly fishes, decapods and mysids, and flounder fed preferentially echinoderms, crustaceans and bivalves. The estimates of growth and asymptotic lengths obtained showed that females were larger than males, for the same age, with the exception of the spotted flounder. Spawning occurred mainly in autumn-winter for common sole, Senegalese sole, four-spotted megrim and flounder, between winter and spring for sand sole, and throughout the year for spotted flounder and bastard sole. Genetic analysis evidenced the existence of population structure in the common sole and Senegalese sole, being evidenced an Atlantic-Mediterranean differentiation and of Eastern and Western Mediterranean populations for common sole and a isolation by distance model of population structure for Senegalese sole. The analysis of fisheries landings revealed that flatfishes were captured mainly during the spawning season, when fishes concentrate in areas near the coast and are particularly vulnerable to fisheries. The fisheries fleet that catch flatfish is mainly composed by small vessels. The capture of soles, flounder, bastard sole, turbot and brill were associated with cuttlefish, octopus and rays. Megrims and spotted flounder were caught with hake, small pelagic fishes and anglerfishes by trawlers. Bycatches associated with flatfish fishery in the central coast of Portugal

represented near 60% of the total catches, being the amount of discards ca. 40%. The management of these fisheries was discussed and some measures proposed.

Keywords: flatfish, small-scale fisheries, multi-species fisheries, stock assessment, management

LIST OF PAPERS

This thesis comprises the papers listed below:

- Teixeira, C.M., Cabral, H.N. (*submitted*). Comparative analysis of the diet, growth and reproduction of the soles, *Solea solea* and *Solea senegalensis*, occurring in sympatry along the Portuguese coast. Journal of the Marine Biological Association of the United Kingdom.
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- Teixeira, C.M., Batista, M.I., Cabral, H.N. (*in revision*). Diet, growth and reproduction of spotted flounder, four-spotted megrim, flounder and bastard sole in the Portuguese coast. Scientia Marina.
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- Teixeira, C.M., Cabral, H.N., 2009. Time series analysis of flatfish landings in the Portuguese coast. Fisheries Research 96, 252–258.
- Teixeira, C.M., Batista, M.I., Cabral, H.N. (*submitted*). Flatfish fisheries in the Portuguese coast. Fisheries Research.
- Batista, M.I., Teixeira, C.M., Cabral, H.N. (*submitted*). Catches of target species and bycatches of an artisanal fishery: the case study of a trammel net fishery in the Portuguese coast. Fisheries Research.

The author of the present thesis had a leading role in the conception, execution, analysis and writing of all the articles listed. All articles published or *in press* were included with the kind permission of the publisher.

Part I

$G_{\text{eneral}}\ Introduction$

Chapter 1

General Introduction

Fisheries Stocks Assessment and Management

Fisheries are an important source of food, with a high economic value and social significance. In the last decades, catches have increased, because a growing human population requires more food and improved technology has simplified all processes involved in fisheries (e.g. captures, processing, distribution). The world fisheries production increased from 18.9 million tonnes, in 1951, to 141.6 million tonnes, in 2005 (source: FAO). Marine fisheries are the largest contributors to the world production, but a decreasing trend has been noticed: marine landings accounted for 90.8% of the total fish production (18.1 million tonnes), in 1951, and this value was reduced to 72.5%, in 2005 (102.7 million tonnes) (source: FAO).

The growing demand for marine products leads to a continuous increase in the exploitation on most marine resources. Many authors (e.g. Jackson et al., 2001; Hutchings and Reynolds, 2004; Caddy and Seijo, 2005; Beddington et al., 2007) recognized problems in the current status of marine fisheries, especially in what regards the collapse of many important fisheries stocks all around the world (Watson and Pauly, 2001; Worm et al., 2006). Overfishing is recognized as the major contributor to observed collapses, which is attributed mainly to technological advancements among the fishermen community to increase the fishing efficiency (e.g. Hall, 1999; Pauly et al., 2002). The present situation of the world fisheries is the result of the interaction of different factors, like, climatic variations, bio-ecological (e.g. natural oscillations), technological innovation, exponential development of fishing capacity, geographic expansion, economic, socio-cultural, institutional and legal (e.g. Garcia and Grainger, 1996; Haddon, 2001).

Fishing activity depends on the state of the resource, and the state of the resource also influences the fishing activity. Thus, knowledge is needed on each of those two components and on their interaction (Hilborn and Walters, 1992). Fisheries science has tried to understand the processes governing the dynamics of fish stocks and to predict sustainable yield levels of exploited marine resources. Fisheries data analysis is useful for stock assessment purposes, for modelling harvested population dynamics and for fisheries management. In fisheries, a quantitative model involves many steps, being the first of all the collection of data from commercial fisheries and research vessel surveys, which are required as input in the modelling (Shepherd, 1988; Hilborn and Walters, 1992; Fournier et al., 1998). Mathematical models were developed based on certain biological, ecological or economical theories to describe the information collected from fisheries (Schnute and Richards, 2001).

Traditionally, fisheries models are based on estimates of parameters characteristics of the population and/or fishing regime (e.g. productivity, growth, maturity, fecundity, recruitment, mortality rate, fishing effort, catches, landings). All different data types used in these models present some limitations:

Groundfish surveys indices – the stock distribution in relation to the survey area may itself vary with abundance (Hutchings, 1996) or with changing environmental conditions, leading to non-proportionality between abundance and catch-per-unit-of-effort (CPUE), catchability of fish that may vary with season, location and other factors (Godø, 1994; Hjellvik et al., 2002);

Landed quantities – the length and age distributions are properties of the total landings, not of the actual fish stock (Hilborn, 1992; Fabrizio and Richards, 1996), because these landings depends on the gear selectivity and discarding practices, and represent a fraction of space and time;

LPUE – reflects changing markets, fisheries techniques, fishing tactics, discarding and stock distribution (Fréon and Misund, 1999; Rose and Kulka, 1999);

Age-length keys – the variation of length-at-age generally is higher among fishing vessels than within the catches of individual vessels (Sen, 1986);

Coefficient of natural mortality – sampling variability and costs are the major problems for the estimation of this parameter. Pope (1979) outlined that the variation of this coefficient with age may be confounded with the exploitation pattern with age, i.e. with catchability, selectivity and discarding, while variation with time may be masked by the variation of mortality due to fishing.

There are many types of assessment models/methods commonly used:

Integrative analysis (Fournier and Archibald, 1982) – including all data into a single analysis. This methodology is usually applied to catch-at-age analysis. Because catch-

at-age data are insufficient to produce reliably estimates of all the parameters of an age-structured model (Doubleday, 1976), and it will be necessary auxiliary information or additional assumptions (Deriso et al., 1985). The most common form of auxiliary information used is an index of relative abundance. This abundance index is usually based on CPUE or survey data;

Bayesian analysis (Gelman et al., 1995; Punt and Hilborn, 1997) – is a convenient method to include prior information into an analysis and represent uncertainty. The prior information can come from analyses of data from previous studies on the same population, from studies on different populations of the same species, from studies on different species, or from expert judgment. Prior information differs from traditional data included in stock assessments, since is typically information relative to certain parameters of the model, rather than observations that can be predicted from the model;

Meta-analysis (Myers et al., 1999) – is a method used to share information among multiple data sets. Data sets with little information about a parameter borrow information from the other data sets. In addition, to provide improved estimates for individual data sets, meta-analysis can be used to describe the distribution of the parameter for all data sets, which can be used as a prior distribution in future analyses. This analysis is a special case of integrated analysis in which each analysis is the same, but each is applied to a different data set. In fisheries applications each data set usually represents a different population or species;

Virtual Population Analysis (VPA) – is typically based in a cohort analysis (Pope, 1972; Siddeek, 1982). VPA involves, for each age, in each year, for every year class, estimation of the catch and the coefficient of instantaneous total mortality. This method fails the basic requirement of an assessment to provide confidence limits for its estimates. This is because the data and the equation are assume to be exact, a subjective decision contrary to the one of the ideals of stock assessment – depend upon the minimum of subjective decisions (Cotter et al., 2004). The main problem with VPA is that it operates retrospectively and provides least information about stock sizes and values of fishing mortality in the last year of the analysis, that is usually the one of most interest for predicting future performance of the fishery.

These models differ greatly in their mathematical structure, assumptions, data requirements, biological and ecological implications, and output. The choice of a model for a given fishery is often decided by the quantity of information available for stock assessment.

For a given model, error structure is assumed, and subsequently an objective function can be established based on this error for the model (e.g. Collie and Sissenwine, 1983; Schnute and Hilborn, 1993; Chen and Paloheimo, 1998). Parameters of the model can then be estimated by optimizing the objective function (Quinn and Deriso, 1999). A combination of the assumed model form and error structure determines the objective function, which in turn dictates the parameter estimation. Therefore, a realistic assumption of the model error structure is essential for reliable parameter estimation in stock assessment. An unrealistic assumption of the model error structure may lead to large errors or even biases in parameter estimation (e.g. Schnute and Hilborn, 1993; Schnute and Richards, 1995; Chen and Andrew, 1998).

Errors in fisheries modelling come from different sources (Hilborn and Walters, 1992; Schnute and Hilborn, 1993). Chen and Paloheimo (1998) referred that four different types of errors have been identified in fisheries modelling:

Measurement errors are consequence from the inability to measure fisheries or ecological variables accurately (Walters and Ludwig, 1981);

Process errors result from the inability to describe the dynamics of fisheries accurately taking into account random variation in the environment (Rosenberg and Restrepo, 1994);

Model errors result from the selection of an inappropriate model to describe the dynamics of fisheries (Schnute and Richards, 2001);

Operating errors are due to the measurement of a variable that is different from the one we intended to measure (Rosenberg and Restrepo, 1994; Chen and Paloheimo, 1998).

All four types of errors are likely to exist in a fisheries model, but it is assumed that model and operating errors are nonexistent to simplify modelling (Helser et al., 2001). Thus, uncertainty considered in fisheries modelling mainly includes measurement and process errors (Restrepo, 1999).

The quality of stock assessment is directly linked to the quality of the catch statistics and negatively affected by illegal or misreported landings, as well as by discarding.

CPUE data are the most important data source in many stock assessments (e.g. Maunder and Punt, 2004; Quirijns et al., 2008), where CPUE is usually assumed to be linearly proportional to abundance. Therefore, CPUE is usually standardized to remove these types of effects when constructing the index of abundance used in the stock assessment (Maunder and Punt, 2004). Many factors other than abundance can influence CPUE. These include environmental factors (e.g. temperature), fishing methods (e.g. trawl versus longline), fishing equipment (e.g. the use of sonar), fishermen behaviour (e.g. experience), management (e.g. the introduction of a quota management system), and economic factors (e.g. the price of fuel) (e.g. Anderson,

1994; Daan, 1997; Horwood and Millner, 1998; Rose and Kulka, 1999; Marchal et al., 2002).

Stock identification is a basic component of modern fisheries stock assessments and population dynamics, because most population models assume that the group of individuals has homogeneous vital rates and a closed life cycle which young fish in group were produced by previous generations in the same group. Stock structure and delineation are uncertain, the reliability of stock assessments, and therefore the effectiveness of fishery management, is severely limited for many fishery resources. Several definitions of the stock concept have been described throughout the fisheries literature (a.e., Parene, 1072). Basks, 1001, Japane, et al., 1001, Japane, et

literature (e.g. Royce, 1972; Booke, 1981; Ihssen et al., 1981; Hilborn and Walters, 1992). Any acceptable stock concept could be applied as long as the markers that are used are inherited and can define a specific fish group associated to a habitat (Booke, 1999).

Fish stocks are identified on the basis of variation in characteristics between stocks, with the strongest influences on stock structure drawn from a suite of complementary techniques that cover multiple aspects of the biology and life history characteristics of a fish species. A holistic approach to stock identification maximizes the likelihood of correctly defining fish stocks (Begg and Waldman, 1999). Overlaying all available information from a range of techniques would enable consistent and definitive patterns of stock structure to be developed, relative to the needs of fisheries management. Genetic variation is useful for determining evolutionary differences between stocks, in contrast to phenotypic variation that is more applicable for studying short-term environmentally induced differences between stocks.

Fisheries management is a broad term that describes the process of administering control of fishing for exploited fish stocks. Fisheries management applies to numerous different fish species that live in various ecosystems and involve different political systems and user groups as well as local and cultural traditions.

Historically, fisheries management has focused on achieving objectives that relate to the well-being of commercially harvested species. The adoption of the 200 nautical miles limit for Exclusive Economic Zones, in 1977, brought nearly all fish stocks under the control of national governments. Most countries currently use quota management systems with an annual Total Allowable Catch (TAC) from a single-species as their main management instrument. In determining the total permissible catch from each stock, some countries follow the precautionary approach.

A commitment to apply a Precautionary Approach (PA) to sustainable use of resources was made at Rio de Janeiro Summit, in 1992 (UN, 1992), and this commitment has been brought into a large number of international policy instruments (Richards and

Maguire, 1998). The implementation details differs widely among jurisdictions, the foundation of a functional PA is a set of explicit objectives and quantitative reference points, as integrated in the Guidelines for a Code of Conduct for Responsible Fishing (FAO, 1997).

Reference points can be defined in any biological currency, with fisheries science and management agencies commonly using fishing mortality and mature biomass (Restrepo et al., 1998; Anonymous, 2002).

The underlying notion of the precautionary principle is to manage the fish stock "within safe biological limits". These safety limits are established by International Council for the Exploration of the Sea (ICES) and other organizations through an assessment of the fish stock biomass. For species with less available data, decision makers act conservatively, so that fewer data translate into more restrictive limits.

The precautionary references points are:

Limit reference points for spawning stock biomass (B_{lim}) – the biomass level below which the possibility of a total breakdown of the stock is very high and the reproductive capacity reduced;

Precautionary level for stock biomass (B_{pa}) – a stock size level such that a short-term reduction in fishing effort is expected to allow the stock to recover above this limit;

Limit reference point for fishing mortality (F_{lim}) – the annual fishing level above which the risk of a total breakdown of the stock is extremely high;

Precautionary level for fishing mortality (Fpa) – the annual fishing level above which the risk that the stock size falls below B_{pa} is high.

Most scientific agencies worldwide, have traditionally given fishery management advice on a stock-by-stock basis. In the Northeast Atlantic the principal form of management is by annual TAC for a limited number of species. TAC is guided by a single-species assessment. A long-term monitoring of fish communities has provided unique insights into the effects of fishing and the environment on marine ecosystems (e.g. Overholtz and Tyler, 1985; Duplisea and Kerr, 1995; Bianchi et al., 2000).

Management has been unsuccessful for multi-species fisheries (Holden, 1994; European Commission, 2001), and several demersal stocks have declined to historically low levels, while exploitation levels remain too high (ICES, 2004). The main problem in managing multi-species fisheries by single-species TAC is that the latter do not restrict the catch but only the landings, the over-quota catch of a species perhaps being discarded or landed illegally (Holden, 1994; Daan, 1997). This occurs because the TAC for different target species may not be depleted in synchrony, so fishing continues when the TAC for one species has been depleted. This has two main effects, fishing mortality is not constrained by the TAC and, deteriorating catch

statistics lead to inaccurate assessments and uncertainty in the advice. Effort management has been proposed as a possible means of resolving these problems and improving the effectiveness of management (Daan, 1997; Ulrich et al., 2002; Shepherd, 2003).

Facing the relative failure of TAC as the main tool for fisheries regulation, there is an increasing interest in alternative management measures. Selective gears are considered in many fisheries to reduce technical interactions (Murawski and Stewart, 1996; Commission of the European Communities, 2001). Marine protected areas (MPA) are also more and more used to protect particular population stages and/or to preserve habitat and food webs (Botsford et al., 1997; Holland, 2000). The effectiveness of MPA is known to depend on the design of the closure (local, size, time) (Sumaila et al., 2000; Jamieson and Levings, 2001) and on interactions with other management measures (Holland, 2003). Evaluating the impact of such alternative management options is essential for fisheries management (Gulland, 1983; Hilborn and Walters, 1992).

Singles-species approach which ignores dependencies between species induced by trophic relationships and by technical interactions in multi-species fisheries (Botsford et al., 1997; Sumaila et al., 2000). The lack of consideration of spatial and seasonal heterogeneities in models of populations and fishing activity dynamics (Pet et al., 1996; Maury and Gascuel, 1999; Holland, 2000). This is particularly critical in a multi-species fisheries context where the diversity of resources and fishing activities make it difficult to estimate resulting fishing mortality (Murawski and Stewart, 1996). Given the complexity of multi-species fisheries dynamics, simulation models are necessary to evaluate the efficiency of alternative management measures.

Fisheries management showed an increasing and renewed interest in the last decade, and have been addressed in may *fora* (e.g. Pauly et al., 1998; Caddy and Cochrane, 2001; Baum et al., 2003; Myers and Worm, 2003). Much of the stimulus for this has come from stock collapses of fisheries, such as cod, haddock, halibut, yellowtail flounder and capelin (e.g. Gjoesaeter, 1995; Hutchings, 2000; Fu et al., 2001), increased awareness of uncertainty and the subsequent incorporation of the precautionary approach into management decisions (e.g. Rosenberg and Brault, 1993; Ward et al., 2000; Stergiou, 2002) and the negative impacts of fishing on marine ecosystems (e.g. Dayton et al., 1995; Turner et al., 1999; Rochet et al., 2005).

Management approaches that actively incorporate the objective of reducing disruption of ecosystems through fisheries are also referred as ecosystem-based management systems. This approach focuses not only on maintaining strong stock levels of the target species, but also on protecting biological diversity. The ecosystem-based approach hence extends beyond the precautionary approach to fish stock management as it incorporates species interactions with other components of the ecosystem. This approach demands not only the sustainable use of fish stocks but also the protection of the aquatic environment by requiring the use of non- or low-impact fishing gear.

An ecosystem-based approach to fisheries management has become a key goal of national and international institutions. A key objective of any ecosystem approach is maintaining the productivity of fish communities, as well as harvests from those communities, within a desired range. Multi-species models (e.g. Collie and Gislason, 2001), ecosystem models (Walters et al., 2005), and general ecosystem principles suggest that the sum of single-species maximum sustainable yields (MSY) can be a poor proxy for the MSY of a multi-species complex, because of ecological interactions and fishing impacts. Simulations of exploited populations and management procedures provide insight into the sensitivities of a system to different management regimes, even if the ultimate management performance cannot be predicted (e.g. Harwood and Stokes, 2003; Kell et al., 2005; Pastoors et al., 2007).

Marine ecosystems are very complex, and one of the major problems with ecosystem approaches is the difficulty of defining operational objectives and performance measures. The main objective of this approach should be to rebuild ecosystems, rather than species-by-species sustainability (Pitcher and Pauly, 1998; Pitcher et al., 1998). There is a need to integrate comprehensive models of key species of interest with comprehensive model of the biological, physical and chemical environment in which they live, while at the same time reducing the dimensions of complexity to manageable levels.

Mace (2001) suggests there are three fundamental prerequisites for successful ecosystem-based management:

Reduce fishing mortality overall - eliminating overfishing on individual target species;

Eliminate overcapacity – controlling the size of fishing fleets and limiting participation levels by regulation, or implicitly by implementation a management system such as Individual Transferable Quotas (ITQ);

Conduct adequate baseline monitoring of marine species and their environment – recommend improved monitoring of all marine species in order to facilitate a comprehensive ecosystem approach.

Trites et al. (1999) pointed out that the main criticism of ecosystem models is that they may not be able to predict changes in community structure.

Portuguese fisheries management is guided by the Common Fisheries Policy (CFP) of the European Union (EU), the present management system includes the establishment

of annual TAC and quotas for some species, technical measures (minimum mesh size, selective fishing gear, closed areas and seasons, minimum landing size for fish and limits on by-or incidental catches) and limitation of fishing effort.

In Portugal, several species (sole, plaice, whiting, Norway lobster, pollack, anglerfishes, hake, megrims, anchovy, blue whiting, mackerel, bluefin tuna, horse mackerel and swordfish) have limitations in fishing (TAC, quotas, and minimum legal size), although only some of them have been assessed (hake, horse mackerel, megrim, anglerfish, sardine, anchovy and Norway lobster). The assessment has been only based on spawning stock and fishing mortality (ICES, 2007).

However, the need to intervene in fisheries became more urgent and the aim of regulatory measures put forward was to make fishers internalize social value of the resource in such a way to as to nullify the external costs and stimulate efficient and conservationist ways of treating the natural resource. Although the main point of departure from the status quo in the fisheries took place on an international scale, with redefinition of international maritime law in the 1960's, these generalized needs for regulations would be transferred to a local level (Garza-Gil et al., 2003). Effective fisheries sustainable development requires a significant investment to collect the needed information (Garcia and Staples, 2000; FAO, 2005).

Multi-species Fisheries

Multi-species fisheries are a complex system characterised by a high spatial and temporal variation, and a high diversity of gears and techniques. The small size of vessels may limit travel distances to areas surrounding homeports, especially in winter when bad weather may put long fishing trips at risk. The target species for these vessels occur mainly in coastal areas. The changing patterns of fishing tactics (fishing location, fishing gear or target species) is based on the multitude of factors, like the weather conditions, the knowledge of fishing grounds, the seasonal availability of resources, the market demand, the recent fishing yield and income, the tradition as well as information and rumours about the yield of other fishermen.

In multi-species fisheries modelling, technical interactions have traditionally been promoted separately from biological interactions among species. The first stage towards identification of technical interactions is a precise description of fishing activity. The concept of the *métier* was introduced in order to categorise the activities of the fishing fleets. *Métier* is usually defined by the use of a given fishing gear in a given area and season, in order to target a single or group of species (e.g. Mesnil and

Shepherd, 1990; Laurec et al., 1991; Lewy and Vinther, 1994). The *métier* concept brings more exact description of the fishing activity than the single term "gear". This concept is commonly used associated to measures of fishing effort (e.g. Biseau, 1998; Ulrich et al., 2001; Alemany and Álvarez, 2003; Tzanatos et al., 2006), however it is sometimes referred as "fishing tactic" or "trip type" (e.g. Pech et al., 2001; Jiménez et al., 2004; Christensen and Raakjær, 2006; Tsitsika and Maravelias, 2008).

Identification of *métiers*, in the majority of cases, has been based on the analysis of datasets on species composition from commercial fisheries or major projects for collecting landings data, in terms of catch or CPUE (e.g. Pelletier and Ferraris, 2000; Ulrich et al., 2001; Silva et al., 2002; Holley and Marchal, 2004).

Understanding the way fishermen select and change *métiers* is an important step for the improvement of fisheries management. It can help to predict the outcome of different management actions and select appropriate management strategies (Cabrera and Defeo, 2001; Salas and Gaertner, 2004).

Fishing gears select catch by size and species (MacLennan, 1992; Dalzell, 1996) and a well-managed fishery is expected to use gear that catch most of the available species at sizes that do not undermine sustainability. Some gears, may take a wide variety of sizes and species with only the smallest individuals avoiding capture (Dalzell, 1996; Gell and Whittington, 2002), other gears, may also take a wide variety of species, but may be very size selective, taking only a relatively narrow range of lengths, depending on the size of the mesh (Acosta, 1994). Gear type and use can therefore affect the efficiency of fish capture, the selectivity and composition of fish resources. The use of gears and understanding the factors that influence use are also vital to determine the interaction between social and ecological change (Stergiou et al., 1996; Glaesel, 2000).

The majority of fishing methods have low species selectivity and result in incidental catches (bycatches) that include species that are unwanted and discarded (discards) and species that are retained and sold (Stobutzki et al., 2003).

Discarding can be highly variable in space and time as a consequence of changing economic, environmental, biological and sociological factors (Crean, 1994; Gillis et al., 1995; Maynou and Sarda, 2001; Bergmann et al., 2002). Discarding usually occurs due to market forces (non-commercial and low value species, low condition and small size) and as a direct result of management measures (minimum landing size and landings restrictions) (Vestergaard, 1996). Discarding patterns are initially influenced by catch compositions, which are determined by environmental and social (regulations and fishermen's behaviour) factors, and are ultimately controlled at the discretion of fishermen, who are influenced by landing constraints and economic forces.

Fishermen discard when they catch the wrong species or the wrong sizes of target species, the catch are damaged, the species quota is reached, or when high grading is practised. These factors decide the fate of the catch; however, it is the processes that occur before fishermen decide on what to land, that have led to the continued high level of discarding. Two processes are identified here as the fundamental causes of high discarding in the European demersal fisheries: the use of unselective fishing techniques, leading to high discard rates, and the failure to restrict fishing effort, leading to high volumes of discards.

Gill nets and trammel nets are fairly size selective but the type and quantity of bycatch is largely dependent on the fishing area and the time of the year (Goñi, 1998). In what regards discards, the extent of this practice is unknown. However, discards estimates are necessary not only for evaluating the impact of fishing on non-commercial species but also on ecosystems as a whole (Alverson et al., 1994). Systematic underestimation of fishing mortality increases the risk of overexploitation (Goñi, 1998).

In addition to official fisheries landings and discards, catches can also include a certain amount of illegal (e.g. such as time or area closures, species quotas, gear restrictions), unreported and unregulated catches. This unknown fraction of catches could profoundly affect estimates of stock abundance and safe removal rates (Ainsworth and Pitcher, 2005). Discard estimations are essential for assessing the full impact of fisheries upon fish populations and upon the ecosystem in which they operate. As the survival of discarded organisms can be low (Evans et al., 1994), discarding can be a substantial component of fishing mortality. Nevertheless, discarding is usually unaccounted for in stock assessments. Furthermore, discards may have an important indirect impact on other species communities, such as seabirds and benthic organisms, by causing changes in prey and predator abundance and species assemblages (e.g. Jennings and Kaiser, 1998; Votier et al., 2004). Discards can also be used in fisheries management in other ways than as a data input in stock assessments: for evaluating conservation measures (minimum landing size, mesh sizes, close areas and seasons) and for identifying the characteristics and behaviours of particular fishing fleets.

Several species are caught by the multi-species fisheries, some of them have been subject of many studies, hake (e.g. Fonseca et al., 2005a; Merino et al., 2008), Norway lobster (e.g. Catchpole et al., 2006; Bahamon et al., 2007), elasmobranchs (e.g. Ellis et al., 2005; Figueiredo et al., 2007), anglerfish (e.g. Revill et al., 2006; Graham et al., 2007), horse mackerel (e.g. Fonseca et al., 2005b; Campos et al.,

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2007) and flatfishes (e.g. Vinther et al., 2004; Rijnsdorp et al., 2006, 2007; Kraak et al., 2008).

Flatfish Fisheries in the Portuguese Coast

Fishing is a traditional and culturally important activity in Portugal, being dominated by small and local fishing vessels (Baeta and Cabral, 2005). According to the 2001 population census, 13837 people are employed in the fisheries sector (source: INE). Of these workers, 57% have between 35 and 54 years of age, often having a low educational level, with 4% being illiterate, and 86% having only primary school education (source: INE).

Between 1992 and 2005 the number of vessels has decreased 38%, but this has been compensated by an increase in power per vessel (source: DGPA). Fishing vessels operate out of 32 ports in Portugal, distributed along the coast.

Flatfish fisheries are widely spread over the Portuguese coast and traditionally have played an important socio-economic role. The economic and social importance of this fleet is evidenced by the contribution to the total landings and revenue, about 4% and 11% of fish landings, respectively, and the number of fishermen involved, nearly 85% of the total (source: DGPA; INE).

A large number of flatfish species occur in the Portuguese coast (e.g. Nielsen, 1986a,b,c,d; Quéro et al., 1986a,b; Cabral, 2000a). This species richness is usually higher than that found in North Europe and similar to that found in the Mediterranean, since many flatfish species are at their southern and northern distribution limits, respectively, along the Portuguese coast (e.g. Quéro et al., 1986a; Desoutter, 1997). The zoogeographic importance of this latitudinal area has long been recognized, representing the transition between northeastern Atlantic warm-temperate and cold-temperate regions (Ekman, 1953; Briggs, 1974).

The water circulation in the Portuguese coast is characterized by a complex current system subject to strong seasonality and mesoscale variability, showing reversing patterns between summer and winter in the upper layers of the slope and outer shelf (e.g. Barton, 1998; Peliz et al., 2005). Upwelling events are common in the Portuguese coast, especially in summer (Fiúza et al., 1982). The occurrence of upwelling pulses during summer is important, since the upwelling process injects nutrients in the surface layer that fuel primary production.

The artisanal fleet is responsible for the largest share of flatfish landings. This fleet consists of almost 6000 vessels, most of them between 5 and 17 m overall length,
with open deck, and operating near the coastline (source: DGPA). The multi-gear fleet is made up of vessels that are licensed to use several different gear types throughout the year. The main fishing gears used in flatfish fisheries are trammel and gill nets and bottom trawl. However, only a fraction of these vessels actively target flatfishes, since many other species are caught in this multi-species fisheries.

The most important species, in terms of landings, are the soles, *Solea solea* (Linnaeus, 1758), *Solea senegalensis* Kaup, 1858, and *Solea lascaris* (Risso, 1810), the bastard sole, *Microchirus azevia* (Capello, 1868), the flounder, *Platichthys flesus* (Linnaeus, 1758), the spotted flounder, *Citharus linguatula* (Linnaeus, 1758), the wedge sole, *Dicologlossa cuneata* (Moreau, 1881), the turbot, *Scophthalmus maximus* (Linnaeus, 1758), the brill, *Scophthalmus rhombus* (Linnaeus, 1758), and the megrims, *Lepidorhombus boscii* (Risso, 1810) and *Lepidorhombus whiffiagonis* (Walbaum, 1792). Flatfish landings present a decreasing trend between 1992 and 2005; soles landings represented near 50% of the total landings of flatfishes; and Leixões is the port with highest flatfish landings (about 20% of total flatfish landings).

Pleuronectiformes of the Portuguese coast have been subject of many studies, which have investigated their feeding ecology (e.g. Cabral, 2000b; Sá et al., 2003; Vinagre et al., 2005), age and growth (e.g. Andrade, 1990; Cabral, 1998, 2003), reproduction (e.g. Santos, 1994; Gomes, 2002; Afonso-Dias et al., 2005), habitat use (e.g. Cabral, 2000a; Cabral et al., 2002; Vinagre, 2007), population genetic (Cabral et al. 2003; Pinheiro et al. 2005; Teixeira, 2007), parasitosis (e.g. Barahona-Fernandes and Dinis 1992; Mateus 2001; Marques, 2006; Marques et al., 2006), as well as fishery (e.g. Ribeiro et al., 1999; Batista, 2005, 2007; Alves, 2008). Despite the large volume of literature, basic knowledge about the biology and ecology of the most important flatfish fisheries resources, as well as detailed analyses of their fisheries, are extremely scarce.

Aims and Importance of this Study

The present work aims to study the feeding ecology, growth, and reproduction of seven flatfish species with commercially importance, define their population structure based on genetic characters, characterize the fleet that catch these resources and assess the state of their stocks.

Several species of Pleuronectiformes occurring off the Portuguese coast have a high commercial value. The quantities landed are not particularly high, but their commercial importance is considerable. Despite the economic importance of these fisheries resources, knowledge about their biology and status of fish stocks is reduced. In the Portuguese coast, the main studies on the ecology of species of Pleuronectiformes have a limited scope and have focused mainly on the juvenile stage, especially in lagoon and estuarine environments (e.g. Dinis, 1986; Andrade, 1990; Cabral, 1998, 2000a, 2000b; Cabral and Costa, 1999; Cabral et al., 2002).

Many studies conducted in northern Europe refer a high pressure on these resources, which caused a decrease of their populations, being some of the stocks outside safe biological limits. Thus, it is extremely important to assess the state of the flatfish stocks in the Portuguese coast.

The reform of the CFP includes a set of measures which aims to ensure the ecologically, economically and socially sustainability of the main fisheries. The CFP reform introduces substantial differences in the way of managing fisheries stocks, emphasizing the role that multi-annual management plans can have and introducing an ecosystem approach to fisheries management. These new approaches require scientific information that is scarce for the majority of flatfish species and for several fisheries in the Portuguese coast.

Structure of the Thesis

This work comprises nine chapters organized in four parts. The General Introduction (Chapter 1) addresses the importance of the assessment and management of fisheries stocks, highlighting the multi-specific fisheries for its complexity, and giving particular attention to flatfish fisheries.

In Chapters 2 to 4 the diet, growth and reproduction of the sole, *Solea solea* (Linnaeus, 1758), Senegalese sole, *Solea senegalensis* Kaup, 1858, sand sole, *Solea lascaris* (Risso, 1810), spotted flounder, *Citharus linguatula* (Linnaeus, 1758), fourspotted megrim, *Lepidorhombus boscii* (Risso, 1810), flounder, *Platichthys flesus* (Linnaeus, 1758), and bastard sole *Microchirus azevia* (de Brito Capello, 1867) were analysed. The analysis of the genetic diversity and population structure of *S. solea* and *S. senegalensis* was addressed in Chapter 5, based on the analysis of the complete sequence of the cytochrome b of mitochondrial DNA of samples collected along the distribution area for the two species.

In Chapter 6 is presented an analysis of the variation of monthly landings of commercial fishing of several species of Pleuronectiformes (soles, bastard sole, flounder, spotted flounder, wedge sole, turbot, brill and megrims) landed along the

Portuguese coast, between 1992 and 2005. Generalized linear models were applied in order to find out which factor influence landings of these species.

Chapter 7 include a characterization of the flatfish fisheries in the Portuguese coast and Chapter 8 evaluate the catches and bycatches of the trammel net fishery operating in the central coast of Portugal. Finally, in Chapter 9, is presented a summary of the main findings and indicated some topics that require further research.

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Part II

Biology and Ecology of Commercially Important Flatfishes

Chapter 2

Comparative analysis of the diet, growth and reproduction of the soles, *Solea solea* and *Solea senegalensis*, occurring in sympatry along the Portuguese coast Journal of the Marine Biological Association of the United Kingdom (*submitted*) By Teixeira, C.M., Cabral, H.N.

Comparative analysis of the diet, growth and reproduction of the soles, *Solea solea* and *Solea senegalensis*, occurring in sympatry along the Portuguese coast

ABSTRACT: Common sole, *Solea solea* and Senegalese sole, *S. senegalensis*, were collected between January 2003 and June 2005 from commercial fishing vessels operating with gill nets and bottom trawls along the Portuguese coast, to examine diet, age and growth and reproduction. Soles fed mainly on crustaceans, polychaetes and bivalves. Feeding activity was highest in spring-summer, for females and for the smallest individuals. Significant differences were found between the proportion of prey items according to season, sex and size class. Common sole presented a wider dietary breadth compared to Senegalese sole. Dietary overlap between the two species was higher for the autumn-winter period and for females. Age of soles was determined from sagittae otoliths readings. The length of fish analysed varied between 187 mm and 462 mm (oldest fish with 9 years), for *S. solea*, and between 199 mm and 472 mm (oldest fish with 8 years), for *S. senegalensis*. The von Bertalanffy growth equation coefficients differed between sexes. For both species, the asymptotic length L_{∞} and growth coefficient *k* obtained for females were higher compared to those estimated for males. The highest values of the gonadosomatic index were obtained for autumn-winter period, when the highest proportion of individuals at spawning stage was recorded.

Keywords: feeding ecology, growth, reproduction, flatfish, *Solea solea*, *Solea senegalensis*, Portuguese coast

INTRODUCTION

Two species of sole, the common sole *Solea solea* (Linnaeus, 1758) and the Senegalese sole *Solea senegalensis* Kaup, 1858, have a wide geographic distribution from the Eastern Atlantic to Mediterranean Sea, and, in Northeastern and Southeastern Atlantic, respectively, inhabiting sandy and muddy bottoms at depths near to 100 and 200 m (Quéro et al., 1986a). These species are very

similar, present a sympatric distribution from North Africa and western Mediterranean up to the Bay of Biscay.

The Portuguese coast is a very important area for several flatfish species (e.g. Nielsen, 1986a,b,c,d; Quéro et al., 1986a,b; Cabral, 2000a), and, some of these species have a high commercial interest. Flatfish fisheries represent 4% of all the fish biomass landed in the Portuguese coast (source: Direcção Geral das Pescas (DGPA)). However, the importance of flatfish fisheries is considerable higher due to the high commercial value of flatfish species, accounting for near 11% of the economical value of the fish landings (source: DGPA). According to official data, soles landings increased from 464.01 tonnes, in 1998, to 510.39 tonnes, in 2005 (source: DGPA).

Studies on the feeding ecology of *S. solea* has been carried in coastal areas of northwestern Europe (e.g. Rijnsdorp and Vingerhoed, 2001; Vinagre et al., 2005) and western Mediterranean (e.g. Molinero et al., 1991; Darnaud et al., 2001). Unlike *S. solea*, the diet of *S. senegalensis* is known only from the western Mediterranean (Molinero et al., 1991; Garcia-Franquesa et al., 1996) and Portugal (Cabral, 2000b; Sá et al., 2003). Most of these studies were focused mainly on juveniles.

Age and growth of *S. solea* has been studied by several authors, in western Europe (e.g. Cabral, 2003; Henderson and Seaby, 2005) and in Mediterranean (e.g. Vianet et al., 1989; Garcia et al., 1991; Türkman, 2003). Few studies were conducted for *S. senegalensis*, and all are relative to the Portuguese coast (Dinis, 1986; Andrade, 1992; Cabral, 2003).

Reproduction study has been conducted on *S. solea* in western Europe (e.g. Baynes et al., 1994; Bromley, 2003) and Mediterranean (e.g. Vallisneri et al., 2002; Türkmen, 2003). Reproduction of *S. senegalensis* has been studied in western Europe (e.g. Dinis, 1986; Andrade, 1990) and in Mediterranean (Ramos, 1982).

Studies conducted along Portuguese coast, revealed that diet of *S. solea* was similar to the diet of *S. senegalensis*, the soles fed a low variability of invertebrates, such as Polychaeta and Amphipoda (Cabral, 2000b; Sá et al., 2003; Vinagre et al., 2005). A longevity of *S. solea* was 15 years and a maximum total length of 500 mm (Dinis, 1986), and Andrade (1990) determined for *S. senegalensis* a maximum total length of 516 mm and a longevity of 11 years. *S. senegalensis* presented a long reproduction period ranges from autumn to spring (Andrade, 1990), or for spring to summer (Dinis, 1986).

Studies on the ecology of these two species in sympatric areas (i.e. from Bay of Biscay to North Africa and western Mediterranean) are scarce. The importance of

these studies is crucial for management purposes. The aim of the present work was to study the feeding ecology, age and growth, and reproduction of *S. solea* and *S. senegalensis* in the Portuguese coast.

MATERIALS AND METHODS

Sampling Surveys and Samples Processing

Bimonthly samples were collected between January 2003 and June 2005 from commercial fishing vessels operating with gill nets and bottom trawls along the Portuguese coast (Figure 2.1). Samples were collected regularly throughout the year and along the coast (a minimum of 40 individuals were obtained per season and sampling area).



Figure 2.1. Location of sampling area.

All fish were measured (total length to nearest 1 mm) and weighed (total and eviscerated wet weight with 0.01 g precision). Gut and gonads were removed and frozen (-20°C) for further analysis. Saggitae otoliths were removed, cleaned and kept dry for later age determination.

Feeding Ecology

The stomach contents of 494 *S. solea* (total length between 187 mm and 465 mm) and 533 *S. senegalensis* (total length between 191 mm and 494 mm) were analysed. Each prey item was identified to the lowest taxonomic level possible, counted and weighed (wet weight with 0.001 g precision).

The relative importance of each prey item in the diet was evaluated by the numerical (NI), occurrence (OI) and gravimetric (GI) indices (Hyslop, 1980). Feeding activity was evaluated by the vacuity index (VI) defined as the percentage of empty stomachs (Hyslop, 1980).

Correspondence analyses (CA) were run to evaluate diet variation with season, sex and fish length according to each of the three index values. Prey items were grouped to a broader taxonomic level, two seasons (autumn-winter and spring-summer) and two size classes (size class 1: <300 mm and size class 2: \geq 300 mm total length) were considered. These analyses were performed using CANOCO (CANOnical Community Ordination) version 4.5 (ter Braack and Šmilauer, 2002).

Diet differences between season, sex and fish size were tested using the χ^2 -test (Zar, 1984) with a 0.05 significance level.

Diet overlap was measured using the Schoener index (I_s), defined as

$$I_{S} = 1 - 0.5 \left(\sum_{i=1}^{n} \left| p_{i_{A}} - p_{i_{B}} \right| \right),$$

where p_{i_A} and p_{i_B} and were the numerical frequencies of item *i* in the diet of species *A* and *B*, respectively (Linton et al., 1981). Values of diet overlap vary from 0, when no food is shared, to 1, when there is the same proportional use of all food resources. Although there are no critical levels with which overlap values can be compared, Wallace (1981) and Wallace and Ramsey (1983) suggested that values higher than 0.6 should be considered as biologically significant.

The degree of feeding specialization of each season, sex and size class of fish was determined using the Shannon–Wiener diversity index H' (Shannon and Weaver, 1949)

$$H' = - \sum_{i=1}^{s} P_i \ln P_i,$$

where P_i is the numerical proportion of the *i*th prey category in the diet and *s* is the total number of different prey categories consumed by predator. This index corresponds to the dietary breadth (Marshall and Elliott, 1997).

Growth

Age was evaluated using otoliths readings. For each specimen, two counts of otolith annuli were made under a dissecting microscope. Whenever the two readings of the same otolith resulted in different age estimates the data were not considered for further analysis.

Estimates of theoretical growth in length were obtained by fitting length-at-age data to the von Bertalanffy growth equation:

$$L_t = L_{\infty} \cdot (1 - e^{-k \cdot (t - t_0)}),$$

where L_t is the total length, L_{∞} is the asymptotic length, k is the growth coefficient and t_0 is the theoretical age at zero length. The growth parameters of this model were estimated iteratively using the least squares method in STATISTICA (Statsoft) software. This analysis was performed separately for males and females.

Reproduction

Gonads were observed macroscopically and a maturation stage was assigned to each individual, according to the scale: I – immature, II – development, III – spawning, IV – post-spawning (Cabral, 1998). For each season (autumn-winter, spring-summer), the percentage of fish in stages II, III and IV was determined.

In order to evaluate gonadal development throughout the year to determine the spawning season, the gonadosomatic index (GSI) was calculated, per sex, for each season. The GSI was expressed as the percentage of the weight of gonads in relation to eviscerated weight of fish. Age and length at first maturity were determined.

RESULTS

Feeding Ecology

Crustacea, Polychaeta and Bivalvia were the most important itens in the diet of *S. solea* and *S. senegalensis* (Table 2.1). Among Crustacea, Amphipoda presented the

highest contribution to the diet of both species, followed by Decapoda and Mysida, for common sole and Senegalese sole, respectively. The most important Polychaeta prey were Nephtyidae and Nereididae to *S. solea* and Nereididae and Cirratullidae to *S. senegalensis. Ensis* spp. and *Tellina distorta* were the most important bivalves in the diet of both species. Vacuity index was higher for Senegalese sole (45.4%) compared to the value obtained for common sole (33.3%). The lowest values of vacuity occurred in spring-summer, for females and for small size individuals (Table 2.2).

The correspondence analysis (CA) performed using data on the three indices for the main prey groups, by season, sex and size class, explained a high percentage of the variance in the first two axes (41.7%, 58.9% and 51.7% for NI, OI and GI, respectively) (Figure 2.2).

When the numerical index was considered in the ordination analysis (Figure 2.2a), the largest individuals of *S. solea* were associated with Decapoda and Paguridae. The diet of small individuals was associated with Bivalvia and Gastropoda for females, and Amphipoda for males. *S. senegalensis* diet was strongly associated with Polychaeta, for small females, in autumn-winter, and for the largest males throughout the year. The small females, in spring-summer, were associated with Gastropoda and the largest females with Mysida. The small males' diet, in autumn-winter, was composed by Perciformes, and, spring-summer, was composed by Gastropoda.

When considering the occurrence data in the ordination diagram (Figure 2.2b) it can be seen that: the size class I of *S. solea* was associated with Echinodermata, Gastropoda and Bivalvia in spring-summer. The largest individuals' diet was composed by Paguridae for females in autumn-winter and for males in spring-summer. Decapoda was associated with small females in autumn-winter and with largest females in spring-summer. Small males were associated with Polychaeta in autumn-winter. *S. senegalensis* was mostly associated with Amphipoda, Isopoda and Mysida. Diet of small males was associated with Perciformes in autumn-winter and with Isopoda in spring-summer.

In the ordination diagram obtained for gravimetrical data (Figure 2.2c), Amphipoda was strongly associated with small females of *S. solea* in autumn-winter, and throughout the year for small males. Females' diet was composed by Bivalvia for class size I in spring-summer, and class size II were associated with Echinodermata in spring-summer, and Paguridae in autumn-winter. The diet of the largest males was composed by Echinodermata in autumn-winter, and Paguridae in spring-summer. *S. senegalensis* was chiefly associated with Polychaeta, Mysida and Crustacea. Amphipoda was associated with females of both class size, and with largest males spring-summer.

For common sole and Senegalese sole, the numbers of each prey item differed between seasons (χ^2 =34.3, d.f.=11, P<0.05 and χ^2 =520.4, d.f.=11, P<0.05, respectively).

The diet of common sole and Senegalese sole was different between sexes (χ^2 =40.3, d.f.=11, P<0.05 and χ^2 =449.2, d.f.=11, P<0.05, respectively).

S. solea and S. senegalesnis presented a different diet between the two size classes (χ^2 =144.0, d.f.=11, P<0.05 and χ^2 =614.0, d.f.=11, P<0.05, respectively).

		S solea			S seneralensis			
Prev itens	NT	NT OT GT		NI OI		GI		
Foraminifera	2.7	17	<0.1	0.1	03	0 1		
Polyplacophora	0.2	0.3	0.1	0.1	0.2	0.2		
Bivalvia	18.2	13.6	9.6	8 1	11 1	4.6		
Dosinia lupinus	0.9	1 0	03	0.2	0.6	0.2		
, Donax vittatus	015	110	010	0.1	0.2	0.2		
Acanthocardia spp.	0.5	0.4	0.8	011	012	012		
Ensis spp.	6.5	4.0	4.3	3.7	4.0	2.6		
Tellina distorta	1.7	1.3	1.2	1.3	1.3	0.3		
Tellina tenius	0.9	0.4	0.8	1.0	1.0	0.0		
Corbula spp.	0.3	0.4	< 0.1					
Chlamys spp.	0.8	0.4	0.1					
Bivalvia n.i.	6.6	5.6	2.1	2.8	5.0	1.3		
Gastropoda	3.7	2.9	1.5	7.9	2.5	5.0		
Gibbula spp.	0.4	0.4	0.1	0.1	0.2	0.2		
Fusinus syracusanus	0.1	0.1	0.1	0.1	0.2	0.2		
Muricopsis cristata	0.1	0.1	< 0.1					
Nucella fragilis	0.1	0.1	0.1					
Nassarius spp.	0.2	0.1	0.1	0.1	0.2	1.4		
Littorina spp.	0.1	0.1	< 0.1	0.1	0.2			
Hydrobia ulvae				0.1	0.2	0.2		
<i>Turritella</i> spp.	0.1	0.1	< 0.1	0.1	0.2	0.2		
Velutina velutina	0.6	0.3	0.8					
Ringicula auriculata	0.5	0.4	0.1	0.1	0.2	0.2		
Scaphander lignarius	1.3	0.9	0.1	7.2	0.9	2.5		
Gastropoda n.i.	0.2	0.3	0.1	0.2	0.6	0.3		
Annelida	28.3	29.4	26.9					
Polychaeta	28.2	29.2	25.5	36.5	37.7	43.8		
Ampharetidae	0.2	0.1	< 0.1	1.9	0.2	0.5		
Cirratulidae				2.6	2.7	1.4		
Flabelligeridae				0.3	0.2	0.2		
Pectinariidae	1.7	0.6	2.4					
Sternaspidae	0.1	0.1	0.3					
Terebellidae	0.3	0.1	0.1					
Trichobranchidae	0.6	0.6	0.8					
Sabellariidae	0.1	0.1	< 0.1	0.9	0.4	1.5		
<i>Lygdamis</i> spp.				0.9	0.4	1.5		
Aphroditidae	0.5	0.5	0.6	0.6	0.8	0.4		
Aphrodita aculeata	0.4	0.4	0.6	0.2	0.4	0.2		
Aphroditidae n.i.	0.1	0.1	< 0.1	0.4	0.4	0.2		
Polynoidae	0.5	0.6	0.3					
Sigalionidae	1.5	1.3	1.6	0.4	0.4	0.6		
Leanira spp.	0.1	0.1	< 0.1					
Sigalion spp.	0.6	0.3	0.2					
Sigalionidae n.i.	0.8	0.9	1.4					
Glyceridae	0.1	0.1	0.1	0.7	1.1	0.8		
Goniadidae	0.1	0.1	0.9					
Nephtyidae	3.1	3.6	4.4	1.8	1.7	0.9		
Nereididae	3.0	0.9	0.6	5.8	2.8	5.5		
Nereis spp.				2.2	0.6	0.7		

Table 2.1. Numerical (NI), occurrence (OI) and gravimetric (GI) indices values of prey found in guts of *S. solea* and *S. senegalensis*, in the Portuguese coast.

(continue)

Table 2.1. (Continued)

	S. solea			S. senegalensis			
Prey itens	NI	OI	GI	NI	OI	GI	
Nereididae n.i.				3.6	2.3	4.8	
Lumbrineridae				0.2	0.2	0.2	
Onuphidae	0.8	03	0.2	0.2	0.2	0.2	
Canitellidae	0.0	0.5	0.2	04	04	04	
Notomastus snn				0.4	0.7	0.7	
Canitellidae n i				0.2	0.2	0.2	
Orbiniidae	03	0 1	04	0.2	0.2	0.2	
Polychaeta n i	15 3	20.0	12.8	20.8	26.6	31.8	
Annelida n i	0 1	0.1	1 2	20.0	20.0	51.0	
Crustacea	34.8	36.6	45.9	36.7	42 7	32.7	
Ostracoda	03	03	< 0.1	0 1	0.4	0 1	
Copenoda	0.1	0.1	< 0.1	0.1	0.1	0.1	
Balanidae	0.3	0.4	< 0.1				
Balanus spp	0.3	0.4	< 0.1				
Decanoda	3.4	2 5	6.5	0.2	05	15	
Alpheus ruber	1.8	1 1	2.4	0.2	0.5	1.5	
Crangon crangon	0.9	0.4	19				
Paguridae	0.1	0.1	0.2				
Pachyaransus marmoratus	0.4	0.6	17				
Carcinus maenas	011	010	117	0.1	0.3	1.3	
Pilumnus hirtellus				0.1	0.2	0.5	
Decapoda n.i.	0.2	0.3	0.3	0.2	0.2	0.0	
Mysida	0.4	0.1	< 0.1	5.5	0.6	0.5	
Cumacea	0.1	0.1	< 0.1				
Tanaidacea				0.1	0.4	0.2	
Isopoda	0.3	0.4	0.2	2.3	3.2	3.3	
Cvathura carinata				0.2	0.2	0.2	
Anthura gracilis				0.1	0.4	0.2	
Eurvdice spp.	0.2	0.3	0.2	1.2	1.3	1.5	
Sphaeromatidae	0.1	0.1	< 0.1	0.2	0.4	0.2	
Isopoda n.i.				0.6	0.9	1.2	
Amphipoda	14.9	13.7	18.7	17.8	14.8	12.5	
Corophium volutator				0.6	0.9	0.3	
Orchestia spp.				1.0	0.2	0.3	
Amphipoda n.i.				16.2	13.7	11.9	
Crustacea n.i.	8.3	12.6	5.1	10.5	22.3	12.8	
Echinodermata	9.8	11.6	14.4	0.6	0.9	1.0	
Crinoidea	0.1	0.1	< 0.1				
Ophiuroidea	5.2	7.4	11.8				
Holothuroidea	0.2	0.3	0.1	0.5	0.8	0.5	
Echinoidea	3.4	2.4	1.7	0.1	0.1	0.5	
Brissus unicolor	3.2	2.1	1.6	0.1	0.1	< 0.1	
Paracentrotus lividus	0.2	0.3	< 0.1				
Echinodermata n.i.	0.9	1.4	0.9				
Enteropneusta	0.5	0.6	0.5	1.5	0.9	1.2	
Balanoglossus clavigerus				0.4	0.2	0.5	
Enteropneusta n.i.				1.1	0.7	0.7	
Perciformes	0.2	0.6	0.2	7.9	1.5	9.2	
Ammodytes tobianus				7.6	0.7	8.9	
Perciformes n.i.				0.3	0.8	0.3	
Unidentified	1.9	2.7	1.1	0.6	2.2	2.2	

Table 2.2.	Vacuity index (VI) for each season (aw – autumn/winter; sps – spring/summer),
	sex (F – females; M – males) and size class (I – size class I; II – size class II),
	for the S. solea and S. senegalensis.

Species	S. solea			S. senegalensis			
	Season (aw/sps)	40.9	26.2		47.3	43.0	
Vacuity Index	Sexes (F/M)	29.2	37.2	33.3	34.8	55.0	45.4
_	Size class(I/II)	27.7	39.2		40.6	50.2	

Both species showed highest dietary diversity in autumn-winter (H'=3.09 to common sole, H'=2.63 to Senegalese sole). Females of common sole showed a higher dietary breadth than males (H'=3.13 and H'=2.98, respectively), and the dietary diversity increased with size (H'=3.13 to class II and H'=2.98 to class I). Senegalese sole showed an opposite tendency relatively to common sole dietary breadth, males Shannon-Wiener index was major than females Shannon-Wiener index (H'=2.63 and H'=2.18), and the dietary breadth decreased with size (H'=2.63 and H'=2.18).

Judged by Schoener index values >0.6, a high diet overlap occurred only in the autumn-winter period (0.61) and between females (0.66).



Figure 2.2. Ordination diagrams of the correspondence analyses performed using numerical (a), occurrence (b) and gravimetric (c) indices values of prey found in guts of *S. solea* (SS) and *S. senegalensis* (SN) (f – females; m – males; aw – autumn/winter; sps – spring/summer; I – size class I; II – size class II).

Age and Growth

Among the 267 individuals collected for age determination to *S. solea*, the otoliths from 154 females and 113 males were used. The total length of fish analysed varied from 224 mm to 462 mm from females and from 187 mm to 415 mm from males. The ages of the samples ranges from 1 to 9 years. A total of 181 individuals were analysed for age determination for *S. senegalensis*, the otoliths from 84 females and 97 males were used. The length of the female specimens analysed varied from 215 mm to 472 mm and that of males from 199 mm to 412 mm. The age of *S. senegalensis* specimens analysed ranged from 2 to 8 years.

The von Bertalanffy growth equation coefficients for common sole differed between sexes (Figure 2.3a). The asymptotic length (L_{∞}) obtained for females was higher compared to males (521.5 mm and 466.9 mm, respectively), while the growth coefficient (k) estimate of females (k=0.23) was higher than that determined for males (k=0.21). The t_0 estimates were -0.11 and 1.57 for females and males, respectively.

Coefficients of von Bertalanffy growth equation for Senegalese sole showed a difference between sexes (Figure 2.3b). The estimated asymptotic lengths were higher for females (L_{∞} =532.3) than for males (L_{∞} =457.2), while growth coefficient was higher to females compared to males (k=0.17 and k=0.15, respectively), and the t_0 estimates were -1.17 to females and -2.91 to males.



Figure 2.3. von Bertalanffy growth curves fitted to length-at-age data of S. solea (a), and S. senegalensis (b) (females - black circles and solid lines; males - empty circles and dashed lines).

Reproduction

The percentage of individuals of S. solea in each maturation developmental stage per season is in agreement with the variation pattern obtained for the GSI (Figures 2.4 and 2.5). The highest values of the GSI were obtained in autumn-winter, which was the period when the highest percentage of individuals in stage III (spawning) was recorded.

Comparing the GSI values of males and females, it can be noticed that the values obtained for females (mean value from 0.79 to 4.52) were extremely high when compared with those determined for males (mean value from 0.06 to 0.12).





The proportion of individuals of *S. senegalensis* according to maturity stages was found in agreement with GSI seasonal changes (Figures 2.4 and 2.5). The highest values of the GSI were obtained in autumn-winter, which was the period when the highest percentage of individuals in stage III (spawning) was recorded.

Comparing the GSI values of males and females, it can be noticed that the values obtained for females (mean value from 2.62 to 3.08) were extremely high when compared with those determined for males (mean value from 0.12 to 0.28).

The age of first maturity of common sole occurred at 4 years for females and males, with the length at first maturity being 370 mm and 348 mm for females and males, respectively. The maturation of Senegalese sole occurred at 3 years for both sexes, females were largest (338 mm) than males (332 mm).



Figure 2.5. Gonadosomatic index mean values, determined for each season and sex for S. solea (a) and S. senegalensis (b) (standard deviation is represented above bars).

DISCUSSION

The present study shows that trophic profile of common sole is characterised by Crustacea, Polychaeta and Mollusca. Our results were similar with the results obtained in several studies made in the western Europe (e.g. Cabral, 2000b; Sá et al., 2003; Vinagre et al., 2005) and in the Mediterranean (Molinero and Flos, 1992). Other authors (e.g. Darnaud et al., 2001; Vallisneri et al., 2002) reported that this species consumes mainly Polychaeta, Crustacea and Mollusca. The variety of habitats – shallow coastal areas, continental shelf, estuarine ecosystems – and the range of fish lengths analysed in these studies probably account for these slight dietary dissimilarities.

The diet of *S. senegalensis* was similar to *S. solea* that of as outline by several authors (e.g. Garcia-Franquesa et al., 1996; Cabral, 2000a; Sá et al., 2003).

The feeding activity of common sole and Senegalese sole varied throughout the year, being highest in spring-summer but lowest in winter-autumn. This feeding behaviour is consistent with the findings reported in several studies (e.g. Molinero and Flos, 1992; Cabral, 2000a), but disagrees with Gracia-Franquesa et al. (1996), Vallisneri et al. (2002) and Sá et al. (2003) that pointed out, for both species, that vacuity was higher in spring-summer. Females of both species presented lower vacuity values than males, which is in agreement with previous studies (Molinero and Flos, 1991; Garcia-Franquesa et al., 1996). Smallest individuals of these two soles species were more

active for feeding than the largest ones (Molinero and Flos, 1991; Garcia-Franquesa et al., 1996). Several factors may explain these differences. Firstly, in spring, environmental conditions are favourable for an increase of prey availability. Secondly, since the process of reproduction was completed, females must recover their energetic resources, as it has been reported for other flatfishes (Pitt, 1973; Lozán, 1992).

Common sole and Senegalese sole presented variations in diet according to season, sex and length, that may be due to many factors, such as changes in space and time of benthic prey, shifts due to life-history patterns of prey and feeding activity of predator (Wootton, 1998). The results relative to diet variation according to fish length were similar to those obtained by other authors for other flatfish species, showing an increase in the importance of larger prey with increasing size of fish (e.g. Belghyti et al., 1993; Cabral, 2000a). This, is consistent with the optimum foraging theory (Gerking, 1994), which states that larger predators tend to consume larger prey in order to maximize the energetic gain relative to capture effort.

Common sole presented a larger dietary breadth compared to Senegalese sole. The diet diversity of both sole species was higher when the vacuity reached a maximum value, which could constitute a compensatory response to a possible decrease of prey availability during those seasons. A study conducted in southern Portugal (Sá et al., 2003) showed than both species presented low dietary variation.

Diet overlap values were high only in the autumn-winter period between the two species and for females. Cabral (2000a) showed that interspecific diet overlap was low for juveniles of the two sole species. Some authors (Moore and Moore, 1976; Poxton et al., 1983; Burke, 1995) report the avoidance of interspecific competition by the adoption of different strategies of resource, which may also be the case of adult soles.

The estimate of von Bertalanffy parameters for *S. solea* obtained in the present study was similar to those reported by Dinis (1986) for the Tagus estuary. The asymptotic length values reported for North Europe and Mediterranean (e.g. Deniel, 1981; Türkman, 2003) were lower than the ones estimated in the present work. Growth coefficient (k) estimates were lower for the Portuguese coast compared to those determined for North Europe and Mediterranean; the highest values were obtained for males by Vianet et al. (1989).

Growth studies for *S. senegalensis* are scarce and all developed in the Portuguese coast. The estimates of von Bertalanffy parameters obtained in this study were similar to those proposed by Andrade (1990), for the southern coast of Portugal.

Andrade (1990) point out that the highest values of total length were relative to females and estimated that the largest individual analysed (516 mm) should have 11
years. The estimates of L_{∞} reported by Dinis (1986), for the Tagus estuary, were lower than our results and also the values determined by Andrade (1990).

Many factors could influence growth. According to Pauly (1994a) latitudinal variation in growth is caused mainly by variations in maintenance metabolism due to latitudinal differences in temperature. The differential growth according to sex, registered for both species, may be due to the distinct maintenance metabolism of the two sexes by different oxygen consumption (Pauly, 1994b), by the different share between reproduction and somatic growth of surplus energy, as Rijnsdorp and Ibelings (1989) and Rijnsdorp (1993a) found in plaice, and different food intake, as Lozán (1992) found in other flatfish.

The results concerning the seasonal variation of gonadal indices suggest that the spawning period of both species is autumn-winter. Previous studies reported that the common sole reproduction period occurred mainly in winter, but it can occur in spring (e.g. Koutsikopoulos et al., 1989; Zaki, 1989). Some authors reported reproduction only in winter (Vallisneri et al., 2002) or in spring (Türkmen, 2003). Previous studies (Ramos, 1982; Türkmen, 2003) reported lower values of length at first maturation than the values obtained in the present work. Studies taking place in the Portuguese coast have showed that the reproduction period of Senegalese sole ranges from autumn to spring (Andrade, 1990), or for spring to summer (Dinis, 1986). Some authors, on the north coast of France (Lagardère et al., 1979) and on the Mediterranean (Ramos, 1982), suggested that reproduction occurs in the spring-summer period. Length at first maturation obtained in the present study was similar to values obtained by Andrade (1990), for the south coast of Portugal.

Natural variation in size and age at maturity within a population of a species can occur in stable populations but is generally small. Several studies (e.g. Walsh, 1994; Bowering et al., 1997; Rijnsdorp and Vethaak, 1997) showed that a large variability in maturation could be related to the declining of populations. Variations in size and age at maturity may be genetical, or associated with changes in environmental conditions on the nursery grounds or later during the juvenile/adult stage (e.g. Stearns and Crandall, 1984; Rijnsdorp, 1993b).

In conclusion, several aspects of *S. solea* and *S. senegalensis* biology remain to be studied, namely the evaluation of prey availability and predation pressure, length-frequency distribution analysis, the characterizion of the reproductive period and gonadal modifications, as well as regarding larvae and juvenile stages.

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Chapter 3

Feeding ecology, growth and sexual cycle of the sand sole, *Solea lascaris*, along the Portuguese coast

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Feeding ecology, growth and sexual cycle of the sand sole, *Solea lascaris*, along the Portuguese coast

ABSTRACT: Sand sole, *Solea lascaris* (Risso, 1810), were collected along the Portuguese coast, between October 2002 and July 2003, to examine feeding habits, age and growth and sexual cycle. The most important prey items were Mysidacea, Amphipoda and Polychaeta. Differences in diet according to season and length size were found: Amphipoda were very important in diet during winter, while Echinodermata were consumed mostly in summer; smaller individuals feed on Amphipoda while larger feed on Decapoda. Age of *S. lascaris* was determined from sagittae otoliths. The length of fish analysed ranged from 61 mm to 340 mm. The von Bertalanffy growth equation parameters differed significantly between sexes (L_{∞} =342.3 mm, k=0.50, t_0 =-0.87 and L_{∞} =264.5 mm, k=0.82, t_0 =0.13, females and males, respectively). The highest values of the gonadosomatic index were obtained in winter and spring, when the highest proportion of individuals at spawning stage was recorded.

Keywords: feeding ecology, growth, reproduction, flatfish, *Solea lascaris,* Portuguese coast

INTRODUCTION

A large number of flatfish species have been reported for the Portuguese coast (e.g. Nielsen, 1986a,b,c,d; Quéro et al., 1986a,b; Cabral, 2000a) and though many of them have a high commercial interest, few studies have been conducted on Pleuronectiformes biology in Portuguese waters (e.g. Dinis, 1986; Andrade, 1990; Cabral, 1998, 2000a,b; Cabral and Costa, 1999; Cabral et al., 2002).

The sand sole, *Solea lascaris* (Risso, 1810) is a Soleidae with a wide geographic distribution, from the southern North Sea to the Gulf of Guinea and the Mediterranean, inhabiting sandy and muddy bottoms at depths of 5 to 350 m (Quéro et al., 1986a).

Despite its broad distribution, most studies on *S. lascaris* biology were conducted along the west coast of Brittany (France) and the Portuguese coast and have

considered essentially its growth and reproduction (e.g. Deniel, 1981; Dinis, 1986; Deniel et al., 1989; Andrade, 1990). The diet of *S. lascaris* was previously studied along the west Brittany (France) and Algerian coasts (Marinaro and Bouabid, 1983; Rodriguez, 1996).

The studies conducted along the Portuguese coast, reported that *S. lascaris* spawns from January to June, southerly populations having an earlier spawning season (Dinis, 1986; Andrade, 1990; Gomes, 2002). A similar latitudinal gradient has been reported for growth parameters with specimens of the central coastal area showing a lower growth coefficient than those collected in Algarve (Andrade, 1990). Dinis (1986) determined a longevity of 11 years and a maximum total length of 355 mm. Cabral et al. (2002) mention that juvenile sand sole fed on small Crustacea and Bivalvia.

S. lascaris is a species with a growing commercial interest. In Portugal, its landings have quadrupled in the last decade (from about 28 tonnes in 1990 to 116 tonnes landed in 2000), coming to represent 6.4% of the landed flatfish. Still less common than other commercially important sole species like *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, with which *S. lascaris* are fished, it reaches high values in auction giving it a great economic importance (DGPA, unpub. data).

As a commercially important species, increasingly exploited and poorly known, the study of *S. lascaris* ecology has become of particular importance for fisheries management purposes. Thus, the aim of the present work was to study the feeding ecology, growth and sexual cycle of *S. lascaris* along the Portuguese coast.

MATERIALS AND METHODS

Sampling Surveys and Samples Processing

A total of 665 individuals were collected seasonally (autumn, winter, spring and summer), between October 2002 and July 2003, from commercial fishing vessels operating with gill nets and bottom trawls along the Portuguese coast (Figure 3.1).

All fish were measured (total length to nearest 1 mm) and weighed (total and eviscerated wet weight with 0.01 g precision). Stomachs and gonads were removed and frozen (-20°C) for further analysis. Then, the stomach contents were removed for identification and gonads were weighed. Each prey item was identified to the lowest taxonomic level possible, counted and weighed (wet weight to 0.001 g). Saggitae otoliths were removed, cleaned and kept dry for later age determination.



Figure 3.1. Map of mainland Portugal landing ports (1. Caminha; 2. Viana do Castelo; 3. Póvoa do Varzim; 4. Leixões; 5. Aveiro; 6. Figueira da Foz; 7. Nazaré; 8. Peniche; 9. Lisboa; 10. Sesimbra; 11. Setúbal; 12. Sines; 13. Sagres; 14. Portimão; 15. Quarteira; 16. Faro; 17. Olhão; 18. Tavira; 19. Vila Real de Santo António).

Feeding Ecology

The relative importance of each item was evaluated by calculating the numerical composition (Cn%), frequency occurrence (F%) and biomass (Cw%) (Hyslop, 1980). Differences in diet composition by sampling season and fish length (two length classes: \leq 250 mm and >250 mm total length) were evaluated by correspondence analyses (CA) that was performed using CANOCO software (ter Braack and Šmilauer, 1998).

Age and Growth

Age was evaluated using otoliths. For each specimen, two counts of otolith annuli were made under a dissecting microscope. Whenever the two readings of the same otolith resulted in different age estimates the data were not considered for further analysis. Estimates of theoretical growth in length were obtained by fitting length-at-age data to the von Bertalanffy growth equation:

$$L_t = L_{\infty} \cdot (1 - e^{-k \cdot (t - t_0)})$$

where L_t is the total length at age t, L_{∞} is the asymptotic length, k is the growth coefficient and t_0 is the theoretical age at zero length. The growth parameters of this model were estimated iteratively using the least squares method in STATISTICA software. This analysis was performed separately for females and males.

Sexual Cycle

Gonads were observed macroscopically and a maturation stage was assigned to each individual, according to a five-stage scale (Table 3.1). For each season the proportion of fish in stages 2 to 5 was determined.

In order to evaluate gonadal development during the annual sexual cycle and to determine the spawning season, the gonadosomatic index (GSI) was calculated per sex for each season. The GSI was expressed as the percentage of the weight of gonads in relation to eviscerated weight of fish.

	Females	Males					
1. Immature	Ovaries small and translucent	Testes small and translucent					
2. Early development	Ovaries larger and opaque,	Testes larger and opaque;					
	small white eggs can be seen	whitish					
3. Late development	Ovaries yellow, both white and	Testes swelling; light-brown					
	translucent eggs can be seen						
4. Spawning or partly spent	Hyaline eggs run from vent on	Testes fully swollen; sperm ca					
	slight pressure; or less swollen	be extruded under light					
	and with red spots but hyaline	pressure					
	eggs are still numerous.						
5. Spent	Ovaries flabby, red coloured	Testes flabby, residual sperm					

Table 3.1. Sexual maturity stages of *Solea lascaris* (adapted from Andrade, 1990, and
Cabral, 1998).

RESULTS

Feeding Ecology

The diet spectrum of *Solea lascaris* was broad, consisting of a variety of Polychaeta, Crustacea, Mollusca, Echinodermata and Cephalochordata (Table 3.2). Crustacea was found to be the most important prey group according to all three indices (Cn=80.7%; F=67.3%; Cw=73.8%). Amongst Crustacea, Mysidacea were the most important prey in both numbers and weight (values of Cn=44.1% and Cw=64.8%), while on occurrence alone Amphipoda was the most important group (F=17.2%). Polychaeta also held a considerable importance in *S. lascaris* diet: when considering occurrence in stomach contents (F=18.8%). Polychaeta were more important than any subgroup of Crustacea and also scored a high value on the basis of weight (Cw=20.5%).

The first two axes of all three CA that were performed explained a high percentage of the total observed variation in diet according to each of the three indices (85.9%, 90.6% and 88.7% for Cn, F and Cw data based analyses, respectively) (Figure 3.2).

Three groups can be identified based on the Cn ordination diagram: one group encompasses the samples relative to autumn diet of the length class 1, that was strongly associated with Polychaeta; winter and spring diets of the length class 1 and winter of the length class 2 formed a second group associated to Amphipoda, Bivalvia and Isopoda; the third group consisted of the spring diet of the length class 2, the summer diet of length class 1 and 2, and the autumn diet of length class 2, which was associated with Cumacea, Decapoda, Echinodermata and Mysidacea.

In the ordination diagram obtained for frequency of occurrence data it can be seen one group relative to winter diet of the length class 1, autumn diet of the length class 1 and 2, and summer diet of the length class 1, associated with Amphipoda and Polychaeta. The diet of individuals of length class 1 in spring and of length class 2 in winter, spring and summer were associated with Crustacea, Decapoda and Echinodermata.

When the Cw was considered in the ordination analysis, Polychaeta was strongly related to spring and winter diets of the length class 1 and 2, respectively. Winter and autumn diets of the length class 1 were associated with Crustacea, Amphipoda and Isopoda; the summer diet of the length size 2 were associated with Cumacea and Decapoda; Echinodermata, Bivalvia and Mysidacea were associated with spring and autumn diets of the length class 2 and summer diet of length class 1.

Table 3.2. Numerical composition (Cn), frequency of occurrence (F) and biomass composition (Cw) indices values of prey found in stomachs of *Solea lascaris* in the Portuguese coast (n – number of stomachs in which prey occurs; p – number of individuals of a specific prey; n.i. – not identified).

Prey Item	n	р	Cn	F	Cw
Polychaeta	139	364	8.4	18.8	20.5
Aphroditidae	1	3	0.1	0.1	0.2
Cirratulidae	2	2	0.1	0.2	0.2
<i>Glycera</i> spp.	1	10	0.3	0.1	1.2
Nereis spp.	7	19	0.5	0.8	0.7
Ophelia bicornis	7	49	1.3	0.9	2.7
Phyllodocidae	3	4	0.1	0.3	0.2
Ephesiella abyssorum	1	1	0.0	0.1	0.0
Syllidae	1	1	0.0	0.1	0.0
Polychaeta n.i.	116	275	6.1	16.1	15.3
Crustacea	413	3099	80.7	67.3	73.8
Cumacea	98	340	9.2	11.8	0.4
Iphinoe trispinosa	3	4	0.3	0.6	0.0
Iphinoe sp.	3	3	0.3	0.7	0.0
Bodotria scorpioides	14	61	1.6	1.6	0.0
Bodotriidae n.i.	5	4	0.1	0.4	0.0
Pseudocuma (Pseudocuma) longicorne	5	8	0.2	0.6	0.0
Diastylis rugosa	20	153	4.0	2.3	0.1
Diastylidae n.i.	9	18	0.5	1.0	0.1
Cumacea n.i.	38	87	2.3	4.7	0.1
Apseudes latreillii	1	2	0.1	0.1	0.0
Mysidacea	85	1689	44.1	12.2	64.8
Isopoda	29	35	0.9	3.2	0.1
Gnathiidae	1	1	0.0	0.1	0.0
Conilera cylindracea	1	1	0.0	0.1	0.0
Eurydice pulchra	3	3	0.1	0.3	0.0
Eurydice sp.	2	2	0.1	0.2	0.0
Idotea balthica	3	4	0.1	0.3	0.0
Idotea sp.	3	3	0.1	0.3	0.0
Isopoda n.i.	10	21	0.5	1.8	0.1
Ampnipoda	121	411	10.7	17.2	1.3
	121	406	10.6	10.8	1.2
Amphipoda n.i.	4	2	0.1	0.4	0.1
Crangen grangen	97	410 2E0	0.7	12.0	5.5
Daguridao	200	220	9.7	9.3	5.0
Pagunuae	10	15	0.1	0.5	0.0
Decanoda n i	10	3/	0.4	2 1	0.1
Crustacea n i	79	214	47	9.8	1.6
Mollusca	88	245	6.4	10.2	47
Antalis entalis	2	5	0 1	0.2	0.0
Gastropoda.	2	2	0.1	0.2	0.0
Bivalvia	84	238	6.2	9.7	4.6
Arca tetragona	1	3	0.1	0.1	0.1
Tapes rhomboides	1	4	0.1	0.1	0.1
Mactra sp.	3	6	0.2	0.3	0.1
Spisula solida	6	10	0.3	0.7	0.1
Tellina tenuis	5	31	0.8	0.7	0.5
Tellina fabula	21	63	1.6	2.5	1.7
Scrobicularia plana	6	9	0.2	0.7	0.6
<i>Abra</i> sp.	1	2	0.1	0.1	0.0
Solecurtinae	2	7	0.2	0.2	0.2
Solenidae	2	4	0.1	0.2	0.0
Bivalvia n.i.	36	99	2.6	4.1	1.3
Echinodermata	25	152	4.0	3.4	0.8
Asteroidea	1	1	0.0	0.1	0.0
Ophiuroidea	16	99	2.6	2.1	0.6
Echinocyamus pusillus	6	50	1.3	0.9	0.2
Echinodermata n.i.	2	2	0.1	0.2	0.0
Cephalochordata	2	17	0.4	0.2	0.2
Branchiostoma lanceolatum	2	17	0.4	0.2	0.2



Figure 3.2. Ordination diagrams of the correspondence analyses performed to numerical composition (a), frequency of occurrence (b) and biomass composition (c) of prey found in stomachs of *Solea lascaris* (1 – length class 1; 2 – length class 2; W – Winter; Sp – Spring; S – Summer; A – Autumn).

Overall, Amphipoda and Echinodermata were the most important element of the winter and summer diets, respectively, according to all three indices. Considering Cn and F, Decapoda were particularly important in spring. The Amphipoda were the major item of the autumn diet according to F and Cw. Winter and autumn diets were very similar according to F and Cw, and spring and summer diets were very similar according to F. Amphipoda were the most important prey item of the length size 1, according to all three indices. The length class 2 fed mainly on Decapoda and Echinodermata, according to all three indices.

Age and Growth

A total of 296 females and 113 males were analysed for age determination. The total length of fish analysed varied from 61 mm to 340 mm, for females, and from 61 mm to 310 mm, for males. The oldest fish was 6 years.

The von Bertalanffy growth equation parameters differed between sexes (Figure 3.3). The asymptotic length (L_{∞}) obtained for females was higher compared to the one obtained for males (342.3 mm and 264.5 mm, respectively), while the growth coefficient (k) estimated for females (k= 0.50) was lower than that determined for males (k=0.82). The t_0 estimates were -0.87 and 0.13 for females and males, respectively.



Figure 3.3. von Bertalanffy growth curves fitted to length-at-age data of *Solea lascaris* (females – black circles and solid lines; males – empty circles and dashed lines).

Sexual Cycle

Percentage of individuals according to maturity stages was in agreement with GSI seasonal changes (Figures 3.4 and 3.5).



Figure 3.4. Gonadossomatic index mean values season, determined for each sex (standard deviation is represented).

The highest values of the GSI were obtained in winter and spring, the seasons when the highest percentage of individuals in spawning were recorded. The lowest GSI values (2.8% of eviscerated weigth for females, 0.2% of eviscerated weigth for males) were recorded in autumn, but a large proportion of partly spent females and spawning males indicated spawning was still taking place.



Figure 3.5. Percentage of individuals in each maturation stage according season, for females (a) and males (b) (maturation stages as in Table 3.1).

Discussion

The diet composition determined for *Solea lascaris* along the Portuguese coast was similar to that described by Rodriguez (1996) for the west coast of Brittany. In both studies, it was found that *S. lascaris* feeds on a wide range of prey belonging to several *taxa*, the most important groups being Mysidacea, Polychaeta and Amphipoda. *S. lascaris* diet reported by Cabral et al. (2002) and Marinaro and Bouabid (1983) is somewhat different: the prey range is much smaller and Polychaeta are not an important prey. However, Cabral et al. (2002) studied the diet of juveniles and Marinaro and Bouabid (1983) had a small sample size (24 full stomachs), which could bias the estimation of prey importance.

The diet of *S. lascaris* is similar to that of *S. solea* and *S. senegalensis* differing mostly on Mollusca importance (e.g. Molinero and Flos, 1991; Garcia-Franquesa et al., 1996; Cabral, 2000b; Darnaude et al., 2001) that seem to be a preferential prey for the former species but not for *S. lascaris*. Overall, *S. lascaris* can be considered an opportunistic and generalist feeder, status that has been recognized for several species of Soleidae (e.g. Cabral, 2000b; Darnaude et al., 2000b; Darnaude et al., 2001).

The differences found in the diet of *S. lascaris* in what regards to season and length size were in agreement with Rodriguez (1996). These seasonal variations were consequence of changes in space- and time-variation of benthic fauna composition, shifts due to life-history patterns of prey and feeding activity of predator (Wootton, 1998). The diet variation according to fish length is consistent with the optimum foraging theory (Gerking, 1994), which states that larger predators tend to consume larger prey in order to maximize the energetic gain relative to capture effort.

The growth pattern found for *S. lascaris* in this study is quite different from what was previously described (e.g. Deniel, 1981; Dinis, 1986; Andrade, 1990). The observed longevities of 6 years for females and 5 years for males are lower than those found by Dinis (1986) (11 years, both for females and males), but similar to that reported by Andrade (1990) (7 and 6 years, respectively for females and males).

The observed pattern in the sexual cycle along the year is in accordance with expectations, and is similar to those reported by Dinis (1986), Andrade (1990) and Gomes (2002) for the Portuguese coast: *S. lascaris* has a winter-summer spawning season and a latitudinal gradient that can be noticed. For the Douarnenez Bay (France), Deniel (1981) found a spawning season from May to September. Deniel (1981) reported a similar gradient for the *S. solea* spawning season.

The observed duration of the spawning period was longer than has been reported (7/8 months in this study against 5 months, Deniel, 1981; Dinis, 1986; Andrade, 1990; Gomes, 2002). These authors have noted that there is an asynchrony in the spawning of older and younger females, the second group spawns later, which could explain this long duration. This trend was not completely evident in this study (data not shown), and the long duration of the spawning season is due perhaps to serial spawning temporally spaced and/or to year fluctuations (e.g. Koutsikopoulos et al., 1995).

Other Soleidae species have a winter-summer spawning season, namely *S. senegalensis* and *Dicologlossa cuneata* (Moreau, 1881) (Dinis, 1986): as *S. lascaris* these are sub-tropical species that attain maturity during the increasing day-light period.

Many aspects of *S. lascaris* biology remain to be studied, namely those regarding larvae and juvenile stages. These have been generally considered as very important life cycle phases, critical for individual survival, and so their knowledge is of extreme importance, both for fisheries and aquaculture purposes.

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CHAPTER 4

Diet, growth and reproduction of spotted flounder, four-spotted megrim, flounder and bastard sole in the Portuguese coast Scientia Marina (*in revision*) By Teixeira, C.M., Batista, M.I., Cabral, H.N.

Diet, growth and reproduction of spotted flounder, four-spotted megrim, flounder and bastard sole in the Portuguese coast

ABSTRACT: Four flatfish species were collected between January 2003 and June 2005 from commercial fishing vessels operating with gill nets and bottom trawls along the Portuguese coast, to examine feeding habits, age and growth and reproduction. *Citharus linguatula* (Linnaeus, 1758), *Lepidorhombus boscii* (Risso, 1810) fed mainly fishes and crustaceans and *Microchirus azevia* (de Brito Capello, 1867), fed chiefly on polychaets and crustaceans. The diet of *Platichthys flesus* (Linnaeus, 1758) was mainly composed by echinoderms and crustaceans. Feeding activity was highest in spring-summer; females and small individuals presented lowest vacuity index values. For all the species, significant differences were found in the proportion of prey items according to season, sex and size class. Ages were determined from sagittae otoliths. The von Bertalanffy growth equation coefficients differed between sexes. The asymptotic length L_{∞} of females was higher compared to males, except for *C. linguatula*. The lowest growth coefficient was obtained for *P. flesus* (k=0.11 for males and k=0.10 for females) and *M. azevia* presented the highest growth coefficient estimates (k=0.40 for females and k=0.30 for males). The highest proportion of individuals were observed throughout the year.

Keywords: flatfish, diet, growth, otoliths, reproduction, Portuguese coast

INTRODUCTION

A large number of flatfish species occur in the Portuguese coast (e.g. Nielsen, 1986a,b,c,d; Quéro et al., 1986a,b; Cabral, 2000a), this species richness is usually higher than that found in North Europe and similar to that found in the Mediterranean, since many flatfish species are at their southern and northern distribution limits, respectively, along the Portuguese coast (e.g. Quéro et al., 1986a; Desoutter, 1997). The majority of flatfish occurring in the Portuguese coast present a high commercial value and are caught by fisheries, as target or bycatch species. Flatfish catches represent 4% of all the fish biomass landed in the Portuguese coast. However, the importance of flatfish fisheries is considerably higher due to the high commercial value

of flatfish species, accounting for near 11% of the economical value of fish landings. According to official data, flatfishes landings increased from 1998 to 2005 (source: DGPA).

Knowledge on the ecology of some flatfish species is extremely scarce, especially for those that are considered fisheries bycatches. Spotted flounder Citharus linguatula (Linnaeus, 1758), four-spotted megrim Lepidorhombus boscii (Risso, 1810), flounder Platichthys flesus (Linnaeus, 1758) and bastard sole Microchirus azevia (de Brito Capello, 1867) are very different flatfish species with distinct distribution areas. Spotted flounder is distributed in the East Atlantic and Mediterranean, inhabiting soft bottoms from the coastline to a depth of about 200 m (Nielsen, 1986a). Four-spotted megrim occurs in the Northeast Atlantic from the British Isles south to Cape Bojador, and it is also found in the Mediterranean. It is common on soft bottoms on depths down to 700-800 m (Nielsen, 1986b). Flounder is common around the coasts of northern Europe and the Mediterranean. This species occurs on soft bottoms from shallow water down to 50 m, it can tolerate brackish or freshwater (Nielsen, 1986d). Bastard sole occurs from the southern range of the Iberian Peninsula to the western part of the Mediterranean Sea and off the African coast, southward to Senegal. It is common on mud and sand of the continental shelf, from the shore down to 250 m (Quéro et al., 1986a).

Diet of *C. lingutula*, as well as age and growth, and reproduction has been studied in the Mediterranean and in the Atlantic coast of Morocco (e.g. Belghyti et al., 1993; Redon et al., 1994; Vassilopoulou and Papaconstantinou, 1994; García-Rodríguez and Esteban, 2000). Studies on four-spotted megrim diet, age and growth, and reproduction were conducted in the western Europe and Mediterranean (e.g. Santos, 1994; Vassilopoulou and Ondrias, 1999; Landa et al., 2002; Vassilopoulou, 2006). Several authors studied the diet, age and growth, and reproduction of *P. flesus* on the north-western Europe, and in the Black Sea (e.g. Summers, 1979; Beaumont and Mann, 1984; Andersen et al., 2005; Çiloğlu, 2005). Age and growth of *M. azevia*, well as reproduction, has been studied in the south coast of Portugal, in the Mediterranean and in the south of Morocco (e.g. Belaid and Marinaro, 1983; Marfin and Hajji, 1988; Andrade, 1998; Afonso-Dias et al., 2005).

Studies conducted in the Portuguese coast, showed that *P. flesus* fed mainly crustaceans, polychaets and molluscs (Vinagre et al., 2005). Santos (1994) determined for a *L. boscii* a maximum total length of 398 mm. Longevity of *M. azevia* was 8 years and a maximum total length of 327 mm (Andrade, 1998). *L. boscii* and *M. azevia* spawns from winter to spring (Santos, 1994; Afonso-Dias et al., 2005).

Despite these studies, knowledge on these species is scarce and integrative and comparative studies are missing. Being a transition between temperate and subtropical waters of the Northeastern Atlantic coasts (Ekman, 1953; Briggs, 1974), studies conducted in the Portuguese coast may provide important information in order to outline those trends. Therefore, the aim of the present work was to study the diet, age and growth and reproduction of spotted flounder, four-spotted megrim, flounder and bastard sole off the Portuguese coast.

MATERIALS AND METHODS

Sampling Surveys and Samples Processing

Samples were collected bimonthly between January 2003 and June 2005 from commercial fishing vessels operating with gill nets and bottom trawls along the Portuguese coast (Figure 4.1). Samples were collected regularly throughout the year and along the coast (a minimum of 40 individuals were obtained per season and sampling area).

Fish were identified, measured (total length to nearest 1 mm) and weighed (total and eviscerated wet weight with 0.01 g precision). Stomachs and gonads were removed and frozen (-20°C) for further analysis. Saggitae otoliths were removed, cleaned and kept dry for later age determination.



Figure 4.1. Location of sampling area.

Diet

The stomach contents of 344 individuals of *C. linguatula* (149–293 mm), 317 individuals of *L. boscii* (149–346 mm), 325 individuals of *P. flesus* (150–430 mm) and 314 individuals of *M. azevia* (149–313 mm) were analysed. Each prey item was identified to the lowest taxonomic level possible, counted and weighed (wet weight to 0.001 g). The relative importance of each prey item in the diet was expressed as a percent of numerical abundance (NI – numerical index), occurrence of food items in stomachs (OI – occurrence index) and weight (GI – gravimetrical index) (Hyslop, 1980). Feeding activity was evaluated by the vacuity index (VI) defined as the percent of empty stomachs (Hyslop, 1980).

Correspondence analyses (CA) were run to evaluate diet variation with season, sex and fish length according to each of the three index values. Prey items were grouped in a broader taxonomic level and two seasons (autumn-winter and spring-summer), and two size classes (size class 1: <250 mm and size class 2: \geq 250 mm total length) were created. These analyses were performed using CANOCO version 4.5 (ter Braak and Šmilauer, 2002).

Diet differences between seasons, sexes and fish size were evaluated using χ^2 tests (Zar, 1984), and with a 0.05 significance level.

Prey diversity was determine for each season, sex and size class using the Shannon– Wiener diversity index H' (Shannon and Weaver, 1949), expressed as

$$H' = -\sum_{i=1}^{s} P_i \ln P_i$$

where P_i is the numerical proportion of the *i*th prey category in the diet and *S* is the total number of different prey categories consumed by the predator. This index corresponds to the dietary breadth (Marshall and Elliott, 1997).

Age and Growth

Age was evaluated based on otolith readings. For each specimen, two counts of otolith annuli were made under a dissecting microscope. Whenever the two readings of the same otolith resulted in different age estimates, the data were not considered for further analysis. A total of 321 (216 females and 105 males) individuals of spotted flounder, 302 (199 females and 103 males) individuals of four-spotted megrim, 314 (209 females and 105 males) individuals of flounder and 292 (147 females and 145 males) individuals of bastard sole were used for age determination.

Estimates of theoretical growth in length were obtained by fitting length-at-age data to the von Bertalanffy growth equation:

$$L_t = L_{\infty} \cdot (1 - e^{-k \cdot (t - t_0)}),$$

where L_t is the total length, L_{∞} is the asymptotic length, k is the growth coefficient and t_0 is the theoretical age at zero length. The growth parameters of this model were estimated iteratively using the least squares method in STATISTICA software. This analysis was performed separately for males and females.

Reproduction

Gonads were observed macroscopically and a maturation stage was assigned to each individual, according to the scale: I – immature, II – development, III – spawning, IV

- post-spawning (Cabral, 1998). For each season (autumn-winter, spring-summer), the percentage of fish in stages I, II, III and IV was determined.

The gonadosomatic index (GSI) was also determined for each sex and season. The GSI was expressed as the percentage of the weight of gonads in relation to eviscerated weight of fish. Age and length at first maturity were determined.

RESULTS

Diet

The diet of *C. linguatula* were mainly composed by mysids and fishes, the most important prey items for *L. boscii* were decapods and fishes, and *M. azevia* fed mainly polychaets and decapods. For *P. flesus,* the most important prey items were echinoderms (particularly Holothuroida), bivalves and crustaceans (amphipods and decapods) (Table 4.1).

	Clinquatula			1	l boscii			D flesus			M azevia		
D	C. /	niyua			. DOSC		<i>г</i>		15 6 7	•••	. azev		
Prey Items	NI	01	GI		01	GI	NI	01	GI		01	GI	
Foraminifera	.0.1	0.0	.0.1	63.7	0.8	<0.1	17.0	22.0	26.2	2.6	1.1	<0.1	
	<0.1	0.2	<0.1				17.8	23.0	26.3	9.0	4.3	3.6	
Pecten spp.							2.0	2 7	4.0	2.1	1.1	1.1	
Ensis spp.	.0.1	0.0	.0.1				2.0	2.7	4.0	6.0	2 2	2 5	
	<0.1	0.2	<0.1				15.2	20.3	22.3	6.9 1 2	3.2	2.5	
Antalis entalis	<0.1	0.2	<0.1	0.1	07	<0.1				1.3	1.1	0.5	
	<0.1	0.2	<0.1	0.1	0.7	<0.1				1.3	3.Z	2.4	
nyulobla ulvae										1.5	1.1	< 0.1	
Scaphander spp.	<0.1	0.2	<0.1							4.5	1.1	1.2	
Gastropoda n.i.	<0.1	0.2	< 0.1	0.1	07	10.0				1.7	1.0	1.1	
Cephalopoda	4.2	11.2	32.9	0.1	0.7	10.0							
	<0.1	0.2	0.2										
	1.5	4.2	27.4										
Alloteutnis spp.	0.7	2.1	20.7										
	<0.1	0.2	0.6										
Loliginidae n.i.	0.7	1.9	6.1										
Octopodidae	0.1	0.3	0.6										
Cephalopoda n.i.	2.5	6.5	4./					054		~~~~		F0 C	
Polychaeta				0.7	4.3	1.9	8.1	25.1	36.5	30.9	33.4	52.6	
Aphrodita aculeata								054		5.6	6.9	5.9	
Polychaeta n.i.					~~ -	<i></i>	8.1	25.1	36.5	25.3	26.5	46.7	
Crustacea	80.9	52.0	20.2	33.1	82.7	64.7	18.2	25.7	6.3	36.5	42.8	31.2	
Balanus spp.							0.2	0.5	<0.1				
Squilla mantis				0.2	1.4	2.1		. – .					
Decapoda	5.7	14.3	9.2	22.8	34.5	42.8	5.5	15.0	5.3	11.6	12.1	11.5	
Paguridae										4.3	4.2	6.0	
Caridea	3.9	9.5	6.6	21.0	26.6	33.0	0.2	0.5	0.3	0.9	1.1	1.5	
Crangonidae	0.9	1.2	0.8	0.4	2.2	0.8							
Crangon crangon	0.1	0.3	0.2	0.4	2.2	0.8							
Philocheras sculptus	<0.1	0.2	<0.1										
Philocheras spp.	0.6	0.8	0.5										
Crangonidae n.i.	0.1	0.7	0.1										
Alpheus glaber	0.7	2.1	3.7										
Palaemonidae	0.8	3.3	1.2										
Palaemon longirostris	<0.1	0.3	0.3										
Palaemonetes varians	<0.1	0.3	0.2										
Processa spp.	0.5	1.5	0.4										
Palaenomidae n.i.	<0.1	1.2	0.3										
Caridea n.i.	1.4	4.2	2.1	20.7	24.5	32.2							
Decapoda n.i.	1.8	4.5	2.6							0.4	0.5	<0.1	
Euphausiacea	1.0	0.3	0.2										
Mysida	63.6	22.5	4.8	2.2	13.7	5.3	0.7	2.1	<0.1				
Leptomysis spp.	26.4	6.9	1.6										
Mysida n.i.	37.2	15.6	3.2				0.7	2.1	<0.1				
Isopoda	0.3	0.8	<0.1	3.8	9.4	9.6	0.5	1.6	0.1				
Eurydice spp.	0.1	0.2	<0.1										
Limnoria spp.	<0.1	0.2	<0.1										
Isopoda n.i.	0.2	0.5	<0.1				0.5	1.6	0.1				
Amphipoda	<0.1	0.2	<0.1	0.1	0.7	<0.1	9.9	2.1	0.1	9.9	12.2	6.4	
Crustacea n.i.	10.4	13.8	4.8	3.9	23.0	4.8	1.4	4.3	0.8	15.0	18.5	13.3	
											(cont	inue)	

Table 4.1.Numerical (NI), occurrence (OI) and gravimetric (GI) indices values of
prey items identified in stomachs of *C. linguatula*, *L. boscii*, *P. flesus* and
M. azevia, off the Portuguese coast (n.i. – not identified).

continue)

	С.	C. linguatula L. b		. bos	cii P. flesus			M	M. azevia			
Prey items	NI	OI	GI	NI	ΟΙ	GI	NI	ΟΙ	GI	NI	01	GI
Echinodermata	< 0.1	0.2	<0.1	0.2	1.4	< 0.1	51.9	15.0	20.7	9.4	10.6	2.2
Crinoidea							0.3	1.1	<0.1			
Ophiuroidea				0.1	0.7	<0.1				3.0	3.7	1.0
Holothuroidea							51.4	13.4	20.7			
Echinoidea	<0.1	0.2	<0.1									
Echinodermata n.i.				0.1	0.7	<0.1	0.2	0.5	<0.1	6.4	6.9	1.2
Actinopterygii	12.5	29.8	44.5	2.1	9.4	23.3	1.0	2.1	3.7			
Pleuronectiformes	0.3	0.5	2.9									
Arnoglossus spp.	<0.1	0.2	0.6									
Pleuronectiformes n.i.	0.3	0.3	2.3									
Ammoditidae							0.3	0.5	2.4			
Callionymus reticulatus	< 0.1	0.2	1.8									
Callionymus spp.	<0.1	0.2	0.1									
Trachinidae	< 0.1	0.2	0.2									
Gobiidae	0.2	0.4	1.8									
Gobius niger	0.1	0.2	1.0									
Pomatoschistus microps	< 0.1	0.1	0.1									
Gobiidae n.i.	< 0.1	0.1	0.7									
Trachurus trachurus	0.1	0.2	5.5									
Lepidotrigla cavillone	< 0.1	0.2	0.5									
Merluccius merluccius	< 0.1	0.2	0.3									
Actinopterygii n.i.	11.6	27.7	31.4				0.7	1.6	1.3			
Unidentified	2.1	6.4	2.1				2.9	9.1	6.5	3.0	3.7	7.8

Vacuity index was higher for spotted flounder (34%) than for bastard sole (29%), flounder (26%) and four-spotted megrim (20%). The lowest values of vacuity occurred in spring-summer, particularly for females and for smallest fishes (Table 4.2).

Table 4.2.Vacuity index (VI) for each season (aw - autumn/winter; sps -
spring/summer), sex (F - females; M - males) and size class (I - size class
I; II - size class II), for the *C. linguatula*, *L. boscii*, *P. flesus* and *M. azevia*.

Vacuity Index									
	Sea	son	Sex	xes	Size	e class			
	aw	sps	F	М	Ι	II			
C. linguatula	40.78	27.72	31.01	38.46	33.87	35.38	34.01		
L. boscii	33.33	15.46	14.29	28.00	17.65	23.81	19.69		
P. flesus	37.93	22.76	19.77	33.33	20.00	27.35	25.66		
M. azevia	30.77	28.43	27.63	30.77	25.77	36.36	29.08		

The correspondence analysis (CA) performed, based on the three indices considered averaged per season, sex and size class, explained ca. 40% the variance in the first two axes (42.0%, 48.6% and 43.2%, respectively for NI, OI and GI). In all three ordination diagrams (NI, OI and GI) for the four species were well discriminated.

In the ordination diagram obtained for numerical data (Figure 4.2a) it can be seen that: Cephalopods were associated with the largest individuals of *C. linguatula* in autumn-winter, and with the largest males in spring-summer. Largest females diet, in spring-summer was composed by fishes. Smallest individuals were associated with mysids. Diet of *L. boscii* was composed largely with decapods and fishes. In autumn-winter the largest females were associated with isopods and the smallest individuals with fishes. *P. flesus*, in the spring-summer, was chiefly related with echinoderms. Amphipods were associated, in spring-summer, with the largest males. Largest females' diet, in autumn-winter, was associated with decapods. *M. azevia* diet was composed mainly by polychaets. In autumn-winter, small females and large males were associated with bivalves and gastropods. Pagurids were associated with small males in spring-summer.

When the OI was considered in the ordination analysis (Figure 4.2b), small individuals of *C. linguatula* was strongly associated with mysids. Cephalopods were mainly associated with largest individuals, and fishes were related with largest males. *L. boscii* was chiefly associated with decapods and isopods. Smallest individuals, in autumn-winter, were related with fishes. *P. flesus* diet was mostly related with echinoderms, bivalves and gastropods. The diet of *M. azevia* was mainly composed by polychaets and amphipods.

Considereding the gravimetrical data in the ordination diagram (Figure 4.2c) it can be seen that: *C. linguatula* was chiefly associated with fishes and cephalopods. The diet of *L. boscii* was mostly related with decapods. In autumn-winter, diet of smallest males was composed by fishes, and largest females were associated with isopods. Mysids were related with smallest females. *P. flesus* diet was largely composed by echinoderms in spring-summer. Largest females, in autumn-winter, were associated with decapods. *M. azevia* was heavily associated with polychaets. Smallest females' diet was composed by amphipods. Males diet, in autumn-winter was associated with pagurids and gatropods for smallest and with decapods for the largest ones.



Figure 4.2. Ordination diagrams of the correspondence analyses performed to numerical (a), occurrence (b) and gravimetric (c) indices values of prey found in guts of *C. linguatula*, *L. boscii*, *P. flesus* and *M. azevia* (CL – *C. linguatula*; LB – *L. boscii*; PF – *P. flesus*; MA – *M. azevia*; f – females; m – males; aw – autumn/winter; sps – spring/summer; I – size class I; II – size class II).

C. linguatula, L. boscii, P. flesus and *M. azevia* showed significant differences in their diets between seasons (χ^2 =30.9, d.f.=11, P<0.05; χ^2 =268.7, d.f.=11, P<0.05; χ^2 =187.7, d.f.=11, P<0.05 and χ^2 =60.0, d.f.=11, P<0.05, respectively).

Significant differences in the diet were observed between sexes in *C. linguatula*, *L. boscii* and *P. flesus* (χ^2 =31.7, d.f.=11, P<0.05; χ^2 =461.6, d.f.=11, P<0.05 and χ^2 =115.9, d.f.=11, P<0.05, respectively).

The diet of individuals of the two size classes also differed significantly in spotted flounder, four-spotted megrim and flounder (χ^2 =294.5, d.f.=11, P<0.05; χ^2 =558.4, d.f.=11, P<0.05 and χ^2 =133.7, d.f.=11, P<0.05, respectively).

The dietary diversification was higher for *C. linguatula* and *P. flesus* in spring-summer period (H'=1.90 and H'=1.58, respectively), and for *L. boscii* and *M. azevia* the higest values were obtained in autumn-winter period (H'=1.30 and H'=2.20, respectively). For the majority of the species, males (*C. linguatula*: H'=1.93; *P. flesus*: H'=2.01; *M. azevia*: H'=2.40) showed a higher dietary breath than females (*C. linguatula*: H'=1.81; *P. flesus*: H'=1.43; *M. azevia*: H'=2.39), *L. boscii* showed an opposite trend, females' diet (H'=1.70) was more diverse than that of males (H'=0.66). For *C. linguatula* and *M. azevia* the dietary diversity increased with size (size class I: H'=1.82; size class II: H'=2.12 and size class I: H'=2.30; size class II: H'=2.49, respectively). *L. boscii* and *P. flesus* presented the opposite trend; dietary diversity was higher for smaller size individuals (size class I: H'=1.43; size class II: H'=0.61 and size class II: H'=1.52; size class III: H'=1.51, respectively).

Age and Growth

The total length of fishes analysed varied between 149 mm and 275 mm for spotted flounder, between 149 mm and 346 mm for four-spotted megrim, between 150 mm and 430 mm for flounder, and between 149 mm and 275 mm for bastard sole. The age ranged from 0 to 7 years in spotted flounder, from 1 to 9 years in four-spotted megrim, from 1 and 14 years in flounder, and from 2 and 7 years in bastard sole.

All species showed a differential growth according to sex. For the majority of the species (*L. boscii, P. flesus* and *M. azevia*) females attained higher lengths than males, except for *C. linguatula* (Figure 4.3 a,b,c,d). The estimated asymptotic lengths for spotted flounder were higher for males than for females (L_{∞} =308.1 mm and L_{∞} =302.0 mm, respectively). For the other species, an opposite trend was noticed: four-spotted megrim: females: L_{∞} =381.1 mm, males: L_{∞} =323.7 mm; flounder: females: L_{∞} =487.9 mm, L_{∞} =456.6mm; and bastard sole: females:

 L_{∞} =339.5 mm; males: L_{∞} =314.8 mm). The growth coefficient of spotted flounder (females: k=0.19; males: k= 0.15) and bastard sole (females: k= 0.40; males: k=0.30) were higher for females than males, while for four-spotted megrim estimated k for males was high that the one determined for females (females: k=0.14; males: k= 0.20). For flounder, growth coefficient estimates were similar for both sexes (females: k=0.10, males: k=0.11). The t_0 estimates for spotted flounder were -4.40, for females, and 3.04, for males; four-spotted megrim were -2.85, for females, and -2.49 for males; flounder were -4.61 for females, and -3.75 for males; and bastard sole were -0.01 for females, and -1.15 for males.


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Reproduction

For all the species studied, the percentage of individuals in each maturation stage per season was in agreement with results relative to GSI values (Figures 4. and 4.5). The highest GSI values were obtained in autumn-winter for *L. boscii* and *P. flesus*, corresponding to period with the highest percentage of individuals in stage III (spawning). The GSI values obtained for *C. linguatula* and *M. azevia* were high throughout the year. Regarding the GSI values of females and males, it can be noticed that the values obtained for females (*C. linguatula*: 1.96 – 2.16; *L. boscii*: 4.90 – 0.90; *P flesus*: 7.41 – 1.38; *M. azevia*: 1.70 – 2.62) were extremely high compared to those determined for males (*C. linguatula*: 0.24 – 0.35; *L. boscii*: 0.17 – 0.14; *P flesus*: 1.11 – 0.20; *M. azevia*: 0.10 – 0.11).



Figure 4.4. Percentage of individual of *C. linguatula* (CL), *L. boscii* (LB), *P. flesus* (PF) and *M. azevia* (MA) in each maturation stage (I - Immature; II - Development; III - Spawning; IV - Post-spawning), according to season (aw - autumn/winter; sps - spring/summer) and to sex (F - females; M - males). (□ Immature; □ Development; ■ Spawning; ☑ Post-spawning).

L. boscii and *C. linguatula* were the species for which the sexual maturation occurred in individuals with lower age and size (2 years for both, and 182 mm and 190 mm respectively). Except for *P. flesus* (females: 3 years and male: 2 years, all the other species presented the same age at first maturity for both sexes. *M. azevia* attained the first sexual maturity at 3 years of age.



Figure 4.5. Gonadosomatic index mean values per season determined for each sex (standard deviation is represented above bars). (a) *C. linguatula*, (b) *L. boscii*, (c) *P. flesus* and (d) *M. azevia*. (□females; ■males).

DISCUSSION

Diet

The present study showed that the diet of the flatfish species considered in this work was mainly composed by mysids and fishes (*C. linguatula*), decapods and fishes (*L. boscii*), polychaets and decapods (*M. azevia*). Flounder fed mainly echinoderms, bivalves and crustaceans. Studies conducted in western Europe (e.g. de Groot, 1971; Serrano et al., 2003; Vinagre et al., 2005), in the Mediterranean (e.g. Morte et al., 1999; de Juan et al., 2007) and in the Atlantic coast of Morocco (Belghyti et al., 1993) reported similar results to those obtained in this work. The present study was the first approach to the feeding ecology of *M. azevia*, and our results revealed that the diet of

this species was similar to other Soleidae, the main prey being crustaceans, polychaets and molluscs (e.g. Cabral, 2000b; Darnaude et al., 2001).

The relative importance of each prey was considerably different according to species. Several authors reported differences in the feeding behaviour between Pleuronectiformes families (de Groot, 1971; Braber and de Groot, 1973; Holmes and Gibson, 1983). Diet composition depends mainly on prey detection mode and gut morphology. Citharidae, Scophthalmidae and Pleuronectidae are visual predators, responding primarily to moving prey, while Soleidae are mainly night feeders consuming less mobile or sedentary prey (de Groot, 1971; Braber and de Groot, 1973).

The present study showed that feeding activity was higher in spring-summer, the females showed lower vacuity values than males and largest individuals had lower feeding activity than smallest. This feeding behaviour is consistent with several authors (Belghyti et al., 1993; Vassilopoulou, 2006; de Juan et al., 2007). Nonetheless, some authors (Redon et al., 1994; Morte et al., 1999; de Juan et al., 2007) reported that vacuity index was highest in the spring-summer. The variations on feeding activity result from the interactions of many factors, like prey availability, and reproductive process. In temperate areas, a highest productivity is registered in the spring and summer which may increase the prey availability. Also, females have higher energetic needs for growth and reproduction which could explain the lower vacuity values (Pitt, 1973; Lozán, 1992). Smaller fish present higher growth rates during the first years of life, which is usually related to a more intense feeding activity during this period to maximize growth (Vassilopoulou and Ondrias, 1999).

The diet of four flatfish species analysed in this study showed seasonal, ontogenic and sexual variations, which were concordant with previous studies (e.g. Redon et al., 1994; Andersen et al., 2005; Vassilopoulou, 2006). These variations could be related to changes in space and time of benthic prey (e.g. Matallanas, 1982; Wootton, 1998). Several authors (e.g. Braber and de Groot, 1973; Gerking, 1994; Platell and Potter, 1998) have showed than an increase in size of fish is associated with an increasing in the consumption of larger prey, this fact reflects, in some species, a combination between increase of mouth size and improved ability to handle prey and to swim faster. This fact is in agreement with the optimum foraging theory (Gerking, 1994), larger predators consume larger prey to maximize the energetic gain relative to capture effort.

M. azevia showed larger diet spectrum compared to the other three flatfish species, according to season, sex and size class. Diet breadth of *L. boscii* and *M. azevia* was higher when the feeding activity was lowest, this fact could correspond to a balance to

compensate the decreasing of prey availability during those seasons (Vassilopoulou, 2006). *C. linguatula* and *P. flesus* presented largest diet diversity on spring-summer, probably due to the availability of a large spectrum of prey.

Age and Growth

The present study showed than the asymptotic length (L_{∞}) obtained for C. linguatula were higher than the values obtained in studies conducted in the eastern Mediterranean (Vassilopoulou and Papaconstantinou, 1994; Turker Çakir et al., 2005). The opposite situation was observed for the growth coefficient (k). The estimate of von Bertalanffy parameters obtained for L. boscii is in agreement with those estimated by several authors in different geographical locations (e.g. Santos, 1994; Vassilopoulou and Ondrias, 1999; Robson et al., 2000). The growth coefficient (k) was similar to those obtained in previous studies, except for Robson et al. (2000), that reported a higher growth coefficient. The values of asymptotic length for P. flesus obtained in this work were higher than on earlier studies. This fact could be related to the smallest length of the fish on the studies conducted in the North Atlantic and the Mediterranean (Deniel, 1981; Vianet et al., 1989). The growth coefficient obtained in this study was smaller than those obtained in previous studies. Growth studies for M. azevia were scarce and all were conducted in the Portuguese coast. The estimate of von Bertalanffy parameters obtained in this work was similar to Andrade (1990, 1998), for the southern coast the of Portugal.

Growth can be influenced by many factors. Latitudinal variations in temperature induce variations in maintenance metabolism (Pauly, 1994a). The different growth patterns between sexes could be explained by the differences in metabolism between females and males, like differences in oxygen consumption (Pauly, 1994b), in the level of surplus energy between reproduction and somatic growth (Rijnsdorp and Ibelings, 1989) and differential food ingestion (Lozán, 1992).

Reproduction

The analysis of seasonal variation of gonadal development suggests that the spawning period of *C. linguatula* and *M. azevia* occurs throughout the year. However, the GSI values of *C. linguatula* were higher during spring-summer which suggested that reproduction occurs mainly in this season. Studies on the reproduction of spotted flounder are scarce and reported only length and age at first maturity. Vassilopoulou and Papaconstantinou (1994) and García-Rodriguez and Esteban (2000) suggested age at first maturity was between the first and the third year of life, being early on

males. Results obtained on the present work were consistent with the previous studies, exception with the length of first maturity, that was highest than earlier studies. The pattern of sexual development pointed out for *M. azevia* is consistent with the existing literature (Andrade, 1990; Afonso-Dias et al., 2005). Age at first maturity obtained on the present work was similar to earlier studies (Belaid and Marinaro, 1983; Andrade, 1990; Afonso-Dias et al., 2005). The results concerning the seasonal variation of gonadal development on the present work for L. boscii were similar to previous studies (Santos, 1994; Anonymous, 2006). The spawning season of P. flesus proposed by several authors outline a latitudinal gradient, with later spawning at higher latitudes. Studies carried out in North Europe showed that flounder spawns at the end of spring (e.g. Cieglewicz, 1962; Solemdal, 1967; Rae, 1971), while in the north of the French and Spanish coast, the spawning occurs mainly in the winter (e.g. Anonymous, 1979; Deniel, 1981; Masson, 1988), similar results were showed in the previous studies conducted while in the Portuguese coast the spawning occurs mainly in the winter (Cunha, 1988), our results were in agreement with earlier work. The length at first maturity obtained in the present study was higher compared to those reported in previous studies (e.g. Masson, 1988; Kosior et al., 1996), which could be explained by the highest growth rates at lower latitudes.

Stable populations can present small variation in size and age at maturity, these variations may be genetical changes, and can be associated with changes on environmental conditions on nursery grounds or during the juvenile and the adult stage (e.g. Stearns and Crandall, 1984; Rijnsdorp, 1993). Some studies have showed that marked variations in length or age at first maturation could indicate population decline (e.g. Bowering *et al.*, 1997; Rijnsdorp and Vethaak, 1997).

Further studies on the bio-ecology of these species, are needed in order to bring new insights into the population dynamics of these commercially important flatfish species.

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Chapter 5

Genetic diversity and population structure of *Solea solea* and *Solea senegalensis* and its relationships with life history patterns

By Teixeira, T.F., Teixeira, C.M., Marques, J.M., Coelho, M.M., Cabral, H.N.

Genetic diversity and population structure of Solea solea and Solea senegalensis and its relationships with life history patterns

ABSTRACT: The genetic diversity and population structure of *Solea solea* and *Solea senegalensis* were analysed, based on the complete cytochrome b sequence of mitochondrial DNA, of samples obtained throughout the species distribution ranges. A low nucleotide diversity and high haplotype diversity was observed in both species (except for Portugal-North population of *S. senegalensis*). The pairwise Φ -statistics and AMOVA of S. solea samples evidenced the genetic divergence of Atlantic and Mediterranean populations and of Eastern and Western Mediterranean populations. Significant differences were also observed between samples of S. senegalensis. Atlantic populations of S. solea, ranging from the Baltic Sea to Portugal-South could be considered as representative of the same panmictic unit. Minimum spanning network analysis revealed star-shaped patterns for populations of both species, suggesting that populations have undergone expansion following bottlenecks. The higher levels of diversity observed in S. senegalensis, compared to S. solea, may be due to differences in the duration of the pelagic larval stage, spawning period and habitat use patterns, with water temperature assuming a major role in restricting gene flow and consequently in the population genetic structure of both species. Although no assessment of soles stocks exploitation status exists for the Portuguese coast, the fact that common populational unit have been recognized for the NE Atlantic, deserves a strong regarding these species, since overexploitation of soles stocks have been reported for the Northern Europe.

Keywords: common sole, Senegalese sole, mitochondrial DNA, genetic population structure, stock identification, fisheries management

INTRODUCTION

The definition of stocks and their boundaries has become an essential part of fisheries management, especially since several studies developed in worldwide have referred high pressure on marine resources, resulting in a decrease of effective population size, being some stocks already out of their biological safety limits. However, the geographic areas considered in the establishment of management policies do not often coincide with the biological stocks' distribution, simply because of seasonal

movements of these stocks between the several management areas, or because different fish stocks may occur simultaneously in a unique area, generally resulting in a mismatch in the spatial scale of management and biological reality (Pawson and Jennings, 1996). The stock concept has numerous meanings in the fisheries context, such as an abstract and undefined unit or a spatial location in which fish are found and can be exploited by a specific fishing technique, and, in the biological context, a genetic unit defined as a group of individuals that mate randomly, with a variable degree of spatial and temporal integrity (Carvalho and Hauser, 1995).

In marine species with high dispersal patterns, which includes most of marine fishes, it is considered that high gene flow leads to population homogeneity, at a large scale, as a result of their life cycle patterns; factors like high fecundity, passive dispersal of larvae and active migration of adults, lead to the lower levels of genetic differentiation exhibited by marine species, relatively to freshwater or anadromous species (Ward et al., 1994; Carvalho and Hauser, 1995; Ward, 2002).

The analysis of genetic variation among fish species allows the discrimination of different stocks and the analyses of their migration patterns and effective size, and to assess individual stock contribution to mixed stock fisheries, evaluating the response of stocks to fisheries exploitation (Wirgin and Waldman, 1994). The assessment of genetic variation and the concept of geographical structure in marine fish populations are fundamental to the understanding of population dynamics and to the conservation and sustainable management of fisheries resources (Carvalho and Hauser, 1995; Bailey, 1997).

The knowledge on population structure is fundamental to manage fisheries of high commercial value species that present a broad-scale distribution. Flatfish species inhabit most of the shelf waters of the world oceans, but the largest quantities are caught in the temperate and boreal zones of the Northern Hemisphere (Pauly, 1994). In 2005, flatfish catches represented 1.46% of the world marine landings and near 70% these were relative to the North Atlantic (721950 tonnes) (source: FAO).

According to the Portuguese fisheries statistics, flatfish fisheries represent 4% of all the fish biomass landed in the Portuguese coast. However, the importance of flatfish fisheries is considerable higher due to the high commercial value of flatfish species, accounting for near 11% of the economical value of fish landings (source: DGPA). According to official data, the most important flatfish species for Portuguese fisheries, in terms of landings, were the soles, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858. Soles landings increased from 464 tonnes, in 1998, to 510 tonnes, in 2005 (source: DGPA).

Few studies of broad-scale geographic range have considered commercially important flatfish, but common sole inhabiting the northern areas of the Eastern Atlantic have been particularly well studied using different kinds of genetic markers: allozymes and nuclear DNA detected significant differentiation between Mediterranean and Atlantic populations, within European Atlantic coast and across Mediterranean (e.g. Koutoulas et al., 1995; Exadactylos et al., 1998; Garoia et al., 2007; Rolland et al., 2007); control region of mitochondrial DNA (mtDNA) revealed the existence of genetic structure among adjacent basins (Guarniero et al., 2002) and RAPDs (random amplification of polymorphic DNA) detected higher levels of genetic variation within and between populations (Exadactylos et al., 2003).

Genetic studies developed with Senegalese sole focused its systematics and phylogeny, particularly in the Mediterranean (e.g. Borsa and Quignard, 2001; Infante et al., 2004), and only recently including a broader-scale area (Pardo et al., 2005). The only population genetic study was developed by Cabral et al. (2003) that analysed nine polymorphic allozyme loci, pointing out a low genetic differentiation and the absence of population structure for *S. senegalensis* inhabiting several estuarine systems along the Portuguese coast.

The aim of the present study is to determine the genetic diversity and population structure of *S. solea* and *S. senegalensis* throughout their distribution range, using mtDNA cytochrome b data, and to evaluate its relationships with species life history patterns, that could be especially useful for the sustainable management of these resources.

MATERIALS AND METHODS

Sampling and DNA Extraction

A total of 172 soles, belonging to both species, were collected from 13 different locations, covering their distribution range, from the Baltic Sea to the Mauritanian coast, and throughout the Mediterranean Sea (Figure 5.1). The number of individuals analysed for each species and the location of sampling areas are presented in Table 5.1.

Total genomic DNA was extracted from tissue samples (fin or muscle), following a phenol-chloroform protocol, as described by Wasko et al. (2003).



Figure 5.1. Sampling areas for each species analysed in this study and their distribution range (blue – *S. solea*; orange – *S. senegalensis*). Codes of sampling areas are defined in Table 5.1.

PCR Amplification and Sequencing

The entire cytochrome b (*cyt b*) mitochondrial DNA gene (about 1141 bp in length) was amplified by PCR, using two specific primers (Infante et al., 2004): GLU1 (5'-GGGGATTTTAACCTCAGGCGTTCAGTTTAC-3') and Thr2 (5'-GGACTAATCGCTTGAAAAAACCACCGTTG-3'). Table 5.1. Geographic location of the sampling areas, their codes, species sampled and number of individuals collected in each area (SS – Solea solea; SN – Solea senegalensis).

Coographical location	Area	Coordinates	Sampled	Sample
Geographical location	Code	Coordinates	Species	size
Denmark (Baltic Sea-North Sea transition-	DBS	N 54.13º E 011.26º	SS	10
Kattegat)				
Denmark (North Sea)	DNS	N 54.80° E 008.13°	SS	10
Belgium (North Sea)	BNS	N 51.57º E 003.24º	SS	10
United Kingdom (Irish Sea)	UKIS	N 53.90° W 003.30°	SS	12
France (Bay of Biscay)	FBBy	N 45.84° W 001.48°	SS/ SN	12/10
Portugal-North (Atlantic Ocean-off Figueira da	PN	N 40.14° W 008.89°	SS/SN	10/10
Foz)				
Portugal-Centre (Atlantic Ocean-off Setúbal)	PC	N 38.45° W 008.99°	SS/SN	10/09
Portugal-South (Atlantic Ocean-off Olhão)	PS	N 37.00° W 007.79°	SS/SN	10/10
Mauritania (Atlantic Ocean)	Maur	N 18.28° W 016.40°	SN	10
France (Gulf of Lyon)	FGL	N 43.45º E 004.01º	SS	10
Italy (Adriatic Sea-off Venice)	IAdS	N 45.36º E 012.44º	SS	10
Greece (Aegean Sea-off Thessaloniki)	GAS	N 40.36º E 022.77º	SS	9
Turkey (Aegean Sea-off Izmir)	TAS	N 38.79º E 026.60º	SS	10

PCR reactions of 25 μ l total volume, containing approximately 50 ng of template DNA, 2 mM of MgCl₂, 0.2 μ M of dNTP's, 0.5 μ M of each primer, 2 U of Taq DNA Polymerase (Fermentas) and 10x Taq buffer (10mM Tris-HCl, ph 9.0; 50 mM KCl) (Fermentas), were conducted as follows: an initial preheat step at 92°C for 120 s, followed by 5 cycles of denaturing at 92°C for 15 s, annealing at 51°C for 45 s and extension at 72°C for 90 s, and 30 cycles of denaturing at 92°C for 15 s, annealing at 52°C for 45 s and extension at 72°C for 90 s, finishing with an extension step at 72°C for 7 min.

PCR products were subjected to electrophoresis in 1% agarose gels, containing ethidium bromide staining, and visualized under UV light. Products were then purified using 10 U of Exonuclease I (Fermentas), 1 U of Shrimp Alkaline Phosphatase (SAP) (Fermentas), and 6.25x SAP buffer (Fermentas). The protocol for purifying PCR products consisted of 30 min at 37°C, 15 min at 80°C and 5 min at 12°C. All products were sequenced in both directions, using the PCR primers and the BigDye Terminator Cycle Ready reaction Kit (Applied Biosystems), and visualized in an AbiPrism 377 Automated Sequencer (Applied Biosystems) (Stabvida[®]).

Sequence Alignment

Contiguous sequences were assembled in Sequencher 4.0 (GeneCodes Corp.) and compared to similar sequences deposited in GenBank, using the Basic Local Alignment Search Tool (BLAST) available on the NCBI website (NCBI, http://www.ncbi.nlm.nih.gov/Genbank). All sequences were aligned using Sequencher 4.0 (GeneCodes Corp.).

Population Genetic Analysis

Intrapopulation diversity was analysed by estimating gene diversity (*h*), and nucleotide diversity (π) (Nei, 1987), using DNASP 4.10.9 (Rozas et al., 2003). Population structure and genetic variation were characterised by Φ -statistics (analogous to the *F*-Statistics of Wright (1969)), which incorporate genetic distance between haplotypes and haplotipic frequencies, using Arlequin 3.11 (Excoffier et al., 2005). The software Modeltest 3.7 (Posada and Crandall, 1998) was used to find the best model of evolution that fitted the data, according with the Akaike criterion. Although resultant models were different for both species (GTR+I+G – general time reversible plus Proportion invariant plus Gamma, for *S. solea*, and GTR – general time reversible for *S. senegalensis*) the pairwise distance method, with γ =0, was considered for both species analyses, since the resultant models were not included in Arlequin 3.11.

Analysis of molecular variance (AMOVA) was used to assess the population configuration and the geographical pattern of population subdivision (Excoffier et al., 1992). For hierarchical analyses, populations were grouped according to their geographic location. Several other rearrangements were tested and the one that maximised among group variation (θ_{CT}) was assumed to be the most probable subdivision. Simulations with 1000 permutations were made to test the statistic significance of results. The isolation by distance (IBD) model was analysed by testing the association between geographic and genetic distances (Smouse et al., 1986) through a Mantel test (Mantel, 1967) with 10000 permutations, as implemented in Arlequin 3.11. Geographical distances between populations were measured as a straight line along the coast between each two areas. A standard Bonferroni *a posteriori* correction was applied to determine the level of significance in multiple tests.

Minimum spanning networks (using the median joining agglomeration method) were constructed with Network 4.201 (Bandelt et al., 1999) based on haplotype data of the sampled populations, and generated with MacClade 4.08 (Maddison and Maddison, 1989). Network 4.201 uses the maximum parsimony method for reconstructing trees,

choosing the smallest and simplest as the best. Median-joining algorithm was used with default parameters, as recommended for this kind of data (Bandelt et al., 1999). The population structure was also investigated using the program BAPS 4.1 (Corander and Tang, 2007), which allows the analysis of sequence data. Given a maximum value of partitions, the algorithm uses a stochastic optimization procedure to find the clustering solution with the highest 'marginal likelihood' of K (i.e., an approximation of the most probable number of differentiated genetic populations conditional on observed data). The maximum number of partitions, K, was set as ranging from 5 to 20 (*S. senegalensis*) and from 12 to 20 (*S. solea*) and, in each case, we the analyses were ran several times, recording the best partition found and the corresponding 'marginal likelihood'.

RESULTS

Solea solea

Genetic diversity was very high, with 75 haplotypes recovered from 123 individuals (77% were unique). The most common haplotype, H7 (15% of the samples), was shared by 18 individuals from Northeast (NE) Atlantic samples (BNS, FBBy, DBS, DNS, PC, PN and UKIS), and did not included any of the Mediterranean samples. Moreover, no haplotypes were shared between individuals from NE Atlantic and Mediterranean samples. The second major common haplotype, H38, was shared by 10 individuals, all from Mediterranean samples (GAS and TAS).

The overall level of haplotype diversity (*h*) was high, ranging from 0.667, in the Denmark North Sea (DNS) and Turkey (TAS) samples, to 1.000, in Portugal-South (PS) and France Gulf of Lyon (FGL) samples. Nucleotide diversity (π) exhibited by all populations was low, ranging from 0.001, in Turkey (TAS), to 0.007, in Portugal-South (PS). The number of haplotypes presented by each population varied, ranging from 5 in the Denmark Baltic Sea (DBS), Denmark North Sea (DNS) and Turkey (TAS) samples to 10 in France Bay of Biscay (FBBy), Portugal-South (PS) and France Gulf of Lyon (FGL) (Table 5.2).

	Nucleotide diversity	Haplotype diversity	Number of
Sample	(π)	(<i>h</i>)	haplotypes
DBS	0.005 (0.003)	0.844 (0.080)	5
DNS	0.003 (0.002)	0.667 (0.163)	5
BNS	0.005 (0.003)	0.978 (0.054)	9
UKIS	0.005 (0.003)	0.909 (0.080)	9
FBBy	0.004 (0.003)	0.970 (0.044)	10
PN	0.004 (0.003)	0.956 (0.059)	8
PC	0.004 (0.003)	0.978 (0.054)	9
PS	0.007 (0.004)	1.000 (0.045)	10
FGL	0.003 (0.002)	1.000 (0.045)	10
IAdS	0.003 (0.002)	0.956 (0.059)	8
GAS	0.002 (0.001)	0.833 (0.127)	6
TAS	0.001 (0.001)	0.667 (0.163)	5

Table 5.2. Genetic diversity of *cyt b* sequences for *S. solea* populations (standard
deviation is presented between brackets) (see Table 5.1 for sample codes).

The hierarchical partition of variance amongst population tested using AMOVA revealed that the proportion of "among groups" variation is large (48.5%) (θ_{CT} =0.485; *P*<0.001), the proportion of variation "within populations" is 47.19% (θ_{ST} =0.528; *P*<0.001). Only a small variance component was attributable to "among populations and within groups" (4.31%) (θ_{SC} =0.084; *P*<0.001).

Low Φ_{ST} values were found in all pairwise analyses within the NE Atlantic samples, while those obtained between the group of NE Atlantic samples and the group of Mediterranean samples were high (0.385-0.771) and significant (*P*<0.001). High and significant Φ_{ST} values (0.191-0.368; *P*<0.001) were also obtained in the pairwise analysis between Western (FGL and IAdS) and Eastern (GAS and TAS) Mediterranean samples (Table 5.3).

	-			_	-		-		-			
Sample	DBS	DNS	BNS	UKIS	FBBy	PN	РС	PS	FGL	IAdS	TAS	GAS
DBS	-											
DNS	0.080	-										
BNS	-0.028	0.013	-									
UKIS	0.023	0.174	0.007	-								
FBBy	0.021	0.021	-0.09	0.083	-							
PN	0.044	0.158	0.027	0.047	0.015	-						
PC	0.030	0.046	-0.003	0.075	0.000	0.007	-					
PS	0.085	0.207	0.085	0.083	0.095	0.001	0.020	-				
FGL	0.462*	0.599*	0.465*	0.414*	0.502*	0.482*	0.488*	0.385*	-			
IAdS	0.528*	0.657*	0.530*	0.480*	0.563*	0.537*	0.531*	0.412*	0.056	-		
TAS	0.627*	0.771*	0.630*	0.566*	0.652*	0.664*	0.664*	0.553*	0.232*	0.368*	-	
GAS	0.587*	0.725*	0.587*	0.533*	0.617*	0.618*	0.618*	0.506*	0.191*	0.285*	0.017	-

Table 5.3. Genetic differentiation (Φ_{ST}) values for *S. solea* samples (* indicates significant values, *P*<0.001) (see Table 5.1 for sample codes).

The Mantel test with sampled populations (from Baltic Sea to Aegean Sea) revealed a clear correlation between genetic and geographical distance (Z=0.63; P<0.05). However, when this test was applied separately to Atlantic and Mediterranean samples, no significant correlations between genetic and geographical distance were found (Z=0.30, P>0.05 and Z=0.44, P>0.05, respectively).

The haplotype network derived from *cyt b* sequences, and using the maximum parsimony method, is presented in Figure 5.2. Size of circles is proportional to the number of individuals within each haplotype. Two major common haplotypes were found, and represent individuals from NE Atlantic samples (H7, shared between 18 individuals) and Mediterranean samples (H38, shared between 10 individuals of Greece (GAS) and Turkey (TAS) samples). These two haplotypes differ from each other by 8 mutations, and 3 haplotypes are missing between them. It is also possible to identify a third haplotype (H27), shared by the individuals from France Gulf of Lyon (FGL) and Italy (IAdS) samples, from which several haplotypes derive by only one mutation.



Figure 5.2. Minimum spanning network analysis of haplotypes identified in samples of *S. solea.* Distances between haplotypes are proportional to the number of mutational steps. Colours correspond to populations as follows: ● Denmark Baltic Sea-North Sea transition (DBS), ● Denmark North Sea coast (DNS), ● United Kingdom Irish Sea (UKIS), ● Belgium North Sea (BNS), ● France Bay of Biscay (FBBy), ● Portugal-North (PN), ● Portugal-Centre (PC), ● Portugal-South (PS), ● France Gulf of Lyon (FGL), ● Itay Adriatic Sea (IAdS), ● Greece Aegean Sea (GAS) and ● Turkey Aegean Sea (TAS).

The population analysis performed in BAPS (Figure 5.3) suggests the existence of three different clusters, represented by the blue, red and green vertical bars. Two of the clusters are present in all Atlantic samples (blue and green), but not in Mediterranean samples, and the red cluster is only found in Mediterranean samples.



Figure 5.3. Population structure obtained with BAPS in *S. solea* populations. Each colour corresponds to a different cluster. Legend: BNS – Belgium North Sea; FBBy – France Bay of Biscay; FGL – France Gulf of Lyon; IAdS – Italy Adriatic Sea; DBS – Denmark Baltic Sea-North Sea transition; GAS – Greece Aegean Sea; DNS – Denmark North Sea coast; PC – Portugal-Centre; PN – Portugal-North; PS – Portugal-South; TAS – Turkey Aegean Sea; UKIS – United Kingdom Irish Sea.

Solea senegalensis

The *cyt b* diversity was relatively high, with 15 haplotypes recovered from the 49 individuals analysed, being 60% of these haplotypes unique. A major haplotype, corresponding to 45% of the samples, was shared between 22 individuals belonging to the France Bay of Biscay (FBBy) and Portugal-North, Centre and South sampes (PN, PC and PS, respectively).

Haplotype diversity (*h*) presented a wide range of values, from 0.378, in the Portugal-North (PN) samples, to 0.778, in France Bay of Biscay (FBBy) sample. However, the nucleotide diversity (π) exhibited by all populations was low (0.001 to 0.002) and the number of haplotypes per population was also low, varying between 3 in Portugal-North (PN) to 5 in Mauritania (Maur) (Table 5.4).

	sample codes).		
	Nucleotide diversity	Haplotype diversity	Number of
Sample	(π)	(<i>h</i>)	haplotypes
FBBy	0.002 (0.001)	0.778 (0.091)	4
PN	0.001 (0.001)	0.378 (0.181)	3
PC	0.001 (0.001)	0.583 (0.183)	4
PS	0.001 (0.001)	0.711 (0.118)	4
Maur	0.001 (0.001)	0.667 (0.163)	5

Table 5.4. Genetic diversity of *cyt b* sequences for *S. senegalensis* populations (standard deviation is presented between brackets) (see Table 5.1 for sample codes).

The AMOVA indicated that a high and significant proportion of the total variance was attributed to "among groups" (48.55%)(θ_{CT} =0.485; *P*<0.001), but the highest, and significant, percentage of variation (52.66%) (θ_{ST} =0.473, *P*<0.001) was obtained "within populations". A small proportion of variance was attributable to "among populations and within groups" (-1.21%) (θ_{SC} =-0.024, *P*<0.001).

The highest levels of genetic differentiation were obtained between the France Bay of Biscay (FBBy) sample and all the others under study (Table 5.5). No genetic differentiation was found between Portuguese coast samples, which were the geographically closest ones, being the highest and significant values of Φ_{ST} presented by a pair of the most geographically distant samples, France Bay of Biscay (FBBy) and Mauritania (Maur) (Φ_{ST} =0.502). The Portugal-Centre (PC) sample did not present significant genetic differentiation from any of the other samples.

Table 5.5.	Genetic differentiation (Φ_{ST}) values for <i>S. senegalensis</i>
	samples (* indicates significant values, $P < 0.01$) (see
	Table 5.1 for sample codes).

Sample	FBBy	PN	PC	PS	Maur
FBBy	-				
PN	0.453*	-			
PC	0.405	-0.032	-		
PS	0.429*	0.095	0.000	-	
Maur	0.502*	0.444*	0.301	0.405*	-

The Mantel test considering all the samples (from France Bay of Biscay (FBBy) to Mauritania (Maur)) failed to show a significant correlation between genetic and geographical distance (P>0.05), despite the high value of the correlation coefficient obtained (Z=0.75).

The haplotype network based on the *cyt b* sequences presented a star-shape (Figure 5.4), suggesting population expansion.



Figure 5.4. Minimum spanning network analysis of haplotypes identified in samples of *S. senegalensis.* Distances between haplotypes are proportional to the number of mutational steps. Colours correspond to samples as follows:
France Bay of Biscay (FBBy), Portugal-North (PN), Portugal-Centre (PC), Portugal-South (PS), Mauritania (Maur).

The most common haplotype, H2, was shared by individuals from four of the five samples analysed – France Bay of Biscay (FBBy), Portugal-North (PN), Portugal-Centre (PC) and Portugal-South (PS). All other haplotypes derived from this one, by one to five mutations, with the highest differentiation being found between the France Bay of Biscay (FBBy) and Mauritania (Maur) samples, which was also evidenced by the absence of shared haplotypes between these samples.

The structure obtained trough BAPS analysis (Figure 5.5) revealed three different clusters, represented by blue, red and green vertical bars. Whereas the green cluster is present in all samples, the blue one occurs only in France Bay of Biscay (FBBy) sample, corroborating the pattern obtained in the network. The presence of the red cluster only in Portugal-Centre (PC) and Mauritania (Maur) samples is concordant with the non-significance of the Φ_{ST} value exhibited in the pairwise analysis of genetic differences between these two samples.



Figure 5.5. Population structure obtained with BAPS in *S. senegalensis* populations. Each colour corresponds to a different cluster. Legend: FBBy – France Bay of Biscay; PC – Portugal-Centre; PN – Portugal-North; PS – Portugal-South; Maur – Mauritania.

DISCUSSION

High genetic variation (*h*) and low to moderate nucleotide diversity (π) were found in all samples of *S. solea* and *S. senegalensis* analysed, except for Portugal-North sample of *S. senegalensis*. The values obtained for both diversity indices were similar to those obtained for another flatfish species, plaice, in the Atlantic area (Hoarau et al., 2004) and are characteristic of species with wide geographic distribution areas.

Significant genetic differentiation was detected, to *S. solea*, at an interregional scale, mainly between two major groups of populations, the Atlantic and the Mediterranean, whereas little or no differentiation could be detected beneath that scale. The geographical distance between these two major areas seems to be the main cause underlying the genetic differentiation found, evidencing a clear relationship between geographical distances and genetic differentiation. These results are, therefore, in agreement with the existence of an isolation by distance model (IBD), as suggested in Kotoulas et al. (1995).

Low levels of differentiation from the Baltic Sea to Portugal-South exhibited by *S. solea* populations, supported by low Φ_{ST} values and high number of shared haplotypes by Atlantic populations, with no particular geographical organization, were also

obtained for other flatfishes (e.g. Borsa et al., 1997; Hoarau et al., 2002; Nielsen et al., 2004), and also for other marine fishes (Viñas et al., 2004). According to Kotoulas et al. (1995), *S. solea* populations from the Atlantic region are expected to constitute panmictic or quasi-panmictic units (structural units that occur in neighbouring localities within a radius of 100 km), presenting high levels of gene flow that, presumably, occur each generation through the gathering of individuals from different areas of spawning, and the passive diffusion of eggs and larvae back to coastal and estuarine nursery areas. In general, marine species seem to be more genetically variable than anadromous and freshwater species (Dewoody and Avise, 2000) and at the same time, less differentiated into populations (Ward, 2002). In the light of these considerations, the absence of genetic differentiation among *S. solea* samples throughout the NE Atlantic was not unexpected, as well as the absence of correlation between geographical distances and genetic differentiation of samples.

Separation between Atlantic and Mediterranean *S. solea* populations can be explained by the colonization of the Mediterranean, from the Atlantic, during the early Pliocene and their settlement there since then, which is consistent with conclusions of Mediterranean biogeographers (Klausewitz, 1973; Quignard, 1978). Thus, an interruption of gene flow between these populations, probably due to the major oceanographic discontinuity between these areas – the Gibraltar Strait-Alboran Sea region – might be the reason for the exhibited pattern, that has already been reported for other marine fish species (Bahri-Sfar et al., 2000; Zardoya et al., 2004; Charrier et al., 2006).

Our results also support an eastward-westward differentiation among Mediterranean populations of common sole, suggested which was in previously studies using allozymes (Kotoulas et al., 1995), control region of mtDNA (Guarniero et al., 2002), nuclear-DNA intronic loci markers (Rolland et al., 2007) and amplified fragment length polymorphisms (AFLPs) (Garoia et al., 2007). Differentiation among such populations could be due to the complex history of the Mediterranean that was strongly impacted during last glacial episodes. During these periods the lower sea level modified coast lines, creating distinct refuges in the Mediterranean and allowing the splitting of the eastern and western basins; since then, they present different hydrographic regimes, the western one being much more uniform than the eastern one because of their respective geographies (Bahri-Sfar et al., 2000). The possible partial recolonization by populations from the Atlantic can, therefore, also be an explanation for the detected differentiation within the Mediterranean (Rolland et al., 2007). These differences have also been attributed to larval temperature tolerances (Kotoulas et al., 1995; Borsa et al., 1997) and to local adaptations to different salinities (Nielsen et al., 2004). The

present study excludes the geographical distances as a structuring force responsible for the genetic differentiation within the Mediterranean, since the Mantel test performed, considering only Mediterranean populations revealed the absence of a significant correlation between genetic and geographical distances. This "Mediterranean division" has also been reported in other marine fishes (Bahri-Sfar et al., 2000; Suzuki et al., 2004).

For S. senegalensis, geographical distance, it seems important for the structuring of populations. This association appeared to be highly related with latitudinal differences between samples, since more geographically distant S. senegalensis populations, such as France Bay of Biscay and Mauritania, showed the highest pairwise Φ_{ST} value, absence of shared haplotypes in the network and unique clusters in BAPS analysis. Results obtained for the geographically closest S. senegalensis populations, such as those from the Portuguese coast - North, Centre and South - presented no genetic differentiation at all, and shared the most common haplotype present in the minimum spanning network of this species. These results are in agreement with those obtained by Cabral et al. (2003) using allozymes, which detected the absence of genetic differentiation throughout the Portuguese coast. Considering the strong association obtained between geographic distances and genetic distances, a significant IBD model, confirmed by the Mantel test would be expected. However, the resulting correlation coefficient was high, but not significant, probably due to the reduced number of populations analysed and/or to a low sample size. The importance of geographical distances per se, acting as a structuring force in NE Atlantic populations, has been found in other marine fishes such as cod (Hutchinson et al., 2001), plaice (Hoarau et al., 2002) and Atlantic herring (Mariani et al., 2005). A weak pattern of isolation by distance along a latitudinal axis was also found in European flounder, from the Western Baltic Sea to Portugal (Borsa et al., 1997).

Considering the NE Atlantic area, *S. solea* and *S. senegalensis* occur in simpatry from Bay of Biscay to North Africa. Since both species present similar life history pattern (a division into a juvenile phase, predominantly estuarine and an adult phase, marine, that may have an impact on the structuring of offshore adult populations, particularly on their genetic differentiation, since a strong association between a particular spawning and nursery area can be expected), similar patterns of genetic differentiation throughout their simpatry area were, somehow, expected. However the level of genetic differentiation obtained for each species was different, with *S. solea* samples presenting genetic homogeneity, conversely to significant genetic differentiation among *S. senegalensis* samples, contradicting the assumption that marine organisms capable of extensive dispersal (those that undergo lengthy planktonic larval development) will necessarily demonstrate widespread genetic homogeneity (Exadactylos et al., 1998).

The low genetic differentiation exhibited by *S. solea* compared to *S. senegalensis*, can be due to a high genetic flow between populations at different stages of their life cycles, namely adult migration between spawning grounds and juvenile dispersion after the estuarine phase. Other authors suggest that the larval period is the most important in this context, with genetic flow increasing with the duration of the pelagic larval period. This latter fact should lead to lower values of genetic differentiation in *S. senegalensis* relatively to *S. solea*, when analysing samples from the same geographical area, since this species presents a wider spawning period (e.g. Dinis et al., 1999; Anguis and Cañavate, 2005).

For the sustainable use of biological resources in the coastal zone are necessary strategies for conservation for maintenance of fisheries in these regions. Because species are not homogeneous, but structured in groups of individuals that are more or less isolated from another, it is necessary that biological sustainable management will be based on knowledge on population genetic structure. The identification of genetically homogeneous groups of individuals constitutes the basic unit for conservation, management and sustainable use.

Soles are the most important species group in Portuguese flatfish fisheries, but are not assessed. This situation is of deep concern, since a decreasing trend in LPUE have been determined for the period between 1992 and 2005 (Teixeira and Cabral, 2009). Also, since the species are not usually sorted at species level at fish houses, it is extremely difficult to evaluate if restrictive measures (net and mesh sizes, minimum legal size and Total Allowable Captures – TAC) are being effective.

In conclusion, because *S. solea* represented one unit in NE Atlantic, and because no evaluations of the exploitation status of flatfish stocks exist in Portugal, there are strong concerns and probably overexploitation of these resources, based on the evidences reported by Teixeira and Cabral (2009) to this fishery in the Portuguese coast, as well as in information for the adjoining areas (North coast of Spain and France), it is urgent to adopt the same management measures to all stock.

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Part III

Stock Assessment and Fisheries

CHAPTER 6

Time series analysis of flatfish landings in the Portuguese coast Fisheries Research 2009, 96, 252–258 By Teixeira, C.M., Cabral, H.N.

Time series analysis of flatfish landings in the Portuguese coast

Abstract: Flatfish fisheries are widely spread over the Portuguese coast and traditionally have a high social and economical relevance in some coastal communities. The artisanal multi-gear fleet is responsible for the largest share of flatfishes landings. However, only a fraction of these vessels actively target flatfishes, since many other species are caught in these multi-species fisheries. To explain the variability of flatfish landings we used the data set of official landings of commercially important flatfish species (monthly landings values, from 1992 to 2005) and applied generalized linear models (GLM) using the NAO index (NAO), sea surface temperature (SST), scalar wind (WIND), rainfall (RAIN) and month as explanatory variables. Flatfish landings did not showed a common trend for different species group between 1992 and 2005. For the majority of the species, significant relationships were found between month and SST and landings per unit effort (LPUE). The seasonal pattern in LPUE reflected migrations to spawning grounds, when flatfishes concentrate in some areas of the continental shelf and become more vulnerable to fishery. Temporal closures should be implemented for the stocks that evidence overexploitation. More studies focused in species abundance and fishing effort estimation are necessary on Portuguese flatfish fisheries in order to determine the status of stocks and adopt adequate management measures.

Keywords: flatfish, landings, stock assessment, environmental factors, fisheries management, Portugal

INTRODUCTION

Fishing is a traditional and culturally important activity in Portugal, being dominated by small fishing vessels (Baeta and Cabral, 2005). Flatfish fisheries are widely spread over the Portuguese coast and traditionally have played and important socio-economic role. Flatfish landings account for less than 4% of all the fish biomass landed in the Portuguese coast. However, the importance of flatfish fisheries is considerable higher due to the high commercial value of flatfish species, accounting for almost 11% of the economical value of fish landings (source: DGPA). The most important species, in terms of landings, are the soles, *Solea solea* (Linnaeus, 1758), *Solea senegalensis* Kaup, 1858, and *Solea lascaris* (Risso, 1810), the bastard sole, *Microchirus azevia* (Capello, 1868), the flounder, *Platichthys flesus* (Linnaeus, 1758), the spotted flounder, *Citharus linguatula* (Linnaeus, 1758), the wedge sole, *Dicologlossa cuneata* (Moreau, 1881), the turbot, *Scophthalmus maximus* (Linnaeus, 1758), the brill, *Scophtahlmus rhombus* (Linnaeus, 1758), and the megrims, *Lepidorhombus boscii* (Risso, 1810) and *Lepidorhombus whiffiagonis* (Walbaum, 1792). The artisanal multi-gears fleet is responsible for the largest share of flatfishes landings. However, only a fraction of these vessels actively target flatfishes, since many other species are caught in this multi-species fishery. The main fishing gears used in flatfish fisheries are trammel and gill nets and bottom trawl.

Regular fluctuations in the stocks and catches of abundant fish species have been documented in many of the world's oceans (e.g. Cushing, 1996; Klyashtorin, 1998; Ménard et al., 2007). Climatic oscillations, anomalies or changes clearly affect population dynamics and several ecological processes in marine ecosystems (e.g. Walther et al., 2002; Stenseth et al., 2004; Duffy-Anderson et al., 2005). The wind regime (e.g. Schwartzlose et al., 1999; Borges et al., 2003) and changes in sea surface temperature (e.g. Fox et al., 2000; Zuur and Pierce, 2004; Désaunay et al., 2006) play a key role in the ecological effects of climate fluctuations. But variations in population abundance are very often associated with large-scale climate indices such as the North Atlantic Oscillation (NAO) or the Southern Oscillation Index (SOI) (Ottersen et al., 2001; Stenseth et al., 2004). They do not have necessarily a strong link with local weather condition (Stenseth et al., 2003), but they can be good predictors of ecological processes (Hallett et al., 2004).

The main aim of this work is to analyse the main trends in commercial flatfish landings in the Portuguese coast, their relationships with key environmental factors and to evaluate possible implications for the management of these resources.

MATERIALS AND METHODS

Data Source

The data set use in this study was composed of LPUE monthly data, by flatfish species group, from 1992 to 2005, obtained from the Governmental Fisheries Bureau (Direcção Geral das Pescas e Aquicultura – DGPA). The species groups considered

were: soles, bastard sole, flounder, spotted flounder, wedge sole, turbot, brill and megrims.

The data analysed were relative to the total flatfish landings for all ports along the Portuguese coast (Figure 6.1). For the data set the fishing effort estimator was the number of vessels according to the fishing gear with major contribution in landings by species/species group. Soles, bastard sole, flounder, wedge sole, turbot and brill were mainly landed by the multi-gear fleet (between 70% and 90% of landings), and consequently the fishing effort was the total number of vessels operating with multi-gear that landed these species; spotted flounder was mainly landed by the trawl fleet (80% of landings), and thus the fishing effort was the total number of vessels operating with trawl that landed this species; and, finally, megrims were landed by multi-gear (near 40%) and trawl (60%) vessels, being the fishing effort estimated based on the total number of vessels that use multi-gear or trawl and landed these species.

Statistical Methods

Simple regression models were used to evaluate the main trend in LPUE throughout the study period, for each species or species group.

We used generalized linear models (GLM) to investigate factors influencing the variation in LPUE of the flatfish species or species groups considered. Several authors applied GLM (McCullagh and Nelder, 1989) in this context, namely Hilborn and Walters (1992), Goñi et al. (1999), Maynou et al. (2003) and García-Rodríguez et al. (2006). GLM are an extension of linear models allowing the incorporation of non-normal distributions of the response variable and transformations of the dependent variables to linearity (McCullagh and Nelder, 1989).

The explanatory variables that were considered in these models were the NAO index (NAO), based on the difference between sea level atmospheric pressure at the Azores and Iceland (obtained from http://www.cgd.ucar.edu/cas/jhurrell/indice.html; Hurrel, 1995), sea surface temperature (SST) and scalar wind (WIND) based on data available on a 1° x 1° grid from ICOADS (http://dss.ucar.edu/pub/coads/forms/msg/), rainfall (RAIN) based on data available on SNIRH (http://snirh.pt/) and month.



Figure 6.1. Map of mainland Portugal landing ports (1. Caminha; 2. Viana do Castelo; 3. Póvoa do Varzim; 4. Leixões; 5. Aveiro; 6. Figueira da Foz; 7. Nazaré; 8. Peniche; 9. Lisboa; 10. Sesimbra; 11. Setúbal; 12. Sines; 13. Sagres; 14. Portimão; 15. Quarteira; 16. Faro; 17. Olhão; 18. Tavira; 19. Vila Real de Santo António).

For the oceanographic and climate variables a time-lag of 2 years was considered, since their effect is mainly registered in early life stages, influencing recruitment success. This time-lag was based on the available knowledge on age at first maturity for each species (e.g. Deniel, 1981; Andrade, 1990; Garcia-Rodriguez and Esteban, 2000; Anonymous, 2006).

The general form of GLM is

$$g(\mu) = \beta^t x$$

whereby a function link $g(\cdot)$ is used to achieve linearity in the parameters β^t of the dependent variable *x*.

A second part of the model is the specification of a variance function (ϕ) that relates the variance of the response variable to the mean:

$$Var(Y) = \varphi V(\mu)$$
, with φ constant

Catch rates (U_{ymtwrn}) were modelled as a function of year (y), month (m), sea surface temperature (t), scalar wind (w), rainfall (r) and North Atlantic oscillation (n). The general model used was:

$$\log(\mu_{ymtwrn}) = \log(E(U_{ymtwrn}))$$
$$= \mu_0 + \beta_y^1 + \beta_m^2 + \beta_t^3 + \beta_w^4 + \beta_r^5 + \beta_n^6$$

where β^t is the parameter set relating the dependent variables to the response, using a log-link function and a gamma distribution for the response variable.

The correct procedure in any analysis to account for the level of catch rates when a species is captured is classified in two categories: zero values and non-zero values.

When zero values are eliminated, it is seen that data may be close to lognormal, which implies that a lognormal or gamma distribution may be appropriate for positive values (Stefánsson, 1996). The results of Myers and Pepin (1990) suggested that the use of the gamma density is preferable to the use of a lognormal density for fisheries data, although this seems to apply mainly when there is a considerable probability of small observations, not dealt with otherwise (Pennington, 1991) and, in other instances, the gain is minor (Firth, 1988). Although other members of the exponential family could be used, the gamma density is used here when the positive values are under consideration.

The goodness-of-fit of the models was assessed by comparing their relative contribution to total deviance explained. The model was fitted in R environment (R Development Core Team, 2005) using a gamma distribution with a log-link function, adding the first order interactions whenever considered adequate.

All statistical analyses were performed using R software (R Development Core Team, 2005). A significance level of 0.05 was considered in all test procedures.

RESULTS

Considering the models that used the monthly landings data, from 1992 to 2005, a decreasing trend was observed for soles landings, with the highest LPUE value registered in 1993 (0.14 tonnes vessel⁻¹) (Figure 6.2a). SST and month were the most

important explanatory variables (29.66% and 15.89% of variance explained, respectively), and the model explained 51.32% of the deviance (Table 6.1). Highest LPUE were obtained for low SST values (Figure 6.3a). These species were mainly landed in winter months (Figure 6.4a).



Figure 6.2. Monthly landings per unit effort (LPUE) of flatfishes species or species groups in the Portuguese coast, between 1992 and 2005. (a) Soles; (b) bastard sole; (c) flounder; (d) spotted flounder; (e) wedge sole; (f) turbot; (g) brill; (h) megrims.

Bastard sole landings showed an increasing trend throughout the period considered. The highest LPUE value was registered in 2000 (0.06 tonnes vessel⁻¹) (Figure 6.2b). SST and rainfall were the most important explanatory variables (23.91% and 4.11% of variance explained, respectively), and the model explained 30.65% of the deviance (Table 6.1). Highest LPUE were obtained for low SST values (Figure 6.3b).

Flounder landings showed a increasing trend between 1992 and 2005. The highest LPUE value was recorded in 2003 (0.07 tonnes vessel⁻¹) (Figure 6.2c). SST and month were the most important explanatory variables (37.92% and 16.08% of variance explained, respectively), and the model explained 65.20% of the deviance (Table 6.1). The highest LPUE were obtained for low SST values (Figure 6.3c). This species was mainly landed in autumn and winter (Figure 6.4b).



Figure 6.3. Relationships between landings per unit effort (LPUE) of soles (a), bastard sole (b), flounder (c), brill (d) and SST.

Spotted flounder landings evidenced a decreasing trend between 1992 and 2005. The LPUE highest value was registered in 1992 (0.51 tonnes vessel⁻¹) (Figure 6.2d). Month was the most important predictor for this species (5.33%), and rainfall was also significant (4.72%). The model explained 12.16% of the deviance (Table 6.1). This species is mainly landed in autumn and winter (Figure 6.4c).

Wedge sole landings showed a decreasing trend between 1995 and 2005. The major LPUE value was registered in 1995 (0.02 tonnes vessel⁻¹) (Figure 6.2e). For this species none of the predictors used showed to be significant.

Table 6.1.Goodness-of-fit statistics for the GLMs fitted to LPUE flatfishes groups (values
of deviance for each factor, residual deviance (Res. Dev.), percentage of the
total deviance explained by each factor (% Expl.), and *p*-values are
presented).

	Predictor	<i>p</i> -value	Res. Dev.	Deviance	% Expl.
	NULL		23.941		
	Main Effects				
	Month	4.38E-07	20.136	3.805	15.893
	NAO	0.003	19.772	4.169	1.520
	SST	<2E-16	12.672	11.269	29.656
Soles	Scalar wind	0.042	12.098	11.843	2.398
	Rainfall	0.007	11.942	11.999	0.652
	Interactions				
	Month : SST	0.012	11.499	12.442	1.849
	NAO : Scalar wind	0.049	11.386	12.555	0.471
	Total explained				51.317
	NULL		34.797		
	Main Effects	1 26 - 11	76 170	0 210	22 007
Bastard sole	Rainfall	3.38E-05	25.047	0.319 9.750	4.112
	Interactions				
	SST : Rainfall	0.013	24.130	10.667	2.635
	i otal explained		00.004		30.654
	NULL Main Effects		90.981		
	Month	9.52E-06	76.350	14.631	16.081
Flounders	NAO	6.49E-04	74.204	16.777	2.359
libunders	Rainfall	7.32E-04	37.588	53.393	2.330
	Interactions		20 502	60.200	
	Month : 551	6.36E-10	30.582	60.399	6.506
			22 030		05.195
	Main Effects		27.039		
Spotted flounder	Month	0.003	25.597	1.442	5.333
	Scalar wind Rainfall	0.040 8.47F-04	25.027 23.750	2.012 3.289	2.108 4.723
	Total explained		_000	2.200	12.164
	NULL		31.732		
Turket	Main Effects				
i urdot	Month SST	9.14E-05 5.11E04	29.000 28 379	2.732	8.609 1.958
	Total explained	5.11L0-f	20.375	5.555	10.567
	NULL		28.499		
	Main Effects				
	Month	7.35E-10	21.853	6.646	23.320
Brill	SST	<2E-16	12.816	15.683	30.941
Di III	Rainfall	0.022	12.815	15.684	0.004
	<i>Interactions</i> Month : SST	0.004	12,107	16,392	2,484
	SST : Rainfall	0.043	12.091	16.408	0.056
	Total explained				56.746
	NULL		95.402		
Megrims	<i>Main Effects</i> Month	0.041	93,631	1.771	1.860
	Total explained	0.071	JJ.0J1	1.//1	1.860

For turbot landings an increasing trend was showed. The highest LPUE value was registered in 2005 (0.01 tonnes vessel⁻¹) (Figure 6.2f). Month (8.61%) and SST (1.96%) were the most important explanatory variable, and the model explained 10.57% of the deviance (Table 6.1). This species was mainly landed in summer and autumn (Figure 6.4d).

A decreasing trend was observed in brill landings values between 1992 and 2005. The highest LPUE value was registered in 2005 (0.01 tonnes vessel⁻¹) (Figure 6.2g). SST was the most important predictor for this species (30.94%) and month was also significant (23.32%). The model explained 56.75% of the deviance (Table 6.1). Highest LPUE were obtained for low SST values (Figure 6.3d). This species was mainly landed in summer and autumn (Figure 6.4e).

Megrims landings presented an increasing trend between 1992 and 2005. The LPUE highest value was registered in 2004 (0.01 tonnes vessel⁻¹) (Figure 6.2h). Month was the most important predictor for this group (1.86%). The model explained 1.86% of the deviance (Table 6.1). These species were mainly caught in summer and autumn (Figure 6.4f).



Time Series Analysis of Flatfish Landings

Figure 6.4. Landings per unit effort (LPUE) of flatfish species or group of species according to month. (a) Soles; (b) flounder; (c) spotted flounder; (d) turbot; (e) brill; (f) megrims.

DISCUSSION

The data used in fisheries management are often of poor quality. This is particularly relevant when assessing and managing fleets that target a large number of resources and use several fishing gears, as is the case of flatfish fisheries in Portugal. In the present study, we used the only possible information to assess the fishing effort (number of boats per fleet component), that, although being a proxy to the real effort, is surely biased. Nonetheless, it is expected that the bias introduced when using this estimator is presumably stable over a large number of years, which do not compromise the trends obtained in the analyses, nor the interpretation of LPUE

variability. Another source of bias was the use of landings, instead of catches. For fishes with a high commercial value, like the majority of flatfishes, the amount of the catches that is not landed, but sold directly in markets, can be high (it may reach 30% to 40% in some seasons and areas). Thus landings are really subestimates of the catches for these resources.

Flatfish landings showed an increasing trend in Portugal in the last decades, which has also been reported all over Europe. In the last 50 years, the increase was near 40% in Europe (228700 to 388783 tonnes) (source: FAO). In Portugal, this increase was even more pronounced, reaching 70%: 394 tonnes, in the 1950; 1270 tonnes, in 2005 (source: DGPA).

Among the environmental factors considered in GLM, month and SST were the ones for which significant relationships with LPUE were found for the large majority of the species. A marked seasonal pattern of LPUE was found for most species or species groups, which should be due to seasonal migrations towards spawning grounds (e.g. Deniel, 1981; Andrade, 1990; Rijnsdorp et al., 1992), usually located at shallower areas comparatively to the habitats used in other periods of the year. Several species concentrate during spawning which can also promote high LPUE values, since fishermen have that empirical knowledge (Petitgas et al., 2003).

SST was the most important predictor explaining soles, bastard sole, flounder and brill landings. Temperature can have direct effects on survival, growth, and distribution of marine organisms. Water temperature can also have indirect effects on production in higher trophic levels by influencing primary or secondary production or may be correlated with other variables that have direct effects on production (e.g. upwelling). Temperature is a limiting factor to maturity and spawning (e.g. Rijnsdorp and Vethaak, 1997; Sims et al., 2005), egg, (e.g. van der Land, 1991; Rijnsdorp and Vingerhoed, 1994), larval (Campos et al., 1994) and juvenile development (e.g. Wegner et al., 2003; Henderson and Seaby, 2005) of flatfish species. The effect of water temperature also influences other trophic levels, namely phytoplankton, zooplankton and benthic communities (Colebrook, 1982; Tunberg and Nelson, 1998). Some authors (e.g. Salen-Picard et al., 2002; Vinagre et al., 2006; Nicolas et al., 2007) suggested that fluctuations in demersal fisheries could be linked to fluctuations in benthic resources. Benthos represent one of the most import prey of the soles, bastard sole and flounder (e.g. Marinaro and Bouabid, 1983; Molinero et al., 1991; Andersen et al., 2005) and an increase in food resources available to these flatfish species may influence their distribution and abundance.

The relationships of LPUE and some of the environmental factors included in the GLM were not significant, although its importance has been reported in other studies.

The NAO influences atmospheric variables such as wind speed and direction, air temperature, sea surface temperature, and rainfall (Hurrell et al., 2003). Marine productivity (Beaugrand and Reid, 2003), abundance of fishes (Attrill and Power, 2002) and recruitment (Ottersen et al., 2001) are also influenced by NAO. During the low NAO periods, higher temperatures result in onshore drift promoting higher larval retention inshore. Some authors have found correlations between NAO and recruitment strength and abundance of sole (Henderson and Seaby, 2005), sardine (Guisande et al., 2001), tuna (Borja and Santiago, 2001) and cod (Dippner and Ottersen, 2001). Other authors who studied the influence of environmental factors on abundance found correlations between upwelling events and larval success (Sanchéz et al., 2003), and between wind and transport of eggs and larvae (Nakata et al., 2000).

For some species considered in the present study, the variability in LPUE explained by GLM was low (e.g. spotted flounder, wedge sole, turbot and megrims), which is probably due to the fact that these species are bycatches of several fisheries and not target species (Fonseca et al., 2005; ICES, 2007).

Scientific studies to sustain an improvement of management measures are scarce for Portuguese flatfish fisheries. The knowledge about these fisheries does not reflect the importance of these resources. Although some regulations exist concerning the total allowable captures (TAC), fishing quotas and minimum legal size (MLS) for some flatfish species and fishing areas, the application of technical conservation measures and the mitigation of fishing effort was never essayed and these fisheries are mainly unassessed and unmanaged.

The main goal of fisheries management has shifted from maximising yields to maintaining sustainable fisheries, and for that purpose a reliable stock assessment and management plan are an urgent need. In Portugal, only megrims have been assessed, based on two indicators: fishing mortality and spawning stock biomass (SSB) (ICES, 2007); although megrims are not extremely important flatfish resources and are a bycatch of anglerfish and hake fisheries (ICES, 2007). Soles are the most important species group in Portuguese flatfish fisheries, but are not assessed. This situation is of deep concern, since a decreasing trend in LPUE has been determined for the period between 1992 and 2005.

Several authors discussed the management of flatfish fisheries (e.g. Daan, 1997; Rice and Cooper, 2003; Kell et al., 2005). Some of the problems in the management of Portuguese flatfish fisheries are the lack of assessment of resource abundance and the limitation of management measures to net and mesh sizes, minimum legal size and TAC. TAC have been proposed only for sole (*S. solea*) and megrims and are relative to

wide geographical areas (ICES areas), being difficult to assign a certain quota to a particular fishing fleet. Also, since the species are not usually sorted at species level at fish houses, it is extremely difficult to evaluate if these restrictive measures are being effective.

In this study we showed some species are mainly caught during the reproduction season, when individuals concentrate in some areas of the continental shelf. Temporal and spatial closures should be implemented for these stocks that evidence overexploitation. The protection of nursery grounds (species such as soles or flounder use estuarine systems and shallow coastal areas as nursery grounds) would also be extremely important to the recovery of overexploited stocks.

Catches trends constitute a conceptually simple and meaningful indicator to characterize fisheries status. Nonetheless, the use of this indicator to define sustainability and to characterize the 'health' of fish stocks might be dangerous and controversial (Mullon et al., 2005). In soles trammel nets fishery, the only possible evaluation of stock is based on official landings data, which are incomplete. Thus, measures to incentive fishermen to land an increasing fraction of fish caught should be implemented. More studies focused in species abundance and fishing effort estimation of Portuguese flatfish fisheries are necessary in order to determine the status of stocks and adopt adequate management measures.

In conclusion, although no evaluations of the exploitation status of flatfish stocks exist in Portugal, there are strong concerns and probably overexploitation of these resources, based on evidences reported in this study, as well as in information for the adjoining (North coast of Spain and France) and more distant geographical areas in the North Atlantic (Rice and Cooper, 2003)

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Chapter 7

Flatfish fisheries in the Portuguese coast Fisheries Research (*submitted*) By Teixeira, C.M., Batista, M.I., Cabral, H.N.

Flatfish fisheries in the Portuguese coast

ABSTRACT: Flatfishes represent an important resource in Portuguese fisheries. Flatfish landings represent a low percentage of total fish weight landed, but its importance is higher when landing commercial value is considered (11%). Official data on flatfish landings from 1992 to 2005 for all landing ports of Portugal was analysed together with vessels characteristics in order to detect patterns in flatfish fisheries. The fleet characteristics of the targeting flatfish are heterogeneous but most of the vessels are multi-gear. Small vessels caught species that occur near coastal areas, mainly soles, bastard sole, flounder, turbot and brill, and octopuses, rays and cuttlefish. Megrims and spotted flounder were caught with others species, like small pelagic fishes, anglerfishes and hake by coastal trawlers. For this fleet component, catches of flatfish are usually bycatch. Generalized Linear Models (GLM) were used to analyze flatfish landings (LPUE landings per unit effort) between 1992 and 2005 and to evaluate its relationships with several variables. Main effects of the models included year, month, landing port and length vessel class. The models explained between 15% and 60% of the variability of the LPUE for the flatfish groups considered, with landing port, length vessel class and month being the more important factors. These results suggest a high spatialtemporal variability. The technical characteristics of the vessel (length) also contributed to LPUE variability. In order to avoid overfishing, a management program should be adopted for which a more in depth knowledge of flatfish stocks is required.

Keywords: flatfish, landings, fleet characteristics, generalized linear models, fisheries management, Portugal

INTRODUCTION

Flatfishes have nearly global occurrence in marine habitats, ranging from the southern Arctic Ocean to continental seas off Antarctica, but the largest quantities are caught in the temperate and boreal zones of the Northern Hemisphere (Munroe, 2005).

In 2005, flatfish catches represented 1.5% of the world marine landings and near 70% of these were in the North Atlantic (721950 tonnes) (source: FAO). Although flatfish

constitute only a minor part of total fish resources, they include highly priced species and support important fisheries.

Fishing is a traditional, culturally important activity in Portugal, and is dominated by small, local fishing vessels (Baeta and Cabral, 2005). Flatfish fisheries are widely spread over the Portuguese coast and traditionally have played and important socioeconomic role. Flatfish landings account for less than 4% of all the fish biomass landed in the Portuguese coast. However, the importance of flatfish fisheries is considerable higher due to the high commercial value of flatfish species, accounting for near 11% of the economical value of fish landings (source: DGPA). The most important species, in terms of landings, are the soles (*Solea solea* (Linnaeus, 1758), *Solea senegalensis* Kaup, 1858 and *Solea lascaris* (Risso, 1810)), the bastard sole (*Microchirus azevia* (Capello, 1868)), the flounder (*Platichthys flesus* (Linnaeus, 1758)), the spotted flounder (*Citharus linguatula* (Linnaeus, 1758)), the wedge sole (*Dicologlossa cuneata* (Moreau, 1881)), the turbot (*Scophthalmus maximus* (Linnaeus, 1758)), the brill (*Scophtahlmus rhombus* (Linnaeus, 1758)), and the megrims (*Lepidorhombus boscii* (Risso, 1810) and *Lepidorhombus whiffiagonis* (Walbaum, 1792)).

Artisanal multi-gears fleet is responsible for the largest share of flatfishes landings. This fleet consists of almost 6000 vessels, most of them between 5 and 17 m overall length with open deck, and thus only fish within a limited distance off the coastline (source: DGPA). However, only a fraction of these vessels actively target flatfishes, since many other species are caught in this multi-species fisheries. The main fishing gears used in flatfish fisheries are trammel and gill nets and bottom trawl. Although an operative artisanal fleet census exists in the Portuguese coast, there is not an exhaustive inventory of gear used by this fleet, thus making it unlikely that landings can be linked to a specific gear. In addition, this fishery activity is wide dispersed that makes it difficult to obtain data.

Most of the regulations in the Portuguese legislation related to flatfish fisheries concern the establishment of the minimum allowed size of captured fish and total admissible captures (TAC). The minimum allowed sizes of captured fish are established for megrims, plaice, soles, bastard sole, brill and flounder. Only the first three species are regulated with TAC in our area (ICES IX, X e CECAF). Although there are no stock evaluations for the flatfish off the Portuguese coast, their status might be a matter of concern given that these resources present strong evidence of overexploitation in close geographic areas in the Northeast Atlantic such as the Bay of Biscay.

Catch and effort data have been used to derive indices of relative abundance for many world fisheries (e.g. Large, 1992; Mejuto and García, 1996; Kimura and Zenger,

1997). Catch and effort data from commercial fisheries provide one of the most readily available sources of information to assess the condition of exploited stocks (Gulland, 1956; Gavaris, 1980).

Multi-species fisheries exhibit space- and time-varying fishing practices, whereby a given fleet, may change its target species, gear type or fishing location over short time scales (weeks to months). These changes may reflect the local abundance of the resource or may be directed by market considerations, such as consumer preferences. The impact of the fleet on the resources mortality is thus, a complex relationship between fishing practice at certain times of the year and locations. To model this relationship it is necessary to identify the fishing tactics or *métiers* (Biseau, 1998) existing in a given fishery. The fishing tactics in a fishery are determined, for example, by target species, gear type, fishing location and fishing season using multivariate ordination and classification methods (e.g. Pelletier and Ferraris, 2000; Pech et al., 2001).

Several authors (e.g. Hilborn and Walters, 1992; Goñi et al., 1999; Mahévas et al., 2004) have used generalized linear models (GLM) (McCullagh and Nelder, 1989) as a means of accounting for technical and biological factors to model landing per unit effort (LPUE) variations. GLM may combine continuous and categorical variables in a single model, helping determine the relative importance of the variables affecting LPUE.

The present study was planned as a combined approach to obtain typologies (Pelletier and Ferraris, 2000) for describing the flatfish fishery of the Portuguese coast. We identify the gears used and determine the LPUE by analysing monthly landings data from an important fraction of the artisanal fleet during a long period (1992–2005). The methodological approach applied, that showed to be useful when studying large amounts of uncharacterised landings data, provides a more complete account of this important fishery in the Portuguese coast and could be the basis for the implementation of management measures.

MATERIALS AND METHODS

Data Source

Commercial fishing data use in these analyses was obtained from the Governmental Fisheries Bureau (Direcção Geral das Pescas e Aquicultura – DGPA). The data used in this study spanned a 14 years period (from January 1992 to December 2005), all

landings data (in weight and in value), included the year, month, vessel number, fishing gear and landing port at which the fish were caught. In addition, the technical characteristics of each vessel (gross tonnage, engine power, length, age and hull material) were also recorded.

The entire flatfish fleet is composed of 5942 vessels operating off the Portuguese coast (Figure 7.1). This fleet catch a wide diversity of species (near 290) and for identifying different métiers several species groups were considered, for flatfish: soles, bastard sole, flounder, spotted flounder, wedge sole, turbot, brill and megrims; and for roundfish and cephalopods: hake (Merluccius merluccius (Linnaeus, 1758)), cuttlefish (Sepia officinalis Linnaeus, 1758), octopuses (Octopus vulgaris Cuvier, 1797 and Eledone cirrhosa (Lamarck, 1798)), seabreams (Boops boops (Linaeus, 1758), Dentex dentex (Linnaeus, 1758), Dentex macrophathalmus (Bloch, 1791), Dentex marroccanus Valenciennes, 1830, Diplodus annularis (Linnaeus, 1758), Diplodus cervinus (Lowe, 1838), Diplodus puntazzo (Cetti, 1777), Diplodus sargus (Linnaeus, 1758), Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817), Diplodus bellottii (Steindachner, 1882), Lithognathus mormyrus (Linnaeus, 1758), Oblada melanura (Linnaeus, 1758), Pagellus acarne (Risso, 1827), Pagellus bogaraveo (Brünnich, 1768), Pagellus erythrinus (Linnaeus, 1758), Pagrus auriga Valenciennes, 1843, Pagrus pagrus (Linnaeus, 1758), Sarpa salpa (Linnaeus, 1758), Sparus aurata Linnaeus, 1758, and Spondyliosoma cantharus (Linnaeus, 1758)), small pelagic fishes (Sardina pilchardus (Walbaum, 1792), Trachurus trachurus (Linnaeus, 1758), Trachurus picturatus (Bowdich, 1825), Trachurus mediterraneus (Steindachner, 1868), Scomber scombrus Linnaeus, 1758, and Scomber japonicus Houttuyn, 1782), rays (Raja brachyura Lafont, 1873, Leucoraja circularis (Couch, 1838), Raja clavata Linnaeus, 1758, Raja montagui Fowler, 1910, Leucoraja naevus (Müller & Henle, 1841) and Raja undulata Lacepède, 1802) and anglerfishes (Lophius budegassa Spinola, 1807 and Lophius piscatorius Linnaeus, 1758), and the remaining species were grouped in "others".



Figure 7.1. Map of mainland Portugal landing ports (1. Caminha; 2. Viana do Castelo; 3. Póvoa do Varzim; 4. Leixões; 5. Aveiro; 6. Figueira da Foz; 7. Nazaré; 8. Peniche; 9. Lisboa; 10. Sesimbra; 11. Setúbal; 12. Sines; 13. Sagres; 14. Portimão; 15. Quarteira; 16. Faro; 17. Olhão; 18. Tavira; 19. Vila Real de Santo António).

The monthly LPUE for each vessel and for each species group was calculated by summing the species total monthly landing per vessel and dividing by the monthly number of landings (number of days at the sea; being landing relative to one day of fishing). Thus, the LPUE for each species group is the monthly average kg per month per vessel and was combined for all vessels.

Statistical Methods

The vessels that landed less than 1000 kg of flatfish per year along the time series were discarded for the analysis. The final data filtering resulting in 375 vessels that represented 73% of total flatfish landing. To avoid an excessive number of vessels,

three groups were established according to the flatfish landings: group 1 (G1) with landings >1000 kg of flatfish per year and \leq 2000 kg of flatfish per year; group 2 (G2) with landings >2000 kg of flatfish per year and \leq 5000 kg of flatfish per year and group 3 (G3) with landings >5000 kg of flatfish per year.

Due to the large amount of compiled data, the first step of the analysis was to obtain a geometrical representation of both cases and variables that could be easily interpreted, thus providing a reduced description of the large data set (Pelletier and Ferraris, 2000). Canonical correspondence analysis (CCA) is a useful tool to describe fishing tactics, since it provides information about the relation between vessel's characteristics (gross tonnage, length, engine power, hull material and fishing gear) and landings (flatfishes and others groups). All calculations were performed using CANOCO (CANOnical Community Ordination) version 4.5 (ter Braack and Šmilauer, 2002).

To investigate the variation in LPUE for flatfishes species (soles, bastard sole, flounder, spotted flounder, wedge sole, turbot, brill and megrims) in relation to year, month, landing port and technical characteristics of vessel (gross tonnage, length and engine power), a GLM (e.g. Chambers and Hastie, 1992; Hilborn and Walters, 1992) was applied. Due to the fact that the technical characteristics of the vessels were highly correlated ($r \ge 0.96$), we use the only vessel length. Based on the frequency distribution of vessel length, were assigned to four vessel length class: class 1: ≤ 7 m; class 2: ≥ 16 and ≤ 8 m, class 3: ≥ 22 and ≤ 17 m and class 4: ≥ 23 m.

Several authors have applied GLM (McCullagh and Nelder, 1989) to the problem of estimating standardised catch rates (e.g. Maynou et al, 2003; Sánchez et al., 2004; García-Rodríguez et al., 2006). GLM are an extension of linear models allowing the incorporation of non-normal distributions of the response variable and transformations of the dependent variables to linearity (McCullagh and Nelder, 1989). The general form of GLM is

$$g(\mu) = \beta^t x$$

whereby a link function $g(\cdot)$ is used to achieve linearity in the parameters β^t of the dependent variables *x*.

A second part of the model involves the specification of a variance function (ϕ) that relates the variance of the response variable to the mean:

$$Var(Y) = \varphi V(\mu)$$
, with φ constant

The general model used was:

$$\ln \mu_{\gamma m p \nu} = \alpha + \beta_{\gamma} + \theta_m + \lambda_p + \delta_{\nu},$$

where μ_{ympv} is the expected LPUE for year y and month m, landing port p and vessel class v and in this work, α the LPUE obtained in January 1992 by vessel class 1 at Aveiro port; β_y the LPUE in the year y relative to 1992; θ_m the LPUE in month m relative to January; λ_p the difference between Aveiro port and the other landing ports; and δ_v the efficiency of vessel class v relative to class 1.

When zero values were eliminated for landings, it is seen that data may be close to lognormal, which implies that a lognormal or gamma distribution may be appropriate for positive values (Stefánsson, 1996). The results of Myers and Pepin (1990) suggested that the use of the gamma density is preferable to the use of a lognormal density for fisheries data, although this seems to apply mainly when there is a considerable probability of small observations, not dealt with otherwise (Pennington, 1991) and, in other instances, the gain is minor (Firth, 1988). Although other members of the exponential family could be used, the gamma density is what will be used here when the positive values are under consideration (Stefánsson, 1996).

The goodness-of-fit of the models was assessed by comparing their relative contribution to total deviance explained. The model was fitted in R environment (R Development Core Team, 2005) using the gamma distribution with a log-link function, adding the first order interactions whenever it result in a better models (high proportion of deviance explained).

All statistical analyses were performed using R software (R Development Core Team, 2005). A significance level of 0.05 was considered in all test procedures.

RESULTS

The flatfish fleet consisted mostly in wood vessels (78%); the fibre vessels represented 18% and only 4% of the vessels were constructed in steel. The average age of these vessels was 27.8 years and the mean values of total length, gross tonnage and engine power were 16.4 m, 54.4 tonnes and 200.2 kW, respectively (Table 7.1).

			Age	(years)			GT (tonnes)			Lengt	(m) (đ	kw)		Hull	naterial	(%)
	Number	min	тах	average	ps	min	max	average	ps	min	лах ал	verage	ps	min	тах	average	ps	Fibre	Wood	Steel
Caminha	7	15.0	49.0	31.0	11.0	2.9	14.7	7.9	4.9	8.0 1	3.4	10.2	1.9	50.0	140.0	91.6	34.7	0.0	100.0	0.0
Viana do Castelo	13	10.0	49.0	29.6	12.8	8.4	223.0	92.1	87.7	11.7 3	2.8	22.0	9.3	64.0	950.0	412.0	314.8	0.0	53.8	46.2
Póvoa do Varzim	112	3.0	49.0	21.2	9.4	2.8	187.0	27.6	21.0	7.7 2	8.6	15.8	2.8	40.0	630.0	193.3	79.3	0.9	93.8	5.4
Leixões	13	16.0	52.0	33.8	9.2	4.3	266.0	91.2	107.7	9.2 3	4.5	19.4	10.6	59.0	1455.0	399.9	446.4	0.0	69.2	30.8
Aveiro	43	6.0	48.0	23.6	11.5	0.9	366.0	179.7	73.7	7.0 3	4.2	26.2	6.7	9.9	1000.0	638.9	230.4	2.4	12.2	85.4
Figueira da Foz	12	8.0	49.0	30.3	13.2	92.0	240.0	189.0	47.0	24.0 3	4.3	30.0	3.3	440.0	1000.0	718.3	183.6	0.0	66.7	33.3
Nazaré	1	'	,	29.0	,	'	,	9.3	ı	ï		12.2	·	ı	ı	111.0	ı	0.0	100.0	0.0
Peniche	25	10.0	56.0	24.3	11.5	2.5	310.0	89.8	104.4	7.3 3	5.0	19.2	9.1	40.0	940.0	353.5	306.1	12.0	64.0	24.0
Lisboa	19	12.0	54.0	27.0	9.6	1.2	275.0	25.1	63.2	5.8 3	4.1	12.9	7.8	35.0	1455.0	210.1	336.9	0.0	100.0	0.0
Sesimbra	15	4.0	59.0	37.9	15.4	2.0	20.0	8.7	4.9	7.1 1	4.7	10.9	2.0	35.0	140.0	89.6	29.4	0.0	93.3	6.7
Setúbal	35	8.0	61.0	35.5	13.8	3.6	15.7	7.8	3.7	7.8 1	3.7	10.3	1.6	35.0	182.0	89.5	38.3	0.0	100.0	0.0
Sines	16	5.0	50.0	22.8	13.6	0.9	192.0	21.1	46.3	5.3 2	4.0	11.5	4.3	40.0	600.0	123.1	134.6	6.3	75.0	18.8
Sagres	17	10.0	56.0	33.3	17.4	2.6	36.7	12.4	10.4	8.1 1	7.0	11.8	3.1	35.0	195.0	104.9	52.8	11.8	82.4	5.9
Portimão	7	7.0	64.0	35.0	24.6	14.2	215.0	46.7	74.3	12.4 3	2.1	16.8	6.9	110.0	630.0	210.6	188.2	0.0	71.4	28.6
Quarteira	2	25.0	30.0	27.5	3.5	4.9	11.3	8.1	4.6	8.3 1	2.2	10.2	2.7	74.8	160.0	117.4	60.3	50.0	50.0	0.0
Faro	9	9.0	48.0	33.0	15.0	3.4	15.2	9.3	5.2	8.9 1	4.8	11.7	2.4	40.0	150.0	96.9	49.1	0.0	100.0	0.0
Olhão	13	3.0	81.0	41.8	21.2	5.3	82.1	20.4	20.4	9.8 1	8.5	13.5	2.7	36.0	461.0	144.1	111.6	7.7	92.3	0.0
Tavira	7	26.0	48.0	36.4	8.1	5.7	22.3	13.7	5.4	9.2 1	4.1	12.4	1.9	60.0	175.0	115.6	43.5	0.0	100.0	0.0
V.R.Santo António	12	3.0	86.0	36.3	25.4	1.4	140.6	30.4	36.8	6.0 2	3.0	14.5	5.6	60.0	494.0	201.5	120.5	25.0	66.7	8.3
Total fleet				27.8				54.4				16.4				200.2		78	18	4

Table 7.1. Main characteristics of vessels that landed flatfish values of age gross tonnages, length, engine power and hull material.

Vessels that caught different flatfish species presented different technical characteristics (Figure 7.2). Soles, bastard sole and turbot were caught by vessels with low gross tonnage, length and engine power.



Figure 7.2. Technical characteristics of flatfish fishery fleet clusters according to main landed species: (a) gross tonnage; (b) vessel length; (c) engine power. (standard deviation above bars).

The CCA identified different fleet components. In the analysis that included the vessels responsible for flatfish landings between 1000 kg and 2000 kg per year, wood/fibre vessels were mainly associated with landings of bastard sole, wedge sole, turbot, brill, spotted flounder, and also of hake, cuttlefish, octopuses, rays and anglerfishes; trawlers caught megrims and small pelagic fishes. Soles, flounder, seabreams and a wide diversity of other fish ("others") were associated with the multi-gear fleet (Figure 7.3a).

In the second group of landings (Figure 7.3b), vessels with flatfish landings between 2000 kg and 5000 kg per year, soles, bastard sole, flounder, turbot, brill, cuttlefish, octopuses, rays and anglerfishes were associated with wood vessels and purse seine/multi-gear. Megrims, spotted flounder, hake and small pelagic fishes were caught mainly by trawlers. Fibre vessels landed higher quantities of wedge sole, seabreams and "others".

The more important vessels in terms of landings, i.e. those with landings of flatfishes higher than 5000 kg per year (Figure 7.3c), showed an association between soles, bastard sole, flounder, turbot, brill, cuttlefish, octopuses, rays and "others" and wood vessels operating with multi-gear/purse seine. Steel vessels operating with trawl landed higher quantities of megrims, spotted flounder, wedge sole, small pelagic fishes, hake, seabreams and anglerfishes. Soles and flounder were caught more intensively by wood vessels operating with several gears, and landings of these species were associated with other flatfish species (bastard sole, turbot and brill) and with cuttlefish, octopuses and rays. Megrims were caught mainly by trawlers, which also landed other flatfish species (spotted flounder and wedge sole) and small pelagic fishes, hake and anglerfishes.



Figure 7.3. Canonical Correspondence Analyses (CCA) ordination diagrams: (a) group 1 (G1) with flatfish landings >1000 kg per year and ≤2000 kg per year, (b) group 2 (G2) with landings >2000 kg per year and ≤5000 kg per year, (c) group 3 (G3) with landings >5000 kg per year. (GT – gross tonnage).

Monthly variation of flatfish LPUE (kg month⁻¹vessel⁻¹) and market price (\in kg⁻¹) are showed in Figure 7.4. This fishery presented a high seasonality, being higher LPUE values registered in colder months (January and February) compared to summer (July), when the lowest LPUE values were recorded. Monthly variation in market price (\in kg⁻¹) also showed a high seasonal variation, being the highest values attained in summer (August) and the lowest in the winter (January and February).

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Figure 7.4. Monthly LPUE (solid line) and market price (dashed line) values, between 1992 and 2005.

The results of the GLM applied to soles LPUE pointed out that the most important explanatory variables were landing port and vessel length (8.28% and 4.80% of deviance explained, respectively), and the model explained 25.46% of the deviance (Table 7.2). LPUE values presented a marked seasonal variation, being peak values obtained in January and February (Figure 7.5a). Leixões was the port where the highest quantities of this flatfish group were landed (Figure 7.6).

The GLM of LPUE of bastard sole showed that landing port was the most important explanatory variable (25.76% of deviance explained), and the model explained 38.76% of the deviance (Table 7.2). LPUE showed also a high seasonality, with highest values registered between November and March (Figure 7.5b). The most important landing port was Olhão (Figure 7.6).

For flounder, landing port was the most important explanatory variable in the applied GLM (28.80% of deviance explained; the model explained 43.03% of the deviance) (Table 7.2). Highest LPUE values were relative to January and February (Figure 7.5a), and Lisboa was the most important landing port (Figure 7.6).

The analysis performed for spotted flounder revealed that landing port and vessel length were the most important explanatory variables (20.34% and 9.44% of deviance explained, respectively), and the model explained 47.83% of the deviance (Table 7.2). Highest LPUE values were registered from March to September (Figure 7.5b), and Olhão was the most important landing port (Figure 7.6).
Year North Year North Perri Year:Toold Perri Perri Perricite Perricite <th< th=""><th></th><th></th><th></th><th></th><th>Main efi</th><th>fects</th><th></th><th></th><th></th><th>Intera</th><th>actions</th><th></th><th></th><th>Total</th></th<>					Main efi	fects				Intera	actions			Total
NUL NUL 0.0001				Year	Month	Port	Length	Year:Month	Year:Port	Year:Length	Month:Port	Month:Length	Port:Length	explained
Pervalue $-2E-16$ $<2E-16$ <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <t< th=""><th></th><th></th><th>NULL</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></t<>			NULL											
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Deviance 755 2583 9290 13173 14960 16329 17249 18548 20603 % Expl. 0.93 2.27 8.28 4.80 2.21 8.28 4.80 2.54 2.54 % Expl. 0.93 2.27 8.28 4.80 0.001 0.001 6.0001 6.0001 0.001 7.50 2.54 P-value 71403 71569 70558 52.20 5001 4053 48232 47900 47550 47449 43758 Periance 134 715 9.010 2.1368 0.01 2.001 40501 4750 47490 4750 47499 4757 Periance 134 715 3.012 2.1463 2.1410 4.7550 4.7490 4.7550 4.7499 4.7575 Periance 1.141 7.158 2.1768 2.3171 2.3532 2.3954 7.5753 Periance 1.141 2.168 2.1768 2.1461 4.0100 <	Soles	Res. Dev.	80924	80169	78341	71634	67751		65964	64595	63675	62376	60321	25.46%
		Deviance	0	755	2583	9290	13173		14960	16329	17249	18548	20603	
p-value p-value 3.88E-06 <2E-16 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <t< td=""><td></td><td>% Expl.</td><td></td><td>0.93</td><td>2.27</td><td>8.28</td><td>4.80</td><td></td><td>2.21</td><td>1.69</td><td>1.14</td><td>1.60</td><td>2.54</td><td></td></t<>		% Expl.		0.93	2.27	8.28	4.80		2.21	1.69	1.14	1.60	2.54	
Bastard bev. Res. bev. 71403 71269 7058 52296 5071 49535 48323 47590 47449 43728 sole 0.ev 134 745 1910 21332 21768 23171 23503 23954 5701 5705 velue 0.19 0.19 2132 21768 2171 23503 23953 23954 5705 velue 0.19 0.19 0.85 25.76 3.08 0.61 1.96 0.760 0.140 5.17 velue 4.21E-05 1.31E-12 0.0001 <0.0001		<i>p</i> -value		3.88E-06	<2E-16	<0.0001	<0.0001	0.0417	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Sole Deviance 134 745 1917 21332 23171 23503 23553 23954 27675 % Expl. 0.19 0.85 25.76 3.08 0.61 1.96 0.50 0.14 5.21 % Expl. 0.19 0.85 25.76 3.08 0.61 1.96 0.50 0.14 5.21 % Expl. 0.19 0.85 25.76 3.08 0.610 <0.001	Bastard	Res. Dev.	71403	71269	70658	52296	50071	49635	48232	47900	47550	47449	43728	38,76%
	sole	Deviance	0	134	745	19107	21332	21768	23171	23503	23853	23954	27675	
p-value 4.21E-05 1.31E-12 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001		% Expl.		0.19	0.85	25.76	3.08	0.61	1.96	0.46	0.50	0.14	5.21	
He. 65259 64782 63844 45047 42132 41431 41302 41099 40190 38593 Flounders Dev. 477 1415 20212 23127 23828 23957 24160 25069 26666 % Expl. 0.73 1.44 28.80 6.64 1.07 0.20 0.31 1.39 2.45 % Expl. 0.73 1.44 28.80 6.64 1.07 0.20 0.31 1.39 2.45 % Expl. 0.73 1.44 28.80 6.64 1.07 0.20 0.31 1.39 2.45 P-value 2E-16 <0.001		<i>p</i> -value		4.21E-05	1.31E-12	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	< 0.0001	
Deviance 477 1415 20212 23127 23828 23957 24160 25069 26666 % Expl. 0.73 1.44 28.80 6.64 1.07 0.20 0.31 1.39 2.45 % Expl. 0.73 1.44 28.80 6.64 1.07 0.20 0.31 1.39 2.45 P-value <2E-16	Flounders	Res. , Dev.	65259	64782	63844	45047	42132		41431	41302	41099	40190	38593	43.03%
% Expl. 0.73 1.44 28.80 6.64 1.07 0.20 0.31 1.39 2.45 P-value <2E-16		Deviance	0	477	1415	20212	23127		23828	23957	24160	25069	26666	
<i>p</i> -value <2E-16 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <		% Expl.		0.73	1.44	28.80	6.64		1.07	0.20	0.31	1.39	2.45	
Spotted Res. Dev. 47613 47305 46004 36321 33721 33228 31821 30221 29397 28981 25231 flounder Deviance 308 1609 11292 13892 14385 15792 17392 18632 23382 % Expl. 0.65 2.45 20.34 9.44 0.43 2.96 3.36 1.73 0.87 5.77		<i>p</i> -value		<2E-16	<2E-16	<0.0001	< 0.0001	7.92E-12	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Hounder Deviance 308 1609 11292 13892 14385 15792 17392 18616 18632 22382 % Expl. 0.65 2.45 20.34 9.44 0.43 2.96 1.73 0.87 5.77	Spotted	Res. Dev.	47613	47305	46004	36321	33721	33228	31821	30221	29397	28981	25231	47.83%
% Expl. 0.65 2.45 20.34 9.44 0.43 2.96 3.36 1.73 0.87 5.77	tlounder	Deviance		308	1609	11292	13892	14385	15792	17392	18216	18632	22382	
		% Expl.		0.65	2.45	20.34	9.44	0.43	2.96	3.36	1.73	0.87	5.77	

Results of the GLM per flatfish groups (soles, bastard sole, flounder, spotted flounder, wedge sole, turbot, brill and megrims) from 1992-Table 7.2. CHAPTER 7

				Main eft	fects				Inter	actions			Total
			Year	Month	Port	Length	Year:Month	Year:Port	Year:Length	Month:Port	Month:Length	Port:Length	explained
		NULL											
	<i>p</i> -value		0.00177	0.0017	< 0.0001	<0.0001			<0.0001	<0.0001	< 0.0001	<0.0001	
Wedge	Res. Dev.	31168	31107	25691	18391	18393			18203	17980	17631	17250	%CC U9
sole	Deviance		61	5477	12777	12775			12965	13188	13537	13918	0/ 77:00
	% Expl.		0.20	15.37	23.42	17.57			0.61	0.71	1.12	1.22	
	<i>p</i> -value		0.00417	<2E-16	< 0.0001	<0.0001		< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Turbot	Res. Dev.	33998	33978	33604	30980	30403		30113	30100	30000	29980	29013	14 60%
	Deviance		20	304	3018	3595		3885	3898	3998	4018	4985	0,00.11
	% Expl.		0.06	1.10	7.66	1.69		0.86	0.04	0.29	0.06	2.84	
	<i>p</i> -value		1.82E-10	<2E-16	<0.0001	<0.0001	1.35E-06	< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Brill	Res. Dev.	37358	37205	36789	30789	28069	27983	27630	27620	27321	26893	26174	70 07%
5	Deviance		153	569	6269	9289	9375	9728	9738	10037	10465	11184	
	% Expl.		0.41	1.11	16.06	7.28	0.23	0.95	0.03	0.80	1.14	1.93	
	<i>p</i> -value		0.01	<0.0001	<0.0001	<0.0001	0.1	< 0.0001	<0.0001	<0.0001	< 0.0001	<0.0001	
Mearims	Res. Dev.	14093	14073	13959	12932	12880	12101	11098	10853	10408	10001	9003	35 27%
n)	Deviance		20	134	1161	1293	1992	2995	3240	3685	4092	5090	
	% Expl.		0.14	0.81	7.29	0.93	4.96	7.12	0.89	3.16	2.91	7.06	

Table 7.2. (Continued)



Figure 7.5. Monthly average values of LPUE (kg month⁻¹ vessel⁻¹) for (a) soles and flounder; (b) bastard sole, spotted flounder and wedge sole, and (c). megrims, turbot and brill.

The analysis of the GLM of LPUE of wedge sole showed that landing port, vessel length and month were the most important explanatory variables (23.42%, 17.57% and 15.37% of deviance explained, respectively), and the model explained 60.22% of the deviance (Table 7.2). The highest LPUE values were obtained from October

to January (Figure 7.5b). Aveiro was the port with the highest landings of this species (Figure 7.6).

For turbot, landing port was the most important explanatory variable in the GLM (7.66% of deviance explained), and the model explained 14.60% of the deviance (Table 7.2). A marked seasonal variation was also noticed, being the highest LPUE values recorded from March to May (Figure 7.5c). Figueira da Foz was the most important landing port for this species (Figure 7.6).



Soles II Bastard sole □ Flounder II Spotted flounder II Wedge sole □ Turbot II Brill II Megrims

Figure 7.6. Average values of flatfish LPUE in the mainly important landing ports on the Portuguese coast, between 1992 and 2005.

Landing port and vessel length were the most important explanatory variables for brill (16.06% and 7.28% of deviance explained, respectively; the model explained 29.94% of the deviance) (Table 7.2). Highest LPUE values were obtained between December and February (Figure 7.5c), and Leixões was the most important landing port (Figure 7.6).

Finally, for megrims, the most important explanatory variables in GLM were landing port and the interaction between year and landing port (7.29% and 7.12% of variance explained, respectively; the model explained 35.27% of the deviance) (Table 7.2). Seasonal variation was also evident, with LPUE peaks from January to March (Figure 7.5c). Highest quatities of this species group were landed at Aveiro (Figure 7.6).

DISCUSSION

The flatfish fisheries in the Portuguese coast are characterised by their multi-species and multi-gear nature. The fishing fleet that catch flatfish is mainly composed by small vessels with low gross tonnage and engine power, and about 70% of the boats constructed more than 20 years ago. This fleet is similar to small-scale fisheries fleet operating in the Mediterranean (e.g. Jiménez et al., 2004; Tzanatos et al., 2005; Piniella et al., 2007), and very different from the one of the North Sea, that is composed by recent and large beam trawlers, with high engine power (Rijsndorp et al., 2006; Hoff and Frost, 2008).

The daily choice of fishing tactics is based on a multitude of factors, including recent fisheries yield and income, knowledge on fishing grounds and the seasonal availability of resources, market demand, weather conditions, tradition, as well as information and rumours about the yield of other fishermen. Changes in the biological or economic conditions and the relative profitability of the *métiers* result in a redistribution of fishing effort (Holland and Sutinen, 1999) leading to tactic diversification.

The results of the ordination analyses that were performed highlighted that the flatfishes that are target species (soles, bastard sole, flounder, turbot and brill) were caught by wood vessels operating with gill nets and trammel nets (multi-gear fleet cluster). The small size of vessels may limit travel distances to areas surrounding homeports, especially in winter when bad weather conditions occur more often. These target species occur near shore, typically in sandy and muddy grounds in the continental shelf, from 10 to 200 m deep (Nielsen, 1986a,b; Quéro et al., 1986). Others species are captured by these vessels, namely cuttlefish, octopuses and rays. Smaller vessels are involved in relatively seasonal fisheries often switching from high income-low risk to lower income-more uncertain *métiers*. This risk-averse behaviour, when followed for many years by the owners of small vessels, can render them "area specialists" (around their homeports), as outlined by Hilborn (1985) and Pet-Soede et al. (2001).

Large vessels with highest gross tonnage and engine power operating with trawl nets landed megrims, spotted flounder, wedge sole, hake, small pelagic fishes and anglerfishes. These flatfish species occur at depths between 300 m and 800 m (Nielsen, 1986a,c; Quero et al., 1986), and are mainly bycatches of this fleet cluster.

The flatfish market prices per weight vary in opposite direction of the landings: the selling price increased during summer due to the lower landings registered in this period and to the highest market demand related with tourism. Nevertheless, the increase in flatfish market prices during summer did not compensate the profits due to

the reduction in landings, which differ from the situation reported for other flatfish fisheries (Wilde, 2003). During summer, the more valuable flatfish species are sold directly to restaurants and local fish markets, promoting parallel markets and a subestimation of landings in this season. As a consequence, the official landings records are indeed underestimates of the catches of commercially important flatfish species. Among the predictors considered in GLM, landing port, vessel length and month were the most important factors for which significant relationships with LPUE were evidenced for the majority of the flatfish species groups considered. This suggest high spatial-temporal variability of the catches of these species and technical characteristics of the vessel also contribute to LPUE variability, which was also outlined by several authors (e.g. Goñi et al., 1999; Ye et al., 2001; Maynou et al., 2003; Mahévas et al., 2004).

The exploitation patterns of flatfish species differ according to latitude, being soles, wedge sole, turbot and megrims caught in higher quantities in the north of Portugal; flounder and brill mainly caught in the central Portuguese coast; and spotted flounder and bastard sole chiefly caught in the southern coast of Portugal. Many *métiers* of the small-scale fisheries are extremely localized being present in only a few ports or a single one (Silva et al., 2002). However, other *métiers* may exist in several locations of a broader geographical area. The specific habitat and migration patterns of certain species are likely to lead to similar exploitation of these species in different locations within a broader area. Also, several flatfish species present their north or south distribution limit along the Portuguese coast which may also explain these differences (Ekman, 1953; Briggs, 1974).

The large variability observed in LPUE of the flatfish species groups considered in this study suggest that resource abundance may also be extremely variable seasonally and interannually. Teixeira and Cabral (2009) showed that these species were caught mainly during the spawning season, when the species concentrate near the coast, which can also induce increase of LPUE values.

Several authors have studied the relationships between vessel characteristics and fishing power or fishing effort (e.g. Houghton, 1977; Biseau, 1991). Fishing effort depends not only on the vessel characteristics, but also on crew (Taylor and Prochaska, 1985; Le Pape and Vigneaux, 2001) and on gear technology and on-board equipment (Marchal et al., 2007).

Effective fisheries sustainable development requires a significant investment to collect the needed information (FAO, 1999; Garcia and Staples 2000). Scientific studies to sustain an improve management measures are scarce for Portuguese fisheries. Some of the problems in the management of flatfish fisheries are the lack of assessment of resource abundance and the limitation of management measures to net and mesh sizes, minimum fish size and TAC. This is of particular concern since there are evidences of flatfish stocks overexploitation: there are 16 flatfish stocks considered overfished in the ICES region and 9 in the NAFO area (Rice et al, 2003). Some management measures are in practice in these areas, namely a recovery plan for sole in Bay of Biscay (COM, 2003). Following a precautionary approach, these measures should probably be expanded to adjoining areas together with the development of management-oriented studies.

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CHAPTER 8

Catches of target species and bycatches of an artisanal fishery: the case study of a trammel net fishery in the Portuguese coast Fisheries Research (*submitted*) By Batista, M.I., Teixeira, C.M., Cabral, H.N.

Catches of target species and bycatches of an artisanal fishery: the case study of a trammel net fishery in the Portuguese coast

ABSTRACT: In this work we studied a small-scale fishery targeting mainly soles (*Solea senegalensis* and *Solea solea*) and cuttlefish (*Sepia officinalis*) with trammel nets. The catches of target species, bycatches and discards of this fishery were studied from October 2004 until August 2005 in the west coast of Portugal. A total of 37 sampling surveys onboard commercial fishing vessels were conducted. The number and weight of individuals of all species caught were registered, as well as the amount discarded and retained by fishermen. A total of 112 species were identified: *Scomber japonicus, Chelidonichthys obscura* and *Callioynimus lyra* were the most discarded fishes, corresponding to 28% in weight and 36% in number of discards. Discards represented 22%, in weight, of the total catches, while the amount retained by fishermen for consumption or direct selling was 12%, also in weight. The overall estimate for the annual discards value due to this fishery was 174 tonnes, comprising 200 fishing vessels. Inconsistencies between the catches recorded onboard and the official landings were detected for the main target species. Some bycatches of species under recovery plans were also found.

Keywords: small-scale fishery, trammel net, bycatch, discards, Portugal

INTRODUCTION

Artisanal or small-scale fisheries are important worldwide because they account for more than a quarter of the world marine catch, contribute about a half of the landings used as human food, and employ about 90% of the world's fishermen (McGoodwin, 1990; FAO, 2003). In Portugal as in other southern European countries, small-scale fishing has a long tradition going back many centuries and is of high socio-economic importance (Stergiou et al., 1997; Baeta and Cabral, 2005). A lot of people from a highly diverse socio-cultural environment are directly and indirectly involved in small-

scale fisheries, which include generally a large number of small vessels exploiting a wide variety of species, using multiple fishing gears. In this context, multi-species catches highlight the necessity for management plans to explicitly recognize the direct and indirect impacts these fisheries have on marine ecosystems (Díaz-Uribe et al., 2007). Although their recognized importance, scientific data on small-scale fisheries is scarce and consequently they are poorly covered by adequate management plans (FAO, 2003, 2004).

Most of the Portuguese fishing vessels are included in the artisanal fisheries group, working mainly with gill and trammel nets, longlines and traps. The artisanal fleet represents near a half of the Portuguese landings, more than 60% of total revenues and near 80% of total fishermen (DGPA, 2006; INE, 2006).

Trammel nets are highly represented in the Portuguese artisanal fisheries. This fishing gear is included in various *métiers* which are characterized by different combinations of mesh sizes, fishing grounds, fishing time, season, markets and consequently target species (e.g. Borges et al., 2001; Stergiou et al., 2006). Thus, trammel net catches along Portuguese coast are composed by a widely range of species, some of them with recognized or highly expected management problems (e.g. European hake, *Merluccius merluccius* (Linnaeus, 1758), anglerfishes, *Lophius* spp., soles, *Solea* spp., cuttlefish, *Sepia officinalis* Linnaeus, 1758, and skates, *Raja* spp.).

Trammel nets fishery for targeting soles is one of the most important *métiers* of trammel net Portuguese fisheries. This fishery is of high importance in Portugal and acquires special relevance in the central coast, since 60% of total soles landings occur in this area being almost all from trammel net fishery (source: DGPA).

Discarding unmarketable, undersized or damaged fish is common practice in most fisheries worldwide, its relevance being extremely variable according to the gear used (Alverson et al., 1994). Discard estimates are necessary, not only for evaluating the impact of fishing on non-commercial species, but also on ecosystems as a whole (Alverson et al., 1994; Hall, 1999). Another relevant fact is that discards are not usually taken into account in stock assessment (Borges et al., 2005). Discarding practices are affected by bycatch composition, which are determined by environmental and social factors but are ultimately controlled by the fishing vessel crews, who are influenced by landings constraints and economic forces (Catchpole et al., 2005).

Despite the large number of studies on bycatch and discards practices worldwide (e.g. Alverson et al., 1994; Rochet et al., 2002; Sánchez et al., 2004; Catchpole et al., 2005; Hall and Mainprize, 2005; Walmsley et al., 2007), few were conducted in small-scale trammel nets fisheries. Tzanatos et al. (2007) reported about 10% for the small-scale fisheries discards in the Patraikos Gulf and Stergiou et al. (1996) in the Evvoikos

Gulf found a discard ratio for gill and trammel nets fishery under 4%. Stergiou et al. (2006) studied the trammel net catch species composition in southern European waters and concluded that both season and depth strongly affected catch species composition and that trammel net fishing occurs in 'hot spots', which present essential habitats of the life history of the targeted and associated species. In Portuguese coastal area also a few studies on trammel net fisheries and their impacts on sea grounds were conducted, but all of them along the southern coast of mainland (Erzini et al., 1997; Borges et al., 2001; Erzini et al., 2002; Erzini et al., 2006; Gonçalves et al., 2007, 2008a).

However, the composition of bycatches, namely of discards, in small-scale fisheries have received few attention and existing studies are scarce. Furthermore, the high variability inherent to small-scale fisheries leads to the need of much more information than the existing in order to apply successful measures in the minimization of the main impact of discards.

In addition to official landings and discards, catches can also include a certain amount of illegal, unreported and unregulated catches, this unknown fraction of catches could profoundly affect estimates of stock abundance and safe removal rates (Ainsworth and Pitcher, 2005).

The main goals of the present study were to characterize the multi-species fishery operating with trammel nets and targeting soles and cuttlefish in the central coast of Portugal and to evaluate the factors affecting variability in catches, bycatches and discards, in order to contribute to the improvement of small-scale fisheries management and to the reduction of bycatches.

MATERIALS AND METHODS

Study Area and Sampling Surveys

The sampling area is located within 38° and 39° North and 8° and 10° West, where depths range between 10 m and 100 m, corresponding to areas from 0.25 to 6 nautical miles off the coast. Winds are predominantly from North and Northeast directions, ranging on average from 8 to 20 knots. Sampling was usually performed with good sea conditions (waves less than 5 m high, most of the time ca. 2 m).

Samplings were carried out in fishing vessels from the two most important fishing harbours of the central coast of Portugal: Setúbal and Sesimbra (Figure 8.1), between October 2004 and August 2005. Ten vessels, randomly chosen amongst a set of voluntary fishermen, were sampled: 4 from Sesimbra and 6 from Setúbal (overall

length between 10 m and 14 m, crew from 3 to 5 fishermen). A total of 37 trips were carried out seasonally (autumn: 5 in Setúbal, 4 in Sesimbra; winter: 4 in Setúbal; 4 in Sesimbra; spring: 5 in Setúbal, 5 in Sesimbra and summer: 5 in Setúbal, 5 in Sesimbra) and 136 net sets were sampled (with a total length of about 204 km), with an average number of 38 sheets and 1.5 km in length per set. Trammel nets sampled were composed of 3 panels, usually made of polyethylene, with a mesh size of the inner panel equal to 100 mm (minimum allowed by Portuguese legislation). Each net sheet had 1.5 to 2.5 m high and ca. 40 m in length. A set of nets was usually composed by a large number of these sheets, usually with more than 2000 m long, with a gap of ca. 1 m between consecutive sheets. Net sets were anchored at each end on the sea bottom. Nets' length, haul location, depth, fishing time (total immersion time of nets) and number of sheets in each set net group were always registed.



Figure 8.1. Location of fishing harbours studied (Sesimbra and Setúbal) and of the fishing sites sampled onboard vessels from each fishing harbour.

In each survey, observers accompanied commercial fishermen during one full-day fishing trip; each trip lasted on average ca. 10 h. Vessels left the fishing harbour at night (before sunrise) and went to the fishing grounds, where the nets were set during the last fishing trip and usually began to retrieve their nets at sunrise.

The observers team was composed of two researchers (one of whom accompanied all fishing trips). When each net was retrieved, each specimen was untangled from the net by fishermen and retained or discarded. During each trip, retained captures were separated by fishermen in specimens for selling and specimens for their own consumption. The observers team identified, measured (total length to the nearest mm) and weighed (with a dynamometer, to the nearest 5 g) all retained individuals.

Discards were preserved in ice and brought to the laboratory to be identified, measured and weighed. The majority of individuals were dead when they were untangled from the nets, at least juvenile skates and rays belonging to discard were processed on board to be returned alive to the sea. All discarded individuals were also classified according to their damage condition following an empirical scale from 1 to 3 in an increasing damage level (1 was attributed to fish in good condition and 3 to severely damage fish).

Data Analysis

Total catches per species (g per 10000 m of net) and ratio between target species catches, retained bycatch and discards in weight and number were determined. Estimates of the retained portion of catches for the fishermen's own consumption and for direct sale (which is forbidden in Portugal) were also calculated. The total annual amount of discards from this trammel nets fishery was estimated based on mean weight of discards per vessel and fishing effort observed. The measure of fishing effort considered took in account number of vessels per fishing harbour, nets length, fishing days and fishing time. This estimate was calculated separately for each season and fishing harbour.

Catches by season were calculated for the eight most important species. The rank of importance was obtained calculating the revenues (in \in) of each species using the mean price of each species in the studied sites during the sample period. Captures was calculated by species, in kg per 10000 m of net. Based on these results, a comparison between Sesimbra and Setúbal vessels was done.

Correspondence Analyses (CA) were performed using Canoco 4.5 software (ter Braak and Šmilauer, 2002) in order to evaluate patterns and relationships between discards and several factors. Two CA with addition of supplementary "environmental data" (latitude, depth, fishing time, total number of species caught and total catches in weight) were performed: one using the species for which the total amount of discards were higher than 10000 kg and the other considering the species for which more than 80 individuals were discarded in overall surveys.

In order to detect the relative importance of the factors affecting discards (low or no marketable value, damage condition and low size) comparative analyses were performed for the 20 most discarded species. Of these, species without marketable value were removed from the analyses as this was the main reason for being discarded.

For each species analysed, percentage of discards was plotted against their mean damage condition. For species with a legislated minimum landing size (MLS) the same approach was applied considering the percentage of discarded individuals below MLS instead of mean damage condition.

In order to estimate the percentage of fish that were not sold by according to national regulations (Portuguese law obligates all captures to be sold in fishing docks or pass there before being sold elsewhere), a comparative analysis between the weight of the most valuable species captured (Senegalese sole, *Solea senegalensis* Kaup, 1858, common sole, *Solea solea* (Linnaeus, 1758) and turbot, *Scophthalmus maximus* (Linnaeus, 1758)) registed by observers in each trip and the weight declared to fishing docks officers by fishermen in the same days. Other possible causes for the differences between both values were negligible.

Finally, the total annual amount of discards from this trammel nets fishery was estimated based on mean weight of discards per vessel and fishing effort (nets length per vessel and number of vessels per fishing harbour). This estimate was calculated separately for each season and fishing harbour.

RESULTS

A total of 87 fish species, 9 molluscs, 5 echinoderms and 11 crustaceans were identified in the catches of this trammel net fishery (Table 8.1). The most important species in terms of weight were *S. officinalis* and *S. senegalensis* which are target species of this trammel net fishery. Captures of this species were almost always landed in fishing docks: 96.8% of *S. senegalensis* captured biomass (94.8% of total number of individuals) and 97.6% of *S. officinalis* biomass captured (96.4% of total number of individuals) were landed in fishing docks. The other target species, *S. solea*, is less important in total captures, however 97.4% of weighted captures were landed in fishing docks (95.1% of total number of individuals).

Table 8.1. Total catches (in biomass and number), retained (R) and discarded bycatch (D) values for each species caught in the trammel nets fishery in the west central coast of Portugal (in brackets values for retained species that are sold at fishing docks).

	Total	Catch	% Byc (Weig	atch ght)	% Byc (Numl	atch ber)
Species	g 10000 m ⁻¹	N 10000 m ⁻¹	R (sold)	D	R (sold)	D
Pisces						
Alosa fallax	47.0	0.1	-	100.0	-	100.0
Ammodytes tobianus	22.9	0.2	77.3 (-)	22.7	25.0 (-)	75.0
Argentina sphyraena	1.4	< 0.1	-	100.0	-	100.0
Argyrosomus regius	239.2	0.1	100.0 (57.6)	0.0	100.0 (33.3)	0.0
Arnoglossus imperialis	93.1	2.2	2.6 (-)	97.4	2.3 (-)	97.7
Arnoglossus laterna	6.4	0.1	-	100.0	-	100.0
Arnoglossus spp.	9.3	0.3	52.7 (-)	47.3	16.7 (-)	83.3
Arnoglossus thori	1.1	< 0.1	-	100.0	-	100.0
Aspitrigla cuculus	14.2	0.1	-	100.0	-	100.0
Balistes capriscus	1702.4	2.4	88.1 (-)	11.9	83.3 (-)	16.7
Belone belone	182.3	0.4	27 (-)	73.0	22.2 (-)	77.8
Boops boops	743.0	5.9	4.8 (-)	95.2	4.2 (-)	95.8
Bothidae	0.5	< 0.1	-	100.0	-	100.0
Bothus podas	14.9	0.1	-	100.0	-	100.0
Callionymus lyra	1411.7	17.5	1.7 (-)	98.3	1.4 (-)	98.6
Callionymus reticulatus	0.2	< 0.1	-	100.0	-	100.0
Capros aper	2.3.0	< 0.1	-	100.0	-	100.0
Centrolabrus exoletus	24.0	< 0.1	-	100.0	-	100.0
Chelidonichthys lastoviza	229.2	1.5	-	100.0	-	100.0
Chelidonichthys lucerna	1244.1	4.2	77.5 (-)	22.5	67.1 (-)	32.9
Chelidonichthys obscurus	2670.6	27.8	28.5 (-)	71.5	14.9 (0.4)	85.1
Chelon labrosus	60.1	< 0.1	-	100.0	-	100.0
Citharus linguatula	228.7	3.3	27.8 (-)	72.2	23.9 (-)	76.1
Conger conger	522.1	0.2	97.8 (34.0)	2.2	75.0 (25.0)	25.0
Coris julis	21.6	0.1	74.1 (-)	25.9	66.7(-)	33.3
Dasyatis pastinaca	216.5	< 0.1	100.0 (100.0)	0.0	100.0 (00.0)	0.0
Dentex dentex	20.2	< 0.1	-	100.0	-	100.0
Dentex macrophthalmus	7.5	0.1	-	100.0	-	100.0
Dentex maroccanus	14.2	0.1	-	100.0	-	100.0
Dentex spp.	10.6	< 0.1	-	100.0		100.0
Dicentrarchus labrax	637.3	0.7	86.2 (20.8)	13.8	78.6 (14.3)	21.4
Dicologlossa cuneata	922.8	11.5	62.1 (-)	37.9	48.3 (-)	51.7
Diplodus annularis	10.9	< 0.1	-	100.0	-	100.0
Diplodus sargus	123.2	0.3	93.7(-)	6.3	85.7(-)	14.3
Diplodus spp.	61.7	0.3	81.4 (-)	18.6	57.1 (-)	42.9
Diplodus vulgaris	680.4	0.4	100.0 (91.1)	0.0	100.0 (37.5)	0.0
Gymnammodytes cicerelus	2.5	< 0.1	-	100.0	-	100.0
Halobatrachus didactylus	88.8	0.1	51.2 (-)	48.8	66.7(-)	33.3
Labridae	97.2	0.2	88.1 (-)	11.9	/5.0 (-)	25.0
Labrus mixtus	12.1	0.1	-	100.0	-	100.0
Labrus spp.	22.1	< 0.1	100.0 (-)	0.0	100.0 (-)	0.0
Lepidorhombus boscii	239.1	1.8	93.4 (-)	6.6	88.9 (-)	11.1
Lepidotrigia cavillone	199.4	2.1	32.0 (-)	68.0	16.3 (-)	83./
Lepidotrigia dieuzeidei	9.5	0.2		100.0	-	100.0
	960.4	1.2	72.5 (64.6)	27.5	50.0 (45.8)	50.0
Liza spp.	45.4	0.1	-	6.8	50.0 (-)	50.0
Macioraniphosus scolopax	0.0	< 0.1	-	100.0	-	100.0
Marluagius marluagius	123.0	< 0.1	100.0(-)	0.0	100.0 (-)	0.0
Microchirus azevia	3305.4 170 C	10.2	01.7 (10.3)	39.3	42.1 (0.9)	5/.9
Microchirus azevia	420.0	2.4	at'o (15'n)	0.4 100.0	83.7 (8.2)	100.0
Microchirus veriegetus	10.1	0.2	-	100.0	-	100.0
Micromosistius poutoess	102 5	2./	2 Q () 2 Q ()	10.9	/4.1 (-) 2 Q ()	25.9 07 7
Mola mola	102.5	1.0	J.O (-)	90.Z	2.0 (-)	9/.Z
mula mula	0.10	0.2	-	100.0	-	100.0

(continue)

Table 1. (Continued)

	Total	Catch	% Byc (Weig	atch (ht)	% Byc (Numb	atch per)
Species	g 10000 m ⁻¹	N 10000 m ⁻¹	R (sold)	D	R (sold)	D
Mugil cephalus	29.6	0.1	-	100.0	-	100.0
Mullus barbatus	115.4	0.6	41.4 (-)	58.6	41.7 (-)	58.3
Mullus spp.	70.7	0.6	13.2 (-)	86.8	8.3 (-)	91.7
Mullus surmuletus	65.4	0.3	76.0 (-)	24.0	83.3 (-)	16.7
Mustelus mustelus	343.3	0.2	100.0 (100.0)	0.0	100.0 (100.0)	0.0
Myliobatis aquila	317.4	0.7	-	100.0	-	100.0
Pagellus acarne	/9/.4	8.5	55.0(-)	45.0	39.0 (-)	61.0
Pagellus erytinnus Pagellus spp	20.1	1.5	51.2 (-)	40.0	30.7 (-)	100.0
Pagrus pagrus	36.3	0.3		100.0		100.0
Pagrus spn	13 3	< 0.1	100 0 (-)	0.0	100.0(-)	0.0
Phycis phycis	265.8	0.3	97.7 (78.1)	2.3	85.7 (71.4)	14.3
Pleuronectiformes	3.7	< 0.1	-	100.0	-	100.0
Raja brachyura	5913.1	2.8	95.9 (95.0)	4.1	83.9 (80.4)	16.1
Raja clavata	6530.7	7.4	94.0 (89.3)	6.0	82.0 (80.7)	18.0
Raja miraletus	1151.8	4.1	70.0 (65.8)	30.0	63.9 (60.2)	36.1
Raja montagui	91.5	0.2	100.0 (100.0)	0.0	100.0 (100.0)	0.0
Raja spp.	154.3	0.1	79.0 (79.7)	20.3	33.3 (33.3)	66.7
Raja undulata	8512.4	4.2	98.6 (94.4)	1.4	94.2 (90.7)	5.8
Sardina pilchardus	666.3	13.1	0.2 (-)	99.8	0.4 (-)	99.6
Scomber japonicus	/524.6	61.6	4.2 (0.1)	95.8	2.9 (0.1)	97.1
Scomber scombrus	1308.4	7.5	27.2(-)	72.8	12.5 (-)	87.5 100.0
Sconhthalmus maximus	3036.7	4.6	- 99 5 (94 9)	100.0	- 97 9 (94 7)	2 1
Scophthalmus rhombus	1945 2	37	98 1 (40 4)	19	96 1 (35 5)	3.9
Scorpaena notata	318.0	3.8	-	100.0	-	100.0
Scorpaena porcus	345.8	0.8	79.4 (-)	20.6	76.5 (-)	23.5
Scyliorhinus canicula	243.7	0.7	57. 4 (26.8)	42.6	42.9 (21.4)	57.1
Serranus cabrilla	166.3	1.4	26.6 (-)	73.4	25.0 (-)	75.0
Serranus hepatus	6.9	0.2	-	100.0	-	100.0
Solea lascaris	5204.8	31.8	83.7 (41.1)	16.3	73.2 (31.0)	26.8
Solea senegalensis	29798.7	92.9	0.6 (-)	2.7	0.7 (-)	4.6
Solea solea	2352.5	5.1	1.5 (-)	1.1	1.9 (-)	2.9
Solea spp.	40.5	0.2	-	100.0	-	100.0
Soleidae	6.5	0.1	-	100.0	-	100.0
Spanuae Spanua aurata	4.2	< 0.1	100.0 (-)	124	100.0(-)	10.0
Sparus aurata Spondyliosoma cantharus	1095 7	55	50.0 (-)	13.4 49.9	37.8 (1.8)	62.2
Symphodus bailloni	2.3	< 0.1	-	100.0	-	100.0
Symphodus spp.	3.9	< 0.1	-	100.0	-	100.0
Torpedo marmorata	233.3	0.1	100.0 (100.0)	0.0	100.0 (100.0)	0.0
Torpedo torpedo	330.0	0.5	-	100.0	-	100.0
Trachinus draco	1369.4	10.4	19.9 (-)	80.1	10.4 (-)	89.6
Trachurus picturatus	10.6	< 0.1	100.0 (-)	0.0	100.0 (-)	0.0
Trachurus trachurus	517.2	5.1	57.7 (-)	42.3	40.8 (-)	59.2
Trigla lyra	176.5	0.4	93.8 (-)	6.2	77.8 (-)	22.2
Trisopterus Iuscus	2765.0	32.1	54.2 (-)	45.8	46.4 (-)	53.6
	9.0	< 0.1	-	100.0	-	100.0
Zeugopierus puncialus Zeus faber	643 3	23	764(-)	23.6	- 617(-)	28.2
Total*	104098.8	427.4	/0.+()	25.0	01.7 ()	50.5
Equinodermata	10105010					
Astropecten aranciacus	4651.0	35.0	-	100.0	-	100.0
Asterias rubens	19.6	0.1	-	100.0	-	100.0
Echinus acutus	19.4	0.1	-	100.0	-	100.0
Holothuroidea	1177.3	2.8	-	100.0	-	100.0
Marthasterias glacialis	28.5	0.4	-	100.0	-	100.0
Paracentrotus lividus	18.8	0.1	-	100.0	-	100.0
Total*	5914.5	38.5				
Crustacea	1 5	. 0 1		100.0		100.0
Carcinus maenas	1.5	< 0.1	-	100.0	-	100.0
Gonenlax rhomboides	23.3 0 4	< 0.0	-	100.0	-	100.0
Hommarus gammurus	57.6	0.1	100.0 (100.0)	0.0	100.0 (100.0)	0.0
Maia goltziana	1.1	< 0.1	-	100.0	-	100.0
Maja squinada	1979.4	2	87.3 (5.2)	12.7	87.8 (2.4)	12.2
Palinurus elephas	83.9	0.2	96.8 (96.8)	3.2	75.0 (75.0)	25.0
Pagurus forbesii	0.8	< 0.1	-	100.0	-	100.0
Liocarcinus holsatus	0.3	<0.1	-	100.0	-	100.0
Calappa granulata	7	0.1	-	100.0	-	100.0

(continue)

	Total	Catch	% Byc (Weig	atch ght)	% Byc (Numl	atch ber)
Species	g 10000 m⁻¹	N 10000 m ⁻¹	R (sold)	D	R (sold)	D
Polybius henslowii Total*	0.6 2157.9	< 0.1 3.3	-	100.0	-	100.0
Mollusca						
Atrina pectinata	283.5	1.9	-	100.0	-	100.0
Aplysia punctata	117.7	1.2	-	100.0	-	100.0
Buccinum undatum	26.8	< 0.1	100.0 (-)	0.0	100.0 (-)	0.0
Cymbium olla	1667.8	9.6		100.0		100.0
Loligo spp.	140.8	0.2	42.0 (42.0)	58.0	20.0 (20.0)	80.0
Nucella lapillus	3.2	< 0.1	-	100.0	-	100.0
Nucella puber	8.4	< 0.1	100.0 (-)	0.0	100.0 (-)	0.0
Octopus vulgaris	8681.2	3.6	100.0 (99.6)	0.0	100.0 (98.6)	0.0
Sepia officinalis	32600.8	38.1	99.4 (97.6)	0.6	2.6 (-)	1.0
Pectin maximus	46.3	0.3	2.1 (-)	97.9	16.7 (-)	83.3
Total*	43576.5	55				
TOTAL	155747.7	524.2	37.7 (12.8)	21.9	22.5 (7.0)	52.8

Table 1. (Continued)

By decreasing order of importance, *Octopus vulgaris* Cuvier, 1797, *Raja undulata* (Lacepède, 1802) and *Scomber japonicus* Houttuyn, 1782, were the most captured bycatches (in weight). However, when the number of individuals was considered, the most abundant species in catches were *S. senegalensis* and *S. japonicus*, followed by *S. officinalis*, *Astropecten aranciacus* (Linnaeus, 1758) and *Trisopterus luscus* (Linnaeus, 1758).

A total of 98 species were discarded (species that were not identified at species level were not considered) (Table 8.1) and 21.9% of catches, in weight, were discarded. Bycatch represented 59.6% of total catches, of which 41% were discarded. *S. japonicus, Chelidonichthys obscurus* (Bloch & Schneider, 1801) and *Callionymus lyra* Linnaeus, 1758, presented the highest values in weight of discards. The main discarded species in number were *S. japonicus, C. obscurus* and *A. aranciacus*. Furthermore, echinoderms were always discarded: *A. aranciacus* and sea cucumbers (Holothuroidea) were the dominant taxa of this group in discards. Crustaceans represent only 1.4% of total catches, being all species discarded, except the ones with high commercial value, i.e. *Homarus gammarus* (Linnaeus, 1758), *Palinurus elephas* (Fabricius, 1787) and *Maja squinado* (Herbst, 1788).

The trammel net fishery showed characteristics quite different in the two studied harbours, which were also variable according to seasons (Table 8.2, Figure 8.2). In general, fishing effort was higher in Setúbal than in Sesimbra and vessels included in the studied *métier* were more numerous in Setúbal. Net's soak time was higher during winter and spring, in Sesimbra, and during autumn and winter, in Setúbal, although Sesimbra's fishermen had left their nets soaked more time continuously. Based in the values, we estimated that the total volume of discards attributed to these vessels is ca. 170 tonnes per year (Table 8.2).

Tabela 8.2. Estimates of discard rates, fishing effort (net length, number of fishing days, number of vessels and mean net's soak time) and total discards per season, for Sesimbra and Setúbal trammel nets fishing fleet (in brackets are indicated the standard deviation calculated values).

Local	Season	Discards ⁻¹ Vessel ⁻¹ day(kg)	Net´s mean length ⁻¹ vessel (m)	Fishing days	N. vessels	Mean soak time (h)	Total discards (kg)
	1						
ā	Autumn	12.1 (8.0)	3882.6 (338.6)	32 (6)	12 (5)	49.5 (7.2)	4664.4
qu	Winter	45.4 (37.6)	4464.7 (801.4)	30 (10)	12 (5)	45.0 (4.3)	16348.2
esi	Spring	19.3 (3.7)	4300.5 (832.0)	30 (8)	12 (5)	35.6 (4.6)	6954.6
0	Summer	11.3 (5.3)	3846.6 (1159.0)	34 (6)	12 (5)	46.9 (3.4)	4628.9
	1						
=	Autumn	38.1 (17.0)	7694.0 (981.5)	60 (8)	30 (5)	20.6 (15.6)	68589.4
úba	Winter	13.9 (5.4)	6310.0 (1026.8)	50 (15)	30 (5)	25.6 (20.2)	20872.7
Set	Spring	16.3 (9.5)	6032.7 (1342,2)	55 (8)	30 (5)	28.2 (13.3)	26865.3
	Summer	14.3 (14.0)	5508.8 (2991.9)	55 (10)	30 (5)	24.2 (13.0)	23673.7
	TOTAL	170.9	42039.8	346	168	275.7	172597.4

R. undulata, Raja clavata (Linnaeus, 1758), *Raja brachyura* (Günther, 1880), *S. maximus, O. vulgaris* and *S. solea* were the most important retained bycatches for this fishery revenues (considering weigh captured and species economic value). Catches of cuttlefish were higher in autumn and winter and attained higher values in Setúbal than in Sesimbra (Figure 8.2a). Catches of this species were negligible, in spring and summer, in both fishing areas. The Senegalese sole was the most captured flatfish species, being caught all over the year. A marked trend with higher values in spring and summer was observed in the catches of *S. senegalensis* in Sesimbra. In Setúbal, catches were more constant and with an opposite trend in respect to Sesimbra, where a marked increase was noticeable in catches from winter to spring (maximum catches); in Setúbal a less evident seasonal variation pattern was outlined, with maximum catches in winter, decreasing in spring and summer. In general, catches of target species (Senegalese sole and cuttlefish) were always higher in Setúbal than in Sesimbra, except Senegalese sole in spring and summer.

Octopus is also captured all over the year, with high catches in Setúbal every season, except in summer (Figure 8.2b). In Sesimbra, its captures are minimal in autumn showing an increase trend to spring when attained the maximum capture rate and a little decrease between spring and summer captures was found. In Setúbal catches of *O. vulgaris* showed a different trend: captures recorded a decrease from winter (maximum captures) to summer (minimum captures); in autumn the captures had also low rates.



🛛 O. vulgaris 🛛 R. brachyura 🖾 R. undulata 🗆 R. clavata 🖿 S. maximus 🖾 S. solea

Figure 8.2. Seasonal variation of total catches of the eight species responsible from higher incomes to fishermen ((a) Solea senegalensis and Sepia officinalis; (b) Octopus vulgaris, Raja brachyura, Raja undulata, Raja clavata, Scophthalmus maximus and Solea solea), in Sesimbra and Setúbal.

As it can be seen in Table 8.1 and Figure 8.2b, the majority of skates' species had true importance as retained bycatch and there was a relevant seasonality in catches. In Setúbal, *R. undulata* was the skate most captured attaining higher captures during winter and spring. In this harbour captures of *R. clavata* were low in winter, increasing in spring to near 9 kg.10000m⁻¹net and turn down to a little bit lower captures in summer; *R. brachyura* showed the lower captures of the three

skates analysed, attaining higher captures in autumn and winter. In contrast, *R. brachyura* was the most captured skate in Sesimbra where captures were higher than those obtained to *R. undulata* in Setúbal, accounting with near 33 kg.10000 m⁻¹ of net in autumn. However, in winter and summer *R. brachyura* was low captured in both studied sites. Catches of *R. undulata* were higher in spring, achieving lower values than in Setúbal. Finally, *R. clavata* presented a near constant trend in captures during the four studied seasons, generally with lower values in Sesimbra than in Setúbal (exception obtained in winter).

S. solea and *S. maximus* were lower captured (in weigh) than the other species considered in the seasonality analyses. However, they attain the highest market values of all species sold in Portuguese fishing docks, being of really economic importance to this fishery. Vessels from Setúbal just caught *S. solea* in autumn while in Sesimbra this species was captured in all seasons, except in spring, but higher captures of *S. solea* were obtained in winter. *S. maximus* showed higher captures in summer, in Setúbal, and revealed short variation along other seasons and sites.

The CA performed regarding discarded species biomass showed that total catches of each trip, fishing time and number of species caught were the principal factors affecting discards (Figure 8.3a). The first two CA ordination axes explained 44.3% of total variance.

The CA performed based on number of individuals discarded (Figure 8.3b) revealed a similar pattern to that obtained using biomass, emphasising the role of total catches per trip, fishing time and number of species caught as important factors affecting discards. In this approach, latitude also appears as an important factor affecting discards. The first two CA ordination axes explained 42.7% of total variance. Depth appears to be a factor with low influence in discards of the studied fishery.

Both ordination diagrams obtained shows a slightly separation between discards from Sesimbra and Setúbal. Discards obtained in Sesimbra seems to be more correlated with higher depths, latitudes, species richness and fishing times and lower total catches than the ones from Setúbal surveys.

Results of CA showed that a separation between seasons occurs also. Thus, samples from autumn and winter are in general closely each other and slightly distant from samples obtained in spring and summer which are closely between them. In the analyses with the number of individuals discarded the seasonal pattern is clearer, although it occurs also in the analyses of discarded biomass. In Figure 8.3a almost all samples from spring and summer appear in the left side of the ordination diagram since they are characterized by higher diversity of species caught per trip, lower total catches in each trip and lower fishing time than in autumn and winter.



Figure 8.3. Correspondence analyses (CA) ordination diagrams based on biomass (a) and number of individuals (b) of most discarded species in the studied trammel net fishery (1. Sardina pilchardus; 2. Trisopterus luscus; 3. Pagellus acarne; 4. Boops boops; 5. Scomber japonicus; 6. Astropecten aranciacus; 7. Trachinus draco; 8. Chelidonichthys obscurus; 9. Merluccius merluccius; 10. Solea lascaris; 11. Callionymus lyra; 12. Dicologlossa cuneata; 13. Solea senegalensis; 14. Scomber scombrus; 15. Spondyliosoma cantharus). (St – Setúbal; Sb – Sesimbra; A – autumn; W – winter; Sp – Spring; Su – Summer; TC – Total catches; N. sps – Number of species; FT – Fishing time; Lat – Latitude; D – Depth).

Discarded species also showed different patterns between seasons and sites. Thus, in the analyses of biomass (Figure 8.3a), the majority of patterns found in the analyses considering number of individuals shall remained, however the interpretation of this diagram is less conclusive. In this analysis, with weight data, *S. japonicus* and *Boops boops* (Linnaeus, 1758) appears to be more correlated with Sesimbra trips than was found in the results of CA with number of individuals.

In the analyses considering the number of individuals (Figure 8.3b) *Scomber scombrus* Linnaeus 1758, *S. senegalensis*, *Dicologlossa cuneata* (Moreau, 1881), *C. lyra*, *Solea lascaris* (Risso, 1810), *M. merluccius*, *C. obscurus*, *Trachinus draco* Linnaeus, 1758 and *A. aranciacus* were the species more characteristic in discards from spring and summer while *T. luscus*, *Pagellus acarne* (Risso, 1827), *B. boops*, *S.*

japonicus and *Sardina pilchardus* (Walbaum, 1792) appear to be more related with autumn and summer fishing trips. Furthermore *S. pilchardus* discards appear to be related with Sesimbra's discards in winter; *S. scombrus*, *S. senegalensis* and *D. cuneata* are more typical in Sesimbra discards during spring and summer.

The analyses of factors influencing discards showed that near 35% of discards were from species without commercial value. Discards of species with some marketable value also occurred and more than 90% of them were in high and moderate damage conditions (damage levels 2 and 3) when discarded. However, for some species (between the 20 most discarded) the individuals discarded were still always in good conditions (e.g. *Scorpaena notata* Rafinesque, 1810 and *Myliobatis aquila* (Linnaeus, 1758)) (Figure 8.4).



Figure 8.4. Mean damage level of the most discarded species during the surveys and corresponding percentage of discards to each species considered in the analyses (in number). Each circle represents one discarded species and the circle sizes correspond to total discarded quantity (in weight).

Discards due low size of individuals caught revealed this factor is important for discards of sand sole (*S. lascaris*) being the principal factor affecting discards of this species (61% of *S. lascaris* discards) (Figure 8.5). Furthermore, discards of *Spondyliosoma cantharus* (Linnaeus, 1758), *T. luscus*, *P. acarne* and *M. merluccius* under the MLS were also significant. Some individuals of *S. senegalensis* were also discarded above the MLS. However, most of the undersized discards were also in moderate or high damage levels (Figures 8.4 and 8.5).



Figure 8.5. Relationship between percentage of individuals under minimum landing size (MLS) and percentages of discards for most discarded species which are under MLS legislation. Each circle represents one discarded species and the circle sizes correspond to total discarded quantity (in weight).

Often the catches landed at fishing docks were not exactly the same as those determined aboard fishing vessels (Figure 8.6). The major discrepancies between catches and landings weights were obtained for *S. senegalensis*: 62% and 31% of fish was not sold at fishing docks, in spring and summer respectively. The higher proportions of the catches retained by fishermen for food consumption or direct sale to local markets or restaurants were registered when mean prices were higher and also in seasons where total catches of the main 3 species (i.e. *S. senegalensis, S. solea, S. maximus*) were higher.



Figure 8.6. Seasonal variation of catches of *S. maximus*, *S. senegalensis* and *S. solea* (in weight) according with their final destiny (landed at fishing docks or other destiny, including own consumption and illegal sales). The dashed line above bars corresponds to the mean price (€ kg⁻¹) for each species in each season.

DISCUSSION

Regarding catch composition of the studied trammel net fishery, we conclude that *S. senegalensis* and *S. officinalis* were the most important species in this fishery, as would be expected since they are its target species. Although *S. solea* had been considered a target species, their significance in total catches is lower than was found to the other target species. This species apparently has lower populations in study area than *S. senegalensis*, and also shows a higher seasonal variance in captures, as well as cuttlefish, whose captures were almost inexistent during spring and summer. Likewise the seasonal increase in landings is also probably related to reproductive strategies. Cuttlefish, for example, assembles in coastal areas outside estuaries (high captures in coastal fisheries) before they enter in estuaries to spawn (Serrano, 1992). The existence of different resources in the study area is of great importance to the studied fishery, since when the principal resources have lower catch rates fishermen can slightly avoid the decline in revenues through the improvement of bycatch

species, maintained their activity economically sustainable. Autumn and winter seems to be the most profitable seasons for fishermen in the studied fishery, with high captures of most valuable species. Thus, the abundances of captures can be due to species life cycle, as highlighted above, but also due fishermen choices and also vessels and gears technical characteristics (Le Pape and Vigneau, 2001). Our results showed that in Sesimbra *S. senegalensis* captures had increased from winter to spring, while in Setúbal they experimented a slightly decrease. From winter to spring, *S. officinalis* is almost inexistent in the study area, meaning that fishermen possibly direct their effort to catch *S. officinalis* during autumn and winter and consequently captures of *S. senegalensis* are lower than they could be if fishermen exclusively had directed their effort to them. Similar considerations can also be made to the other target and relevant non-target species (e.g. skates, octopus), which emphasize that although controlled by biological and environmental factors, human economic-social constrains are extremely important in these type of fisheries (Merino et al., 2008; Reglero and Morales-Nin, 2008).

Furthermore, our results showed that there are some retained bycatch species whose relevance in final revenues is also high, such as skates (*R. clavata*, *R. undulata*, *R. brachyura*), octopus (*O. vulgaris*) and some flatfish species (*S. maximus*). Fishermen retained all species that have some commercial value but when only one or a few individuals of one valuable species were captured, fishermen kept them for personal consumption, due to the low selling value that they would have at the fishing dock, which was also concluded by Gonçalves et al. (2007) for a similar fishery in the southern coast of the country.

The results obtained in this study revealed there were a great number of species caught in this trammel net fishery. Of all species caught, 98 was discarded once at least. In the Algarve fisheries (southern Portugal), Erzini et al. (2002) found that 78 species were discarded in trammel nets fishery, while Gonçalves et al. (2007, 2008a) identified a higher number of discarded species for the same area, 105 and 156, respectively. However, lower diversity of discarded species was found in some studies regarding trammel nets fishery in the Mediterranean (Stergiou et al., 2006; Gonçalves et al., 2007).

In which respect to discards ratio, the present study found one of the highest values for percentage of discards in trammel nets fisheries (21.9% in weight and 52.8% of the total number of individuals). Percentage of discards found in this study is higher than reported in other works regarding trammel nets in the Algarve coast: Borges et al. (2001) found a discard ratio of 13% (in weight) and Gonçalves et al. (2007) determined a discard ratio of 49% (in number of individuals). Thus, differences

between discarded rates using the same gear could be related to local species diversity, to environmental constrains, social-economic aspects or simply due random factors. The south coast of Portugal (Algarve) usually has lower hydrodynamics and less storm days at sea than the west coast (source: Instituto Hidrográfico), which can contribute to lower bycatch rates found in that area. During our observations after storming days, the species entangled usually showed higher degradation signs and, on the other hand, storming days lead to higher soak times since fishermen left nets soaked during those periods.

However, species discarded from trammel nets have been far greater than that of other static gears such as longlines or gill nets (Borges et al., 2001). Thus, we can conclude that although trammel nets are considered a selective gear, they are not species selective and they present lower selectivity than other gears with the same classification.

The discard ratio of the studied fishery is similar to that found by Borges et al. (2001) for demersal purse-seine fisheries in southern Portugal (near 20%) and showed lower rates than was found in fish trawl fishery (62%), crustacean trawl fishery (70%) (Borges et al., 2001) and demersal purse-seine (near 51%) (Gonçalves et al., 2008b) in south Portuguese coast and than it was estimated for beach seine fishery (44%) in the central coast of Portugal (Cabral et al., 2003). In the North Sea, soles are mainly caught by beam trawlers, for which a high level of discards is also reported: per each kilogram of *S. solea* caught, 11.4 kilograms are discarded, mainly other flatfishes (Garthe and Damm, 1997).

The differences found in discard ratios of several fisheries, even using the same gear and in adjacent areas, indicate the need to evaluate discards for each fishery. Thus, long-term monitoring is required to improve our understanding of the factors affecting discarding and of the implications of such levels of discarding on a fragile and stressed environment (Hollingworth, 2000; Kaiser and de Groot, 2000). The discards of the studied fishery were estimated as about 174 tonnes per year. However, we can not assess what this means in which respects to ecosystem level impacts because although the great number of studies about bycatch composition or bycatch rates in fisheries around the world, little is known about the fate of these discards and their real impact on ecosystems (ICES, 1998; Tingley et al., 2000; Monteiro et al., 2001). However, it is likely that the type of discarding associated with purse seines that fish in relatively shallow water, often involving large quantities released over a short period of time in a small area may have a greater potential impact on the ecosystem than trawl discarding that takes place as the trawler is moving, over periods of time. Thus, trawl discards are dispersed over a much wider area and the continuous stream of discards going overboard allows sea birds more opportunity to scavenge than when a large catch is slipped (Erzini et al., 2002). Follow this assumption, discards from trammel nets fishery can be considered as having less impact in the ecosystem than seiners or trawlers, since their discards are made in little amounts along all net length and during a long time period. However, the high importance of small-scale fisheries, which contribute to most of the total captures worldwide, lead us to think that their global capacity to disturbing ecosystems could match large-scale fisheries (Bundy and Pauly, 2001; Díaz-Uribe et al., 2007).

There are many reasons for discards (Clucas, 1997), however, in most situations, the decision by fishermen to discard components of their catch is driven by economic factors (FAO, 1999). In this study the main reasons for discards were found to be the low or inexistent selling price of the species and fishes' damage condition. Borges et al. (2001) reported that poor condition of the catch after the nets have been set for periods of up to 12 h or more is the main reason for discards of trammel nets. Acosta (1994) showed that higher soak times does not represent higher efficiency and the proportion of dead fish and the spoiled degree increases with the increase of soak time. Restrictions on the fish landings and undersized fish are also strong motives for discards in other fisheries, namely the North Sea flatfish fishery, where undersized plaice is highly discarded (Clucas, 1997) although in the present study the capture of undersized fishes is rare due to size selective characteristics of the gear.

So, in order to reduce discards of the trammel net fishery existing regulation could be enforced, namely in which respects to nets soak time. In Sesimbra, almost all vessels included in the studied *métier* also had traps for catching cephalopods, hauling each kind of gear in alternating days. Consequently, fishermen from Sesimbra left them nets at sea more than 48 hours and nets usually became soaked during all weekend in both studied harbours. Those behaviours should be avoided in order to minimize the amount of damage fishes, allowing them to be sold. Another measure to reduce discards could be the development of more appropriate net configurations, independent of mesh size, as suggested by Gray (2002) for a multi-species gill net fishery. Besides this, some adjustment on fisheries legislation could also minimize bycatch, namely in which respect to minimum landing sizes. In the present analyses of bycatch, we found that 60% of *S. lascaris* captures are under the MLS (some of them were retained to fishermen own consumption). However, this species are under the same legislation as S. senegalensis and S. solea (MLS is 240 mm). We considerer that the MLS for S. lascaris should be revised since individuals became mature at lower lengths than the other to species of genus Solea spp. (Cabral et al., 2007; Teixeira et al., in press).

In addition to all impacts in the marine ecosystems due discard practices, all bycatches constitute a higher amount of unreported data that could be of great importance in the stock evaluation or in the analyses of the results of recovery programs applied to species that are bycatch of other fisheries. In fisheries where there is not a continuous monitoring, especially in fisheries as the studied one, whose captures are so variable and influenced by so many factors, great amounts of important data are unknown. Mettling et al. (1995) estimated that 40% of French catches are unreported and we think that in Portugal the percentage is not lower certainly.

Most of the small-scale fisheries around the world has no management and when it exist is based in landing data and consequently without taking in consideration bycatch or discards neither continuous monitoring (Lleonart and Maynou, 2003; Merino et al., 2008).

Moreover, bycatch captures are not the only reason for incorrect fisheries statistics, also target species records are underestimations of the catches of commercially important species because there are an important fraction of their catches that is not declared and sold at the fishing docks, as it should be, and consequently does not part of the fisheries statistics. Concerning the most valuable species, namely *S. senegalensis*, *S. solea* and *S. maximus*, the highest differences between landings and captures corresponds to periods when prices and total catches are higher. This happens essentially in spring and summer, when catches and landings differ 62% and 31%, respectively. In these seasons selling prices for these species are much higher as a result of an increase in demand. Fishes are often sold directly to restaurants, which pay more than fishing docks. This is a common practice in other Portuguese fisheries, such as beach seine fishery, where a large proportion of the catches with higher commercial value is sold directly after capture at the beach (Cabral et al., 2003).

Catch trends constitutes a conceptually simple and meaningful indicator to characterise fisheries status. Nonetheless, the use of this indicator to define sustainability and to characterize the 'health' of fish stocks might be dangerous and controversial (Mullon et al., 2005). In the studied trammel net fishery, the only available evaluation of stock possible is based on official data landings, which are incomplete as outlined above. Aiming for a better management of fisheries resources, the use of effective total catches would be of great importance. Thus, incentives for fishermen (such as more competitive prices and better market strategies in fishing docks) to land an increasing fraction of fish caught should be implemented. Some EU countries, including Portugal, have in practice sampling programmes to determine and

monitor bycatches (Tingley et al., 2000). However, the Portuguese sampling surveys in the scope of this programme are quite limited, which constrains its use on a broader fisheries context.

Thus, the lack of quantitative data on a spatial and temporal scale strongly reduces the chance of developing management measures to make fishing methods sustainable in the long term (Colloca et al., 2004). Moreover, it is necessary to develop standardized data collection routines and indicators of fishing effort for Portuguese artisanal fisheries that allow scientists and fisheries managers the possibility of work with data that consider all catches, bycatches and other usually unreported captures.

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Part IV

Main Conclusions and Final Remarks

Chapter 9

Main Conclusions and Final Remarks

The present work analysed several aspects of the bio-ecology and fisheries of the most commercially important flatfishes in the Portuguese coast. The study of the feeding habits revealed that soles and bastard sole fed mainly on crustaceans, polychaetes and molluscs, while spotted flounder and four-spotted megrim consumed more intensively fishes, decapods and mysids, and founder fed preferentially echinoderms, crustaceans and bivalves (Chapters 2, 3 and 4). Diet composition varied with season, sex and size class. All species considered in this study showed a differential growth according to sex (Chapters 2, 3 and 4). The estimated asymptotic lengths were highest for females than males, with the exception of spotted flounder. Spawning occurred mainly in autumn-winter for common sole, Senegalese sole, four-spotted flounder and flounder, spanning into spring for the sand sole, and throughout the year for spotted flounder and bastard sole (Chapters 2, 3 and 4). Our results showed that age at first maturity of females and males was similar, except for flounder.

The genetic analysis of two sympatric species, common sole and Senegalese sole, revealed that both have a low haplotype diversity and a moderate to high nucleotide diversity (Chapter 5). Common sole presented a population structure with a genetic divergence between Atlantic and Mediterranean populations, and between west and east Mediterranean populations. For the Senegalese sole a pattern of genetic heterogeneity among populations separated geographically was evidenced.

Multi-species fisheries, as is the case of flatfish fisheries in Portugal, are extremely complex to evaluate and manage. Catches result from a variety of fishing grounds, and several species are targeted by these fisheries which use different gears. Furthermore factors like environmental conditions, economics or individual fishers' behaviour also influence catches. The artisanal flatfish fisheries in the Portuguese coast exhibited a marked spatial and temporal variation of fishing practices, whereby a given fleet may change its target species, gear type or fishing location in short time scales. A marked seasonal variation was found for these fisheries (Chapter 6) and different fleet components, targeting some particular species, were identified (Chapter 7). The variety of species captured, gears used, and the use of a wide range of landing sites make it difficult to evaluate the stocks exploitation status of the resources and the fishing intensity exerted. Efficiency varies among individual vessels owing to differences in the skill of the fishers and vessel characteristics (e.g. Squires and Kirkley, 1999), and it may increase over time through continuous developments in the fishing industry (e.g. Ulrich et al., 2002; O'Neill et al., 2003).

In the Portuguese multi-species fisheries only a fraction of the catches are relative to target species, and, thus, a wide variety of non-target species are also captured. Some of the bycatch species have commercial value and can be sold, while others are discarded (e.g. fish are damaged, prohibited species, less than legal minimum landing size). Bycatches represented near 60% of total catches of the trammel net fishery in the central coast of Portugal, of which about 40% were discarded (Chapter 8). This study revealed that this fleet, composed by ca. 200 vessels, discarded 174 tonnes per year. Solutions to discard problems in multi-species fisheries elsewhere include the development of more selective fishing gear and practices that minimize the capture of non-target species and undersize individuals of the target species (Gray et al., 2001). Bycatch contributes to changing the structure of marine communities and/or ecosystems and to biological overfishing, with serious implications for marine populations and the overall health and sustainability of ecosystems (e.g. Kaiser and De Groot, 2000; Kelleher, 2005).

The present work represents the first integrative approach to the study of flatfish subjected to fisheries in the Portuguese coast, but further studies are needed in order to provide a good scientific knowledge on these species and for management purposes. Some of the topics that need to be addressed are length-frequency distribution analysis of landings, characterization of the reproductive period through the evaluation of the seasonal variation in gonads development, fecundity, relationships between larvae, juvenile and adult stages, flatfish distribution and abundance patterns, fishing effort and *métiers* involved in these small-scale fisheries.

Furthermore, it would be particularly important to evaluate climate change and other anthropogenic impacts on flatfish populations and fisheries.

Sustainable fisheries management is the challenge in nowadays fisheries, which is, however, extremely difficult to reach, especially in these complex multi-species and multi-gear fisheries and with scarce knowledge.

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