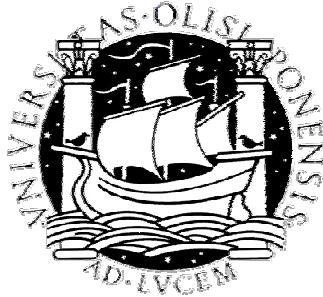


UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL



Ecology of the juveniles of the soles, *Solea solea* (Linnaeus, 1758)
and *Solea senegalensis* Kaup, 1858, in the Tagus estuary.

CATARINA MARIA BATISTA VINAGRE

DOUTORAMENTO EM BIOLOGIA
ESPECIALIDADE - BIOLOGIA MARINHA E AQUACULTURA

2007

UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL



Ecology of the juveniles of the soles, *Solea solea* (Linnaeus, 1758)
and *Solea senegalensis* Kaup, 1858, in the Tagus estuary.

CATARINA MARIA BATISTA VINAGRE

Tese orientada pelo Professor Doutor Henrique Nogueira Cabral
Professor Auxiliar com Agregação da
Faculdade de Ciências da Universidade
de Lisboa, Portugal

DOUTORAMENTO EM BIOLOGIA
ESPECIALIDADE - BIOLOGIA MARINHA E AQUACULTURA

2007

TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	i
RESUMO.....	iii
SUMMARY.....	vii
LIST OF SCIENTIFIC PUBLICATIONS.....	ix
CHAPTER 1 – GENERAL INTRODUCTION.....	1
CHAPTER 2 – HABITAT USE.....	13
Introduction.....	15
Habitat Suitability Index Models for the sympatric soles <i>Solea solea</i> and <i>Solea senegalensis</i> , in the Tagus estuary: defining variables for management.....	19
Nursery fidelity, primary sources of nutrition and food web interactions of the juveniles of <i>Solea solea</i> and <i>S. senegalensis</i> in the Tagus estuary (Portugal): a stable isotope approach.....	37
Diel and semi-lunar patterns in the use of an intertidal mudflat by juveniles of Senegal sole, <i>Solea senegalensis</i> Kaup, 1858.....	56
Conclusions.....	71
CHAPTER 3 – FOOD CONSUMPTION.....	73
Introduction.....	75
Effect of temperature and salinity on the gastric evacuation of the juvenile soles <i>Solea solea</i> and <i>Solea senegalensis</i>	80
Foraging behaviour of <i>Solea senegalensis</i> Kaup, 1858, in the presence of a potential predator, <i>Carcinus maenas</i> Linnaeus, 1758.....	93

Prey consumption by the juveniles of <i>Solea solea</i> (Linnaeus, 1758) and <i>S. senegalensis</i> Kaup 1858, in the Tagus estuary, Portugal.....	102
Conclusions.....	114
CHAPTER 4 – GROWTH AND CONDITION.....	117
Introduction.....	119
Growth variability of juvenile soles <i>Solea solea</i> (Linnaeus, 1758) and <i>Solea senegalensis</i> Kaup, 1858, based on condition indices in the Tagus estuary, Portugal.....	122
Habitat specific growth rates and condition indices for the sympatric soles populations of <i>Solea solea</i> (Linnaeus, 1758) and <i>S. senegalensis</i> Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA:DNA ratio.....	135
Latitudinal variation in spawning period and growth of 0-group sole, <i>Solea solea</i>	150
Conclusions.....	164
CHAPTER 5 – RECRUITMENT AND MORTALITY.....	167
Introduction.....	169
Impact of climate and hydrodynamics on soles' larval imigration into the Tagus estuary, Portugal.....	172
Fishing mortality of the juvenile soles, <i>Solea solea</i> and <i>Solea senegalensis</i> , in the the Tagus estuary, Portugal.....	190
Conclusions.....	208
CHAPTER 6 – GENERAL CONCLUSIONS AND FINAL REMARKS.....	209

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Prof. Dr. Henrique Cabral for taking the supervision of this work, for the invaluable guidance, encouragement and friendship.

I am thankful to Prof. Dra. Maria José Costa for taking me on the “Instituto de Oceanografia” marine zoology working group and for all the support granted to this work.

I am very grateful to Prof. Dr. João Catalão for guiding me into the world of the geographic information systems and for all the help with the habitat suitability models.

I wish to thank Prof. Dr. Rachid Amara for the opportunity to carry out the otolith microstructure analysis in his lab, for giving me the opportunity to analyse his data and for teaching me so much on this subject.

I am most grateful to Anabela Maia for being such a great colleague, for her help in the most crucial moments of this work, enthusiasm, encouragement and friendship. I am also very thankful to Marisa Batista, for her willingness to help, friendly support and for the collaboration in the aquariums maintenance at a crucial point in this work. I wish to thank Patrick Reis-Santos for the collaboration in the field work and Miguel Ruano for the support in the field work and especially for teaching me the RNA-DNA determination methodologies.

A warm, thank you, to Inês Cardoso for her constant encouragement and friendly support through the ups and downs of the last four years. I thank Susana França, Tadeu Pereira and Vanessa Fonseca for the help in the field, in the initial stages of this work, when we came into close contact with the Alcochete mudflats, and all the colleagues at the “Instituto de Oceanografia” who supported and encouraged me.

A warm “Merci”, to Jonathan, Natasha and Dominique, whom I had the pleasure to know during my stay at the environmental station of Wimereux (Université de la Cote D’Opale), in France.

I wish to thank the fishermen I worked with, Sr. Manuel Lourenço and Sr. Domingos Chefe, for their guidance in the quest for juvenile soles, and for all the interesting discussions that added to my knowledge of the Tagus estuary nurseries.

My personal thanks go to Joana Moreno for the friendship that goes beyond years and for being there when I most needed. Warm thanks, to Violante Medeiros, for the long-distance support and funny chats, to Patrícia Palma, Miguel Lopes and Joana Macedo for the many joyful moments and enthusiastic spirit, to Vanda, Nuno, Domingos, Sofia, Sónia, João, Hélder, Xana, Hugo and Pedro, whose presence in my life was important in many phases of this work.

To my parents, for the unconditional support that allowed me to venture this far and for instilling me with scientific curiosity from the very beginning.

This thesis was supported by Fundação para a Ciência e a Tecnologia through the Ph. D. grant SFRH/BD/12259/2003.

RESUMO

Os linguados *Solea solea* (Linnaeus, 1758) e *Solea senegalensis* Kaup, 1858, estão entre os peixes com maior valor comercial, em Portugal. Os indivíduos adultos destas espécies habitam a plataforma continental, enquanto que os juvenis se concentram em áreas costeiras e em particular em estuários. Duas importantes áreas de viveiro para os juvenis destas espécies foram identificadas no estuário do Tejo: a área de Vila Franca de Xira e a de Alcochete. Enquanto que *S. solea* apenas coloniza a área de viveiro de Vila Franca de Xira, *S. senegalensis* coloniza ambas as áreas de viveiro. O presente trabalho tem como objectivo aprofundar o conhecimento no que se refere à ecologia de juvenis destas espécies nas áreas de viveiro do estuário do Tejo.

O uso do habitat pelos juvenis destas espécies foi analisado em diferentes escalas espaciais, recorrendo a técnicas de análise espacial, análises isotópicas e a um programa de amostragem na zona intertidal que teve em conta os ciclos circun-diário e lunar.

Foram desenvolvidos modelos espaciais de qualidade do habitat para ambas as espécies de forma investigar quais as variáveis que definem as áreas onde os juvenis se concentram e que, por isso, deverão ser levadas em conta na elaboração de planos de gestão que visem as populações de linguado ou as áreas estuarinas em questão. A importância da salinidade, temperatura, substrato, profundidade e presença de plataformas vasosas foi confirmada, no entanto, a inclusão da abundância de presas provou ser crucial para a definição das áreas de elevada qualidade ambiental onde as grandes concentrações destas espécies ocorrem de forma consistente.

As análises isotópicas aos vários elos das cadeias alimentares de ambas as áreas de viveiro, revelaram que os juvenis de *S. senegalensis* com idade inferior a 1 ano exibem elevada fidelidade à zona de viveiro onde vivem, enquanto que uma fracção considerável de juvenis com mais de um ano de idade explora ambas as áreas de viveiro. Concluiu-se também, que as cadeias tróficas das duas áreas de viveiro têm uma dependência diferencial das fontes de água-doce.

A investigação simultânea do efeito do ciclo circun-diário e lunar sobre uso da zona intertidal por parte dos juvenis de *S. senegalensis*, permitiu detectar um padrão no qual durante as marés vivas a abundância de juvenis atinge o pico no crepúsculo, enquanto que nas marés mortas o pico é diurno. O maior pico de abundância ocorre no crepúsculo durante a lua-cheia. A análise dos padrões de actividade dos seus principais predadores e informação prévia sobre os efeitos dos ciclos circun-diário e lunar sobre as suas presas indicam que os padrões de actividade encontrados para *S. senegalensis* têm uma relação estreita com a dos seus predadores e presas.

Foram conduzidas experiências em cativeiro de forma a determinar a influência da temperatura e salinidade sobre a evacuação gástrica de ambas as espécies, assim como para a detecção de alterações no comportamento alimentar devido a pressão predatória.

Observou-se que a evacuação gástrica aumenta com a temperatura, para ambas as espécies (com excepção da temperatura experimental de 26°C, em *S. solea*). No caso da salinidade, verificou-se um efeito diferente, de acordo com a espécie. A baixas salinidades, *S. solea* manifestou um aumento da taxa de evacuação gástrica, enquanto que para *S. senegalensis* registou-se uma diminuição desta taxa. O efeito da temperatura experimental de 26°C em *S. solea* foi discutido, concluindo-se que este é possivelmente um indício de que esta espécie se encontra perto do seu limite superior térmico. Este efeito não foi observado em *S. senegalensis*, provavelmente porque, sendo esta uma espécie com afinidades subtropicais, possuirá um limite superior térmico mais elevado. A diferença detectada nas taxas de evacuação gástrica a 26°C terá importantes implicações competitivas para *S. solea* durante os meses mais quentes, quando ambas as espécies de linguado se concentram em águas pouco profundas, ricas em presas, mas onde as temperaturas são consideravelmente mais elevadas do que o óptimo metabólico de *S. solea*. A experiência de observação comportamental revelou um decréscimo de 10% na actividade de *S. senegalensis*, quando na presença de um potencial predador.

As taxas de evacuação gástrica, juntamente com ciclos de amostragem conduzidos na área de estudo, foram integradas num modelo, de forma a calcular o consumo alimentar de ambas as espécies. O consumo diário de *S. senegalensis* foi consideravelmente superior ao de *S. solea*. Os padrões alimentares observados evidenciaram dois picos distintos de actividade alimentar, ao anoitecer e ao amanhecer, estes foram, no entanto, muito mais pronunciados para *S. senegalensis* do que para *S. solea*. Uma vez que estudos a latitudes mais elevadas verificaram picos de actividade alimentar em *S. solea* mais pronunciados e da ordem de magnitude da encontrada no estuário do Tejo para *S. senegalensis*, concluiu-se ser este mais um indício de que o metabolismo de *S. solea* está próximo do seu limite térmico, estando, por isso, diminuído. O cálculo do consumo alimentar total, durante os meses de maior abundância destas espécies nas áreas de viveiro, e da disponibilidade alimentar do meio levou à conclusão de que a disponibilidade de alimento não é um factor limitante para os juvenis de linguado do estuário do Tejo. Considerou-se, no entanto, que será necessária mais informação sobre a variabilidade de abundância das comunidades de presas e sobre o consumo por parte de outros predadores para determinar com segurança a capacidade de suporte das áreas de viveiro do estuário do Tejo. A elevada variabilidade inter-anual da abundância de ambas as espécies de linguado deverá, também, ser levada em conta.

O estudo do crescimento e condição dos juvenis de *S. solea* e *S. senegalensis* focou a variação das taxas de crescimento e da razão RNA-DNA nas sucessivas coortes que colonizam o estuário do Tejo. Focou também, a comparação da qualidade do habitat entre as duas áreas de viveiro e a comparação do crescimento e período de desova num contexto latitudinal.

Foram observados padrões de variação do crescimento e da condição que reflectem o processo de colonização estuarina. O crescimento e a condição são mais elevados no início da

colonização, diminuindo com o tempo, e a primeira coorte apresenta valores mais elevados que as coortes subsequentes.

A comparação das taxas de crescimento calculadas para as duas áreas de viveiro evidenciou valores mais elevados para a área de Alcochete do que para a área de Vila Franca de Xira. A condição geral dos indivíduos de ambas as espécies foi considerada boa, não tendo sido encontrada uma diferença significativa entre as duas áreas. Concluiu-se que, dada a elevada dinâmica característica das áreas de viveiro estuarinas, será necessária a determinação da qualidade ambiental destas áreas durante um período de tempo mais alargado, de forma a investigar a sua variação inter-anual. As taxas de crescimento de *S. solea* no estuário do Tejo foram superiores às estimadas para latitudes mais elevadas. O uso de ambas as metodologias para monitorização de qualidade ambiental das áreas de viveiro do estuário do Tejo, o cálculo de taxas de crescimento com base nos anéis diários dos otólitos e a determinação da razão RNA-DNA, foi discutido.

A determinação da época de desova de *S. solea* através de contagem retrógrada dos anéis diários dos otólitos e a sua comparação com outras áreas de viveiro ao longo da costa ocidental Europeia, revelou existir uma variação latitudinal na qual a desova é iniciada mais cedo quanto menor a latitude.

O impacto de factores climáticos e oceanográficos sobre a imigração larvar para as áreas de viveiro foi investigado. Da análise de uma série temporal descontínua de densidades de juvenis de ambas as espécies (1988-2006) e da sua relação com o índice de oscilação do Atlântico Norte, a prevalência de ventos do quadrante Norte e o caudal do rio Tejo, durante o período larvar, resultou que só para esta última variável foi encontrada uma correlação significativa. A extensão das plumas fluviais na zona costeira será, assim, muito importante, provavelmente devido ao seu papel no transporte de pistas químicas que são utilizadas pelas larvas para direccionar o seu movimento. Isto significa que em anos chuvosos estas pistas químicas atingirão uma área maior aumentando a probabilidade de detecção por parte das larvas. Concluiu-se que a diminuição e maior concentração temporal da precipitação, previstas devido a alterações climáticas, terão um efeito significativo sobre a imigração larvar de ambas as espécies, mas em particular sobre *S. senegalensis*, uma vez que o seu período de desova é mais alargado. Foi, desta forma, evidenciada a importância da monitorização dos caudais fluviais e do seu efeito sobre a imigração de linguados para áreas de viveiro estuarinas.

A estimativa do impacto da pesca sobre estes juvenis, permitiu concluir que esta tem um impacto considerável sobre os efectivos populacionais de ambas as espécies, sendo este, no entanto, mais acentuado para *S. solea*. Verificou-se que a fracção da população de *S. senegalensis* afectada por mortalidade por pesca é menor, uma vez que, esta espécie coloniza também a área de Alcochete, onde o esforço de pesca é bastante menor do que em Vila Franca de Xira. Desta forma a área de viveiro de Alcochete funciona como uma zona mais protegida para esta espécie. Foi confirmada a necessidade de revisão da legislação que permite a pesca com arrasto de vara no estuário do Tejo. Foi dado ênfase à importância da

integração da informação obtida neste trabalho em planos de gestão dos mananciais de ambas as espécies de linguado, assim como, na gestão do estuário do Tejo.

Foram sugeridos vários estudos futuros, nos quais se incluem, projecções dos efeitos de alterações climáticas sobre as populações de linguado, a monitorização da qualidade dos habitats, a recolha de séries temporais contínuas de dados referentes às densidades de juvenis e adultos e a determinação das áreas de desova dos linguados na costa Portuguesa.

PALAVRAS-CHAVE: SOLEA; ESTUÁRIOS; DISTRIBUIÇÃO; ALIMENTAÇÃO E CRESCIMENTO; RECRUTAMENTO.

SUMMARY

The soles, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, are among the most valuable commercial fish in Portugal. Two important nursery areas for the juveniles of these species have been identified in the upper area of the Tagus estuary. The present work aimed at investigating the ecology of these species in the Tagus estuary nursery areas. Habitat use was analysed at different spatial scales, using spatial modelling, isotopic analysis and a complex sampling program accounting for the diel and lunar cycles. Prey abundance proved crucial in the prediction of high densities of juveniles. The stable isotope approach revealed low connectivity between nursery areas and different levels of dependence upon the freshwater energy pathway. A diel and semi-lunar activity pattern was detected for *S. senegalensis*. Experimental work on gastric evacuation and feeding behaviour and its application to the wild populations allowed the estimation of food consumption by juvenile soles. Temperature, salinity and predation pressure were found to affect prey consumption. Otolith daily increments and RNA-DNA ratio analyses, showed that growth rates and condition variation reflects estuarine colonization patterns. The Tagus estuary soles were found to be in good overall condition and their growth rates were higher than at higher latitudes. The use of these methodologies for habitat quality monitoring was discussed. Estimation of spawning time through back-counting of otolith daily increments and comparison with other areas revealed a latitudinal variation in *S. solea* spawning in that it tends to occur earlier with decreasing latitude. Investigation on the effect of climate and hydrodynamics upon migrating sole larvae emphasized the importance of river drainage in this process. The magnitude of the mortality caused by fishing conducted within the nursery areas was considerable for *S. solea* and lower for *S. senegalensis*. Several management measures were suggested and discussed. Future studies were proposed.

KEY-WORDS: SOLEA; ESTUARIES; DISTRIBUTION; FEEDING AND GROWTH; RECRUITMENT.

LIST OF SCIENTIFIC PUBLICATIONS IN INTERNATIONAL JOURNALS

This thesis comprises the scientific publications listed below.

- Vinagre, C., Fonseca, V., Cabral, H., Costa, M.J. 2006. Habitat suitability index models for the juvenile soles, *Solea solea* and *Solea senegalensis*: defining variables for management. *Fisheries Research* 82, 140-149.
- Vinagre, C., Salgado, J., Costa, M.J., Cabral, H.N. (*in press*). Nursery fidelity, food web interactions and primary sources of nutrition of the juveniles of *Solea solea* and *S. senegalensis* in the Tagus estuary (Portugal): a stable isotope approach. *Estuarine, Coastal and Shelf Science*.
- Vinagre, C., França, S., Cabral, H.N., 2006. Diel and semi-lunar patterns in the use of an intertidal mudflat by juveniles of Senegal sole, *Solea senegalensis* Kaup, 1858. *Estuarine, Coastal and Shelf Science* 69: 246-254.
- Vinagre, C., Maia, A., Cabral, H.N., 2007. Effect of temperature and salinity on the gastric evacuation of the juvenile soles *Solea solea* and *Solea senegalensis*. *Journal of Applied Ichthyology* 23, 240-245.
- Maia, A., Vinagre, C., Cabral, H.N. (*submitted*). Foraging behaviour of *Solea senegalensis* Kaup, 1858, in the presence of a potential predator, *Carcinus maenas* Linnaeus, 1758.
- Vinagre, C., Cabral, H.N. (*submitted*) Prey consumption by the juveniles of *Solea solea* (Linnaeus, 1758) and *S. senegalensis* Kaup 1858, in the Tagus estuary, Portugal.
- Fonseca, V., C. Vinagre, H. Cabral. 2006. Growth variability of juvenile soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, based on condition indices in the Tagus estuary, Portugal. *Journal of Fish Biology* 68: 1551-1562.
- Vinagre, C., Fonseca, V., Maia, A., Amara, R., Cabral, H.N. (*in press*). Habitat specific growth rates and condition indices for the sympatric soles populations of *Solea solea* (Linnaeus, 1758) and *S. senegalensis* Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA:DNA ratio. *Journal of Applied Ichthyology*.
- Vinagre, C., Amara, R., Maia, A., Cabral, H.N. (*submitted*) Latitudinal variation in spawning period and growth of 0-group sole, *Solea solea* (L.).
- Vinagre, C., Costa, M.J., Cabral, H.N. (*in press*) Impact of climate and hydrodynamics upon soles' larval immigration into the Tagus estuary. *Estuarine Coastal and Shelf Science*.
- Vinagre, C., Costa, M.J., Cabral, H.N. (*submitted*) Fishing mortality of the juvenile soles, *Solea solea* and *Solea senegalensis*, in the the Tagus estuary, Portugal. *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 permission of the publisher.

CHAPTER 1

- GENERAL INTRODUCTION -

The common sole, *Solea solea* (Linnaeus 1758), and the Senegal sole, *Solea senegalensis*, Kaup 1858, are flatfishes with sympatric distribution from the Bay of Biscay to Senegal and the western Mediterranean (Quéro *et al.*, 1986). *S. solea* extends its distribution from Norway and the western Baltic, to Senegal, including the Mediterranean, while *S. senegalensis* is distributed from the Gulf of Biscay to South Africa, and is rare in the Mediterranean (Quéro *et al.*, 1986).

These two species are very similar morphologically, as well as, in ecological needs. While adults live in coastal waters up to 200m (Quéro *et al.*, 1986), 0-group juveniles concentrate in shallow coastal and estuarine waters, where they live for about 2 years (Koutsikopoulos *et al.*, 1989).

These species distribution within nursery areas has been mainly associated with low salinities (Riley *et al.*, 1981; Jager, 1993; Cabral and Costa, 1999) and fine substrates, such as sand and mud (Riley *et al.*, 1981; Rogers, 1992; Cabral and Costa, 1999). Yet, various authors concluded that substrate preferences among flatfishes are mainly related to prey availability (Gibson and Robb, 1992, Amezcua and Nash, 2001, Vinagre *et al.*, 2005).

While small scale movements and activity patterns of juveniles have been thoroughly studied in other flatfish, such as plaice *Pleuronectes platessa* (Linnaeus, 1758) and flounder *Platichthys flesus* (Linnaeus, 1758) (e.g. Gibson, 1973; 2003; Wirjoatmodjo and Pitcher, 1980; van der Veer and Bergman, 1987; Raffaelli *et al.*, 1990; Burrows, 1994; Gibson *et al.*, 1998), little work has been carried out on soles. Lagardère (1987) reported that *S. solea* juveniles' activity is mainly nocturnal and presents two peaks of feeding activity, at dawn and dusk, which was later confirmed by Cabral (1998), not only for *S. solea* but also for *S. senegalensis*, in the Tagus estuary. Studies on *S. solea* and *S. senegalensis*, in the Tagus estuary (Cabral, 1998; 2000), as well as, on other flatfish in other areas, suggest that some flatfish species perform tidal migrations driven by feeding and predator avoidance (e.g. Wirjoatmodjo and Pitcher, 1980; Gibson *et al.*, 1998). Although some studies indicate that the semi-lunar cycle may also be an important factor determining the use of intertidal areas by flatfish (Rafaelli *et al.*, 1990) the full effect of this cycle is still scarcely understood.

Little is known about the connectivity among the habitats occupied by these species along the Portuguese coast. Cabral *et al.* (2003) concluded that genetic differentiation is of low magnitude, yet it is higher for *S. solea* than for *S. senegalensis*, probably due to the more

extended period of occurrence of larval stages of *S. senegalensis* in the plankton (Cabral *et al.*, 2003).

The feeding ecology of *S. solea* has been thoroughly investigated in coastal areas of North-west Europe (deGroot, 1971; Braber and Degroot, 1973; Quiniou, 1978; Lagardère, 1987, Henderson *et al.*, 1992) and Western Mediterranean (Ramos, 1981; Molinero and Flos, 1991; 1992; Molinero *et al.*, 1991). In the Portuguese coast, studies on the feeding ecology of *S. solea* have been carried out in the Sado estuary (Sobral, 1981), at Lagoa de Santo André (Bernardo, 1990), in the Tagus estuary (Costa, 1982; 1988; Gonçalves, 1990; Cabral, 1998; Cabral, 2000) and in the Douro estuary (Vinagre *et al.*, 2005). It has been concluded that polychaetes, molluscs and crustaceans are the most important groups in this species diet. Literature on the feeding ecology of *S. senegalensis* is scarce. Molinero *et al.* (1991) reported on this species' diet in the Western Mediterranean, Bernardo (1990) at Lagoa de Santo André and Cabral (1998; 2000) in the Tagus estuary. Diets of the two sole species are very similar. Juvenile flatfish generally consume the most abundant food resources in a generalist and opportunistic manner (e.g. Lasiak and McLachlan, 1987; Beyst *et al.*, 1999; Cabral, 2000).

A large number of studies have been carried out on growth of *S. solea*, mostly in North-west Europe (e.g. Amara *et al.*, 1994; Rogers, 1994; Jager *et al.*, 1995; Amara *et al.*, 2001). Several studies have described growth of *S. solea* in the Portuguese coast. Dinis (1986), Costa (1990) and Cabral (2003) investigated growth in the Tagus estuary, Bernardo (1990) focused on the Lagoa de Santo André and Andrade (1990, 1992) on Ria Formosa. A considerable variation in growth rates and length at the end of the first year was observed, with generally higher growth rates being reported for the Portuguese coast in comparison with the northern European coast. Again, studies on *S. senegalensis* are scarcer. Garcia *et al.* (1991) investigated growth in *S. senegalensis* in the Mediterranean West coast, while in the Portuguese coast Bernardo (1990) focused on the Lagoa de Santo André, Andrade (1990, 1992) on Ria Formosa and Cabral (2003) in the Tagus estuary.

Studies on the condition of these species are still scarce. Gilliers *et al.* (2004) applied various condition indexes (RNA-DNA ratio, Fulton's *K*, marginal otolith increment width) to *S. solea* in the Northern French coast and found that all habitats provided equivalent conditions for juvenile sole. However, Gilliers *et al.* (2006), working on a broader spatial scale, found interesting relations with anthropogenic disturbance. Amara and Galois (2004) found that the fastest growing *S. solea* larvae in the Northern French coast presented higher levels of triacylglycerol and sterol.

In the Portuguese coast, *S. solea* and *S. senegalensis* attain sexual maturity when they reach 3 to 4 years old (Cabral *et al.*, 2007). While coastal spawning grounds of *S. solea* in Northern European waters are well known and are generally located at depths between 40m and 100m (Koutsikopoulos *et al.*, 1991; Wegner *et al.* 2003), such information is not available for Southern European waters and for *S. senegalensis* over its entire distribution range. Spawning of *S. solea* takes place in spring at the highest latitudes and winter at lower latitudes (Wegner *et al.*, 2003). Evidence from multi-cohorts of juveniles colonizing the nursery grounds

indicates that *S. senegalensis* spawns later, during a broader period and has one main spawning period in spring and a secondary period in summer-autumn (Cabral, 1998). Gonad maturation in the wild and emission of eggs in captivity corroborates these evidences (Dinis, 1986; Andrade, 1990; Anguis and Cañavate, 2005; Garcia-Lopez *et al.*, 2006).

Since spawning grounds are distant from the nursery areas, transport of eggs and larvae is an important issue. Unfavourable climate and hydrodynamic circulation may lead to high mortality rates at this stage (e.g. Marchand, 1991; Van der Veer *et al.*, 2000; Wegner *et al.*, 2003). In fact, it is generally agreed that recruitment variation in flatfish stocks is dominated by density independent factors operating on the eggs and larvae (Leggett and Frank, 1997; Van der Veer *et al.*, 2000). Although an important body of literature has been gathered on the transport of *S. solea* eggs and larvae and several models have been constructed (Miller *et al.*, 1984; Boelhert and Mundy, 1988; Rijisdorp *et al.*, 1985; Bergman *et al.*, 1989; Marchand and Masson, 1989; Champalbert *et al.*, 1989; Champalbert and Koutsikopoulos, 1995; Arino *et al.*, 1996; Ramzi *et al.*, 2001; deGraaf, 2004), no data exists for the Portuguese coast, nor for *S. senegalensis* over its entire distribution range.

Both soles have high commercial value, *S. senegalensis* is a species of increasing interest in aquaculture and is currently cultured in the Portuguese and Spanish southern coasts (Dinis *et al.*, 1999; Imsland *et al.*, 2003).

Fishing pressure upon soles has been increasing in the Portuguese coast, while a decrease in the captures per fishing effort has been recorded (Cabral *et al.*, 2007). Several issues hinder the investigation on soles' fisheries in the Portuguese coast. One of the most important ones is that an important portion of captures is sold directly to restaurants or to the final consumer, escaping any kind of control. Furthermore, the official data on fisheries does not distinguish *Solea* species (Cabral *et al.*, 2007). Capture of undersized juveniles that are sold out of the official commercial circuits is also an important problem concerning sole fisheries. In the Tagus estuary, there is an important fishery that targets sole juveniles of both species (Cabral *et al.*, 2002). Baeta *et al.* (2005) concluded that this fishery is not environmentally sustainable.

The Tagus estuary is an important area for *S. solea* and *S. senegalensis* juveniles (Costa and Bruxelas, 1989; Cabral and Costa, 1999). With approximately 320 km², it is the largest estuarine system in Portugal and one of the largest in Europe. Water residency time is of ca. 23 days in average flow conditions, yet ranges from 6 to 140 days in extreme flow conditions (Lemos, 1964; Rodrigues *et al.*, 1988). Mean river flow is 400 m³s⁻¹, though it is highly variable both seasonally and interannually, tidal range is ca. 4 m (Loureiro, 1979). According to Hayes (1979) this is a mesotidal estuary. Salinity varies from 0 ‰, 50 km upstream from the mouth, to ca. 35‰ at the mouth of the estuary, within the estuary it is very variable and depends on the tidal phase and flow regime (Cabrita and Moita, 1995). It is a partially mixed estuary with a mean depth lower than 10 m. Although its bottom is composed of a heterogeneous assortment of substrates, its prevalent sediment is muddy sand in the upper and middle estuary and sand in the low estuary and adjoining coastal area (Cabral and Costa, 1999). Around 40% of this estuarine area is intertidal (Bettencourt, 1979), composed by mudflats, fringed by saltmarshes.

Two main nurseries for sole have been identified in the upper estuarine areas of the Tagus estuary (near Vila Franca de Xira and Alcochete) by Costa and Bruxelas (1989) and Cabral and Costa (1999). Although most of the environmental factors present a wide and similar range in these two areas, some differences can be outlined. The area near Vila Franca de Xira is deeper (mean depth 4.4 m), presents lower and highly variable salinity and has a higher proportion of fine sand in the substrate. The nursery area near Alcochete is shallower (mean depth 1.9 m), and more saline, with lower variability in salinity, while substrate is mainly composed of mud (Cabral and Costa, 1999). While in the Vila Franca de Xira nursery the two sole species, *S. solea* and *S. senegalensis* can be found, in the Alcochete nursery only *S. senegalensis* is present (Cabral and Costa, 1999).

Important work has been carried out concerning the ecology of *S. solea* and *S. senegalensis* in this estuarine area. There is now information on these species distribution and abundance (Costa, 1982; 1986; Costa and Bruxelas, 1989; Cabral and Costa, 1999), diets (Costa, 1988; Cabral, 2000) and growth (Costa, 1990; Cabral, 2003).

Still, there are several open questions regarding these species dynamics in the Tagus estuary and life-cycles in the Portuguese coast, that need to be investigated so that future decision on these species management can be based on sound scientific knowledge. The present work aims at investigating relevant issues concerning the ecology of the juveniles of *S. solea* and *S. senegalensis* in the Tagus estuary, narrowing the existing information gaps.

This thesis comprises of six chapters, while chapter 1 provides a general view on the main subjects, introduces the scientific themes that will be dealt and presents the structure of the present work, chapters 2 to 5, each, concern a major ecological theme or two closely related themes. The themes focused are: habitat use, food consumption, growth and condition, and recruitment and mortality. Chapter 6 presents the general conclusions and final remarks.

Chapter 2 - Habitat use - investigated the following main questions: What variables should be taken into account to model these species habitat use? Is there connectivity between the two nurseries? What factors affect the use of mudflats by these species?

In order to investigate these questions, the use of the estuarine habitat by the two sole species was analysed at three spatial scales. The first work "Habitat suitability index models for the juvenile soles, *Solea solea* and *Solea senegalensis*: Defining variables for management" looked at broad patterns of habitat use at an estuarine scale, the second work "Nursery fidelity, food web interactions and primary sources of nutrition of the juveniles of *Solea solea* and *Solea senegalensis* in the Tagus estuary (Portugal): a stable isotope approach" investigated connectivity between the nurseries and energy source dependence and the third work "Diel and semi-lunar patterns in the use of an intertidal mudflat by juveniles of Senegal sole, *Solea senegalensis*" investigated activity patterns occurring within one of the most important components of the Tagus estuary nurseries, its extensive mudflats.

Chapter 3 – Food consumption - focused on the questions: What affects prey consumption by these species? How much prey do soles consume? Is soles' abundance limited by the amount of prey available at the nurseries?

To investigate these questions, the effect of temperature and salinity in the gastric evacuation rates of *S. solea* and *S. senegalensis* and the impact of predation pressure in foraging behaviour was investigated. The information obtained was used to produce a first estimation of food consumption by the two sole species in the Tagus estuary nursery grounds during the period of most intense use by juveniles and compare it to the total prey available in the sediment.

Chapter 4 – Growth and condition - looked at the following main questions: Are there growth and condition patterns related to the estuarine colonization undertaken by these juveniles? Which of the nurseries offers better conditions to these juveniles? Can growth rates based on otolith daily increments and condition based on RNA-DNA ratio be used for habitat quality monitoring of soles' nurseries? Does *S. solea* grow faster in the Tagus estuary than at higher latitudes? Are there latitudinal trends in the spawning period of *S. solea*?

In order to answer these questions growth and condition in the successive cohorts of *S. solea* and *S. senegalensis* colonizing the Tagus estuarine nurseries were determined, habitat quality of the two nurseries was compared, and growth and spawning were analysed in a latitudinal perspective. Growth based on otolith daily increments and condition on the RNA-DNA ratio was estimated.

Chapter 5 – Recruitment and mortality - investigated the following main questions: What is the impact of climate and hydrodynamics on the larval immigration of sole towards the Tagus estuary? What is the impact of fishing mortality upon soles' juveniles of the Tagus estuary?

To answer these questions the relation between river drainage, the North Atlantic Oscillation index (NAO index) and the North-South wind component intensity over the three months prior to the end of the estuarine colonization and the densities of *S. solea* and *S. senegalensis* in the nursery grounds were investigated for both species based on a discontinuous historical dataset (from 1988 to 2006) for the Tagus estuary, the catches of *S. solea* and *S. senegalensis* of the beam trawl fishery within the nursery areas of the Tagus estuary were estimated, the mortality of discards of sole juveniles was evaluated, and the impact of this fishery in year-class strength of both sole species was determined, taking into account the various cohorts colonizing the estuary over time.

Chapter 6 - General conclusion and final remarks - presents the main conclusions of the present work, as well as, its contribution for future species and estuarine management. Future studies are also suggested.

References

- Amara, R., Galois, R., 2004. Nutritional condition of metamorphosing sole: spatial and temporal analyses. *Journal of Fish Biology* 64, 72-88.
- Amara, R., Desaunay, Y., Lagardère, F., 1994. Seasonal variation in growth of larval sole *Solea solea* (L.) and consequences on the success of larval immigration. *Netherlands Journal of Sea Research* 32, 287-298.
- Amara, R., Laffargue, P., Dewarumez, J.M., Maryniak, C., Lagardère, F., Luczac, C., 2001. Feeding ecology and growth of 0-group flatfish (sole, dab and plaice) on a nursery ground (Southern Bight of the North Sea). *Journal of Fish Biology* 58, 788-803.
- Amezcuca, F., Nash, R.D.M., 2001. Distribution of the order Pleuronectiformes in relation to sediment type in the North Irish Sea. *Journal of Sea Research* 45, 293-301.
- Andrade, J.P., 1990. A importância da Ria Formosa no ciclo biológico de *Solea senegalensis* Kaup 1858, *Solea vulgaris* Quensel 1806, e *Solea lascaris* (Risso, 1810). Tese de Doutoramento, Universidade do Algarve, Faro.
- Andrade, J.P., 1992. Age, growth and population structure of *Solea senegalensis* Kaup, 1858 (Pisces, Soleidae) in the Ria Formosa (Algarve, Portugal). *Scientia Marina* 56, 35-41.
- Anguis, V., Cañavate, J.P., 2005. Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. *Aquaculture* 243, 133-145.
- Arino, O., Koutsikopoulos, C., Ramzi, A., 1996. Elements of mathematical modelling of evolution in number of a sole population. *Journal of Biological Systems* 4, 445-458.
- Baeta, F., Pinheiro, A., Corte-Real, M., Costa, J.L., Almeida, P.R., Cabral, H., Costa, M.J., 2005. Are the fisheries in the Tagus estuary sustainable? *Fisheries Research* 76, 243-251.
- Bergman, M.J.N., van der Veer, A., Stam, A., Zuidema, D., 1989. Transport mechanisms of larval plaice (*Pleuronectes platessa* L.) from the coastal zone into the Wadden Sea nursery area. *Rapport P.-v. Réunion Conseil international pour l'Exploration de la Mer* 191, 43-49.
- Bernardo, J.M., 1990. Dinâmica de uma lagoa costeira eutrófica (Lagoa de Santo André). Tese de Doutoramento, Faculdade de Ciências de Lisboa, Lisboa.
- Bettencourt, A., 1979. Estuário do Tejo. Documento Preliminar. Hidroprojecto, Drena, Lisboa.
- Beyst, B., Cattrijsse, A., Mees, J., 1999. Feeding ecology of juvenile flatfishes of the surf zone of a sandy beach. *Journal of Fish Biology* 55, 1171-1186.
- Boelhart, G.W., Mundy, B.C., 1988. Roles of behavioural and physical factors in larval and juvenile fish recruitment to estuarine nursery areas. *American Fisheries Society Symposium* 3, 51-67.
- Braber, L., de Groot, S.J., 1973. The food of five flatfish species (Pleuronectiformes) in the southern North Sea. *Netherlands Journal of Sea Research* 6, 163-172.
- Burrows, M.T., 1994. An optimal foraging and migration model for juvenile plaice. *Evolutionary Ecology* 8, 125-149.

- Cabral, H.N., 1998. Utilização do estuário do Tejo como área de viveiro pelos linguados, *Solea solea* (L., 1758) e *Solea senegalensis* Kaup, 1858, e robalo, *Dicentrarchus labrax* (L., 1758). PhD Dissertation, University of Lisbon, Portugal.
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the U.K.* 83, 861-868.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Cabral, H.N., Teixeira, C.M., Gamito, R., Costa, M.J., 2002. Importance of discards of a beam trawl fishery as input of organic matter into nursery areas within the Tagus estuary. *Hydrobiologia* 475/476, 449-455.
- Cabral H.N., Castro, F., Linhares, D., Alexandrino, P., 2003. Genetic differentiation among *Solea solea* (L., 1758) and *Solea senegalensis* Kaup, 1858, (Pisces: Pleuronectiformes) in several estuarine systems of the Portuguese coast. *Scientia Marina* 67, 43-52.
- Cabral, H.N., Teixeira, C.M., Marques, J.F., Batista, M., Baeta, F., Teixeira, T., Costa M.J., 2007. *Biologia e estado de exploração dos stocks de espécies de linguados e solhas com interesse comercial da costa portuguesa: contributos para a gestão sustentável dos recursos (Projecto SOLEA). Relatório Final. Instituto de Oceanografia, FCUL, Lisboa, 207 pp.*
- Cabrita, M.T., Moita, M.T., 1995. Spatial and temporal variation of physico-chemical conditions and phytoplankton during a dry year in the Tagus estuary, Portugal. *Netherlands Journal of Aquatic Ecology* 29, 323-332.
- Champalbert, G., Bourdillon, A., Castelbon, C., Chikhi, D., Direach-Boursier, L., Maquart-Moulin, C., Patriti, G., 1989. Determinisme des migrations des larves et juveniles de soles. *Océanis* 15, 171-180.
- Champalbert, G., Koutsikopoulos, C., 1995. Behavior, transport and recruitment of Bay of Biscay sole (*Solea solea* (L.)): laboratory and field studies. *Journal of the Marine Biological Association of the U.K.* 75, 93-108.
- Costa, M.J., 1982. Contribution à l'étude de l'écologie des poissons de l'estuaire Tage (Portugal). PhD Dissertation, Université Paris VII, Paris.
- Costa, M.J., 1986. Les poissons de l'estuaire du Tage. *Cybium* 10, 57-75.
- Costa, M.J., 1988. Écologie alimentaire des poissons de l'estuaire du Tage. *Cybium* 12, 301-320.
- Costa, M.J., 1990. Age and growth studies of the sole (*Solea vulgaris vulgaris* Quensel, 1806) in the Tagus estuary, Portugal. *Boletim do Instituto Nacional de Investigação das Pescas* 15, 63-67.

- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial species. *Scientia Marina* 53, 561-566.
- deGraaf, M., Jager, Z., Vreugdenhil, C.B., Elorche, M., 2004. Numerical simulations of tidally cued vertical migrations of flatfish larvae in the North Sea. *Estuarine, Coastal and Shelf Science* 59, 295-305.
- deGroot, S.J., 1971. On the interrelationships between morphology of the alimentary tract food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). *Netherlands Journal of Sea Research* 5, 121-196.
- Dinis, M.T., 1986. Quatre Soleidae de l'estuaire du Tage. Reproduction et croissance. Essai d'élevage de *Solea senegalensis* Kaup. Tese de Douturamento. Université de Bretagne Occidentale, Brest
- Dinis, M.T., Ribeiro, L., Soares, F. and Sarasquete, C., 1999. A review of the cultivation potential of *Solea senegalensis* in Spain and Portugal. *Aquaculture* 176, 27-38.
- Garcia, E., Molinero, A. & Flos, R., 1991. Growth comparative study in two species of sole *Solea vulgaris* and *Solea senegalensis* in the Mediterranean Sea. *Aquaculture and Environment. Special Publication of the European Society of Aquaculture* 14, 37-38
- Garcia-Lopez, Anguis, V., Couto, E., Canário, A.V.M., Cañavate, J.P., Sarasquete, C., Martinez-Rodriguez, G., 2006. Non-invasive assessment of reproductive status and cycle sex steroid levels in a captive wild broodstock of Senegalese sole *Solea senegalensis* (Kaup). *Aquaculture* 254, 583-593. doi:10.1016/j.aquaculture.2005.10.007.
- Gibson, R.N., 1973. The intertidal movements and distribution of young fish on a sandy beach with special reference to the plaice (*Pleuronectes platessa* L.). *Journal of Experimental Marine Biology and Ecology* 12, 79-102.
- Gibson, R.N., 2003. Go with the flow. Tidal migration in marine animals. *Hydrobiologia* 503, 153-161.
- Gibson, R.N., Robb, L., 1992. The relationship between body size, sediment grain size and the burying ability of juvenile plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* 40, 771-778.
- Gibson, R.N., Pihl, L., Burrows, M.T., Modin, J., Wennhage, H., Nickell, L.A., 1998. Movement and homing of juvenile plaice (*Pleuronectes platessa*) in relation to predators, competitors, food availability and abiotic factors on a microtidal nursery ground. *Marine Ecology Progress Series* 165, 145-159.
- Gilliers, C., Amara, R., Bergeron, J.P., Le Pape, O., 2004. Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. *Environmental Biology of Fishes* 71, 189-198.
- Gilliers C., Le Pape O., Desaunay Y., Morin J., Guerault D., Amara R., 2006. Are growth and density quantitative indicators of essential fish habitat quality? An application to the common sole *Solea solea* nursery grounds. *Estuarine, Coastal and Shelf Science* 69, 96-106.

- Gonçalves, J., 1990. Fluxos de energia na cadeia trófica do estuário do Tejo. Estudo da ecofase estuarina do elo *Solea solea* (Linnaeus, 1758) (Pisces, Soleidae). Relatório de estágio. Faculdade de Ciências de Lisboa, Lisboa.
- Hayes, M.O., 1979. Barrier island morphology as a function of tidal and wave regime. In: Leatherman S.P. (ed.) Barrier islands from the Gulf of St. Lawrence to the Gulf of Mexico. Academic Press, New York, 1-27.
- Henderson, P.A., James, D., Holmes, R.H.A., 1992. Trophic structure within the Bristol channel: seasonality and stability in Bridgewater Bay. Journal of the Marine Biology Association of the United Kingdom 72, 675-690.
- Imsland, A.K., Foss, A., Conceição, L.E.C., Dinis, M.T., Delbare, D., Schram, E., Kamstra, A., Rema, P., White, P., 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. Reviews in Fish Biology and Fisheries 13, 379-407.
- Jager, Z., Kleef, H.L., Tydeman, P., 1993. The distribution of 0-group flatfish in relation to abiotic factors on the tidal flats in the brackish Dollard (Ems estuary, Wadden Sea). Journal of Fish Biology 43, 31-43.
- Jager, Z., Kleef, H., Tydeman, P., 1995. Mortality and growth of 0-group flatfish in the brackish Dollard (Ems estuary, Wadden Sea). Journal of Fish Biology 34, 119-129.
- Koutsikopoulos, C., Desaunay, Y., Dorel, D., Marchand, J., 1989. The role of coastal areas in the life history of sole (*Solea solea* L.) in the Bay of Biscay. Scientia Marina 53, 567-575.
- Koutsikopoulos, C., Fortier, L., Gagné, J., 1991. Cross-shelf dispersion of Dover sole eggs and larvae (*Solea solea*) in Biscay Bay and recruitment to inshore nurseries. Journal of Plankton Research 13, 923-945.
- Lagardère, J.P., 1987. Feeding ecology and daily food consumption of common sole, *Solea vulgaris* Quensel, juveniles of the french atlantic coast. Journal of Fish Biology 30, 91-104.
- Lasiak, T.A., McLachlan, A., 1987. Opportunistic utilization of mysid shoals by surf-zone teleosts. Marine Ecology Progress Series 37, 1-7.
- Leggett, W.C., Frank, K.T., 1997. A comparative analysis of recruitment variability in North Atlantic Flatfishes – testing the species range hypothesis. Journal of Sea Research 37, 281-299.
- Lemos, P.A.F., 1964. Estuário do Tejo. Administração Geral do Porto de Lisboa, Lisboa.
- Loureiro, J.M., 1979. Curvas de duração dos caudais médios diários no rio Tejo. Direcção Geral de Recursos e Aproveitamentos Hidráulicos, Lisboa.
- Marchand, J., 1991. The influence of environmental conditions on the settlement, distribution and growth of 0-group sole (*Solea solea* (L.)) in a macrotidal estuary (Vilaine, France). Netherlands Journal of Sea Research 27, 307-316.
- Marchand, J., Masson, G., 1989. Process of estuarine colonization by 0-group sole (*Solea solea*): hydrological conditions, behaviour and feeding activity in the Vilaine estuary. Rapp. P.-v. Réun. Cons. Int. Expl. Mer 191, 287-295.

- Miller, J., Reed, J.P., Pietrafesa, L.J., 1984. Patterns, mechanisms and approaches to the study of migration of estuarine-dependent fish larvae and juveniles. In: McCleave, J.D., Arnold, G.P., Dodson, J.J., Neills, W.H. (Eds.), *Mechanisms of migration in fishes*, Plenum Press, New York and London, pp 209-225.
- Molinero, A., Flos, R., 1991. Influence of age and sex on the feeding habits of the common sole *Solea solea*. *Marine Biology* 111, 493-501.
- Molinero, A., Flos, R., 1992. Influence of season on the feeding habits of the common sole *Solea solea*. *Marine Biology* 113, 499-507.
- Molinero, A., Garcia, E., Flos, R., 1991. Comparative study of feeding habits for two species of sole: *Solea vulgaris* and *Solea senegalensis*. *Aquaculture and the environment*, Special publication of the European Society of Aquaculture 14, 219-220.
- Quero, J.C., Dessouter, M., Lagardère, F., 1986. Soleidae In: *Fishes in the North-eastern Atlantic and Mediterranean*. Whitehead, P., Bauchot, M.-L., Hureau, J.-C., Nielsen, J., Tortonese, E., (Eds.), 1986. UNESCO, Paris, pp. 1308-1342.
- Quiniou, L., 1978. Les poisons demersaux de la Baie de Dournenez. Alimentation et ecologie. Tese de Doutorado, Université de Bretagne Occidentale.
- Raffaelli, D., Richner, H., Summers, R., Northott, S., 1990. Tidal migrations in the flounder (*Platichthys flesus*). *Marine Behaviour Physiology* 16, 249-260.
- Ramzi, A., Arino, O., Koutsikopoulos, C., Boussovar, A., Lazure, P., 2001. Modelling and numerical simulations of larval migration of the sole (*Solea solea* (L.)) of the Bay of Biscay. Part 1: modelling. *Oceanologica acta* 24, 101-112.
- Richard, P., Bergeron, J.P., Boulhic, M., Galois, R., Ruyet, J., 1991. Effect of starvation on RNA, DNA and protein content of laboratory reared larvae and juveniles of *Solea solea*. *Marine Ecology Progress Series* 72, 69-77.
- Rijdsdorp, A.D., van Stralen, M., van der Veer, H., 1985. Selective tidal transport of North Sea plaice (*Pleuronectes platessa*) in coastal nursery areas. *Transactions of the American Fisheries Society* 114, 461-470.
- Riley, J.D., Symonds, D.J., Woolner, L., 1981. On the factors influencing the distribution of 0-group demersal fish in coastal waters. *Rapp. P.-v. Réun. Conseil International de exploration de la Mer* 178, 223-228.
- Rodrigues, A.C., Silva, M.C., Câmara, A., Fernandes, T.F., Ferreira, J.G., 1988. Dispersion modelling for a complex estuary: the case of the Tagus. *Water, Science and Technology* 20, 271-276.
- Rogers, S.I., 1992. Environmental factors affecting the distribution of sole (*Solea solea* (L.)) within a nursery area. *Netherlands Journal of Sea Research* 29, 153-161.
- Rogers, S.I., 1994. Population density and growth rate of juvenile sole *Solea solea* (L.) *Netherlands Journal Sea Research* 32, 353-360.
- Sobral, D., 1981. Contribuição para o estudo da ictiofauna bentónica do estuário do rio Sado. Relatório de estágio. Faculdade de Ciências de Lisboa, Lisboa.

- van der Veer, H.W., Bergman, M.J.N., 1987. Development of tidally related behaviour of a newly settled 0-group plaice (*Pleuronectes platessa*) population in the western Wadden Sea. *Marine Ecology Progress Series* 31, 121-129.
- Van der Veer, H.W., Berghahn, R., Miller, J.M., Rijnsdorp, A.D., 2000. Recruitment in flatfish, with special emphasis on North Atlantic species: Progress made by the Flatfish Symposia. *ICES Journal of Marine Science* 57, 202-215.
- Vinagre, C., França, S., Costa, M.J., Cabral, H., 2005. Niche overlap between juvenile flatfishes, *Platichthys flesus* and *Solea solea*, in a southern European estuary and adjacent coastal waters. *Journal of Applied Ichthyology* 21, 114-120.
- Wegner, G.W., Damm, U., Purps, M., 2003. Physical influences on the stock dynamics of plaice and sole in the North Sea. *Scientia Marina* 67, 219-234.
- Wirjatmodjo, S., Pitcher, T.J., 1980. Flounders follow the tides to feed: Evidence from ultrasonic tracking in an estuary. *Estuarine, Coastal and Shelf Science* 19, 231-241.

CHAPTER 2

- HABITAT USE -

Habitat suitability index models for the juvenile soles, *Solea solea* and *Solea senegalensis*: defining variables for management. Fisheries Research. 2006, 82, 140-149.

By Vinagre, C., Fonseca, V., Cabral, H., Costa, M.J.

Nursery fidelity, food web interactions and primary sources of nutrition of the juveniles of *Solea solea* and *Solea senegalensis* in the Tagus estuary (Portugal): a stable isotope approach. Estuarine, Coastal and Shelf Science (*in press*).

By Vinagre, C., Salgado, J., Costa, M.J., Cabral, H.N.

Diel and semi-lunar patterns in the use of an intertidal mudflat by juveniles of Senegal sole, *Solea senegalensis* Kaup, 1858. Estuarine, Coastal and Shelf Science. 2006, 69, 246-254.

By Vinagre, C., França, S., Cabral, H.N.

Introduction

The study of habitat use by fish has long been an important subject area within fish ecology. However, in the 1980's ecologists' attention focused mainly on the conservation of commercial fish stocks, in an attempt to recover target species subjected to over-fishing in the 1970's (Fluharty, 2000). In the 1990's, as concerns over biodiversity and habitats increased, there was a greater need for the integration of large amounts of data.

Research on habitat use by fish became, and still is, particularly intense in the United States of America, after the approval of the "Sustainable Fisheries Act" (SFA, 1996), which addresses the importance of sustaining adequate habitats for fish species. This legislation demands the determination of the "Essential Fish Habitat", defined as "those waters and substrate necessary to fish for spawning, breeding, feeding, or growth to maturity" (SFA, 1996). The recent European Water Framework (Directive 2000/60/EC; EC, 2000) follows a similar philosophy, concentrating on the need for the identification and protection of specific water bodies as a whole for the conservation of biodiversity.

In order to simultaneously have a broad and in-depth knowledge of the habitat use by fish, studies at different spatial scales are crucial. In the present chapter the use of the estuarine habitat by the soles, *Solea solea* (Linnaeus 1758) and *Solea senegalensis* Kaup, 1858, was analysed at three spatial scales. While the first work "Habitat suitability index models for the juvenile soles, *Solea solea* and *Solea senegalensis*: defining variables for management" looks at broad patterns of habitat use at an estuarine scale, the second work entitled "Nursery fidelity, food web interactions and primary sources of nutrition of the juveniles of *Solea solea* and *Solea senegalensis* in the Tagus estuary (Portugal): a stable isotope approach" investigates relations and processes occurring in the nurseries, while the third work "Diel and semi-lunar patterns in the use of an intertidal mudflat by juveniles of Senegal sole, *Solea senegalensis*" focuses on the activity patterns occurring within one of the most important components of the Tagus estuary nurseries: its extensive mudflats.

The first work aims at producing simple and effective habitat models to predict the distribution of both sole species in the Tagus estuary, while investigating which key variables determine habitat quality for soles.

Several studies have produced important information on sole distribution within estuaries throughout Europe, although the majority were conducted in areas where only *S. solea* exists. Most of these studies indicate that juvenile sole occur in higher densities in shallow areas, with fine sediment (e.g. Dorel *et al.*, 1991; Koutsikopoulos *et al.*, 1989; Rogers, 1992; Dorel and Desaunay, 1991) and low salinity (e.g. Marchand and Mason, 1989; Marchand, 1991). Previous studies in the Tagus estuary, from 1978 to 2002, identified two nursery areas in the upper Tagus estuary, where juvenile sole concentrate in similar conditions to those reported for other estuaries (Costa, 1982; Costa, 1986; Costa, 1988; Costa and Bruxelas, 1989, Cabral

and Costa, 1999). All previous information had to be taken into account in the construction of the habitat suitability models for the Tagus estuary. In this context a Geographic Information System (GIS) was used to effectively collate, archive, display, analyse and model spatial and temporal data originated by the different scientific studies produced over time.

The second work aims at assessing the site fidelity of soles inhabiting the two nursery areas, at investigating food web interactions and at determining the dominant nutrient pathways, using stable isotopes. There have been several attempts at assessing site fidelity of soles in the Tagus estuary through mark-recapture experiments, yet none was successful due to the low level of individuals recaptured (Cabral, *personal communication*). Stable isotopes are powerful tools that allow not only the determination of site fidelity (Fry *et al.*, 1999; Talley, 2000; Fry *et al.*, 2003), but also the investigation of nutrient pathways, food-web interactions and energy sources (e.g. Simenstad and Wissmar, 1985; France, 1995; Paterson and Whitfield, 1997; Riera *et al.*, 1999; Darnaude *et al.*, 2004). These issues are still unexplored in the Tagus estuary, yet in-depth scientific knowledge on the functioning of the Tagus estuary food-webs will be needed to face current and prospective management challenges that present itself today and in the near future due to growing anthropogenic pressure and the many impacts that will arise from climate warming.

The third work aims at evaluating the diel and semi-lunar patterns in the use of the intertidal mudflats of the Tagus estuary by *S. senegalensis*. The extensive mudflat areas that dominate the upper Tagus estuary are one of the most important components of these nurseries, since they play a very important role as feeding areas for birds and fish. Studies on other fish suggest that these movements may be strongly structured by tidal and day-night cycles (Naylor, 2001; Morrison *et al.*, 2002; Krumme *et al.*, 2004), yet the effect of the lunar cycle is still scarcely understood. Cabral (2000) reported that intertidal mudflats are very important feeding grounds for *S. senegalensis* juveniles, however spatial and temporal patterns of this species use of the intertidal zone are still unknown.

References

- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Costa, M.J., 1982. Contribution à l'étude de l'écologie des poissons de l'estuaire Tage (Portugal). PhD Dissertation, Université Paris VII, Paris.
- Costa, M.J., 1986. Les poissons de l'estuaire du Tage. *Cybiurn* 10, 57-75.
- Costa, M.J., 1988. Écologie alimentaire des poissons de l'estuaire du Tage. *Cybiurn* 12, 301-320.

- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial species. *Scientia Marina* 53, 561-566.
- Costa, M.J., Cabral, H.N., 1999. Changes in the Tagus estuary nursery function for commercial fish species: some perspectives for management. *Aquatic Ecology* 33, 53-74.
- Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., Harmelin-Vivien, M.L., 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* 138, 325-332.
- Dorel, D., Desaunay, Y., 1991. Comparison of three *Solea solea* (L.) nursery grounds of the Bay of Biscay: distribution, density and abundance of 0-group and I-group. ICES CM, G, 75.
- EC (European Communities), 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23rd October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L. 327, 1-72
- France, R., 1995. Stable nitrogen isotopes in fish: literature synthesis on the influence of ecotonal coupling. *Estuarine, Coastal and Shelf Science* 41, 737-742.
- Fluharty, D., 2000. Habitat protection, Ecological issues, and Implementation of the Sustainable Fisheries Act. *Ecological Applications* 10, 325-337.
- Fry, B., Mumford, P., Robblee, M., 1999. Stable isotope studies of pink shrimp (*Farfantepenaeus duorarum* Burkenroad) migrations on the southwestern Florida Shelf. *Bulletin of Marine Science* 65, 419-430.
- Fry, B., Baltz, D.M., Benfield, M., Fleeger, J., Gace, A., Haas, H., Quiñones-Rivera, Z., 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. *Estuaries* 26, 82-97.
- Koutsikopoulos, C., Desaunay, Y., Dorel, D., Marchand, J., 1989. The role of coastal areas in the life history of sole (*Solea solea* L.) in the Bay of Biscay. *Scientia marina* 53, 567-575.
- Krumme, U., Saint-Paul, U., Rosenthal, H., 2004. Tidal and diel changes in the structure of a nekton assemblage in small intertidal mangrove creeks in Northern Brazil. *Aquatic Living Resources* 17, 215-229.
- Marchand, J., 1991. The influence of environmental conditions on the settlement, distribution and growth of 0-group sole (*Solea solea* (L.)) in a macrotidal estuary (Vilaine, France). *Netherlands Journal of Sea Research* 27, 307-316.
- Marchand, J., Masson, G., 1989. Process of estuarine colonization by 0-group sole (*Solea solea*): hydrological conditions, behaviour and feeding activity in the Vilaine estuary. *Rapp. P.-v. Réun. Cons. Int. Expl. Mer* 191, 287-295.
- Morrison, M.A., Francis, M.P., Hartill, B.W., Parkinson, D.M., 2002. Diurnal and Tidal variation in the abundance of the fish fauna of a temperate tidal mudflat. *Estuarine, Coastal and Shelf Science* 54, 793-807.
- Naylor, E., 2001. Marine animal behaviour in relation to lunar phase. *Earth, Moon and Planets* 85-86, 291-302.

- Paterson, A.W., Whitfield, A.K., 1997. A stable carbon isotope study of the food-web in a freshwater-deprived South African estuary, with particular emphasis on the ichthyofauna. *Estuarine, Coastal and Shelf Science* 45, 705-715.
- Riera, P., Stal, L.J., Nieuwenhuize, J., Richard, P., Blanchard, G., Gentil, F., 1999. Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources. *Marine Ecology Progress Series* 187, 301-307.
- Rogers, S.I., 1992. Environmental factors affecting the distribution of sole (*Solea solea* L.) within a nursery area. *Netherlands Journal of Sea Research* 29, 153-161.
- Simenstad, C.A., Wissmar, R.C., 1985. $\delta^{13}\text{C}$ evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. *Marine Ecology Progress Series* 22, 141-152.
- SFA (Sustainable Fisheries Act) US Senate 23 May, 1996. Report of the committee on commerce, science and transportation on S.39: Sustainable Fisheries Act. Report 104-276, 104th Congress, Second session. US Government printing, Washington D.C. U.S.A.
- Talley, D., 2000. Ichthyofaunal utilization of newly-created versus natural salt marsh creeks in Mission Bay, California. *Wetlands Ecology and Management* 8, 117-132.

Habitat suitability index models for the juvenile soles, *Solea solea* and *Solea senegalensis*: defining variables for management

Abstract: Habitat Suitability Index (HSI) models were used to map habitat quality for the sympatric soles *Solea solea* (Linnaeus, 1758) and *S. senegalensis* Kaup, 1858, in the Tagus estuary, Portugal. The selection of input variables to be used in these models is crucial since the recollection of such data involves important human and time resources. Various combinations of variables were developed and compared. Habitat maps were constructed for the months of peak abundance of *S. solea* and *S. senegalensis* consisting of grid maps for depth, temperature, salinity, substrate type, presence of intertidal mudflats, density of amphipods, density of polychaetes and density of bivalves. The HSI models were run in a Geographic Information System by reclassifying the habitat maps to a 0-1 suitability index scale. Following reclassification, the geometric mean of the suitability index values for each variable was calculated by grid cell, using different combinations of variables, and the results were mapped. Models performance was evaluated by comparing model outputs to data on species' densities in the field surveys at the time. Further model testing was performed using independent data. Results show that there are two areas that provide the highest habitat quality. The model that combined density of amphipods and the abiotic variables had the highest correlation with the distribution of *S. solea* while the combination of density of polychaetes and the abiotic variables had the highest correlation with *S. senegalensis* distribution. These variables should be taken into account in future management plans, since they indicate the main nursery grounds for these species.

Key-words: Habitat suitability; Flatfish; *Solea solea*; *Solea senegalensis*; Estuarine nurseries; Fisheries management.

Introduction

There is a growing need to adopt ecosystem concepts into management plans and it is generally agreed that habitat quality assessment should play a decisive role in the environmental decision process. Yet field studies often fail to completely cover the available habitat or do not provide comprehensive temporal coverage. This can lead to management decisions based on scarce and inadequately integrated information. In this context, Geographic Information Systems (GIS) can be used to effectively integrate and model spatial and temporal data.

Habitat suitability index modelling (HSI) is a valuable tool in ecology. It can be used in combination with GIS technology providing maps and information upon which environmental managers can make informed decisions (Terrel, 1984; Bovee and Zuboy, 1988). These models are based on suitability indices that reflect habitat quality as a function of one or more environmental variables. The HSI modelling method used in this study was based on the U.S. Fish and Wildlife Service Habitat Evaluation Procedures Program (Terrel, 1984; Bovee and

Zuboy, 1988) which was primarily used in terrestrial and freshwater environments, but has also been applied to estuaries (e.g. Gibson, 1994; Reyes *et al.*, 1994; Brown *et al.*, 2000).

One of the most important issues when developing species management models is that of selecting which variables should be taken into account. The ideal management model should be simple and rely on a few key variables. It is also fundamental to make the time spent on field and laboratory work, as well as on integrating the data, compatible with the requests of managers and decision-makers. However, it is well known that species dynamics are complex and dependent on the combined effect of several variables.

In the present study habitat maps were constructed using different combinations of pertinent variables and compared with *Solea solea* (Linnaeus, 1758) and *S. senegalensis* Kaup, 1858, densities.

The soles, *S. solea* and *S. senegalensis*, were chosen for this study because of their importance in management terms, regarding both ecosystem and commercial perspectives. These species are top benthic predators that usually do not occur in high densities in the same nurseries. In fact previous studies indicate that the Tagus estuary (Portugal) may be unique in the simultaneous occurrence of both species in high abundance (e.g. Costa and Bruxelles, 1989; Cabral and Costa, 1999).

Baeta *et al.* (2005) concluded that sole fisheries in the Tagus estuary are not environmentally sustainable. Beam trawling is illegal in all Portuguese estuaries except the Tagus where it is quite common in the uppermost areas and has juvenile soles as its main target (Baeta *et al.*, 2005). A defence period (when fishing is forbidden) between the 1st of May and the 31st of July is in place, as well as a minimum length at capture (24 cm), however these regulations are not fully respected and sole 0-group juveniles are captured during the defence period to be sold for aquaculture and local restaurants. Their high commercial value, the increasing market demand for adults and juveniles and the fishing pressure not restricted to the coast but also present in the estuary, make sole fisheries an interesting ecological and socio-economical subject.

The zoogeographic importance of the latitudinal area where the Tagus estuary is located has long been recognized, representing the transition between the North-eastern Atlantic warm-temperate and cold-temperate regions (Ekman, 1953; Briggs, 1974). This estuary plays an important role as an over-wintering area and feeding ground for birds and part of its upper portion is a nature reserve (The Tagus Estuary Nature Reserve). In addition, some areas have special protection status (Birds Directive 79/409/EEC). Its importance as a nursery area for several fish species, including *S. solea* and *S. senegalensis*, has also been documented by several studies (e.g. Costa and Bruxelles, 1989; Cabral and Costa, 1999; Cabral 2000).

The aim of this study is to produce a simple, yet effective, model to predict *S. solea* and *S. senegalensis* juveniles' distribution in the Tagus estuary, in order to contribute to future management of fisheries in this estuarine system. Comparison of the different models produced in the present study will allow us to decide which variables should be taken into account while managing these species.

Material and methods

Study area

The Tagus estuary (Fig. 1), with an area of 320 km², is a partially mixed estuary with a tidal range of 4 m. This estuarine system has a mean depth lower than 10 m and about 40% of its area is composed of intertidal mudflats (Cabral and Costa, 1999).

Although its bottom is composed of a heterogeneous assortment of substrates, its prevalent sediment is muddy sand in the upper and middle estuary and sand in the low estuary and adjoining coastal area (Cabral and Costa, 1999). The mean river flow is 400 m³s⁻¹, though it is highly variable both seasonally and interannually. Salinity varies from 0‰, 50 km upstream from the mouth, to 35‰ at the mouth of the estuary (Cabral *et al.*, 2001). Water temperature ranges from 8°C to 26°C (Cabral *et al.*, 2001).

In the summer the average water temperature is 24°C in the upper estuary and 17°C in the adjacent coast. In winter mean water temperatures range from 16°C in the upper estuary to 15°C in the adjacent coast (e.g. Cabrita and Moita, 1995). Wind induced upwelling occurs in coastal areas during summer (Fiúza *et al.*, 1982).

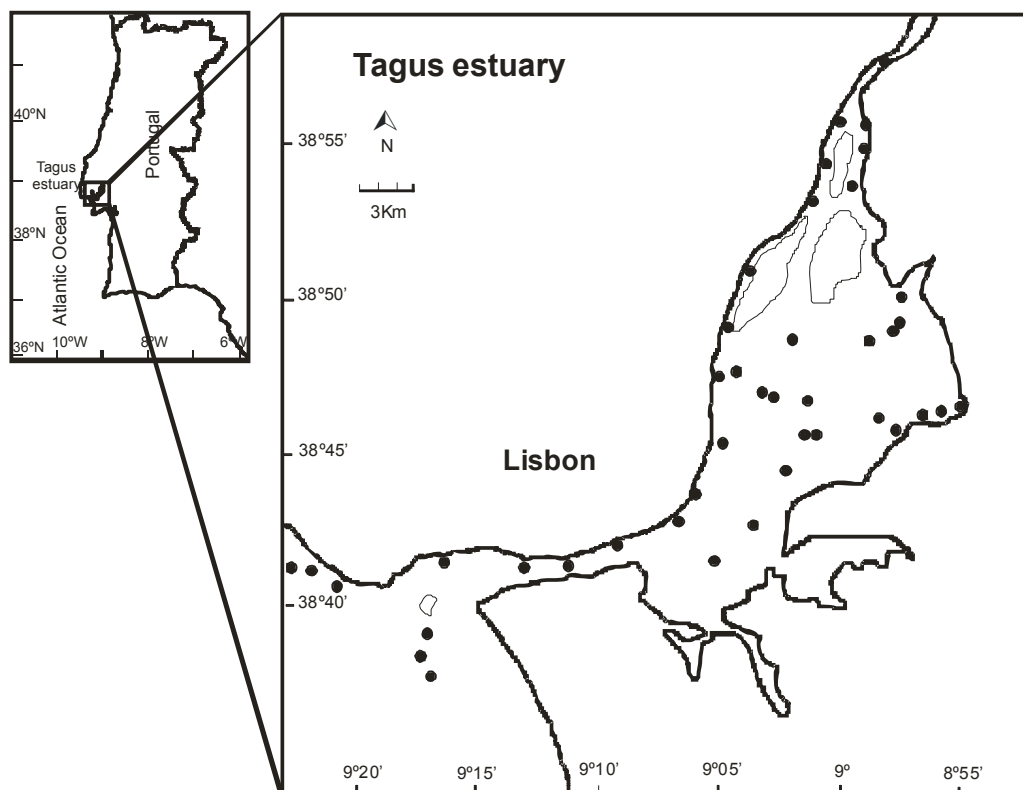


Figure 1 - Map of the Tagus estuary and location of the sampling sites used in the models.

Database

Various sources of data that describe the temporal and spatial variation of depth, temperature, salinity, substrate, etc, are available for the Tagus estuary. Several surveys have been conducted in the Tagus estuary since 1978 (e.g. Bettencourt, 1979; Costa, 1982; Costa,

1988; Costa and Bruxelas, 1989; Cabral, 1998; Cabral and Costa, 1999; Costa and Cabral, 1999; Cabral 2000; Cabral *et al.*, 2001). These studies provide useful information on the environmental variables and on the species abundance, as well as its seasonal variation and spatial occurrence. Maps and depth charts were obtained from the latest maps and charts developed by the Portuguese Hydrographical Institute. All information was assembled in a Geographic Information System.

Data Analysis

Data from the database of the Instituto de Oceanografia that comprises most studies on the Tagus estuary referred above were explored. Data from different years were analysed, as well as from the selected surveys. A visual display of the datasets was performed in order to detect abnormal values. Histograms and summary statistics (measures of location, spread and shape) were calculated to better understand the statistical properties associated to the datasets.

Data were interpolated using the inverse distance to a power method and digital environmental maps were developed in a grid format using the software Surfer 7.0[®]. The inverse distance to a power method was used since it is a local interpolation method (only a subset of observational points is used to estimate the values of each interpolated point). Local interpolation methods are appropriate for branched systems with complex hydrology such as the Tagus estuary, where global interpolation would not make sense (Isaaks and Srivastava, 1989; Bailey and Gatrell, 1996). In this method observational points are weighted such as the influence of one point declines with distance from the point to be interpolated. Again, in a highly branched estuary, such as the Tagus, proximity should be weighted when interpolating new points (Isaaks and Srivastava, 1989; Bailey and Gatrell, 1996). The appropriate range radius of interpolation was determined through exploration of the dataset for each variable.

For each species data from its month of 0-group juveniles peak abundance of the 2001 surveys were selected, and information on environmental variables at 42 sampling stations throughout the Tagus estuary and adjoining coastal area was collated (Fig. 1). The month of peak abundance was chosen since it is at this time that the estuary assumes nursery function. Maps of *S. solea* density in May 2001 and *S. senegalensis* density in November 2001 were developed based on survey data (Figs. 2, 3). The inverse distance to a power was used to create these maps, for the reasons already mentioned for the environmental variables and because 0-group juveniles of both species concentrate in small areas (Cabral and Costa, 1999). In May 2001 water temperature ranged from 14 to 17°C, while salinity ranged from 0 to 30 ‰ (inside the estuary). In November 2001 water temperature ranged from 13 to 18°C, while salinity ranged from 2 to 30 ‰ (inside the estuary).

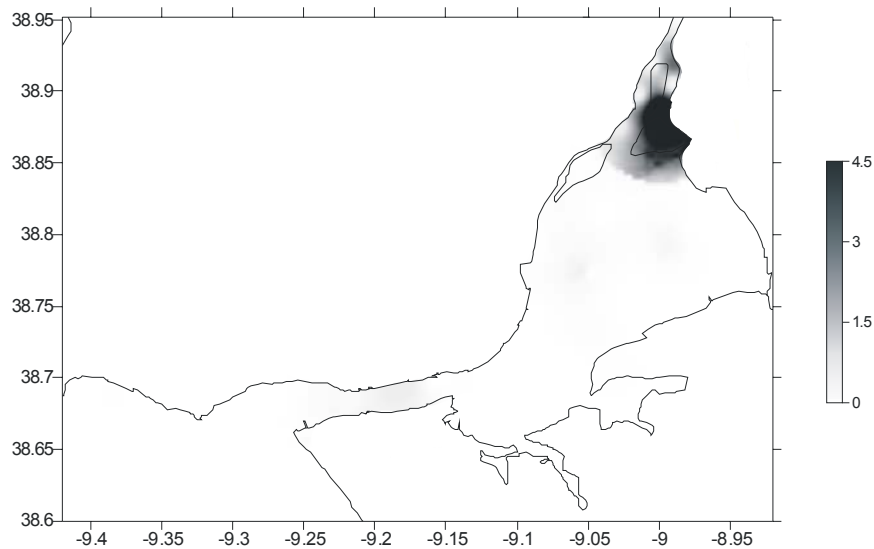


Figure 2 – *S. solea* 0-group juveniles density (ind. 1000 m⁻²) in the Tagus estuary in May 2001.

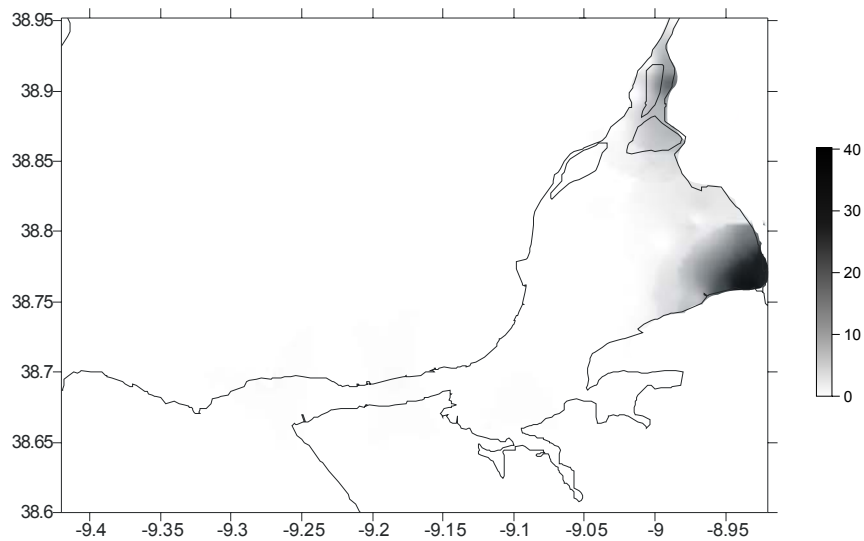


Figure 3 - *S. senegalensis* 0-group juveniles density (ind. 1000 m⁻²) in the Tagus estuary in November 2001.

For each model, Suitability Index (SI) values between 0 and 1 were assigned to ranges of each environmental variable, depending on how favourable the range is for survival, growth and reproduction (Table 1). An SI value of 1 was assigned to the most favourable conditions, an SI value of 0,5 was assigned to average suitability, an SI of 0,1 intended to represent the range of conditions where a species can occur but is rare and an SI of 0 was assigned to environmental conditions outside the survival range of the species.

Table 1 – Definitions of suitability index values (adapted from Brown *et al.*, 2000).

Suitability Index Value	Description of habitat use
1	High density or relative abundance in field studies; high growth potential; active preference in behavioural studies.
0.5	Common occurrence or average density in field studies; average growth potential.
0.1	Rare occurrence or low density in field studies; tolerance documented in field or laboratory studies; little growth potential.
0	Little or no occurrence in field studies; mortality may occur in laboratory or field studies; active avoidance in behavioural studies.

Each environmental variable was reclassified by grid-cell to the suitability index scale (e.g. temperature in degrees centigrade was converted to a 0-1 suitability scale), based on habitat affinities of both species derived from published information on the species biology as well as on our database from the surveys conducted in the Tagus since 1978 and expert review (Table 2).

In order to adapt Brown *et al.*, (2000) models to these flatfish species a variable called intertidal was added. Sampling stations were given an index of 0 or 1, depending on its location in subtidal environments or over intertidal mudflats, respectively. Intertidal mudflats cover around 40% of the Tagus estuary, and have been recognized as important feeding grounds for these benthic species (Cabral, 2000). Intertidal areas are also important settling areas for metamorphosing sole juveniles (van der Veer *et al.*, 2001).

Concerning the variables related to prey presence, namely density of amphipods, density of bivalves and density of polychaetes (Cabral, 2000), its 90th and 70th percentile were determined. Densities under the 70th percentile were given an index of 0.1, densities between this value and the 90th percentile value were given an index of 0.5 and densities above the 90th percentile value were given an index of 1. The geometric mean of the suitability index values was calculated by grid cell, overlaying the environmental maps. This resulted in a map of the composite habitat suitability index value (Figs. 4 and 5). The model was first run using temperature, depth, salinity and substrate type, as well as absence / presence of intertidal mudflats (with a value of 0 and 1, respectively). Secondly a sixth variable was added to the calculation: the major prey items (density of amphipods, polychaetes and bivalves) were considered as separate variables, in order to understand which one was the most important in defining the distribution of soles' juveniles, if any. An additional model was calculated using all abiotic and biotic variables.

Table 2 - Definitions of suitability index values for abiotic variables and most relevant references

	Suitability Index Value		Most relevant references
	<i>S. solea</i>	<i>S. senegalensis</i>	
Sediment	Mud or fine sand = 1 Sand = 0,5 Coarse sand and gravel = 0,1		De Groot (1971), Rogers (1992), Gibson (1994), Cabral and Costa (1999), Amezcua and Nash (2001).
Depth (m)	≤ 10 m = 1 > 10 and < 14 m = 0,5 ≥ 14 m = 0,1		Riley <i>et al.</i> (1981), Rijinsdorp <i>et al</i> (1992), Rogers (1993), Symonds and Rogers (1995), Cabral and Costa (1999), LePape <i>et al</i> (2003), Eastwood <i>et al</i> (2003)
Temperature (°C)	≥ 16 °C and < 24 °C = 1 ≥ 24 °C and ≤ 26 °C = 0,5 ≥ 11 °C and < 16 °C = 0,5 ≥ 26 °C and ≤ 28°C = 0,1 < 11 °C = 0,1	> 13°C and < 28 °C = 1 ≤ 13 °C = 0,5 ≥ 28 °C = 0,1	Irwin (1973), Fonds (1976), Fonds (1979), Cabral and Costa (1999), LeFrançois and Claireaux (2003), Imsland <i>et al.</i> (2003).
Salinity (‰)	≥ 10 ‰ and < 33 ‰ = 1 < 10 ‰ and > 7 ‰ = 0,5 ≥ 33 ‰ = 0,5 ≤ 7 ‰ and ≥ 1 ‰ = 0,1 < 1 ‰ = 0	≥ 4 ‰ = 1 < 4 ‰ and ≥ 1 ‰ = 0,5 < 1 ‰ = 0	Riley <i>et al.</i> (1981), Marchand (1991), Cabral and Costa (1999).
Intertidal presence	Presence = 1 Absence = 0		Cabral and Costa (1999), Cabral (2000) Van der Veer <i>et al</i> (2001).

The calculation of the habitat suitability indices was based on an unweighted geometric mean for each species, and the following five models were tested:

$$HSI_{abiotic} = (SI_{salinity} \cdot SI_{temperature} \cdot SI_{substrate} \cdot SI_{depth} \cdot SI_{intertidal})^{1/5}$$

$$HSI_{amphipods} = (SI_{salinity} \cdot SI_{temperature} \cdot SI_{substrate} \cdot SI_{depth} \cdot SI_{intertidal} \cdot SI_{amphipods})^{1/6}$$

$$HSI_{polychaetes} = (SI_{salinity} \cdot SI_{temperature} \cdot SI_{substrate} \cdot SI_{depth} \cdot SI_{intertidal} \cdot SI_{polychaetes})^{1/6}$$

$$HSI_{bivalves} = (SI_{salinity} \cdot SI_{temperature} \cdot SI_{substrate} \cdot SI_{depth} \cdot SI_{intertidal} \cdot SI_{bivalves})^{1/6}$$

$$HSI_{all} = (SI_{salinity} \cdot SI_{temperature} \cdot SI_{substrate} \cdot SI_{depth} \cdot SI_{intertidal} \cdot SI_{bivalves} \cdot SI_{polychaetes} \cdot SI_{amphipods})^{1/8}$$

Because all terms in the models ranged from 0 to 1, models output also ranged from 0 to 1. One of the most important advantages of using the geometric mean is that if any term equals zero so will the output since, it's a multiplicative calculation. Therefore it takes only one

of the variables to be totally unsuitable for the output to be zero. As a result, habitat with any single environmental characteristic outside the range of the species will be identified as unsuitable, regardless of the values of the other environmental variables.

The habitat suitability maps were compared to the data from the fish sampling surveys. Model performance was evaluated with the Spearman correlation test, which compared model outputs for both species to data on its densities in the field surveys at the same time. Histograms of the density of each species (from sampling) according to ranges of SI values calculated (for the same grid cells) were produced and Kruskal-Wallis tests between the range groups ([0-0,2[;[0,2-0,4[;[0,4-0,6[;[0,6-0,8[;[0,8-1,0[) performed, using SYSTAT 10.0. To prevent bias inherent to the use of the same data for both model estimation and validation the final model was tested with independent data from 2002.

Results

Model outputs show that the upper estuary has the highest habitat quality for juveniles of both species and exclude the coastal area and lower estuary, as well as most of the middle estuary (Figs. 4, 5). The abiotic variables model, $HSI_{abiotic}$ (temperature, salinity, substrate, type, depth, presence of intertidal areas) (Figs. 4a, 5a), indicates a broad area of high habitat quality for both soles, located in the upper estuary. The inclusion of prey density as a sixth variable greatly restricted that area to smaller areas within the upper estuary. The model that included density of amphipods, $HSI_{amphipods}$, yielded very good results in predicting *S. solea* densities in its month of peak abundance (Figs. 4b, 6), while the model that included polychaetes, $HSI_{polychaetes}$, had the best results for *S. senegalensis* (Figs. 5c, 7). The Kruskal-Wallis test indicated that the distribution of density values of both species differed significantly across SI groups ($p < 0,0005$) (Figs. 6 and 7).

$HSI_{amphipods}$ model (Fig. 4b) predicted areas of high habitat quality for *S. solea* in the northwest of the upper estuary in an area dominated by intertidal islands and canals. This is the estuarine area with the highest fresh water inflow. Salinity varies between 0‰ and 20‰ while temperature varies between 14°C and 22°C (all year range).

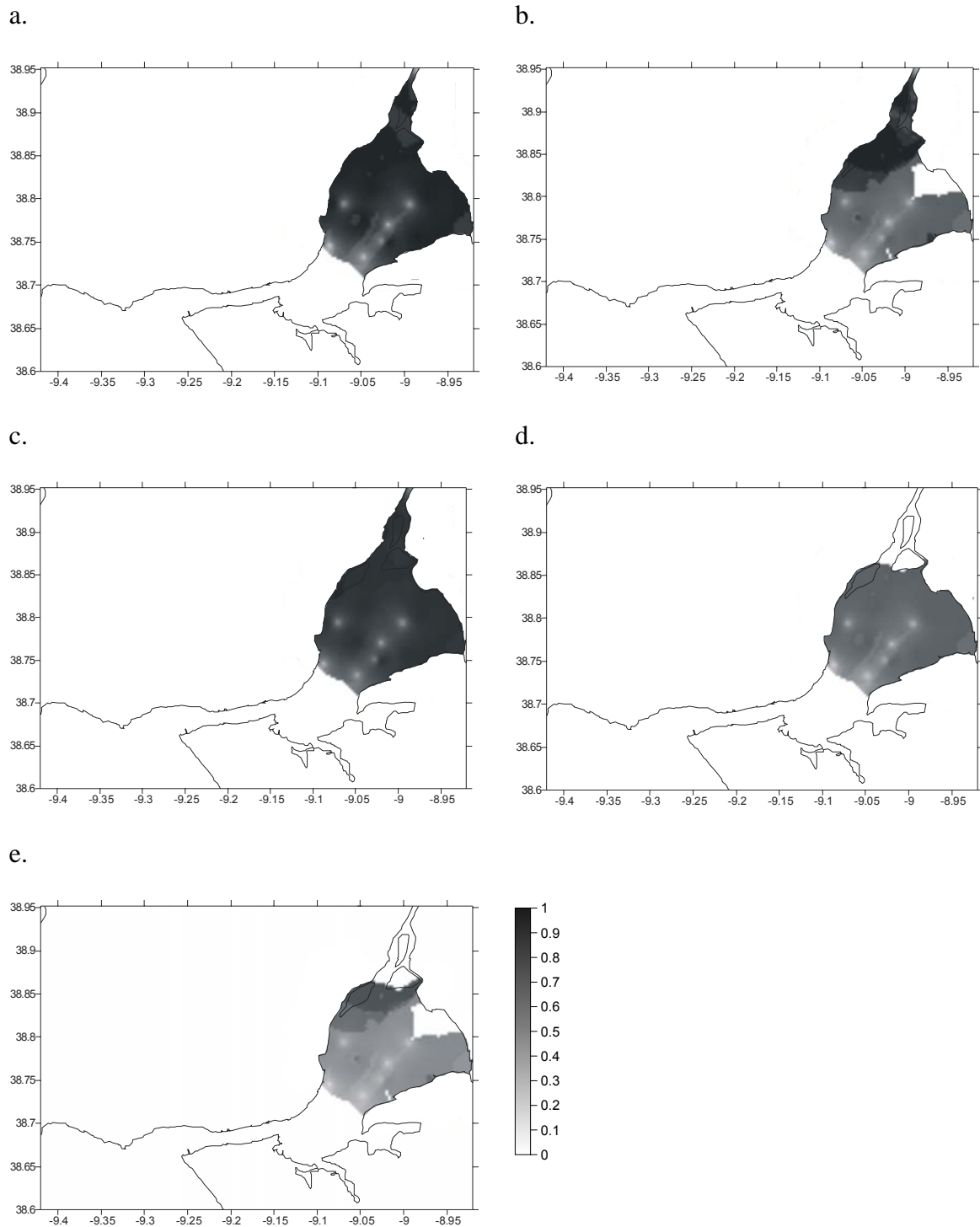


Figure 4 – Mapped habitat suitability for *S. solea* 0-group juveniles according to the different models tested ((a) $HSI_{abiatic}$; (b) $HSI_{amphipods}$; (c) $HSI_{polychaetes}$; (d) $HSI_{bivalves}$; (e) HSI_{all}).

$HSI_{polychaetes}$ model (Fig. 5c) predicted an area of high habitat quality for *S. senegalensis* in the east of the upper estuary. This is a low depth area dominated by intertidal mudflats. Salinity varies between 6‰, in the winter, and 25‰ and temperature between 10°C and 26°C.

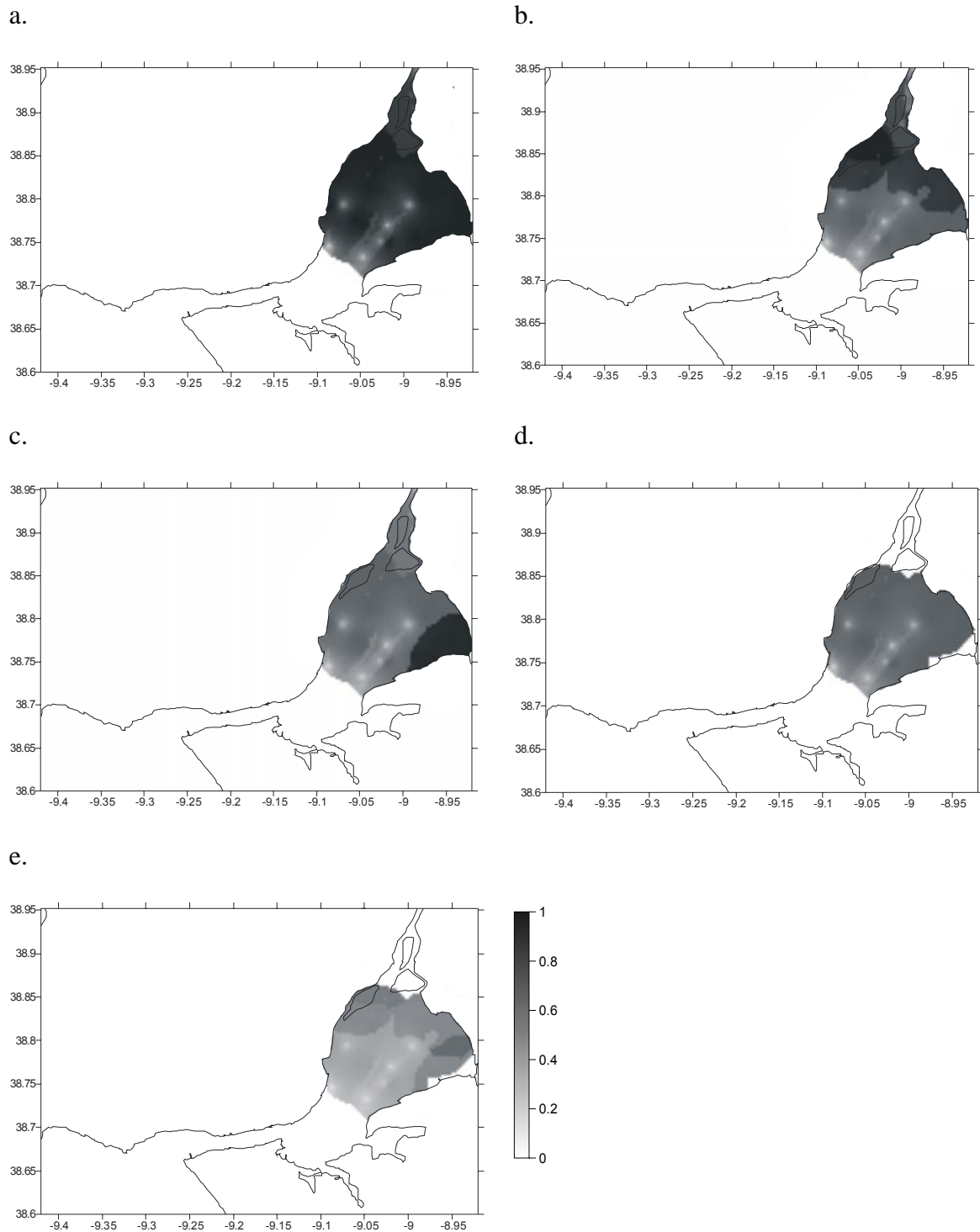


Figure 5 - Mapped habitat suitability for *S. senegalensis* 0-group juveniles according to the different models tested ((a) $HSI_{abiotic}$; (b) $HSI_{amphipods}$; (c) $HSI_{polychaetes}$; (d) $HSI_{bivalves}$; (e) HSI_{all}).

The models which included all abiotic variables as well as all prey types, HSI_{all} (Figs. 4e, 5e), predicted areas with the highest habitat quality located in the upper estuary. This model yielded SI values generally under 0.5 and excluded most of the nursery areas of both soles.

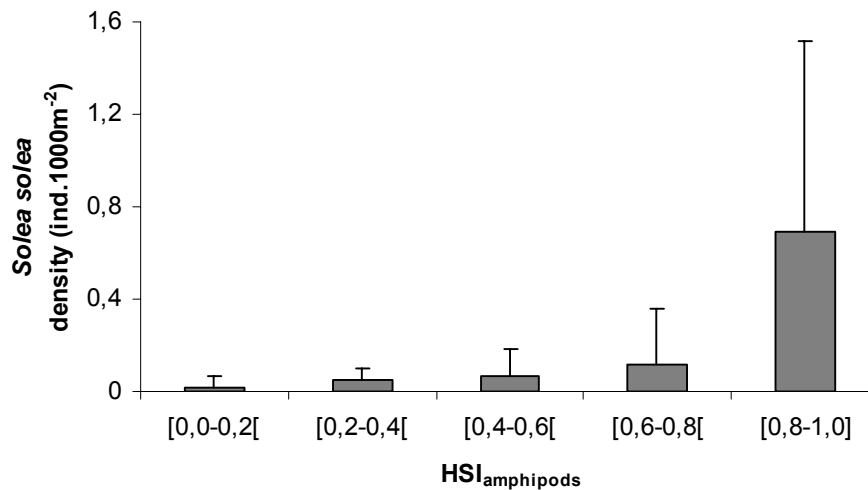


Figure 6 - *S. solea* 0-group juveniles density prediction of the HSI_{amphipods} model $(SI_{\text{salinity}} SI_{\text{temperature}} SI_{\text{substrate}} SI_{\text{depth}} SI_{\text{intertidal}} SI_{\text{amphipods}})^{1/6}$ for the month of May 2001. Mean density and standard deviation.

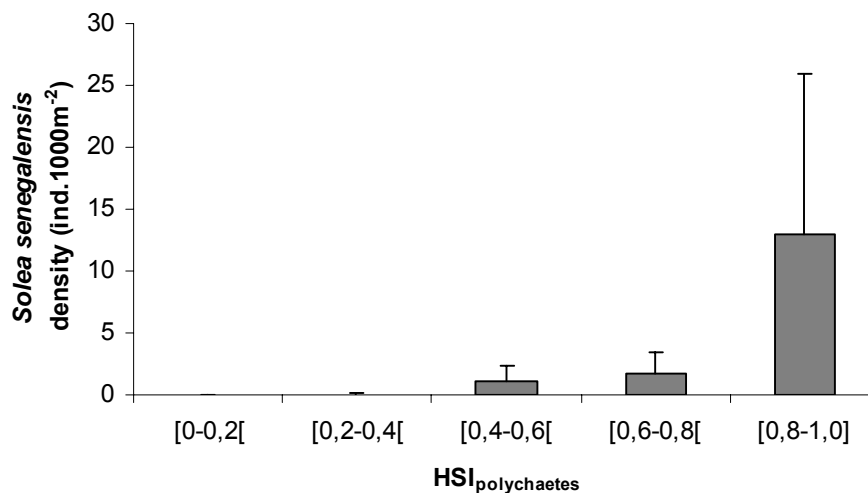


Figure 7 - *S. senegalensis* 0-group juveniles density prediction of the HSI_{amphipods} model $(SI_{\text{salinity}} SI_{\text{temperature}} SI_{\text{substrate}} SI_{\text{depth}} SI_{\text{intertidal}} SI_{\text{polychaetes}})^{1/6}$ for the month of November 2001. Mean density and standard deviation.

The Spearman test revealed that the spatial distribution of *S. solea* in May 2001 and the distribution predicted by the HSI_{amphipods} model, in which density of amphipods was used as a sixth variable, had the highest correlation ($r = 0,43$; $p < 0,05$). Concerning *S. senegalensis*, the Spearman test revealed that its spatial distribution in November 2001 and the HSI_{polychaetes} model, which used polychaetes as the sixth variable, had the highest correlation value ($r = 0,59$; $p < 0,05$).

The model which included all abiotic variables as well as all prey types yielded lower values of correlation for both species, $r = 0,07$ ($p < 0,05$) for *S. solea* and $r = 0,19$ ($p < 0,05$) for *S. senegalensis*. Further model testing with independent data from the 2002 survey produced similar results.

Discussion

The HSI modelling method used in the present study was generally successful for its intended use of mapping habitat quality for *S. solea* and *S. senegalensis*. The inclusion of prey abundance data proved to be very important in the definition of high suitability habitat for both sole species and in the prediction of high density areas. One of the main conclusions of this study is that although the majority of the Tagus estuary upper areas have an overall high habitat quality for soles; both species' juveniles concentrate in rather small areas, probably due to foraging opportunities. This has important implications for fisheries management. These areas should be regarded as crucial for these species lifecycle and protected from disturbing activities, especially during the months when they are used as nursery areas by soles.

Regulations on a defence period and minimum length at capture are already in place, yet they are not fully respected by fishermen. The demand by aquacultures and local restaurants for soles juveniles (both illegal demands) and the lack of regulation supervision and enforcement make this illegal activity a profitable occupation. Also, although the defence period protects *S. solea* 0-group juveniles, to effectively protect *S. senegalensis* it would have to be extended to the month of December.

The present study is fisheries focused, and since fisheries management is still not fully developed in Portugal the modelling technique should be simple enough to accommodate the goals and constraints related to the decision making process. Rather than wishing to obtain accurate maps of the maximum extent of suitable habitats, managers (usually with no scientific background) are more likely concerned with identifying the most important areas in the species life cycle (Langton and Auster, 1999). We acknowledge, however, that fisheries science should rely heavily in statistics and modelling and that knowledge on the full extent of species suitable habitats is an important goal for fisheries management.

Other authors have approached the issue of flatfish habitat modelling in different ways. Using regression tree analysis, Norcross *et al.*, (1997) modelled habitat suitability for flatfish in Alaska, while Swartzman *et al.*, (1992) and Stoner *et al.*, (2001) used generalized additive models for modelling flatfish distribution in the Bering Sea and winter flounder in New Jersey, respectively. Le Pape *et al.* (2003) characterised the distribution of *S. solea* using a general linear model. Eastwood *et al.* (2003) applied regression quantiles to estimate the limits to the spatial extent and suitability of *S. solea* nursery grounds, producing maps of the upper limits of suitability and this way minimizing its underestimation, which is important in a conservationist perspective but would hardly be applied to an highly urbanized estuary such as the Tagus where impacts are diverse and protection of the whole suitable area for any species is generally

not possible. Bearing this in mind this work intended to detect crucial estuarine areas for the life cycle of both soles and to determine which variables should be taken into account in future management actions.

Presence of prey appears to be a major factor affecting soles' distribution. It is generally recognized that prey availability is determinant in the distribution of flatfish (e.g. Miller *et al.*, 1991; Sogard, 1992; Gibson, 1994; 1997; Matilla and Bonsdorff, 1998) and it is also well known that macrobenthic species can use less than half of the suitable habitat due to limitations in settlement and/or juvenile survival (Armonies and Reise, 2003). As such, habitat classified as highly suitable, according to abiotic variables, can actually be empty habitat in terms of prey (e.g. Buttman, 1986; Eckman, 1990; Armonies and Reise, 2003). On the other hand, intermittent stagnant conditions that facilitate larval settlement, as well as higher than average survival rates can result in local accumulations of various macrobenthic organisms (Gross *et al.*, 1992; Snelgrove, 1994; Hsieh and Hsu, 1999).

In order to take these phenomena into account, density of amphipods, polychaetes and bivalves were chosen as variables since they are the most important groups in both species diet (Lagardère, 1987; Costa, 1988; Molinero and Flos, 1991; Beyst *et al.*, 1999; Cabral, 2000). This way, from the five distinct models created for each species, the one which included only abiotic variables indicated, as expected, a broad area of high suitability for both soles, while the other models, that also included prey density, greatly restricted the areas of high habitat quality. Prey abundance data proved to be of the utmost importance for the prediction areas of high juvenile soles density. The models which included all prey items had lower correlations with species distributions. This is due to the fact that being the model multiplicative if any term is equal to zero so will the output. Yet this does not make biological sense, because the lack of one or two of the prey items will not make an area necessarily unsuitable to a species if there is abundance of another prey group. This is possibly the biggest limitation of this method.

Few attempts have been made to assess the influence of prey density in flatfish distribution because of the difficulty in quantifying the abundance of prey items. While Pihl and van der Veer (1992) found no correlation between plaice density and biomass of macrofauna, Stoner *et al.*, (2001) reported that the abundance of prey contributed significantly to the generalized additive model for winter flounder with total length between 25 and 55mm. Yet, various studies, that did not quantify prey density, concluded that substrate preference is probably indirect and linked to prey availability (e.g. Gibson and Robb, 1992; Jager *et al.*, 1993; Gibson, R.N., 1994)

The model that included density of amphipods yielded very good results in predicting *S. solea* densities in its month of higher abundance estimates, while the model that included polychaetes had the best results for *S. senegalensis*. The models determined that the areas of highest habitat quality are located in the upper estuary which is dominated by large extensions of intertidal mudflats.

Sole juveniles of the 0-1 group of both species feed primarily on amphipods, polychaetes and bivalves (e.g. Lagardère, 1987; Costa, 1988; Molinero and Flos, 1991; Beyst *et al.*, 1999;

Cabral, 2000), all of which are found in close association with muddy and sandy substrates. Sole are also known to bury into fine grained sediments, probably to avoid predation (Dorel *et al.*, 1991; Rogers 1992). The models produced here clearly confirm these assertions. It was also confirmed that some areas classified by the abiotic model ($HSI_{abiotic}$) as highly suitable are in fact habitat empty of prey and have therefore low quality for fish juveniles, the models that take into account prey density classify this areas with a zero suitability index.

Only marginal differences in the spatial distributions of both soles could be attributed to variations in temperature and salinity. This is probably a facet of the sampling regime, in that had samples been collected over a broader temperature and salinity range then a stronger relationship may have emerged.

In other studies authors have concluded that the models developed may be constrained to defining habitat quality at a particular season and geographic location, due to the lack of model testing in other situations (e.g. Brown *et al.*, 2000; Eastwood *et al.*, 2003). In the case of the Tagus estuary previous studies report that soles have concentrated in the upper estuary in all surveys throughout the year, so we can conclude that although conditions in this area vary and the number of soles fluctuates, these are preferential areas all year long. Concerning geographic location, differences are expected since these species habitat preferences seem to differ between estuaries (Cabral and Costa, 1999; Cabral, 2000). Cabral (1998) compared the influence of several environmental factors in the distribution of these species and concluded that it is difficult to generalize to different estuarine systems, since many of the factors are correlated and/or have an indirect effect on distribution, as previously reported by Riley *et al.*, (1981). Further testing of the present models would allow an assessment of the models applicability to a broader set of time periods and geographic locations.

Because of the dynamic nature of habitat features nursery grounds can expand, contract and shift in location over time (Stoner *et al.*, 2001). Distribution patterns can be altered due to natural and anthropogenic changes. The identification and protection of these critical habitats is essential for the long term conservation of commercial fish stocks.

Acknowledgements

This study had the support of Fundação para a Ciência e a Tecnologia (FCT) that financed several of the research projects related to this work and the PhD grant given to C. Vinagre. The authors would like to thank everyone involved in the sampling surveys, Dr. J. Catalão for providing assistance in the GIS procedures and two anonymous referees that commented on the draft version of the manuscript.

References

- Amezcuca, F., Nash, R.D.M., 2001. Distribution of the order Pleuronectiformes in relation to sediment type in the North Irish Sea. *Journal of Sea Research* 45, 293-301.
- Armonies, W., Reise, K., 2003. Empty habitat in coastal sediments for populations of macrozoobenthos. *Helgoland Marine Research* 56, 279-287.

- Baeta, F., Pinheiro, A., Corte-Real, M., Costa, J.L., Almeida, P.R., Cabral, H., Costa, M.J., 2005. Are the fisheries in the Tagus estuary sustainable? *Fisheries Research* 76, 243-251.
- Bailey, T.C., Gatrell, A.C., 1996. *Interactive Spatial Data Analysis*. Longman.
- Beyst B., Cattrijsse A., Mees, J., 1999. Feeding ecology of juvenile flatfishes of the surf zone of a sandy beach. *Journal of Fish Biology* 55, 1171-1186.
- Bettencourt, A., 1979. Estuário do Tejo. Documento preliminar. Hidroprojecto, Drena, Lisboa.
- Bovee, K., Zuboy, J.R., 1988. Biological Report 88 (11). In: *Proceedings of the workshop Development and Evaluation of habitat suitability criteria*. US Fish and Wildlife Service
- Briggs, J.C., 1974. *Marine Zoogeography*. McGraw-Hill, New York.
- Brown, S., Buja, K., Jury, S., Monaco, M., Banner, A., 2000. Habitat suitability index models for eight fish and invertebrate species in Casco and Sheepscot Bays, Maine. *North American Journal of Fish Management* 20, 408-435.
- Buttman., C.A., 1986. Larval settlement of soft sediment invertebrates: some predictions based on an analysis of near bottom profiles. In: Nihoul J.C.L. (ed) *Marine interfaces: ecohydrodynamics*. Elsevier, Amsterdam, p 487-513.
- Cabral, H.N., 1998. Utilização do estuário do Tejo como área de viveiro pelos linguados, *Solea solea* (L., 1758) e *Solea senegalensis* Kaup, 1858, e robalo, *Dicentrarchus labrax* (L., 1758). PhD Dissertation, University of Lisbon, Portugal.
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect environmental changes? *Climate Research* 18, 119-126.
- Cabrita, M.T., Moita, M.T., 1995. Spatial and temporal variation of physico-chemical conditions and phytoplankton during a dry year in the Tagus estuary (Portugal). *Aquatic Ecology* 29, 323-332.
- Costa, M.J., 1982. Contribution à l'étude de l'écologie des poissons de l'estuaire Tage (Portugal). PhD Dissertation, Université Paris VII, Paris.
- Costa, M.J., 1988. Écologie alimentaire des poissons de l'estuaire du Tage. *Cybiurn* 12, 301-320.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial species. *Scientia Marina* 53, 561-566.
- Costa, M.J., Cabral, H.N., 1999. Changes in the Tagus estuary nursery function for commercial fish species: some perspectives for management. *Aquatic Ecology* 33, 53-74.

- Dorel, D., Koutsikopoulos, C., Desaunay, Y., Marchand, J., 1991. Seasonal distribution of young sole (*Solea solea* (L.)) in the nursery of the Bay of Vilaine (Northern Bay of Biscay) Netherlands Journal of Sea Research 27, 297-306.
- Eastwood, P.D., Meaden, G.J., Carpentier, A., Rogers, S.I., 2003. Estimating limits to the spatial extent and suitability of sole (*Solea solea*) nursery grounds in the Dover Strait. Journal of Sea Research 50, 151-165.
- Eckman, J.E., 1990. A model of passive settlement by planktonic larvae onto bottoms of differing roughness. Limnol. Oceanogr. 35, 887-901.
- Ekman, S., 1953. Zoogeography of the Sea. Sidgwick, Jackson, London.
- Fiúza, A.F.G., Macedo, M.E., Guerreiro, M.R., 1982. Climatological space and time variation of the Portuguese coastal upwelling. Oceanol. Acta 5, 31-40.
- Fonds, M. 1976. The influence of T and salinity on the growth of young sole *Solea solea* L. 10th Eur. Ecol. Prog. Ser. 1, 91-99.
- Fonds, M. 1979. A seasonal fluctuation of in growth rate of young plaice (*Pleuronectes platessa*) and sole (*Solea solea*) in the laboratory at constant temperatures and natural daylight cycle. In: Naylor, E., Hartnoll, R.G. (Eds.), Cyclic phenomena in marine plants and animals. Proceedings of the 13th European Marine Biology Symposium. Pergamon Press, Oxford, pp. 151-156.
- Gibson, R.N., Robb, L., 1992. The relationship between body size, sediment grain size and burying ability of juvenile plaice *Pleuronectes platessa*. L. Journal of Fish Biology 40, 771-778.
- Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Netherlands Journal Sea Research 32, 191-206.
- Gibson, R.N., 1997. Behaviour and the distribution of flatfishes. Journal of Sea Research 37, 241-256.
- Gross, T.F., Werner, F.E., Eckman, J.E., 1992. Numerical modelling of larval settlement in turbulent bottom boundary layers. Journal of Marine Research 50, 611-642.
- Groot, S.J. De, 1971. On the interrelationships between morphology of the alimentary tract food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). Netherlands Journal of Sea Research 5, 121-196.
- Hsieh, H.L., Hsu, C.-F., 1999. Differential recruitment of annelids onto tidal elevations in an estuarine mudflat. Marine Ecology Progress Series 177, 93-126.
- Imsland, A.K., Foss, A., Conceição, L.E.C., Dinis, M.T., Delbare, D., Schram, E., Kamstra, A., Rema, P., White, P., 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. Reviews in Fish Biology and Fisheries 13, 379-407.
- Irwin, D.N., 1973. The growth and survival of Dover sole, *Solea solea*, and some observations on the growth of plaice, *Pleuronectes platessa*, considered at various temperatures. PhD Dissertation, University of Liverpool, UK.
- Isaaks, E.H., Srivastava, M., 1989. Introduction to Applied Geostatistics. Oxford University Press, New York.

- Jager, Z., Kleef, H.L., Tydeman, P., 1993. The distribution of 0-group flatfish in relation to abiotic factors on the tidal flats in the brackish Dollard (Ems estuary, Wadden Sea). *Journal of Fish Biology* 43, 31-43.
- Lagardère, J.P., 1987. Feeding ecology and daily food consumption of common sole, *Solea vulgaris* Quensel, juveniles of the french atlantic coast. *Journal of Fish Biology* 30, 91-104.
- Langton, R.W., Auster, P.J., 1999. Managing essential fish habitats: What are the next steps? *Fisheries* 24, 30-31.
- LeFrançois, C., Claireaux G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole, *Solea solea*. *Marine Ecology Progress Series* 259, 273-284.
- Le Pape, O., Chauvet, F., Mahévas, S., Lazure, P., Guérault, D., Désaunay, Y., 2003. Quantitative description of habitat suitability for the juvenile common sole (*Solea solea*, L.) in the Bay of Biscay (France) and the contribution of different habitats to the adult population. *Journal of Sea Research* 50, 139-149.
- Matilla, J., Bonsdorff, E., 1998. Predation by juvenile flounder (*Platichthys flesus* L.): a test of prey vulnerability, predator preference, switching behaviour and functional response. *Journal of Experimental Marine Biology Ecology* 227, 221-236.
- Miller, J.M., Burke, J.S., Fitzhugh, G.R., 1991. Early life history patterns of Atlantic North American flatfish: Likely (and unlikely) factors controlling recruitment. *Netherlands Journal of Sea Research* 27, 261-275.
- Moliner, A., Flos, R., 1991. Influence of age and sex on the feeding habits of the common sole *Solea solea*. *Marine Biology* 111, 493-501.
- Norcross, B.L., Mutter, F., Holladay, B.A., 1997. Habitat models for juvenile pleuronectids around Kodiak Island, Alaska. *Fisheries Bulletin* 95, 504-520.
- Pihl, L., van der Veer, H.W., 1992. Importance of exposure and habitat structure for the population density of 0-group plaice, *Pleuronectes platessa* L., in coastal nursery areas. *Netherlands Journal of Sea Research* 29, 145-152.
- Reyes, E., Sklar, F.H., Day, J.W., 1994. A regional organism exchange model for simulating fish migration. *Ecology Modelling* 74, 255-276.
- Rijnsdorp, A.D., Van Beek, F.A., Flatman, S., Millner, R.M., Riley, J.D., Giret, M., De Clerck, R., 1992. Recruitment of sole, *Solea solea* (L.), stocks to the Northeast Atlantic. *Netherlands Journal of Sea Research* 29, 173-192.
- Riley, J.D., Symonds, D.J., Woolner, L., 1981. On the factors influencing the distribution of 0-group demersal fish in coastal waters. *Rapp. P.-v Reun. Cons. Int. Expl. Mer* 178, 223-228.
- Rogers, S.I., 1992. Environmental factors affecting the distribution of sole (*Solea solea* L.) within a nursery area. *Netherlands Journal of Sea Research* 29, 153-161.

- Rogers, S.I., 1993. The dispersion of sole, *Solea solea*, and plaice, *Pleuronectes platessa*, within and away from a nursery ground in the Irish Sea. *Journal of Fish Biology* 43, 275-288.
- Snelgrove, P.V.R., 1994. Hydrodynamic enhancement of invertebrate larval settlement in midrodepositional environments: colonisation tray experiments in a muddy habitat. *Journal of Experimental Marine Biology and Ecology* 176, 149-166.
- Sogard, S.M., 1992. Variability in growth rates of juvenile fishes in different estuarine habitats. *Marine Ecology Progress Series* 85, 35-53.
- Stoner, A.W., Manderson J.P., Pessuti, J.P., 2001. Spatially explicit analysis of estuarine habitat for juvenile winter flounder: combining generalized additive models and geographic information systems. *Marine Ecology Progress Series* 213, 253-271.
- Swartzman, G.L., Huang, C., Kaluzny, S., 1992. Analysis of Bering Sea groundfish survey data using generalized additive models. *Canadian Journal of Fisheries and Aquatic Science* 49, 1366-1378.
- Symonds, D.J., Rogers, S.I., 1995. The influence of spawning and nursery grounds on the distribution of sole *Solea solea* (L.) in the Irish Sea, Bristol Channel and adjacent areas. *Journal of Experimental Marine Biology and Ecology* 190, 243-261.
- Terrel, J.W., 1984. Biological Report 85 (6) In: Proceedings of the workshop: Fish habitat suitability index models. U.S. Fish and Wildlife Service.
- Van der Veer, H.W., Dapper, R., Witte, J.I.J., 2001. The nursery function of the intertidal areas in the western Wadden Sea for 0-group sole *Solea solea* (L.). *Journal of Sea Research* 45, 271-27.

Nursery fidelity, food web interactions and primary sources of nutrition of the juveniles of *Solea solea* and *Solea senegalensis* in the Tagus estuary (Portugal): a stable isotope approach

Abstract: Stable carbon and nitrogen isotopes were used to assess site fidelity of *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, juveniles, to investigate food web interactions and to determine the dominant nutrient pathways in two nursery areas in the Tagus estuary, Portugal. Samples of water from the main sources and from the nursery areas and respective saltmarsh creeks were collected for isotope analysis, as well as sediment, benthic microalgae, saltmarsh halophytes, *S. solea*, *S. senegalensis* and its main prey, *Nereis diversicolor*, *Scrobicularia plana* and *Corophium* spp. While site fidelity was high in 0-group juveniles, it was lower for 1-group juveniles, possibly due to an increase in mobility and energy demands with increasing size. Analysis of the food web revealed a complex net of relations. Particulate organic matter from the freshwater sources, from each nursery's waters and saltmarsh creeks presented similar isotopic composition. Sediment isotopic composition and saltmarsh halophytes also did not differentiate the two areas. All components of the food web from the benthic microalgae upwards were isotopically different between the nursery areas. These components were always more enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the lower nursery area than at the nursery located upstream, appearing as if there were two parallel trophic chains with little trophic interaction between each other. A mixture of carbon and nitrogen sources is probably being incorporated into the food web. The lower nursery area is more dependent upon an isotopically enriched energy pathway, composed of marine particulate organic matter, marine benthic microalgae and detritus of the C4 saltmarsh halophyte *Spartina maritima*. The two nursery areas present a different level of dependence upon the freshwater and marine energy pathways, due to hydrological features, which should be taken into account for *S. solea* and *S. senegalensis* fisheries and habitat management.

Key-words: Connectivity; Stable isotopes; Estuarine fishes; Flatfish; Sole; Eastern Atlantic; Portugal; Tagus estuary.

Introduction

The soles, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, are among the most important commercial fishes in Portugal (Costa and Bruxelas, 1989). The Tagus estuary, one of the largest estuaries in Western Europe, has two main nursery areas for fish, where soles can be found (Figure 1) (Costa and Bruxelas, 1989; Cabral and Costa, 1999). A differential multi-cohort immigration process towards estuaries has been described for these species, associated with different spawning periods that induce several pulses of new recruits (Dinis, 1986; Andrade, 1992; Cabral, 2003).

S. solea 0-group juveniles colonize nursery A in one or two pulses from April to June leaving the estuary towards the coast around October-November (Cabral and Costa, 1999; Cabral, 2003; Fonseca, 2006). *S. senegalensis* colonise the upper Tagus nurseries latter and in

several pulses (the first pulse colonises only nursery A, while the following pulses can colonise both areas) (Cabral and Costa, 1999, Fonseca *et al.*, 2006) resulting from a prolonged spawning period with two major peaks (Spring and Summer) (Anguis and Cañavate, 2005). While one first cohort arrives at the estuary in Spring, another cohort arrives later in Summer and a third cohort has also been observed in some years in Autumn (*personal observation*). Individuals from the latter cohorts will stay in the estuary during the winter, only emigrating towards coastal waters in the following year (Cabral, 2003).

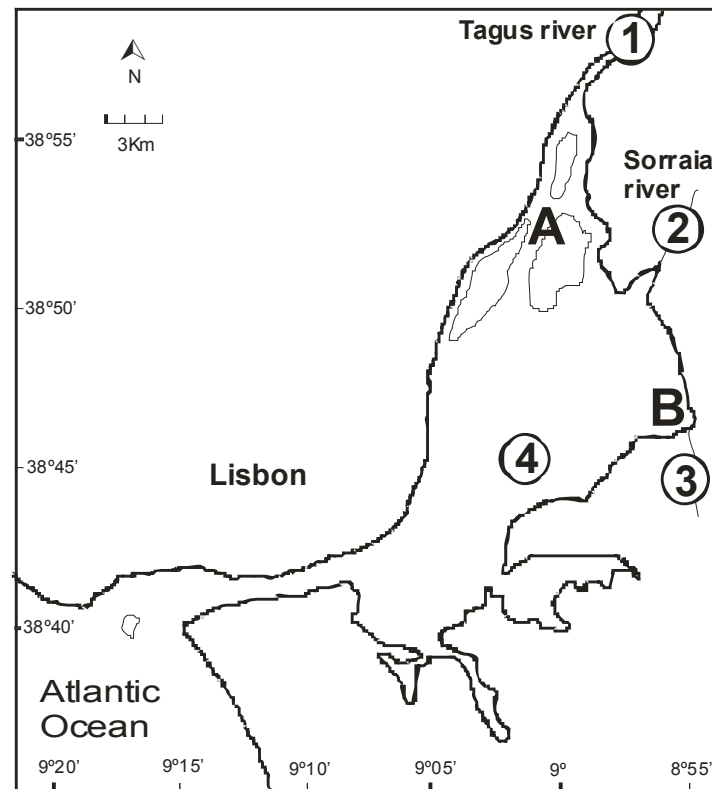


Figure 1 – Location of the nursery areas (A and B) within the Tagus estuary. Numbers indicate water sources sampled (1- Tagus river; 2- Sorraia river; 3- Ribeira das Enguias river and 4- Samouco area).

Soles are the main target of the beam-trawl fisheries inside the estuary, and the most important species in juvenile numbers and commercial value. Beam trawling is illegal in all Portuguese estuaries except the Tagus where it is quite common in the uppermost areas. Baeta *et al.* (2005) investigated sole fisheries in the Tagus estuary and concluded that they are not environmentally sustainable.

S. solea and *S. senegalensis* are very similar in aspect. The features that distinguish both species are not obvious, even to fishermen and fisheries technicians. The two species have traditionally and up to today been treated as one item for management. Fisheries data from official sources treat these species as *Solea* sp. Yet, as mentioned above the two species

have very different life-cycles and habitat use patterns. This way the current management approach is inadequate, e.g. the no-fishing period put in place for the Tagus estuary only protects *S. solea* juveniles while much of the juvenile period of *S. senegalensis* is left unprotected and a thorough analysis of the fisheries data is impossible since the data is not species specific. If the two species continue to be managed as one item several misconceptions may arise. Nursery B may be regarded as secondary habitat or alternative habitat. Managers may consider that impacts in one of the nursery areas may be minimized by the existence of another nursery, yet *S. solea* is only present at one of the nurseries and the connectivity level between *S. senegalensis* populations using both nurseries is unknown.

Attempts have been made at assessing the connectivity between the two nurseries through mark-recapture experiments, yet the low percentage of recaptured individuals have made it impossible to draw any conclusions (Cabral, *personal communication*). For the good management of soles in this estuarine system they must be analysed as two species with different life cycles and protection needs, yet several other issues need to be addressed, such as, site fidelity of *S. senegalensis* juveniles, food web interactions for both species and the energy sources from which their populations depend.

In depth knowledge on these issues is particularly urgent given the important management challenges that will arise in this and other estuarine systems due to the fast increasing density of human populations and the effects of global climate change.

Human pressure is one of the main threats to the Tagus estuary fish nurseries. While in nursery A there is a project for the urbanization of the islands with the construction of a large tourist resort, the area around nursery B turned into one of the fastest growing population agglomerates in the country after the building of a new bridge that connects it to Lisbon.

Climate change will also alter the environmental conditions for these species. Recent trends show that there has been a decrease in rainfall in these area, and that rain tends to be is more concentrated in time (Miranda *et al.*, 2002), which can have important impacts in the complex hydrology of these nursery areas. Recent trends also show an increase in temperature and in the duration of heat waves (Miranda *et al.*, 2002). This could have an important impact on *S. solea*, since its optimal metabolic temperature is estimated at 18,8 °C (LeFrançois and Claireaux, 2003), and during heat waves water temperature in these areas are today higher than 25°C. *S. senegalensis* being a tropical species will potentially fare better than *S. solea*, a temperate species, under higher temperatures (there are no studies on its optimum metabolic temperature).

Sea level rise may be one of the most important consequences of climate warming impacting soles in the future. An important portion of the food available to these species concentrates in the large intertidal mudflat platforms that encompass *circa* 40% of the estuarine area of the Tagus estuary (Cabral, 2000). Since the river banks are urbanized most of the intertidal area will be lost, with the consequence steep decrease in available food to soles.

Already, there are studies that show alterations in these estuarine fish assemblage due to climate change and river flow fluctuations (Costa and Cabral, 1999; Cabral *et al.*, 2001; Costa

et al., *in press*). Costa and Cabral (1999) and Cabral *et al.* (2001) reported that in the last thirty years typically cold water species such as *Platichthys flesus*, and *Ciliata mustela* presented a steep decrease in abundance while species with tropical affinities, such as *Diplodus bellottii*, *Halobatrachus didactylus*, *Sparus aurata* and *Argyrosomus regius* have increased their abundance. Freshwater input is highly variable in the Tagus estuary and has been shown to have an important effect on the estuarine fish community composition by Costa *et al.*, (*in press*), yet soles were analyzed as *Solea* sp., making conclusions on a specific level impossible.

This reinforces the need for a more accurate understanding of the estuarine food webs and energy sources on which the two species of sole depend. The aim of the present study was to (1) assess the site fidelity of the sole populations inhabiting the two nursery areas, to (2) investigate food web interactions and to (3) determine the dominant nutrient pathways in both nurseries.

An isotope analysis approach was chosen because studies ranging for two decades have proved that stable isotopes are powerful tools for ecological studies. They were used for discriminating nutrient pathways and energy sources in complex systems such as estuaries (e.g. Simenstad and Wissmar, 1985; France, 1995; Paterson and Whitfield, 1997; Riera *et al.*, 1999; Darnaude *et al.*, 2004), for elucidating food web interactions and changes in trophic position (e.g. Nichols *et al.*, 1985; Hanson *et al.*, 1997; Cabana and Rasmussen, 2002), as well as for the reconstruction of migration routes and life histories of fish (e.g. Kline *et al.*, 1998; Cunjak *et al.*, 2005; Herzka, 2005; Phillips and Eldridge, 2006). Several authors have successfully used stable isotopes to study the connectivity between habitats (Fry *et al.*, 1999; Talley, 2000; Fry *et al.*, 2003).

The combined use of stable carbon and nitrogen isotopes provides an accurate picture of food web structure and nutrient pathways (Peterson *et al.*, 1985; Owens, 1987). Since terrestrial primary producers generally have lower $\delta^{13}\text{C}$ than marine producers (Haines and Montague, 1979; Riera and Richard, 1996; Bouillon *et al.*, 2000), and the increase in $\delta^{13}\text{C}$ from prey to predator is of only 0-1 ‰ (De Niro and Epstein, 1978; Fry and Sherr, 1984; Peterson and Fry, 1987), this isotope is particularly useful in estuarine systems, since it allows the identification of the primary source of organic carbon in the diet of fish and also the evaluation of its dependence on the freshwater and marine energy pathways (Simenstad and Wissmar, 1985; Paterson and Whitfield, 1997; Darnaude *et al.*, 2004). The nitrogen isotope signature is generally used as a marker of trophic position, since $\delta^{15}\text{N}$ increases by 2.5-4.5 ‰ from prey to predator (Owens, 1987; Peterson and Fry, 1987). Yet, this isotope can also be used as a tracer of organic material across ecotones, since marine organisms are enriched in ^{15}N relative to freshwater organisms, and estuarine and anadromous fish present intermediate $\delta^{15}\text{N}$ values depending on their time feeding in either fresh- or saltwater (e.g. France, 1995; Doucett *et al.*, 1999).

For the movement of fish to be traced there must be a switch to prey with a different isotopic signature in the new habitat, if that is the case it will gradually be reflected by the fish tissues (Fry, 1983; Herzka *et al.*, 2001), enabling the identification of migrant individuals if they

are caught before equilibrating to the isotopic composition of the food sources in the new habitat (Fry *et al.*, 1999; 2003).

Materials and methods

Study area

The Tagus estuary (Figure 1), with an area of 320 km², is a partially mixed estuary with a tidal range of ca. 4 m. This estuarine system has a mean depth lower than 10 m and about 40% of its area is composed of intertidal mudflats (Cabral and Costa, 1999) fringed by extensive areas of saltmarshes dominated by *Spartina maritima*, *Halimione portulacoides* and *Sarcocornia fruticosa* (Caçador *et al.*, 1996). Although its bottom is composed of a heterogeneous assortment of substrates, its prevalent sediment is muddy sand in the upper and middle estuary and sand in the low estuary and adjoining coastal area (Cabral and Costa, 1999). The mean river flow is ca. 400 m³s⁻¹, though it is highly variable both seasonally and inter-annually (Loureiro, 1979). Salinity varies from 0, 50 km upstream from the mouth, to ca. 35 at the mouth of the estuary (in practical salinity units) (Cabral *et al.*, 2001). Water temperature ranges from 8°C to 26°C (Cabral *et al.*, 2001). Wind induced upwelling occurs in the adjoining coastal areas during summer (Fiúza *et al.*, 1982).

Two important nurseries for sole were identified in the Tagus estuary in previous studies (A, Vila Franca de Xira, and B, Alcochete; Figure 1) by Costa and Bruxelas (1989) and Cabral and Costa (1999). Although most of the environmental factors present a wide and similar range in these two areas, some differences can be outlined. The uppermost area, A, is deeper (mean depth 4.4 m), presents lower and highly variable salinity and has a higher proportion of fine sand in the substrate. Nursery B is shallower (mean depth 1.9 m), and more saline, with lower variability in salinity, while substrate is mainly composed of mud (Cabral and Costa, 1999) (distance between the two nurseries is *circa* 10 km). While in nursery A the two sole species, *S. solea* and *S. senegalensis* can be found, in nursery B only *S. senegalensis* is present (Cabral and Costa, 1999). At immigration *S. solea*'s length varies between 11 mm and 20 mm (Russel, 1976), such information is not yet available for *S. senegalensis*.

Sampling

Beam trawls were conducted in both nursery areas in May, July and September of 2001 in order to capture *S. solea* and *S. senegalensis*. All soles were measured (total length with 1 mm precision). Three samples of water, sediment, saltmarsh plants, benthic microalgae, and soles' main prey species were collected in May, July and September of 2001 in both nursery areas. Water samples for POM analysis were collected at high tide at both nurseries in saltmarsh tidal creeks, in the water adjacent to the saltmarsh and in the subtidal area and at low tide in its' main fresh water sources, the Tagus river for nursery A (source number 1; Figure 1) and the Sorraia river (source number 2; Figure 1) and Ribeira das Enguias (source number 3; Figure 1) for nursery B. The waters adjacent to Samouco were sampled at high tide in order to

analyze the estuarine water coming into the nurseries (source number 4; Figure 1). Three replicates were collected from each source. Three replicates of surface sediment were collected in nursery A and B. Tissues of saltmarsh plants, *S. maritima*, *H. portulacoides* and *S. fruticosa* were cleaned of mud and when present, epiphytes were removed by scraping with a razor blade. Pools of 10 plants of the same species were used to produce 3 replicate samples for each saltmarsh. Three replicates of benthic microalgae samples were collected in nursery A and B, in the intertidal mudflats at low tide. Textile panels of 20 cm by 20 cm were laid in the sediment surface in order to collect the benthic microalgae that concentrate in the surface during low tide. The panels were rinsed with distilled water that was later decanted in order to separate the microalgae from the sediment that was also attached to the panels.

The supernatant was then filtered onto precombusted filters. The main prey for both sole species in the Tagus estuary are the amphipod *Corophium* spp, the bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor* (Cabral, 2000). While for *S. plana* only the valves muscle was used for isotopic analysis, for *Corophium* spp. and *N. diversicolor* the whole animals were used after rinsed with distilled water. The dried tissues were ground to fine powder with a mortar and a pestle and added into pools for analysis.

Zooplankton was not sampled since its numbers are very low in the upper Tagus estuary due to the high turbidity of the system. Previous studies have shown that much of the organic matter in the water column is composed of suspended benthic microalgae (Vale and Sundy, 1987).

Stable isotope analysis

Muscle tissue samples of *S. solea* and *S. senegalensis* for C and N stable isotope analysis were dissected and dried at 60 °C. Dorsal white muscle samples were taken since this tissue tends to be less variable in terms of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Pinnegar and Polunin, 1999). The dried tissues were ground to fine powder with a mortar and a pestle. Isotopic analysis was carried out on an individual basis.

Water samples were filtered until clogged onto precombusted filters. Sediment samples were dried at 60°C and ground to a fine powder. Subsamples of the ground samples of water POM and sediment were acidified with several drops of 10% HCl while being observed under a dissecting microscope. If bubbling occurred the subsample was acidified, rinsed with distilled water, redried at 60 °C and stored in glass vials. A separate subsample was used for nitrogen isotope analysis. The acidification procedure was carried out to detect contamination by carbonates, as they present higher $\delta^{13}\text{C}$ values than organic carbon (DeNiro and Epstein, 1978), yet carbonates were not detected in none of the samples of the present work. Samples of saltmarsh plants, *S. maritima*, *H. portulacoides* and *S. fruticosa* were dried to constant weight at 60 °C. The dried tissues were ground to a fine powder with a mortar and a pestle. Benthic microalgae samples were dried at 60°C and ground to a fine powder.

For prey species isotope analysis a subsample with a minimum of 5g was analyzed from a pooled sample of several individuals (the number of individuals needed to have the

minimum of 5g was very variable). The acidification procedure described above was used to detect carbonate contamination, yet none of the samples was contaminated.

$^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in the samples were determined by continuous flow isotope mass spectrometry (CF-IRMS) (Preston and Owens, 1983). The standards used were Peedee Belemite for carbon and atmospheric N_2 for nitrogen. Precision of the mass spectrometer, calculated using values from duplicate samples, was $\leq 0.2\%$.

Isotope ratios were expressed as parts per thousand (‰) differences from a standard reference material:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$$

where X is ^{13}C or ^{15}N , R is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ and δ is the measure of heavy to light isotopes in the sample.

Data analysis

T tests were performed in order to investigate differences of isotopic composition of *S. senegalensis* between nurseries A and B, according to sampling month. This procedure was carried out in order to investigate site fidelity from the different sized juveniles that were caught throughout the sampling period. The percentage of individuals from both nurseries with overlapping isotopic values was calculated for all months.

Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the particulate organic matter from the water sources were tested with a one-way ANOVA. Whenever the null hypothesis was rejected Tukey *post hoc* tests were conducted. To test for differences in the isotopic composition of the surface sediment of the two nurseries a t-test was performed. In order to investigate food web interactions a one-way ANOVA was conducted for both species of sole and both nurseries (*S. solea* versus *S. senegalensis* from nursery A versus *S. senegalensis* from nursery B). For all other components of the food web separate t-tests were carried out in order to compare isotopic signatures from the two nurseries. All the statistical tests performed were carried out separately for each isotope.

The relation between length and isotopic values for both species and in both nurseries was investigated since it could be a confounding factor in the interpretation of movement among nurseries, yet such a relationship was not found.

Results

S. solea and *S. senegalensis* nursery fidelity

While *S. solea* was captured only at nursery A, *S. senegalensis* was present at both nurseries. For *S. solea* a steady increase in length occurred throughout the study period, as would be expected in a nursery area (Table 1).

Table 1 – Mean length (in mm) of *S. solea* and *S. senegalensis* collected in Nursery A and B for isotopic analysis (standard deviation values in brackets; sample size indicated below values).

	May	July	September
<i>S. solea</i>	81 (± 9)	125 (± 10)	153 (± 14)
(Nursery A)	n=10	n=12	n=12
<i>S. senegalensis</i>	169 (± 21)	69 (± 33)	107 (± 39)
(Nursery A)	n=20	n=13	n=11
<i>S. senegalensis</i>	143 (± 11)	83 (± 26)	114 (± 27)
(Nursery B)	n=20	n=12	n=12

The size of the *S. senegalensis* individuals captured in our samples in May in both nurseries indicates that they are from the last cohort of this species from the previous year (that stays in the estuary during the winter) (Table 1). Captures of *S. senegalensis* in July were dominated by the first cohort of the year of 0-group juveniles, with much smaller lengths, as were the samples from September, the later with a predictable increase in length (Table 1).

The t test applied to the isotopic analysis of *S. senegalensis* 1-group juveniles captured in May revealed that fish from nursery B tend to have higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than fish from nursery A. Although there is some overlap (Figure 2a) there is a significant difference in isotopic signature between the two nurseries ($P < 0.05$ for both isotopes). Individual data analysis showed that 35.5% of the individuals (15 in a total of 40) had an overlapping isotopic composition in the $\delta^{13}\text{C}$ range between -16.8‰ and -16.2‰.

0-group juveniles of *S. senegalensis* captured in July and September presented very distinct isotopic signatures for the two nursery areas ($P < 0.05$ for both isotopes) (Figures 2b, 2c), while presenting the same trend as the larger juveniles from the May samples, with nursery B *S. senegalensis* assuming higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Stable isotope values of particulate organic matter (POM) and sediment

Particulate organic matter (POM) from all freshwater sources (1, 2, 3; Figure 1) were depleted in $\delta^{13}\text{C}$ (mean values between -24.8 and -24.6‰) (Figure 3). POM from the middle estuary (source 4; Figure 1) that is expected to flow into the nurseries at high tide was comparatively enriched in $\delta^{13}\text{C}$ (mean value of -21.3). While no significant differences were detected for the isotopic composition among the POM from the freshwater sources ($P > 0.05$ for both isotopes) (sources 1, 2, 3; Figure 1) A significant difference in $\delta^{13}\text{C}$ was found between the POM from the middle estuary (source 4; Figure 1) and the POM from all the freshwater sources.

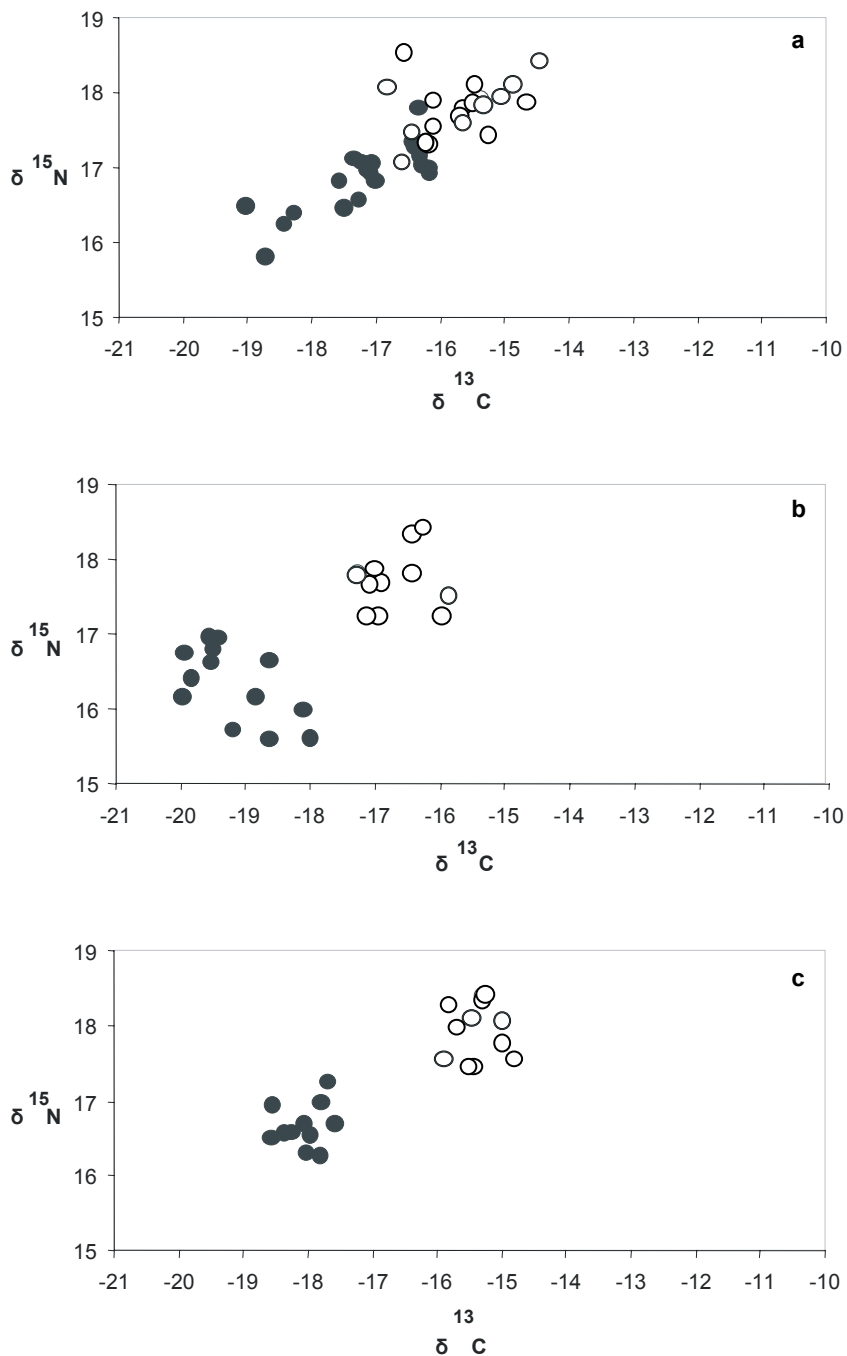


Figure 2 – Distributions of stable isotope ratio values for *rr* in nursery A (black dots) and B (white dots) (in May (a), July (b) and September (c)). Symbols represent individual fish.

No significant difference in $\delta^{15}\text{N}$ was found between the POM from all water sources ($P > 0.05$). Intermediate $\delta^{13}\text{C}$ values were found for the POM in the water collected in the nurseries (mean value -23.2 for nursery A and -23.5‰ for nursery B), as well as in each respective saltmarsh creeks (mean value -23.2 for nursery A and -23.4‰ for nursery B). Isotopic values for POM from each nurseries' waters and saltmarsh creeks were not significantly different ($P > 0.05$ for both isotopes) (Figure 3).

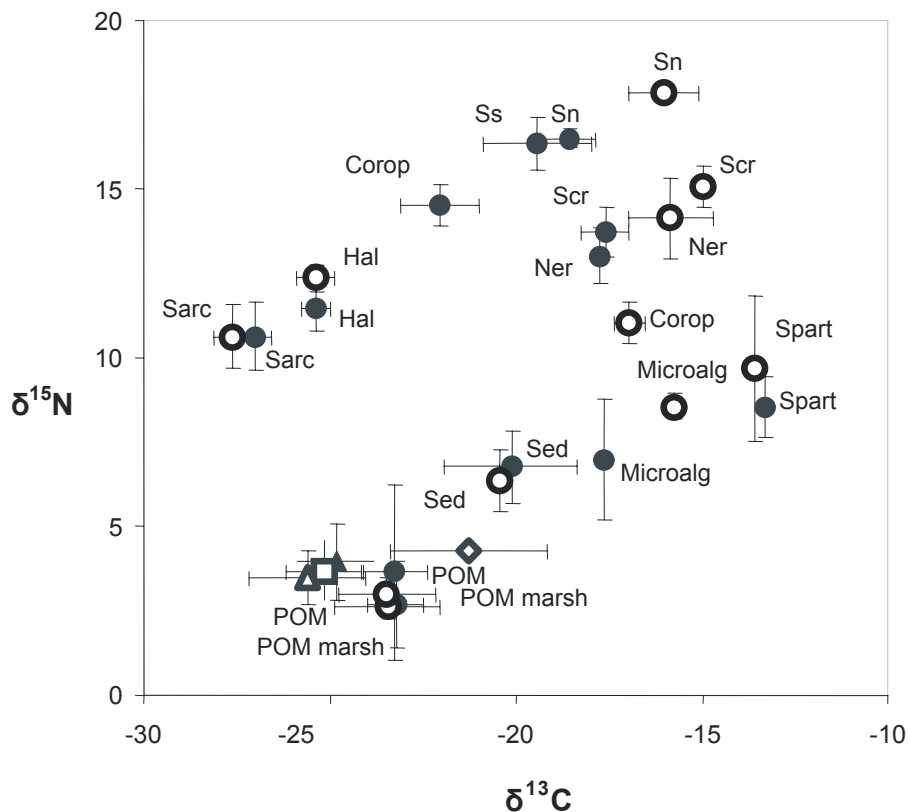


Figure 3 – Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the dominant carbon and nitrogen sources (\blacktriangle as the Tagus estuary POM input; \square as the Sorraia River POM input; \triangle as the Ribeira das Enguias POM input and \diamond as the Samouco POM input); POM from each nursery area (POM); POM saltmarsh creeks (POM marsh); sediment (Sed); benthic microalgae (Microalg); *Sarcocornia fruticosa* (Sarc); *Halimione portulacoides* (Hal); *Spartina maritima* (Spart); *Corophium* spp. (Corop); *Nereis diversicolor* (Ner); *Scrobicularia plana* (Scr); *Solea solea* (Ss) and *S. senegalensis* (Sn). Black dots stand for nursery A items, white dots stand for nursery B items.

No significant difference was found between the isotopic signatures of the surface sediment from the two nurseries, both in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($P > 0.05$). Surface sediments were more enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than the suspended POM (Figure 3).

Stable isotope values of producers

The $\delta^{13}\text{C}$ values of the saltmarsh halophytes differed greatly among species with *S. maritima* being enriched (between -13.6‰ and -13.3‰) and *S. fruticosa* and *H. portulacoides* (between -25.3 and -27.7‰) being depleted in $\delta^{13}\text{C}$ (Figure 3). The $\delta^{15}\text{N}$ values of the saltmarsh halophytes did not differ among species with *S. maritima* being more depleted in $\delta^{13}\text{C}$ (between

7.63‰ and 11.82‰) than *S. fruticosa* and *H. portulacoides* (between 9.61 and 12.77‰) (Figure 3).

No significant difference was found in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the halophytes from the two nursery areas ($P > 0.05$). It is at the benthic microalgae level that the two nurseries start to differentiate (mean values of -17.6 for nursery A and -15.7 for nursery B), with a significant difference in the isotopic composition ($P < 0.05$ for both isotopes). A tendency for more enriched $\delta^{13}\text{C}$ levels in nursery B can be noticed from this food web level upwards in the trophic chain (Figure 3).

Stable isotope values of soles from both nurseries and its' prey

Significant differences in isotopic signatures were found among soles. Differences in $\delta^{13}\text{C}$ were found between *S. solea*, *S. senegalensis* from nursery A and *S. senegalensis* from nursery B ($P < 0.05$). For $\delta^{15}\text{N}$ there was no difference for the two sole species living in nursery A, but there was a significant difference between soles from different nurseries, with *S. solea* and *S. senegalensis* from nursery A being different from *S. senegalensis* from nursery B ($P < 0.05$).

Soles' main prey, *N. diversicolor*, *S. plana* and *Corophium* spp., presented significant differences in their isotopic signatures between nursery areas both in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($P < 0.05$). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were generally higher in nursery B than in nursery A, with the exception of $\delta^{15}\text{N}$ in *Corophium* sp. (Figure 3). $\delta^{13}\text{C}$ values from both nurseries presented intermediate levels between those of its freshwater sources and the more enriched seawater. An increase in $\delta^{15}\text{N}$ with increasing trophic level can be observed (Figure 3).

Discussion

S. solea and S. senegalensis nursery fidelity

Distinct isotopic signatures between nursery areas were found for 0-group soles. No individual presented intermediate isotopic values reflecting migration between nurseries (Fry *et al.*, 1999; 2003; Herzka *et al.*, 2002). Therefore it can be concluded that there is low connectivity between the two sites for 0-group soles due to the high site fidelity exhibited by these fish. Limited movement range had already been reported for marked juvenile *S. solea* (0-group and 1-group) (Coggan and Dando, 1988), as well as for other 0-group flatfish, such as winter flounder, *Pseudopleuronectes americanus* (Walbaum, 1792) and plaice, *Pleuronectes platessa* (Linnaeus, 1758) (ca. 100 m of range for 90% of marked individuals for both species) (Saucerman and Deegan, 1991; Burrows *et al.*, 2004).

1-group sole also presented different isotopic signatures between nursery areas, yet they exhibited lower site fidelity, with 35.5% of migrant individuals identified and consequently a larger connectivity between the two nursery areas. This is probably due to an increase in locomotory capacity with increasing size, coupled with larger energy demands that lead 1-group sole to forage in wider areas and search for alternative feeding opportunities within the estuary.

Although a good distinction among isotopic signatures was found for soles from the two nurseries, it must be taken into account that isotopic turnover rates, the speed at which an individual will reach equilibrium following a shift to an isotopically distinct prey (Herzka, 2005), are not yet determined for these species, so the length of time an immigrant will be distinguishable from a long time resident is still unknown. However, it has been reported that young fishes with faster growth rates will equilibrate within days or weeks, while larger fish may take well over a year (Herzka, 2005). This means that 0-group sole presumably reach the equilibrium faster than 1-group individuals, making migrants hard to detect. We should conclude that our estimations on the high site fidelity of 0-group soles need the support of isotopic turnover experiments in order to gather information regarding their projection in time.

Food web interactions

The analysis of the food web through its isotopic composition reveals a complex net of relations between its components. As it is common in ecotones, such as estuaries, energy paths are complex and there are various sources contributing to the energy flow throughout the food web (Riera *et al.*, 1999; Wainright *et al.*, 2000; Weinsten *et al.*, 2000; Alfaro *et al.*, 2006), with fluctuating inputs due to diel and semilunar cycles associated with the tides.

While POM from freshwater sources presented similar isotopic signatures, the more saline incoming water presented a different signature, more enriched in ^{13}C and ^{15}N . The input of these enriched marine waters will be especially important every high tide (twice a day in this region) and especially during spring tides, carrying enriched POM, as well as suspended benthic microalgae from down stream that will be incorporated into the local food web. The relative importance of these inputs will also depend on the river inflow, which in the Tagus is regulated by dams and strongly depends on the frequency and intensity of the seasonal rains which are quite variable in this region.

Water POM at the nursery areas and respective saltmarsh creeks was depleted in ^{13}C and ^{15}N and did not show any isotopic differences from the freshwater sources. This is probably due to the fact that 2001 was a very rainy year with strong river inflow into the estuary (Costa *et al.*, *in press*). However, sediment was isotopically more enriched which complied with the presence of a marine influence. Sediment isotopic composition did not differentiate the nurseries. It is only from the benthic microalgae level upwards that the nurseries start to differentiate, with the consumers from nursery B being always more enriched in $\delta^{13}\text{C}$, than the ones from nursery A.

The $\delta^{13}\text{C}$ values of the saltmarsh halophytes differed greatly with *S. maritima* being enriched and *S. fruticosa* and *H. portulacoides* being depleted in $\delta^{13}\text{C}$. The range of $\delta^{13}\text{C}$ for *S. maritima* (-13.6‰ and -13.3‰) fell well within the range reported by other authors for other C_4 species and *Spartina* species (Haines, 1976; Currin *et al.*, 1995; Paterson and Whitfield, 1997). The $\delta^{13}\text{C}$ values for *S. fruticosa* and *H. portulacoides* (between -25.3 and -27.7‰) are comparable to other C_3 saltmarsh plants (Paterson and Whitfield, 1997) and within the range of -23 to -30‰ reported by Smith and Epstein (1971) for terrestrial C_3 plants.

As stated above, consumers' isotopic signatures differed between nurseries. This is indicative of a low level of connectivity between the two nursery areas. It appears as if there are two parallel trophic chains with little trophic interaction between each other. This has also been reported by other authors in other estuaries (Paterson and Whitfield, 1997). It is possibly due to the low mobility of 0-group sole and its' prey, as well as hydrological features of this area that do not facilitate the connectivity between sites.

Concerning the soles, there were different isotopic signatures in $\delta^{13}\text{C}$ for all groups considered, *S. solea*, *S. senegalensis* from nursery A and *S. senegalensis* from nursery B. Yet, there was no difference in $\delta^{15}\text{N}$ in soles from the same nursery (nursery A). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were different between nursery areas, with *S. senegalensis* from nursery B exhibiting more enriched values. The results for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ reflect the feeding ecology of soles in these nursery grounds and the fact that there are no other nursery habitats within this system. Differences in the isotopic composition on the base of the food-web among habitats are patent in the benthic microalgae that are taken up by the main prey of soles.

Cabral (2000) reported that the diets of *S. solea* and *S. senegalensis* in nursery A were quite similar and correlated with prey availability, indicating opportunistic utilization of food resources and low selectivity (Miller *et al.*, 1985). Similar diets, of closely related species foraging in the same site, presumably lead to very similar isotopic signatures, as could be observed in the present study. Cabral (2000) also concluded that the feeding pattern of *S. senegalensis* was different at nursery B, with a positive selection of intertidal areas as feeding grounds, and *S. plana* as its main prey, while in nursery A *Corophium* spp. were the main prey.

Feeding ecology is possibly an additional factor leading to the differentiation of isotopic signatures of sole from the two nursery areas, observed in the present study. The two species of sole inhabiting nursery A are probably feeding on a combination of the prey items analysed, hence $\delta^{13}\text{C}$ values fall between the more depleted values found for *Corophium* spp. and the more enriched values found for both *S. plana* and *N. diversicolor*. *S. senegalensis* from nursery B should also be feeding on a combination of prey since its $\delta^{13}\text{C}$ values are within the distributions of $\delta^{13}\text{C}$ of its' three main prey.

Primary sources of nutrition

The isotopic signatures observed in the consumers suggest that they are dependent on more than one energy path. They are dependant on depleted sources of carbon but other enriched sources cause values of consumers to be greater than what would be expected due to normal trophic shift. Among the possible enriched sources that may be contributing to the food web there is the saltmarsh C_4 halophyte, *S. maritima* that incorporates the trophic web through a detritus pathway (Teal, 1962; Odum, 1980), the marine POM and also the suspended benthic microalgae that are washed in with the rising tides. *Spartina* species have been shown to produce detritus more depleted in $\delta^{13}\text{C}$ than its' living tissues (Benner *et al.*, 1987; Currin *et al.*, 1995), so these should be closer in $\delta^{13}\text{C}$ values to the consumers isotopic signatures.

The role of saltmarshes as carbon sources in estuarine food webs has been thoroughly discussed, especially the link between macrophytes and marine transients (e.g. Teal, 1962; Odum, 1968; Odum, 1980; Haines and Montague, 1979; Nixon, 1980; Litvin and Weinstein, 2003). Teal's (1962) hypotheses that saltmarsh may drive much of the secondary production in estuaries, has been gradually altered to accommodate new findings such as, that finfish, phytoplankton, benthic microalgae, and organic matter exports are also important contributors to nutrient flux in estuarine waters (Haines and Montague 1979; Nixon, 1980; Sullivan and Montcrieff, 1990; Eldridge and Cifuentes, 2000). These contributions were first quantified by Deegan (1993) for Gulf menhaden *Brevoortia patronus*, but also apply to other marine transients (Weinstein *et al.*, 2000; Litvin and Weinstein, 2003). Further research has shown that saltmarshes vary in their ability to export organic matter (Roman and Daiber, 1989; Dame *et al.*, 1991). In fact, Riera *et al.* (1999) reported that despite the wide availability of saltmarsh plants they may not be significant contributors of carbon and nitrogen to the local food web

A mixture of sources is probably being incorporated into the food web. Previous studies have shown that benthic microalgae may contribute significantly to saltmarsh primary production (Sullivan and Montcrieff, 1988; Pickney and Zingmark, 1993), as well as being important components in saltmarsh food webs (Sullivan and Montcrieff, 1990; Currin *et al.*, 1995). Since isotopic values of benthic microalgae tend to be spatially variable, a wider sampling strategy encompassing large areas in both nurseries may bring new insights into their importance in these areas.

Isotopic signatures of the consumers reveal that there is a different dependence on each energy pathway according to nursery area, with nursery B being relatively more dependent on the $\delta^{13}\text{C}$ enriched energy pathway. This is probably related to the complex hydrology of this estuary. Nursery B presents higher salinity values and has therefore a greater marine influence than nursery A (Cabral and Costa, 1999). Nursery A receives the direct influence of the Tagus river inflow, by far the largest freshwater input to this estuary. Nursery B is located in a more sheltered area, where the Tagus flow has lost much of its current and other freshwater inputs came from much smaller rivers. The extension of saltmarshes is also larger in nursery B, which, as stated above, can be an important source of $\delta^{13}\text{C}$ enriched material to the local food web.

It can thus be concluded that two important fish species for commercial fisheries in Portugal, *S. solea* and *S. senegalensis*, have important nursery areas in the Tagus estuary, whose energy pathways are dependent on complex hydrological features. These nurseries present low connectivity and different levels of dependence upon freshwater and marine energy pathways. The two nurseries should be managed as independent habitats, one which provides habitat for the juveniles of two sole species and another that provides habitat for only one of the species juveniles, yet it is where cohorts develop over its' first year of life independent of the population that colonizes the other nursery.

Both species populations are dependent upon fluctuations in freshwater and marine water inflow, both natural and man controlled. Further research into the contribution of each nursery to the adult stocks should produce useful information for fish stock management.

Acknowledgements

Authors would like to thank everyone involved in the field work. This study had the support of the Portuguese Fundação para a Ciência e a Tecnologia (FCT) and the European Union which financed several of the research projects related to this work.

References

- Alfaro, A.C., Thomas, F., Sergent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (Northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine, Coastal and Shelf Science* 70, 271-286.
- Anguis, V., Cañavate, J. P., 2005. Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. *Aquaculture* 243, 133-145.
- Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. *Nature* 22, 708-710
- Bouillon, S., Mohan, P.C., Sreenivas, N., Dehairs, F., 2000. Sources of suspended organic matter and selective feeding by zooplankton in an estuarine mangrove ecosystem as traced by stable isotopes. *Marine Ecology Progress Series* 208, 79-92.
- Burrows, M.T., Gibson, R.N., Robb L., MacLean A., 2004. Alongshore dispersal and site fidelity of juvenile plaice from tagging and transplants. *Journal of Fish Biology* 65, 620-634. doi:10.1111/j.1095-8649.2004.00467.x
- Cabana, G., Rasmussen, J.B., 2002. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 372, 255-257. doi:10.1038/372255a
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect environmental changes? *Climate Research* 18, 119-126.
- Caçador, I., Vale, C., Catarino, F., 1996. Accumulation of Zn, Pb, Cu and Ni in sediments between roots of the Tagus estuary salt marshes, Portugal. *Estuarine Coastal Shelf Science* 42, 393-403.
- Coggan, R.A., Dando, P.R., 1988. Movements of juvenile Dover sole, *Solea solea* (L.), in the Tamar Estuary, South-western England. *Journal of Fish Biology* 33, 177-184.

- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Scientia Marina* 53, 561-566.
- Costa, M.J., Cabral, H.N., 1999. Changes in the Tagus estuary nursery function for commercial fish species: some perspectives for management. *Aquatic Ecology* 33, 53-74.
- Costa, M.J., Vasconcelos, R., Costa, J.L., Cabral, H.N., *in press*. River flow influence on the fish community of the Tagus estuary (Portugal). *Hydrobiologia*.
- Cunjak, R.A., Roussel, J.-M., Gray, M.A., Dietrich, J.P., Cartwright, D.F., Munkittrick, K.R., Jardine, T.D., 2005. Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. *Oecologia* 144, 636-646.
- Currin, C.A., Newell, S.Y., Paerl, H.W., 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Marine Ecology Progress Series* 121, 99-116.
- Dame, R.F., Spurrier, J., Williams, T.M., Kjerfve, B., Zingmark, R.G., Wolaver, T.G., Chrzanowski, T.H., McKellar, H.N., Vernberg, F.J., 1991. Annual material processing by a salt marsh estuarine basin in South Carolina, USA. *Marine Ecology Progress Series* 72, 153-166.
- Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., Harmelin-Vivien, M.L., 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* 138, 325-332.
- De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42, 495-506.
- Deegan, L.A., 1993. Nutrient and energy transport between estuaries and coastal marine ecosystems by fish migration. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 74-79.
- Doucett, R.R., Power, M., Power, G., Caron, F., Reist, J. D., 1999. Evidence for anadromy in a southern relict population of Arctic charr from North America. *Journal of Fish Biology* 55, 84-93. doi:10.1111/j.1095-8649.1999.tb00658.x
- Eldridge, P.M., Cifuentes, L.A., 2000. A stable isotope model approach to estimating the contribution of organic matter from marshes to estuaries. In: Weinstein M. P., Kreeger, D. A. (Eds.), *Concepts and Controversies in Tidal Marsh Ecology*. Kluwer Academic, Dordrecht, The Netherlands, pp. 495-514.
- Fiúza, A.F.G., Macedo, M.E., Guerreiro, M.R., 1982. Climatological space and time variation of the Portuguese coastal upwelling. *Oceanologica Acta* 5, 31-40.
- Fonseca, V.F., Vinagre, C., Cabral, H., 2006. Growth variability of juvenile sole *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, and comparison with RNA-DNA ratios in the Tagus estuary, Portugal. *Journal Fish Biology* 68, 1551-1562. doi:10.1111/j.0022-1112.2006.001042.x
- France, R., 1995. Stable nitrogen isotopes in fish: literature synthesis on the influence of ecotonal coupling. *Estuarine, Coastal and Shelf Science* 41, 737-742.

- Fry, B., 1983. Fish and shrimp migrations in the northern Gulf of Mexico analyzed using stable C, N, and S isotope ratios. *Fishery Bulletin* 81, 789-801.
- Fry, B., Sherr, E.B., 1984. $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* 27, 13-47.
- Fry, B., Mumford, P., Robblee, M., 1999. Stable isotope studies of pink shrimp (*Farfantepenaeus duorarum* Burkenroad) migrations on the southwestern Florida Shelf. *Bulletin of Marine Science* 65, 419-430.
- Fry, B., Baltz, D.M., Benfield, M., Fleeger, J., Gace, A., Haas, H., Quiñones-Rivera, Z., 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. *Estuaries* 26, 82-97.
- Haines, E.B., 1976. Stable carbon isotope ratios in the biota, soils and tidal water of a Georgia saltmarsh. *Estuarine and Coastal Marine Science* 4, 609-616.
- Haines, E.B., Montague, C.I., 1979. Food sources of estuarine invertebrates analysed using $^{13}\text{C}/^{12}\text{C}$ ratios. *Ecology* 60, 48-56.
- Hanson, S., Hobbie, J.E., Elmgren, R., Larsson, U., Fry, B., Johansson, S., 1997. The stable nitrogen ratio as a marker of food-web interactions and fish migration. *Ecology* 78, 2249-2257.
- Herzka, S., 2005. Assessing connectivity of estuarine fishes based on stable isotopes ratio analysis. *Estuarine, Coastal and Shelf Science* 64, 58-69.
- Herzka, S.Z., Holt, S.A., Holt, G.J., 2001. Documenting the settlement history of individual fish larvae using stable isotope ratios: model development and validation. *Journal of Experimental Marine Biology and Ecology* 265, 49-74.
- Herzka, S.Z., Holt, S.A., Holt, G.J., 2002. Characterization of settlement patterns of red drum (*Sciaenops ocellatus*) larvae to estuarine nursery habitat: a stable isotope approach. *Marine Ecology Progress Series* 266, 143-156.
- Kline, T.C., Wilson, W.J., Goering J.J., 1998. Natural isotope indicators of fish migration at Prudhoe Bay, Alaska. *Canadian Journal of Fisheries and Aquatic Science* 55, 1494-1502.
- LeFrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole, *Solea solea*. *Marine Ecology Progress Series* 259, 273-284.
- Litvin, S.Y., Weinstein, M.P., 2003. Life history strategies of estuarine nekton: the role of marsh macrophytes, benthic microalgae, and phytoplankton in the trophic spectrum. *Estuaries* 26, 552-562.
- Miller, J.M., Crowder, L.B., Moser, M.L., 1985. Migration and utilization of estuarine nurseries by juvenile fishes: an evolutionary perspective. *Contributions in Marine Science* 27, 338-352.
- Miranda, P.M.A, Coelho, F.E.S., Tomé, A.R., Valente, M.A., 2002. 20th Century Portuguese Climate and Climate Scenarios. In: Santos, F.D., Forbes, K., Moita, R. (Eds.), *Climate change in Portugal. Scenarios, Impacts and Adaptation measures – SIAM Project*. Gradiva, Lisboa, Portugal, pp. 27-83.

- Nichols, P.D., Klumpp, D.W., Johns, R.B., 1985. A study of food chains in seagrass communities III.* Stable carbon isotope ratios. *Australian Journal of Marine and Freshwater Research* 36, 683-690.
- Nixon, S.W., 1980. Between coastal marshes and coastal waters—a review of twenty years of speculation and research on the role of salt marshes in estuarine productivity and water chemistry. In: Hamilton R., Macdonald, K. B. (Eds.), *Estuarine and wetland processes*. Plenum, NY. pp. 437–525.
- Odum, E.P., 1968. A research challenge: evaluating the productivity of coastal and estuarine water. *Proceedings of the Second Sea Grant Conference, Graduate School of Oceanography, University of Rhode Island, Newport, USA*, pp. 63-64.
- Odum, E.P., 1980 The status of three ecosystem level hypotheses regarding salt marsh estuaries: tidal subsidy, outwelling, and detritus based food chains. In: Kennedy (Ed.) *Estuarine Perspectives*. Academic Press, NY, pp. 485-495.
- Owens, N.J.P., 1987. Natural variations in ^{15}N in the marine environment. *Advances in Marine Biology* 24, 389-451.
- Paterson, A.W., Whitfield, A.K., 1997. A stable carbon isotope study of the food-web in a freshwater-deprived South African estuary, with particular emphasis on the ichthyofauna. *Estuarine, Coastal and Shelf Science* 45, 705-715.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecological Systematics* 18, 293-320.
- Peterson, B.J., Howarth, R.W., Garrit, R.H., 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227, 1361-1363.
- Phillips, D.L., Eldridge, P.M., 2006. Estimating the timing of diet shifts using stable isotopes. *Oecologia* 147, 195-203.
- Preston, T., Owens, N.J.P., 1983. Interfacing an automatic elemental analyser with an isotope ratio mass spectrometer: the potential for fully automated total nitrogen and nitrogen-15 analysis. *Analyst* 108, 971–977.
- Pickney, J., Zingmark, R.J., 1993. Modelling intertidal benthic microalgal annual production in an estuarine ecosystem. *Journal of Phycology* 29, 396–407.
- Pinnegar, J.K., Polunin, N.V.C., 1999. Differential fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among fish tissues: implications for the study of trophic interactions. *Functional Ecology* 13, 225–231.
- Riera, P., Richard P., 1996. Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. *Estuarine Coastal and Shelf Science* 42, 347-360.
- Riera, P., Stal, L.J., Nieuwenhuize, J., Richard, P., Blanchard, G., Gentil, F., 1999. Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources. *Marine Ecology Progress Series* 187, 301-307.

- Roman, C. T., Daiber, F. C., 1989. Organic carbon flux through a Delaware Bay salt marsh: tidal exchange, particle size distribution and storms. *Marine Ecology Progress Series* 54, 149-156.
- Russell, F. S., 1976. *The Eggs and Planktonic Stages of British Marine Fishes*. Academic Press, London, 524 pp.
- Saucerman, S.E., Deegan, L.A., 1991. Lateral and cross-channel movement of young-of-the-year winter flounder (*Pseudopleuronectes americanus*) in Waiquoit Bay, Massachusetts. *Estuaries* 14, 440-446.
- Simenstad, C.A., Wissmar, R.C., 1985. $\delta^{13}\text{C}$ evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. *Marine Ecology Progress Series* 22, 141-152.
- Smith, B.N., Epstein, S., 1971. Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant Physiology* 47, 380-384
- Sullivan, M.J., Moncreiff, C.A., 1988. Primary production of edaphic algal communities in a Mississippi salt marsh. *Journal of Phycology* 24, 49-58.
- Sullivan, M.J., Moncreiff, C.A., 1990. Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analyses. *Marine Ecology Progress Series* 62, 149-159.
- Talley, D., 2000. Ichthyofaunal utilization of newly-created versus natural salt marsh creeks in Mission Bay, California. *Wetlands Ecology and Management* 8, 117-132.
- Teal, J.M., 1962. Energy flow in the salt marsh ecosystem of Georgia. *Ecology* 43, 614-624.
- Vale, C., Sundby, B., 1987. Suspended sediment fluctuations in the Tagus estuary on semidiurnal and fortnightly time scales. *Estuarine, Coastal and Shelf Science* 25, 495-508.
- Wainright, S.C., Weinstein, M.P., Able, K.W., Currin, C.A., 2000. Relative importance of benthic microalgae, phytoplankton and the detritus of smooth cordgrass *Spartina alterniflora* and the common reed *Phragmites australis* to brackish-marsh food webs. *Marine Ecology Progress Series* 200, 77-91.
- Weinstein, M.P., Litvin, S.Y., Bosley, K.L., Fuller, C.M., Wainright, S.C., 2000. The role of tidal salt marsh as an energy source for marine transient and resident finfishes: a stable isotope approach. *Transactions of the American Fisheries Society* 129, 797-810.

Diel and semi-lunar patterns in the use of an intertidal mudflat by juveniles of Senegal sole, *Solea senegalensis*

Abstract: Intertidal mudflats are a dominant feature in many estuarine systems and may comprise a significant component of the feeding grounds available to fish. The Senegal sole, *Solea senegalensis* Kaup, 1858, is one of the most important flatfishes in the Tagus estuary (Portugal) and its juveniles feed in the large intertidal flats. Many aspects of this species ecology and lifecycle are still unknown, namely its behaviour adaptations to predictable environmental variations like day-night and semi-lunar cycles. Such activity patterns may strongly influence its' use of mudflat habitats. Two encircling nets were deployed in an intertidal flat, one in the lower and the other in the upper mudflat. Nets were placed during high tide and organisms collected when the ebbing tide left the flats dry. Sampling took place in June-July 2004, covering all possible combinations of the diel and semi-lunar cycles with six replicates. Monthly beam trawls were carried out to determine density and average length of *S. senegalensis* predators in the intertidal and subtidal areas. Sediment samples were also taken, to determine prey density in the lower intertidal, upper intertidal and subtidal areas. *S. senegalensis* captured were mostly 0-group juveniles. *Crangon crangon* (Linnaeus, 1758) (one of the main predators) density and average length was higher in the subtidal than in the intertidal. Prey density decreased from the upper intertidal to the subtidal area. The highest average density of *S. senegalensis* occurred during full moon at dawn/dusk. A semi-lunar activity pattern was detected. At spring tides abundance peaked at dusk/dawn, while at neap tides abundance peaked during the day. Predators' densities over these periods were analysed and predator avoidance discussed. While during quarter and full-moon nights *S. senegalensis* extended its distribution over the lower and upper mudflat, during new-moon colonisation was restricted to the lower mudflat. It was concluded that, while diel patterns of activity are well studied and are likely associated with feeding rhythms, the influence of the moon cycle despite its importance is a more complex phenomena that needs further investigation.

Key-words: Intertidal environment; Lunar cycles; Day-night cycle; Sole; Feeding behaviour; Activity rhythms; Eastern Atlantic; Portugal; Tagus estuary.

Introduction

Estuarine intertidal mudflats are very important in the functioning of estuarine systems and it is generally recognized that they have a disproportionately high productivity when compared to subtidal areas (Elliot and Taylor, 1989, Elliott and Dewailly, 1995). Moreover, these sheltered shallow waters provide important feeding grounds for juvenile fishes (e.g. Hædrich, 1983; Able *et al.*, 1990; Costa and Elliott, 1991). However, intertidal mudflats are only available to fish during tidal inundation which means that the use of this habitat implies tidal migrations.

It is assumed that fish exhibit movements during their life cycles at various spatial scales, ranging from daily habitat shifts to larger movements between systems (Morisson *et al.*, 2002). Morisson *et al.* (2002) remarked that while long migrations have been reported during the life cycles of many fish; comparatively little work has been done on smaller scale movements over short temporal and spatial scales for estuarine fishes. However, such small scale movements have been thoroughly studied in flatfish that use coastal and estuarine intertidal

areas such as plaice *Pleuronectes platessa* and flounder *Platichthys flesus* and conclusions point at feeding and predator avoidance as the main driving forces behind tidal migrations (e.g. Gibson, 1973; 1980; 1982; 1999; 2003; Burrows, 1994; Ellis and Gibson, 1995; Wirjoatmodjo and Pitcher, 1980; Summers, 1980; van der Veer and Bergman, 1986; Raffaelli *et al.*, 1990; Gibson *et al.*, 1998). Studies on other fish species suggest that these movements may be strongly structured by tidal and day-night cycles (Naylor, 2001; Morrison *et al.*, 2002; Krumme *et al.*, 2004), although Quinn *et al.* (1981) found no differences in fish assemblages between nights of full and new moon. Raffaelli *et al.* (1990) observed marked differences in the number and length of *P. flesus* using the intertidal in successive day and night tides, yet the full effect of the semi-lunar cycle is still scarcely understood in flatfish. Estuarine organisms are adapted to predictable environmental cycles and show rhythmic activity synchronized with tidal cycles (Neumann, 1981, Palmer, 1995). While some of these patterns are controlled endogenously, others seem to be controlled by direct response to environmental variations associated with the tidal cycle (Naylor, 1982; Naylor and Williams, 1984; Saigusa and Kawagoye, 1997).

The Senegal sole, *Solea senegalensis*, Kaup 1858, is a benthonic fish distributed from the Bay of Biscay to Senegal and western Mediterranean (Quero *et al.*, 1986). It is a species of increasing interest in aquaculture and is commonly cultured in the Portuguese and Spanish southern coasts (Dinis *et al.*, 1999). This species is one of the most important flatfishes in the Tagus estuary (Cabral and Costa, 1999). Exactly where settlement of this species' larvae occurs is still unknown, yet it is possible that it takes place in the intertidal as it happens for the very similar species *Solea solea* (Linnaeus, 1758) (van der Veer *et al.*, 2001).

The main predators of other 0-group juveniles flatfishes are the crab *Carcinus maenas* (Linnaeus, 1758) and the shrimp *Crangon crangon* (Linnaeus, 1758) (Pihl and Van der Veer, 1992; Modin and Pihl, 1996). These are also thought to be the main predators of *S. senegalensis* because of similarity in size and form and based on our own aquarium observations (unpublished data).

S. senegalensis is an important predator of amphipods, polychaetes and bivalves (Cabral, 2000) and is therefore of great importance for the dynamics and composition of the biological communities in many of the estuarine and coastal systems where it occurs. Cabral (2000) reported that intertidal mudflats are very important feeding grounds for *S. senegalensis* juveniles, yet many features of this species' use of the intertidal are still unknown. Observation of this species distribution over the mudflats in all combinations of diel and semi-lunar cycles will advance our understanding of its ecology as well as of estuarine fish dynamics. The present study aims to evaluate the diel and semi-lunar patterns in the use of the intertidal mudflats by *S. senegalensis*.

Materials and Methods

Study area

The Tagus estuary (Fig. 1), with an area of 320 km², is a partially mixed estuary with a tidal range of about 4 m. This estuarine system has a mean depth <10 m and about 40% of its area is composed of intertidal areas, which are predominantly mudflats. Although its bottom is composed of a heterogeneous assortment of substrates, its prevalent sediment is muddy sand in the upper and middle estuary and sand in the lower estuary. The mean river flow is 400 m³s⁻¹, though it is highly variable both seasonally and annually.

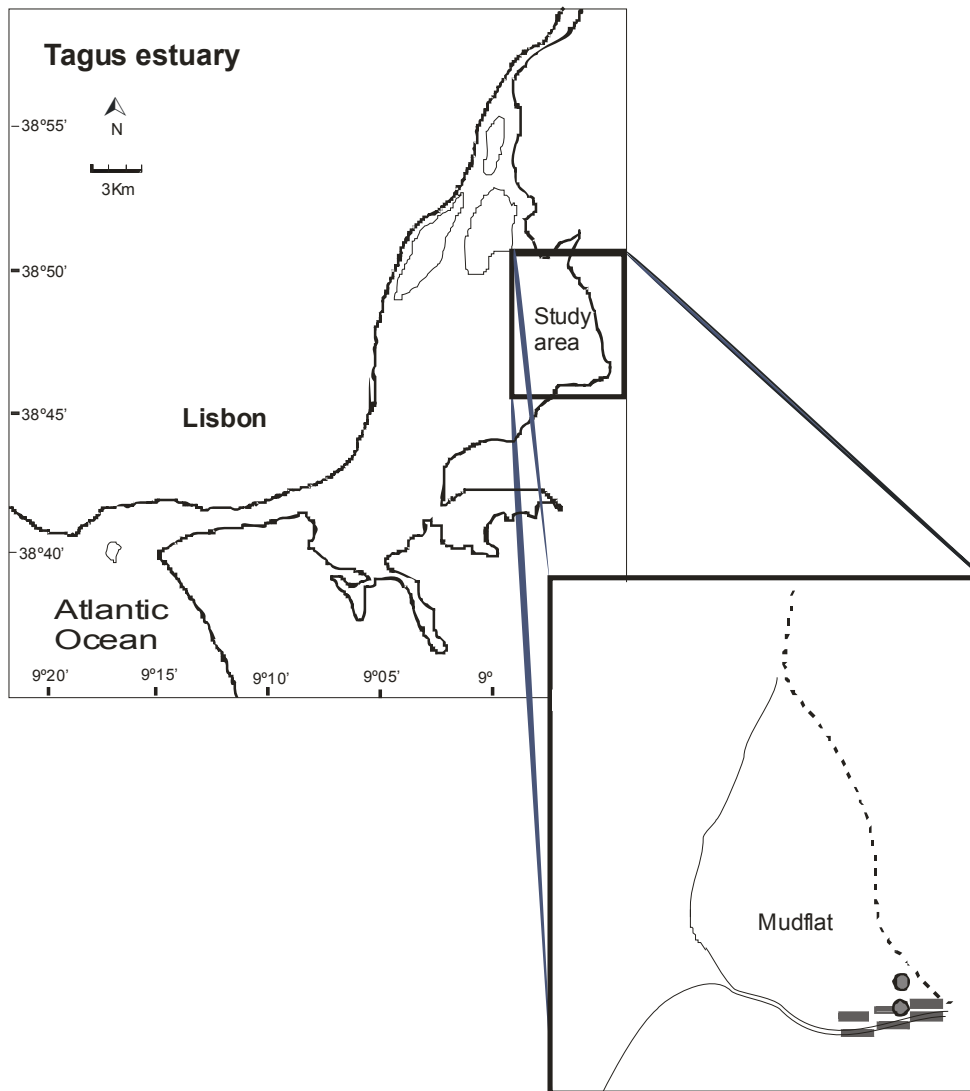


Figure 1 – Location of the study area in the Tagus estuary, Portugal. Insert shows the study area in detail, the encircling nets are represented by dots (●), while trawls are represented by rectangles (▬). The dotted line (---) represents the limit of the saltmarsh.

The mudflat where this study took place is located in the upper estuary in a sheltered south bank branch (Fig. 1). The tidal regime is semidiurnal, daily the intertidal mudflat is inundated during two periods of approximately 3.5 hours at its upper part, and 5 hours at its

lower part. The subtidal area (channel) is always submerged. Salinity in this area varies from 4, in winter, to nearly 30 in summer, while water temperature ranges from 8°C to 26°C (Cabral *et al.*, 2001). Maximum depth in the mudflats during high tide is 2.5 m.

Sampling

In order to study the distribution of Senegal sole across the tidal flats during high tide, two encircling nets were placed in the mudflats, one in the lower mudflat (closer to the channel), the other in the upper mudflat (closer to the salt marsh) and distanced approximately 150 m (always at the same intertidal level). Preliminary work in this area had shown that captures were considerably higher using this technique comparing to bottom trawl.

Nets had a perimeter of 100 m and a mesh size of 5 mm. They were supported by wooden sticks and deployed (simultaneously) by boat at high tide peak. The operation took the shape of a closed circle trapping the nekton inside. Metal weights were attached to the bottom of the nets so that they would be naturally buried in the mud when deployed. Twenty wooden sticks supported each net. In order to avoid the scaring of the fish the boat was operated with sticks, motor was turned off and silence was kept. At low tide the mudflat drains completely leaving the organisms trapped in the nets. Organisms were hand collected, frozen at -20°C and later identified and measured. Net perimeter was used to calculate the area of the sampled circle in order to estimate densities of the organisms captured.

All possible combinations of diel and semi-lunar cycles were covered in June-July 2004. Six replicates of each combination of these cycles were carried out (three in each month, whenever they existed); samples were taken on three consecutive days in each lunar phase (table 1). Since the tidal regime is semidiurnal two surveys per day could be conducted.

Table 1 - Sampling days when encircling nets were deployed in the lower and upper mudflat, with information on tide height, tide phase and diel cycle component.

	Tidal height	Day	Night	Dawn/Dusk
Full moon	3.59-3.99	4th June 2004		
		5th June 2004	31th July 2004	4th June 2004
		3rd July 2004	3rd June 2004	5th June 2004
		4th July 2004	3rd July 2004	4th July 2004
		5th July 2004		5th July 2004
		31th July 2004		
New moon	3.30-3.54	16th June 2004		
		17th June 2004	16th June 2004	17th June 2004
		18th June 2004	16th July 2004	18th June 2004
		16th July 2004	17th July 2004	18th July 2004
		17th July 2004		
		18th July 2004		
Quarter moon	2.95-3.28	9th June 2004	9th June 2004	23rd June 2004
		10th June 2004	10th June 2004	24th June 2004
		9th July 2004	9th July 2004	23rd June 2004
		10th July 2004	10th July 2004	24th June 2004
		23rd June 2004	11th July 2004	
		24th June 2004		
		24th June 2004	25th June 2004	

Three beam trawl replicates (Fig. 1) of ten minutes' and covering a length of approximately 600 m (3 m of opening and 3 mm mesh size) were carried out by boat in the intertidal and in the channel (on the same day, just minutes apart), each month, in order to determine density and average length of *S. senegalensis* predators (because encircling nets were impossible to use in the channel, trawls were necessary in order to obtain comparable densities). At the beginning and at the end of each trawl coordinates were registered with a GPS (Global Positioning System) in order to calculate the distance traveled. Trawl opening and the distance traveled allowed us to determine the area trawled for densities calculation. In the starting point of each trawl, sediment samples were collected using a Van Veen grab to determine prey density. A preliminary experiment was carried out in order to determine the adequate number of replicates. Ten replicates were carried out in the upper and lower mudflat, as well as in the subtidal channel. Benthic organisms were preserved in 4% buffered formalin and identified to the species level.

Data analysis

S. senegalensis were aged according to Cabral (2003). Differences in density were tested using a factorial ANOVA according to the semi-lunar and diel cycles, as well its location over the mudflat (upper versus lower mudflat). Tukey *post-hoc* tests were performed whenever the null hypotheses were rejected. A significance level of 0.05 was considered in all test procedures.

Densities and average length of *C. maenas* and *C. crangon* caught in the beam trawls were calculated in order to assess predation pressure in the intertidal and subtidal habitats. Similar ANOVA tests with Tukey *post-hoc* were applied to *C. maenas* and *C. crangon* densities (caught in the encircling nets), the major predators of *S. senegalensis* in the study area. Organisms were classified as prey items according to Cabral (2000). Prey density was calculated in the subtidal, lower intertidal and upper intertidal for the months of June and July 2004. Major prey groups were used in the prey density comparison.

Results

Species composition in the trawls

Ten species were caught in the trawls. The most abundant species was *C. crangon* both in the intertidal (overall mean density: 0.18 ind.m⁻²) and in the subtidal (overall mean density: 2.02 ind.m⁻²). Other species caught in considerably lower numbers were: *Pomatochistus microps*, *Palaemon longirostris*, *Palaemon serratus*, *C. maenas*, *Pomatochistus minutus*, *Dicentrarchus labrax*, *Engraulis encrasicolus*, *Liza ramada*, *Sardina pilchardus*, *Diplodus bellottii* and *Palaemon elegans*. *D. bellottii* and *P. elegans* were absent from the intertidal, while *E. encrasicolus* and *S. pilchardus* were absent from the subtidal.

Species composition in the encircling nets

Sixteen species were caught in the surrounding nets. The most abundant species was *C. crangon*, both in the lower (overall mean density 1.17 ind.m⁻²) and in the upper encircling nets (overall mean density 0.72 ind.m⁻²). Other species caught in lower numbers in the encircling nets were, in the lower encircling nets: *P. longirostris*, *P. serratus*, *P. microps*, *C. maenas*, *S. pilchardus*, *L. ramada*, *D. labrax*, *S. senegalensis*, *P. minutus*, *Syngnathus* sp., *Atherina presbiter*, *E. encrasicolus*, while in the upper encircling nets, all of the above species were caught except *Syngnathus* sp. Four species were only present in the upper encircling nets: *D. bellottii*, *Chelon labrosus* and *Argyrosomus regius*.

Solea senegalensis distribution

S. senegalensis caught in the encircling nets belonged to two age groups, however mostly 0-group juveniles (size range: 18 to 72 mm; mean: 34.4 mm; standard deviation: 12.05 mm). Only 7.3% of *S. senegalensis* were aged 1 year or older (mean size was 171.3 mm and standard deviation 30.60 mm). *S. senegalensis* occurred both in the lower and upper mudflats. However, this species was more abundant in the lower mudflat than in the upper mudflat. A significant difference in the densities of *S. senegalensis* over the mudflats was found ($F=25.7$; $P<0.05$).

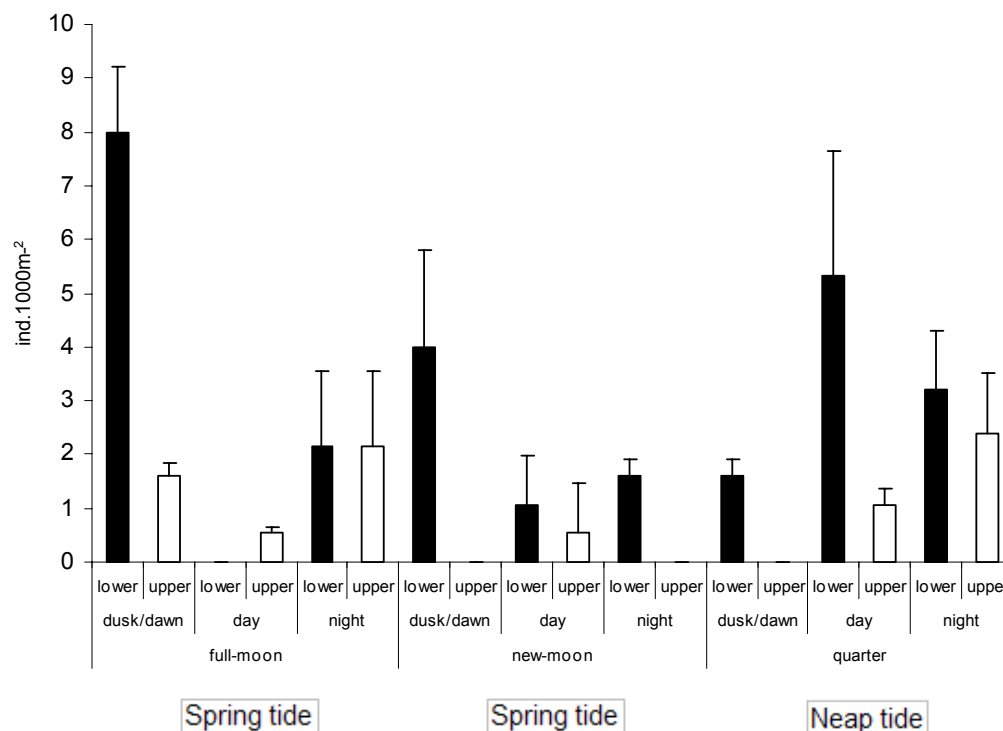


Figure 2 - Mean density and standard deviation values of *S. senegalensis* (ind.1000 m⁻²) caught in the encircling nets over all combinations of the diel and semilunar cycles, as well as its distribution over the lower and upper mudflat.

Although no significant differences in *S. senegalensis* density throughout the diel cycle were found ($F=2.6$; $P>0.05$), a combined effect of the diel and the semi-lunar cycle was detected ($F=8.0$; $P<0.05$). The *post-hoc* Tukey test revealed that the full moon, dusk/dawn, lower mudflat combination was significantly different from all other combinations, except for new moon, dusk/dawn, lower mudflat and quarter moon, day, lower mudflat. These three combinations of variables correspond to the three major density peaks of *S. senegalensis* (Fig. 2).

Density of *S. senegalensis* was significantly different according to the semi-lunar cycle ($F=2.9$; $P<0.05$). Mean abundance was highest in the mudflats during full-moon and quarter-moon. Mean abundance of this species during new-moon was considerably lower (Fig. 2). A significant combined effect was also detected between the semi-lunar cycle and the distribution of *S. senegalensis* over the mudflats.

A semi-lunar pattern was observed. During full and new moon (spring tides) abundance peaked at dawn/dusk; this did not happen during quarter moon (neap tides), when abundance peaked at daytime. During quarter and full-moon nights *S. senegalensis* extended its distribution over the lower and upper mudflat, while during new-moon colonisation by this species was restricted to the lower mudflat. In fact, an important decrease in the use of the upper mudflat in new-moon was observed during all periods of the diel cycle.

Predators' distribution

No other potential predators other than *C. maenas* and *C. crangon* was observed in the trawls and encircling nets. Mean density of *C. maenas* caught in the intertidal trawls was 0.01 ind.m^{-2} , while in the subtidal it was 0.01 ind.m^{-2} . *C. maenas* mean carapace length in the intertidal area was 38.0 mm (standard deviation: 8.39 mm) while in the subtidal area it was 40.2 mm (standard deviation: 11.41 mm). Mean density of *C. crangon* collected in the intertidal trawls was 0.18 ind.m^{-2} , while in the subtidal it was 2.02 ind.m^{-2} . *C. crangon* mean length in the intertidal was 34.9 mm (standard deviation: 20.43 mm) while in the subtidal it was 36.0 mm (standard deviation: 27.38 mm).

Data from the encircling nets experiment revealed a significant difference in the distribution of *C. maenas* over the mudflats ($F=5.9$; $P<0.05$), while no significant difference in the distribution of *C. crangon* was found.

Although no significant differences in *C. maenas* density throughout the diel cycle were found ($F=2.3$; $P>0.05$), a combined effect of the diel and semi-lunar cycle was detected ($F=6.3$; $P<0.05$). Regarding *C. crangon*, significant differences in density were detected throughout the diel cycle ($F=9.8$; $P<0.05$) and a combined effect of the diel and the semi-lunar cycle was also detected ($F=11.7$; $P<0.05$).

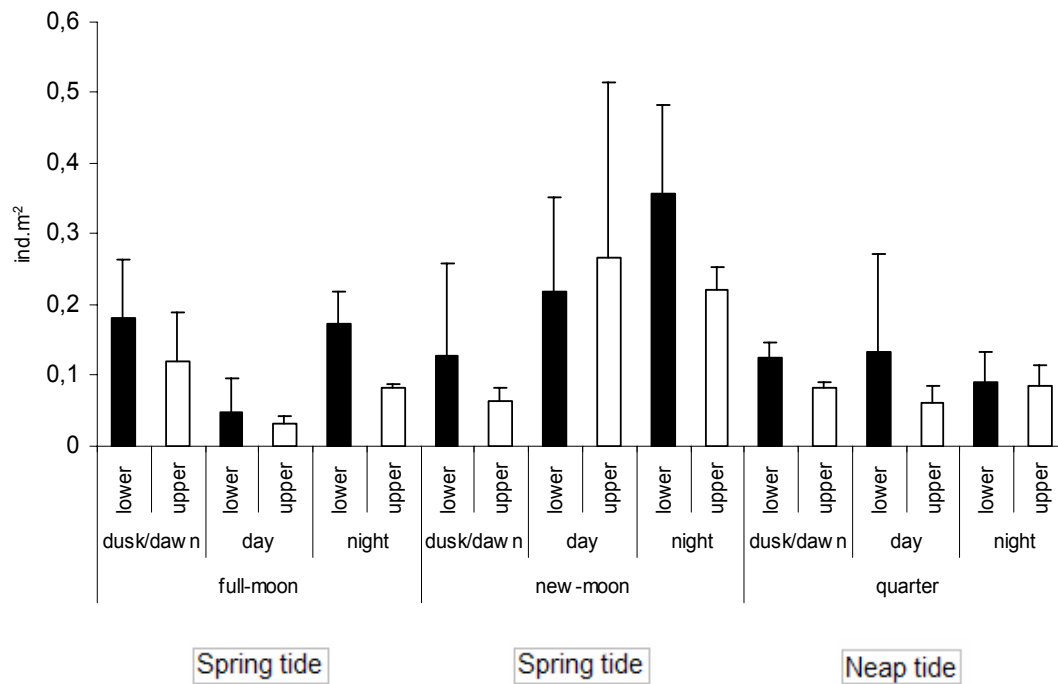


Figure 3 – Mean density and standard deviation values of *C. maenas* (ind.m⁻²) caught in the encircling nets over all combinations of the diel and semilunar cycles, as well as its distribution over the lower and upper mudflat.

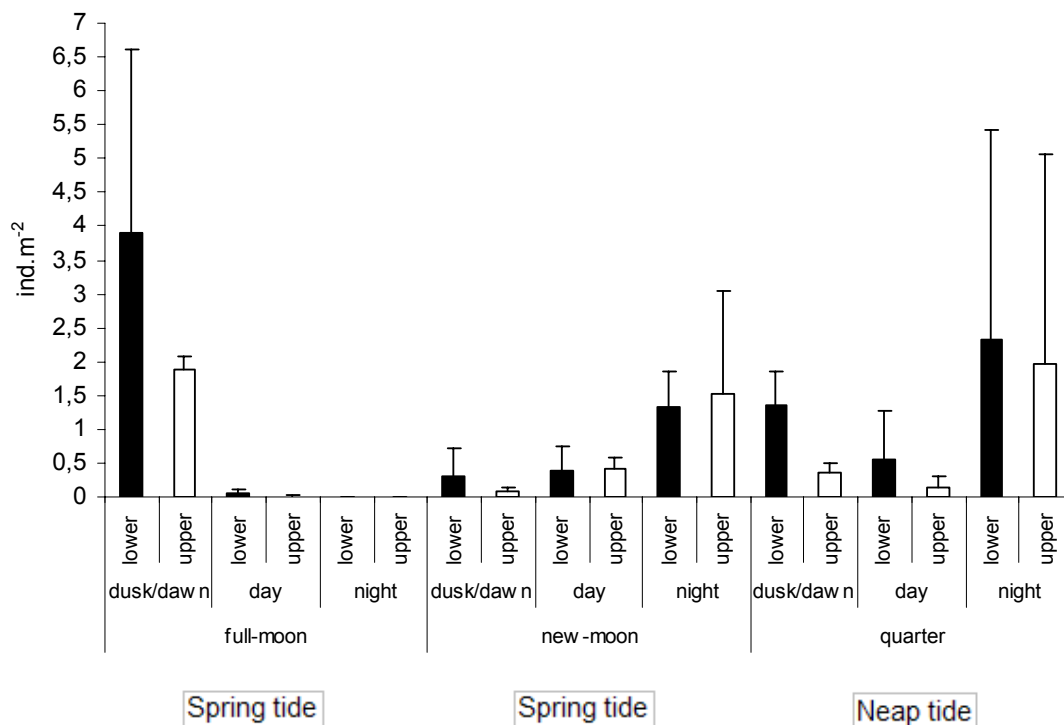


Figure 4 – Mean density and standard deviation values of *C. crangon* (ind.m⁻²) caught in the encircling nets over all combinations of the diel and semilunar cycles, as well as its distribution over the lower and upper mudflat.

Density of *C. maenas* was significantly different according to the semi-lunar cycle ($F=13.0$; $P<0.05$), while that of *C. crangon* was not ($F=2.9$; $P<0.05$), but as already referred a combined effect of the diel and semilunar cycles was detected.

C. maenas major activity peaks were observed during new-moon, mainly at night but also during the day (Fig. 3). *C. crangon* major activity peaks occurred during full-moon, at dusk/dawn. Important peaks were also registered during quarter moon nights (Fig. 4).

Prey distribution

Several species of *S. senegalensis*' prey were found in the sediment samples, mainly Polychaeta and Bivalvia. Amphipoda were also present but at very low densities. The analysis of these two prey groups mean densities in the subtidal (392 ind.m⁻² and 96 ind.m⁻², for Polychaeta and Bivalvia, respectively), lower intertidal (3008 ind.m⁻² and 552 ind.m⁻², for Polychaeta and Bivalvia, respectively) and upper intertidal (4856 ind.m⁻² and 1964 ind.m⁻², for Polychaeta and Bivalvia, respectively) clearly shows a decrease in prey densities from the upper intertidal to the subtidal area (Fig. 5).

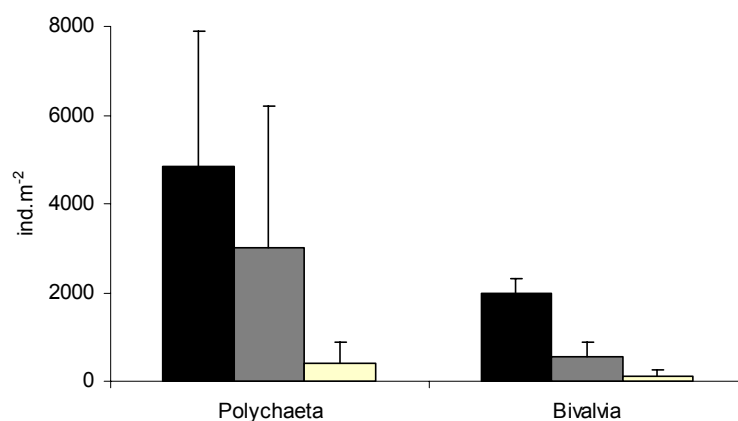


Figure 5 - *S. Senegalensis* prey in the sediment (upper intertidal in black; lower intertidal in grey; subtidal in white; mean density and standard deviation values).

Discussion

As the mudflats totally drain during the ebb, fish can only migrate when the rising tide floods the intertidal flats. This study concluded that *S. senegalensis* migrate with the rising tide towards both lower and upper mudflats. This behavior is most likely driven by the search for food in the rich flats as reported for other flatfish species such as the European flounder, *Platichthys flesus* (Linnaeus, 1758) (Wirjatmodjo and Pitcher, 1980; Ansell and Gibson, 1990) and the plaice *Pleuronectes platessa* (Linnaeus, 1758) (Kuipers, 1973; Ansell and Gibson, 1990; Burrows, 1994). Studies on the nursery function of intertidal mudflats for *Solea solea*

(Linnaeus, 1758), a very similar species, have shown that 0-group juveniles use the intertidal during the first months of settlement (Van der Veer *et al.*, 2001), after that period the population stops migrating to the intertidal (Wolff *et al.*, 1981). However, Van der Veer *et al.* (2001) reported that a small portion of the 0-group population may adopt a tidal migration strategy similar to that of plaice.

Avoidance of subtidal predators may also be an important function of intertidal incursions. Various authors have observed that predators of intertidal migrant species are larger, more numerous and more varied subtidally (e.g. Hunter and Naylor, 1993; Ellis and Gibson, 1995). In the present work, only *C. crangon* was larger and presented higher densities in the subtidal channel. *C. maenas* was larger in the subtidal area but its densities were not higher in the intertidal area. It should also be pointed out that the proportion of predators in relation to sole is very high in the study area.

S. senegalensis seems to stay mainly in the lower part of the mudflat. Many factors may be involved in this kind of distribution over the mudflats. The benefits of migrating further upshore may be counteracted by the energetic costs of swimming a longer distance, the danger of stranding by the ebbing tide and the increased risk of predation by terrestrial predators, such as fish eating birds. Fish eating birds are abundant in this area, which is one of the most important wetlands for birds in Europe (Moreira, 1997).

Separate analysis on the effect of environmental cycles fails to show the full picture of a species rhythmic migratory behavior; therefore it is crucial to analyse the abundance over the combination of all cycles, as performed in the present study. The distribution of *S. senegalensis* over all possible combinations of the environmental cycles shows three major abundance peaks, all taking place in the lower mudflat.

The diel activity pattern of this species was confirmed when combined with the semilunar cycle, with peak activity over the mudflats concentrated in the dawn/dusk and night periods. Preliminary laboratory studies on *S. senegalensis* behaviour have shown that this species is more active at dawn/dusk and night than during the day (personal observation), similarly to what Lagardère (1987) observed in *S. solea* in the field.

Yet, an important abundance peak was registered during the day, in the lower mudflat, on quarter moon. This peak could be related to rhythmic patterns of predator abundance. Many animals sacrifice foraging opportunities to avoid predation risk (Krebs and Kacelnik, 1991; Burrows *et al.*, 1994). *S. senegalensis* may avoid foraging in dusk/dawns and nights of higher predation pressure and, on the other hand, take advantage of periods of lower predation pressure to forage, regardless of its endogenous diel rhythm. Our data shows that during the quarter moon period *C. crangon* presented higher densities over the flats during the night and dusk/dawn, while its densities were much lower during the day. This could be the main reason why during quarter moon *S. senegalensis* prefers to forage during the day.

The biggest density peak of *S. senegalensis* during new-moon (the third peak overall) also matches periods of lower abundance of the two main predators. In this period, both predators' densities were higher during the night and day, than during dusk/dawn. *S.*

senegalensis again seems to cease the opportunity to forage when predation pressure is lowest. This opportunist behaviour is not always clear when looking at the other combinations of cycles, possibly since other factors such as prey abundance also play an important role in the cost-benefit relation underlying foraging behaviour. When looking at the full-moon period *S. senegalensis* prefers concentrating its foraging activity exactly when predation pressure is at its highest, during dusk/dawn. Yet it must be pointed out that full moon dusk/dawn and nights are probably when prey availability is at its highest too. As remarked by Ansell and Gibson (1990), prey availability is more important than prey absolute abundance since the later may not be an accurate reflection of prey encounter rate. While it is likely that prey availability is higher at higher tide levels, some polychaetes and amphipods are also known to synchronize their reproductive cycles with the semilunar cycle and many come out of the benthos during full moon (e.g. Lawrie and Raffaelli, 1998; Naylor, 1988) further exposing themselves to predation by *S. senegalensis*. The dusk/dawn, full moon peak was the major abundance peak registered for *S. senegalensis*.

A general semi-lunar pattern can be recognized for *S. senegalensis* in the lower mudflat. During spring tides abundance peaked at dusk/dawn, while in neap tides it peaked during the day. As previously discussed, *S. senegalensis* seems to alter its diel rhythm during quarter-moon, possibly to avoid high abundance of predators such as *C. crangon* during neap tide dusk/dawn.

Lunar and semi-lunar rhythms have been recognized in various species (Munro Fox, 1923; Palmer, 1995; Naylor, 2001; Bentley *et al.*, 2001; Hampel *et al.*, 2003). However, whether such rhythms are directly controlled by environmental variables or have an endogenous component is usually a matter of discussion (Morgan, 2001; Naylor, 2001). The effects of the Moon on earth are various, and while some are evident like moonlight and ocean tides, others like barometric pressure and electromagnetic radiation are more subtle (Morgan, 2001). The advantages of peak foraging coinciding with the most beneficial stage of the environmental cycle may have been favoured by natural selection leading to the development of endogenous “clocks” of semi-lunar periodicity. While tidal level is likely to influence the level to which the fish move up shore, the direct effect of moonlight may also play an important role in fish behaviour.

It was observed that while during full-moon *S. senegalensis* extended its distribution over the lower and upper mudflat, during new-moon colonisation by this species was restricted to the lower mudflat. This is probably related to the amount of light provided by the full-moon, which enables *S. senegalensis* to better escape its predators, as well as chase its own prey. In the absence of moonlight, predation risk increases and it is harder to catch prey. While it is true that in the absence of moonlight flatfish can not be seen by predators it will still be harder to escape especially in an area where predator density is so high. It is generally assumed that migration gives a selective advantage to the individual that migrates (Gibson, 2003). The benefit of venturing over the upper mudflat may be too small when compared to the risk of predation and increased energy cost of preying in total darkness. During the dusk/dawn period *S.*

senegalensis was absent from the upper mudflats, with the exception of the full-moon period. Again, this could be related to the light intensity, which is greater in dawn/dusk during full-moon.

It is interesting to note that an important decrease in the use of the upper mudflat in new-moon was observed during all periods of the day. This observation suggests that the absence of moonlight is not the only factor restraining this species activity over the mudflats during new-moon.

This study provides the first insight into the effect of the day-night and semi-lunar cycles in the activity of *S. senegalensis*. The highest densities of this species over the mudflat take place at full-moon during the dusk/dawn period. A semi-lunar activity pattern was detected. At spring tides abundance peaked at dusk/dawn, while at neap tides abundance peaked during the day. Activity patterns of this species seem to have a close relation with the activity patterns of its prey and predators.

Future studies on *S. senegalensis* rhythmic behavior are needed in order to fully understand the factors determining such patterns. Further application of cost-benefit analysis to migrating fish species, the development of energetic models and the investigation of “endogenous clocks” will certainly provide new insights into the functions of intertidal migrations.

Acknowledgements

Authors would like to thank everyone involved in the field work. This study had the support of Fundação para a Ciência e a Tecnologia (FCT) which financed several of the research projects related to this work.

References

- Able, K.W., Matheson, K.W., Morse, W.W., Fahay, M.P. and Shepherd, G., 1990. Patterns of summer flounder *Paralichthys dentatus* early life history in the mid-Atlantic Bight and New Jersey estuaries. U.S. Fisheries Bulletin 88, 1-12.
- Ansell, A.D.; Gibson, R.N., 1990. Patterns of feeding and movement of juvenile flatfishes on an open sandy beach. In: Barnes, M., Gibson, R.N. (Eds.) Trophic Relationships in the Marine Environment. Proceedings of the 24th European Marine Biology Symposium. Aberdeen University Press, Aberdeen, United Kingdom, pp 191-207.
- Bentley, M.G., Olive, P.J.W. and Last, K., 2001. Sexual satellites, Moonlight and the Nuptial dances of Worms: The Influence of the Moon on the Reproduction of Marine Animals. Earth, Moon and Planets 85-86, 67-84.
- Burrows, M.T., 1994. An optimal foraging and migration model for juvenile plaice. Evolutionary Ecology 8, 125-149.
- Burrows, M.T., Gibson, R.N. and MacLean, A., 1994. Effects of endogenous rhythms and light conditions on foraging and predator-avoidance in juvenile plaice. Journal of Fish Biology 45, 171-180.

- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the U.K.* 83, 861-868.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect environmental changes? *Climate Research* 18, 119-126.
- Costa, M.J., Elliott, M., 1991. Fish usage and feeding in two industrialised estuaries: The Tagus, Portugal and the Forth, Scotland. In: Elliot, M., Ducrotoy J.-P. (Eds), *Estuaries and Coasts: Spatial and Temporal Comparisons*. Olsen & Olsen, Frenenburg, pp. 289-297.
- Dinis, M.T., Ribeiro, L., Soares, F. and Sarasquete, C., 1999. A review of the cultivation potential of *Solea senegalensis* in Spain and Portugal. *Aquaculture* 176, 27-38.
- Elliott, M., Taylor, C.J.L., 1989. The production ecology of the subtidal benthos of the Forth estuary, Scotland. *Scientia Marina* 53, 531-541.
- Elliott, M., Dewailly, F., 1995. The structure and components of European estuarine fish assemblages. *Netherlands Journal of Aquatic Ecology* 29, 397-417.
- Ellis, T.R., Gibson, R.N., 1995. Size selective predation of 0-group flatfishes on a Scottish coastal nursery ground. *Marine Ecology Progress Series* 127, 27-37.
- Gibson, R.N., 1973. The intertidal movements and distribution of young fish on a sandy beach with special reference to the plaice (*Pleuronectes platessa* L.). *Journal of Experimental Marine Biology and Ecology* 12, 79-102.
- Gibson, R.N., 1980. A quantitative description of the behaviour of the wild juvenile plaice *Pleuronectes platessa*. *Animal Behaviour* 28, 1202-1216.
- Gibson, R.N., 1982. The effect of hydrostatic pressure cycles on the activity of young plaice *Pleuronectes platessa*. *Journal of the Marine Biological Association of the U.K.* 62, 621-635.
- Gibson, R.N., 1999. Movement and homing in intertidal fishes. In: Horn, M.H., Martin K.L.M., Chotkowski M.A. (Eds.), *Intertidal fishes. Life in two worlds* Academic Press. London, pp. 97-125.
- Gibson, R.N., 2003. Go with the flow. Tidal migration in marine animals. *Hydrobiologia* 503, 153-161.
- Gibson, R.N., Pihl, L., Burrows, M.T., Modin, J., Wennhage, H., Nickell, L.A., 1998. Movement and homing of juvenile plaice (*Pleuronectes platessa*) in relation to predators, competitors, food availability and abiotic factors on a microtidal nursery ground. *Marine Ecology Progress Series* 165: 145-159.

- Haedrich, R.L., 1983. Estuarine Fishes. In: Ketchum B. (Ed.), *Ecosystems of the World*. 26. Estuaries and Enclosed Seas. Elsevier, Amsterdam, pp. 183-207.
- Hampel, H., Cattrijsse, A., Vincx, M., 2003. Tidal, diel and semi-lunar changes in the faunal assemblage of an intertidal salt marsh creek. *Estuarine, Coastal and Shelf Science* 56, 795-805.
- Hunter, E., Naylor, E., 1993. Intertidal migration by shore crab *Carcinus maenas*. *Marine Ecology Progress Series* 101, 131-138.
- Krebs, J.R., Kacelnik, A., 1991. Decision making. In: Krebs J.R., Davies N.B. (Eds) *Behavioural Ecology*. Blackwell Scientific, Oxford, pp. 105-136.
- Krumme, U., Saint-Paul, U., Rosenthal, H., 2004. Tidal and diel changes in the structure of a nekton assemblage in small intertidal mangrove creeks in Northern Brazil. *Aquatic Living Resources* 17, 215-229.
- Kuipers, B., 1973. On the tidal migration of young plaice (*Pleuronectes platessa*) in the Wadden Sea. *Netherlands Journal of Sea Research* 6, 376-388.
- Lagardère, J.P., 1987. Feeding ecology and daily food consumption of common sole *Solea vulgaris* Quensel, juveniles on the French Atlantic coast. *Journal of Fish Biology* 30, 91-104.
- Lawrie, S. M., Raffaelli, D. G., 1998. In situ swimming behaviour of the amphipod *Corophium volutator* (Pallas). *Journal of Experimental Marine Biology and Ecology* 224, 237-251.
- Modin, J., Pihl, L., 1996. Small-scale distribution of juvenile plaice and flounder in relation to predatory shrimp in a shallow Swedish bay. *Journal of Fish Biology* 49, 1070-1085.
- Moreira, F., 1997. The importance of shorebirds to energy fluxes in a food web of a southern European estuary. *Estuarine, Coastal and Shelf Science* 44, 67-78.
- Morgan, E., 2001. The Moon and life on Earth. *Earth, Moon and Planets* 85-86, 279-290.
- Morrison, M.A., Francis, M.P., Hartill, B.W., Parkinson, D.M., 2002. Diurnal and Tidal variation in the abundance of the fish fauna of a temperate tidal mudflat. *Estuarine, Coastal and Shelf Science* 54, 793-807.
- Munro Fox, H., 1923. Lunar Periodicity in Reproduction. *Proceedings of the Royal Society of Biology* 155, 523-549.
- Naylor, E., 1982. Tidal and Lunar Rhythms in Animals and Plants. In: Brady J. (Ed), *Biological timekeeping*, Society of Experimental Biology Seminar Series 14. Cambridge University Press, Cambridge, pp. 33-48.
- Naylor, E., 2001. Marine animal behaviour in relation to lunar phase. *Earth, Moon and Planets* 85-86, 291-302.
- Naylor, E., Williams, B.G., 1984. Environmental Determination of Tidally Rhythmic Behaviour in Marine Animals. *Zoology Journal of the Linnean Society* 80, 201-208.
- Neumann, D., 1981. Tidal and lunar rhythms. In: Aschoof, J. (Ed.) *Biological Rhythms*. Vol 4. *Handbook of Behavioural Neurobiology*. Plenum, London, pp. 351-390.
- Palmer, J.D., 1995. *The Biological Rhythms and Clocks of Intertidal Animals*. Oxford University Press. New York and Oxford.

- Pihl, L., Van der Veer, H.W., 1992. Importance of exposure and habitat structure for the population density of 0-group plaice, *Pleuronectes platessa* L., in coastal nursery areas. *Netherlands Journal of Sea Research* 29, 145-152.
- Quéro, J.-C., Dessouter, M., Lagardère, F., 1986. Soleidae. In: Whitehead, P.J.P., Bauchot, M.-L., Hureau, J.-C., Nielsen J., Tortonese E. (Eds). *Fishes of the North-eastern Atlantic and the Mediterranean*. UNESCO, Bungay, pp. 1308-1324.
- Quinn, N.J., Kojis, B.L., 1981. The lack of changes in nocturnal estuarine fish assemblages between new and full moon phases in Serpentine Creek, Queensland. *Environmental Biology of Fishes* 6, 213-218.
- Raffaelli, D., Richner, H., Summers, R., Northott, S., 1990. Tidal migrations in the flounder (*Platichthys flesus*). *Marine Behaviour Physiology* 16, 249-260.
- Saigusa, M., Kawagoye, O., 1997. Circatidal rhythm of an intertidal crab *Hemigrapsus sanguineus*: synchrony with unequal tidal height and involvement of a light-response mechanism. *Marine Biology* 129, 87-96.
- Summers, R. W., 1980. The diet and feeding behaviour of the flounder *Platichthys flesus* (L.) in the Ythan estuary, Aberdeenshire, Scotland. *Estuarine, Coastal and Shelf Science* 11, 217-232.
- van der Veer, H.W., Bergman, M.J.N., 1987. Development of tidally related behaviour of a newly settled 0-group plaice (*Pleuronectes platessa*) population in the western Wadden Sea. *Marine Ecology Progress Series* 31, 121-129.
- van der Veer, H.W., Dapper R., Witte J.I.J., 2001. The nursery function of the intertidal areas in the western Wadden Sea for 0-group sole *Solea solea* (L.). *Journal of Sea Research* 45, 271-279.
- Wirjatmodjo, S., Pitcher, T.J., 1980. Flounders follow the tracks to feed: Evidence from ultrasonic tracking in an estuary. *Estuarine, Coastal and Shelf Science* 19, 231-241.
- Wolff, W.J., Manders, M.A., Sandee, A.J.J., 1981. Tidal migration of plaice and flounders as a feeding strategy. In: Jones, N.V., Wolff, W.J. (Eds), *Feeding and Survival Strategies of Estuarine Organisms*. Plenum Press, London, pp. 159-171.

Conclusions

The present analysis of habitat use by *S. solea* and *S. senegalensis* at different spatial scales revealed highly complex processes and patterns.

The Habitat Suitability models developed were successful in their intended goal of mapping habitat quality for *S. solea* and *S. senegalensis* in a simple, yet effective way. The importance of salinity, temperature, substrate, depth and presence of intertidal mudflats in the distribution of both species was confirmed, nonetheless the inclusion of prey abundance data proved crucial in the definition of high suitability areas and in the prediction of high densities of juveniles.

The stable isotope approach revealed that 0-group *S. senegalensis* present high site fidelity and do not move between nurseries, while a considerable amount 1-group individuals explore the two feeding grounds. It was also concluded that the food-webs from each of the nursery areas have low connectivity and show different levels of dependence upon freshwater and marine energy pathways, with nursery A more dependent on the freshwater energy pathway and nursery B having a greater contribution from the marine energy pathway.

The first insight into the effect of the day-night and semi-lunar cycles in the activity of *S. senegalensis* was presented in this work. It was concluded that the highest densities of this species over the mudflats take place at full-moon during the dusk/dawn period. A semi-lunar activity pattern was also detected. While at spring tides abundance peaks at dusk/dawn, at neap tides abundance peaks during the day. The analysis of the effect of day-night and semi-lunar cycles upon its predators along with literature information on that effect upon its prey strongly suggests that *S. senegalensis* activity pattern is closely related to that of its predators and prey.

Future management of these nursery areas should take into account their importance as two independent nurseries for soles. Although the majority of the Tagus estuary upper areas have an overall high habitat quality for soles; both species' juveniles concentrate in rather small areas, probably due to foraging opportunities, with important implications for fisheries management. These areas should be regarded as crucial for these species lifecycle and protected from disturbing activities, especially during the months when they are used as nursery areas by soles. The low connectivity and different levels of dependence upon freshwater and marine energy pathways of the two nurseries indicate that they should be managed as independent habitats, one which provides habitat for the juveniles of two sole species and another that provides habitat for only one of the species juveniles, yet does so independently of the cohorts that colonize the other nursery area. Nursery B does not represent a complement or an alternative area for soles from nursery A, and vice-versa. Dependency of these nurseries upon freshwater and marine water inflow as energy pathways should also be carefully looked at. Provisions on rainfall alteration due to climate change indicate that freshwater input into the

estuarine food-webs will be scarcer and more concentrated in time. Special attention should also be placed on the upper estuary mudflats. These areas are vital to the ecology of soles' nursery grounds, yet they are subjected to increasing human pressure from rapid urbanization of its surrounding areas, as well as, to the threat of sea level rise due to climate change. Future studies on soles rhythmic behavior are needed in order to fully understand the patterns of intertidal use reported here. Application of cost-benefit analysis to fish migrations, development of energetic models and investigation of "endogenous clocks" will certainly provide new insights into the driving forces behind intertidal migrations.

CHAPTER 3

- FOOD CONSUMPTION -

Effect of temperature and salinity on the gastric evacuation of the juvenile soles *Solea solea* and *Solea senegalensis*. *Journal of Applied Ichthyology*. 2007, 23, 240-245.

By Vinagre, C., Maia, A., Cabral, H.N.

Foraging behaviour of *Solea senegalensis* Kaup, 1858, in the presence of a potential predator, *Carcinus maenas* Linnaeus, 1758. (*submitted*).

By Maia, A., Vinagre, C., Cabral, H.N.

Prey consumption by the juveniles of *Solea solea* (Linnaeus, 1758) and *S. senegalensis* Kaup 1858, in the Tagus estuary, Portugal (*submitted*).

By Vinagre, C., Cabral, H.N.

Introduction

Fish must have an energy source to meet metabolic demands. Essential amino-acids, fatty acids, minerals and vitamins are also essential to maintain health and promote growth (Moyle and Cech, 1996). This assumes particular importance during the juvenile period, when fast growth implies increased energy demands (Moyle and Cech, 1996). Juvenile fish concentrate in areas where prey availability is high. In fact, various studies have shown that juvenile flatfish distribution within nursery areas is predominantly determined by food availability, rather than by other environmental variables (Gibson, 1994, 1997; Matilla and Bonsdorff, 1998; Cabral and Costa, 1999; Amezcua and Nash, 2001; Vinagre *et al.*, 2005, 2006).

The high benthic productivity of estuaries is one of the main reasons why several flatfish species spend their juvenile phase in estuarine nursery grounds (*e.g.* McLusky, 1989). Flatfishes are a major energy pathway for the conversion of benthic production into a form suitable for consumption by higher predators, such as humans (Link *et al.*, 2005). The need to monitor and manage commercial fish stocks, as well as, sensitive areas, such as estuaries, has driven the investigation of food consumption in wild fish. Various models have been developed combining information on gastric evacuation rates, determined experimentally, with that of stomach contents of wild fish (*e.g.* Thorpe, 1977; Elliott and Persson, 1978; Eggers, 1979; Jobling, 1981; Bromley, 1987). Food consumption models generally assume that the rate at which food is evacuated from the stomach is equal to the rate at which food is ingested (Bromley, 1994).

Soon, investigators realized that gastric evacuation rates were affected by several factors, temperature was among the most relevant ones. It was concluded that there was generally an exponential relationship between temperature and gastric evacuation rate (*e.g.* Elliot and Person, 1978; Elliott, 1992; Bromley, 1994).

Salinity should also be an important factor affecting gastric evacuation rates, albeit scarcely studied. Drinking rate (Smith, 1930; Evans, 1993), food intake (*e.g.* Buckley *et al.*, 1995; Peterson-Curtis, 1997; Imsland *et al.*, 2001), food conversion efficiency (Lambert *et al.*, 1994; Likongwe *et al.*, 1996; Alava *et al.*, 1998; Imsland *et al.*, 2001), hormone balance (McCormick, 1996; Bjornsson *et al.*, 1998) and metabolic rate of fish (Woo and Kelly, 1995; Swanson, 1996; Dutil *et al.*, 1997), are affected by salinity, all of which will likely affect gastric evacuation.

Another factor that affects the feeding behaviour of fish is predation pressure (Jones and Paszkowski, 1997; Turner *et al.*, 1999). In the presence of a potential predator many flatfish decrease their activity in order to avoid detection, thus decreasing their time allocated to the search for prey (Burrows *et al.*, 1994; Burrows and Gibson, 1995).

Previous studies on the feeding ecology of juvenile sole in the Tagus estuary focused on diet composition (Costa, 1982; 1988; Gonçalves, 1990; Cabral, 2000).

The present chapter investigated the effect of temperature and salinity in the gastric evacuation rates of *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, and the impact of predation pressure in foraging behaviour, such information was used to produce a first estimation of food consumption by the two sole species in the Tagus estuary nursery grounds during the period of most intense use by juveniles.

The first work, "Effect of temperature and salinity on the gastric evacuation of the juvenile soles *Solea solea* and *Solea senegalensis*", aims to investigate the effects of temperature and salinity in the gastric evacuation of *S. solea* and *S. senegalensis* juveniles fed discrete meals of *Nereis diversicolor* (Müller, 1776), to relate it to the estuarine environment where they spend their early life and to compare estuarine and coastal nurseries' habitat use constrains for juvenile soles. The estimation of gastric evacuation rates through experimental work in captivity has been carried out for many commercial fish species, but not for *S. solea* or *S. senegalensis*. The gastric evacuation values incorporated into food consumption models that encompass species where this information is not available will generally use values estimated for other species, leading to rough estimations that potentially yield large errors. It is thus very important to add gastric evacuation rates for these species into the available scientific literature. It is also important to understand how differences in gastric evacuation rates may influence species distribution and habitat use, since this will possibly be an explaining factor driving habitat preferences.

The second work, "Foraging behaviour of *Solea senegalensis* in the presence of a potential predator, *C. maenas*", aims to investigate the interaction of the juvenile *S. senegalensis*, with its natural predator *C. maenas* and assess its impact on sole's foraging behaviour. The incorporation of species interactions into food consumption models is very important, and although some work has been carried out into predator-prey relations, impacts on feeding behaviour have been scarcely studied in flatfish.

The third work, "Prey consumption by the juvenile soles, *Solea solea* (L., 1758) and *Solea senegalensis* Kaup, 1858, in the Tagus estuary, Portugal", aims to estimate food consumption of *S. solea* and *S. senegalensis* juveniles in the two nursery areas of the Tagus estuary, taking into account water temperature and diel patterns of feeding activity, and determine the total food consumed by soles over the summer versus estimated total prey present in the sediment. Data on gastric evacuation rates produced by the first study of this chapter, along with 24h sampling cycles in the wild were incorporated into the Elliott and Persson (1978) model, one of the most widely used of all the food consumption models. This is the first time a model incorporating both experimentally determined gastric evacuation rates and field data is applied to the estimation of food consumption by both these species.

The investigation of gastric evacuation rates, food consumption and its relations with environmental variables is crucial for the inclusion of these species into broader multi-species food-web models that will ultimately need to be constructed for an in-depth understanding of trophic relations in estuarine systems.

References

- Alava, V.R., 1998. Effect of salinity, dietary lipid source and level on growth of milkfish (*Chanos chanos*) fry. *Aquaculture* 167,229-236.
- Amezcuca, F., Nash, R.D.M., 2001. Distribution of the order Pleuronectiformes in relation to sediment type in the North Irish Sea. *Journal of Sea Research* 45, 293–301.
- Bjornsson, B.T., Stefansson, G.V., Berge, A.I., Hansen, T., Stefansson, S.O., 1998. Circulating growth hormone levels in Atlantic salmon smolts following saewater transfer: effects of photoperiod regime, salinity, duration of exposure and season. *Aquaculture* 168, 121-137.
- Bromley, P.J., 1987. The effects of food type, meal size and body weight on digestion and gastric evacuation in turbot *Scophthalmus maximus* L. *Journal of Fish Biology* 30, 501-512.
- Bromley, P.J., 1994. The role of gastric evacuation experiments in quantifying the feeding rates of predatory fish. *Reviews of Fish Biology and Fisheries* 4, 36-66.
- Buckley, J. A.; Steinberg, N. D.; Conover, D. O., 1995. Effects of temperature, salinity and fish size on growth and consumption of juvenile bluefish. *Journal of Fish Biology* 47, 696–706.
- Burrows, M.T., Gibson, R.N., 1995. The effects of food, predation risk and endogenous rhythmicity on the behaviour of juvenile plaice, *Pleuronectes platessa* L.. *Animal Behaviour* 50(1), 41-52.
- Burrows, M.T., Gibson, R.N., MacLean, A., 1994. Effect of endogenous rhythms and light conditions on foraging and predator avoidance in juvenile plaice. *Journal of Fish Biology* 45, 171-180.
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Costa, M.J., 1982. Contribution à l'étude de l'écologie des poissons de l'estuaire Tage (Portugal). PhD Dissertation, Université Paris VII, Paris.
- Costa, M.J., 1988. Écologie alimentaire des poissons de l'estuaire du Tage. *Cybiurn* 12, 301-320.
- Dutil, J. D., Lambert, Y., Boucher, E., 1997. Does higher growth rate in Atlantic cod (*Gadus morhua*) at low salinity result from lower standard metabolic rate or increased protein digestibility? *Canadian Journal of Fisheries and Aquatic Science* 54, 99-103.
- Eggers, D.M., 1979. Comment on some recent methods for estimating food consumption by fish. *Journal of the Fisheries Research Board of Canada* 36, 1018-1020.
- Elliott, J. M., 1992. Rates of gastric evacuation in piscivorous brown trout, *Salmo trutta* L. *Freshwater Biology* 25, 297-305.
- Elliott, J.M., Persson, L., 1978. The estimation of daily rates of food consumption for fish. *Journal of Animal Ecology* 47, 977-993.

- Evans, D.H., 1993. Osmotic and ionic regulation. In: *The Physiology of Fishes*. Eds: D.H. Evans, New York, USA. pp. 315-341.
- Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. *Netherlands Journal of Sea Research* 32, 191–206.
- Gibson, R.N., 1997. Behaviour and the distribution of flatfishes. *Journal of Sea Research* 37, 241–256.
- Gonçalves, 1990. Fluxos de energia na cadeia trófica do estuário do Tejo: estudo da ecofase estuarina do elo *Solea solea* (Linnaeus, 1758) (Pisces: Soleidae). Degree thesis, Faculdade de Ciências da Universidade de Lisboa, Lisboa.
- Imsland, A. K., Foss, A., Gunnarson, S., Berntssen, M. H. G., Gerald, R. F., Bonga, S. W., 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 198, 353-367.
- Jobling, M., 1981. Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. *Journal of Fish Biology* 19, 245-257.
- Jones, H. M., Paszkowski, C. A., 1997. Effects of exposure to predatory cues on territorial behaviour of male fathead minnows. *Environmental Biology of Fishes* 49, 97–109.
- Lambert, Y., Dutil, J. -D., Munro, J., 1994. Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries Science* 51, 1569-1576.
- Likongwe, J. S., Stecko, T. D., Stauffer, J. R., Carline, R. F., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus). *Aquaculture* 146, 37-46.
- Link, J.S., Fogarty, M.J., Langton, R.W., 2005. The trophic ecology of flatfishes In: *Flatfishes, biology and exploitation*. Gibson, R.N. (Eds.) Blackwell Publishing Ltd, 185-204.
- Matilla, J., Bonsdorff, E., 1998. Predation by juvenile flounder (*Platichthys flesus* L.): a test of prey vulnerability, predator preference, switching behaviour and functional response. *Journal of Experimental Marine Biology and Ecology* 227, 221–236.
- McCormick, S. D., 1996. Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na^+ , K^+ -ATPase in Atlantic salmon (*Salmo salar*): interaction with cortisol. *Genetics and Comparative Endocrinology* 101, 3-11.
- McLusky, D.S., 1989. *The estuarine ecosystem*. 2nd edition. Blakie, London, UK.
- Moyle, P.B., Cech, J.J.J., 1996. *Fishes an introduction to Ichthyology*, 3rd edn. Prentice Hall, N.J.
- Peterson-Curtis, T.L., 1997. Effects of salinity on survival, growth, metabolism and behavior in juvenile hogchockers, *Trinectes maculatus* (Achiridae). *Environmental Biology of Fishes* 49,323-331.
- Smith, H. W., 1930. The absorption and excretion of water and salts by marine teleosts. *American Journal of Physiology* 93, 480.
- Swanson, C., 1996. Early development of milkfish: effects of salinity on embryonic and larval metabolism, yolk absorption and growth. *Journal of Fish Biology* 48, 405-21.

- Thorpe, J.E., 1977. Daily ration of adult perch, *Perca fluviatilis* L. during summer in Loch Leven, Scotland. *Journal of Fish Biology* 11, 55-68.
- Turner, A.M., Fetterolf, S.A., Bernot, R.J., 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* 118, 242-247.
- Vinagre, C., França, S., Costa, M.J., Cabral H.N., 2005. Niche overlap between the juvenile flatfishes, *Platichthys flesus* and *Solea solea*, in a southern European estuary and adjoining coastal waters. *Journal of Applied Ichthyology* 21, 114-120.
- Vinagre, C., Fonseca, V., Cabral, H., Costa, M.J. 2006. Habitat suitability index models for the juvenile soles, *Solea solea* and *Solea senegalensis*: defining variables for management. *Fisheries Research* 82, 140-149.
- Woo, N. Y. S., Kelly, S. P., 1995. Effects of salinity and nutritional status on growth and metabolism of *Sparus sarba* in a closed seawater system. *Aquaculture* 135, 229-238.

Effect of temperature and salinity on the gastric evacuation of the juvenile soles *Solea solea* and *Solea senegalensis*

Abstract: Gastric evacuation experiments were performed on juveniles of Senegal sole, *Solea senegalensis*, Kaup1858, and Common sole, *Solea solea* (Linnaeus 1758). Three temperatures were tested, 26°C, 20°C and 14°C at a salinity of 35 ‰. A low salinity experiment was also carried out at 15 ‰, at 26°C. Experimental conditions intended to reflect conditions in estuarine and coastal nurseries where juveniles of these species spend their first years of life. The relation between stomach contents and time was best described by exponential regression models for both species. An analysis of covariance (ANCOVA) was performed in order to test differences in evacuation rate due to temperature and salinity (slope of evacuation time against stomach contents) for each species. While temperature increased evacuation rates in both species (although not at 26°C in *S. solea*), the effect of low salinity differed among species, leading to a decrease in gastric evacuation rate in that of *S. senegalensis* and an increase in *S. solea*. Differences in gastric evacuation rate between species were related to its metabolic optimums and to its distribution in the nursery area where fish were captured. Implications for the use of estuarine and coastal nurseries are discussed.

Key-words: Flatfish; *Solea solea*; *Solea senegalensis*; Nursery areas; Gastric evacuation; Temperature; Salinity.

Introduction

The evaluation of feeding interactions between species and quantification of predation requires food consumption estimates. A common approach to estimating food consumption in the wild is the combination of field data on stomach contents and information on gastric evacuation rates (e.g. Bajkov, 1935; Bromley, 1994). Food consumption models generally assume that over long time periods the rate at which food is evacuated from the stomach is equal to the rate at which food is ingested.

The determination of gastric evacuation rates in commercial fish species is also important for aquaculture purposes. Much of the fish cultured in Portugal and Spain comes from semi-extensive multi-species fish-farms, where estimation of food consumption by each species is crucial for management purposes.

This study focuses on 0-group juveniles of Senegal Sole *Solea senegalensis*, Kaup1858, and Common sole, *Solea solea*, (Linnaeus 1758). These are benthic flatfishes with sympatric distribution from the Bay of Biscay to Senegal and western Mediterranean (Quero *et al.* 1986). They are very similar morphologically as well as in ecological needs. Both species are important predators and therefore can be of great importance to the dynamics and composition of the biological communities in the estuarine and coastal systems where they occur. Both soles have high commercial value and *S. senegalensis* is a species of increasing interest in aquaculture and is commonly cultured in the Portuguese and Spanish southern coasts (Dinis *et al.* 1999; Imsland *et al.*, 2003).

The first year of life is a key stage in fish development, particularly for species, like the soles, that concentrate in large densities in estuarine and coastal nurseries where space and food partitioning become an issue (e.g. Schoener, 1974; Ross, 1986). In these nurseries, fish juveniles benefit from the reduced number of predators and conditions favourable to rapid growth, such as high temperatures and prey abundance. However, they must withstand temperature and salinity amplitudes much wider than those found in the sea (Haedrich, 1983). Therefore, the estimation of food consumption in estuarine and coastal waters must take into account the influence of temperature and salinity in the gastric evacuation of fish.

Temperature is probably the most studied variable influencing digestion and gastric evacuation (Bromley, 1994). Although there are some exceptions, most studies found an exponential relationship between temperature and gastric evacuation rate (e.g. Elliot and Person, 1978; Elliott, 1992; Bromley, 1994).

Salinity is not usually addressed in gastric evacuation studies, yet there is evidence that this factor should be taken into account when dealing with euryhaline fish. It is well known that teleost fish hypo-osmoregulate in marine environments, and are therefore faced with osmotic water loss and passive gain of many ions (Smith, 1930; Evans, 1993). To avoid dehydration they constantly drink ambient water, absorbing the majority of the imbibed volume within the intestine. Euryhaline fish when faced with low salinities will lower their drinking rate and eventually stop drinking (Smith, 1930; Evans, 1993). Drinking rate has an important influence on gastric evacuation, because every time fish drink part of the stomach content will be flushed to the intestine. Osmoregulation is also known to affect food intake (e.g. Buckley *et al.*, 1995; Peterson-Curtis, 1997; Imsland *et al.*, 2001), food conversion efficiency (Lambert *et al.*, 1994; Likongwe *et al.*, 1996; Alava *et al.*, 1998; Imsland *et al.*, 2001), hormone balance (McCormick, 1996; Bjornsson *et al.*, 1998) and metabolic rate (Woo and Kelly, 1995; Dutil *et al.*, 1997; Swanson, 1996).

The present study aims to 1) investigate the effects of temperature and salinity in the gastric evacuation of *S. solea* and *S. senegalensis* juveniles fed discrete meals of *Nereis diversicolor* (Müller, 1776), to 2) relate it to the estuarine environment where they spend their early life and to 3) compare estuarine and coastal nurseries' habitat use constrains for juvenile soles.

Materials and methods

Study area

The Tagus estuary (Fig.1), where the fish used in the experiments were captured, is one of the largest estuaries in Western Europe (320 km²). It is a partially mixed estuary with a tidal range of ca. 4 m. Approximately 40% of the estuarine area is intertidal. Two important sole nurseries were identified in the Tagus estuary in previous studies (A, Vila Franca de Xira, and B, Alcochete; Fig. 1) by Costa and Bruxelles (1989) and Cabral and Costa (1999). The uppermost area, A, is deeper (mean depth 4.4 m), presents lower and highly variable salinity

and has a higher proportion of fine sand in the substract (approximately 40%). Nursery B is shallower (mean depth 1.9 m), and more saline, with lower variability in salinity, while substrate is mainly composed of mud (mean value 60.4%) (Cabral, 1998; Cabral and Costa, 1999). In the uppermost nursery (A) both species are present yet *S. solea*'s highest densities occur at the lowest salinity area, closest to the freshwater input, while *S. senegalensis* presents high densities over a wider salinity range within the nursery (Cabral and Costa, 1999). At nursery B only *S. senegalensis* is present (Cabral and Costa, 1999).

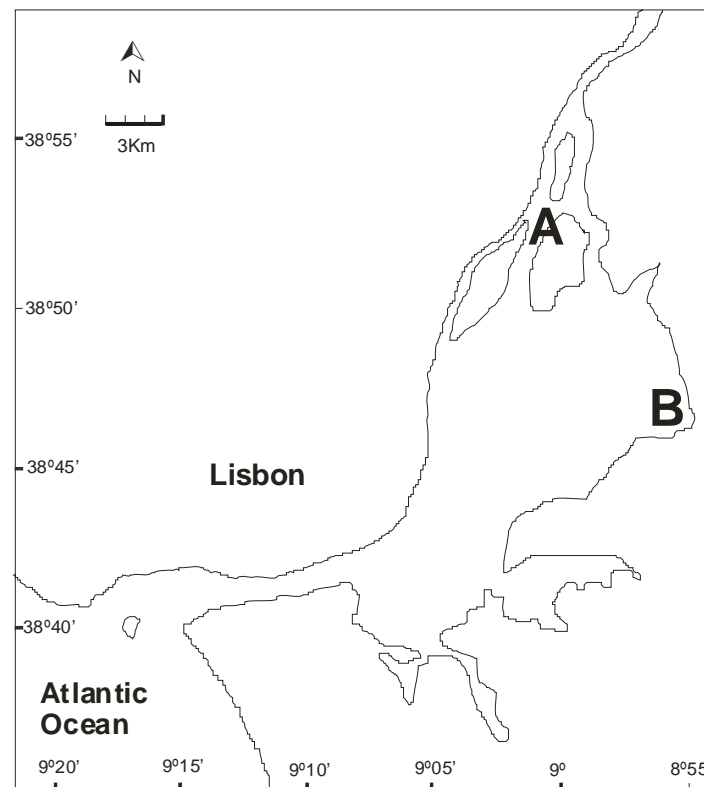


Figure 1 - Location of the nursery areas where juvenile sole were captured.

Gastric evacuation experiments

S. senegalensis and *S. solea* were captured in the Tagus estuary and selected according to their size. Prior to experiments fish were held in circular tanks with capacities of 350 l for a minimum of 4 weeks. Fish were then transferred to 160 l aquariums equipped with mechanical and biological filter units. Temperature was regulated with a precision of ± 0.1 °C. Salinity was regulated with a precision of 0.1 ‰. Temperature and salinity were monitored daily. Fish were exposed to a day length of 12 hours.

For the gastric evacuation experiments fish were transferred into compartments to allow controlled feeding of each individual. Compartments measured 25 × 30 × 40 cm (length, width, depth). Experimental fish weighted between 3.00 g and 5.00 g and measured between 70 and 85 mm (total length). Fish were kept for 3 weeks at these compartments prior to the gastric

evacuation experiments. During this period they were fed with the same prey given during the experiment.

A preliminary experiment was carried out in order to determine period of complete stomach and digestive tube emptying and assess the appropriate interval between observations. In the preliminary experiment observations were made 30 min after feeding and every hour for 48 hours.

Prior to the evacuation experiments fish were not fed for 24 hours to allow a complete emptying of the stomachs. The experimental meal was offered to the fish for 15 min, as in the 3 week feeding period. After feeding, fish were anaesthetized in 1:3000 solution of tricaine methanesulphonate (MS 222 Sandoz) and sacrificed with a cut on the anterior spine. Observations were made at 0h, 2h, 3h, 4h, 8h, 14h, 16h and 20h after feeding. Fish were measured (total length with 1 mm precision) and weighted (wet weight with 0.01 g precision). Stomach contents were weighted (wet weight with a 0.001 precision).

Ragworm, *N. diversicolor*, a natural prey of *S. senegalensis* and *S. solea* (Cabral, 2000a), was reared in aquariums at the laboratory. Immediately before the experiment, worms were weighted (wet weight with a 0.001g precision) and the ones that weighted 0.300 g were selected, according to experimental needs. Prey weight was determined from previous experience of stomach contents analysis of specimens captured in the Tagus estuary.

This experiment intended to mimic the temperatures that these species finds in nursery areas where both soles are sympatric, during its first year of life. Three temperatures were tested; 26°C a water temperature commonly found in estuarine nursery areas during Summer; 20°C, common in Spring and Autumn; and 14°C, common during winter (Cabral, 2000a). Since the period of most intense growth of these species occurs during Summer (Cabral, 2003), 26°C was the temperature chosen for the salinity experiment. The salinity experiment intended to compare gastric evacuation in coastal and estuarine nurseries and was therefore conducted at 35 ‰, typical salinity in coastal waters and 15 ‰ a common salinity in the Tagus estuary nursery areas, as well as other estuarine nurseries where these species occur.

Data analysis

Mean and standard deviation values were calculated for each set of replicates in order to determine time of total stomach emptying. A regression procedure was conducted in each of the datasets and it was concluded that the relation between stomach content and time was exponential. Since the exact time in which stomachs became empty cannot be determined, experiments in which empty stomach occurred were excluded from the regression analysis to avoid bias (Bromley, 1994; Temming and Andersen, 1994). Stomachs were considered empty when their contents weight was less than 1% of the initial meal weight.

An analysis of covariance (ANCOVA) was conducted in the software STATISTICA to test differences in evacuation rate due to temperature and salinity (slope of evacuation time against stomach contents) for each species. In order to fulfil the requirements of this analysis the data on stomach contents was log transformed. A significance level of 0.05 was considered.

Results

Total stomach emptying time decreased with temperature in *S. senegalensis*, as a result of the observed increase in gastric evacuation rate with temperature ($P < 0.05$). The lower evacuation rate observed at a lower salinity (15 ‰) ($P < 0.05$), when compared to seawater salinities (35 ‰), resulted in an increase of total stomach emptying time.

The period for total stomach emptying for *S. senegalensis* was 14 hours for fish held at 26°C and 16 hours for fish held at 20°C and 14°C (Fig. 2). The period for total stomach emptying for individuals held at 15 ‰ (at 26°C) was 16 hours (Fig. 3). The relation between stomach contents and time was best described by an exponential regression.

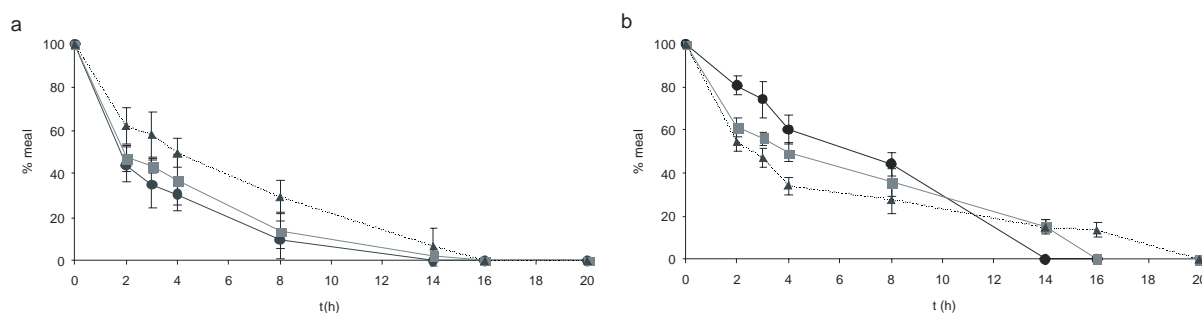


Figure 2 – Percentage of meal (mean and standard deviation values) in the stomachs of *S. senegalensis* (a) and *S. solea* (b) according to experiment time at 26°C (●), 20°C (■) and 14°C (▲).

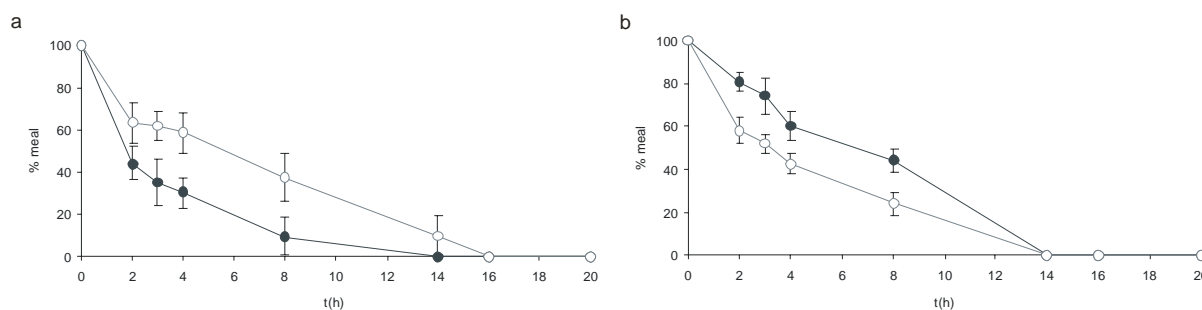


Figure 3 – Percentage of meal (mean and standard deviation values) in the stomachs of *S. senegalensis* (a) and *S. solea* (b) according to experiment time at a salinity of 35 ‰ (●) and 15 ‰ (○).

Gastric evacuation rate at 26°C was 0.325 gh^{-1} , at 20°C was 0.259 gh^{-1} and at 14°C was 0.152 gh^{-1} (Fig. 4). Gastric evacuation rate at 15‰ was 0.118 gh^{-1} (Fig. 5); considerably lower than at 35 ‰ (Fig. 5).

Total stomach emptying time decreased with temperature in *S. solea*. Gastric evacuation rate increased with temperature from 14°C to 20°C, yet at 26°C a decline was observed ($P < 0.05$). Contrary to the observed in *S. senegalensis*, the evacuation rate in

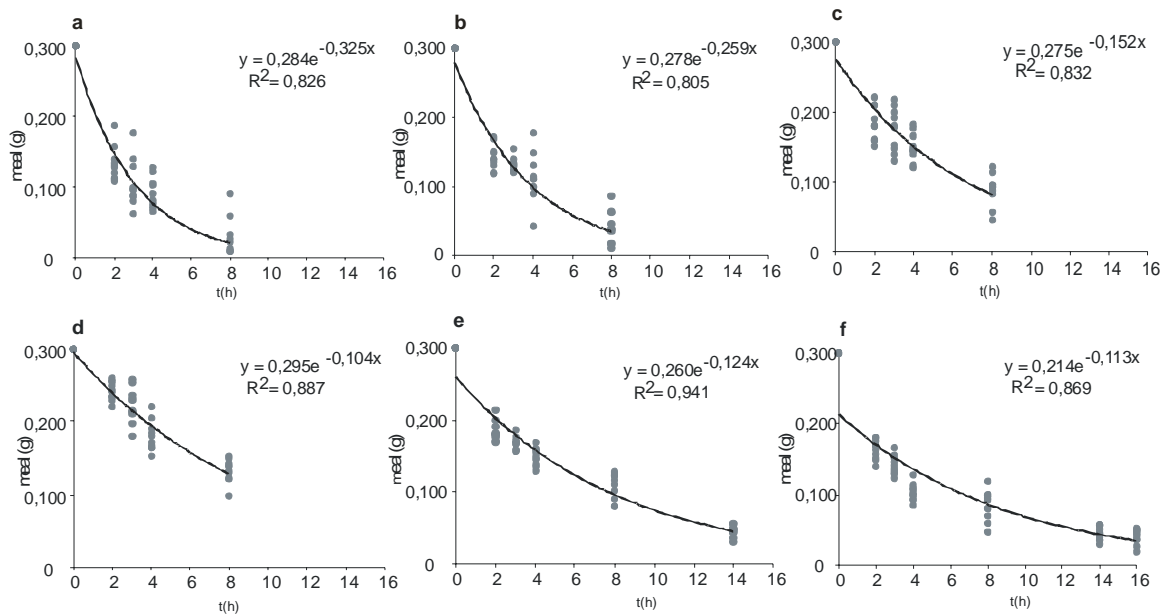


Figure 4 – Exponential regressions of gastric evacuation for *S. senegalensis* at 26°C (a), 20°C (b) and 14°C (c) and for *S. solea* at 26°C (d), 20°C (e) and 14°C (f) (grey dots ● indicate replicates).

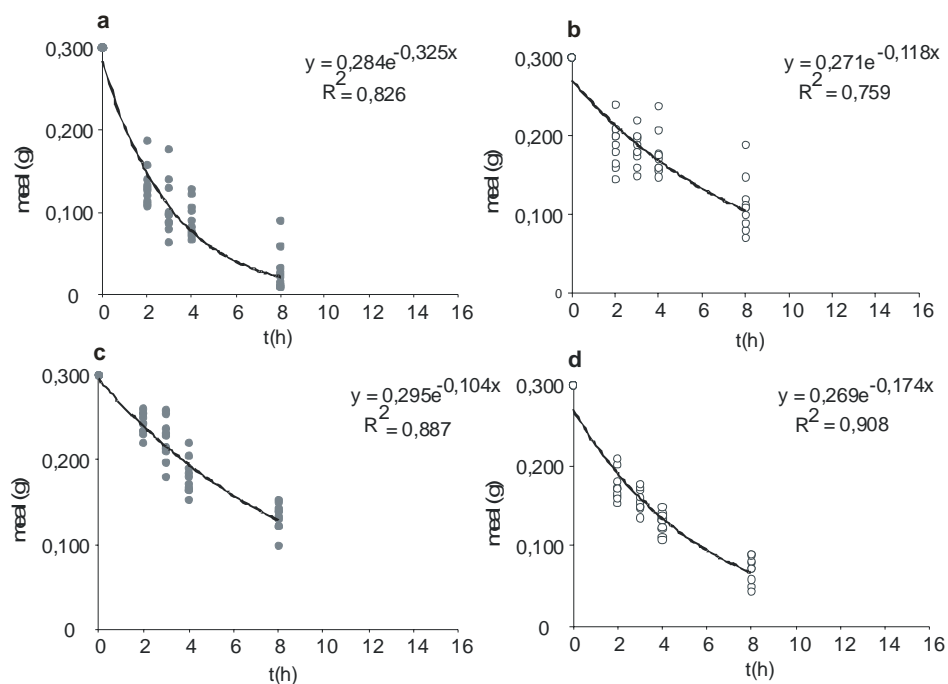


Figure 5– Exponential regressions of gastric evacuation for *S. senegalensis* at salinity 35 ‰ (a) and 15 ‰ (b) and for *S. solea* at 35 ‰ (c) and 15 ‰ (d) (grey dots ● indicate replicates).

S. solea was higher at the lower salinity (15 ‰) ($P < 0.05$), when compared to the seawater salinity (35 ‰), yet the time for total stomach emptying was the same.

The period for total stomach emptying for *S. solea* was 14 hours for fish held at 26°C, 16 hours at 20°C and 20h at 14°C (Fig. 2). The period for total stomach emptying for individuals held at 15 ‰ (at 26°C) was 14 hours (Fig. 3). The relationship between stomach contents and time was also best described as an exponential regression. Gastric evacuation rate at 26°C was 0.104 gh⁻¹, at 20°C was 0.124 gh⁻¹ and at 14°C was 0.113 gh⁻¹ (Fig. 4). Gastric evacuation rate at 15 ‰ (26°C) was 0.174 gh⁻¹, considerably higher than at 35 ‰ (Fig. 5).

Discussion

Both temperature and salinity have an important effect on gastric evacuation in *S. solea* and *S. senegalensis* and should be addressed when estimating food consumption in natural and semi-natural systems. While temperature increased evacuation rates in both species (although not at 26°C in *S. solea*), the effect of low salinity differed among species, leading to an increase in gastric evacuation rate in *S. solea* and a decrease in *S. senegalensis*.

Several studies have focused on the effect of temperature over gastric evacuation in several fish species (e.g. Elliot and Person, 1978; Elliott, 1992; Bromley, 1994) including flatfish (e.g. Jobling *et al.*, 1977; Flowerdew and Grove, 1979; Jobling, 1980; Hurst, 2004). The works of Kruuk (1963) and De Groot (1971) showed that evacuation rates of *S. solea* increase with increasing temperature. The effect of temperature on gastric evacuation is a well known phenomenon that enables fish to have higher daily food consumption rates at higher temperatures.

Factors that affect gastric evacuation may also affect appetite and food intake. A correlation between gastric emptiness and food intake was found for salmonids (Brett, 1971; Elliot 1975a, 1975b), *Gasterosteus aculeatus* (Linnaeus 1758) (Tugendhat, 1960; Beukema, 1968) and *Euthynnus pelamis* (Linnaeus 1758) (Magnunson, 1969). It is thought that appetite is mediated by stretch receptors in the stomach wall similar to those of higher animals (Stevenson, 1969). It is also well known that when food is unlimited ingestion increases with increasing temperature reaching a peak at the optimum temperature before declining steeply as the temperature approaches the species thermal limit (Jobling, 1993; Yamashita *et al.*, 2001). The same should happen for gastric evacuation since these processes are strongly related.

Because *S. senegalensis* is a subtropical species the highest temperature tested, 26°C, is probably well below its thermal upper limit and that is reflected by the steadily increasing evacuation rates with temperature. *S. solea*, however, is a temperate species with a metabolic optimum temperature of approximately 19°C (LeFrançois and Claireaux, 2003). Thus, the observed decline in *S. solea* evacuation rate at 26°C is quite probably due to thermal stress, meaning that in estuarine nurseries where these soles are sympatric, such as the Tagus estuary, *S. solea* is at a disadvantage during the summer months when juveniles of both sole species concentrate in shallow waters, rich in prey but where temperature warms up well above its metabolic optimum.

Salinity is usually not addressed in gastric evacuation studies, since most of the work on gastric evacuation has aimed at incorporating food consumption of adult fish stocks in multi-

species models for fisheries management, such as the North Sea multi-species model (Gislason and Helgason, 1935; Pope, 1991). Yet, in order to study food consumption of juvenile marine fish that spend their first two years in estuarine nurseries, salinity must be taken into account, since it is the single most important factor determining teleost fish drinking rates (Smith, 1930; Evans, 1993). Antibiotic evacuation studies reported stomach content leakage in marine fish held at 35‰ due to permanent drinking (Guichard, 2000). This doesn't happen in freshwater fish since they don't drink (Smith, 1930; Evans, 1993). Euryhaline fish such as soles, when held at low salinities will lower their drinking rate and therefore present higher retention of stomach contents and lower evacuation rates.

Furthermore, several studies show that although feeding rates increase with salinity, food conversion efficiency decreases with increasing salinity (Saillant *et al.*, 2003; Wuenschel *et al.*, 2004), possibly due to higher metabolic costs at seawater salinity. This means that fish at lower salinities operate at higher efficiency being able to maintain high growth rates at lower ration levels. This has important implications when assessing habitat use constraints, in that estuarine nurseries will provide conditions that allow higher food conversion than coastal nurseries.

Interestingly, low salinity had a different effect according to the sole species studied. *S. solea* seems to be better adapted to low salinities, which is reflected in its higher evacuation rates at 15 ‰ than at seawater. The opposite was observed in *S. senegalensis*. *S. solea* and *S. senegalensis* are very similar and are considered sister species, yet when in sympatry *S. solea* seems to prefer lower salinity habitats than *S. senegalensis*, as has been observed in the Tagus estuary (Cabral and Costa, 1999) as well as in other estuaries (Dorel *et al.* 1991; Marchand, 1993; Cabral, 2000b; Cabral *et al.*, 2007). A different level of adaptation to low salinity, has evacuation rates seem to indicate, is probably the most important factor determining these species partition of space within the nursery area.

Other authors have observed species specific use of nursery habitats concerning salinity, for other flatfish species such as southern flounder and summer flounder in North America (Powell and Schwartz, 1977; Burke *et al.*, 1991), among the flatfish community in North Carolina (North America) (Walsh *et al.*, 1999), for Japanese flounder in Japan (Yamashita *et al.*, 2001), among the flatfish community of the Sado estuary (Portugal) (Cabral, 2000b) and in the flatfish community of the Ems estuary (Netherlands) (Jager *et al.*, 1993).

Although many factors other than temperature and salinity are important for the evaluation of habitat quality, our results indicate that for *S. solea* estuarine nurseries provide salinity conditions more favourable than coastal nurseries, since this species has higher evacuation rates at low salinities, yet in many estuarine systems it will endure summer temperatures that lead to thermal stress, which would not happen in the coast. For *S. senegalensis* estuarine nurseries provide favourable temperatures during the nursery period. The decrease in gastric evacuation rate at low salinities is probably compensated by higher food conversion, as observed in other euryhaline species (Saillant *et al.*, 2003; Wuenschel *et al.*, 2004).

One important factor that may influence food uptake in fish nurseries is predator pressure. Predation is now recognized as one of the main factors influencing prey behaviour (review in Lima 1990) and predator avoidance is known to lead to changes in habitat use, feeding, morphology and growth of prey (Turner *et al.*, 1999; Jones and Paszkowski, 1997). Maia *et al.* (*submitted*) reported that predator presence lead to a decrease of 10% in the foraging activity of *S. senegalensis*, meaning that nurseries that provide appropriate temperature and salinity levels may have its habitat quality potential hampered by high densities of juvenile fish predators.

The present work provides important information upon which food consumption models can be estimated for *S. senegalensis* and *S. solea*. Further experimental research using a broader range of temperature and salinity will bring new insights into the effect of these important factors over these species dynamics. Studies on the effect of other factors such as fish size and prey type will allow for a more comprehensive outlook on the gastric evacuation process in soles.

Acknowledgements

Authors would like to thank Dr. Ana Passos for providing the ragworms and everyone involved in the aquariums maintenance. This study was co-funded by the European Union through the FEDER-Fisheries Programme (MARE), as well as the Fundação para a Ciência e a Tecnologia (FCT) which financed several of the research projects related to this work.

References

- Alava, V.R., 1998. Effect of salinity, dietary lipid source and level on growth of milkfish (*Chanos chanos*) fry. *Aquaculture* 167,229-236.
- Bajkov, A.D., 1935. How to estimate the daily food consumption of fish under natural conditions. *Transactions of the American Fisheries Society* 65, 288-289.
- Beukema, J.J., 1968. Predation by the 3-spined stickleback (*Gasterosteus aculeatus*): the influence of hunger and experience. *Behaviour* 31, 1-126.
- Bjornsson, B.T., Stefansson, G.V., Berge, A.I., Hansen, T., Stefansson, S.O., 1998. Circulating growth hormone levels in Atlantic salmon smolts following saewater transfer: effects of photoperiod regime, salinity, duration of exposure and season. *Aquaculture* 168, 121-137.
- Brett, J.R., 1971. Satiation time, appetite and maximum food intake of sockeye salmon (*Onkorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* 28, 409-415.
- Bromley, P.J., 1994. The role of gastric evacuation experiments in quantifying the feeding rates of predatory fish. *Reviews of Fish Biology and Fisheries* 4, 36-66.
- Buckley, J.A., Steinberg, N.D., Conover, D.O., 1995. Effects of temperature, salinity and fish size on growth and consumption of juvenile bluefish. *Journal of Fish Biology* 47, 696-706.

- Burke, J.S., Miller, J.M., Hess, D.E., 1991. Immigration and settlement pattern of *Paralichthys dentatus* and *P. lethostigma* in a estuarine nursery ground, North Carolina, USA. *Netherlands Journal of Sea Research* 27, 393-405.
- Cabral, H.N., 2000a. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., 2000b. Distribution and abundance patterns of flatfishes in the Sado estuary, Portugal. *Estuaries* 23, 351-358.
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the U.K.* 83, 861-868.
- Cabral, H., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P., Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P.T., Costa, M.J., 2007. Relative importance of estuarine flatfish nurseries along the Portuguese coast. *Journal of Sea Research* 57, 209-217. doi:10.1016/j.seares.2006.08.007.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fish* 56, 389-397.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial species. *Scientia Marina* 53,561-566.
- Cabral, H., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the U.K.* 83, 861-868.
- De Groot, S.J., 1971. On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). *Netherlands Journal of Sea Research* 5, 121-196.
- Dinis, M.T., Ribeiro, L., Soares, F., Sarasquete, C., Stickney, R.R., McVey, J.P., 1999. A review of the cultivation potential of *Solea senegalensis* in Spain and in Portugal. *Flatfish cultivation* 176, 27-38.
- Dorel, D., Desaunay, Y., 1991. Comparison of three *Solea solea* (L.) nursery grounds of the bay of Biscay: distribution, density and abundance of 0-group and 1-group. *ICES CM, G*, 75
- Dutil, J.D., Lambert, Y., Boucher, E., 1997. Does higher growth rate in Atlantic cod (*Gadus morhua*) at low salinity result from lower standard metabolic rate or increased protein digestibility? *Canadian Journal of Fisheries and Aquatic Science* 54, 99-103.
- Elliot, J.M., 1975a. The growth of brown trout (*Salmo trutta* L.) fed on maximum rations. *Journal of Animal Ecology* 44, 805-821.
- Elliot, J.M., 1975b. The growth of brown trout (*Salmo trutta* L.) fed on reduced rations. *Journal of Animal Ecology* 44, 823-842.
- Elliott, J.M., 1992. Rates of gastric evacuation in piscivorous brown trout, *Salmo trutta* L. *Freshwater Biology* 25, 297-305.

- Elliot, J.M., Person, L., 1978. The estimation of daily rates of food consumption for fish. *Animal Ecology* 47, 977-993.
- Evans, D.H., 1993. Osmotic and ionic regulation. In: *The Physiology of Fishes*. Eds: D.H. Evans, New York, USA. pp. 315-341.
- Flowerdew, M.W., Grove, D.J., 1979. Some observations of the effects of body weight, temperature, meal size and quality on gastric evacuation emptying time in the turbot, *Scophthalmus maximus* (L.) using radiography. *Journal of Fish Biology* 14, 229-238.
- Gislason, H., Helgason, T., 1985. Species interaction in assessment of fish stocks with special application to the North Sea. *Dana* 5, 1-44.
- Guichard, B., 2000. Devenir d'un aliment médicamenteux dans le tractus digestif du turbot (*Psetta maxima*, L.1758), pour l'étude des rejets d'un antibactérien en pisciculture marine. PhD thesis, Nantes, France.
- Haedrich, R.L., 1983. Estuarine Fishes. In: *Ecosystems of the world 26. Estuaries and Enclosed Seas*. Eds: B., Ketchum. Amsterdam. pp. 183-207.
- Hurst, T.P., 2004. Temperature and state-dependence of feeding and gastric evacuation in juvenile Pacific halibut. *Journal of Fish Biology* 65,157-169.
- Imsland, A. K., Foss, A., Gunnarson, S., Berntssen, M.H.G., Gerald, R.F., Bonga, S.W., 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 198, 353-367.
- Imsland, A. K., Foss, A., Conceição, L. E. C., Dinis, M. T., Delbare, D., Schram, E., Kamstra, A., Rema, P., White, P., 2003. A review of the culture potential of *Solea solea* and *Solea senegalensis*. *Reviews in Fish Biology and Fisheries* 13, 379-407.
- Jager, Z., Kleef, H.L., Tydeman, P., 1993. The distribution of 0-group flatfish in relation to abiotic factors on the tidal flats in the brackish Dollard (Ems estuary, Wadden Sea). *Journal of Fish Biology* 43, 31-43.
- Jobling, M., Gwyther, D., Grove, D.J., 1977. Some effects of temperature, meal size and body weight on gastric evacuation time in the dab *Limanda limanda* (L). *Journal of Fish Biology* 10, 291-298.
- Jobling, M., 1993. Bioenergetics: feed intake and energy partitioning. In: *Fish Ecophysiology* Eds: J.C., Rankin, F.B., Jensen, London, U.K. 309pp.
- Jones, H.M., Paszkowski, C.A., 1997. Effects of exposure to predatory cues on territorial behaviour of male fathead minnows. *Environmental Biology of Fishes* 49, 97-109.
- Lima, S.L., 1990. Evolutionarily stable antipredator behavior in isolated foragers: on the consequences of successful escape. *Journal of Theoretical Biology* 143, 77-89.
- Kruuk, H., 1963. Diurnal periodicity in the activity of the common sole, *Solea vulgaris* Quensel. *Netherlands Journal of Sea Research* 2, 1-28.
- Lambert, Y., Dutil, J.-D., Munro, J., 1994. Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries Science* 51, 1569-1576.

- LeFrançois C., Claireaux G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole, *Solea solea*. Marine Ecology Progress Series 259, 273-284.
- Likongwe, J.S., Stecko, T.D., Stauffer, J.R., Carline, R.F., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus). Aquaculture 146, 37-46.
- Maia, A., Vinagre, C., Cabral, H.N., (submitted) Foraging behaviour of *Solea senegalensis* Kaup, 1858, in the presence of a potential predator, *Carcinus maenas* Linnaeus, 1758.
- Magnuson, J.J., 1969. Digestion and food consumption by skipjack tuna. Transactions of the American Fisheries Society 98, 379-392.
- Marchand, J., 1993. The influence of seasonal salinity and turbidity maximum variations on the nursery function of the Loire estuary (France). Netherlands Journal of Aquatic Ecology 27, 427-436.
- McCormick, S.D., 1996. Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na^+ , K^+ -ATPase in Atlantic salmon (*Salmo salar*): interaction with cortisol. Gen. Comp. Endocrinol. 101, 3-11.
- Peterson-Curtis, T.L., 1997. Effects of salinity on survival, growth, metabolism and behavior in juvenile hogchockers, *Trinectes maculatus* (Achiridae). Environmental Biology of Fishes 49, 323-331.
- Pope, J., 1991. The ICES multispecies assessment working group: evolution, insights, and future problems. ICES Marine Science Symposia 193, 22-33.
- Powell, A.B., Schwartz, F.J., 1977. Distribution of paralichthid flounders (Bothidae: Paralichthys) in North Carolina estuaries. Chesapeake Science 18, 334-339.
- Quero, J.C., Dessoutter, M., Lagardere, F., 1986. Soleidae. In: Fishes of the North-eastern Atlantic and Mediterranean. Eds: P., Whitehead, M.-L., Bauchot, J.-C., Hureau, J., Nielsen, E., Tortonese, Paris, France. pp. 1308-1324
- Ross, S.T., 1986. Resource partitioning in fish assemblages: a review of field studies. Copeia 19, 352-388.
- Saillant, E., Fostier, A., Haffray, P., Menu, B., Chatain, B., 2003. Saline preferendum for the European sea bass, *Dicentrarchus labrax*, larvae and juveniles: effect of salinity in early development and sex determination. Journal of Experimental Marine Biology and Ecology 287, 103-117.
- Schoener, T.W., 1974. Resource partitioning in ecological communities. Science 185, 27-39.
- Smith, H.W., 1930. The absorption and excretion of water and salts by marine teleosts. American Journal of Physiology 93, 480.
- Stevenson, J.A.F., 1969. Mechanisms in the control of food and water intake in neural regulation of food and water intake (Ed. Morgane, P.J.) Ann. N.Y. Acad. Sci. 157, 1069-1083.
- Swanson, C., 1996. Early development of milkfish: effects of salinity on embryonic and larval metabolism, yolk absorption and growth. Journal of Fish Biology 48, 405-21.

- Temming, A., Andersen, N.G., 1994. Modelling gastric evacuation in cod. A model applicable to the estimation of the daily ration of cod in the field. ICES Journal of Marine Science 51, 429-438.
- Tugendhat, B., 1960. The normal feeding behaviour of the three spined stickleback *Gasterosteus aculeatus*. Behaviour 15, 284-318.
- Turner, A.M., Fetterolf, S.A., Bernot, R.J., 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. Oecologia 118, 242-247.
- Walsh, H.J., Peters, D.S., Cyrus, D.P., 1999. Habitat utilization by small flatfishes in a North Carolina estuary. Estuaries 22, 803-813.
- Woo, N.Y. S., Kelly, S.P., 1995. Effects of salinity and nutritional status on growth and metabolism of *Sparus sarba* in a closed seawater system. Aquaculture 135, 229-238.
- Wuenschel, M.J., Joguvich A.R., Hare, J.A., 2004. Effect of temperature and salinity on the energetics of juvenile gray snapper (*Lutjanus griseus*): implications for nursery habitat value. Journal of Experimental Marine Biology and Ecology 312, 333-347.
- Yamashita, Y., Tanaka, M., Miller, J.M., 2001. Ecophysiology of juvenile flatfish in nursery grounds. Journal of Sea Research. 45, 205-218.

Foraging behaviour of *Solea senegalensis* in the presence of a potential predator, *Carcinus maenas*

Abstract: Habitat modelling requires incorporation of both biotic and abiotic data. For juvenile flatfish the factors that most influence growth are water temperature, food abundance and predatory pressure. This study focuses on the impact of a predator, shore crab, *Carcinus maenas* (Linnaeus, 1758) in the foraging activity of sole, *Solea senegalensis* Kaup, 1858, while feeding on the ragworm, *Nereis diversicolor* Müller (1776). The results show that in the presence of both prey and predator, the foraging activity of sole is strongly impacted with a 10% decrease in overall activity, when compared to the sole in the presence of only food. Crawling and tapping were the behaviours most correlated with foraging and these activities were also strongly impacted by the presence of both food and predator. The rapid escape response occurred when the predator was present independently of the presence of food. This study also provides further support to visual recognition of predators and olfactory prey recognition in the Senegalese sole.

Key-words: *Carcinus maenas*; Foraging behaviour; Potential predator; *Solea senegalensis*.

Introduction

In order to model habitat usage of a species, in addition to abiotic factors, other conditions such as food availability and predatory pressure must be evaluated. Suitable habitats in estuarine systems that are high in prey abundance and lack predators do not abound, so the predator impact in the feeding rate of a prey must be taken into account in habitat modelling.

For flatfish the key factors affecting growth and survival of juveniles are water temperature, food abundance and predation pressure (Gibson, 1994).

Several studies have focused on the behaviour of flatfish in their natural conditions (e.g. van der Veer and Bergman, 1986; Cabral and Costa, 1999; Cabral, 2000; Amezcua and Nash, 2001) and some even did so in experimental conditions (Ansell and Gibson, 1993; Aarnio *et al.*, 1996; Gibson and Robb, 2000; Burrows and Gibson, 1995). Predator-prey interactions have also been extensively studied in flatfish through experimental design (Gibson *et al.*, 1995; Fairchild and Howell, 2000; Kellison *et al.*, 2000; Hossain *et al.*, 2002; Taylor, 2004; Breves and Specker, 2005; Taylor, 2005; Lemke and Ryer, 2006). However, none was able to quantify the impact of the presence of a predator in the feeding rate.

Predation is now recognized as one of the main factors influencing prey behaviour (review in Lima, 1990) and predator avoidance is known to lead to changes in habitat use, feeding, morphology and growth of prey (Turner *et al.*, 1999; Jones and Paszkowski, 1997). Also, despite the obvious fitness benefits of prey ingestion, antipredator behaviours can be costly, strongly impacting activities like feeding and breeding (Wong *et al.*, 2005). For plaice, *Pleuronectes platessa* (Linnaeus, 1758), predation by the crustaceans *C. crangon* and *Carcinus maenas* (Linnaeus, 1758) have been identified as key factors in regulation of density within the

nursery areas (Van der Veer, 1986; Van der Veer and Bergman, 1987; Van der Veer *et al.*, 1990). *C. maenas* preys heavily on juvenile *Solea senegalensis* Kaup, 1858, in Portuguese estuaries where they cohabit (Cabral, unpublished data).

The Senegalese sole, *S. senegalensis*, is a benthic fish distributed from the Bay of Biscay to Senegal and western Mediterranean (Quero *et al.*, 1986). It is a species of increasing interest in aquaculture and is commonly cultured in the Portuguese and Spanish southern coasts (Dinis *et al.*, 1999). The ragworm, *Nereis diversicolor* Müller (1776), is a natural prey of *S. senegalensis* (Cabral, 2000)

The first year of life is a key stage in fish development, particularly for species, like the soles, that concentrate in large densities in estuarine and coastal nurseries where space and food partitioning become an issue (e.g. Schoener, 1974; Ross, 1986).

The portunid shore crab *C. maenas* has a wide distribution in coastal and estuarine shallow waters of temperate areas (Udekem d'Acoz, 1993).

This study focuses on 0-group juveniles of *S. senegalensis*, since this is when the individuals are most susceptible to *C. maenas* predation and it is also when natural mortality is most common in natural and semi-natural systems (Houde, 1987). It aims to investigate the interaction of the juvenile Senegalese sole, *S. senegalensis*, with its natural predator *C. maenas* and assess its impact on the sole's foraging behaviour.

Materials and methods

Prior to experiments fish were held in circular tanks with capacities of 350 l for a minimum of 4 weeks (maximum stocking density was 120 fish per 350 l). Fish were then transferred to 160 l aquariums equipped with mechanical and biological filter units. Temperature was regulated with a precision of ± 0.1 °C. Salinity was regulated with a precision of 0.1 ‰. Temperature and salinity were monitored daily. Fish were exposed to a day length of 12 hours.

Eight months old juvenile soles, *S. senegalensis*, were used in this experiment, kept in a natural light cycle. All treatments were carried out in aquaria 50x25x30 cm (l x w x h), with salinity 35 (PSU) and temperature 25°C. Ragworms, *N. diversicolor*, were reared in aquariums at the laboratory.

For the behaviour experiments, fish were transferred into experimental compartments where they were kept for 3 weeks prior to experiments (Figure 1). Prior to the experiment there were 45 hours of *ad libitum* observations to determine the most common behaviours.

All soles were conditioned, two days earlier, with 48h fasting, freely moving *C. maenas*. They were therefore non-naïve to this predator. For prey simulation, living *N. diversicolor* was used, since this is one of their favourite prey items (Cabral, 2000) and soles were kept in aquaria for the previous 6 months feeding on living *N. diversicolor*. Soles ranged in size from 65 to 132mm TL, averaging 94.9mm.

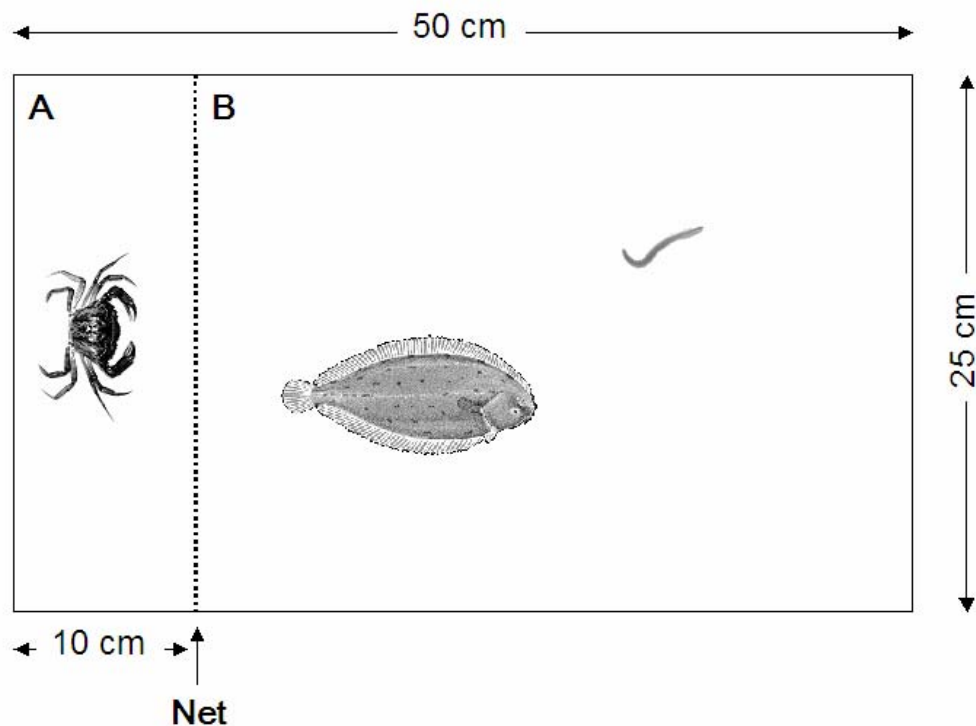


Figure 1 – Schematic representation of experimental setup, the negative stimulus, *C. maenas* was kept in compartment A, while *S. senegalensis* and *N. diversicolor* (positive stimulus) were in compartment B.

C. maenas used in the treatments were subjected to 48h fasting and soles to 24h fasting. Crabs ranged in size from 46 to 70mm carapace width, with an average size of 55.2mm.

There were four different treatments (see figure 1): a) *C. maenas* in A; *S. senegalensis* in B (visual and chemical predatory stimulus only) – negative treatment; b) *C. maenas* in A; *S. senegalensis* in B and one *N. diversicolor* near the net on B side (visual and chemical predatory stimulus only, complete prey stimulus) – interaction treatment; c) *S. senegalensis* in B and one *N. diversicolor* near the net on B side (no predatory stimulus, complete prey stimulus) – positive treatment; d) *S. senegalensis* in B – control treatment. Each treatment had at least 12 replicates. To make sure the net was not a source of variability, treatment a) was repeated 6 times with the crab and the sole on the same side of the net and no differences were found.

Observations were carried out for 10 minutes without stimulus and 30 minutes with stimulus, starting at dusk and under red light. These conditions were chosen based on other experiments conducted in *S. senegalensis* (Pais *et al.*, 2004) that show the species to be more active and forage mostly at this time of the day. Every individual was used only once to avoid learned behaviours.

Decrease or increase in percent activity after stimuli was analysed using Mann-Whitney U Test, to accommodate for potential individual variability. Mean percentage of time spent for every of the seven most common behaviours was computed along with mean frequency (times per minute) for the four treatments. Non-parametric ANOVA was used to test for differences in

the treatments using both time and frequency of the most common behaviours. In all test procedures a significance level of 0.05 was considered.

Results

The Senegalese sole behaviour was dominated by resting. Only 6.5% of activity was observed in the control group (Figure 2). Active behaviours included crawling on the substrate, swimming, “head-up” movement, eating, rapid escape, tapping and burrowing. Crawling is characterized by the individual moving over the substrate keeping the body in contact with it; while in swimming, the individual moves undulating the body, without touching the substrate. In the “head-up” movement the individual lifts its head while static on the substrate. Burrowing is characterized by rapid undulation of the body in an attempt to burry itself. Rapid escape occurs when the individual dashes away from a threat. In tapping, the individual taps several times its head on the substrate. And finally, eating is when the individual bites and chews food items.

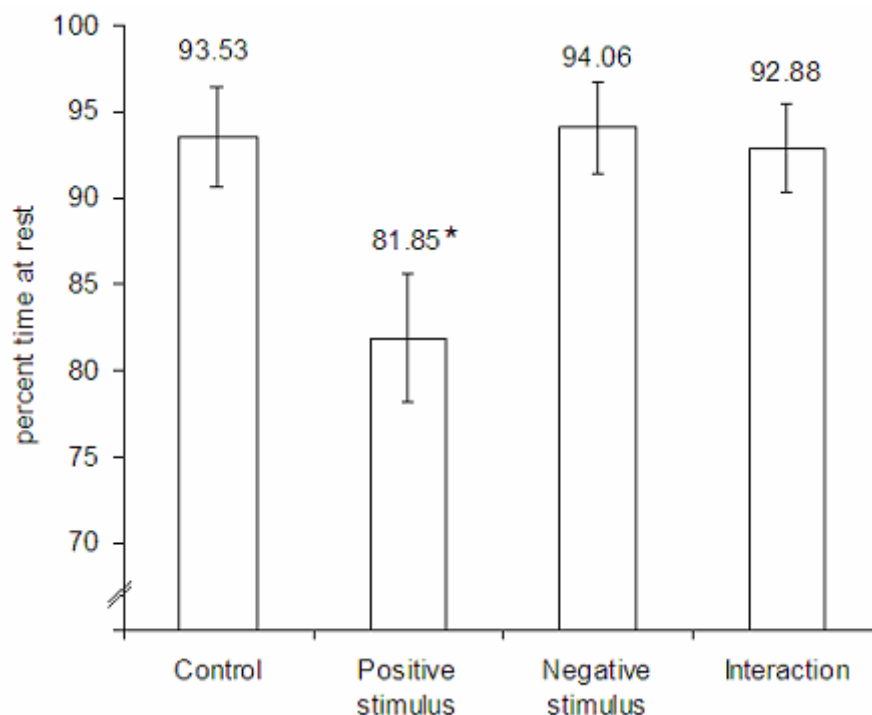


Figure 2 – Percent time spent resting by *S. senegalensis* in the different treatments (control, positive stimulus, interaction and negative stimulus), bars represent standard error.

It was observed a decrease in the overall activity of soles in the presence of *C. maenas* and *Nereis diversicolor* by an order of magnitude of 10% (Figure 2, H (3, N=80), $p=0.0096$), similar to the activity in the presence of only the *C. maenas*. Also significant, was the number of rapid escapes in the presence of *C. maenas*, especially in the absence of *N. diversicolor* (H (2,

N=60), $p=0.0032$). On the contrary, tapping only occurred in the presence of prey (Figure 3, H (2, N=60), $p=0.014$).

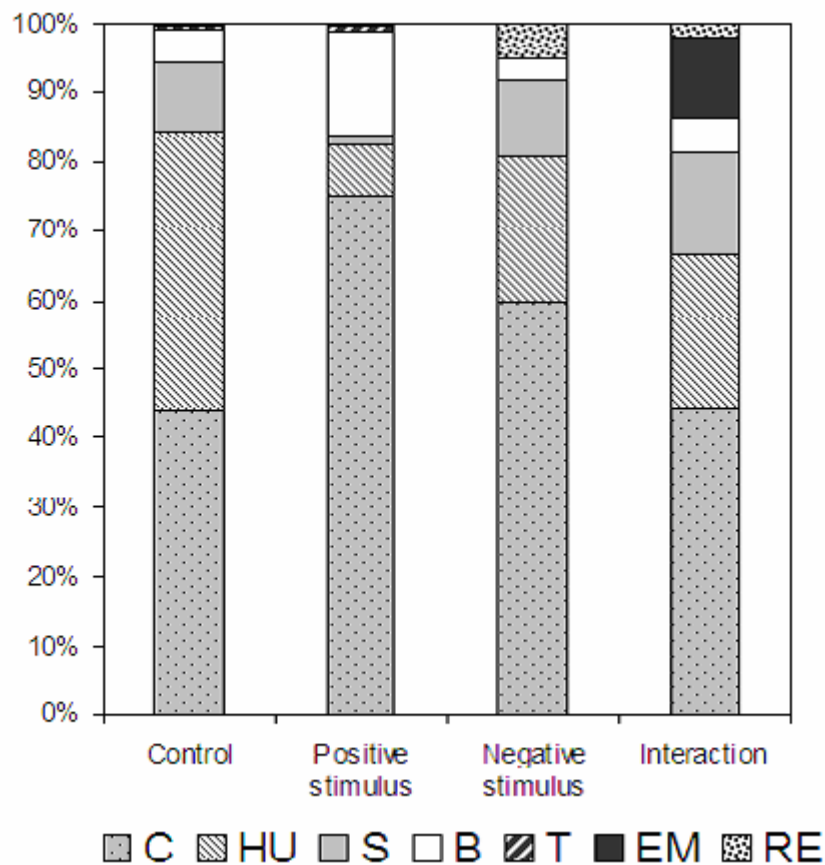


Figure 3 – Time (in percentage of total observed time) that *S. senegalensis* spent in each active behaviour (C – crawling, HU – head up, S – swimming, B – burrow, T – tapping, EM – eating movements, RE – rapid escape), associated with the four different treatments. Sn – *S. senegalensis*, Cm – *C. maenas*, Nd – *N. diversicolor*.

When food was present and predator absent, time spent in crawling and burrowing was greater (Figure 3, H (3, N=80), $p=0.0042$). In terms of variation in activity prior and after stimulus, it can be observed that the negative stimulus is correlated with an overall decrease in activity; while the positive stimulus with an increase in activity by 8% on average (H (2, N=17), $p=0.003$).

Discussion

Soles are known to have a strong relationship with benthos (De Groot, 1971), thus it is not surprising the low activity of this species. Their behaviours are also simple, especially when social interaction is not being analysed. Crawling, burrowing and tapping were most frequent in

the presence of food and as such appear to be related to foraging habits. Crawling was also related to the negative stimulus (presence of predator) and together with rapid escape and swimming can represent the typical predator like threat evasion behaviour. Other option of the sole, though observed less often was the attempt to burry itself to evade the detection from the predator.

It was not possible to exactly ascertain if predator recognition was visual, chemical or both. However, all naïve soles were successful in avoiding crab touching or grabbing them and the “crab over fence” setup elicited a predator like threat escape behaviour, thus dismissing tactile recognition. In a study directed to investigate the feeding stimuli, De Groot (1971) found that the presence of an 8 cm ball elicited a flight response by the common sole, *Solea solea* (Linnaeus, 1758), suggesting visual predator recognition. It was also noted that an attack is not necessary to elicit rapid escape behaviour by the common sole. That, along with the findings of Appelbaum and Schemmel (1983) which concluded that chemoreception in *S. solea* is not as important as previously thought, indicates that predator recognition must be mainly visual.

This study also allowed some insight into what is the main foraging pattern of the Senegal sole. Prey recognition is typically olfactory, similarly to what has previously been described for *S. solea* (De Groot, 1971; Appelbaum and Schemmel, 1983; Harvey, 1996), since the individual will increase its activity in the presence of food, moving randomly to the prey, searching in the substrate, as seen by the increase in tapping behaviour in the presence of just food. The tapping movement in relation to foraging has also been previously described for *S. solea* (De Groot, 1971). This behaviour might enhance the water circulation around the individual allowing for better prey detection. Also, being *S. senegalensis* morphologically very similar to *S. solea*, the presence of taste buds in the oral cavity, pharynx, gill rakers and lips (Appelbaum and Schemmel, 1983) the tapping behaviour would strongly enable the chemical food detection.

There is a quantifiable impact on the Senegal sole foraging by the presence of a predator. The 10% decrease in activity puts the interaction sole-crab-worm close to the sole-crab situation. It is well documented that *C. maenas* impacts the population of other juvenile flatfishes, especially *S. solea* and *P. platessa* (e.g. Modin and Pihl, 1994; Fairchild and Howell, 2000). However, apart from the direct risk of predation it has to be taken into account the trade-off between escape from a predator and foraging. Suitable nursery grounds for sole in terms of water temperature, salinity and food supply in Portuguese estuaries are also the areas where the green crab is more abundant (Cabral, unpublished data). It is also important to refer that since *C. maenas* is a generalist feeder it also competes with soles for food resources such as polychaetes and amphipods (Cohen *et al.*, 1995).

The next step will be to adjust the existing habitat models to incorporate this interaction of predator-sole-prey. This information is of the uttermost importance for delimiting marine reserves, since *C. maenas* is a species with very high reproductive potential (Cohen *et al.*, 1995), and their numbers would likely increase to pose a threat to soles. Further studies should focus on the comparison of sites with different crab densities and cross that information with

soles' stomach contents. Also knowledge of predator-prey behaviour is important in releasing of hatchery reared fish for stock enhancement purposes (Fairchild and Howell, 2000).

Acknowledgments

Authors would like to thank Pedro Pousão and the CRIP-Sul Aquaculture station for providing the fish. This study was co-funded by the European Union through the FEDER-Fisheries Programme (MARE), as well as the Fundação para a Ciência e a Tecnologia (FCT) which financed several of the research projects related to this work.

References

- Aarnio, K., Bonsdorff, E., Rosenback, N., 1996. Food and feeding habits of juvenile flounder *Platichthys flesus* (L.), and turbot *Scophthalmus maximus* L. in the Aaland Archipelago, northern Baltic Sea. *Journal of Sea Research* 36, 311-320.
- Amezcuca, F, Nash, R.D.M., 2001. Distribution of the order Pleuronectiformes in relation to the sediment type in the North Irish Sea. *Journal of Sea Research* 45, 293-301.
- Ansell, A.D., Gibson, R.N., 1993. The effect of sand and light on predation of juvenile plaice (*Pleuronectes platessa*) by fishes and crustaceans. *Journal of Fish Biology* 43, 837-845.
- Appelbaum, S., Schemmel C., 1983. Dermal sense organs and their significance in the feeding behaviour of the common sole *Solea vulgaris*. *Marine Ecology Progress Series* 13, 29-36.
- Breves, J.P., Specker, J.L., 2005. Cortisol stress response of juvenile winter flounder (*Pseudopleuronectes americanus*, Walbaum) to predators. *Journal of Experimental Marine Biology and Ecology* 325, 1-7.
- Burrows, M.T., Gibson, R.N., 1995. The effects of food, predation risk and endogenous rhythmicity on the behaviour of juvenile plaice, *Pleuronectes platessa* L.. *Animal Behaviour* 50(1), 41-52.
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *S. senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57(6), 1550-1562.
- Cabral, H., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental. Biology of Fishes* 56, 389-397.
- Cohen, A.N., Carlton, J.T., Fountain, M.C., 1995. Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. *Marine Biology* 122(2), 225-237.
- De Groot, S.J., 1971. On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces. Pleuronectiformes). *Netherlands Journal of Sea Research* 5(2), 121-196.
- Dinis, M.T., Ribeiro, L., Soares, F., Sarasquete, C., 1999. A review on the cultivation potential of *Solea senegalensis* in Spain and in Portugal. *Aquaculture* 176(1-2), 27-38.

- Fairchild, E.A., Howell, W.H., 2000. Predator-prey size relationship between *Pseudopleuronectes americanus* and *Carcinus maenas*. *Journal of Sea Research* 44, 81-90.
- Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. *Netherlands Journal of Sea Research* 32(2), 191-206.
- Gibson, R. N., Robb, L., 2001. Sediment selection in juvenile plaice and its behavioural basis. *Journal of Fish Biology* 56(5), 1258-1275.
- Gibson, R. N., Yin, M. C., Robb, L., 1995. The behavioural basis of predator-prey size relationships between shrimp (*Crangon crangon*) and juvenile plaice (*Pleuronectes platessa*). *Journal of the Marine Biological Association of the United Kingdom* 75, 337-349.
- Harvey, R., 1996. The olfactory epithelium in plaice (*Pleuronectes platessa*) and sole (*Solea solea*), two flatfishes with contrasting feeding behaviour. *Journal of the Marine Biological Association of the United Kingdom* 76, 127-139.
- Hossain, M.A.R., Tanaka, M., Masuda, R., 2002. Predator-prey interaction between hatchery-reared Japanese flounder juvenile, *Paralichthys olivaceus*, and sandy shore crab, *Matuta lunaris*: daily rhythms, anti-predator conditioning and starvation. *Journal of Experimental Marine Biology and Ecology* 267, 1-14.
- Houde, E.D., 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium*.
- Jones, H.M., Paszkowski, C.A., 1997. Effects of exposure to predatory cues on territorial behaviour of male fathead minnows. *Environmental Biology of Fishes* 49, 97-109.
- Lagardère, J. P., 1987. Feeding ecology and daily food consumption of common sole, *Solea vulgaris* Quensel, juveniles on the French Atlantic coast. *Journal of Fish Biology* 30, 91-104.
- Lemke, J.L., Ryer, C.H., 2006. Risk sensitivity in three juvenile (Age-0) flatfish species: Does estuarine dependence promote risk-prone behavior? *Journal of Experimental Marine Biology and Ecology* 333, 172-180.
- Lima, S. L., 1990. Evolutionarily stable antipredator behavior in iso-lated foragers: on the consequences of successful escape. *Journal of Theoretical Biology* 143, 77-89.
- Modin, J., Pihl, L., 1994. Differences in growth and mortality of juvenile plaice, *Pleuronectes platessa* L., following normal and extremely high settlement. *Netherlands Journal of Sea Research* 32(3/4), 331-341.
- Pais, M., Castro, R., Fonseca, V., Vinagre C., Cabral H., 2004. Behavior of the Senegalese sole, *Solea senegalensis* Kaup, 1858, in experimental conditions. *Proceedings of the VI National Congress of Etology*. Universidade de Coimbra, Coimbra, Portugal, pp. 57.
- Quero, J.C., Delmas, G., Du Buit, M.H., Fonteneau, J., Lafon, A., 1986. Observations ichtyologiques effectuees en 1985. *Annales de la Societe des Sciences naturelles de la Charente-Maritime*. La Rochelle. Vol. 7, no. 4, pp. 493-498.

-
- Ross, S. T., 1986. Resource Partitioning in Fish Assemblages: A Review of Field Studies. *Copeia* 1986 (2) 352-388.
- Schoener, T. W., 1974. Resource Partitioning in Ecological Communities. *Science* 185, 27-39.
- Taylor, D. L., 2005. Predation on post-settlement winter flounder *Pseudopleuronectes americanus* by sand shrimp *Crangon septemspinosa* in NW Atlantic estuaries. *Marine Ecology Progress Series* 289, 245–262.
- Turner, A. M., Fetterolf, S. A., Bernot, R. J., 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* 118(2), 242-247.
- Van Der Veer, H.W., 1986. Immigration, settlement, and density-dependent mortality of a larval and early postlarval 0-group plaice (*Pleuronectes platessa*) population in the western Wadden Sea. *Marine Ecology Progress Series* 29, 223-236.
- Van der Veer, H.W., Bergman, M.J.N., 1987. Development of tidally related behaviour of a newly settled 0-group plaice (*Pleuronectes platessa*) population in the western Wadden Sea. *Marine Ecology Progress Series* 31, 121-129.
- Van der Veer, H.W., Pihl, L., Bergman, M.J.N., 1990. Recruitment mechanisms in North Sea plaice *Pleuronectes platessa*. *Marine Ecology Progress Series* 64(1-2), 1-12.
- Wong, B. B. M., Bibeau, C., Bishop, K. A., Rosenthal, G. G., 2005. Response to perceived predation threat in fiddler crabs: trust thy neighbor as thyself? *Behaviour Ecology and Sociobiology* 58, 345-350.

Prey consumption of the juvenile soles *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal

Abstract: The soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, are marine flatfish that use coastal and estuarine nursery grounds, which usually present high food availability, refuge from predators and favourable conditions for rapid growth. Two important nursery grounds for these species juveniles have been identified in the Tagus estuary, one in the upper part of the estuary (nursery A) and another in the south bank (nursery B). While *S. solea* is only present at the uppermost nursery area, *S. senegalensis* is present at both nurseries. Although they are among the most important predators in these nursery grounds, there are no estimates on their food consumption or on the carrying capacity of the system for soles. The Elliott and Persson (1978) model was used to estimate food consumption of both species juveniles in both nursery areas, taking into account gastric evacuation rates previously determined and 24 h sampling surveys, based on beam-trawl catches carried out every 3 hours, in the summer of 1995. Monthly beam trawls were performed to determine sole densities over the summer. Density estimates and daily food consumption values were used to calculate total consumption over the summer period. Sediment samples were taken for the estimation of prey densities and total biomass in the nursery areas. Daily food consumption was lower for *S. solea* (0.030 g wet weight d⁻¹) than for *S. senegalensis* (0.075 g wet weight d⁻¹). It was concluded that thermal stress may be an important factor hindering *S. solea*'s food consumption in the warmer months. Total consumption of *S. solea* over the summer (90 days) was estimated to be 97 kg. *S. senegalensis* total consumption in nursery A was estimated to be 103 kg, while in nursery B it was 528 kg. Total prey biomass estimated for nursery A was 300 tonnes, while for nursery B it was 58 tonnes. This suggests that food is not a limiting factor for sole in the Tagus estuary. However it was concluded that more in depth studies into the food consumption of other species and prey availability are needed in order to determine the carrying capacity of this system for sole juveniles.

Key-words: Prey consumption; Prey availability, Carrying capacity, Estuarine nurseries, Flatfish, Sole, Feeding ecology.

Introduction

Estuaries have long been recognized as important nursery areas for many fish species (Haedrich, 1983; Miller *et al.*, 1985; Beck *et al.*, 2001). One of the main reasons why the estuarine environment is favourable for the growth of juvenile fish lies in its high food availability.

While some authors refer that predatory pressure by fish does not impact prey communities and that food availability is never a limiting factor for juvenile fish populations living in estuaries (Gee *et al.*, 1985; Rafaelli, 1989), other authors suggest that impact is not only high but that fish are in fact the main biotic regulators of estuarine endofauna communities (Phil, 1985; Jaquet and Rafaelli, 1989).

Several approaches to the estimation of the feeding rates of fish populations in the wild have been put forward, driven by the need to construct food webs to be used in the

management of fish stocks (Bromley, 1994). In this context, the quantification of predation and feeding interactions among species is a key issue.

Since the observation of feeding in wild populations is generally impracticable, several indirect methods have been developed. A considerable number of experimental studies have investigated the feeding rates required to produce growth rates similar to those measured in wild fish (Gerking, 1962; Elliott, 1975a; b; Jones and Hislop 1978; Jobling, 1982), and the energetics of growth (Mann, 1965; Solomon and Brafield, 1972; Jobling, 1988; Hewett, 1989). Others have looked at nitrogen requirements for growth (Smith and Thorpe, 1976; Bowen, 1987) and a more limited number investigated both energy and nitrogen requirements (Cowey and Sargent, 1972; Bromley, 1974; 1980).

These methods require in-depth knowledge of each species feeding and growth and depend on the assumption that feeding, digestion, food conversion and metabolic expenditure of captive fish is similar to that of wild fish.

Various models have been developed following a different approach that combines information on gastric evacuation rates, determined experimentally, with that of stomach contents of wild fish (e.g. Thorpe, 1977; Elliott and Persson, 1978; Eggers, 1979; Jobling, 1981; Bromley, 1987). The only assumption being that food passes through the stomach at the same rate in experimental fish as in wild fish, so that the amount of food evacuated mirrors the amount of food consumed (Tyler, 1970; Bromley, 1987).

The soles, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, are marine flatfish that use coastal and estuarine nursery grounds, which usually present high food availability, refuge from predators and favourable conditions for rapid growth (e.g. Haedrich, 1983). Two important nursery grounds for these very similar species have been identified in the Tagus estuary, one of the largest estuaries in west Europe (Costa and Bruxelas, 1989; Cabral and Costa, 1999). Several studies have investigated these juveniles habitat use (Costa and Bruxelas, 1989; Cabral and Costa, 1999; Vinagre *et al.*, 2006a), diet (Cabral, 2000), feeding rhythms (Cabral, 1998; 2000; Vinagre *et al.*, 2006b), impact of predatory pressure on feeding (Maia *et al.*, unpublished) and gastric evacuation at different temperatures and salinities (Vinagre *et al.*, 2007). There is now enough information to be incorporated into a food consumption model and apply it to the Tagus estuary nursery areas. This is will be the first time a model incorporating both experimentally determined gastric evacuation rates and field data is applied to both sole species.

Assessment of the daily rations of soles allows the estimation of total food consumed over the summer period, when densities of these species juveniles are highest and thus have more potential impact upon their prey densities. There are no studies on the carrying capacity of these nursery grounds for juvenile sole. Estimation of the total food consumed and of the total prey in the nursery areas should give us a first insight into this matter.

The aim of the present study is to (1) estimate food consumption of *S. solea* and *S. senegalensis* juveniles in the two nursery areas of the Tagus estuary, taking into account water

temperature and diel patterns of feeding activity, and (2) determine the total food consumed by soles over the summer versus the total prey present in the sediment.

Material and Methods

Study area

The Tagus estuary, with an area of 320 km², is a partially mixed estuary with a tidal range of ca. 4 m. About 40% of the estuarine area is intertidal. The upper part of the estuary is shallow and fringed by saltmarshes. The two main nursery areas for fish (A – Vila Franca de Xira and B – Alcochete) identified by Costa and Bruxelles (1989) and Cabral and Costa (1999) are located in the upper estuary (Figure 1).

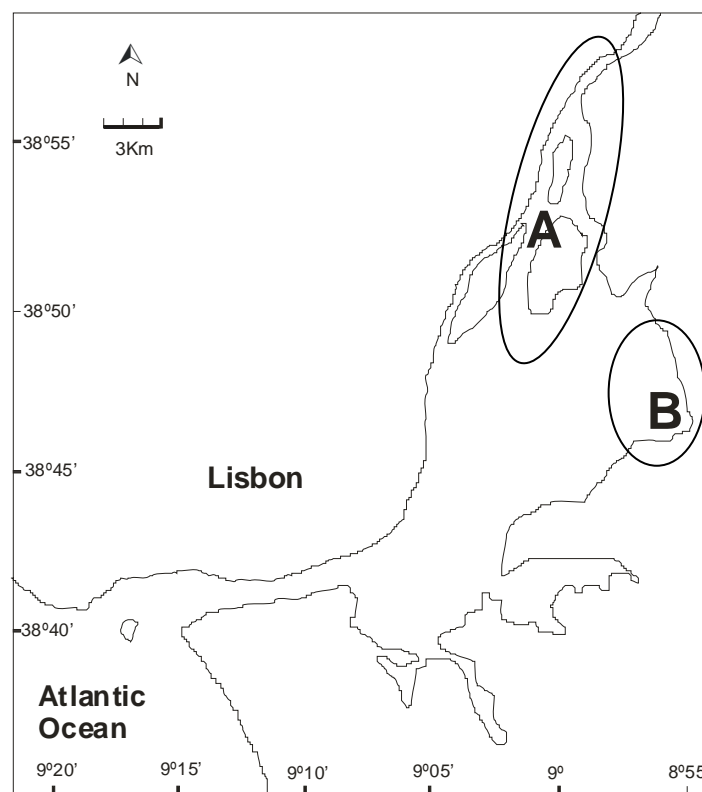


Figure 1 – The Tagus estuary and its main nursery areas.

Although most of the environmental factors vary widely within the estuary, their ranges are similar in these two areas. However, the uppermost area (A) is deeper (mean value 4.4 m), has lower salinity (mean value 5‰) and a higher proportion of fine sand in the sediment, while in the other area (B) the mean values of depth and salinity are 1.9 m and 20.7‰, respectively, and the sediment is mainly composed of mud (Cabral and Costa 1999). The area of each nursery determined from nautical maps is 46.46 km² for area A and 24.75 km² for area B. Intertidal mudflats encompass 23% of area A and , and 87% of area B. While in nursery A both sole species are present, in nursery B only *S. senegalensis* exists Cabral and Costa (1999)

Food consumption estimation

The model applied in the present study was the one developed by Elliott and Persson (1978):

$$F_t = [(S_t - S_0 e^{-Rt}) R t] / (1 - e^{-Rt})$$

where F_t is food consumption after t hours, S_t is stomach content after t hours, S_0 is the stomach content at the start of the observation period and R is the gastric evacuation rate constant determined experimentally.

This is one of the most widely used of all the food consumption models. This model allows the estimation of food consumption to be carried out in separate time periods (generally of 3 hours). Feeding is assumed to be constant over each period of observation.

The evacuation rate (R) used in the model was calculated by Vinagre *et al.* (2007). Field data used is from monthly sampling conducted in the summer of 1996 (June, July and August), as well as on a 24 h sampling cycle, carried out in July 1996. The sampling method used was based on beam trawls (10 in area A and 5 hauls in area B, every month in the monthly sampling program and one every 3 hours in the 24 h cycle). A 4 m beam trawl with 1 tickler chain, 10 mm mesh size and 5 mm stretched mesh at the codend, was used. Hauls had 15 min duration and the distance travelled was registered using a GPS. Estimation of the area swept was carried out using the beam length and the distance travelled.

Individuals caught in the beam trawls were identified, counted weighted (wet weight with 0.01 g precision) and their total length measured to the nearest mm. Soles stomachs were excised, contents were removed and preserved in 4% buffered formalin. Stomach contents were analysed. Each prey item was identified to the lowest taxonomic level possible, counted, and weighed (wet weight with 0.001 g precision). The amount of food ingested in relation to total body weight was estimated for each individual.

Carrying capacity of the nursery areas

Sediment samples were randomly collected at each site, 20 samples in area A and 10 samples in area B, half on the subtidal and half on the intertidal, using a van Veen grab (0.05 m²). Sediments were transported to the laboratory and then sieved through a 0.5 mm nylon mesh to collect macrofauna specimens. Organisms were preserved in 4% buffered formalin and later identified and weighted (wet weight with 0.001 g precision).

The wet weight of soles' prey (polychaetes, *Scrobicularia plana* and amphipods) identified in the intertidal and subtidal sediment was averaged for each nursery area. For *S. plana* only the siphon was weighted, because only the siphons are consumed by soles. The total amount of prey in the sediment was estimated taking into account the area of each nursery, as well as the proportion of intertidal *versus* subtidal area.

The daily prey consumption calculated for each sole species was used to calculate total consumption over the summer (90 days, corresponding to the 3 months considered), taking into account the average density of both species in both nursery areas in the three months

considered. The summer months chosen for the estimation correspond to the period when densities of both soles are higher in the nursery areas.

Results

Two daily peaks in feeding activity were identified for both species (Figure 2). While, *S. solea* presents a narrow feeding peak at 9h and a broader peak between 21h and 0h, *S. senegalensis* presents two broad peaks, one between 6h and 9h and another between 21h and 0h.

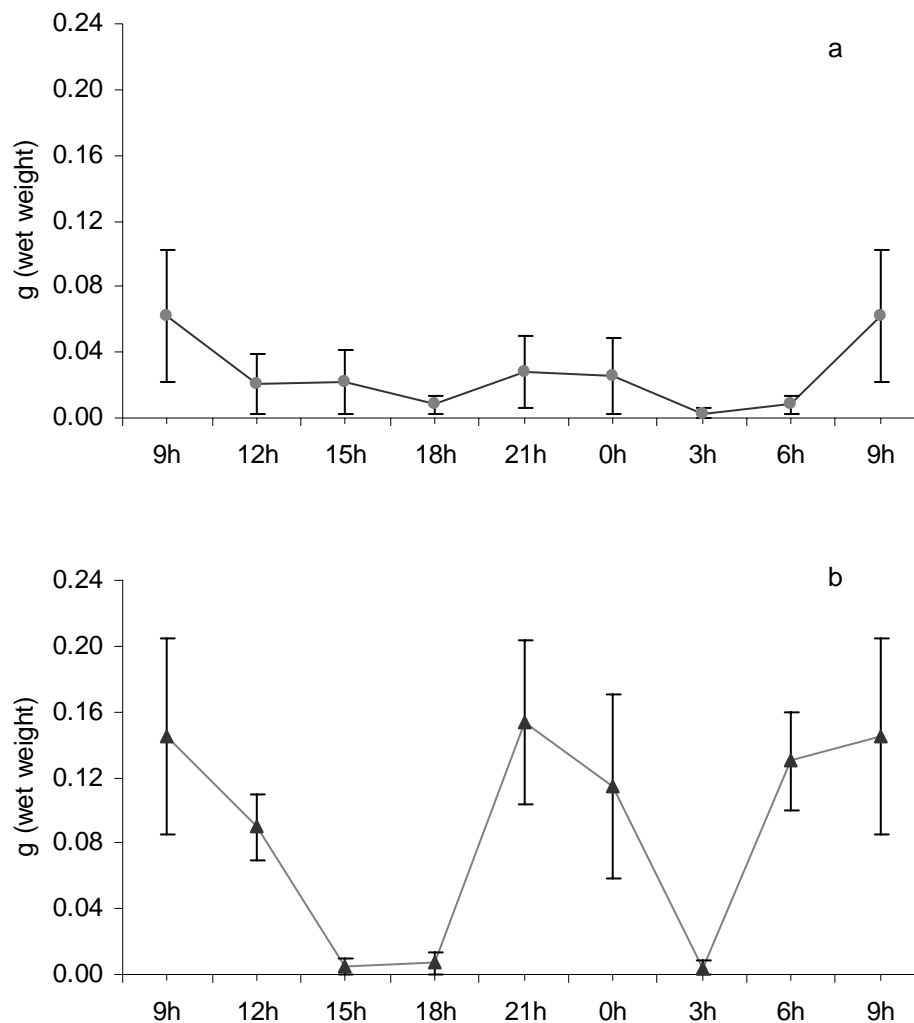


Figure 2 – Mean stomach contents (and standard deviations) of *S. solea* (n = 110 individuals) (a) and *S. senegalensis* (n = 100 individuals) (b) over a 24 h sampling period.

While in *S. solea* the feeding peak registered in the morning is more pronounced than the night peak, in *S. senegalensis* both peaks seem to have the same importance. Peaks were followed by periods when stomach contents were very low. Weight of stomach contents was generally higher in *S. senegalensis* than in *S. solea*. While in *S. solea* the peaks in mean weight

of stomach contents were 0.062 g at 9h and 0.028 at 21h, in *S. senegalensis* they were 0.145 g at 9h and 0.145 at 21h.

Total length of *S. solea* considered in this cycle varied between 81 mm and 150 mm, while *S. senegalensis* total length varied between 95 mm and 150 mm. Mean length of *S. solea* was 119 mm, while that of *S. senegalensis* was 138 mm. Stomach contents were mainly composed of various polychaetes (mainly *Nereis diversicolor*), *S. plana* siphons and amphipods (mainly *Corophium* spp.). Stomach contents amounted to 0.28% of mean total weight for *S. solea* and 0.40% of mean total weight for *S. senegalensis* during the peak consumption periods.

The daily food consumption values estimated using Elliott and Persson (1978) model were 0.030 gd^{-1} (wet weight) for *S. solea* and 0.075 gd^{-1} for *S. senegalensis* (wet weight).

Table 1 – Mean 0-group sole densities (ind.1000 m^{-2}) in June, July and August 1996.

	June	July	August
<i>S. solea</i> (nursery A)	0.18	0.40	1.70
<i>S. senegalensis</i> (nursery A)	0.19	0.31	0.51
<i>S. senegalensis</i> (nursery B)	6.72	0.40	2.41

Mean *S. solea* densities over the summer months varied between 0.18 ind.1000 m^{-2} and 1.70 ind.1000 m^{-2} , while that of *S. senegalensis* varied between 0.19 ind.1000 m^{-2} and 0.51 at nursery A and between 0.40 ind.1000 m^{-2} and 6.72 ind.1000 m^{-2} at nursery B (Table 1). Total consumption of *S. solea* over the three months considered was estimated to be 97 kg. *S. senegalensis* total consumption in area A was estimated to be 103 kg, while in area B it was 528 kg.

Table 2 – Mean prey biomass (g.m^{-2}) in the Tagus estuary nursery areas.

	Subtidal			Intertidal		
	Polychaeta	<i>S. plana</i>	Amphipoda	Polychaeta	<i>S. plana</i>	Amphipoda
Nursery A	4.388	0.010	3.480	1.080	0.424	0.192
Nursery B	4.093	0.005	0.011	1.404	0.720	0.040

Mean prey biomass ranged from 0.040 gm⁻² and 1.404 gm⁻² in the intertidal, and from 0.005 gm⁻² and 4.388 gm⁻² in the subtidal (Table 2). Total prey biomass estimated for area A was 300 tonnes, while for area B it was 58 tonnes.

Discussion

The feeding activity patterns observed in the present study that encompass two distinct peaks of activity, confirm the findings of Lagardère (1987) for 0-group *S. solea*. The peaks reported by Lagardère (1987) were, however, more pronounced, more similar to the ones found for *S. senegalensis* in the present study. This seems to indicate that consumption of *S. solea* in the summer months in the Tagus estuary does not present has intense episodes as at higher latitudes.

Daily food consumption estimated in the present study was considerably higher for *S. senegalensis* than for *S. solea*. Vinagre *et al.* (2007) reported steadily increasing gastric evacuation rates with temperature for *S. senegalensis* and *S. solea*, yet for the late a decline was observed for the highest temperature tested (26°C). It was concluded that *S. solea*, being a temperate species with a metabolic optimum temperature of approximately 19°C (LeFrançois and Claireaux, 2003), possibly suffered thermal stress at this temperature. It is well known that ingestion increases with increasing temperature, reaching a peak at the optimum temperature and declining as temperature approaches the species thermal limit (Jobling, 1993; Yamashita *et al.*, 2001). The low consumption observed in the field in the present study reinforces the idea that *S. solea* may be under thermal stress during the warmer months at subtropical latitudes.

There are no other studies on *S. senegalensis* food consumption in the wild because this species ecology has not yet been thoroughly studied as *S. solea*'s. Other studies exist for *S. solea* and although they followed different experimental approaches, some comparisons can be made. Fonds and Saksena (1977) produced a food consumption model based on excess feeding of captive sole at various temperatures. According to this model daily consumption for 80 mm sole at 26°C is 1.2 g (wet weight of mussel meat). Based on a similar experimental design Fonds *et al.* (1992) produced food consumption models for plaice, *Pleuronectes platessa* (Linnaeus, 1758), and flounder, *Platichthys flesus* (Linnaeus, 1758). Based on these models 5 g plaice daily consumption at 22°C (the highest experimental temperature) would be 3.5 g (wet weight of mussel meat), while that of 5 g flounder would be 0.64 g (wet weight of mussel meat).

The values estimated by Fonds and Saksena (1977) and Fonds *et al.* (1992), albeit for different species are still of the same magnitude, as expected since a similar experimental approach was followed in both studies and because these species are flatfish and have therefore important morphological and physiological similarities.

The daily consumption values provided by the above mentioned studies are considerably higher than those found in the present study. One important issue is that in both studies fish were given excess food. It has been reported that during intense feeding periods *S.*

solea (Lagardère, 1987) and *Pleuronectes platessa* (Kuipers, 1975; Basimi and Grove, 1985) may use the anterior portion of the intestine as an additional food reservoir, where newly-ingested food is transferred, enabling high rates of food intake. This effect was not tested by Vinagre *et al.* (2007) for soles, since the gastric evacuation experiment carried out was based on single meals. Another important issue is that these approaches did not have a field component. Several factors may hinder food consumption in the wild, thus lowering the estimates given by studies with a field component. One of such factors is predator pressure. It has been shown that the presence of a predator may lower up to 10% the feeding activity in *S. senegalensis* (Maia *et al.* unpublished).

The daily consumption value estimated by Lagardère (1987) (0.041 mg dry weight) for 0-group *S. solea* following the Elliott and Persson model using a R of 0.366 and a field component similar to the present study was not very different to the values presented here. A direct comparison is not possible since the values from Lagardère (1987) are given in dry weight and the ones from the present study in wet weight, yet even if we account for a water content higher than 90 %, the values are still of the same magnitude.

The R value used by Lagardère (1987) was estimated experimentally by Durbin *et al.* (1983) for *Merluccius bilinearis*, Mitchell, 1814, and *Gadus morhua* (Linnaeus, 1758). The incorporation into the model of an R estimated for other species, with marked morphological and physiological differences from the species being analysed, as probably lead to some overestimation of food consumption. Another issue that may account for differences is fish size, there's only information on the age group but not on its average size, which could be considerably different.

Prey densities in the sediment of both nurseries were within the ranges reported by other studies, concerning the Tagus estuary (Rodrigues *et al.*, 2006; Silva *et al.*, 2006). Our results seem to indicate that food is not a limiting factor for soles, in the Tagus estuary. Other authors had reached the some conclusion concerning other fish communities (Gee *et al.*, 1985; Raffaelli, 1989). Yet, a more in depth investigation is necessary in order to account for other species food consumption and prey densities fluctuations. Fluctuations in soles densities should also be taken into account. In the present study, important variability was reported for soles densities, ranging from 0.18 ind.1000 m⁻² to 1.70 ind.1000 m⁻² for *S. solea* and from 0.40 ind.1000 m⁻² to 6.72 ind.1000 m⁻² for *S. senegalensis*. This confirms previous investigations that concluded that these species populations present important abundance fluctuations, Cabral and Costa (1999) reported maximum mean densities of 26.0 ind.1000 m⁻² for *S. solea* and 61.6 ind.1000 m⁻² for *S. senegalensis* over a three year period. Prey availability is also an important issue, since some prey may be present in the substrate but not available for all its predators. For instance, it is well known that amphipds, such as *Corophium* spp. have semilunar activity rhythms that affect their probability of being captured by fish (Lawrie and Raffaelli, 1998).

Recent studies indicate that sole feeding rhythms are also influenced by the semilunar cycle (Vinagre *et al.*, 2006b), it would therefore be interesting to conduct 24h sampling in the

different phases of this cycle in order to assess food consumption variation and incorporate it in total food consumption estimates over the broad periods when nurseries are used by juveniles.

These and other contributions to the study of sole juveniles' ecology will certainly provide the necessary information for the fine estimation of food consumption to be incorporated into multi-species food-web models for stock and estuarine management.

Acknowledgements

This study was co-funded by the European Union through the FEDER—Portuguese Fisheries Programme (MARE), as well as by the 'Fundação para a Ciência e a Tecnologia'. Authors would like to thank everyone involved in the field work.

References

- Basimi, R.A., Grove, D.J., 1985. Estimates of daily food intake by an inshore population of *Pleuronectes platessa* L. off eastern Anglesey, North Wales. *Journal of Fish Biology* 27, 505-520.
- Beck, M., Heck, K., Able, K., Childers, D., Egglestone, D., Gillanders, B., Halpern, B., Hays, C., Hoshino, K., Minello, T., Orth, R., Sheridan, P., Weinstein, M., 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* 51, 633-641
- Bowen, S.H., 1987. Dietary protein requirements of fishes – a reassessment. *Canadian Journal of Fisheries and Aquatic Science* 44, 1995-2001.
- Bromley, P.J., 1974. The effects of temperature and feeding on the energetics, nitrogen metabolism and growth of juvenile sole (*Solea solea* (L.)). PhD thesis, University of East Anglia, Norwich. 94 pp.
- Bromley, P.J., 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). *Aquaculture* 19, 359-369.
- Bromley, P.J., 1987. The effects of food type, meal size and body weight on digestion and gastric evacuation in turbot *Scophthalmus maximus* L. *Journal of Fish Biology* 30, 501-512.
- Bromley, P.J., 1994. The role of gastric evacuation experiments in quantifying the feeding rates of predatory fish. *Reviews in Fish Biology and Fisheries* 4, 36-66.
- Cabral, H.N., 1998. Utilização do estuário do Tejo como área de viveiro pelos linguados, *Solea solea* (L., 1758) e *Solea senegalensis* Kaup, 1858, e robalo, *Dicentrarchus labrax* (L., 1758). PhD Dissertation, University of Lisbon, Portugal.
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H., Costa, M.J., 1999. Differential use of nursery within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.

- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Scientia Marina* 53, 561–566.
- Cowey, C.B., Sargent, J.R., 1972. Fish nutrition. *Adv. Mar. Biol.* 10, 383-492.
- Durbin, E.G., Durbin, A.G., Langton, R.W., Bowman, R.E., 1983. Stomach contents of silver hake, *Merluccius bilinearis*, and Atlantic cod, *Gadus morhua*, and estimation of their daily rations. *Fisheries Bulletin* 81, 437-454.
- Elliott, J.M., 1975a. The growth of brown trout (*Salmo trutta* L.) fed on maximum rations. *Journal of Animal Ecology* 44, 805-821.
- Elliott, J.M., 1975b. The growth of brown trout (*Salmo trutta* L.) fed on reduced rations. *Journal of Animal Ecology* 44, 823-842.
- Elliott, J.M., Persson, L., 1978. The estimation of daily rates of food consumption for fish. *Journal of Animal Ecology* 47, 977-993.
- Eggers, D.M., 1979. Comment on some recent methods for estimating food consumption by fish. *Journal of the Fisheries Research Board of Canada* 36, 1018-1020.
- Fonds, M., Saksena, V.P., 1977. The daily food intake of young sole (*Solea solea* L.) in relation to their size and the water temperature. 3rd Meeting of the ICES working group on mariculture, Brest, France. *Actes de colloques du CNEXO* 4, 51-58.
- Gee, J.M., Warwick, R.M., Davey, J.T., George, C.L., 1985. Field experiments on the role of epibenthic predators in determining prey densities in an estuarine mudflat. *Estuarine, Coastal and Shelf Science* 21, 429-448.
- Gerking, S.D., 1962. Production and food utilization in a population of bluegill sunfish. *Ecol. Monogr.* 32, 31-78.
- Haedrich, R.L., 1983. Estuarine Fishes. In: Ketchum B (ed) *Ecosystems of the World* 26 Estuarine and Enclosed Seas. Elsevier, Amsterdam.
- Hewett, S.W., 1989. Ecological applications of bioenergetics models. *Am. Fish. Soc. Symp.* 6, 113-120.
- Jaquet, N., Rafaelli, D., 1989. The ecological importance of the sand goby *Pomatochistus minutus* (Pallas). *Journal of Experimental Marine Biology and Ecology* 128, 147-156.
- Jobling, M., 1981. Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. *Journal of Fish Biology* 19, 245-257.
- Jobling, M., 1982. Food and growth relationships of the cod, *Gadus morhua* L., with special reference to Balsfjorden, north Norway. *Journal of Fish Biology* 21, 357-371.
- Jobling, M., 1988. A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. *Aquaculture* 70, 1-19.
- Jobling, M., 1993. Bioenergetics: feed intake and energy partitioning. In: *Fish Ecophysiology* Eds: J.C., Rankin, F.B., Jensen, London, U.K. 309pp.
- Jones, R., Hislop, J.R.G., 1978. Further observations on the relation between food intake and growth of gadoids in captivity. *J. Cons. int. Explor. Mer* 38, 244-251.

- Lawrie, S.M., Raffaelli, D.G., 1998. In situ swimming behaviour of the amphipod *Corophium volutator* (Pallas). *Journal of Experimental Marine Biology and Ecology* 224, 237-251.
- LeFrançois C., Claireaux G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole, *Solea solea*. *Marine Ecology Progress Series* 259, 273-284.
- Kuipers, B., 1975. Experiments and field observations on the daily food intake of juvenile plaice, *Pleuronectes platessa* L., In Proc. 9th Europ. mar. biol. Symp. (H. Barnes ed.), pp. 1-12, Aberdeen: Aberdeen University Press.
- Maia, A., Vinagre, C., Cabral, H.N. (submitted) Foraging behaviour of *Solea senegalensis* Kaup, 1858, in the presence of a potential predator, *Carcinus maenas* Linnaeus, 1758.
- Mann, K.H., 1965. Energy transformation by a population of fish in the River Thames. *Journal of Animal Ecology* 34, 253-275.
- Miller, J.M., Crowder, L.B., Moser, M.L., 1985. Migration and utilization of estuarine nurseries by juvenile fishes: an evolutionary perspective. *Contrib. Mar. Sci.* 27, 338-352.
- Phil, L., 1985. Food selection and consumption of mobile epibenthic fauna in shallow marine areas. *Marine Ecology Progress Series* 22, 169-179.
- Rafaelli, D., Conacher, A., McLachlan, H., Emes, C., 1989. The role of epibenthic crustacean predators in an estuarine food web. *Estuarine, Coastal and Shelf Science* 28, 149-160.
- Rodrigues, A.M., Meireles, S., Pereira, T., Gama, A., Quintino, V., 2006. Spatial patterns of benthic macroinvertebrates in intertidal areas of a southern European estuary: the Tagus, Portugal. *Hydrobiologia* 555, 99-113.
- Silva, G., Costa, J.L., Almeida, P.R., Costa, M.J., 2006. Structure and Dynamics of a Benthic Invertebrate Community in an Intertidal Area of the Tagus Estuary, Western Portugal: A Six Year Data Series. *Hydrobiologia* 555, 115-128.
- Smith, M.A.K., Thorpe, A., 1976. Nitrogen metabolism and trophic input in relation to growth in freshwater and saltwater *Salmo gairdneri*. *Biol. Bull. mar. biol. Lab. Woods Hole* 150, 139, 151.
- Solomon, D.J., Bradfield, A.E., 1972. The energetics of feeding, metabolism and growth of perch (*Perca fluviatilis* L.). *Journal of Animal Ecology* 41, 699-718.
- Thorpe, J.E., 1977. Daily ration of adult perch, *Perca fluviatilis* L. during summer in Loch Leven, Scotland. *Journal of Fish Biology* 11, 55-68.
- Tyler, A.V., 1970. Rates of gastric emptying in young cod. *Journal of the Fisheries Research Board of Canada* 27, 1177-1189.
- Vinagre, C., Fonseca, V., Cabral, H., Costa, M.J. 2006a. Habitat suitability index models for the juvenile soles, *Solea solea* and *Solea senegalensis*: defining variables for management. *Fisheries Research* 82, 140-149.
- Vinagre, C., França, S., Cabral, H.N. 2006b. Diel and semi-lunar patterns in the use of an intertidal mudflat by juveniles of Senegal sole, *Solea senegalensis* Kaup, 1858. *Estuarine, Coastal and Shelf Science* 69, 246-254.

- Vinagre, C., Maia, A., Cabral, H.N. 2007. Effect of temperature and salinity on the gastric evacuation of the juvenile soles *Solea solea* and *Solea senegalensis*. *Journal of Applied Ichthyology* 23, 240-245.
- Yamashita, Y., Tanaka, M., Miller, J. M., 2001. Ecophysiology of juvenile flatfish in nursery grounds. *Journal of Sea Research* 45, 205-218.

Conclusions

The experimental work on gastric evacuation and feeding behaviour included in this chapter, and its application to wild populations allowed for the first estimation of food consumption of the juvenile sole using the Tagus estuary nursery grounds.

It was also concluded that both temperature and salinity have an important effect on gastric evacuation in *S. solea* and *S. senegalensis*. While temperature increased evacuation rates in both species (although not at 26°C in *S. solea*), the effect of low salinity differed among species, leading to a decrease in gastric evacuation rate in that of *S. senegalensis* and an increase in *S. solea*.

The effect of the 26°C experimental temperature on *S. solea*'s gastric evacuation was discussed and it was concluded that it was possibly evidence of thermal stress, since the metabolic optimum temperature for this species is much lower (approximately 19°C). This effect was not observed in *S. senegalensis*, probably because this is a tropical species with different thermal limits. Thus, *S. solea* may be at a disadvantage during the summer months when juveniles of both sole species concentrate in shallow waters, rich in prey but where temperature warms up well above its metabolic optimum.

The results concerning the effect of salinity were quite interesting given that, when in sympatry, *S. solea* seems to prefer lower salinity habitats than *S. senegalensis*. A different level of adaptation to low salinity is probably the most important factor determining these species partition of space within the nursery area.

Results indicate that estuarine nurseries provide better conditions than coastal nurseries for *S. solea* in terms of salinity, yet in southern European estuarine systems it will endure summer temperatures that lead to thermal stress, since estuarine waters are warmer than coastal waters in that period. For *S. senegalensis* estuarine nurseries provide favourable temperatures during the nursery period, yet low salinity decreases its' gastric evacuation rate. Habitat choice by both species will depend on the cost-benefit relation of the available habitats. The sum of all advantages provided by estuarine nurseries will certainly play an important role in this process.

The behaviour experiment revealed that the presence of a predator strongly impacts the foraging activity of sole in the presence of prey with a 10% decrease in overall activity, when compared to sole's activity in the presence of only food. Crawling and tapping were the activities most correlated with foraging. Rapid escape response occurred when the predator was present independently of the presence of food. There was also evidence that in *S. senegalensis* the recognition of predators is visual, while that of prey is mainly olfactory.

The estimated daily food consumption was considerably higher for *S. senegalensis* than for *S. solea*. The feeding activity patterns observed encompassed two distinct peaks of activity, they were, however, more pronounced for *S. senegalensis* than for *S. solea*. Studies with *S.*

solea at higher latitudes found pronounced peaks of feeding activity, which seems to indicate that consumption of *S. solea* in the summer months in the Tagus estuary is hindered, possibly by thermal stress, like observed in the gastric evacuation experiments. Prey abundance estimations indicate that food is not a limiting factor for soles, in the Tagus estuary, similarly to what had been observed in other estuarine systems. Yet, more studies concerning prey density variation, as well as, consumption by other benthic predators are needed in order to accurately determine food availability and partitioning in the Tagus estuary. The high inter-annual variation in soles' densities should also be taken into account.

The information on sole juveniles' feeding ecology provided in this chapter can be incorporated into future multi-species food-web models for stock and estuarine management.

CHAPTER 4

- GROWTH AND CONDITION -

Growth variability of juvenile soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, based on condition indices in the Tagus estuary, Portugal. *Journal of Fish Biology*, 2006, 68: 1551-1562.
By Fonseca, V., C. Vinagre, H. Cabral

Habitat specific growth rates and condition indices for the sympatric soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA-DNA ratio. *Journal of Applied Ichthyology (in press)*
By Vinagre, C., Fonseca, V., Maia, A., Amara, R., Cabral, H.N.

Latitudinal variation in spawning period and growth of 0-group sole, *Solea solea* (L.). (*submitted*)
By Vinagre, C., Amara, R., Maia, A., Cabral, H.N.

Introduction

Growth has been one of the most intensely studied aspects of fish biology. Growth is continuous in most fish species and is regarded as a good indicator of fish health (Lagler *et al.*, 1977; Moyle and Cech, 1996). Because growth is influenced by many factors it is considered to be an integrative indicator of the overall health of the individuals or populations and a reflection of the environmental conditions of the habitat where fish live. Fish growth is thus also a good indicator of habitat quality (Gibson, 1994; Phelan *et al.*, 2000).

Positive growth (an increase in length or weight over time) indicates a positive energy balance in metabolism, meaning that the rate of anabolism exceeds that of catabolism (Moyle and Cech, 1996). Anabolic processes are regulated by hormones; however growth rates of fish are greatly dependent on various interacting environmental factors, such as temperature, dissolved oxygen, salinity, ammonia, photoperiod, among others. These environmental factors will, in turn, interact with other factors such as competition, quantity and quality of food, toxicity of dissolved chemicals, as well as, the age and state of maturity of the fish (Moyle and Cech, 1996).

In the marine environment, predation is mainly related to the size of the individuals (Cushing, 1975). Fast growth of juvenile fish potentially increases individuals' survival chances because less time is spent at the more vulnerable sizes (Sogard, 1992, 1997; Able *et al.*, 1999). It is in this context that estuaries play an important role in the life cycle of many fish species, because of their high food availability, low number of predators and in particular high water temperature, during the most important period for juvenile fish (e.g. McLusky, 1989).

Growth of juvenile sole in the Tagus estuary nursery grounds was previously investigated by Dinis (1986), Costa (1990) and Cabral (2003), the later reported higher growth rates than found at higher latitudes (Rogers, 1994, Amara *et al.*, 2001, Amara, 2004), certainly because of the higher temperatures of the Tagus estuary waters. Previous studies in the Tagus also referred the existence of various cohorts colonizing the estuary over time, which generally does not happen in northern European areas.

The present chapter focused on the study of growth and condition in the successive cohorts of *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, colonizing the Tagus estuarine nurseries, in the comparison of habitat quality of the two nurseries and in the analysis of growth and spawning in a latitudinal perspective.

The first work "Growth variability of juvenile soles *Solea solea* and *Solea senegalensis*, and comparison with RNA-DNA ratios in the Tagus estuary, Portugal" aims to determine the growth variability of juvenile soles *S. solea* and *S. senegalensis* based on absolute growth rates, estimated by modal progression analyses, and compare it to RNA-DNA ratios. Little is known about multi-cohort colonization of estuarine nurseries, mainly because in the northern European areas, where most of the investigation on flatfish has been carried out, there is only a

narrow peak of spawning that results in just one cohort. Yet, the different cohorts immigrating towards the Tagus nurseries will face quite different environmental conditions in the nurseries depending on the time of arrival.

The second work “Habitat specific growth rates and condition indices for the sympatric soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA-DNA ratio” assessed habitat quality through the comparison of growth rates and condition in the two estuarine nurseries and discussed the use of both methodologies as tools for habitat quality monitoring. Ecological monitoring will be crucial in the achievement of informed estuarine management in the future. Examination of otolith daily increments for the estimation of growth rates was applied to the juveniles of both species. This method had been previously validated for *S. senegalensis* by Ré *et al.* (1988) and for *S. solea* by Lagardère and Troadec (1997), it had also been already applied to other sole populations (*e.g.* Amara *et al.*, 1994; Amara, 1995). It allows a more precise estimation of growth rate when compared to modal progression analyses of length, since the later presents some problems, such as, difficulties in clearly identifying modal components and the misclassification of slow growers.

The third work “Latitudinal comparison of spawning period and growth of 0-group sole, *Solea solea* (L.)” aims at assessing latitudinal differences in timing of spawning, and growth rates of *S. solea* juveniles following settlement in the nursery grounds. The general assumption that the main factors contributing to higher growth rates and earlier spawning are higher temperatures and photoperiod make it pertinent to investigate if there is a latitudinal trend. Studies using the exact same methodology for the determination of growth rates and back-calculation of spawning dates were carried out in the Tagus estuary (38°N), in the Douro estuary (41°N), in the Vilaine estuary (47°N) and in the eastern English Channel (49°N). Data from these studies was further compared with data from a revision of published studies, in order to investigate the existence of a latitudinal trend in growth rates and spawning time.

References

- Able, K. W., Manderson, J. P., Studholme, A., 1999. Habitat quality for shallow water fishes in an urban estuary: the effects of man-made structures on growth. *Marine Ecology Progress Series* 187, 227–235.
- Amara, R., 1995. La sole, *Solea solea* (L.), du Golfe de Gascogne: métamorphose, transfert des larves vers la cote et colonisation des nourriceries. Ph.D. Thesis, Université de Paris, Paris.
- Amara, R. 2004. 0-group flatfish growth conditions on a nursery ground (Bay of Canche, Eastern English Channel). *Hydrobiologia* 518, 23-32.
- Amara, R., Desaunay, Y., Lagardère, F., 1994. Seasonal variation in growth of larval sole *Solea solea* (L.) and consequences on the success of larval immigration. *Netherlands Journal of Sea Research* 32, 287-298.

- Amara, R., Laffargue, P., Dewarumez, J.M., Maryniak, C., Lagardère, F., Luczac, C., 2001. Feeding ecology and growth of 0-group flatfish (sole, dab and plaice) on a nursery ground (Southern Bight of the North Sea). *Journal of Fish Biology* 58, 788-803.
- Andrade, J.P., 1992. Age, growth and population structure of *Solea senegalensis* Kaup 1858 (Pisces, Soleidae) in the Ria Formosa (Algarve, Portugal). *Scientia Marina* 56, 35–41.
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the United Kingdom* 83, 861–868.
- Costa, M.J., 1990. Age and growth studies of the sole (*Solea vulgaris vulgaris* Quensel, 1806) in the Tagus estuary, Portugal. *Boletim do Instituto Nacional de Investigação das Pescas* 15, 63-67.
- Dinis, M.T., 1986. Quatre soleidae de l'estuaire du Tage. Reproduction et croissance. Essai d'élevage de *Solea senegalensis* Kaup. PhD Thesis, Université de Bretagne Occidentale, Brest, France.
- Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. *Netherlands Journal of Sea Research* 32, 191-206.
- Lagardère, F., Troadec, H., 1997. Age estimation in common sole *Solea solea* larvae: validation of daily increments and evaluation of a pattern recognition technique. *Marine Ecology Progress Series* 155, 223-237.
- McLusky, D.S., 1989. *The estuarine ecosystem*. 2nd edition. Blakie, London, UK.
- Moyle, P.B., Cech, J.J.J., 1996. *Fishes, an introduction to Ichthyology*, 3rd edn. Prentice Hall, N.J.
- Ré, P., Rosa, H.C., Dinis, M.T., 1988. Daily microgrowth increments in otoliths of *Solea senegalensis* Kaup larvae *Ciênc. Biol. Ecol. Syst.* 8, 37-41.
- Rogers, S.I., 1994. Population density and growth rate of juvenile sole *Solea solea* (L.) *Neth J Sea Res* 32, 353-360.
- Sogard, S.M., 1992. Variability in growth rates of juvenile fishes in different estuarine habitats. *Marine Ecology Progress Series* 85, 35–53.
- Sogard, S.M., 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* 60, 1129–1157.

Growth variability of juvenile soles *Solea solea* and *Solea senegalensis*, and comparison with RNA-DNA ratios in the Tagus estuary, Portugal

Abstract: Growth variability and condition of juvenile soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, were assessed through RNA:DNA estimates and compared to absolute growth rates. Higher mean cohort RNA:DNA ratios were observed for cohort I at the beginning of estuarine occurrence for both species (4.42 and 4.87, for *S. solea* and *S. senegalensis* respectively). Despite different estuarine colonization habits, no significant differences were observed between RNA:DNA monthly variation for both sole species within the same year ($P > 0.05$ for 2003 and 2004). Juvenile *S. senegalensis* showed significant differences between RNA-DNA ratios obtained for the two nursery areas ($P < 0.001$). The decrease of seasonal growth rates with fish age was similar to seasonal variation of mean RNA:DNA values. Thus the RNA:DNA pattern of juvenile *S. solea* and *S. senegalensis* reflected growth and estuarine colonization patterns.

Key-words: Growth variability; Nutritional condition; RNA-DNA ratio; *Solea senegalensis*; *Solea solea*.

Introduction

Early life stages of marine fishes are generally characterized by high and variable mortality rates, which will affect recruitment to the adult population. Small fluctuations in growth and survival rates during this period can be magnified when considering year-class strength (Houde, 1987; Van der Veer *et al.*, 1990; Myers and Cardigan, 1993). Therefore suitable biotic and abiotic conditions for young fishes to settle and grow are essential to ensure that a significant number of individuals enter the reproductive population. Habitats such as estuaries that provide such suitable growth conditions, namely high food abundance, refuge from predators and high water temperature, serve as important nursery grounds for many marine fish species of commercial interest (Haedrich, 1983; Beck *et al.*, 2001).

Faster growth of juvenile fishes potentially increases individuals' survival probability, because less time is spent at more vulnerable sizes and therefore, they are more likely to overcome the hardship of the following less favourable season (Sogard, 1992, 1997; Able *et al.*, 1999). How environmental variability influences individual fishes has been linked to several individual changes at a molecular level (e.g. cortisol levels, nucleic acid content and enzymatic activity) (Weber *et al.*, 2003). Hence, it is of great importance to directly determine the individual-environmental linkage on short time scales, especially for estuarine dependent species, because of the dynamic nature of these environments (e.g. river flow and tidal cycle) (Haedrich, 1983). Nucleic acid quantification and subsequent RNA-DNA ratios have been used

in numerous studies as condition indices, in order to assess nutritional condition and growth of larvae and juvenile fishes (Buckley, 1984; Richard *et al.*, 1991; Gwak and Tanaka, 2001).

This biochemical index reflects variations in protein synthesis rates (thus recent growth) as RNA concentration fluctuates both with food accessibility and protein requirement, while DNA somatic content remains relatively constant for each species, thus providing a recent picture of overall fish condition (Bulow, 1970; Buckley and Bulow, 1987). Mainly for larval stages, RNA:DNA values have been positively correlated with recent growth (Westerman and Holt, 1994), food availability (Clemmesen, 1994) and water temperature (Rooker and Holt, 1996), and through estimated growth rate, were used to assess the suitability of different estuarine habitats (Yamashita *et al.*, 2003).

Two species of sole, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, use the Tagus estuary as a nursery ground and represent c. 60% of juvenile fish abundance in this area. Both species are commercially important, being highly exploited by local and coastal fisheries (Costa and Bruxelles, 1989). A multicohort population structure for both sole species has been described in several studies (Dinis, 1986; Andrade, 1992; Cabral, 2003) and is usually associated with different nursery colonization processes (such as river flow and wind regime) and with prolonged spawning periods that induce several pulses of new recruits over 1 year (Marchand, 1991).

S. solea 0-group juveniles enter the estuary in early spring, grow fast until late summer when they migrate to coastal areas, while 0-group *S. senegalensis* colonize the estuary from June to August and only leave the nursery grounds generally in the following spring or summer (Cabral, 2003). Although niche overlap occurs, it is limited to a short period and a small area, and the differential pattern of habitat usage minimizes interspecific competition between juveniles (Cabral and Costa, 1999; Cabral, 2000, 2003).

Previous studies reported higher growth rates of juvenile soles in the Tagus estuary when compared to other important European estuaries (Cabral, 2003). Cabral (2003) also outlined differences in growth rates between cohorts and period of estuarine use. Juveniles of both species had higher growth rates for cohort I and for the beginning of the estuarine colonization period. The aim of the present study was to determine the growth variability of juvenile soles *S. solea* and *S. senegalensis* based on absolute growth rates, estimated by modal progression analyses, and compare it to RNA-DNA ratios.

Materials and methods

Study area and sampling procedures

The Tagus estuary is a partially mixed estuary, located in the north-eastern Atlantic temperate region. With a total area of 320 km² and a tidal range of 4 m, this system serves as a nursery ground for numerous marine fish species of commercial interest (Cabral and Costa, 1999). Two main nursery areas (A and B) have been identified for juvenile soles, located in the

upper shallower section of the estuary (<10 m deep) and bordered by salt marshes (Fig. 1) (Costa and Bruxelles, 1989; Cabral and Costa, 1999).

Juvenile soles were captured monthly from May 2003 to July 2004. The two nursery grounds were sampled using a 4 m beam trawl, with a 10 mm mesh-size and a bottom tickler chain to increase capture efficiency. Trawls were conducted at daylight and at low water at a speed of 1.85 km h^{-1} (1 knot) for 15 min. A minimum of five trawls were conducted per month at each nursery area. All *S. solea* and *S. Senegalensis* individuals captured were measured (total length, LT, to the nearest mm) and weighed (wet mass to 0.01 g).

A section of the posterior white muscle was removed (except for very small sized fishes, where all the muscle was extracted) and immediately frozen in liquid nitrogen. The muscle samples were later freeze dried and kept sealed in a freezer at -20°C . Based on the monthly-length distribution, 30 individuals were selected for nucleic acid quantification (except for months when juvenile soles were not captured and for monthly captures of <30 individuals, in which case all individuals collected were analysed).

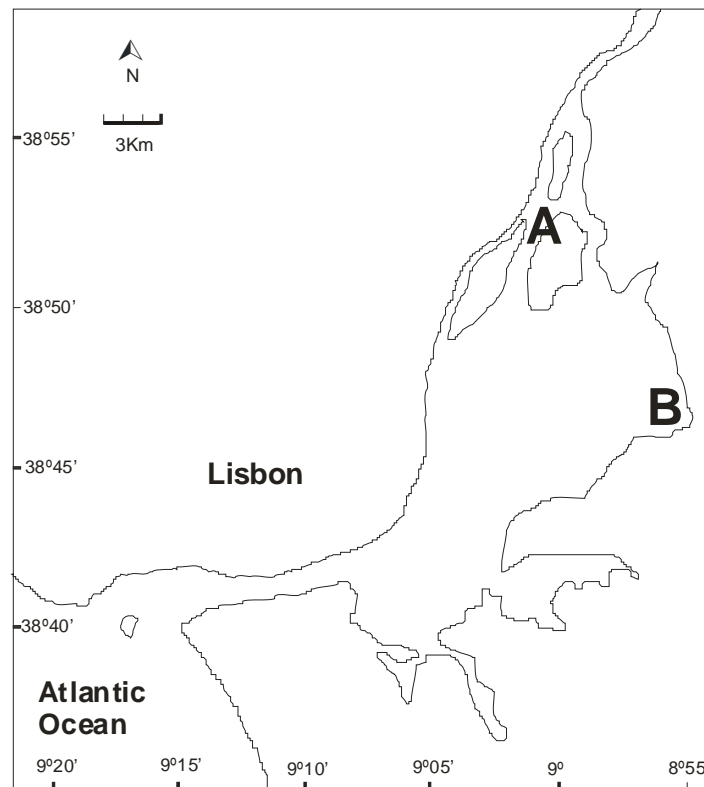


Figure 1 - Location of the nursery areas where juvenile sole were captured.

Nucleic acid determination

Nucleic acid quantification was carried out with the fluorometric method described by Caldaroni *et al.* (2001). Prior to the routine use of this procedure, several assays were performed to calibrate and standardize the method to the species being studied and to the

equipment used. Thus, detection limits, standard calibration curves for RNA and DNA and spike recovery of homogenate samples ($n = 3$), were first determined with a series of dilutions of pure calf thymus DNA (Calbiochem) and 18S- and 28S-rRNA (Sigma). Tissue sample autofluorescence and residual fluorescence were also analysed, the latter by adding 1 U ml⁻¹ DNase ($n = 3$) (Sigma). Concentration of stock standard RNA and DNA solutions were first checked with an UV-spectrophotometer. To ensure sample reproducibility, two 20 mg (dry mass) replicates of each juvenile sole were analysed. Reagents and sample volumes were adjusted to the cuvette spectrofluorometre used. The white muscle was homogenized through short-term ice-sonication with 200 ml of 1% sarcosine solution (N-lauroylsarcosine), and then diluted with 1.8 ml Tris-EDTA buffer (Trizma, pH 7.5) (sarcosine final concentration of 0.1%).

Total nucleic acid fluorescence (RNA and DNA) was measured by adding 300 ml sample homogenate, 1.8 ml Tris-EDTA and 150 ml ethidium bromide (EB, 1 mg ml⁻¹) to the first vial. DNA fluorescence was determined by digesting RNA content with 150 ml RNase (A from bovine pancreas, 20 U ml⁻¹ incubated at 37°C for 30 min, Sigma) in the second vial containing 300 ml sample homogenate, 1.65 ml Tris-EDTA and 150 ml EB. Excitation and emission wavelengths used were 360 and 600 nm, respectively. RNA fluorescence was determined by subtracting the DNA fluorescence reading (second reading) from the total fluorescence value (first reading). RNA and DNA content in tissue samples was calculated through calibration curves constructed previously plus the dilution factors used.

Statistical analysis

Monthly L_T -frequency distributions were determined for both species. Growth was estimated through modal progression analysis of L_T distributions, based on the Bhattacharya method (Bhattacharya, 1967). The software used in this analysis was FISAT II, version 1.1.2, (FAO, 2002). Absolute growth rate (G_A) of young soles was determined as follows: $G_A = (L_{T2} - L_{T1}) / (t_2 - t_1)$ where L_{T1} and L_{T2} correspond to total length at times t_1 and t_2 .

Mean monthly condition indices of cohorts were determined based on individual RNA:DNA values of the juveniles included in each cohort. Mean \pm S.D. cohort L_T was the length range considered for each cohort, without overlap between different cohorts. Tukey-type multiple comparisons tests were used to compare RNA-DNA ratios, according to procedures described by Zar (1996). Comparisons were made for intra-cohort variation (RNA-DNA ratios from juvenile sole belonging to one species and to the same cohort for the time interval considered), for inter-cohort variation (within different cohorts of juvenile sole from one species), and finally between the two species for the 2 years. The null hypothesis was the equality of RNA-DNA ratios, for a significance level of 0.05.

Results

Several cohorts were identified for both species, although modal components were not the same for the 2 years considered (Figs 2 and 3). Juvenile *S. solea* entered the Tagus estuary in

April to May and returned to the sea in late September. In the first year, only in August were two cohorts identified, while in the second year three cohorts were observed (Fig. 2). For *S. senegalensis*, two age-groups (0-group and 1-group) were present in both years (September 2003 and April 2004). Juvenile estuarine usage occurred during a wider period, from April to September, with higher juvenile abundance towards the end of summer (September and October). Once again a polymodal composition was observed for L_T frequency distributions, with three 0-group cohorts identified in 2004.

Difficulties with following the monthly progression of some cohorts in both species were caused by low sample sizes and also by close mean L_T of modal components, which restricted the absolute growth rate estimates. *S. solea* absolute growth rates ranged from 0.53 to 1.19 mm day⁻¹, whereas for *S. senegalensis* they varied from 0.40 to 1.38 mm day⁻¹ (Fig. 4a).

Higher growth estimates were observed for 0-group cohort I in both species, namely from April to May for *S. solea* and September to October for *S. senegalensis*. During the second year, G values for the other two *S. solea* cohorts were lower for the second cohort in comparison to cohort III, despite initial similar L_T (Fig. 2).

For *S. senegalensis* there was a considerable difference between cohorts I with similar mean L_T in the two consecutive years, although they correspond to different months. RNA-DNA ratios varied with cohort, month and year for both sole species. The higher mean cohort RNA-DNA ratios were observed for cohort I in the first month of juvenile colonization for both species (4.42 and 4.87 in April 2004, for *S. solea* and *S. senegalensis*, respectively (Fig. 4b, c)).

In subsequent months RNA-DNA ratios decreased for the first cohort, as new cohorts that entered the estuary had higher values by comparison with cohort I. Hence, on a monthly basis, whenever more than one cohort was present, younger juveniles belonging to newly arrived cohorts, had higher RNA-DNA ratios than earlier cohorts (August 2003 and May, and June and July 2004).

This trend always occurred except in November 2003 for juvenile *S. senegalensis*, when cohort II had a lower RNA:DNA mean value than cohort I, probably due to the small sample size of cohort II. Juvenile (1-group) *S. senegalensis* showed quite low RNA-DNA mean ratios that ranged from 1.79 to 0.05. *S. solea* RNA:DNA intra-cohort variation was assessed for cohort I for both years, and the first month considered (May 2003 and April 2004, Fig. 4b) was always significantly different from the following months (Tukey HSD homogeneous tests, d.f. = 36 and 22, $P < 0.001$ and $P < 0.05$).

Intra-cohort RNA-DNA ratio for cohorts II and III in 2004 also showed significant differences between the 2 months when these cohorts were present (Tukey tests, d.f. = 33 and 25, $P < 0.001$ and < 0.05). Significant differences between the three *S. solea* cohorts in 2004 were observed only for cohort I (Tukey test, d.f. = 84, $P < 0.001$), but not between cohorts II and III.

When comparing mean RNA:DNA values for *S. solea* and for the 2 years, they also differed significantly (Tukey test, d.f. = 208, $P < 0.001$). For *S. senegalensis* RNA-DNA ratios

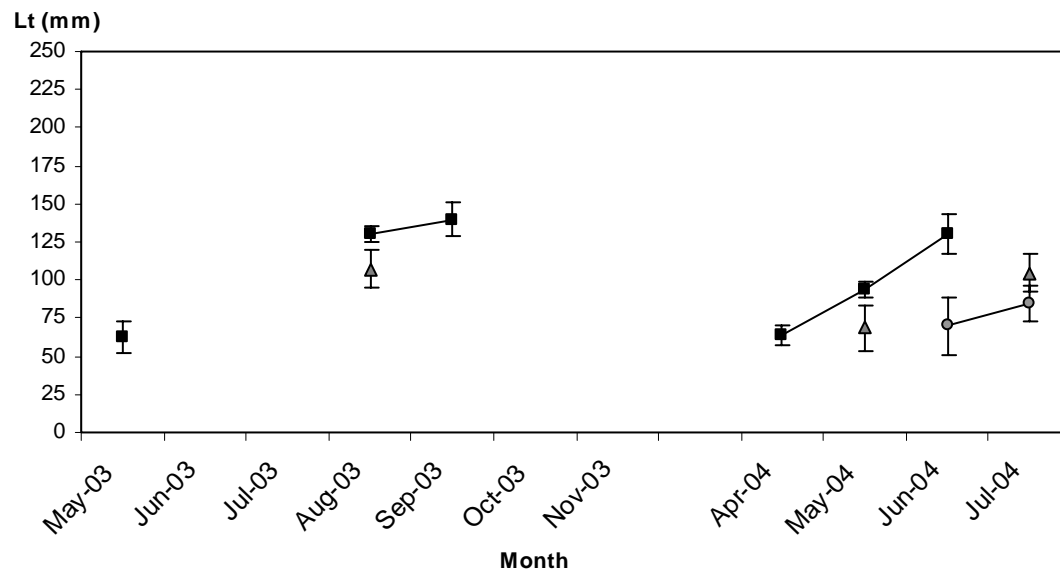


Figure 2 - Cohort mean total length (mm) and standard deviation *per* month of 0-group *Solea solea* juveniles: —■— cohort I, —▲— cohort II and —●— cohort III.

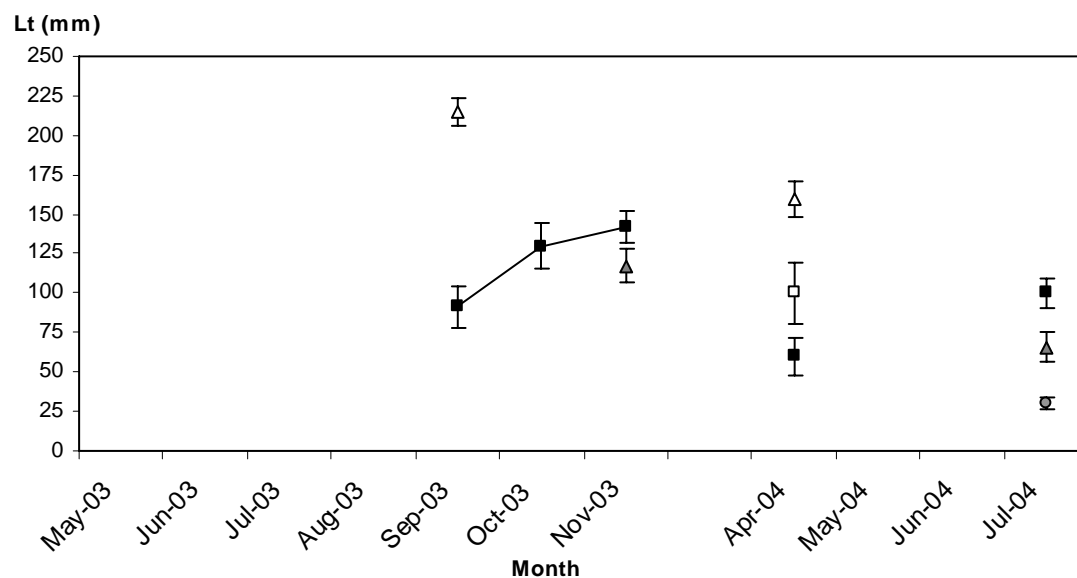


Figure 3 – Cohort mean total length (mm) and standard deviation *per* month, of juvenile *Solea senegalensis*: —■— 0-group/cohort I, —▲— 0-group/cohort II, —●— 0-group/cohort III, —□— 1-group/cohort I and —△— 1-group/cohort II.

also revealed intra-cohort variation for cohorts I and II in 2003 and 2004 respectively (Tukey HSD test, d.f. = 40 and 14, $P < 0.05$ and $P < 0.01$). As for *S. solea* the first month of estuarine occurrence had different mean RNA-DNA ratios (higher values, Fig. 4c) compared with subsequent months. As noticed for *S. solea*, variation of RNA-DNA ratios in *S. senegalensis* between years was significantly different (Tukey tests, d.f. = 124, $P < 0.001$).

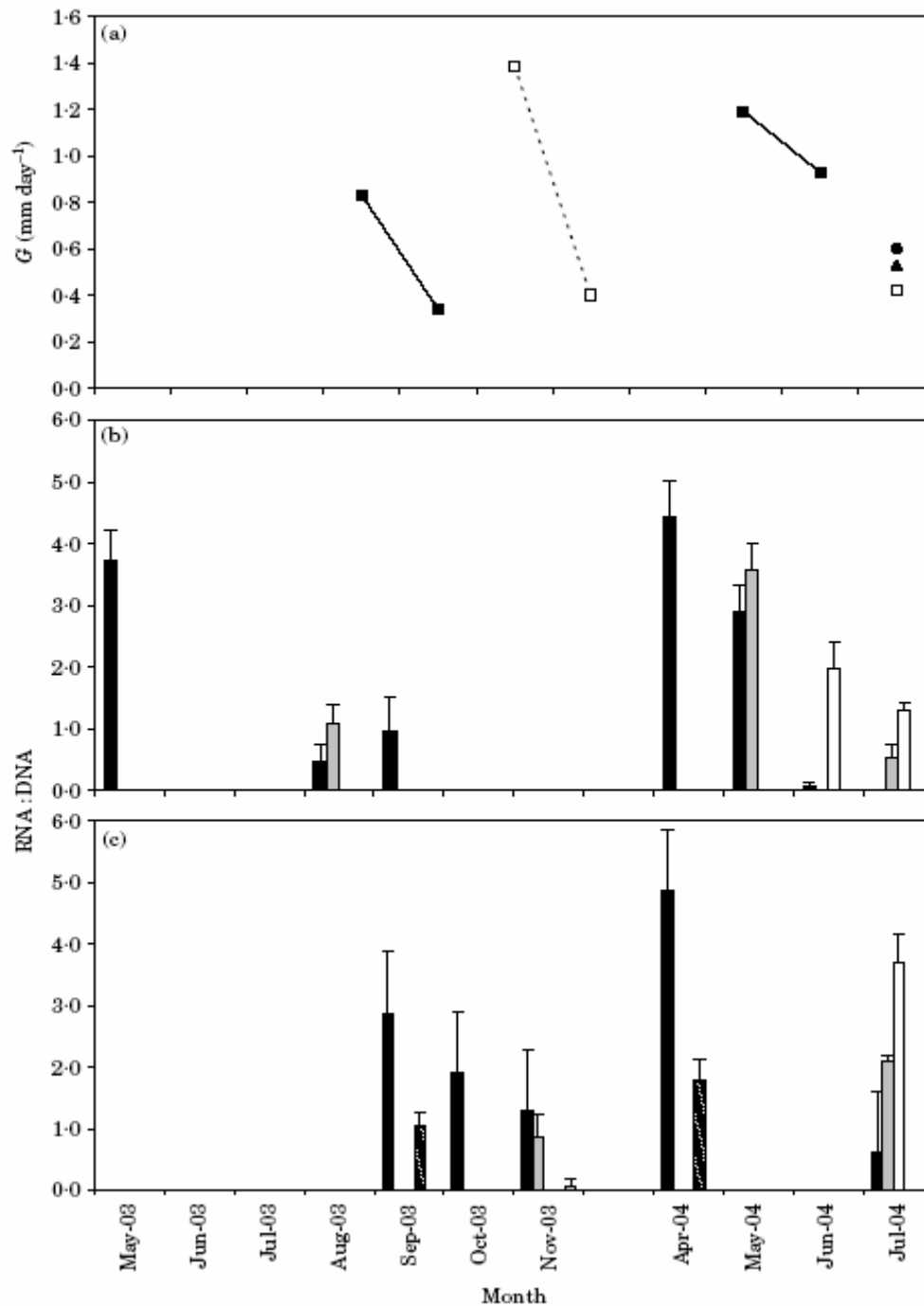


Figure 4 – Absolute growth rates (mm day^{-1}) and RNA-DNA mean ratios (\pm s.d.) for the different cohorts identified for both sole species in 2003 and 2004: **4.a** AGR values (mm day^{-1}) for \blacksquare *S. solea* cohort I, \square *S. senegalensis* cohort I, \blacktriangle *S. solea* cohort II and \bullet *S. solea* cohort III; **4.b** RNA-DNA mean ratios (\pm s.d.) for 0-group *S. solea* juveniles: \blacksquare cohort I, \square cohort II and \square cohort III; **4.c** RNA-DNA mean ratios (\pm s.d.) for juvenile *S. senegalensis*: \blacksquare 0-group/cohort I, \square 0-group/cohort II, \square 0-group/cohort III, \blacksquare 1-group/cohort I and \square 1-group/cohort II.

The periods compared for both years however were not the same. For both species equality of RNA-DNA ratios was tested for each year, and since no significant differences were observed, RNA:DNA-based condition indices revealed a similar trend over a 1 year period (Tukey tests, d.f. = 184 and 148 for 2003 and 2004 respectively, $P > 0.05$).

The mean RNA:DNA monthly variation over the 2 years considered (Fig. 5), reveals a similar pattern of mean RNA:DNA variation with estuarine occurrence, for both *S. solea* and *S. senegalensis*. The observed trend reflects the decrease in RNA-DNA ratios with time spent in estuarine areas, therefore with fish age and L_T .

The mean RNA-DNA of *S. senegalensis* is also compared between the two different nursery habitats used by these juveniles. Although it refers only to two months in different years (September 2003 and July 2004), in both periods juvenile sole from Vila Franca de Xira had a higher RNA-DNA mean ratio, which was significantly different from juveniles present in Alcochete (Tukey tests, d.f. = 35 and 16, $P < 0.05$).

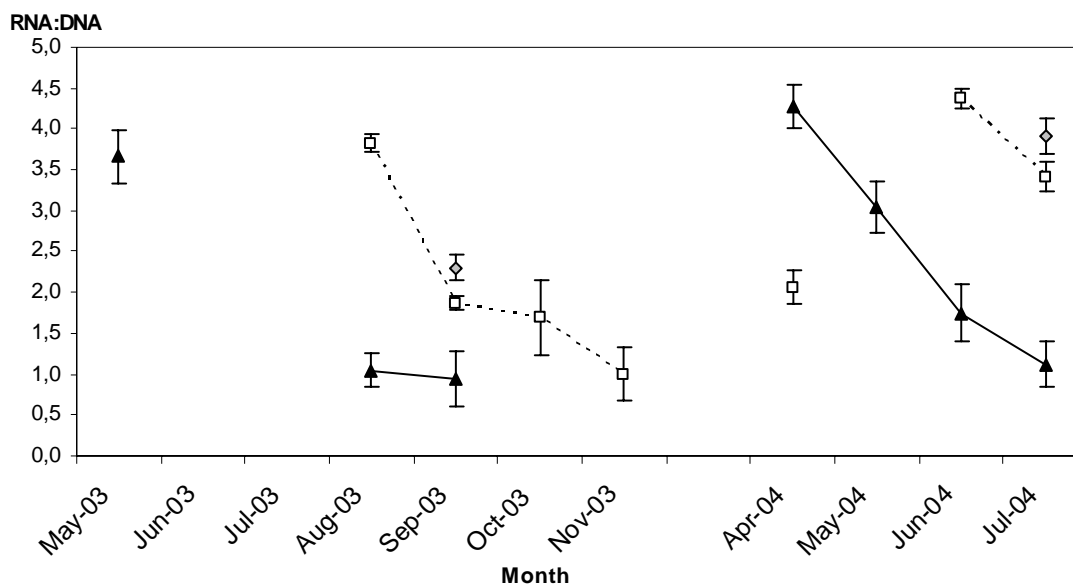


Figure 5 – Mean RNA-DNA monthly variation and standard deviation of juvenile soles over the two years considered. \blacktriangle represents young *S. solea*, while for *S. senegalensis* the two nursery areas in the Tagus estuary are distinguished, $-\square-$ Alcochete and $-\circ-$ Vila Franca de Xira, in September 2003 and July 2004.

Discussion

The polymodal structure of the L_T -frequency distributions of *S. solea* and *S. senegalensis* reflected the occurrence of several cohorts according to species and year in the Tagus estuary, and is in agreement with previous studies (Andrade, 1992; Cabral, 2003). The number of cohorts entering the nursery areas is determined by spawning period and spawning behaviour (i.e. longer spawning periods and several oocyte emission events favour a larger

number of cohorts), which along with environmental conditions influence the pattern of habitat usage of both species (Cabral, 2003).

These differences in habitat use, that consist of earlier and shorter estuarine occurrence for *S. solea* (April to August), as well as a more restricted distribution in the nursery grounds when compared to *S. senegalensis* (June to the following summer) (Cabral and Costa, 1999; Cabral, 2000), were also observed in the present study. Cabral (2003) concluded that differences in growth patterns of *S. solea* and *S. senegalensis* reflected their differences in habitat use in the Tagus estuary.

Absolute growth rate estimations were limited by the discontinuous data; nonetheless, G calculated were within the range of previous studies for this area and for northern European areas (Cabral, 2003). The first cohorts of 0-group juveniles for both species and at the beginning of the estuarine colonization period had the highest growth values, and also the highest mean RNA-DNA ratios. Intra-cohort RNA:DNA variation was significantly different for the estuarine settlement period for both sole species.

Successive cohorts did not reach the maximum RNA-DNA ratios reported for the first cohort, suggesting that the first individuals to arrive, at least for *S. solea*, had lower competitor pressure (lower fish densities). Better food quality and quantity in certain periods could also justify higher RNA-DNA ratios for young soles, however, during the main period of their occurrence (from May to September), prey abundance and quality do not vary significantly in the Tagus estuary (Cabral, 2000).

Also, whenever more than one cohort occurred, simultaneous newly arrived juveniles had higher RNA-DNA ratios and growth rates than earlier settled juvenile soles. RNA-DNA ratios for juvenile soles were within range (-1.1 to 8.2) of other studies of juvenile flatfish species (Mathers *et al.*, 1992; Gwak and Tanaka, 2001; Yamashita *et al.*, 2003), including a recent study on *S. solea* collected in 1 month in several sites of the northern French coast and where RNA:DNA varied from 1.4 to 4.3 (Gilliers *et al.*, 2004). Although growth estimates were obtained for a time gap of nearly 1 month (between captures) and the RNA:DNA condition indices are a recent growth indicator, similar patterns were observed on both time scales.

The decrease of seasonal growth rates with fish age was concurrent with mean RNA:DNA values seasonal variation. Previous studies with food deprivation of larval and juvenile fishes, including *S. solea* larvae (Richard *et al.*, 1991), indicated that starving larvae had RNA-DNA ratios of 1 while fed larvae had values of c. 3 to 4 (Clemmesen, 1996). Despite high individual variation within replicate experiments observed in some of these studies, and also reported by Bergeron and Boulhic (1994) and Bergeron (1997) for early larval *S. solea*, temperature was found to directly influence total RNA-DNA ratios (Buckley, 1984).

In the present study, nutritional condition indices of wild soles assessed by RNA-DNA ratios indicated that juvenile soles were in a fairly good condition status in the first 2 months of estuarine colonization, with mean ratio values >3 . Hence higher protein synthesis during this period reflected the higher growth rates estimated for both species. As the nursery period advanced, mean RNA:DNA values diminished indicating a decrease in growth rate.

Variability among monthly cohorts and RNA : DNA estimates was expected and can be explained by individual variability, either on a performance level or by their being subject to unsuitable conditions. All fishes were collected from nursery areas, where there are suitable growth conditions as suggested in the present work, since *S. senegalensis* had high RNA-DNA ratios from August to September (near 3.5) when *S. solea* showed fairly lower indices (c. 1.0).

Therefore, a pattern in RNA-DNA ratios for species and cohorts was observed. When young juvenile soles enter the estuary they have faster growth rates that decrease with fish age and L_T as RNA-DNA ratios decrease, due to lower nucleic acid concentration in somatic tissue of older fishes (Buckley and Bulow, 1987; Buckley *et al.*, 1999). Lower RNA-DNA ratios were observed for the end of colonization periods, when juveniles migrate to marine coastal areas, or as in the case of some juvenile *S. senegalensis*, remain in the nursery areas until the following year (with low condition indices).

This suggests that the different habitat usage pattern described earlier for both sole species is reflected by the RNA:DNA based condition indices as well as growth rates. The mean RNA:DNA values of *S. senegalensis* for the two different nursery habitats used by juveniles of this species were different in 2 months for both years. Individuals collected in the upper northern area had higher RNA-DNA mean ratios, which could suggest that this area may have better growth conditions than the other area. Data available, however, are insufficient and further analysis is necessary to verify this possibility.

Further studies on quantitative determination of growth of wild juvenile soles based on RNA-DNA ratios and other environmental variables would be a valuable tool for rapid growth assessment and prediction (Malloy *et al.*, 1996; Gwak and Tanaka, 2001; Yamashita *et al.*, 2003). Recently, Weber *et al.* (2003) reported the advantages of multiple biochemical indices, namely total lipid, RNA-DNA ratio and triglyceride content, in juvenile fish growth estimates. The present study verified that the pattern of RNA:DNA variation of juvenile *S. solea* and *S. senegalensis* during the estuarine colonization period reflects growth patterns and estuarine movements of young sole.

Acknowledgements

The present study had the financial support of the Fundação para a Ciência e a Tecnologia (FCT) which financed several of the research projects related to this work. The authors would also like to thank the referees for their important contribution to improving earlier versions of the manuscript

References

- Able, K.W., Manderson, J.P., Studholme, A., 1999. Habitat quality for shallow water fishes in an urban estuary: the effects of man-made structures on growth. *Marine Ecology Progress Series* 187, 227–235.
- Andrade, J.P., 1992. Age, growth and population structure of *Solea senegalensis* Kaup 1858 (Pisces, Soleidae) in the Ria Formosa (Algarve, Portugal). *Scientia Marina* 56, 35–41.

- Beck, M., Heck, K., Able, K., Childers, D., Egglestone, D., Gillanders, B., Halpern, B., Hays, C., Hoshino, K., Minello, T., Orth, R., Sheridan, P., Weinstein, M., 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* 51, 633–641.
- Bergeron, J.-P., 1997. Nucleic acids in ichthyoplankton ecology: a review, with emphasis on recent advances for new perspectives. *Journal of Fish Biology* 51, 284–302.
- Bergeron, J.-P., Boulhic, M., 1994. Rapport ARN/ADN et évaluation de l'état nutritionnel et de la croissance des larves de poissons marins: un essai de mise au point expérimentale chez la sole (*Solea solea* L.). *ICES Journal of Marine Science* 51, 181–190.
- Bhattacharya, C.G., 1967. A simple method of resolution of a distribution into Gaussian components. *Biometrics* 23, 115–135.
- Buckley, L.J., 1984. RNA-DNA ratio: an index of larval fish growth in the sea. *Marine Biology* 80, 291–298.
- Buckley, L.J., Bulow, F.J., 1987. Techniques for estimation of RNA, DNA, and protein in fish. In *Age and Growth of Fish* (Summerfelt, R. C., Hall, G. E., eds), pp. 345–354. Ames, IA: Iowa State University Press.
- Buckley, L.J., Caldarone, E., Ong, T.L., 1999. RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiology* 401, 265–277.
- Bulow, F. J., 1970. RNA-DNA ratios as indicators of recent growth rates of a fish. *Journal of the Fisheries Research Board of Canada* 27, 2343–2349.
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis* within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550–1562. doi: 10.1006/jfbi.2000.1408
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the United Kingdom* 83, 861–868.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389–397.
- Caldarone, E.M., Wagner, M., St Onge-Burns, J., Buckley, L.J., 2001. Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. *Northeast Fisheries Science Center (Reference Document) 01–11*, 1–22.
- Clemmesen, C., 1996. Importance and limits of RNA/DNA ratios as a measure of nutritional condition in fish larvae. In *Survival Strategies in Early Life Stages of Marine Resources* (Watanabe, Y., Yamashita, Y. and Oozeki, Y., eds), pp. 67–82. Rotterdam: A. A. Balkema.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Scientia Marina* 53, 561–566.

- Dinis, M.T., 1986. Quatre soleidae de l'estuaire du Tage. Reproduction et croissance. Essai d'élevage de *Solea senegalensis* Kaup. PhD Thesis, Université de Bretagne Occidentale, Brest, France.
- FAO, 2002. FISAT-II, Software Version 1.1.2 FAO-ICLARM Stock Assessment Tools. Rome: FAO
- Gilliers, C., Amara, R., Bergeron, J. P., Le Pape, O., 2004. Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. *Environmental Biology of Fishes* 71, 189–198.
- Gwak, W.S., Tanaka, M., 2001. Developmental changes in RNA:DNA ratios of fed and starved laboratory-reared Japanese flounder larvae and juveniles, and its application to assessment of nutritional condition for wild fish. *Journal of Fish Biology* 59, 902–915. doi: 10.1006/jfbi.2001.1703
- Haedrich, R.L., 1983. Estuarine Fishes. In *Ecosystems of the World: Estuarine and Enclosed Seas* (Ketchum, B., ed.), pp. 183–207. Amsterdam: Elsevier.
- Houde, E.D., 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2, 17–29.
- Malloy, K.D., Yamashita, Y., Yamada, H., Targett, T.E., 1996. Spatial and temporal patterns of juvenile stone flounder *Kareius bicoloratus* growth rates during and after settlement. *Marine Ecology Progress Series* 131, 49–59.
- Marchand, J., 1991. The influence of environmental conditions on settlement, distribution and growth of 0-group sole (*Solea solea* (L.)) in a microtidal estuary (Vilaine, France). *Netherlands Journal of Sea Research* 27, 307–316.
- Mathers, E.M., Houlihan, D.F., Cunningham, M.J., 1992. Nucleic acid concentrations and enzyme activities as correlates of growth rate of the saithe *Pollachius virens*: growth-rate estimates of open-sea fish. *Marine Biology* 112, 363–369.
- Myers, R.A., Cardigan, N.G., 1993. Density-dependent juvenile mortality in marine demersal fish. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 1576–1590.
- Richard, P., Bergeron, J.P., Boulhic, M., Galois, R., Ruyet, J., 1991. Effect of starvation on RNA:DNA and protein content of laboratory-reared larvae and juveniles of *Solea solea*. *Marine Ecology Progress Series* 72, 69–77.
- Rooker, J.R., Holt, G.J., 1996. Application of RNA:DNA ratios to evaluate the condition and growth of larval and juvenile red drum (*Sciaenops ocellatus*). *Marine and Freshwater Research* 47, 283–290.
- Sogard, S.M., 1992. Variability in growth rates of juvenile fishes in different estuarine habitats. *Marine Ecology Progress Series* 85, 35–53.
- Sogard, S.M., 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* 60, 1129–1157.
- Van der Veer, H.W., Phil, L., Bergman, M.J.N., 1990. Recruitment mechanisms in North Sea Plaice *Pleuronectes platessa*. *Marine Ecology Progress Series* 64, 1–12.

- Weber, L.P., Higgins, P.S., Carlson, R.I., Janz, D., 2003. Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. *Journal of Fish Biology* 63, 637–658. doi: 10.1046/j.1095-8649.2003.00178.x
- Westerman, M., Holt, G., 1994. RNA : DNA during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. *Marine Biology* 121, 1–9.
- Yamashita Y., Tominaga O., Takani, H., Yamada, H., 2003. Comparison of growth, feeding and cortisol level in *Platichthys bicoloratus* juveniles between estuarine and nearshore nursery grounds. *Journal of Fish Biology* 63, 617–630. doi: 10.1046/ j.1095-8649.2003.00175.x
- Zar, J.H., 1996. *Biostatistical Analysis*, 3rd edn. New Jersey: Prentice Hall.

Habitat specific growth rates and condition indices for the sympatric soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA-DNA ratio.

Abstract: Habitat specific growth rates and condition indices were estimated for *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, in two nursery areas within the Tagus estuary. While in the uppermost nursery area the two species of sole live in sympatry, in the lower nursery only *S. senegalensis* is present. Daily increments of left lapillar otoliths were used to estimate age and determine growth rates. Condition indices were assessed through RNA-DNA ratio in muscle samples. Growth rates were higher for *S. senegalensis* than for *S. solea*. Growth rates of *S. senegalensis* from the uppermost nursery area were lower when compared to those obtained for the other nursery. The RNA/DNA condition index followed the general trend given by the growth rate estimates, i.e. values were higher for *S. senegalensis* than for *S. solea*. However, no significant differences were detected between the condition of *S. senegalensis* from the two nurseries. Larger variations in salinity and highest pollution loads may be important factors lowering the habitat quality of the uppermost nursery in comparison to the lower nursery. The use of growth rate estimates based on otolith readings and the RNA/DNA index as tools for habitat quality assessment was discussed.

Key-words: Growth variability; Nutritional condition; RNA-DNA ratio; *Solea senegalensis*; *Solea solea*.

Introduction

Growth and survival in early life stages strongly influence successful recruitment to the adult populations (Houde, 1987, van der Veer *et al.*, 1990). Rapid growth means that less time is spent in the most vulnerable size ranges and that larger individuals will prevail by the end of the nursery period, along with the competitive advantages related to it (van der Veer and Bergman, 1987; Ellis and Gibson, 1995; Sogard, 1992; 1997).

Fish nurseries are generally located in areas, such as estuaries and shallow coastal waters, which provide suitable conditions for survival and enhancement of growth, namely high food abundance, refuge from predators and higher water temperature (Haedrich, 1983; Miller *et al.*, 1985; Beck *et al.*, 2001). Such areas can be considered of higher habitat quality for juvenile fish than surrounding waters.

Assessing habitat quality of nursery areas has been a long pursued goal for estuarine and marine biologists due to its importance for the identification of essential fish habitats in species life cycles (e.g. Sustainable Fisheries Act, 1996; Brown *et al.*, 2000; Eastwood *et al.*, 2003; LePape *et al.*, 2003). The recent European Water Framework (Directive 2000/60/EC; EC,

2000) follows a similar philosophy, concentrating on the need for identification and protection of specific water bodies (e.g. estuaries).

Habitat quality cannot be measured directly and should always be assessed on a comparative basis (Gibson, 1994; Adams, 2002). Also, the comparison of several indices is advised for this purpose (Ferron and Legget, 1994).

The estimation of habitat specific growth rates is a key step for the determination of habitat quality (Able *et al.*, 1999). Growth rates based on otolith daily rings provide an accurate measure of growth that integrates the whole life of the fish.

Because of the dynamic nature of estuarine environments it is also of great importance to assess the individual-environmental linkage on short time scales. Nucleic acid quantification and subsequent RNA-DNA ratios has been used in numerous studies as indices for nutritional condition and growth assessment in larvae and juvenile fish (e.g. Buckley, 1984; Richard *et al.*, 1991; Gwak and Tanaka, 2001). This biochemical index reflects variations in growth related protein synthesis, since RNA concentration fluctuates both with food intake and protein requirement, while DNA somatic content remains constant, providing a recent picture of overall fish condition and growth (Bullow, 1970; Buckley and Bulow, 1987).

Few studies have assessed habitat quality and compared different sites. Habitat quality differences have been found along pollution gradients (Burke *et al.*, 1993), in areas impacted by man-made structures (Able *et al.*, 1999), in protected marine reserves (Lloret and Planes, 2003) and between estuarine and nearshore flatfish nurseries (Yamashita *et al.*, 2003; Gilliers *et al.*, 2004).

The Tagus estuary is used as a nursery area by two commercially important species of sole, the common sole *Solea solea* (Linnaeus, 1758) and the Senegal sole, *Solea senegalensis* Kaup 1858 (Costa and Bruxelas, 1989; Cabral and Costa, 1999). Two nursery areas have been identified within the estuary, one in the uppermost section that is used by both species juveniles, and another in the upper eastern section (also in the upper estuary but at a lower location), used only by *S. senegalensis* (Costa and Bruxelas, 1989; Cabral and Costa, 1999).

Niche overlap has been reported, albeit for a short period (Cabral, 2000). Cabral (2003) and Fonseca *et al.* (2006) reported higher growth rates for soles in the Tagus estuary than in other important North-European nurseries, using modal progression analysis. Fonseca *et al.* (2006) concluded that RNA/DNA variation patterns over the nursery period reflected growth and estuarine colonization patterns. Yet, both authors pointed at the limitations of length frequency progression methods and called out for the application of a more accurate growth rate determination method.

While *S. solea* is a temperate species with a distribution that ranges from the Baltic Sea to Senegal, *S. senegalensis* is a tropical species that ranges from South Africa to the Bay of Biscay (Quéro *et al.*, 1986). The Tagus estuary is one of the few nurseries where both sole species are present in high abundance (Cabral and Costa, 1999).

Studies on *S. senegalensis* ecology are scarce (Dinis, 1986; Andrade, 1992; Cabral and Costa, 1999; Cabral 2000; 2003; Anguis and Cañavate, 2005) and do not allow for conclusive

remarks about recruitment variability, while for *S. solea* an important body of literature has already been developed. It is generally agreed that recruitment of *S. solea* is determined before the end of the first year of life, and that water temperature plays an important role (e.g. Rijnsdorp *et al.*, 1992; Wegner *et al.*, 2003; Henderson and Seaby, 2005).

However, most studies were conducted in temperate waters. In fact, Van der Veer *et al.* (1994) concluded that the restricted latitude range where most knowledge on flatfish was gathered may have biased the conclusions. The understanding of the factors controlling recruitment in flatfish, and soles in particular, is hampered by the lack of studies in subtropical and tropical areas (van der Veer *et al.*, 1994; Pauly, 1994), where longer photoperiod, higher temperatures, and a wider period of high primary productivity allow longer spawning and settlement periods, along with higher growth rates.

Understanding the role of habitat quality in the early life of fish over its full range of distribution is very important for essential fish habitat determination, particularly for species such as the soles that are the main target of fisheries over a wide geographical area.

The present paper aims at: (1) estimating habitat specific growth rates and condition indices in *S. solea* and *S. senegalensis*, in two nursery areas of the Tagus estuary (Portugal) based on otolith daily rings and RNA-DNA ratio, respectively; and at (2) discussing the use of both methodologies as tools for habitat quality monitoring.

Material and Methods

Study areas

The Tagus estuary (Fig.1), one of the largest estuaries in Western Europe (320 km²), is a partially mixed estuary with a tidal range of ca. 4 m. Approximately 40% of the estuarine area is intertidal. Much of its upper area is composed by extensive intertidal mudflats fringed by saltmarshes (Caçador and Vale, 2001). Two important sole nurseries were identified in the Tagus estuary in previous studies (A, Vila Franca de Xira, and B, Alcochete; Fig. 1) by Costa and Bruxelles (1989) and Cabral and Costa (1999).

Although most of the environmental factors present a wide and similar range in these two areas, some differences can be outlined. The uppermost area, A, is deeper (mean depth 4.4 m), presents lower and highly variable salinity and has a higher proportion of fine sand in the substrate (approximately 40%). Nursery B is shallower (mean depth 1.9 m), and more saline, with lower variability in salinity, while substrate is mainly composed of mud (mean value 60.4%) (Cabral, 1998; Cabral and Costa, 1999). Nursery A is located in an industrialised area that receives important quantities of industrial and urban sewage, while nursery B is located in an area with much lower human pressure and no important industries (Vale, 1986). Previous studies on heavy metals presence in subtidal sediments have also revealed that nursery A presents a higher concentration of heavy metals than nursery B (França *et al.*, 2005).

Climate in this area is Mediterranean with mild winters and warm and dry summers (Aschmann, 1973).

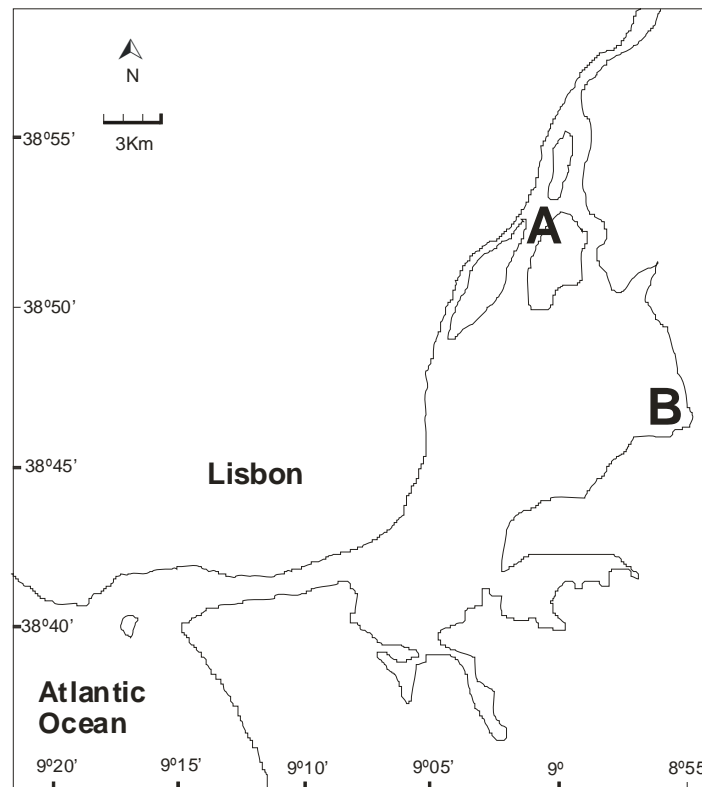


Figure 1 – Location of the nursery areas in the Tagus estuary

Environmental data

In each trawl environmental data, such as water temperature and salinity, were registered with a multiparameter probe. Environmental data were statistically explored with SYSTAT 10.0. Mean values and standard deviations were estimated for water temperature and salinity in both nursery areas during the June-July period.

Juvenile collections

Both nurseries were surveyed monthly from March to October 2005 in order to determine the beginning and the end of estuarine colonization by soles' 0-group juveniles. From late June (when the first 0-group juveniles were detected in the nursery areas) and during July (when colonization ended) surveys were intensified, taking place at approximately two weeks intervals, in order to better determine the end of the estuarine immigration process of the first cohort of each species.

S. solea is a temperate species and thus has a temporally restricted spawning period leading to a concentrated in time estuarine colonization. *S. senegalensis*, however, has a very wide spawning period (Anguis and Cañavate, 2005) which is characteristic of tropical species and leads to several successive cohorts. Cabral (2003) and Fonseca *et al.* (2006) observed that growth and condition is higher for the first cohort of both species entering the estuary, indicating that direct comparisons should take into account the estuarine colonisation process. In 2005, the first cohort of both species occurred at approximately the same time, presenting the highest

densities when compared to subsequent cohorts. In order to work with comparable samples containing enough number of individuals for growth and condition assessment, we chose to study the first cohort of each species.

Length frequency of the first cohorts of 0-group juveniles was analysed at the end of the colonization period for each nursery area and for each species (Fig. 2). Age and condition were determined in 0-group *S. solea* and *S. senegalensis* collected at 8 stations in nursery A and in 0-group *S. senegalensis* collected at 6 stations in nursery B (at the end of the estuarine colonization). Trawls were conducted with a 2.5 m beam trawl with 5 mm stretched mesh at the codend.

All samples were frozen immediately after collection. In the laboratory individuals were identified, counted and their total length measured to the nearest mm.

Growth rate estimation

Otoliths of a subsample of juveniles chosen randomly from each length category (5 mm length categories) were examined. The daily nature of the otoliths increments were validated by Lagardère and Troadec (1997) for *S. solea* and by Ré *et al.* (1988) for *S. senegalensis*. The left lapillus, which has the longest axis due to the bilateral asymmetry between the right and left lapillus, was used for all age estimates. Lapillar otoliths were used because they are relatively thin and have well-defined increments that are spatially more uniform than in sagittae otoliths which have accessory primordia (Amara *et al.*, 1994). Otoliths were removed and mounted with glue on microscope slides. They were polished in the sagittal plane to the central primordial with an aluminium oxide polishing bar.

Otoliths were analysed under transmitted light at x400 or x1000 magnification, using a video system fitted to a compound microscope. Otolith counts were made along the posterior axis. Otolith increments were counted three times, and the age was regarded as the mean of the three counts. Precision was estimated by computing the coefficient of variation. Otoliths were eliminated whenever the reading variation was above 5%.

Age was estimated for 151 *S. solea* and 59 *S. senegalensis* from nursery A, and for 52 *S. senegalensis* from nursery B.

Growth was described by a linear model. An analysis of covariance (ANCOVA) was conducted to test differences in growth between nursery areas and species (slope of age against length).

RNA-DNA ratio determination

Nucleic acid determination was carried out following the fluorometric method described by Caldarone *et al.* (2001) and adapted to a cuvette spectrofluorometer, as described in Fonseca *et al.* (2006). Detection limits, standard calibration curves for RNA, DNA and spike recovery of homogenate samples ($n = 3$) were first determined with a series of dilutions of pure calf-thymus DNA (Calbiochem) and 18S- and 28S-rRNA (Sigma). Tissue sample autofluorescence and residual fluorescence were analysed, the later by adding $1 \text{ U } \mu\text{l}^{-1}$ DNase

(n = 3) (Sigma). Concentrations of stock standard RNA and DNA solutions were first checked with and UV-spectrophotometer.

To ensure reproducibility, two 20 mg (dry weight) replicates of each juvenile sole were analysed. White muscle was homogenised through short term ice-sonication with 200 µl of 1% sarcosine solution (N-lauroylsarcosine), and then diluted with 1.8 ml Tris-EDTA buffer (Trizma, pH 7.5) (sarcosine final concentration of 0.1 %). Total nucleic acid fluorescence (RNA and DNA) was measured by adding 300 µl sample homogenate, 1.8 ml Tris-EDTA and 150 µl Ethidium Bromide (EB, 1 mg ml⁻¹) to the first vial. DNA fluorescence was determined by digesting RNA content with 150 µl RNase (A from bovine pancreas, 20 U ml⁻¹ incubated at 37°C for 30 min, Sigma) in the second vial containing 300 µl sample homogenate, 1.65 ml Tris-EDTA and 150 µl EB. Excitation and emission wavelengths used were 360 nm and 600 nm, respectively. RNA fluorescence value was determined by subtracting the DNA fluorescence reading (second reading) from the total fluorescence value (first reading). RNA and DNA content in tissue samples was calculated through calibration curves constructed previously plus the dilution factors used.

T tests were performed in order to compare the condition between the two nursery areas, and between both species. Interspecific comparison is generally not carried out since RNA-DNA ratio is species specific (Bullow, 1987). Yet, *S. solea* and *S. senegalensis* are genetically very closely related and are thus regarded as sister-species (Ben-Tuvia, 1990; Tinti and Picinetti, 2000), for that reason we found that between species comparison of this condition index was both interesting and justified. Since the RNA-DNA ratio is dependent on the individuals age tests were performed only between overlapping length ranges. Comparisons were made between both species at nursery A and between *S. senegalensis* from nursery A and B. The software used for the test procedures was STATISTICA.

Results

In the June-July period, mean salinity in nursery A was 12.9 ‰ (standard deviation = 3.0; minimum = 6.9 ‰; maximum = 16.9 ‰), while in nursery B it was 32.5 ‰ (standard deviation = 0.1; minimum = 32.4 ‰; maximum = 32.6 ‰). Mean water temperature in nursery A was 24.4°C (standard deviation = 0.9; minimum = 23.5°C; maximum = 25.7°C), while in nursery B it was 25.0°C (standard deviation = 0.5; minimum = 24.3°C; maximum = 25.9°C).

The first cohorts of both soles colonized the estuary in June-July, establishing spatial and temporal sympatry in the upper nursery area, but not in the lower nursery where only *S. senegalensis* was present, as previously observed (Cabral and Costa, 1999; Cabral, 2003). As expected the first cohort of *S. senegalensis* was followed by new cohorts entering the estuary in the following months. *S. solea* presented only one cohort.

Length frequency distribution of 0-group juveniles at the end of the colonization period showed approximately normal distributions for both species and nurseries studied (Fig. 2).

Growth during the first months following settlement was best described by a linear model (Fig. 3). *S. solea* 0-group juveniles growth rate was estimated to be 0.767 mm/d (range of total length of individuals analysed, TL : 57-109 mm; n = 215) in nursery A. *S. senegalensis* 0-group juveniles growth rate was estimated as 0.970 mm/d (range of total length

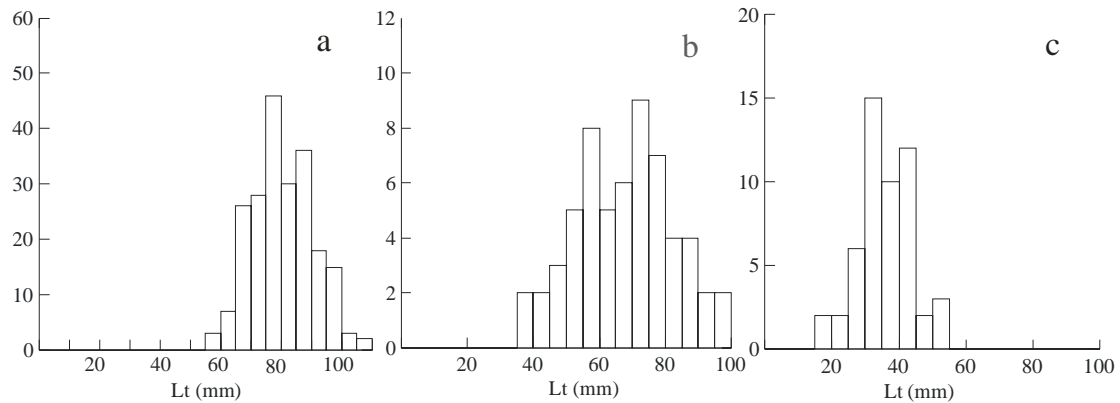


Figure 2 – Length-frequency distribution of 0-group soles caught in the Tagus estuary: a) *S. solea* caught at nursery A; b) *S. senegalensis* caught at nursery A; c) *S. senegalensis* caught at nursery B.

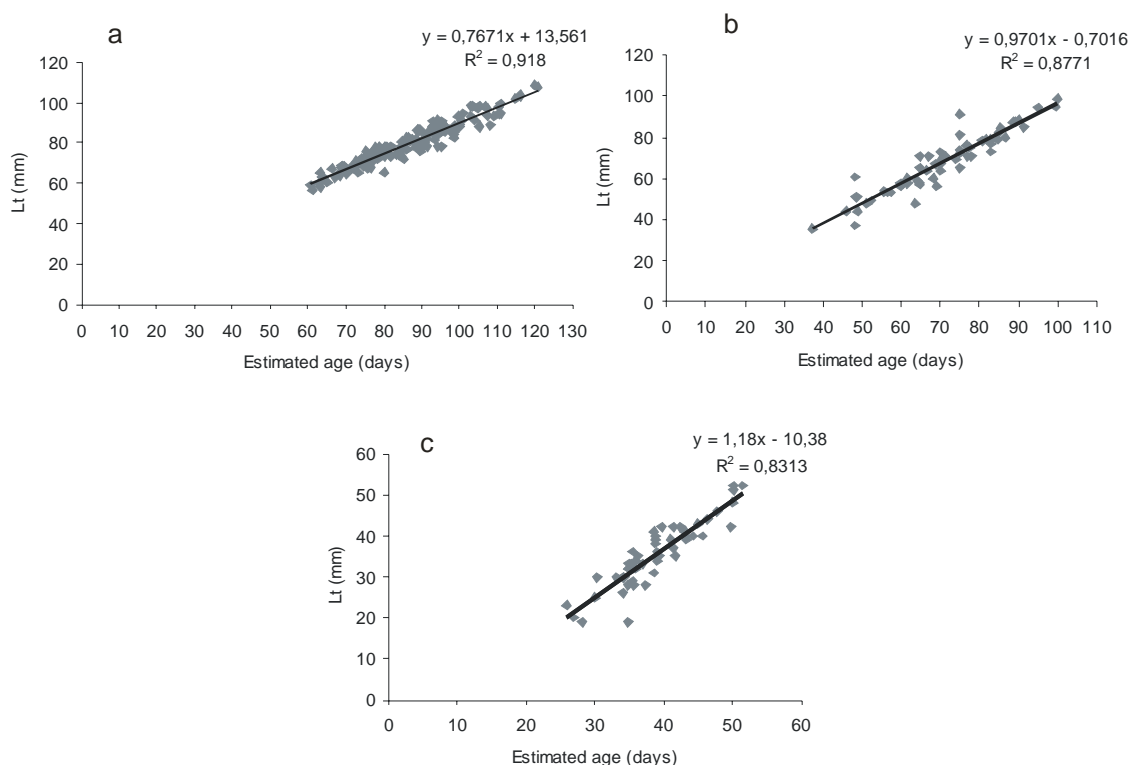


Figure 3 - Regression of soles total length (mm) against estimated age (days) by daily otolith increments: a) *S. solea* caught at nursery A; b) *S. senegalensis* caught at nursery A; c) *S. senegalensis* caught at nursery B.

of individuals analysed, TL : 36-99 mm; n = 59) in nursery A, while in nursery B growth rate was estimated as 1.180 mm/d (range of total length of individuals analysed, TL : 19-52 mm; n = 52).

Thus, *S. solea* presented a slower growth rate than *S. senegalensis* from both nurseries ($p < 0.05$), while *S. senegalensis* from nursery B presented the fastest growth rate ($p < 0.05$). Mean RNA-DNA ratio was 2.90 for *S. solea* (nursery A), while for *S. senegalensis* it was 3.50 in nursery A and 4.01 in nursery B. Condition was significantly different between the two species in nursery A (t test = -3.81, $p < 0.05$), while no significant differences were detected between *S. senegalensis* from nursery A and B ($t = 0.25$, $p > 0.05$).

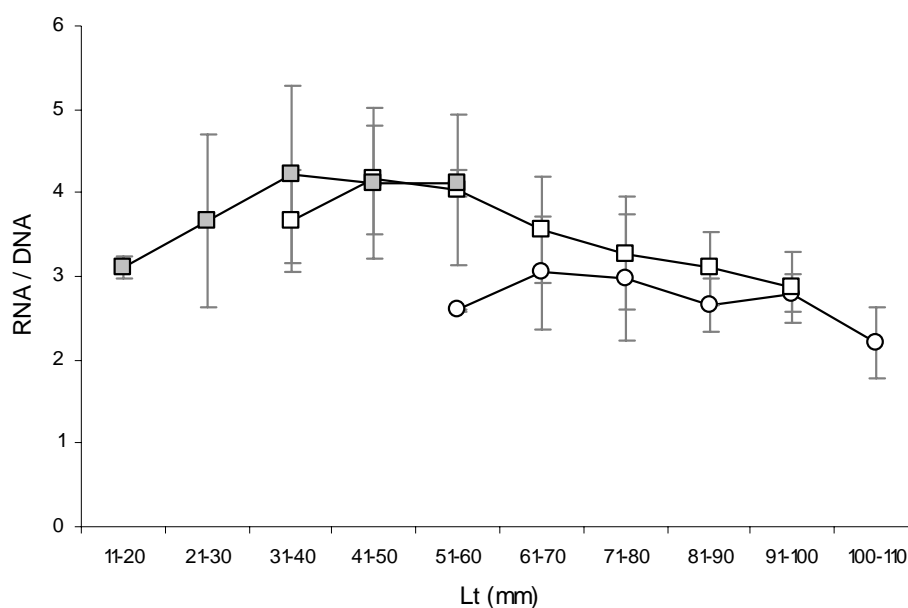


Figure 4 - RNA-DNA mean ratios (and standard deviations) for 0-group *S. solea* from nursery A \circ ; for *S. senegalensis* from nursery A \square and for *S. senegalensis* from nursery B \square .

Condition peaked in the second length class in both species from nursery A, while in nursery B peak condition was observed in the third length class (Fig.4). After reaching a peak RNA-DNA ratio declined with fish length in both species. The smaller category lengths presented low values, especially in *S. senegalensis* from nursery B.

Discussion

Habitat specific growth rates estimated through otolith readings revealed differences between nurseries and sole species, while habitat specific condition based on RNA-DNA ratio revealed differences between species but not between nurseries.

Higher growth rates were found in *S. senegalensis* from nursery B than from nursery A. RNA-DNA ratios didn't reveal differences between nursery areas, but were higher for *S.*

senegalensis than for *S. solea*, both inhabiting nursery A. Interspecific comparison of growth rates within nursery A also revealed a higher value for *S. senegalensis*. These results seem to indicate that nursery B has a higher habitat quality than nursery A. This is in accordance with Fonseca *et al.* (2006) that also observed higher RNA-DNA ratios for the first cohort of *S. senegalensis* in nursery B than in nursery A, for two consecutive years (2003-2004). Previous studies on soles growth in the Tagus estuary (Costa, 1990; Cabral, 2003; Fonseca *et al.* 2006) did not aim to compare the different nursery areas and made no distinction between them, using the population as a whole.

Differences between nursery areas depend on multiple factors not always clearly identifiable due to the highly complex and variable nature of estuarine systems. Yet, differences between the sole nurseries in the Tagus estuary are possibly related to salinity and pollution levels. Nursery B has more stable salinity levels than nursery A, implying that an important amount of energy, that would be used for constant adjustment to salinity variation, can be diverted to growth (Evans, 1993; Moyle and Cech, 1996). Another important aspect that differentiates nursery A from B is the pollution load and human pressure (França *et al.*, 2005). The lower levels of pollution stress that fish are exposed to in nursery B should be important for the general health and growth of individuals.

Growth rates for *S. solea* were higher in the Tagus than in Northern European nursery areas (e.g. Rogers, 1994; Amara *et al.*, 2001; Amara, 2004). This was also reported by Cabral (2003) and Fonseca *et al.* (2006) using modal progression analysis of length-frequency data. Higher growth rates are to be expected in southern Europe due to higher water temperature (Yamashita *et al.*, 2001; Henderson and Seaby, 2005) as well as longer photoperiod throughout the year (Devauchelle *et al.*, 1987; Boeuf, H. and Le Bail, 1999).

S. senegalensis growth rates were higher than reported by Andrade (1992) in the Ria Formosa and Cabral (2003) in the Tagus estuary but similar to those reported by Fonseca *et al.* (2006) for the first cohort of this species in the Tagus estuary.

RNA-DNA ratios for juvenile soles were within the range of other studies on juvenile flatfishes (-1.1 to 8.2) (e.g. Mathers *et al.*, 1992; Gwak and Tanaka, 2001; Yamashita *et al.*, 2003; Fonseca *et al.*, 2006). For *S. solea*, Gilliers *et al.* (2004) estimated RNA-DNA ratios between 1.40 and 4.30 in the Northern French coast. Starvation experiments with reared *S. solea* concluded that RNA-DNA ratio of fed fish was around 2, while that of starved fish usually dropped below this value (Richard *et al.*, 1991). Richard *et al.* (1991) pointed out that indices from reared and wild fish must be compared cautiously, since food offered to captive fish may be of lower nutritional value than wild prey. Keeping this important issue in mind it can be concluded that soles from the Tagus estuary were in a fairly good nutritional status, since mean RNA-DNA ratios were always above 2 and varied between 2.21 and 4.22.

As reported by other authors, the RNA-DNA ratio was found to be dependent on age (Buckley and Bulow, 1987; Buckley *et al.*, 1999). A distinctive pattern of decreasing RNA-DNA ratio with fish length was observed for both species in nursery A, but not in nursery B. Lower condition values were noticeable in the first length classes for both species and nursery areas,

and were especially evident in nursery B. Since the RNA-DNA ratio reflects recent growth, this could be due to temporarily unfavourable conditions that affected the smaller individuals of both nurseries and species.

The higher growth rates and condition of *S. senegalensis* when compared to *S. solea* can have important implications in a warming climate scenario. *S. senegalensis* appears to be better adapted than *S. solea* to the present environmental conditions of the Tagus estuary. Temperature is one of the most important factors determining growth and this estuary has higher temperatures than the northern European estuaries where *S. solea* thrives but *S. senegalensis* is not present. Water temperature in the upper Tagus estuary is usually above 23°C during the Summer months, well above the *S. solea* metabolic optimum temperature, which is approximately 19°C (LeFrançois and Claireaux, 2003). *S. senegalensis* metabolic optimum temperature has not yet been determined, but being this a subtropical species it will probably be higher than that of *S. solea*. Also, spawning, egg incubation and rearing temperatures for *S. senegalensis* are considerably higher than for *S. solea* (Imslund *et al.*, 2003). This way, in a warming climate scenario lower densities of temperate species such as *S. solea* and higher densities of subtropical species such as *S. senegalensis* are to be expected, like pointed out by Cabral *et al.* (2001).

Both methods used in the present study provided valuable information concerning habitat quality. While growth rates estimated from daily otolith rings provide long term information on growth throughout the whole life of the fish, RNA-DNA ratios only inform about recent growth. Thus growth rates based on otolith readings are influenced not just by the habitat quality of past months in the nursery, but also by the marine environment prior to immigration to the nursery areas. This may be a limitation when the objective is to estimate habitat quality solely in an estuarine nursery area. Nonetheless, the integration of information over the months spent in the nursery is very important for quality assessment.

Recent growth assessed through RNA-DNA ratios is quite valuable since it is based solely on the conditions provided by the nursery area, yet it can be influenced by unusual events that do not reflect the average habitat quality of the area. Intensive sampling for RNA-DNA ratios determination starting at the beginning of the estuarine colonization could yield very interesting results yet since this index only reflects the nutritional condition of the fish over a period of about 3 days the assessment of habitat quality over a period of ca. two months would be quite costly and time consuming.

Therefore, the combination of both indices used in this study integrating habitat quality over a long period with recent condition is quite interesting for habitat quality determination in highly variable environments such as estuarine nurseries, since the information given by both methods complements each other.

Other methods such as recent growth estimation based on marginal otolith increment width (*e.g.* Amara and Galois, 2004; Gilliers *et al.*, 2004), condition based on protein concentration (*e.g.* Peragón *et al.*, 2001; Weber *et al.*, 2003), condition based on lipid content (*e.g.* Galois *et al.*, 1990; Lloret and Planes, 2003; Weber *et al.*, 2003; Amara and Galois, 2004)

and the use of molecular biomarkers in areas subjected to pollution (e.g. Nunes *et al.*, 2005; Rendón-von Osten *et al.*, 2005) are also very promising. Further research will certainly determine the most appropriate combinations of indices for each species and habitat type.

Acknowledgements

This study was co-funded by the European Union through the FEDER – Portuguese Fisheries Programme (MARE), as well as by the Fundação para a Ciência e a Tecnologia. The authors thank all people involved in the sampling surveys.

References

- Able, K.W., Manderson, J.P., Studholme, A., 1999. Habitat quality for shallow water fishes in an urban estuary: the effects of man-made structures on growth. *Marine Ecology Progress Series* 187, 227-235.
- Adams, S.M., 2002. Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland. p.621.
- Amara, R., 2004. 0-group flatfish growth conditions on a nursery ground (Bay of Canche, Eastern English Channel). *Hydrobiologia* 518, 23-32.
- Amara, R., Galois, R., 2004. Nutritional condition of metamorphosing sole: spatial and temporal analyses. *Journal of Fish Biology* 64, 72-88.
- Amara, R., Desaunay, Y., Lagardère, F., 1994. Seasonal variation in growth of larval sole *Solea solea* (L.) and consequences on the success of larval immigration. *Netherlands Journal of Sea Research* 32, 287-298.
- Amara, R., Laffargue, P., Dewarumez, J.M., Maryniak, C., Lagardère, F., Luczac, C., 2001. Feeding ecology and growth of 0-group flatfish (sole, dab and plaice) on a nursery ground (Southern Bight of the North Sea). *Journal of Fish Biology* 58, 788-803.
- Andrade, J.P. 1992. Age, growth and population structure of *Solea senegalensis* Kaup 1858, (Pisces, soleidae) in the Ria Formosa (Algarve, Portugal). *Scientia Marina* 56, 35-41.
- Anguis, V., Cañavate, J.P., 2005. Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. *Aquaculture* 243, 133-145.
- Aschmann, H., 1973. Distribution and peculiarity of Mediterranean Ecosystems. Origins and Structure. In: Castri F, Mooney HA (eds.) *Ecological studies*. Springer-Verlag, Berlin.
- Beck, M., Heck, K., Able, K., Childers, D., Egglestone, D., Gillanders, B., Halpern, B., Hays, C., Hoshino, K., Minello, T., Orth, R., Sheridan, P., Weinstein, M., 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* 51, 633-641.
- Ben-Tuvia, A., 1990. A taxonomic reappraisal of the Atlanto-Mediterranean soles *Solea solea*, *Solea senegalensis* and *Solea lascaris*. *Journal of Fish Biology* 36, 947-960.
- Boeuf, G., Le Bail, P.-Y., 1999. Does light have an influence on fish growth? *Aquaculture* 177, 129-152.

- Brown, S., Buja, K., Jury, S., Monaco, M., Banner, A., 2000. Habitat suitability index models for eight fish and invertebrate species in Casco and Sheepscot Bays, Maine. *North American Journal of Fisheries Management* 20, 408-435.
- Buckley, L.J., 1984. RNA-DNA ratio: an index of larval fish growth in the sea. *Marine Biology* 80, 291-298
- Buckley, L.J., Bullow, F.J., 1987. Techniques for estimation of RNA:DNA and protein in fish. In: Summerfelt RC, Hall GE (eds) *Age and growth of fish*. Iowa State University Press, Ames.
- Buckley, L.J., Caldarone, E., Ong, T.L., 1999. RNA-DNA ratio and other nucleic acid based indicators for growth and condition of marine fishes. *Hydrobiology* 401:265-277.
- Bullow, F.J., 1987. RNA-DNA ratios as indicators of growth in fish: A review. In: Summerfelt RC, Hall GE (eds) *Age and growth of fish*. Iowa State University Press, Ames.
- Cabral, H.N., 1998. Utilização do estuário do Tejo como área de viveiro pelos linguados, *Solea solea* (L., 1758) e *Solea senegalensis* Kaup, 1858, e robalo, *Dicentrarchus labrax* (L., 1758). PhD dissertation, Universidade de Lisboa, Lisboa, Portugal.
- Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis* within the nursery areas of the Tagus estuary, Portugal. *Journal of Fish Biology* 57, 1550-1562.
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the U.K.* 83, 861-868.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by the sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56,389-397.
- Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect environmental changes? *Climate research* 18, 119-126.
- Caçador, I., Vale, C., 2001. Salt marshes. In: Prasad MNV (ed) *Metals in the environment*. University of Hyderabad. Hyderabad.
- Caldarone, E.M., Wagner, M., St. Onge-Burns, J., Buckley, L.J., 2001. Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. *Northeast Fisheries Science Center, Ref Doc 01-11*, p 1-22.
- Costa, M.J., 1990. Age and growth studies of the sole (*Solea vulgaris vulgaris* Quensel, 1806) in the Tagus estuary, Portugal. *Boletim do Instituto Nacional de Investigação das Pescas* 15, 63-67.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Scientia Marina* 53, 561-566.
- Devauchelle, N., Alexandre, J.C., Le Corre, N., Letty, Y., 1987. Spawning of sole (*Solea solea*) in captivity. *Aquaculture* 66, 125-147.

- Dinis, M.T., 1986. Quatre soleidae de l'estuaire du Tage. Reproduction et croissance. Essai d'élevage de *Solea senegalensis* Kaup. PhD dissertation, Université de Bretagne Occidentale, Brest, France.
- Eastwood, P.D., Meaden, G.J., Carpentier, A., Rogers, S.I., 2003. Estimating limits to the spatial extent and suitability of sole (*Solea solea*) nursery grounds in the Dover Strait. *J. Sea Res.* 50, 151-165.
- EC (European Communities), 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23rd October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities L.* 327, 1-72.
- Ellis, T., Gibson, R.N., 1995. Size selective predation of 0-group flatfishes on a Scottish coastal nursery ground. *Marine Ecology Progress Series* 127, 27-37.
- Evans, D.H., 1993. Osmotic and ionic regulation. In: Evans DH (ed) *The Physiology of Fishes*. CRC Press, Boca Raton, FL.
- Ferron, A., Leggett, W.C., 1994. An appraisal of condition measures of marine fish larvae. *Advances in Marine Biology* 30, 217-303.
- Fonseca, V.F., Vinagre, C., Cabral, H., 2006. Growth variability of juvenile sole *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, and comparison with RNA-DNA ratios in the Tagus estuary, Portugal. *Journal Fish Biology* 68, 1551-1562. doi:10.1111/j.0022-1112.2006.001042.x
- França, S., Vinagre, C., Caçador, I., Cabral, H.N., 2005. Heavy metal concentrations in sediment, benthic invertebrates and fish in three saltmarsh areas subjected to different pollution loads in the Tagus estuary (Portugal). *Marine Pollution Bulletin* 50, 993-1018.
- Galois, R., Lagardère, F., Richard, P., 1990. Changes in biochemical composition and otolith microstructure of larval common sole, *Solea solea* (L.) under experimental starvation. *La Mer* 28, 273-285.
- Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. *Netherlands Journal of Sea Research* 32, 191-206.
- Gilliers, C., Amara, R., Bergeron, J.-P., Le Pape, O., 2004. Comparison of growth and condition indices on juvenile flatfish in different coastal nursery grounds. *Environmental Biology of Fishes* 71, 189-198.
- Gwack, W.S., Tanaka, M., 2001. Developmental changes in RNA:DNA ratios of fed and starved laboratory-reared Japanese flounder larvae and juveniles, and its application to assessment of nutritional condition for wild fish. *Journal of Fish Biology* 59, 902-915.
- Haedrich, R.L., 1983. Estuarine Fishes. In: Ketchum B (ed) *Ecosystems of the World* 26 Estuarine and Enclosed Seas. Elsevier, Amsterdam.
- Henderson, P.A., Seaby, R.M., 2005. The role of climate in determining the temporal variation in abundance, recruitment and growth of sole *Solea solea* in the Bristol Channel. *Journal of the Marine Biological Association of the UK* 85, 197-204.
- Houde, E.D., 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2, 17-29.

- Lagardère, F., Troadec, H., 1997. Age estimation in common sole *Solea solea* larvae: validation of daily increments and evaluation of a pattern recognition technique. Marine Ecology Progress Series 155, 223-237.
- LeFrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole, *Solea solea*. Marine Ecology Progress Series 259, 273-284.
- Le Pape, O., Chauvet, F., Mahévas, S., Lazure, P., Guérault, D., Désaunay, Y., 2003. Quantitative description of habitat suitability for the juvenile common sole (*Solea solea*, L.) in the Bay of Biscay (France) and the contribution of different habitats to the adult population. Journal of Sea. Reserach 50, 139-149.
- Lloret, J., Planes, S., 2003. Condition, feeding and reproductive potential of white sea bream (*Diplodus sargus*) as indicators of habitat quality and the effect of reserve protection in the northwestern Mediterranean. Marine Ecology Progress Series 248, 197-208.
- Imsland, A.K., Foss, A., Conceição, L.E.C., Dinis, M.T., Delbare, D., Schram, E., Kamstra, A., Rema, P., White, P., 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. Reviews in Fish Biology and Fisheries 13, 379-407.
- Miller, J.M., Burke, J.S., Fitzhugh, G.R., 1991. Early life history patterns of Atlantic North American flatfish: likely (and unlikely) factors controlling recruitment. Netherlands Journal of Sea Research 27, 261-275.
- Moyle, P.B., Cech, J.J.J., 1996. Fishes, an introduction to Ichthyology, 3rd edn. Prentice Hall, N.J.
- Nunes, B., Carvalho, F., Guilhermino, L., 2005. Acute toxicity of widely used pharmaceuticals in aquatic species: *Gambusia holbrooki*, *Artemia parthenogenica*, *Tetraselmis chuii*. Ecotoxicology and Environmental Safety 61, 413-419.
- Pauly, D., 1994. A framework for latitudinal comparisons of flatfish recruitment. Netherlands Journal of Sea Research 32, 107-118.
- Peragón, J., Barroso, J.B., Garcia-Salguero, L., de la Higuera, M., Lupiáñez, J.A., 2001. Growth, protein turnover rate and nucleic acid concentration in the white muscle of rainbow trout during development. The International Journal of Biochemistry and Cell Biology 33, 1227-1238.
- Quéro, J.-C., Dessouter, M., Lagardère, F., 1986. Soleidae. In : Whitehead, Bauchot M-L, Hureau J-C, Nielsen J, Tortonese E (eds) Fishes of the North-eastern Atlantic and the Mediterranean, Vol.III, UNESCO, Bungay.
- Ré, P., Rosa, H.C., Dinis, M.T., 1988. Daily microgrowth increments in otoliths of *Solea senegalensis* Kaup larvae Ciênc. Biolog. Ecol. Syst. 8, 37-41.
- Rendón-von Osten, J., Ortiz-Arana, A., Guilhermino, L., Soares, A.M.V.M., 2005. In vivo evaluation of three biomarkers in mosquitofish (*Gambusia yacatana*) exposed to pesticides. Chemosphere 58, 627-636.

- Richard, P., Bergeron, J.P., Boulhic, M., Galois, R., Ruyet, J., 1991. Effect of starvation on RNA, DNA and protein content of laboratory reared larvae and juveniles of *Solea solea*. Marine Ecology Progress Series 72, 69-77.
- Rijnsdorp, A.D., Van Beek, F.A., Flatmann, S., Millner, R.M., Riley, J.D., Giret, M., De Clerck, R., 1992. Recruitment in sole stocks, *Solea solea* (L.) in the northeast Atlantic. Netherlands Journal of Sea Research 29, 173-192.
- Rogers, S.I., 1994. Population density and growth rate of juvenile sole *Solea solea* (L.) Netherlands Journal Sea Research 32, 353-360.
- Sogard, S.M., 1992. Variability in growth rates of juvenile fishes in different estuarine habitats. Marine Ecology Progress Series 85, 35-53.
- Sogard, S.M., 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin Marine Science 60, 1129-1157.
- SFA (Sustainable Fisheries Act) US Senate 23 May., 1996. Report of the committee on Commerce, Science and Transportation on S.39: Sustainable Fisheries Act. Report 104-276, 104th Congress, Second Session. US Government Printing, Washinton, D.C., USA.
- Tinti, F., Picinetti, C., 2000. Molecular systematics of the Atlanto-Mediterranean *Solea* species. Journal of Fish Biology 56, 604-614.
- Vale, C., 1986. Distribuição de metais e matéria particulada em suspensão no sistema estuarino do Tejo. Dissert. Invest. Inst. Nacional Investigação Pesca, Lisboa.
- Van der Veer, H.W., Bergman, M.J.N., 1987. Predation by crustaceans on a newly settled 0-group plaice, *Pleuronectes platessa*, population in the Western Wadden Sea. Marine Ecology Progress Series 35, 203-215.
- Van der Veer, H.W., Bergman, M.J.N., Rijnsdorp, A.D., 1994. Impact of juvenile growth on recruitment in flatfish. Netherlands Journal of Sea Research 32, 153-173.
- Van der Veer, H.W., Phil, L., Bergman, M.J.N., 1990. Recruitment mechanisms in North Sea Plaice *Pleuronectes platessa*. Marine Ecology Progress Series 64, 1-12.
- Weber, L.P., Higgins, P.S., Carlson, R.I., Janz, D.M., 2003. Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. Journal of Fish Biology 63, 637-658.
- Wegner, G., Damm, U., Purps, M., 2003. Physical influences on the stock dynamics of plaice and sole in the North Sea. Scientia Marina 67, 219-234.
- Yamashita, Y., Tominaga, O., Takani, H., Yamada, H., 2003. Comparison of growth, feeding and cortisol level in *Platichthys bicoloratus* juveniles between estuarine and nearshore nursery grounds. Journal of Fish Biology 63, 617-630.

Latitudinal variation in spawning period and growth of 0-group sole, *Solea solea* (L.).

Abstract: 0-group sole, *Solea solea* (Linnaeus, 1758) were sampled in four nursery grounds: two in the Northern French coast and two in the Portuguese coast. Juvenile sole were collected at the Vilaine estuary (Northern Bay of Biscay) in 1992, in the Authie estuary (Eastern English Channel) in 1997, and in the Douro and Tagus estuary (Northern and central Portugal, respectively) in 2005. Left lapilli otoliths were used to estimate age and investigate variability in growth rates and hatch dates. In the French study areas nursery colonisation ended in early June in the Vilaine estuary and in late June in the Authie estuary. In the Portuguese estuaries nursery colonisation ended in May in the Douro estuary and in late June in the Tagus estuary. Growth rates were higher in the Portuguese estuaries, 0.767 mm.d^{-1} in the Tagus estuary and 0.903 mm.d^{-1} in the Douro estuary. In the French nurseries, growth rates were estimated to be 0.473 mm.d^{-1} in the Villaine estuary and 0.460 mm.d^{-1} in the Authie estuary. Data on growth rates from other studies shows that growth rates are higher at lower latitudes, probably due to higher water temperature. Spawning took place between early January and early April in the Villaine estuary's coastal area in 1992. In 1997, in the Authie estuary spawning started in late January and ended in early April. In the Douro estuary's adjacent coast spawning started in mid-January and ended in late-March, in 2005, while in the Tagus estuary's adjacent coast spawning started in mid-February and ended in mid-April, in the same year. Literature analysis of the spawning period of sole along a latitudinal gradient ranging from 38°N to 55°N in Northeast Atlantic indicated that there is a latitudinal trend, in that spawning starts sooner at lower latitudes. Results support that local conditions, particularly hydrodynamics, may overrule general latitudinal trends.

Key-words: Latitudinal variation; Growth; Spawning; *Solea solea*; Juvenile nursery grounds; Northeast Atlantic

Introduction

Determination of spawning period and 0-group juveniles' growth in fish is very important for the study of fish recruitment. Temporal changes in spawning can contribute to variations in year-class strength by influencing the spatial and temporal coexistence of larvae, prey availability, predator abundance, and favourable environmental conditions. Growth during the first months of life is also crucial for fish survival, since faster growth implies improved predator avoidance and a wider choice in prey (Van der Veer and Bergman, 1987; Ellis and Gibson, 1995; Sogard, 1992; 1997). However, the study of spawning in fish is generally difficult and time consuming, since it requires previous knowledge of the main spawning areas and several successive egg sampling surveys throughout the spawning period which generally extends over several months.

The discovery of daily increments in the otoliths of marine fish (Pannella, 1971) provided a powerful tool to study the early life history of fish. Counts of such increments have been used to examine temporal and spatial variability in spawning and growth rates (Methot, 1983 ; Yoklavich and Bailey, 1990). Hatch-dates of the young juveniles collected in coastal

nursery grounds at the end of the settlement period, can thus be back-calculated, overcoming the difficulties of traditional successive egg sampling.

The study of fish recruitment requires not only the determination of spawning periods and 0-group juveniles' growth but also the identification of the factors which govern their dynamics. Several studies suggest that the factors controlling recruitment of a species vary over its geographic range, e.g. along a latitudinal gradient (Houde, 1989; Miller *et al.*, 1991; Pauly, 1994).

Miller *et al.* (1991) developed the latter called "species range hypotheses" (Leggett and Frank, 1997) which assumes that species differ in their susceptibility to different controls on recruitment due to different life history traits, and that species life history traits vary over their distribution range. Controlling factors will, this way, differ over latitudinal and inshore-offshore gradients. Looking at the latitudinal and inshore-offshore variation in food, predation and abiotic factors these assumptions lead to the following implications: (1) abiotic factors are most important at the edges of the species range; (2) predation plus abiotic factors control recruitment at the polar edge of the range; (3) food plus abiotic factors control recruitment at the equatorial edge. Miller *et al.* (1991) also predicted that recruitment would be more variable at the polar edge of the species range, least near the centre of the range, and be intermediate near the equatorial edge. However, they pointed out that inshore-offshore environmental gradients may swamp latitudinal effects.

Since then, several studies observed variation patterns that do not correspond to the "species range hypotheses" expectation (Walsh, 1994; Leggett and Frank, 1997; Phillipart *et al.* 1998). Van der Veer *et al.* (2000) concluded that the likely trends in food, predation and abiotic factors, on which Miller *et al.* (1991) based their hypotheses, will probably act only in the juvenile stage, while year-class strength appears to be established already in the pelagic phase (Leggett and Frank, 1997; Van der Veer *et al.* 2000). The dominance of density independent factors operating at a local scale on the eggs and larvae stresses the importance of hydrodynamic circulation as a key factor in determining recruitment in flatfish (Leggett and Frank, 1997).

In a global perspective on flatfish distribution, Pauly (1994) analysed major latitudinal trends in recruitment concluding that their ultimate cause was a temperature mediated difference in metabolic rate (Pauly, 1978, 1979). This author also noted that while in temperate waters there is one narrow peak of flatfish spawning and recruitment (Cushing, 1975) consequence of the single annual peak of primary production, in tropical waters there is not one spawning peak but two extended spawning periods of unequal importance that reflect primary production dynamics in warm waters (Pauly and Navaluna, 1983). The difference in the importance of these two spawning and recruitment periods increases gradually towards higher latitudes until it is reduced to the narrow peak observed in temperate waters (Pauly, 1994).

The common sole, *Solea solea* (Linnaeus, 1758), is a flatfish of high commercial importance in Northwest Europe. This species is found in coastal waters of the eastern North Atlantic, from western Scotland and the western Baltic Sea to Southern Western Europe,

including the Mediterranean and extending southwards along the African coast as far as Senegal (Whitehead *et al.*, 1986). Sole spawns over winter and spring generally at depths between 40m and 100m (Koutsikopoulos *et al.*, 1991; Wegner *et al.* 2003). Several studies have assessed the factors affecting recruitment in sole and, although some conclusions may seem contradictory (e.g. Henderson and Holmes, 1991; Henderson and Seaby, 1994 and Rijnsdorp *et al.* 1992), it is generally agreed that recruitment of sole is determined before the end of the first year of life and that water temperature plays an important role (e.g. Rijnsdorp *et al.*, 1992; Van der Veer *et al.* 2000; Wegner *et al.*, 2003; Henderson and Seaby, 2005). However, all of these studies were carried out in temperate waters; in fact Van der Veer *et al.* (1994) concluded that most studies on flatfish recruitment were conducted in temperate systems which may have biased the conclusions. They also referred that recruitment variability increases towards lower latitudes. Due to more prolonged spawning and settlement periods, variability in juvenile size increases and therefore size-selective mortality becomes an important factor. The understanding of the factors controlling recruitment in flatfish, and sole in particular, is hampered by the lack of studies in (sub)tropical areas (Van der Veer *et al.*, 1994; Pauly, 1994). The understanding of the recruitment process over the whole distribution area of sole will bring new insights into the population dynamics of this species.

The main objectives of the present work were to assess geographical differences in 1) timing of spawning, and 2) growth rates of *S. solea* juveniles during their first months following settlement, in the Northeast Atlantic.

Materials and Methods

Study areas

Nursery grounds studied in France are located on the Northern French coast (Fig.1). The Villaine and the Authie estuary were chosen for this study because they are located at latitudes where climate is temperate and sole population dynamics is well documented (e.g. Lagardère, 1987; Koutsikopoulos *et al.*, 1989; Marchand, 1991; Koutsikopoulos and Lacroix, 1992; Amara *et al.*, 1993; 1994; Amara, 2004; Le Pape *et al.*, 2003). Climate in this area is temperate.

Nursery areas studied in Portugal are located in the Portuguese West coast (Fig.1). The Douro and Tagus estuaries were chosen for this study because they are two of the most important nursery areas for this species at its subtropical range (Cabral *et al.*, 2007) and also because they are located at different latitudes and at a considerable distance (ca. 300 km) (Fig.1). Climate in this area is Mediterranean with mild winters and warm, dry summers (Aschmann, 1973).

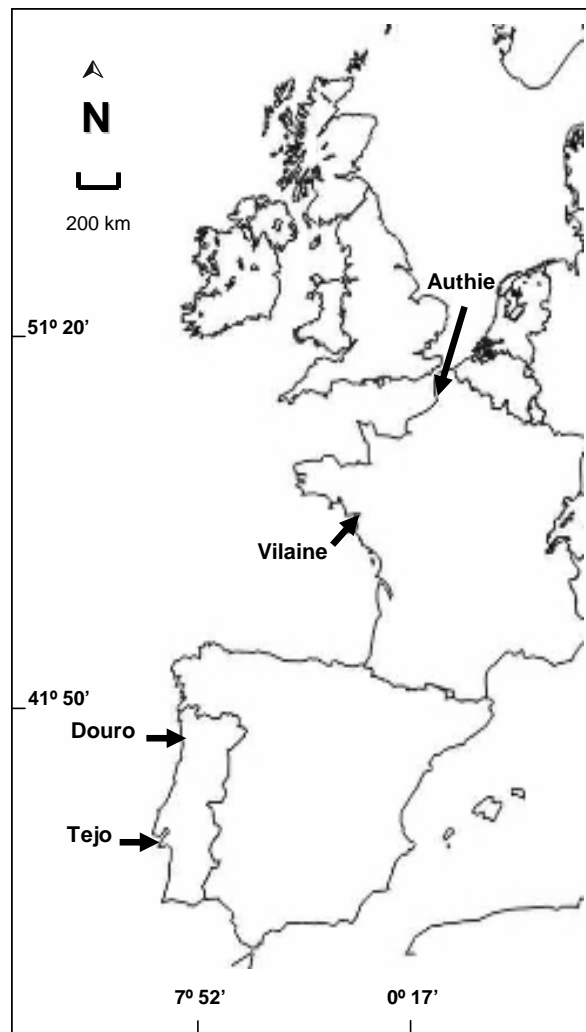


Figure 1 – Map of Western Europe (arrows point at the study areas).

Water temperatures in the adjacent coast of the study areas, during a broad period that encompasses the spawning period (in the area between the 50 m and the 100 m bathymetric), were accessed at the World Data Center for Remote Sensing of the Atmosphere (WDC-RSAT) and consist on Sea Surface Temperature derived from NOAA-AVHRR data. The range of SST values in this database is scaled between 0.125°C and 31.75°C (maximum temperature). The radiometric resolution is 0.125°C. Data from all six of the passes that the satellite makes over Europe in each 24 hour period are used. The SST maps are composed according the maximum temperature value given at every pixel's position to minimize cloud coverage. Weekly values were derived from the daily maximum images using the average at every pixel's position (Fig.2 and 3).

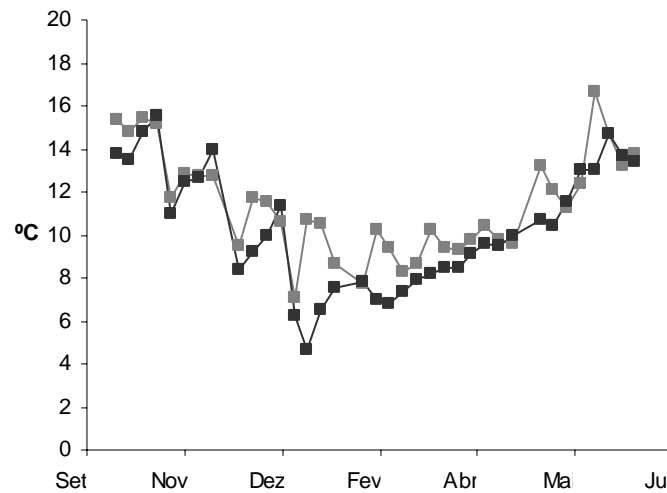


Figure 2 – Surface sea water water temperatures in the study areas of France (Authie estuary data (1996-97) presented in black, Vilaine coastal area data (1991-92) presented in grey).

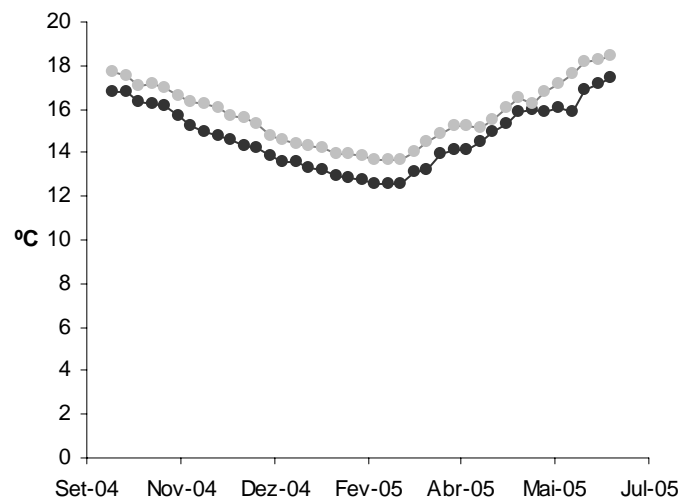


Figure 3 – Surface sea water water temperatures in the study areas of Portugal (Douro estuary's adjacent coastal area data presented in black, Tagus estuary's adjacent coastal area data presented in grey).

Juvenile collections

0-group sole were collected at the end of the settlement period on four important and geographically distant nursery grounds of the French and Portuguese coasts. Samples were collected throughout the immigration period and length frequency distributions were analysed in

order to determine the end of estuarine colonization. French estuaries were surveyed year around, every two weeks.

Both Portuguese estuaries were surveyed monthly from March to September 2005. Surveys were intensified from early May in the Douro and from early June in the Tagus (when the first 0-group juveniles were detected in the nursery areas) until July, taking place at approximately two weeks intervals, in order to better determine the end of the estuarine immigration process of the first cohort. Length frequency of 0-group juveniles at the end of the colonization period was analysed for each study area.

In the Vilaine estuary, estuarine colonization ended in June in 1992. 0-group sole were collected from 13 stations on the 2nd of June 1992, with a small sledge (1 m wide by 0.3 m high, 4.1 m length) without tickler chains, fitted with a 5 mm mesh mouth and a 1.5 mm codend (Marchand and Masson, 1988). The average depth in this area is 6 m at mean tide. In the Authie estuary, estuarine colonization ended in June, in 1997. Samples were carried out at 8 stations parallel to the coast (the average depth is 5 m at mean tide) on 24 June 1997. Juveniles were collected with a 3 m beam trawl with one tickler chain and fitted with 14 mm mesh mouth and 6 mm codend. In the Douro estuary, estuarine colonization ended in May, in 2005. 0-group sole were collected at 10 stations on 7th May 2005. Trawls were conducted with a 12 m otter-trawl with 10 mm mesh size (stretched mesh) and a 5 mm codend (beam-trawling is not possible in the Douro estuary due to its bottom morphology). To ensure that the trawl would not lose contact with the bottom, and thereby maintaining a high catching efficiency for flatfish, the ground rope of the trawl was equipped with a heavy metal chain. In the Tagus estuary colonization ended in late June in 2005. 0-group sole were collected at 10 stations on the 27th June 2005. Trawls were conducted with a 2.5 m beam trawl with 10 mm mesh size (stretched mesh) and a 5 mm codend. Differences in fishing methods are not important, since the aim of the present study was to analyse the populations structure in terms of age and length and not to directly compare densities.

All samples were preserved in 95% ethanol (in France) or immediately frozen (in Portugal). In the laboratory all soles were counted and total length (TL) measured to the nearest 1 mm.

Age and growth determination

Otoliths of a subsample of juveniles chosen randomly from each length category (5 mm) were examined. The left lapillus, which has the longest axis due to the bilateral asymmetry between the right and left lapillus, was used for all age estimates. Lapilli otoliths were used because they are relatively thin and have well-defined increments spatially more uniform than in sagittae otoliths which have accessory primordia (Amara *et al.*, 1994).

Otoliths were analysed under transmitted light at X400 or X1000 magnification, using a video system fitted to a compound microscope. Otolith counts and measurements were made along the posterior axis. Otolith increments were counted three times, and the age was regarded as the mean of the three counts.

Growth was described by a linear model. An analysis of covariance (ANCOVA) was done to test among geographic area differences in growth (slope of age against length) over the first months of the juvenile life.

Back-calculation of spawning date distributions

Hatch-dates were estimated from age and date of capture. Duration of the embryonic period was calculated based on Fonds (1979), according to the water temperature. Length-frequency distributions were converted to age using separate age-length keys developed from sub-samples of fish within each year and for each of the four areas. Spawning periods along a latitudinal gradient in the Northeast Atlantic were compared based on the present work and published literature.

Results

Length frequency distribution of 0-group sole at the end of the colonization period showed a normal distribution fit in all nurseries studied (Fig. 4). Growth during the first months following settlement was best described by a linear model (Fig. 5). In the Vilaine estuary, growth rates of 0-group juveniles were estimated to be 0.473 mm.d^{-1} (range of total length of individuals analysed, TL : 20-66 mm; $n = 198$) in 1992, while in the Eastern Channel growth rate was estimated to be 0.460 mm.d^{-1} (LT : 19-65 mm; $n = 226$) in 1997. In the Douro estuary, 0-group juveniles growth rate was estimated to be 0.903 mm.d^{-1} (range of total length of individuals analysed, TL : 31-91 mm; $n = 60$) in 2005, while in the Tagus estuary 0-group juveniles growth rate was estimated to be 0.767 mm.d^{-1} (range of total length of individuals analysed, TL : 57-109 mm; $n = 215$) in 2005. Significant differences were found in the growth rates between all sites analysed ($P < 0.05$).

The spawning period of the Vilaine estuary juveniles took place from early January to early April in 1992 (Fig.6). For the Eastern Channel juvenile sole population spawning period was estimated to be from late January to mid April, in 1997 (Fig.6). Spawning of Portuguese sole juveniles took place from the 23rd of January to the 30th of March and from the 12th of February to the 21th April, for the Douro and Tagus estuaries, respectively (Fig.6). The analysis of *S. solea* spawning period at different latitudes based on the present study and published literature shows a latitudinal trend in spawning dates, with spawning starting earlier at lower latitudes (Fig. 6). Both French estuaries followed this trend. In the Vilaine estuary hatch dates indicated earlier spawning, from December to early April, than in the Authie estuary, from late January to mid April. In the Douro estuary spawning started in mid-January and ended in late-March, in 2005, while in the Tagus estuary spawning started in mid-February and ended in mid-April, in the same year. The Portuguese estuaries agree with the latitudinal trend when compared to the higher latitudes but not when compared to the French estuaries.

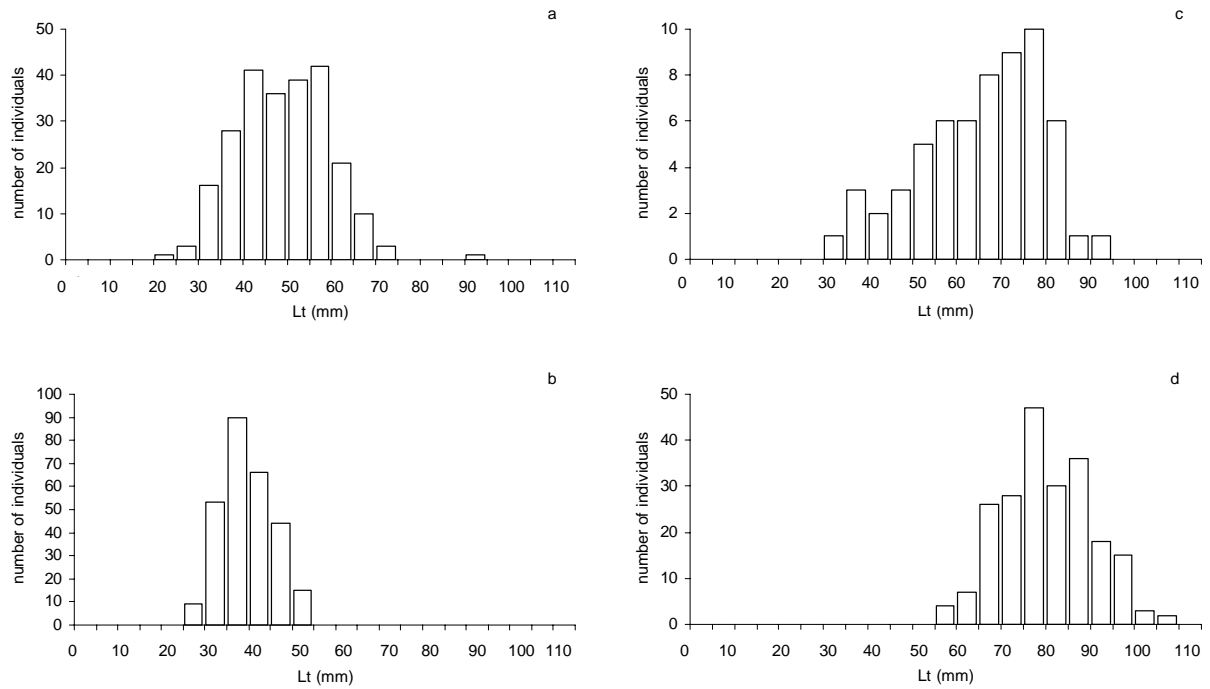


Figure 4 – Length frequency of 0-group juvenile *S. solea* in each study area (a- Vilaine estuary 1992; c- Authie estuary 1997; d- Douro estuary 2005; e- Tagus estuary 2005).

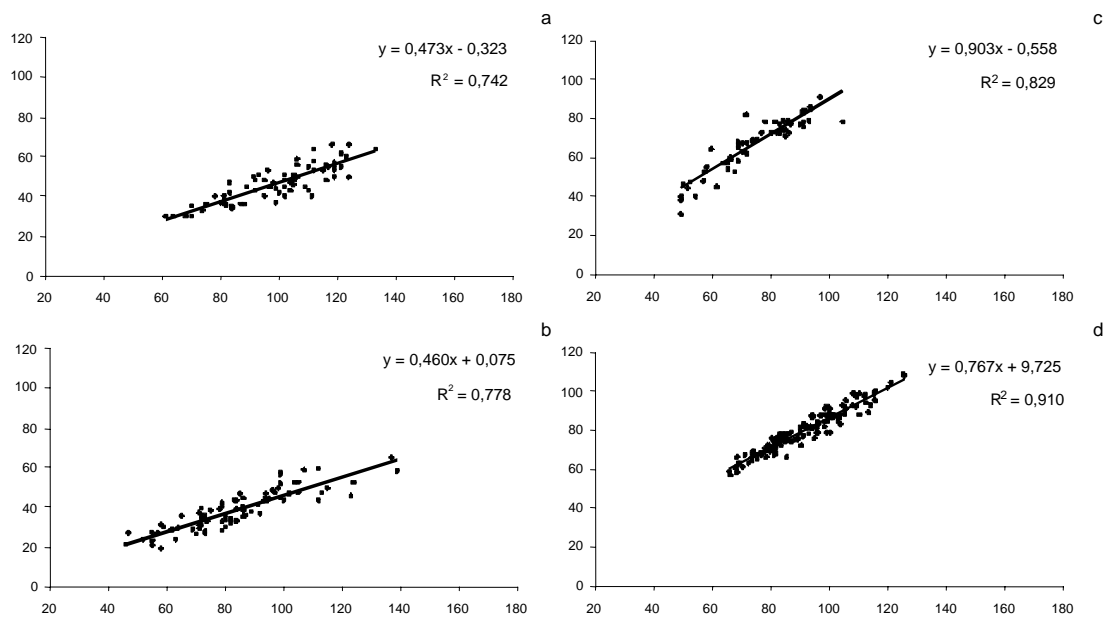


Figure 5 – Linear regression describing growth in each study area (a- Vilaine estuary 1992; b- Vilaine estuary 1993; c- Authie estuary 1997; d- Douro estuary 2005; e- Tagus estuary 2005).

Discussion

Comparison of spawning periods

Results from the present study and literature analysis on the *S. solea* spawning period at different latitudes indicates that there is a latitudinal trend, in that spawning starts sooner at lower latitudes (Fig 6).

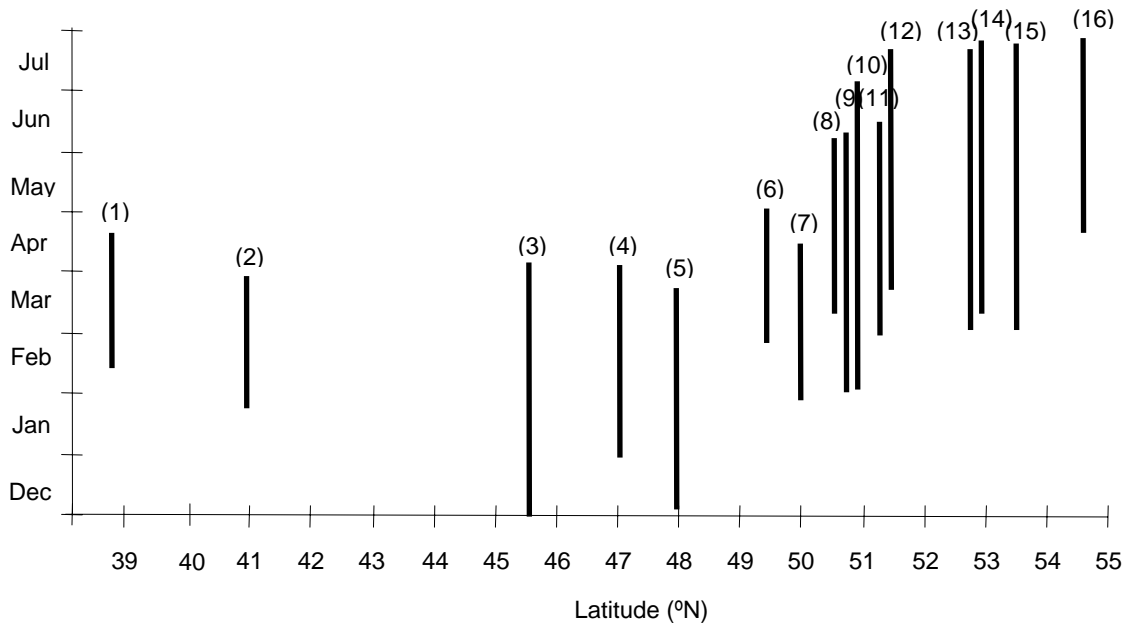


Figure 6 - *S. solea* spawning period at different latitudes based on the present study (1 – Tagus estuary, 2 – Douro estuary, 4 – Vilaine estuary and 7 – Authie estuary) and published literature (LeBec, 1983 (3), Deniel, 1981 (5), ICES, 1992 (6, 8, 11, 12, 13, 14, 15, 16, 17), Horwood, 1993 (9), Woerling and Lehoerff, 1993 (10)).

Warmer water temperatures during the winter at lower latitudes are expected to have a strong influence on the onset of spawning in fish, leading to earlier colonisation of nursery grounds (Amara *et al.*, 1993; 1994). Along with temperature, photoperiod can also be an important spawning triggering factor, as suggested by Devauchelle *et al.* (1987).

The Portuguese estuaries agree with this trend when compared to the higher latitudes but not so much when compared to the French estuaries. More information about the population dynamics of *S. solea* at these latitudes would be needed in order to fully understand the observed results, yet some considerations may be done. In the case of the Portuguese coast special attention should be paid to the local hydrodynamics, due to the occurrence of coastal upwelling of cold water.

Northerly trade winds created by a latitudinal displacement of the Azores anticyclone favour offshore Ekman transport of surface water (Wooster *et al.*, 1976; Peliz *et al.*, 2002). Although upwelling is more frequent between March and September, it is generally considered

that winds that favour this phenomena are a recurrent feature of the Portuguese coast (Huthnance *et al.*, 2002). Offshore Ekman transport of surface water will likely direct the eggs and larvae of flatfish away from the coastal nurseries, resulting in high mortality rates that will confound analysis based on otolith readings from the survivors.

Results from the Portuguese nurseries support that local conditions, particularly hydrodynamics, may overrule general latitudinal trends, as suggested by Leggett and Frank (1997) and Van der Veer *et al.* (2000).

Comparison of growth rates

Significant growth variations were observed between the two French nursery grounds studied due probably to the different temperature exposure histories (Fig.2). In the Vilaine and in the Authie estuary growth rate estimates were in the range of those recorded for other northern European juvenile sole populations (e.g. Rogers, 1994; Amara, 1994; Amara *et al.*, 2001).

S. solea growth rates were significantly higher in both Portuguese estuaries studied. This was expected since temperature is generally considered the most important factor affecting growth and ocean water temperatures are higher at this latitude, throughout the year (despite cold water upwelling) and temperatures inside the estuarine nurseries are considerably higher than in the coast during the nursery period. The longer photoperiod may also contribute to this result. The Tagus estuary did not fully comply with the latitudinal trend. Although growth rate in this estuary was considerably higher than in France, it was also lower than that found in the Douro, a more northerly estuary. As already mentioned more information on the population dynamics of *S. solea* at this latitude would be needed in order to fully understand the observed results, yet some considerations may be put forward.

S. solea may be facing thermal stress in the Tagus estuary, since water temperature in the nursery grounds largely exceeds its metabolic optimum temperature, which is estimated to be 18.8°C (LeFrançois and Claireaux, 2003). Energy spent on facing adverse conditions will be diverted from growth, thus hindering growth rates. Another important aspect that may affect fish growth is pollution. Heavy metal contamination is much higher in the Tagus than in the Douro, due to higher concentration of polluting industries and human pressure (Vinagre *et al.* 2004; França *et al.* 2005). Since the *S. solea* nursery in the Tagus is located in one of the most polluted areas of the estuary (Vale, 1986), pollution may be hindering sole juveniles growth.

S. solea growth has already been estimated for the Tagus estuaries by Cabral (2003) in 1995 and 1996 and by Fonseca *et al.* (2006) in 2003 and 2004, both using length progression analysis. In 1995 estimated growth rate at the first month of nursery residency was 0,70 mmd⁻¹, in 1996 it was 1,51 mmd⁻¹, in 2003 it was 0,80 mmd⁻¹ and in 2004 it was 1,19 mmd⁻¹ (Cabral, 2003; Fonseca *et al.*, 2006). The growth rate determined for 2005 in the present study (0,767 mmd⁻¹) is within the range of estimations obtained in previous years and thus it may be concluded that growth rates are quite variable in this estuarine system.

This study shows that although major gradients affect spawning and growth of sole, local conditions may overrule the latitudinal trend. The development of longer data time-series in the southern distribution range of sole is needed in order to fully understand recruitment dynamics of this species.

Further investigation on the role of hydrodynamics in the pelagic stage of flatfish and on the metabolic scope and genetic variation within the *S. solea* distribution range will certainly provide new insights into the factors controlling recruitment in sole.

Acknowledgements

This study had the support of Fundação para a Ciência e a Tecnologia (FCT) that financed several of the research projects related to this work and the PhD grant given to C. Vinagre. The authors would like to thank everyone involved in the sampling surveys.

References

- Amara, R., 2004. 0-group flatfish growth conditions on a nursery ground (Bay of Canche, Eastern English Channel). *Hydrobiologia* 518, 23-32.
- Amara, R., Lagardère, F., Desaunay, Y., 1993. Seasonal distribution and duration of the planctonic stage of Dover sole, *Solea solea*, larvae in the Bay of Biscay: an hypothesis. *Journal of Fish Biology* 43, 17-30
- Amara, R., Desaunay, Y., Lagardère, F., 1994. Seasonal variation in growth of larval sole *Solea solea* (L.) and consequences on the success of larval immigration. *Netherlands Journal of Sea Research* 32, 287-298.
- Amara, R., Laffargue, P., Dewarumez, J.M., Maryniak, C., Lagardère, F., Luczac, C., 2001. Feeding ecology and growth of 0-group flatfish (sole, dab and plaice) on a nursery ground (Southern Bight of the North Sea). *Journal of Fish Biology* 58, 788-803.
- Aschmann, H. 1973. Distribution and peculiarity of Mediterranean Ecosystems. Origins and Structure. F. Castri and H.A. Mooney (eds.) *Ecological studies* 7, 11-19. Springer-Verlag, Berlin.
- Beverton, R.J.H., Iles, T.C., 1992. Mortality rates of 0-group plaice (*Pleuronectes platessa* L.), dab (*Limanda limanda* L.) and turbot (*Scophthalmus maximus* L.) in European waters II. Comparison of mortality rates and construction of life table for 0-group plaice. *Netherlands Journal of Sea Research*, 29, 49-59.
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the United Kingdom* 83, 861-868.
- Cabral, H., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P., Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P.T., Costa, M.J., 2007. Relative importance of estuarine flatfish nurseries along the Portuguese coast. *Journal of Sea Research* 57, 209-217. doi:10.1016/j.seares.2006.08.007.

- Cushing, D.H., 1975. Marine ecology and fisheries. Cambridge University Press, Cambridge, 1-278.
- Deniel, C. 1981. Les poissons plats (Téléostéens, Pleuronectiformes) en baie de Douarnenez. Reproduction, croissance et migration des Bothidae, Scophthalmidae, Pleuronectidae et Soleidae. Thèse d'Etat. Université de Bretagne Occidentale, Brest, pp.474.
- Devauchelle, N., Alexandre, J.C., Le Corre, N., Letty, Y., 1987. Spawning of sole (*Solea solea*) in captivity. *Aquaculture* 66, 125-147.
- Ellis, T., Gibson, R.N., 1995. Size selective predation of 0-group flatfishes on a Scottish coastal nursery ground. *Marine Ecology Progress Series* 127, 27-37.
- Fonds, M., 1979. Laboratory observations on the influence of temperature and salinity on development of the eggs and growth of the larvae of *Solea solea* (Pisces). *Marine Ecology Progress Series* 1, 91-99.
- Fonseca, V.F., Vinagre, C., Cabral, H., 2006. Growth variability of juvenile sole *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, and comparison with RNA-DNA ratios in the Tagus estuary, Portugal. *Journal Fish Biology* 68, 1551-1562. doi:10.1111/j.0022-1112.2006.001042.x
- França, S., Vinagre, C., Caçador, I., Cabral, H.N., 2005. Heavy metal concentrations oin sediment, benthic invertebrates and fish in three saltmarsh areas subjected to different pollution loads in the Tagus estuary (Portugal). *Marine Pollution Bulletin* 50, 993-1018.
- Henderson, P.A., Holmes, R.H.A., 1991. On the population dynamics of dab, sole and flounder in the lower Severn estuary, England. *Netherlands Journal of Sea Research* 27, 337-344.
- Henderson, P.A., Seaby, R.M.H., 1994. On the factors influencing juvenile flatfish abundance in the lower Severn estuary, England. *Netherlands Journal of Sea Research* 32, 321-330.
- Henderson, P.A., Seaby, R.M., 2005. The role of climate in determining the temporal variation in abundance, recruitment and growth of sole *Solea solea* in the Bristol Channel. *Journal of the Marine Biological Association of the United Kingdom* 85, 197-204.
- Horwood, 1993. The Bristol channel sole (*Solea solea* (L.)): a fisheries case-study. *Advances in Marine Biology* 29, 216-367.
- Houde, E.D., 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2, 17-29
- Huthnance, J.M., Aken, H.M.V., White, M., Barton, E.D. and 5 others., 2002. Ocean margin exchange – water flux estimates. *Journal of Marine Systems* 32, 107-137.
- ICES, 1992. Report on the study group on the fecundity of sole and plaice in sub-areas IV, VII and VIII. Lowestoft, 6-10 July 1992.-ICES C.M. 1992/G :16, 1-73.
- Koutsikopoulos, C., N. Lacroix., 1992. Distribution and abundance of sole (*Solea solea*) eggs and larvae in the bay of Biscay between 1986 and 1989. *Netherlands Journal of Sea Research* 29, 81-97.
- Koutsikopoulos, C., Desaunay, Y., Dorel, D., Marchand, J., 1989. The role of coastal areas in the life history of sole (*Solea solea* L.) in the Bay of Biscay. *Scientia marina* 53, 567-575.

- Koutsikopoulos, C., Fortier, L., Gagné, J., 1991. Cross-shelf dispersion of Dover sole eggs and larvae (*Solea solea*) in Biscay Bay and recruitment to inshore nurseries. *Journal of Plankton Research* 13, 923-945.
- Lagardère, J.P., 1987. Feeding ecology and daily food consumption of common sole, *Solea vulgaris* Quensel, juveniles of the french atlantic coast. *Journal of Fish Biology* 30, 91-104.
- Le Bec, C. 1983. Fécondité de la sole *Solea solea* (Quensel, 1806) du golfe de Gascogne. ICES. G53, 16 pp.
- LeFrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole, *Solea solea*. *Marine Ecology Progress Series* 259, 273-284.
- Leggett, W.C., Frank, K.T. 1997. A comparative analysis of recruitment variability in North Atlantic Flatfishes – testing the species range hypothesis. *Journal of Sea Research*, 37, 281-299.
- Marchand, J., 1991. The influence of environmental conditions on the settlement, distribution and growth of 0-group sole (*Solea solea* (L.)) in a macrotidal estuary (Vilaine, France). *Netherlands Journal of Sea Research* 27, 307-316.
- Method, R.D. Jr. 1983. Seasonal variation in survival of larval northern anchovy, *Engraulis mordax*, estimated from the age distribution of juveniles. *Fisheries Bulletin U.S.* 81, 741-750.
- Miller, J.M., Burke, J.S. Fitzhugh, G.R. 1991. Early life history patterns of Atlantic North American flatfish: likely (and unlikely) factors controlling recruitment. *Netherlands Journal of Sea Research*, 27, 261-275.
- Panella, G., 1971. Fish Otoliths: daily growth rates and periodical patterns. *Science* 173, 1124-1127.
- Pauly, D., 1978. A preliminary compilation of fish length and growth parameters. *Ber. Inst. Meeresk. Kiel* 55, 1-200.
- Pauly, D., 1979. Gill size and temperature as governing factors in fish growth: a generalization of von Bertalanffy's growth formula. *Ber. Inst. Meeresk. Kiel* 63, 1-200.
- Pauly, D., 1994. A framework for latitudinal comparisons of flatfish recruitment. *Netherlands Journal of Sea Research* 32 (2), 107-118.
- Pauly, D., Navaluna, N.A., 1983. Monsoon-induced seasonality in the recruitment of Phillipine fishes – *FAO Fisheries Report* 291, 823-833.
- Peliz, Á., Rosa, T.L., Santos, A.M.P., Pissarra, J.L., 2002. Fronts, jets and counter-flows in the Western Iberian upwelling system. *Journal of Marine Systems* 35, 61-77.
- Phippart, C.J.M., Henderson, P.A., Joahnnessen, T., Rijnsdorp, A.D., Rogers, S.I. 1998. Latitudinal variation in fish recruits in Northwest Europe. *Journal of Sea Research* 39, 69-77.

- Rijdsdorp, A.D., Van Beek, F.A., Flatman, S., Millner, R.M., Riley, J.D., Giret, M., De Clerck, R., 1992. Recruitment in sole stocks, *Solea solea* (L.) in the northeast Atlantic. Netherlands Journal of Sea Research 29, 173-192.
- Rogers, S.I., 1994. Population density and growth rate of juvenile sole *Solea solea* (L.) Netherlands Journal of Sea Research 32, 353-360.
- Sogard, S.M., 1992. Variability in growth rates of juvenile fishes in different estuarine habitats. Marine Ecology Progress Series 85, 35-53
- Sogard, S.M., 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science 60, 1129-1157.
- Vale, C., 1986. Distribuição de metais e matéria particulada em suspensão no sistema estuarino do Tejo. Dissert. Invest. Inst. Nacional Investigação e Pescas, Lisboa.
- Van der Veer, H.W., Bergman, M.J.N., 1987. Predation by crustaceans on a newly settled 0-group plaice, *Pleuronectes platessa*, population in the Western Wadden Sea. Marine Ecology Progress Series 35, 203-215.
- Van der Veer, H.W., Berghahn, R., Rijnsdorp, A.D., 1994. Impact of juvenile growth on recruitment in flatfish. Netherlands Journal of Sea Research 32, 153-173.
- Van der Veer, H.W., Berghahn, R., Miller, J.M., Rijnsdorp, A.D., 2000. Recruitment in flatfish, with special emphasis on North Atlantic species: Progress made by the Flatfish Symposia. ICES Journal of Marine Science 57, 202-215.
- Vinagre, C., França, S., Costa, M.J., Cabral H.N. 2004. Accumulation of heavy metals by flounder, *Platichthys flesus* (L. 1758) in a heterogeneously contaminated nursery area. Marine Pollution Bulletin 49, 1109-1126
- Walsh, S.J., 1994. Recruitment variability in populations of lough rough dab (American plaice) *Hippoglossoides platessoides* (Fabricius) in the North Atlantic. Netherlands Journal of Sea Research, 32, 421-431.
- Wegner, G., Damm, U., Purps, M., 2003. Physical influences on the stock dynamics of plaice and sole in the North Sea. Scientia Marina 67, 219-234
- Whitehead, P., Bauchot, M-L., Hureau, J.-C., Nielsen, J., Tortonese, E. (1986) Fishes of the North-eastern Atlantic and Mediterranean, UNESCO, Paris, Vol III
- Woerling, D., G. Le Fevre Lehoerff. 1993. Fluctuations pluriannuelles de la ponte de la sole *Solea solea* (L.) sur le littoral du sud de la mer du Nord. J. Rech. océanogr. 18, 74-79.
- Wooster, W.S., Bakun, A., McLain, D.R., 1976. The seasonal upwelling cycle along the eastern boundary of the North Atlantic. Journal of Marine Research 34, 131-141.
- Yakovlich, M.M., Bailey, K.M., 1990. Hatching period, growth and survival of young walleye Pollock *Theragra chalcogramma* as determined from otolith analysis. Marine Ecology Progress Series 64, 13-23.

Conclusion

The introduction of new tools to the investigation of growth and condition of *S. solea* and *S. senegalensis* in the Tagus estuary, revealed previously unknown patterns related to estuarine colonization, allowed the differentiation of the two nursery areas and the analysis of growth and spawning in a wide geographical perspective.

The assessment of growth and condition variability in these two species showed that growth rate and condition depend heavily on the estuarine colonization process. When young juvenile soles enter the estuary they present fast growth rates and high RNA-DNA ratios that decrease over time. The decrease of growth rate with age has already been reported for other species, it seems to be predetermined and, to a certain degree, providing that basic needs are met, independent of environmental factors. This was observed in all cohorts and in both species. The first cohort to colonize the estuary presents higher growth and condition than subsequent cohorts, possibly due to higher availability of food and less competition. Higher growth rates were found for *S. senegalensis* when compared to *S. solea*. Higher growth rates were found for the Tagus soles than reported for northern European areas, it was also concluded that soles from the Tagus estuary are in good overall condition.

Differences in habitat specific growth rates were found among the two nursery areas of the Tagus estuary. Results indicated that in 2005 nursery B provided higher habitat quality for *S. senegalensis* than nursery A. This may have important implications in a warming climate scenario, since water temperatures will likely be more appropriate for subtropical species, such as *S. senegalensis*, than for temperate species such as *S. solea* that will probably suffer thermal stress. It was concluded that the simultaneous use of habitat specific growth rates, that integrate the whole life of the fish, and RNA-DNA ratios that only inform about recent conditions, would be interesting for environmental monitoring purposes since the information provided by the two methods is complementary.

A latitudinal variation was found, in that growth rates are higher and spawning takes place earlier at lower latitudes. This is mainly due to latitudinal trends in water temperature and photoperiod. The Tagus estuary was slightly off trend in a local context, although growth was higher than in the French estuaries studied, it was lower than in the Douro estuary. Temperature is possibly a key factor hindering *S. solea* growth rates in the Tagus estuary, since water temperatures in the Tagus over the juvenile period of this species are higher than its optimum metabolic temperature (approximately 19°C), meaning that *S. solea* may be in thermal stress. Temperature also plays an important role in the toxicity of dissolved chemicals which in turn may affect growth. Spawning was in accordance to the latitudinal trend, in that it took place earlier in the Tagus and Douro estuaries than in northern European estuaries, yet it took place even earlier in the French estuaries. This supports recent theories that state that local conditions, particularly the oceanographic conditions, may overrule general latitudinal trends.

The Portuguese coast is located in a very complex upwelling system in comparison with other major eastern continental boundaries such as the North American West Coast, the Peru-Chile upwelling system or the Benguela upwelling system. It was concluded that upwelling may be an important factor interfering with larval immigration towards nursery areas and thus confounding the back-calculation of spawning based on the survivors that reach the nursery grounds.

More studies are needed in order to clearly establish some of the results putted forward for the first time, in this chapter. A longer and continuous data series on growth and condition patterns in the Tagus estuary, as well as in other estuaries, would allow a more general outlook on growth and condition patterns related to estuarine colonization.

Due to the variability associated with estuarine nurseries more studies are needed to assess habitat quality and find out if one of the nurseries is consistently better than the other, for *S. senegalensis*. Future studies should focus on determining the contribution of each nursery towards the adult stock. Analysis of otoliths' microchemistry has shown promising results in the identification of the original nursery grounds of the individuals composing the adult stocks in other coastal areas and should be applied to the Portuguese sole stocks.

In what concerns habitat quality monitoring, several methods are currently being investigated with interesting results, such as, recent growth estimation based on marginal otolith increment width, condition based on protein concentration, condition based on lipid content and the use of molecular biomarkers in areas subjected to pollution. Future research will certainly determine which are the most appropriate for each species and habitat.

Further investigation on latitudinal trends is urgent in a time when global warming effects are already being reported in several bio-geographical regions of the world, since it will allow for some changes to be predictable and management actions to be taken ahead of time (e.g. to predict earlier spawning and take measures to protect the spawning stock).

Studies on the complex hydrology of the Portuguese coast will certainly provide new insights into its effect upon larval migration, estuarine colonization timing, number of cohorts reaching the nurseries and condition of the newly arrived immigrants. It would also be very important to conduct sampling surveys to collect eggs and larvae in order to determine the location of the spawning areas for sole off the Portuguese coast. This would enable the protection of these areas, and also provide important information for the construction of mathematical models of the movements of eggs and larvae.

CHAPTER 5

- MORTALITY AND RECRUITMENT -

Impact of climate and hydrodynamics on soles' larval immigration into the Tagus estuary, Portugal.
Estuarine, Coastal and Shelf Science (*in press*).

By Vinagre, C., Costa, M.J., Cabral, H.N.

Fishing mortality of the juvenile soles, *Solea solea* and *Solea senegalensis*, in the the Tagus estuary,
Portugal (*submitted*).

By Vinagre, C., Costa, M.J., Cabral, H.N.

Introduction

Mortality and recruitment are key issues in population and fisheries management. These concepts are intertwined since recruitment to a particular stage or area depends on the mortality in the previous stages or areas of the species life cycle (Cushing, 1974; Rothschild, 1986; Zijlstra *et al.*, 1982).

The decline, and in some cases collapse, of some of the world's most important fisheries (e.g. North Sea fisheries, Georges Bank) (Grainger and Garcia, 1996) has captured the attention of biologists all around the world. Investigation has focused on the extent of mortality caused by fishing activities and on the phenomena ruling natural mortality, in an effort to understand the changes taking place. The main aim of such studies has been the management of fish stocks in a sustainable way (e.g. Kawasaki, 1983, Garcia and Staples, 2000).

It has been concluded that overexploitation of target species results in major changes in the ecosystem, since repercussions reach all trophic levels. Substitution of target species by other species that are able to explore the same ecological niches have been widely reported because of its visibility, yet this is surely accompanied by unrecorded and poorly known effects on other levels, such as the benthos and plankton (Moyle and Cech, 1996). Reversibility of these effects is still scarcely understood (Ludwig *et al.*, 1993).

Research on mortality and recruitment become even more complex when scientists in various parts of the world realized that changes had to be analysed against a background of climate change (McFarlane *et al.*, 2000). The geographical location of Portugal, in transition waters between subtropical and temperate regions, makes it particularly vulnerable to climatic changes. An increase in the occurrence of species with tropical affinities and a decrease in the occurrence of species with temperate affinities has been reported (Cabral *et al.*, 2001). Climate and hydrodynamics have been recognised as the main controllers of recruitment variation in flatfish stocks, through their effect upon the eggs and larvae stages (e.g. Marchand, 1991; Van der Veer *et al.*, 2000; Wegner *et al.*, 2003).

In the present chapter, the effect of climate and hydrodynamics on sole larval immigration towards the Tagus estuary was investigated, as well as, the magnitude of the mortality caused by fishing upon the juveniles that reach these nursery grounds.

In order to understand the potential impacts of climate change on the various stages of soles' life cycles it is crucial to look back on existing data to investigate what have been the most important climatic features influencing juvenile soles populations. This investigation focused on the process of larval immigration towards nursery areas, since it is assumed that mortality rate during this process is high, driven by density-independent factors, and that small variations in mortality rate at this point may result in large differences in the number of survivors (Rijnsdorp *et al.*, 1995). The climatic and hydrodynamic features investigated were: river

drainage, because larvae are known to follow chemical cues to direct their migrations; the North Atlantic Oscillation (NAO) index, since it is a good indicator of the prevalent climate conditions in the Portuguese coast; and wind direction, because it is an important factor in larval transport.

The assessment of fishing mortality affecting soles in the Tagus estuary is particularly important because this is the only Portuguese estuary where beam trawling is legal. The Tagus estuary has an exception regime due to its traditional brown shrimp fishery. Yet, since brown shrimp market value suffered a drastic decrease, fishing effort has been re-directed towards soles and other fish species that are caught as juveniles. Beam-trawl is conducted in the nursery areas because 0-group sole are part of the local gastronomy and are also sold to aquacultures. The importance of discards from this fishery was studied by Cabral *et al.* (2002) that concluded that they constituted an important input of organic matter to the estuarine ecosystem. Soles are not discarded in high quantities, because they are in fact the main target species. The investigation of soles' mortality is very complex in this estuary since there are various cohorts colonizing the nursery grounds throughout time, a situation that does not happen in northern European areas, where most studies have deemed secondary cohorts has not significant, leaving them out of mortality estimations (Zijlstra *et al.*, 1982; Desaunay *et al.*, 1987; Jager *et al.*, 1995). The present study is the first to take into account the impact of mortality in the different cohorts colonizing nursery grounds.

References

- Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect environmental changes? *Climate Research* 18, 119-126.
- Cabral, H.N., Teixeira, C.M., Gamito, R., Costa, M.J., 2002. Importance of discards of a beam trawl fishery as input of organic matter into nursery areas within the Tagus estuary. *Hydrobiologia* 475/476, 449-455.
- Costa, M.J., Cabral, H.N., 1999. Changes in the Tagus estuary nursery function for commercial fish species: some perspectives for management. *Aquatic Ecology* 33, 53-74.
- Cushing, D.H., 1974. The possible density-dependence of larval mortality and adult mortality in fishes. In: J.H.S. Blaxter. *The early life of fish*. Springer-Verlag, Berlin, 103-112.
- Desaunay, Y., Koutsikopoulos, C., Dorel, D., 1987. Evaluation de la mortalité saisonnière des jeunes soles durant la phase de pre-recrutment. *Conseil International pour L'Exploration de la Mer* 46, 1-8.
- Garcia, S.M., Staples, D., 2000. Sustainability reference systems and indicators for responsible marine capture fisheries: a review of concepts and elements for a set of guidelines. *Marine and Freshwater Research* 51, 385-426.
- Grainger, R.J.R. and Garcia, S.M., 1996. *Chronicles of Marine Fishery Landings (1950-94): Trend Analysis and Fisheries Potential*. FAO Fisheries Technical Paper No. 359. Rome, Italy: FAO.

- Kawasaki, T., 1983. Why do some pelagic fishes have wide fluctuation in their numbers? Biological basis of fluctuation from the viewpoint of evolutionary ecology. *FAO Fish. Rep.* 29/1 (3), 1065-1080.
- Jager, K., Kleef, H.L., Tydeman, P., 1995. Mortality and growth of 0-group flatfish in the brackish Dollard (Ems estuary, Wadden Sea). *Netherlands Journal of Sea Research* 34, 119-129.
- Ludwig, D., Hilborn, R., Walters, C., 1993. Uncertainty, resource exploitation, and conservation: lessons from history. *Science* 260, 17-36. Reprinted in *Ecological Applications* Vol. 3, no 4, pages 547-549.
- McFarlane, G.A., King, J.R., Beamish, R.J., 2000. Have there been recent changes in climate? Ask the fish. *Progress in Oceanography* 47, 147-169.
- Moyle, P.B., Cech, J.J.J., 1996. *Fishes, an introduction to Ichthyology*, 3rd edn. Prentice Hall, N.J.
- Rijnsdorp, A.D., Berghahn, R., Miller, J.M., van der Veer, H.W., 1995. Recruitment mechanisms in flatfish: what did we learn and where do we go. *Netherlands Journal of Sea Research* 34, 237-242.
- Rothschild, B.J., 1986. *Dynamics of marine fish populations*. Harvard University Press, Cambridge, 1-277.
- Zijlstra, J.J., Dapper, R., Witte, J.I.J., 1982. Settlement, growth and mortality of post-larval plaice (*Pleuronectes platessa* L.) in the western Wadden Sea. *Netherlands Journal of Sea Research* 15, 250-272.

Impact of climate and hydrodynamics on soles' larval immigration towards the Tagus estuary, Portugal

Abstract: Spawning grounds of the soles, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, are distant from the estuarine nurseries where juveniles concentrate. Recruitment of these species is highly dependent on the success of the larval migration towards the inshore nursery grounds. Yet, unfavourable climate and hydrodynamic circulation may lead to high mortality rates at this stage. The relation between river drainage, NAO index and the North-South wind component intensity over the three months prior to the end of the estuarine colonization and the densities of *S. solea* and *S. senegalensis* in the nursery grounds were investigated for both species based on a discontinuous historical dataset (from 1988 to 2006) for the Tagus estuary. Multiple linear regression models were developed for sole density and environmental data (separately for each species). Results showed that river drainage is positively correlated with juveniles densities of both species, possibly due to the existence of chemical cues used by larvae for movement orientation. NAO index and the North-South wind component intensity relations with soles densities were not significant. It was concluded that the high complexity of the Portuguese upwelling system makes it hard to detect causal relations of the environmental variables tested. The importance of river flow for coastal ecosystems was stressed. Since climate change scenarios predict a strong decrease in rain fall over the Portuguese river basins, as well as a concentrated period of heavy rain in winter, it was hypothesised that future river drainage decrease over much of the year may lead to lower recruitment success for soles, especially for *S. senegalensis*.

Key-words: Climate; Recruitment; River drainage; Larvae; Sole; Flatfish, Nursery.

Introduction

Although juvenile nurseries of *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858) are located inshore, spawning takes place offshore (Russel, 1976; Whittames *et al.*, 1995). Thus, larvae must migrate from the spawning grounds along the continental shelf towards shallow coastal areas, and particularly estuaries (Russel, 1976; Norcross and Shaw, 1984). It is generally agreed that recruitment variation in flatfish stocks is dominated by density independent factors operating at a local scale on the eggs and larvae (Leggett and Frank, 1997; Van der Veer *et al.*, 2000), meaning that climate and hydrodynamic circulation are key factors in these species distribution and abundance (*e.g.* Marchand, 1991; Van der Veer *et al.*, 2000; Wegner *et al.*, 2003).

The soles, *S. solea* and *S. senegalensis*, are among the most important commercial fishes using the Tagus estuary as a nursery area (Costa and Bruxelas, 1989; Cabral and Costa, 1999). Data on juvenile sole abundance dating back from 1978 reveals years of high densities contrasting with years where juvenile soles were very scarce in the main Portuguese estuaries (review in Cabral, *et al.*, 2007). Previous studies in the Tagus estuary have reported trends in the fish assemblage related to climatic change. While cold water fish species have been disappearing from the estuary, fish species with tropical affinities have been increasing in abundance (Cabral *et al.*, 2001). Costa *et al.* (*in press*) has reported an important effect of river

flow on the fish assemblage of the Tagus estuary, yet analysed these two species as one item, *Solea sp.*

Several authors have pointed out that larvae of various organisms follow chemical cues from estuaries in order to direct their movement towards nursery areas (Creutzberg *et al.*, 1978; Tanaka, 1985; Tamburri *et al.*, 1996; Forward *et al.*, 2003). Drought and the consequent decrease in river drainage will lower the concentration of estuarine chemical cues reaching coastal waters making it less detectable by larvae. Variation in river drainage also has important consequences within the estuary, leading to changes in organic matter input, salinity, water currents and in the concentration of pollutants.

Several authors have shown that the North Atlantic Oscillation (NAO) index is correlated with precipitation in the west of the Iberian Peninsula since it interferes with the trajectory of depressions in the North Atlantic (Zhang *et al.*, 1997; Trigo *et al.*, 2002), it may therefore be a good indicator of the prevalent climate conditions in the Portuguese coast that will affect soles larvae migration, as it has been shown for sardine larvae transport (Guisande *et al.*, 2001; Borges *et al.*, 2003).

In the case of the Portuguese coast special attention should be paid to the occurrence of coastal upwelling. Offshore Ekman transport of surface water will likely direct the eggs and larvae away from the coastal nurseries, resulting in high mortality rates at these stages. This effect has been observed in sardine off the Portuguese coast (Santos *et al.*, 2001; Borges *et al.*, 2003). Although upwelling is more frequent between March and September, it is generally considered that winds that favour this phenomenon are a recurrent feature of the Portuguese coast and can occur in winter as well (Huthnance *et al.*, 2002). While *S. solea* spawning period takes place from late January to mid-April in the Portuguese coast (Vinagre, *unpublished data*), the spawning period of *S. senegalensis* is very variable and consists of two periods, from January to June and in Autumn around October-November (Anguis and Cañavate, 2005; García-Lopes *et al.*, 2006). Thus, there is an overlap between the spawning periods of both species and the upwelling season.

Evidence of climate change makes the understanding of the effect of these climatic features on fish larvae migration an urgent issue. Global mean temperature has increased since the beginning of the twentieth century, yet this increase has not been homogeneous throughout the globe, temperatures have risen more in some areas. One of such areas is the Iberian Peninsula (IPPC, 2001). Precipitation patterns also changed around the world, with increasing intensity in some parts of the high and medium latitudes of the Northern hemisphere and decreasing intensity and frequency in parts of Europe (including Portugal), Africa and Asia (IPPC, 2001).

Trends in several climatic indices, such as an increase in the Su index (summer days per year), in the HWD index (heat wave duration), and in the PSD index (drought duration) have been reported for Portugal (Miranda *et al.*, 2002; Pires, 2004). Several responses to climate change in the fisheries of bluefin tuna, sardines and octopus off the Portuguese coast have been identified dating back from the twentieth century (Reis *et al.*, 2006).

The understanding of the conditions that affect sole 0-group abundance will bring new insights into the dynamics of these species recruitment in the recent past, thus allowing an improved understanding of natural variation and fisheries impact against a background of climate change. The aim of the present work is to investigate the impact of hydrodynamic and climatic features such as river drainage, the NAO index and wind direction in 0-group *S. solea* and *S. senegalensis* densities within the Tagus estuary, through the analysis of historical data.

Materials and methods

Study area

The Tagus estuary (Figure 1), one of the largest estuaries in Western Europe (320 km²), is a partially mixed estuary with a tidal range of ca. 4 m. Approximately 40% of the estuarine area is intertidal. The upper area of the estuary has been identified a nursery ground for *S. solea* and *S. senegalensis* by Costa and Bruxelles (1989) and Cabral and Costa (1999).

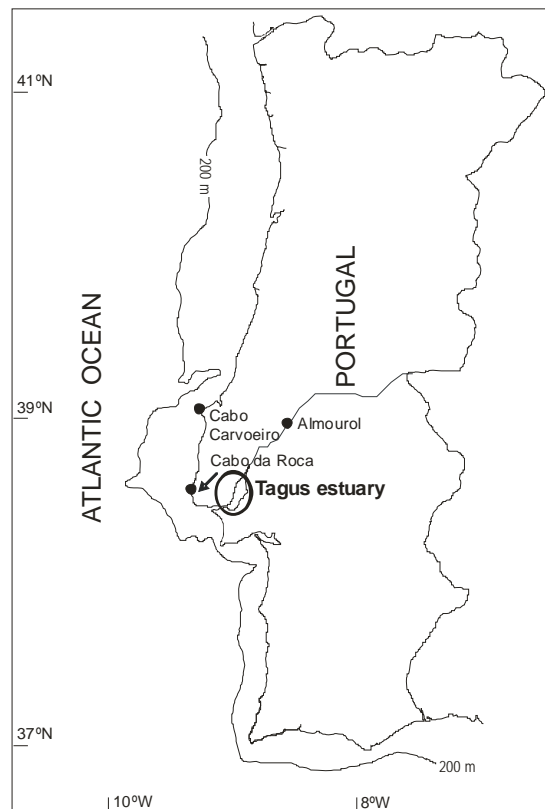


Figure 1 – The Portuguese coast and the Tagus estuary.

The adjacent coast is meridionally oriented and lies in the west of a continental margin. During spring and summer the predominant, north-easterly trade winds cause persistent upwelling of cooler water from about 100-300 m depth, along the entire western Iberian coastal

margin (Fiúza, 1983; Haynes *et al.*, 1993; Smyth *et al.*, 2001). Upwelling events usually begin, and remain particularly intense, off Cabo da Roca (Figure 1), the nearest area to the Tagus estuary of intense upwelling. Upwelling filaments often form at this cape, extending more than 100 km offshore (Haynes *et al.*, 1993) and reaching velocities of 0.28 m s^{-1} (Smyth *et al.*, 2001). In winter the winds relax, with intermittent periods of both upwelling- and downwelling-favourable winds (e.g. Santos *et al.*, 2004; Mason *et al.*, 2005a).

Below the surface, a poleward flowing undercurrent is consistently present over the slope, the Iberian Poleward Current (Huthnance *et al.*, 2002). This is a relatively narrow and weak flow that often extends to the surface during winter (e.g. Haynes and Barton, 1990; Frouin *et al.*, 1990; Mazé *et al.*, 1997).

Data analysis

Fish data analysed are a part of the “Instituto de Oceanografia” database (Faculty of Sciences of the University of Lisbon). Due to the fragmentation of the time-series and the unreliability of fisheries data (species misidentification and unreported catches) the fish density data presented in the present work is not continuous. Beam trawls were conducted monthly or bimonthly in both nurseries areas of the Tagus estuary in all years considered (1988, 1994, 1995, 1996, 2000, 2001, 2002, 2005 and 2006). A four meter beam trawl with one tickler chain and 5 mm stretched mesh at the codend was used. All samples were frozen immediately after collection. In the laboratory individuals were identified, counted and their total length measured to the nearest mm. The distance travelled in each tow was determined based on a global positioning system device (GPS) and the headline length was used as a measure of width in the swept area calculations. Fish abundance was expressed as density (number of individuals per 1000 m^2). Data on 0-group soles was selected for analysis. Monthly 0-group density averages were calculated in order to determine the month of peak abundance of each species for each of the years studied. For the purpose of investigating larval immigration into the estuary, environmental variables that could have affected this process were analysed in the 3 months prior to the peak abundance month. The peak abundance month reflects the end of estuarine immigration of the most successful cohort. We have decided to analyse only the most successful cohort since all years present a pattern of one first very successful cohort that presents much higher densities than all others and is responsible for the most part of juvenile soles living in the nurseries that year (Cabral and Costa, 1999). At the time of peak density fish are approximately 3 months old, since the peak is reached at the end of estuarine colonization after which densities gradually decrease due to mortality within the system.

We have, thus, explored inter-annual differences in soles densities and their relation to several environmental variables that acted upon larvae in the three months prior to the end of estuarine colonization. Since *S. solea* and *S. senegalensis* have considerably different life-cycles separate analysis were carried out for the two species.

It was considered that the data series was not long or continuous enough to enable the analysis of sole density trends during the period considered.

Factors related to spawning, such as spawning biomass and eggs abundance were not used since there is no available data. The spawning areas of these species have not yet been determined for the Portuguese coast. It was assumed that they should be located at depths from 40 to 100 m like in other coastal areas (Koutsikopoulos *et al.*, 1991; Wegner *et al.* 2003).

Monthly mean river drainage for the three months before each density peak was averaged. Data were provided by the National Water Institute (INAG) and were taken at the Almourol data station (Fig. 1). Data on pollution loads that may affect the coast areas during high river discharges as well as the nursery's quality was not taken into account, since only punctual studies exist for this area.

Monthly data on the North Atlantic Oscillation (NAO) index (defined as the pressure difference between Lisbon and Reykjavik) were taken from the United States of America NOAA National Weather Service database, available at <http://www.cpc.noaa.gov>. The average value of this index for the three months prior to each density peak was calculated.

Daily wind data were provided by the Portuguese Meteorological Institute (Instituto de Meteorologia). The station chosen was the Cabo Carvoeiro station (Fig. 1) since this is considered to be the station that better reflects wind in Portugal's west coast. The North-South wind component intensity was calculated (to infer upwelling favourable winds that cause offshore transport of eggs and larvae), as well as its average in the three months prior to each density peak.

Data were explored on Brodgar software (Highland Statistics Lda). Due to the existence of extreme values, fish density data was square root transformed. Data were pair plotted in order to investigate multi-collinearity between the independent variables.

A multiple linear regression was carried out using the 0-group sole density data (separate analysis for each species) as the dependent variable and the environmental variables as the independent variables. Residuals were tested for trends. Multi-collinearity was once more checked with the variance inflation factor (VIF) diagnostic.

Results

S. solea density over the month of peak abundance in the study period presented a distinct peak in 1988 and very low levels from 2000 onwards (Fig. 2a). An important abundance peak in 1988 was also detected for *S. senegalensis* along with very low levels from 2000 to 2002 and 2005 (Fig. 2b).

S. solea densities varied between 0.001 ind.1000 m⁻² and 143 ind.1000 m⁻², while *S. senegalensis* varied between 0.001 ind.1000 m⁻² and 46 ind.1000 m⁻².

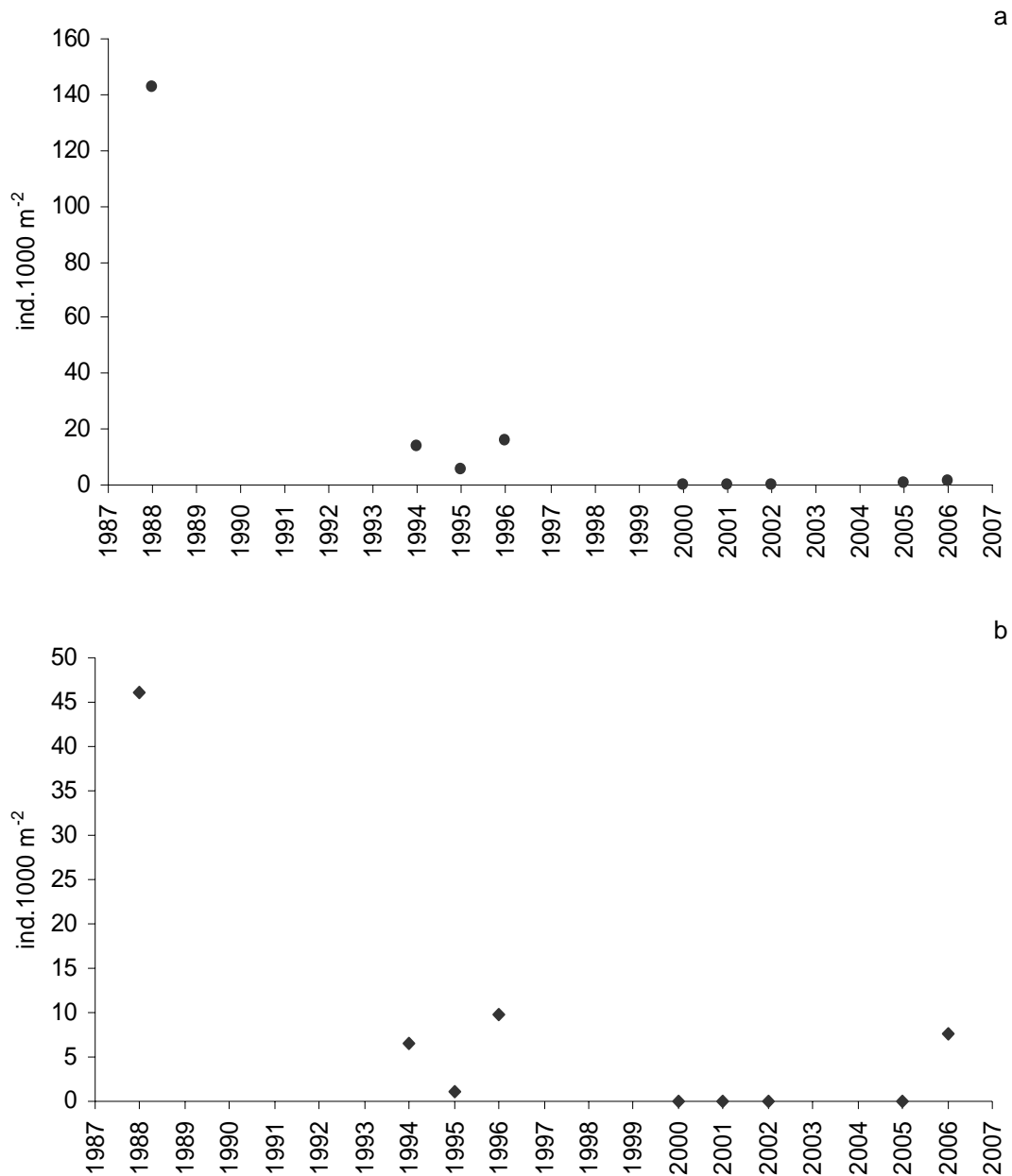


Figure 2 – Soles 0-group density in the month of peak abundance in the study period (a- *S. solea*; b – *S. senegalensis*).

Mean monthly river drainage from 1988 to 2006 was highly variable both seasonally and yearly, with several high peaks and drought periods (Fig. 3). Mean drainage over the three months prior to 0-group sole peak abundance in the estuary presented high values in 1988, 1996 and 2001 for *S. solea* and in 1988, 1994, 1996 and 2006 for *S. senegalensis* (Fig. 4a, 4b). Low values were detected in 2000 and 2005 for *S. solea* and in 1995 and 2005 for *S. senegalensis* (Fig. 4a, 4b). Mean drainage over the periods studied varied between $194 \times 10^6 \text{ m}^3$ and $1378 \times 10^6 \text{ m}^3$ for *S. solea* and $117 \times 10^6 \text{ m}^3$ and $827 \times 10^6 \text{ m}^3$ for *S. senegalensis*.

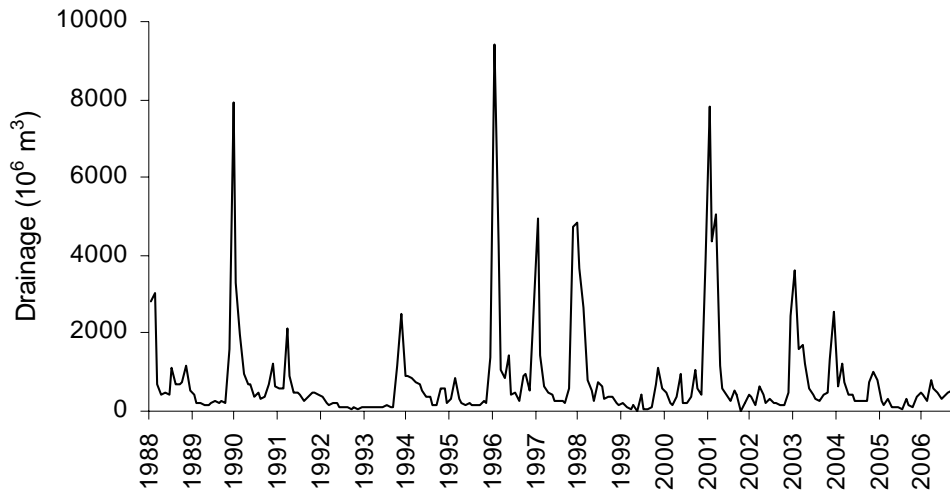


Figure 3 – Mean monthly drainage of the Tagus river from 1988 to 2006.

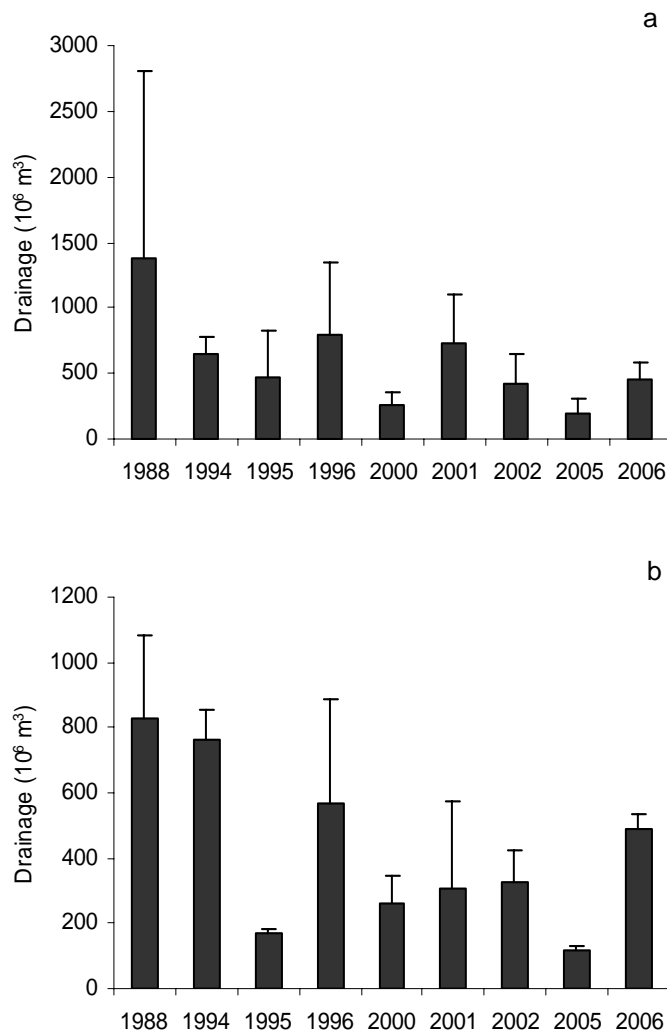


Figure 4 – Mean drainage over the three months prior to 0-group sole peak abundance in the estuary (a- *S. solea*; b – *S. senegalensis*).

NAO index over the three months prior to 0-group sole peak abundance in the estuary was positive over most of the years considered, except for 1988 and 2005 for *S. solea* (Fig. 5a). For *S. senegalensis* there were positive NAO index values for 1994, 1995, 1996 and 2002 and negative values for 1988, 2000, 2001, 2005 and 2006 (Fig. 5b). For both species there was a reduction of wind favourable for upwelling in the months considered after 2000-2001. NAO index over the periods studied varied between -1.13 and 0.99 for *S. solea*, and -0.82 and 0.95 for *S. senegalensis*.

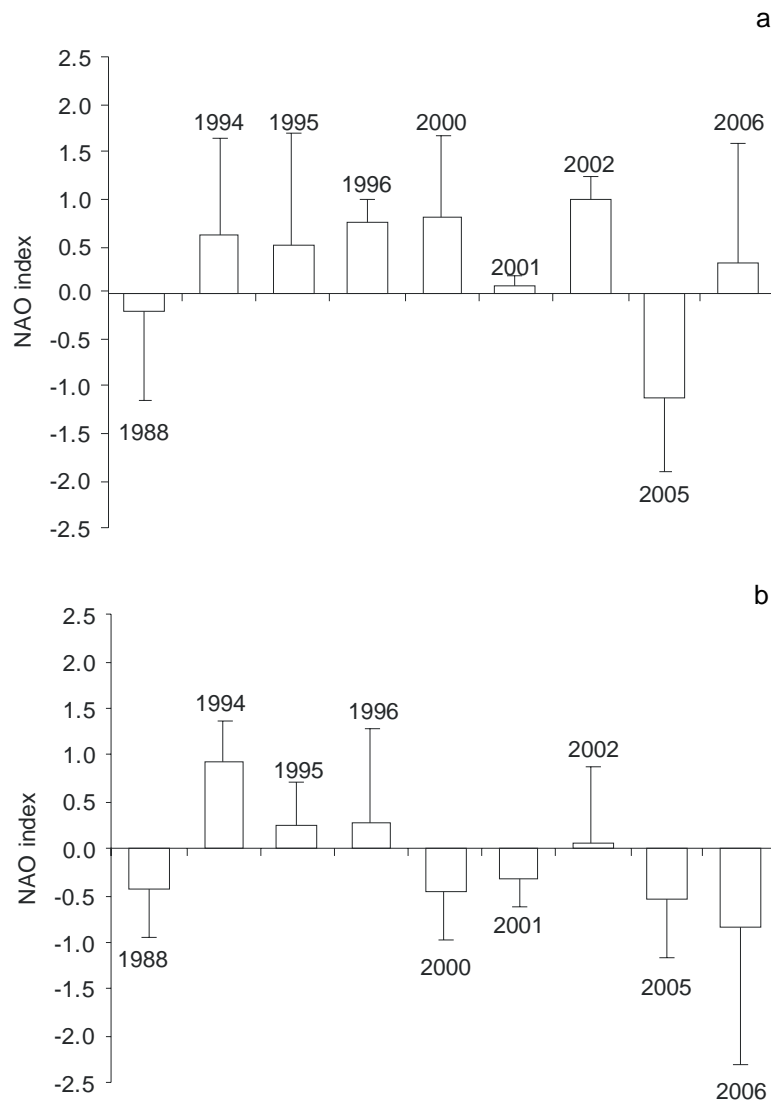


Figure 5 - Mean North Atlantic Oscillation (NAO) index over the three months prior to 0-group sole peak abundance in the estuary (a- *S. solea*; b – *S. senegalensis*).

Mean North-South wind component intensity (negative for northerly winds) over the three months prior to 0-group sole peak abundance was negative in most of the years studied for *S. solea*, with the exception of 2002 and 2005 (Fig. 6a). For *S. senegalensis* the mean

North-South wind component was also negative for most of the years with the exception of 2001, 2002 and 2005 (Fig. 6b). This means that northerly winds prevailed over the larval stage period of both soles in most of the years investigated.

Mean winds over the periods considered varied between -3.03 ms^{-1} and 1.20 ms^{-1} for *S. solea* and -3.74 ms^{-1} and 0.20 ms^{-1} for *S. senegalensis*.

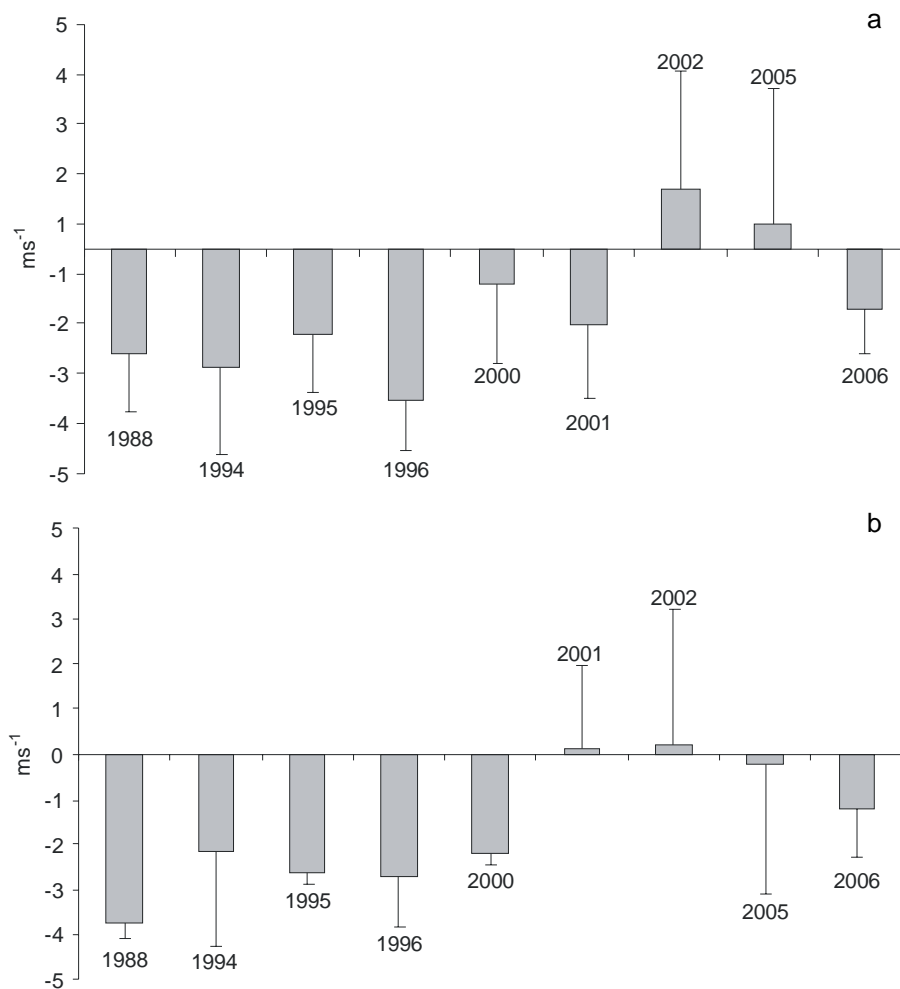


Figure 6 - Mean North-South wind component intensity over the three months prior to 0-group sole peak abundance in the estuary (a- *S. solea*; b – *S. senegalensis*).

Multi-collinearity was not detected for the independent variables for both species, through the pair plots analysis.

Only river drainage presented a significant relation with density of both sole species ($P < 0.05$) (Table 1, Table 2) in the multiple regression analyses. The mean NAO index and mean North-South wind component intensity for the three months before the estuarine peak of soles densities did not present a significant relation with the peak density data for both sole species ($P > 0.05$ for both variables in the two multiple regressions) (Table 1, Table 2) (Fig. 3, Fig. 4). No variable had a VIF > 2 (values > 10 indicate serious problems with multi-collinearity) in both multiple regressions.

There is a tendency for higher sole density values in years with higher river drainage over the larval stage of both species (Fig. 7a, 7b).

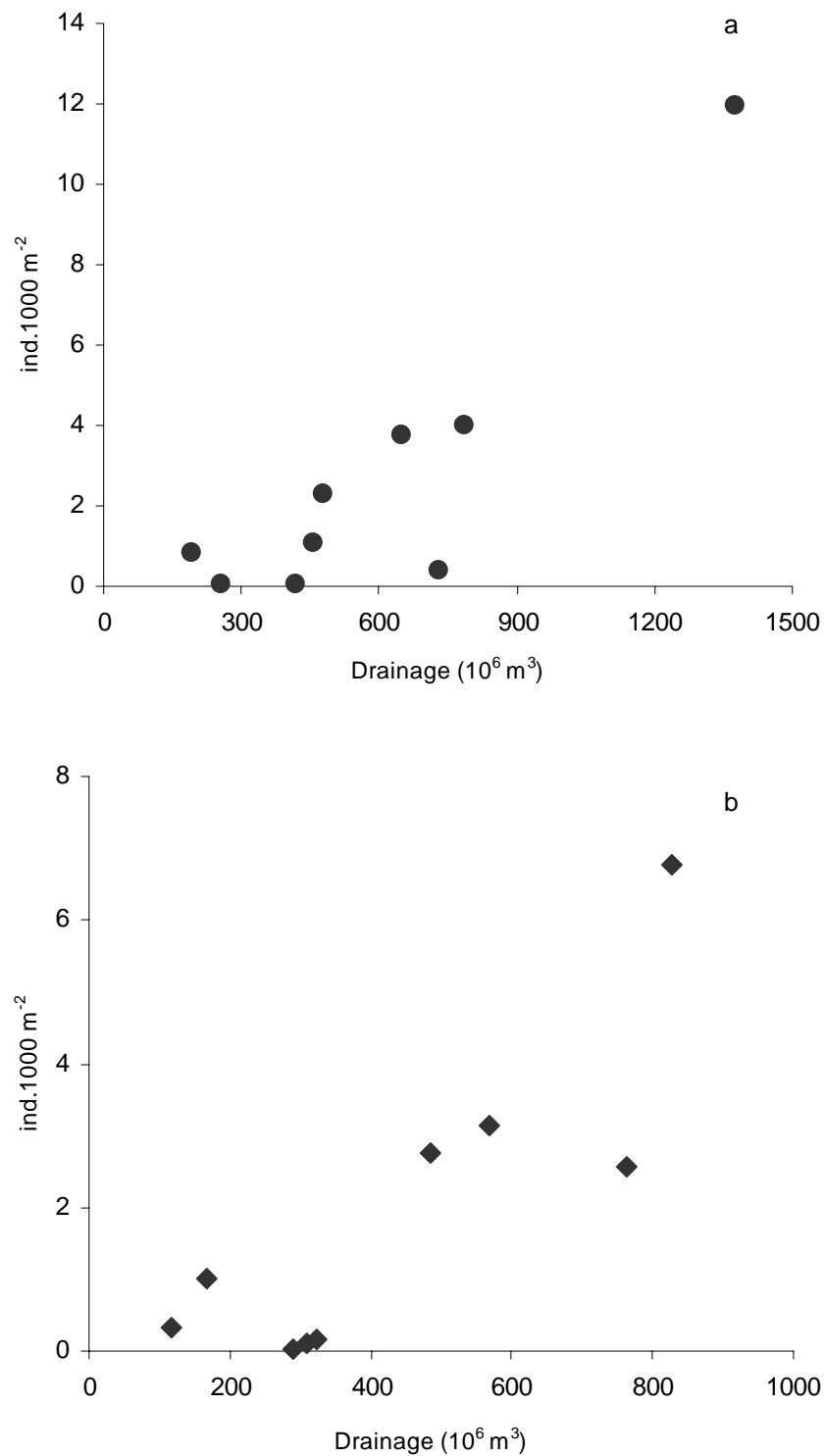


Figure 7 – 0-group sole densities (square root transformed) in relation to river drainage over the three months prior to peak abundance in the estuary (a- *S. solea*; b – *S. senegalensis*).

Discussion

The present investigation reveals the high importance of river drainage in the estuarine colonization process undertaken by the soles, *S. solea* and *S. senegalensis*.

One of the ways that river flow drainage may positively affect the estuarine immigration of these species is through the extension of river plumes throughout the adjacent coastal areas. It is generally agreed that river plumes may have a crucial role as indicators of the proximity of nursery areas for fish larvae. This means that in years of high river drainage these plumes extend to a wider area, increasing the probability of being detected by fish larvae spawned in the coast that will then direct their movement towards the nursery grounds. Miller (1988) described the cues to water masses that might be used by immature fish for orientation. He suggested that odour, temperature, salinity, turbidity and pH could be such cues, yet concluded that odour and salinity were the most likely ones. Creutzberg *et al.* (1978) had already observed that captive plaice and sole larvae did not respond to changes in salinity, temperature and odour (estuarine water), yet the smell of food elicited a strong swimming response from unfed larvae, concluding that wild larvae direct their movement as a response to food odour cues from the rich intertidal flats. Tanaka (1985) reported that a gradient of food availability seem to lead the early juveniles of red sea bream towards their nursery grounds. A chemical which is known to attract juveniles of *S. solea* is glycine-betaine, a compound present in its main prey, polychaetes, molluscs and crustaceans (Konosu and Hayashi, 1975), and thus probably involved in the cueing effect of the river plume.

River drainage is also important as an input of organic matter to the estuary and adjacent coastal areas, meaning more food availability for larvae and juveniles, as well as for the adults living in the coast (Cushing, 1995; Lloret *et al.*, 2001; Salen-Picard *et al.*, 2002; Darnaude *et al.*, 2004).

Weather is probably also important in the regulation of larval survival and movement, yet none of the weather features tested had a significant effect on density of soles during their abundance peak. The complexity inherent to weather factors makes it harder to clearly identify causal effects.

Studies on flatfish larval migration in nurseries located in non-upwelling systems have found that inshore winds are important forces directing larval movement towards nursery areas (e.g. Koutsikopoulos *et al.*, 1991; Marchand, 1991; Bailey and Picquelle, 2002; Wegner *et al.*, 2003). The opposite phenomenon, offshore advection of fish larvae has also been reported, in areas subjected to upwelling (e.g. Guisande *et al.*, 2001; Landaeta and Castro, 2002). Yet, the eastern North Atlantic boundary is highly complex in comparison with other major eastern boundaries such as the North American West Coast, the Peru-Chile upwelling system or the Benguela upwelling system (Mason *et al.*, 2005b). The primary difference between this region and the other major upwelling systems is its highly irregular topography and coastline (Mason *et al.*, 2005b).

Some phenomena that occur in the Portuguese coast may, in fact, favour larval immigration towards the shore during upwelling events. Their occurrence will add to the

complexity of the forces acting upon the larvae and confound statistical analysis. The probable existence of an upwelling shadow zone in the lee of Cabo da Roca (Figure 1) has been reported by Moita *et al.*, 2003 and may be an important factor favouring larval immigration during upwelling events. Upwelling shadow zones are characteristic small scale features of upwelling regions, which may play a disproportionately significant role in biological productivity since their circulation and stratification promote retention of propagules (eggs and larvae) that otherwise may be advected offshore (e.g. Wing *et al.*, 1998; Mason *et al.*, 2005b).

Water mass variability along the coast may also play an important role in larval movement. Huthnance (1995) suggested that the circulation off the western Iberia shelf edge may consist of a number of distinct horizontal cells, with poleward flow (the Iberian Poleward Current), but with limited continuity between them. Thus, individual water parcels containing eggs and larvae may be trapped in eddies that will hamper their movement. Mesoscale eddies are common in this area due to the interaction of the Iberian Poleward Current with topography (e.g. Peliz *et al.*, 2002; Serra and Ambar, 2002; Peliz *et al.*, 2003; Míguez *et al.*, 2005).

The combination of various phenomena may also occur, Santos *et al.* (2004) demonstrated that the interaction of a strong winter upwelling event, the Iberian Poleward Current and the buoyant river plume (resulting from river discharge) off western Iberia in February 2000 lead to the retention of sardine larvae. Evidence of high pollution input into the Portuguese coastal area during river floods resulting in the death of fish may also play an important role in some years (Vale, *personal communication*), although that was not detected in the present study.

Early investigations on flatfish, and particularly *S. solea* larvae, often explained its movement towards inshore areas as passive transport by drift (Cushing, 1975; Miller *et al.*, 1984; Boelhart and Mundy, 1988). Arino *et al.* (1996) proposed a one dimensional mathematical model for *S. solea* larvae inshore immigration that took eggs and larvae as passive elements. Yet, several studies had reported that this species larvae are active swimmers that perform circadian and tidal migrations in the water column (Champalbert *et al.*, 1989; Marchand and Masson., 1989; Champalbert and Koutsikopoulos, 1995). Several studies on *S. solea* and other flatfishes concluded that estuarine immigration depends on the active tidal behaviour of larvae, which stay near the bottom during ebbing currents and migrate into the water column at flood tides, thus using the most favourable tides to penetrate the estuary (Rijisdorp *et al.*, 1985; Bergman *et al.*, 1989; Marchand and Masson, 1989). Ramzi *et al.* (2001) constructed a two dimensional model for *S. solea* larvae inshore immigration that did not encompass larvae active behaviour, yet concluded that a three dimensional model was necessary to account for the vertical migrations performed by this species. Miller (1988) had already emphasised the need for such a model since small differences in vertical distribution of larvae can result in large differences in horizontal transport.

Vertical migrations may also play a role in the avoidance of the upper layer of water that suffers offshore advection during upwelling events. Such movements have been observed in

larval stages of several invertebrate species in upwelling areas, including the Portuguese coast (Alexander and Roughgarden, 1996; Marta-Almeida *et al.*, 2006; dos Santos *et al.*, 2007).

De Graaf (2004) constructed a three dimensional model for flatfish larval immigration and concluded that tidally cued vertical migration was the main factor directing transport towards the nearest coast in the North Sea.

Three dimensional models will be particularly important in order to predict the effect of climate change in the larvae immigration process and consequent recruitment. Changes in river drainage magnitude and seasonal pattern are expected due to the alteration of precipitation over the Iberian river basins. Miranda *et al.* (2006) precipitation model for 2100, using the IS92a scenario (Leggett *et al.*, 1992), based upon the assumption that greenhouse gases emissions will double by the end of the XXI century (in comparison to 1990), predicts a decrease in annual precipitation for Portugal. This model also predicts that precipitation will be more concentrated in time, with an increase of 30-40 % of rain fall in the Tagus basin during winter and a decrease in the rest of the year, particularly in the summer when this decrease will be between 70 and 85 % for most of the country. While an increase in river drainage in the winter may be beneficial for *S. solea* larvae that are spawned partly in this period, *S. senegalensis* larvae will be faced with much lower river plumes reaching the coastal area, during the most part of its spawning, particularly the second period, this could have an important effect in their immigration to the Portuguese estuaries in the future. Rain fall decrease over spring, summer and autumn will lead to a decrease in nutrient input of terrestrial origin into the estuarine system and adjacent coastal areas, leading to a decrease in productivity over the period when both soles use the estuary as a nursery ground. This will potentially affect food availability leading to lower fitness of the juveniles.

This decrease in rain fall will lead to water shortage (for irrigation and human consumption) with the consequent water retention in the upriver dams, many of them in Spanish territory. Although minimum ecological river drainage has already been agreed in international treaties between Portugal and Spain, close monitoring will be crucial in order to keep the impact of summer draughts in coastal ecosystems to a minimum.

The present work indicates that river drainage has an important effect upon *S. solea* and *S. senegalensis* larval immigration towards the Tagus estuary. There is clearly a need for a broader and continuous dataset on these species densities within estuaries. Studies on these species larval ecology in the Portuguese coast are also lacking. A continuous density dataset along with improved knowledge on larval ecology and on the complex hydrodynamics of the Portuguese coast will quite possibly reveal the effects of other variables influencing this process

Acknowledgements

Authors would like to thank everyone involved in the field work and Ricardo Lemos for the helpful guidance in many aspects of this manuscript. This study had the support of Fundação para a Ciência e a Tecnologia (FCT) which financed several of the research projects related to this work.

References

- Alexander, S.E., Roughgarden, J., 1996. Larval transport and population dynamics of intertidal barnacles: a coupled benthic/oceanic model. *Ecological Monographs* 66, 259-275.
- Anguis, V., Cañavate, J.P., 2005. Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. *Aquaculture* 243, 133-145.
- Arino, O., Koutsikopoulos, C., Ramzi, A., 1996. Elements of mathematical modelling of evolution in number of a sole population. *Journal of Biological Systems* 4, 445-458.
- Bailey, K.M., Picquelle S.J., 2002. Larval distribution of offshore spawning flatfish in the gulf of Alaska: potential transport pathways and enhanced onshore transport during ENSO events. *Marine Ecology Progress Series* 236, 205-217.
- Bergman, M.J.N., van der Veer, A., Stam, A., Zuidema, D., 1989. Transport mechanisms of larval plaice (*Pleuronectes platessa* L.) from the coastal zone into the Wadden Sea nursery area. *Rapport P.-v. Réunion Conseil international pour l'Exploration de la Mer* 191, 43-49.
- Boelhart, G.W., Mundy, B.C., 1988. Roles of behavioural and physical factors in larval and juvenile fish recruitment to estuarine nursery areas. *American Fisheries Society Symposium* 3, 51-67.
- Borges, M.F., Santos, A.M.P., Crato, N., Mendes, H., Mota, B., 2003. Sardine regime shifts off Portugal: a time-series analysis of catches and wind conditions. *Scientia Marina* 67, 235-244.
- Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect environmental changes? *Climate Research* 18, 119-126.
- Cabral, H., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P., Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P.T., Costa, M.J., 2007. Relative importance of estuarine flatfish nurseries along the Portuguese coast. *Journal of Sea Research* 57, 209-217. doi:10.1016/j.seares.2006.08.007.
- Champalbert, G., Bourdillon, A., Castelbon, C., Chikhi, D., Direach-Boursier, L., Maquart-Moulin, C., Patrity, G., 1989. Determinisme des migrations des larves et juveniles de soles. *Océanis* 15, 171-180.
- Champalbert, G., Koutsikopoulos, C., 1995. Behavior, transport and recruitment of Bay of Biscay sole (*Solea solea* (L.)): laboratory and field studies. *Journal of the Marine Biological Association of the U.K.* 75, 93-108.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Scientia Marina* 53, 561-566.
- Costa, M.J., Cabral, H.N., 1999. Changes in the Tagus estuary nursery function for commercial fish species: some perspectives for management. *Aquatic Ecology* 33, 53-74.

- Costa, M.J., Vasconcelos, R., Costa, J.L., Cabral, H.N., *in press*. River flow influence on the fish community of the Tagus estuary (Portugal). *Hydrobiologia*.
- Creutzberg, F.A., Eltink, G.W., van Noort, G.J., 1978. The migration of plaice larvae *Pleuronectes platessa* into the western Wadden Sea. In: McLusky, D.S., Berry, A.J. (Eds.) *Physiology and behavior of marine organisms*. Pergamon Press, New York, pp 243-251.
- Cushing, D.H., 1975. *Marine ecology and fisheries*. Cambridge University Press, 278 pp.
- Cushing, D.H., 1995. The long-term relationship between zooplankton and fish. *ICES Journal of Marine Science* 52, 611-626.
- Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., Harmelin-Vivien, M.L., 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* 138, 325-332.
- De Graaf, M., Jager, Z., Vreugdenhil, C.B., Elorche, M., 2004. Numerical simulations of tidally cued vertical migrations of flatfish larvae in the North Sea. *Estuarine, Coastal and Shelf Science* 59, 295-305.
- Dos Santos, A., Santos, A.M.P., Conway, P., 2007. Horizontal and vertical distribution of cirripede larvae in an upwelling system off the Portuguese coast. *Marine Ecology Progress Series* 329, 145-155.
- Fiúza, A.F.G., 1983. Upwelling patterns off Portugal. In: Suess, E., Thiede, J. (Eds.), *Coastal upwelling: its sediment record*, Plenum Publishing, New York, pp. 85-98.
- Forward, R., Tankersley, R., Smith, K., Welch, J., 2003. Effects of chemical cues on orientation of blue crab, *Callinectes sapidus*, megalopae in flow: implications for location of nursery. *Marine Biology* 142, 747-756, doi: 10.1007/s00227-002-0966-7.
- Frouin, R.; Fiúza, A.F.G.; Ambar, I.; Boyd, T.J., 1990. Observations of a poleward surface current off the coast of Portugal and Spain during winter. *Journal of Geophysical Research* 95, 679-691.
- García-Lopez, Anguis, V., Couto, E., Canário, A.V.M., Cañavate, J.P., Sarasquete, C., Martínez-Rodríguez, G., 2006. Non-invasive assessment of reproductive status and cycle sex steroid levels in a captive wild broodstock of Senegalese sole *Solea senegalensis* (Kaup). *Aquaculture* 254, 583-593. doi:10.1016/j.aquaculture.2005.10.007.
- Guisande, C., Cabanas, J.M., Vergara, A.R., Riveiro, I., 2001. Effect of climate on recruitment success in Atlantic Iberian sardine *Sardina pilchardus*. *Marine Ecology Progress Series* 223, 243-250.
- Haynes, R.; Barton, E.D., 1990. A poleward flow along the Atlantic coast of the Iberian Peninsula. *Journal of Geophysical Research* 95, 11425-11441.
- Haynes, R.; Barton, E.D.; Pilling, I., 1993. Development, persistence and variability of upwelling filaments off the Atlantic coast of the Iberian Peninsula. *Journal of Geophysical Research*, 98, 22681-22692.
- Huthnance, J.M., 1995. Circulation, exchange and water masses at the ocean margin: the role of physical processes at the shelf edge. *Progress in Oceanography* 35, 353-431.

- Huthnance, J.M., Aken, H.M.V., White, M., Barton, E.D., 2002. Ocean margin exchange – water flux estimates. *Journal of Marine Systems* 32, 107-137.
- IPCC, 2001. Third assessment report of the working group I: The science of climate change. J. T. Houghton *et al.* (Eds). Cambridge University Press, Cambridge, 578pp.
- Konosu, S., Hayashi, T., 1975. Determination of α -alanine betaine and glycine betaine in some marine invertebrates. *Bulletin of the Japanese Society of Scientific Fisheries* 41, 743-746.
- Koutsikopoulos, C., Fortier, L., Gagné, J., 1991. Cross-shelf dispersion of Dover sole eggs and larvae (*Solea solea*) in Biscay Bay and recruitment to inshore nurseries. *Journal of Plankton Research* 13, 923-945.
- Landaeta, M.F., Castro, L.R., 2002. Spring spawning and early nursery zone of the mesopelagic fish *Maurolicus parvipinnis* at the coastal upwelling zone off Talcahuano, central Chile. *Marine Ecology Progress Series* 226, 179-191.
- Leggett, J., Pepper, W.J., Swart, R.J., 1992. Emissions scenarios for the IPCC: an update. P.69-96 In: Houghton, J.T., Callander, B.A., Varney, S.K (Eds.), *Climate change 1992: The supplementary report to the IPCC scientific assessment*, Cambridge University Press, Cambridge,
- Leggett, W.C., Frank, K.T., 1997. A comparative analysis of recruitment variability in North Atlantic Flatfishes – testing the species range hypothesis. *Journal of Sea Research* 37, 281-299.
- Lloret, J., Leonard, J., Solé, I., Fromentin, J.M., 2001. Fluctuations of landings and environmental conditions in the north-west Mediterranean Sea. *Fisheries Oceanography* 10, 33-50.
- Marchand, J., Masson, G., 1989. Process of estuarine colonization by 0-group (*Solea solea*): hydrological conditions, behaviour, and feeding activity in the Vilaine estuary. *Rapport P.-v. Réunion Conseil international pour l'Exploration de la Mer* 191, 287-295.
- Marchand, J., 1991. The influence of environmental conditions on settlement, distribution and growth of 0-group sole (*Solea solea* (L.)) in a macrotidal estuary (Vilaine, France). *Netherlands Journal of Sea Research* 27, 307-316.
- Marta-Almeida, M., Dubert, J., Peliz, A., Queiroga, H., 2006. Influence of vertical migration pattern on retention of crab larvae in a seasonal upwelling system. *Marine Ecology Progress Series* 307, 1-19.
- Mason, E., Santos, A.M.P., Peliz, Á.J., 2005a. Western Iberian winter wind indices based on significant wind events. *Ocean Science Discussions* 2, 105-127.
- Mason, E., Coombs, S., Oliveira, P. B., 2005b. An overview of the literature concerning the oceanography of the eastern North Atlantic region. *Relatório Científico-Técnico do Instituto de Investigação das Pescas e do Mar*, 58 pp.
- Mazé, J.P., Arhan, M., Mercier, H., 1997. Volume budget of the eastern boundary layer off the Iberian Peninsula. *Deep-Sea Research Part I-Oceanographic Research Papers*, 44, 1543-1574.

- Miller, J., 1988. Physical processes and the mechanisms of coastal migrations of immature marine fishes. American Society Symposium 3, 68-76.
- Miller, J., Reed, J.P., Pietrafesa, L.J., 1984. Patterns, mechanisms and approaches to the study of migration of estuarine-dependent fish larvae and juveniles. In: McCleave, J.D., Arnold, G.P., Dodson, J.J., Neills, W.H. (Eds.), Mechanisms of migration in fishes, Plenum Press, New York and London, pp 209-225.
- Miguez, B.M., Varela, R.A., Roson, G., Souto, C., Cabanas, J.M., Farina-Busto, L., 2005. Physical and biogeochemical fluxes in shelf waters of the NW Iberian upwelling system. Hydrography and dynamics. Journal of Marine Systems 54, 127-138.
- Miranda, P.M.A, Coelho, F.E.S., Tomé, A.R., Valente, M.A., 2002. 20th Century Portuguese Climate and Climate Scenarios. In: Santos, F.D., Forbes, K., Moita, R. (Eds.), Climate change in Portugal. Scenarios, Impacts and adaptation measures – SIAM Project, Gradiva, Lisboa, 454 pp.
- Miranda, P.M.A, Valente, M.A., Tomé, A.R., Trigo, R., Coelho, F.E.S., Aguiar, A., Azevedo, E.B., 2006. O Clima de Portugal nos séculos XX e XXI. In: Santos, F.D., Miranda, P. (Eds.), Alterações climáticas em Portugal, cenários, impactos e medidas de adaptação – SIAM II project, Gradiva, Lisboa, 506 pp.
- Moita, T.; Oliveira, P.B.; Mendes, J.C.; Palma, A.S., 2003. Distribution of chlorophyll-a and *Gymnodinium catenatum* associated with coastal upwelling plumes off central Portugal. Acta Oecologica 24, 125-132.
- Norcross, B.L., Shaw, R.F., 1984. Oceanic and estuarine transport of fish eggs and larvae: a review. Transactions of the American Fisheries Society 113, 153-165.
- Peliz, Á.J.; Rosa, T.L.; Santos, A.M.P.; Pissarra, J.L., 2002. Fronts, jets, and counter-flows in the Western Iberian upwelling system. Journal of Marine Systems 35, 61-77.
- Peliz, Á.J.; Dubert, J.; Haidvogel, D.B.; Le Cann, B., 2003. Generation and unstable evolution of a density-driven Eastern Poleward Current: The Iberian Poleward Current. Journal of Geophysical Research-Oceans 108, 3268.
- Pires, V., 2004. Evolução histórica do Índice Palmer (PDSI) em Portugal Continental. Instituto de Meteorologia, Monografia nº47.
- Ramzi, A., Arino, O., Koutsikopoulos, C., Boussouar, A., Lazure, P., 2001. Modelling and numerical simulations of larval migration of the sole (*Solea solea* (L.)) of the Bay of Biscay. Part 1: modelling. Oceanologica acta 24, 101-112.
- Reis, C.S., Lemos, R., Alagador, D., 2006. Pescas. In: Santos, F.D., Miranda, P. (Eds.) Alterações climáticas em Portugal, cenários, impactos e medidas de adaptação – SIAM II project, Gradiva, Lisboa, 506 pp.
- Rijdsdorp, A.D., van Stralen, M., van der Veer, H., 1985. Selective tidal transport of North Sea plaice (*Pleuronectes platessa*) in coastal nursery areas. Transactions of the American Fisheries Society 114, 461-470.
- Russell, F. S., 1976. The Eggs and Planktonic Stages of British Marine Fishes. Academic Press, London, 524 pp.

- Santos A.M.P., Borges M.F., Groom S. 2001. Sardine and horse mackerel recruitment and upwelling off Portugal. *ICES Journal of Marine Science* 58, 589-596.
- Salen-Picard, C., Darnaude, A.M., Arlhac, D., Harmelin-Vivien, M.L., 2002. Fluctuations of macrobenthic populations: a link between climate-driven river run-off and sole fishery yields in the Gulf of Lions. *Oecologia* 133, 380-388.
- Santos, A.M.P., Peliz, Á.J., Dubert, J., Oliveira, P.B., Angelico, M.M., Ré, P., 2004. Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. *Continental Shelf Research*, 24, 149-165.
- Serra, N; Ambar, A., 2002. Eddy generation in the Mediterranean undercurrent. *Deep-Sea Research Part II* 49, 4225-4243.
- Smyth, T.J.; Miller, P.I.; Groom, S.B.; Lavender, S.J., 2001. Remote sensing of sea surface temperature and chlorophyll during Lagrangian experiments at the Iberian margin. *Progress in Oceanography* 51, 269-281.
- Tanaka, M., 1985. Factors affecting the inshore migration of pelagic larval and demersal juvenile red sea bream *Pagrus major* to a nursery ground. *Transactions of the American Fisheries Society* 114, 471-477.
- Tamburri, M. N., Finelli, C. M., Wethey, D. S., Zimmer-Faust, R. K., 1996. Chemical Induction of Larval Settlement Behavior in Flow. *The Biological Bulletin* 191, 367-373.
- Trigo, R.M., Osborn, T.J., Corte-Real, J., 2002. The North Atlantic Oscillation influence on Europe: climate impacts and associated physical mechanisms. *Climate research* 20, 9-17.
- Van der Veer, H.W., Berghahn, R., Miller, J.M., Rijnsdorp, A.D., 2000. Recruitment in flatfish, with special emphasis on North Atlantic species: Progress made by the Flatfish Symposia. *ICES Journal of Marine Science* 57, 202-215.
- Wegner, G.W., Damm, U., Purps, M., 2003. Physical influences on the stock dynamics of plaice and sole in the North Sea. *Scientia Marina* 67, 219-234.
- Whittames, E.R., Walker, M.G., Dinis, M.T., Whiting C.L., 1995. The geographical variation in the potential annual fecundity of Dover sole *Solea solea* (L.) from European shelf waters during 1991. *Netherlands Journal of Sea Research* 34, 45-58.
- Wing, S.R., Largier, J.L., Bostford, L.W., 1998. Coastal retention and longshore displacement of meroplankton near capes in eastern boundary currents: Examples from the California Current. *South African Journal of Marine Science* 19, 119-127.
- Zhang, X., Wang, X.L., Corte-Real, J., 1997. On the relationships between daily circulation patterns and precipitation in Portugal. *Journal of Geophysical Research* 102, 13495-13507.

Fishing mortality of the juvenile soles, *Solea solea* and *Solea senegalensis*, in the the Tagus estuary, Portugal

Abstract: In the Tagus estuary, the brown-shrimp beam trawl fishery is mainly carried out within the nursery grounds for the soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858. In 1995 and 1996, monthly sampling surveys were performed in the two major fishing areas within the Tagus estuary, to estimate fishing effort, catches and discards relative to sole juveniles, as well as, the impact on year class strength. Proportion of discards was assessed according to species and fish size. A survival of discards experiment was carried out on board, for periods of 30 minutes, taking into account species and fish size. A decomposition of composite distributions of length frequencies was carried out in order to identify the various cohorts colonizing the nursery areas. Proportion of sole discarded varied according to month, which was mainly related to fish size. Mortality of juveniles discarded decreased with increasing fish size. Mean estimates of the number of sole juveniles within the nursery areas of the Tagus estuary were higher for *S. senegalensis* than for *S. solea*, 13.26×10^6 and 7.50×10^6 , respectively. Yet, estimates of the total amount of sole catches were higher for *S. solea*, approximately 30 tonnes.year⁻¹, relative to *S. senegalensis*, with approximately 21 tonnes.year⁻¹. Fishing mortality was considerably higher for *S. solea*, 28% to 39%, than for *S. senegalensis*, 4% to 10%. It was concluded, that this is probably due to faster growth by *S. senegalensis*, that decreases the time spent at the most vulnerable size range, and to the use of both nurseries by this species, since nursery B presents much lower fishing pressure.

Key-words: Fisheries; Beam trawl; Discards; Fishing mortality; Multi-cohorts; Flatfish; Sole.

Introduction

The use of beam-trawl is forbidden in all Portuguese estuaries, due to its high impact upon the many species that use estuarine areas as nursery grounds, yet the Tagus estuary has an exception regime due to its traditional brown shrimp, *Crangon crangon* (Linnaeus, 1758), fishery. The commercial value of brown shrimp has however dropped drastically leading fishermen to direct their activities to more profitable target species, like the soles, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858.

The beam trawl fishery is conducted intensively in the most important nursery areas for *S. solea* and *S. senegalensis* within the estuary (Cabral and Costa, 1999). This way, most of the soles caught are 0-group juveniles, well below the minimum length at capture (24 cm). While in other sole fisheries such small individuals have no commercial value, in the areas around the Tagus estuary they are valued by local restaurants, because 0-group sole juveniles are part of the traditional gastronomy. Juvenile soles are also sold to fish-farms. These commercial demands, both illegal, and the lack of regulation supervision and enforcement result in high fishing pressure at a period when vulnerability is high, possibly affecting year-class strength.

Two important sole nurseries were identified in the Tagus estuary in previous studies (A, Vila Franca de Xira, and B, Alcochete; Figure 1) by Costa and Bruxelles (1989) and Cabral and Costa (1999). While in nursery A the two sole species, *S. solea* and *S. senegalensis* can be

found, in nursery B only *S. senegalensis* is present (Costa and Bruxelas, 1989; Cabral and Costa, 1999).

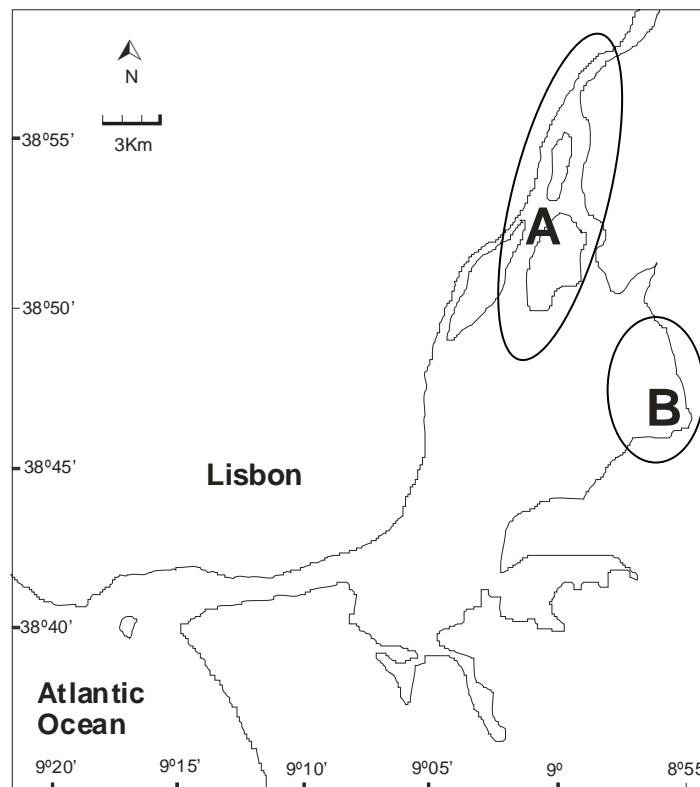


Figure 1 – Location of the study area in the Tagus estuary (nursery A- Vila Franca de Xira, nursery B – Alcochete).

S. solea 0-group juveniles are known to colonize nursery A around April-May, in one or more cohorts, leaving the estuary towards the coast around October-November (Cabral and Costa, 1999). *S. senegalensis* colonise the upper Tagus nurseries latter and in several pulses (Cabral and Costa, 1999, Fonseca *et al.*, 2006) resulting from a prolonged and variable spawning period with two major peaks (Spring and Summer) (Anguis and Cañavate, 2005). While one first cohort arrives at the estuary in late spring, another cohort arrives in late summer and a third cohort has also been observed in some years in Autumn (Cabral, 2003). Individuals from the latter cohorts will stay in the estuary during the winter, only emigrating towards coastal waters in the following year (Cabral and Costa, 1999). The temporal pattern of nursery habitat use by soles adds to the complexity of estimating population parameters, such as fishing mortality, in the Tagus estuary. In fact, while studies on northern European flatfish nurseries consider only one major 0-group cohort in the estimation of mortality (Zijlstra *et al.*, 1982; Desaunay *et al.*, 1987; Jager *et al.*, 1995), this is clearly not appropriate in subtropical and tropical flatfish nurseries, since secondary cohorts encompass an important part of the population.

Another important issue besides mortality due to fishing, is mortality of discards. Beam trawl fisheries are characterised by a considerable bycatch of fish and invertebrates, which is

discarded immediately after sorting on board (van Beek *et al.*, 1990; Ross and Hokenson, 1997; Cabral *et al.*, 2002). In the Tagus estuary, the estimate of the annual catch of the beam trawl fishery is ca. 1750 tonnes, of which approximately 90% is discarded (Cabral *et al.*, 2002). The main fish and crustacean species discarded after capture are *C. crangon* (50%), *Liza ramada* (Risso, 1826) (19%), *Carcinus maenas* (Linnaeus, 1758) (13%) and *Pomatoschistus minutus* (Pallas, 1770) (8%) (Cabral *et al.*, 2002). Although in lower numbers compared to these species, juveniles of *S. solea* and *S. senegalensis* are also among the discards of this fishery (Cabral *et al.*, 2002).

Mortality of discards differs according to species and is influenced by several conditions, either environmental or inherent to the catching and sorting methods, namely type of gear, haul duration, total volume of the catch and the sorting process used (e.g. Van Beek *et al.*, 1990; Richards *et al.*, 1995; Ross and Hokenson, 1997; Gamito and Cabral, 2003).

Although it is generally accepted that the beam trawl fishery, as other trawl fisheries, produces considerable disturbance on benthic environments (e.g. Bergman and Hup, 1992; Jennings *et al.*, 2001), the question whether this fishery presents a large impact on fish populations is controversial. In fact, some authors, based on studies conducted in the beam trawl fishery in the North Sea, suggested that the effects on fish stocks are negligible (Kennelly, 1995; Berghahn and Purps, 1998). Other studies emphasize the role of discards as organic matter inputs into the estuarine food web that can result in the enhancement of consumer populations (Ramsay *et al.*, 1997, Groenewold and Fonds, 2000). Flatfishes are among the consumers that have been reported as having a strong response to fisheries discards (Groenewold and Fonds, 2000).

The present study aims (1) to estimate the catches of *S. solea* and *S. senegalensis* of the beam trawl fishery within the nursery areas of the Tagus estuary, (2) to evaluate the mortality of discards of sole juveniles, and (3) to evaluate the impact of this fishery in year-class strength of both soles species.

Material and Methods

Study area

The Tagus estuary, with an area of 320 km², is a partially mixed estuary with a tidal range of ca. 4 m. About 40% of the estuarine area is intertidal. The upper part of the estuary is shallow and fringed by saltmarshes. The two main nursery areas for fish (A – Vila Franca de Xira, B – Alcochete) identified by Costa and Bruxelles (1989) and Cabral and Costa (1999) are located in the upper estuary (Figure 1). Although most of the environmental factors vary widely within the estuary, their ranges are similar in these two areas. However, the uppermost area (A) is deeper (mean value 4.4 m), has lower salinity (mean value 5‰) and a higher proportion of fine sand in the sediment. In the other area (B) the mean values of depth and salinity are 1.9 m and 20.7‰, respectively, and the sediment is mainly composed of mud (Cabral and Costa 1999).

Sampling procedures and data analysis

Fishing effort (in h.day⁻¹) had been previously determined by Cabral *et al.* (2002) from interviews to local fishermen. According to these authors, the fishing effort is approximately constant all over the year, being the estimates different according to fishing area: 25 vessels, 4.01 h.day⁻¹ for area A and 15 vessels, 1.48 h.day⁻¹ for area B (see Figure 1). Juvenile abundance and catches estimates were based on monthly sampling surveys aboard of commercial fishing vessels, performed in 1995 and 1996. During these surveys, 5 to 10 hauls were performed per month in each area using a 4 m beam trawl with 1 tickler chain and 10 mm mesh size. Hauls had 15 min duration and the distance travelled was registered using a GPS. Estimation of the area swept was carried out using the beam length and the distance travelled. Catches of each haul were sorted by fishermen and the number and weight of *S. solea* and *S. senegalensis* caught and discarded were determined. Proportion of discards was further analysed according to three length classes, <101 mm, 101 mm to 150 mm and >150 mm total length.

Experiments to estimate the survival of beam trawl discards were also carried out aboard commercial fishing vessels operating under normal conditions in 10 different dates during June and July 2000. After capture *S. solea* and *S. senegalensis* juveniles were placed in different plastic tanks (60x40x50 cm) filled with water, according to size of fish. Three length classes were considered: <101 mm, 101 mm to 150 mm and >150 mm total length. The tanks were checked every 5 min and the dead specimens were recorded and removed. The experiments were terminated after 30 min, when the remaining dead and live specimens were removed. For each species, the values obtained for each length class (in the proportion of discards according to length and in the survival experiment) were compared using the Kruskal-Wallis test at a significance level of 0.05 (Zar, 1996). Whenever the null hypothesis was rejected, a posteriori multiple comparisons were performed. The Mann-Whitney test was used to evaluate the differences in the mortality rates determined for *S. solea* and *S. senegalensis*.

Based on the monthly sampling surveys and on the experiments of discards mortality several estimates were calculated, for each species and year, as follows:

(1) Total number of juveniles (N):

$$N = PD \cdot A / FGE,$$

where PD is the peak density registered at the beginning of the recruitment to nursery areas, A is the area of the fishing zone and FGE is the fishing gear efficiency. PD was obtained from the sampling surveys, A was determined from nautical maps (area of zone A= 46.46 km²; area of zone B=24.75 km²) and a FGE of 0.3 was considered based on Kuipers (1975) and Creutzberg *et al.* (1987);

(2) Catch (C):

$$C = \sum MC_i \cdot FE_i$$

where MC_i is the mean value of the number (CN, in number) or biomass (CB, in weight) of juveniles in the catches of month i and FE_i is the fishing effort for month i ;

(3) Discards (D):

$$D = \sum C_i \cdot PD_i$$

where C_i is the estimate in number (DN, in number) or in biomass (DB, in weight) of the catches of month i and PD_i is the proportion of soles in the discards for month i , determined in the present study;

(4) Fish discarded dead (Dd):

$$D_d = \sum D_i \cdot MR_i$$

where D_i is the estimate of the number (DdN, in number) or biomass (DdB, in weight) of juveniles in the discards of month i and MR_i is the mortality rate. Since the mortality rates were determined according to fish length, the mean length of fish per month was used to assign a mortality rate to each month.

(5) Mortality due to fishing (MF, in percentage):

$$MF = \frac{\sum C_i - (\sum D_i - \sum D_{d_i})}{N} \cdot 100$$

where C_i , D_i , D_{d_i} and N are determined as described above.

These estimates were calculated based on a sub-set of the original database including the period between the month of peak density and the last month before emigration (after which a steep decrease in density is detected, along with a break-up of the normal distribution of fish length). Calculations were based only on this period to meet the assumption of a negligible flux of individuals migrating into and out of the nurseries.

Due to the possibility of several 0-group cohorts, data was analysed in order to identify the various cohorts. A preliminary analysis of length frequency distribution revealed that multi-cohorts only occurred in *S. senegalensis* in both years studied. For this purpose, a decomposition of composite distributions of *S. senegalensis* length was carried out on FISAT II (FAO, 2002), using the Bhattacharya's method. This allowed the isolation of each cohort and its follow up through the study period, until emigration was detected (rapid decrease in the number

of individuals with break-up of the normal distribution). Densities of each cohort were estimated throughout the study period. The estimation of the parameters N, C, D, Dd and MF, described above, was carried out separately for each cohort.

Results

While for *S. solea* only one cohort was identified in each year, *S. senegalensis* data clearly reflected a multi-cohort pattern in both years (Figure 2). In June 1995, the first cohort of *S. senegalensis* was detected in nursery A, composed of individuals with a mean length of 58 mm. The first cohort progression was identified in July and August, with increasing mean length, in September 1995 this cohort was no longer identifiable, probably because most individuals had attained emigration length and were already out of the system. Analysis of this cohort's densities throughout time shows that July was the peak abundance month (Figure 3).

Calculations done for this cohort refer to the period between July and August. A second cohort was detected in July 1995 in nursery A, with a mean length of 57 mm, this cohort numbers density peaked in September (Figure 3) and continued within the nursery until January, when a rapid decrease and break-up of the normal distribution indicated emigration out of the nursery. Calculations done for this cohort refer to the period between September and December. A third cohort was identified in September in nursery B, with a mean length of 85 mm; this cohort's density peaked in November 1995 (Figure 3) and was also detected in December 1995, yet in January 1996 its numbers decreased drastically (although the normality of the distribution was kept, January was not included in the calculations due to its' very low densities. Calculations done for this cohort referred to the period between November and December.

Numbers of *S. senegalensis* were very low during January and absent in February 1996. In March 1996 two cohorts were detected, one with a mean length of 120 mm in nursery B, possibly one of the cohorts that entered the system in late 1995 (which was called cohort I), and another with a mean length of 85 mm (which we called cohort II) in nursery A (Figure 2). Cohort I increased its numbers until reaching a density peak in May 1996 (Figure 3), indicating that the cohorts that immigrate into the nursery area late in the year and emigrate during winter, gradually come back into the nursery during spring. This cohort was detected within the nursery until July, after which it emigrated out of the system. Calculations done for this cohort refer to the period between May and July. Cohort II, which was first detected in March (Figure 2), peaked in May (Figure 3) and was present until August, in September its numbers decreased steeply and there are a break-up in the normal distribution indicating emigration. Calculations done for this cohort referred to the period between May and August. Two new cohorts were identified in October and a few individuals in November, yet its numbers were deemed to low for calculations.

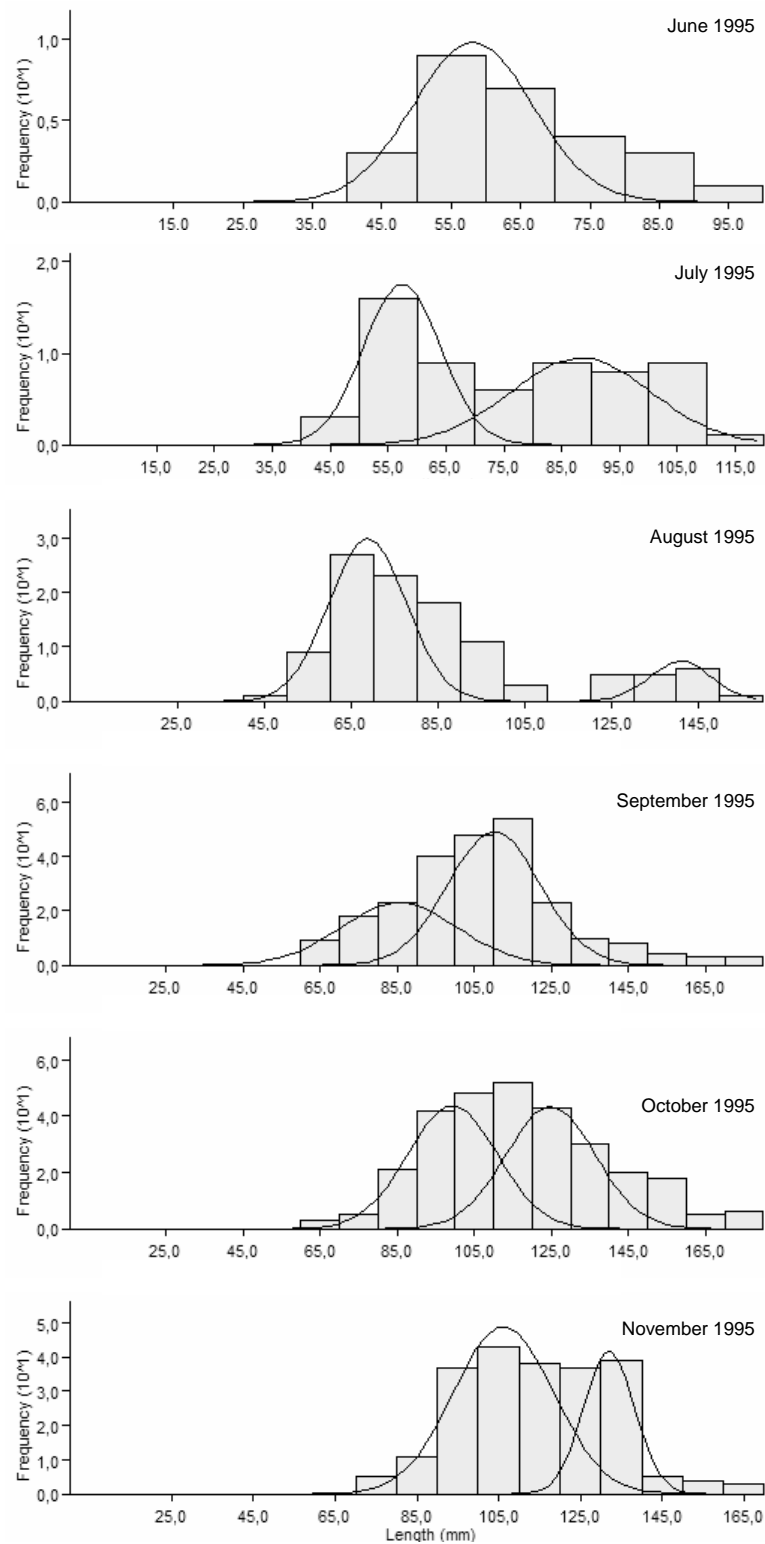


Figure 2 – Frequency of length distributions of *S. senegalensis* and decomposition into cohorts for 1995 and 1996.

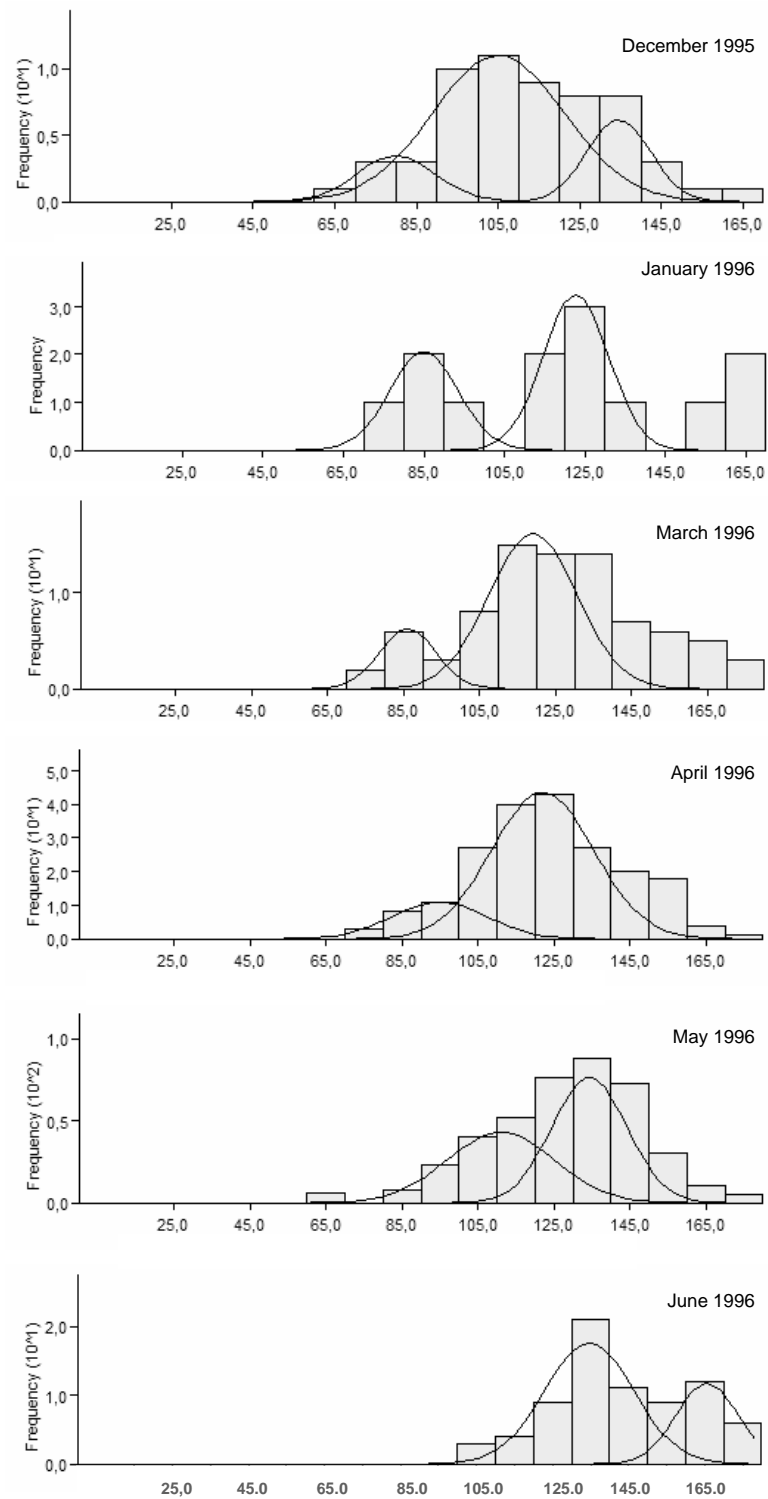


Figure 2 – Frequency of length distributions of *S. senegalensis* and decomposition into cohorts for 1995 and 1996 (continuation).

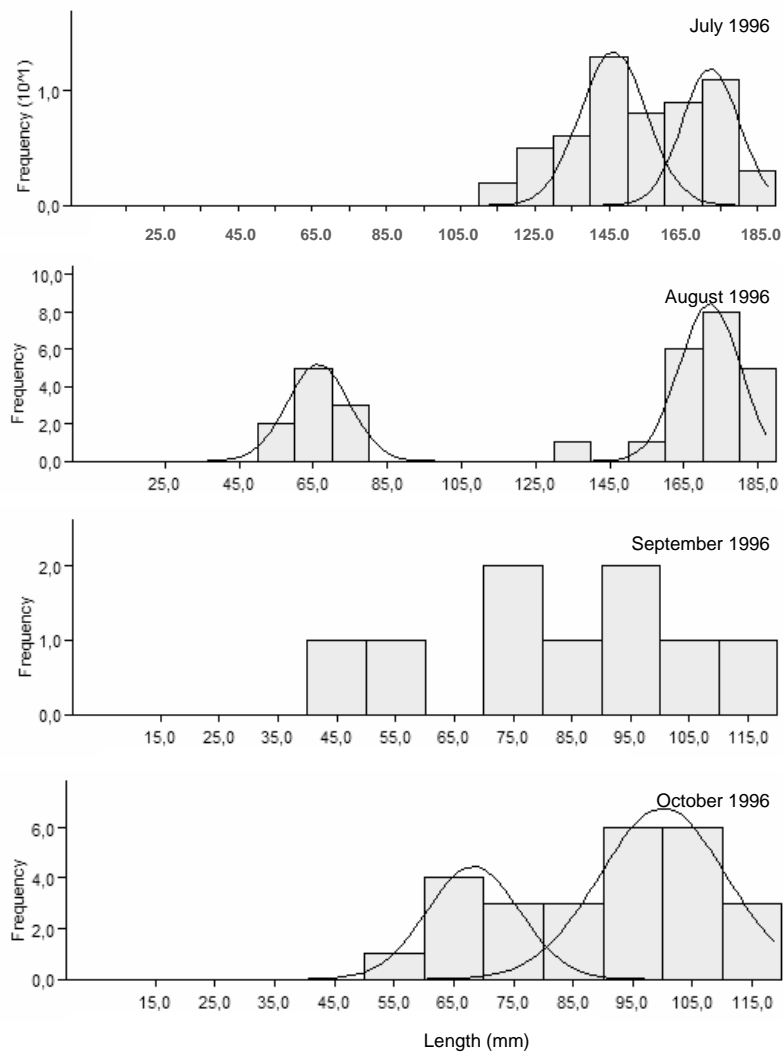


Figure 2 – Frequency of length distributions of *S. senegalensis* and decomposition into cohorts for 1995 and 1996 (continuation).

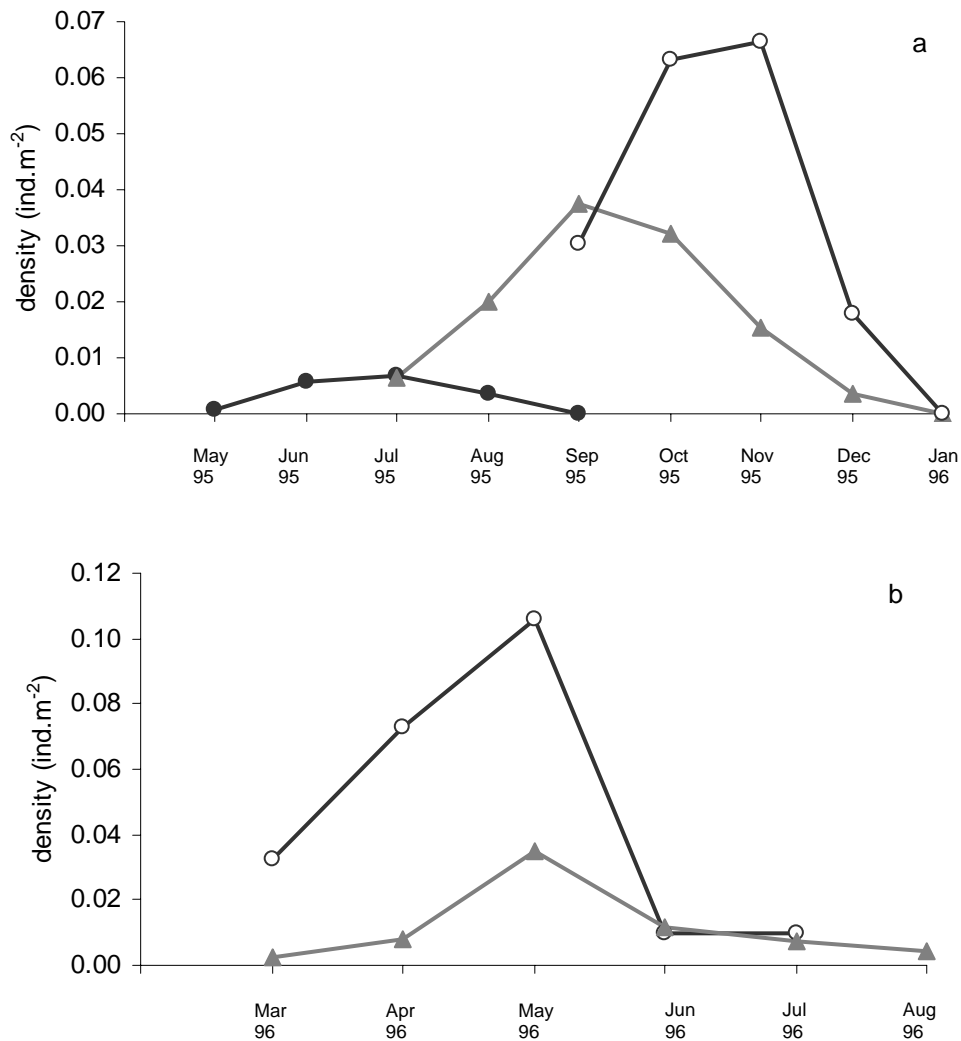


Figure 3 – Densities of *S. senegalensis* cohorts throughout the study period in 1995 and 1996. Graphic “a” presents the 1995 densities: black circles for the first cohort (nursery A); grey triangles for the second cohort (nursery A); white circles for the third cohort (nursery B). Graphic “b” presents the 1996 densities: white circles for the first cohort (nursery B); grey triangles for the second cohort (nursery A).

The mean estimates of the number of *S. solea* and *S. senegalensis* juveniles within the nursery areas of the Tagus estuary varied considerably according to the year (Table 1 and 2). For the same two years, the estimates obtained for *S. senegalensis* were higher compared to those obtained for *S. solea* (mean value of 6.30×10^6 and 12.30×10^6 , for 1995, respectively, and mean value of 2.39×10^6 and 14.17×10^6 , for 1996, respectively).

Table 1 - Estimates of number, catches, discards and mortality due to beam trawl fishing of 0-group *S. solea* within nursery A of the Tagus estuary.

	1995	1996	Mean values
Number (x10 ⁶)	6.30	2.39	7.50
Catch (in number x10 ⁶)	3.34	0.81	2.08
Catch (in tonnes)	37.37	22.64	30.01
Discarded (in number x10 ⁶)	0.95	0.14	0.55
Discarded (in tonnes)	8.77	2.83	5.80
% Discarded (number)	29	17	23
% Discarded (weight)	23	13	18
Discarded dead (in number x10 ⁶)	0.05	0.01	0.03
Discarded dead (in tonnes)	0.32	0.07	0.20
Fish mortality due to fishing (%)	39	28	34

Table 2 - Estimates of number, catches, discards and mortality due to beam trawl fishing of the *S. senegalensis* cohorts within the nursery areas of the Tagus estuary.

	1995			1996		Total		Mean values
	cohort I	cohort II	cohort III	cohort I	cohort II	1995	1996	
	nursery A	nursery A	nursery B	nursery B	nursery A			
Number (x10 ⁶)	1.04	5.83	5.49	8.75	5.42	12.36	14.17	13.26
Catch (number x10 ⁶)	0.14	1.23	0.13	0.19	0.81	1.50	1.00	1.25
Catch (in tonnes)	2.27	19.85	1.68	4.95	13.69	23.80	18.64	21.22
Discarded (number x10 ⁶)	0.07	0.20	< 0.01	0.10	0.40	0.26	0.50	0.38
Discarded (in tonnes)	1.05	3.20	< 0.01	2.48	6.74	4.25	9.22	6.74
% Discarded (number)	47	16	0.04	50	49	18	49	34
% Discarded (weight)	46	16	0.05	51	51	18	51	35
Discarded dead (number x10 ⁶)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Discarded dead (in tonnes)	0.05	0.03	< 0.01	0.05	0.07	0.08	0.11	0.09
Fish mortality due to fishing (%)	7	18	2	1	8	10	4	7

The mean values of the catch estimates, expressed in terms of number of individuals, were higher for *S. solea* (2.08×10^6 for *S. solea* and 1.25×10^6 for *S. senegalensis*). The same occurred in terms of biomass (mean value of 30.01 tonnes for *S. solea* and 21.22 tonnes for *S. senegalensis*) (Table 1 and 2).

The proportion of soles juveniles discarded in the beam trawl fishery varied considerably according to the month studied (Figure 4, Figure 5).

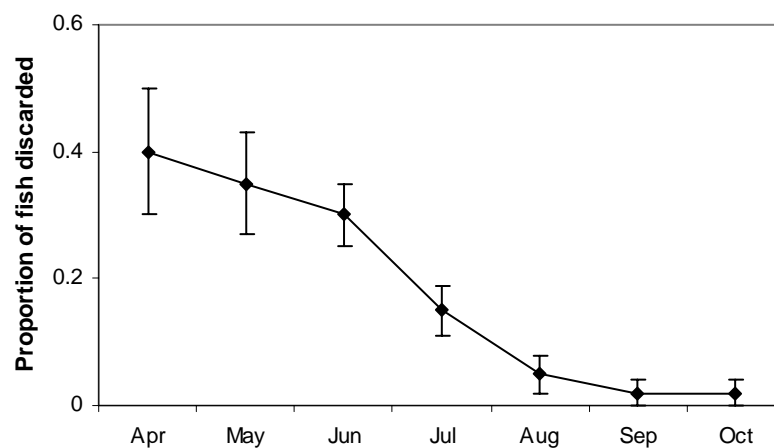


Figure 4 - Monthly mean values of the proportion of 0-group *S. solea* (in number) discarded in relation to catches, from April to October, based on the 1995 and 1996 surveys.

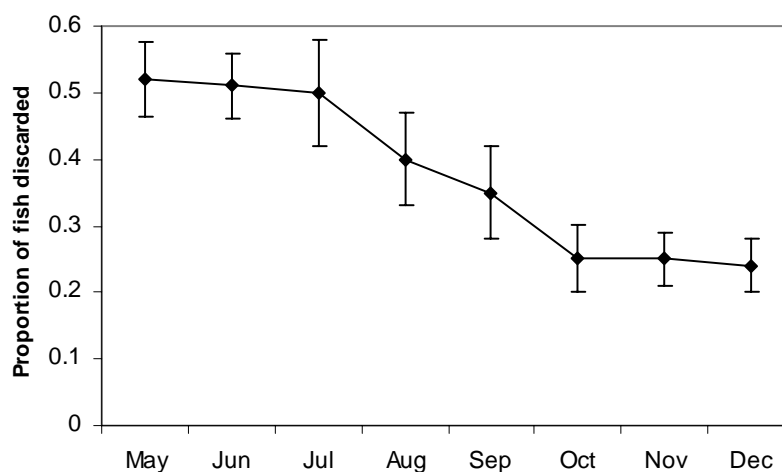


Figure 5 – Monthly mean values of the proportion of 0-group *S. senegalensis* (in number) discarded in relation to catches, from May to December, based on the 1995 and 1996 surveys.

For *S. solea*, the proportion of fish discarded was relatively high between April and June (mean values higher than 30%), with a rapid decrease from June on (Figure 4). From August to October less than 5% of the juveniles caught were discarded. For *S. senegalensis*, a constant decrease in the proportion of fish discarded was obtained for 0-group juveniles between May and December (Figure 5), yet the values were always higher than 20%.

High levels of discarding were registered for sole smaller than 100 mm, with 40% of fish discarded, while for 101 mm to 150 mm discards amounted to 25% and for sole larger than 150 mm the amount discarded was lower than 2%. Variation of discards according to month is clearly related to the size composition of the catches.

The overall estimates of discards determined for *S. solea* (mean value of 5.80 tonnes) were lower than those relative to *S. senegalensis* (mean value of 6.74 tonnes) (Table 1 and 2). These values represented 18% and 35% of the sole catches (in weight), respectively for *S. solea* and *S. senegalensis*.

The mortality rates of the discarded *S. solea* and *S. senegalensis* juveniles were significantly different according to length of fish ($H=9.93$, $p<0.05$; and $H=10.78$, $p<0.05$; respectively for *S. solea* and *S. senegalensis*). For both species, the values determined for fish smaller than 100 mm total length were considerable higher than those obtained for fish larger than 101 mm (Figure 6). Post-hoc comparisons revealed no significant differences between the mortality rates determined for both species of all length classes considered.

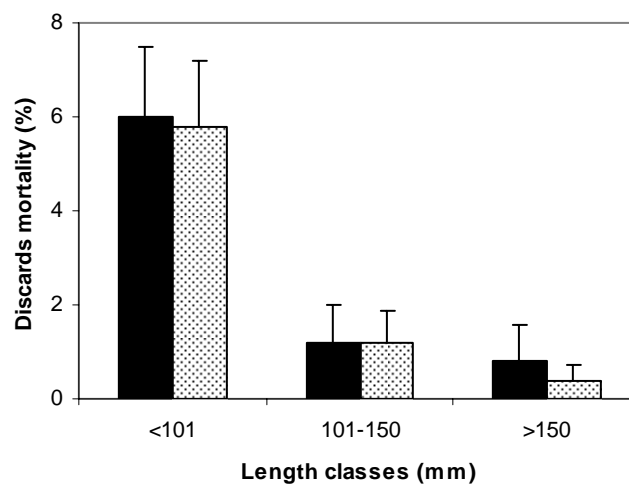


Figure 6 – Mortality estimates (in percentage of the number of individuals) determined for *S. solea* (in black) and *S. senegalensis* (dot pattern) juveniles according to fish length.

Although the estimates of fishes discarded dead were extremely low when compared to the discards estimates, the values determined for *S. solea* in weight were almost 50% higher compared to those obtained for *S. senegalensis* (Table 1 and 2).

The overall estimates of juvenile mortality due to the beam trawl fishery in the Tagus estuary varied between 39% and 28% of the number of individuals, for *S. solea* in 1995 and 1996, respectively, while the overall estimates of fishing mortality for *S. senegalensis* were much lower, 10% and 4%, in 1995 and 1996, respectively.

Discussion

The present study allowed for a first estimation of the impact of mortality due to fishing upon a multi-cohort population of juvenile sole. The decomposition of composite length distributions performed, allowed for an effective identification of the various cohorts colonizing the nursery grounds resulting in a fine estimation of the total number of individuals and ultimately in more accurate mortality estimates.

A higher number of *S. senegalensis* relative to *S. solea* within the Tagus estuary nursery areas, was estimated in the present study. Higher densities of *S. senegalensis* in comparison to *S. solea* had been previously reported by Cabral and Costa (1999). Also, *S. senegalensis* uses a wider area of habitat that includes the two nurseries, while *S. solea* only colonizes one of the nurseries.

The catch estimates reported in the literature (e.g. Fonds, 1994; Garthe and Damm, 1997; Berghahn and Purps, 1998) are considerably higher than the ca. 51 tonnes.year⁻¹ (*S. solea* and *S. senegalensis*) estimated for the Tagus estuary. This was expectable since most studies refer to the North Sea, one of the most heavily fished areas in the world and one of the most important feeding grounds for adult *S. solea* (Catchpole *et al.*, 2005), with a larger area and a better equipped fleet that can operate at higher velocities than the Tagus estuary fleet, which works in a confined area and is composed mainly by artisanal boats (Garth and Damm, 1997; Berghahn and Purps, 1998; Lopes, 2004).

Catch numbers relative to the estimated total numbers of *S. senegalensis* were lower than for *S. solea*, this is due to the fact that some cohorts of *S. senegalensis* immigrate into nursery B, where the fishing effort is much lower than in nursery A.

The estimates of the biomass of *S. solea* and *S. senegalensis* in the discards obtained in the present work were lower than those previously pointed out by Cabral *et al.* (2002). This was certainly due to the fact that in the present study the estimates incorporated the proportion of sole discarded determined on a monthly basis, while in Cabral *et al.* (2002) these values were averaged per season.

The proportion of discards varied considerably according to month for both species. This variation should be mainly related to fish size, which is the most important selection criteria when fishermen sort fish catches, with fishes smaller than 100 mm being discarded in very high quantities, since there is no market for such small soles.

The particular commercial demands that act upon sole fisheries in the Tagus estuary determine a very different composition of discards compared to the North Sea fisheries, where only adults have commercial value and most juveniles are discarded, meaning that the proportion of flatfish in discards is extremely high when compared to the Tagus estuary fishery.

Garthe (1993) estimated a proportion of 93% of flatfish and 7% of roundfish in the discards of the beam trawl fishery in the North Sea. Considering the discards estimates obtained by Cabral *et al.* (2002) for the Tagus estuary, flatfish discards represent less than 5% of all fish discards.

The survival of discards experiment revealed that survival increased with increasing fish length. That is to be expected due to an increase of resilience with age. Differences in mortality among the two species were not detected in the present study, which indicates that the ca. 50% lower dead discards estimated for *S. senegalensis* relative to *S. solea*, are not related to higher survival ability in adverse conditions. The higher survival of this species is possibly due to higher growth rates, previously reported by Cabral (2003), which result in less time spent in the more vulnerable length range, leading to higher survival in discards.

The experiments for evaluating the mortality of the discards conducted in the present study were performed in similar conditions. However, discards mortality is influenced by several factors, namely temperature, sorting process and haul duration (van Beek *et al.*, 1990; Richards *et al.*, 1995; Ross and Hokenson, 1997; Gamito and Cabral, 2003) and thus the estimates obtained in these experiments should present a higher variability if a more diverse set of conditions was considered.

The duration of these experiments is also a key factor that has a considerable impact on the mortality estimates. Keeping fishes in a controlled environment such as a tank for a long time period may overestimate discards mortality. On the opposite, if the time frame is too short, mortality estimates may be also biased and, in this case, lower than the real values. In literature, the time scales used in these kind of experiments varied from 30 min to 6 days (van Beek *et al.*, 1990; Ross and Hokenson, 1997; Kaiser and Spencer, 1995; Gamito and Cabral, 2003). In the present work, fish were kept in experimental tanks for 30 min, in order to avoid overestimating the mortality of soles juveniles in the discards. The experimental design used was based on the previous work performed by Gamito and Cabral (2003).

The mortality rates determined in the present work were quite low, both for *S. solea* and *S. senegalensis*. Similar studies conducted in the North Sea obtained a wider variation of the mortality rate estimates. Walter and Becker (1997), outlined that mortality of fish discards, such as *Pleuronectes platessa* Linnaeus, 1758, *Platichthys flesus* (Linnaeus, 1758), *S. solea* and *Limanda limanda* (Linnaeus, 1758) varies between 17% and 100%. According to these authors, the values determined for these species were extremely lower than those obtained for roundfish species, for which the mortality rates were approximately 100%. Other authors, such as van Beek *et al.* (1990), reported that the mortality of sole discards in the shrimp fishery in the North Sea employing a light beam trawl without tickler chains but with rollers attached to the ground rope, was estimated at about 40% to 50%. These differences between the estimates obtained in the North Sea and in the Tagus estuary could be due in large extent to a different fishing practice, namely in what concerns haul duration and the sorting process that have a considerable impact on discards mortality (Kelle, 1976; van Beek *et al.*, 1990; Richards *et al.*, 1995; Ross and Hokenson, 1997; Gamito and Cabral, 2003). In the North Sea beam trawl fishery hauls are generally longer (ca. 120 min) compared to those performed in this fishery in

the Tagus estuary (ca. 30 min). Also, several sorting devices are commonly used in the North Sea fisheries, namely shaking sieves, which drastically increase discards mortality (Kelle, 1976, van Beek *et al.*, 1990).

The results obtained in the present study suggest that the beam trawl fishery in the Tagus estuary has a considerable impact on *S. solea* and a lower impact on *S. senegalensis* stocks, affecting the year class strength from 28% to 39% and from 4% to 10%, respectively. The main factors that lower *S. senegalensis* fishing mortality are lower catches and death of discards. Analysis of the various cohorts of this species clearly shows low fishing mortality for the cohorts that colonize nursery B (Table 2), where they are subjected to much lower fishing pressure. It can thus be concluded that nursery B acts as an area where *S. senegalensis* can grow with less human interference.

These findings clearly contrast with studies conducted in the North Sea shrimp fisheries, which have shrimp as its main target and therefore reject most of the sole catches. According to Bergham and Purps (1998), even if the German shrimp fisheries were to stop completely, it would probably not have any detectable beneficial effect on flatfish stocks. Only if all fleets, German, Danish and Dutch, were to stop completely would a significant effect be produced.

The unique legislative status of the Tagus estuary, concerning the use of beam trawl, should be reviewed and further studies on the monitoring and modelling of the beam trawl fishing activity should be promoted.

Acknowledgements

Authors would like to thank everyone involved in the field work. This study had the support of Fundação para a Ciência e a Tecnologia (FCT) which financed several of the research projects related to this work.

References

- Anguis, V., Cañavate, J.P., 2005. Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. *Aquaculture* 243, 133-145.
- Berghahn, R., Purps, M., 1998. Impact of discard mortality in Crangon fisheries on year-class strength of North Sea flatfish species. *Journal of Sea Research* 40, 83-91.
- Bergman, M.J.N., Hup, M., 1992. Direct effects of beamtrawling on macrofauna in a sandy sediment in the southern North Sea. *ICES Journal of Marine Science* 49, 5-11.
- Cabral, H., Costa, M.J., 1999. Differential use of nursery within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* 56, 389-397.
- Cabral, H.N., Teixeira, C.M., Gamito, R., Costa, M.J., 2002. Importance of discards of a beam trawl fishery as input of organic matter into nursery areas within the Tagus estuary. *Hydrobiologia* 475/476, 449-455.
- Cabral, H.N., 2003. Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. *Journal of the Marine Biological Association of the U.K.* 83, 861-868.

- Catchpole, T.L., Frid, C.L.J., Gray, T.S., 2005. Discards in North Sea fisheries: causes, consequences and solutions. *Marine Policy* 29, 421-430.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Scientia Marina* 53, 561–566.
- Creutzberg, F., Duineveld, G.C.A., van Noort, G.J., 1987. The effects of different numbers of tickler chains on beam trawl catches. *Journal du Conseil International Exploitation de la Mer* 43, 159-168.
- Desaunay, Y., Koutsikopoulos, C., Dorel, D., 1987. Evaluation de la mortalité saisonnière des jeunes soles durant la phase de pre-recrutement. *Conseil International pour L'Exploration de la Mer* 46, 1-8.
- FAO, 2002. FISAT-II, Software Version 1.1.2 FAO-ICLARM Stock Assessment Tools. Rome: FAO
- Fonds, M., 1994. Catch composition of 12-m beam trawl and 4-m beam trawl for sole fishing. In: de Groot, S.J., Lindeboom, H.J. (Eds.), *Environmental impact of bottom gears on benthic fauna in relation to natural resources management and protection of the North Sea*. Netherlands Institute of Sea Research, Texel, pp. 95-130.
- Fonseca, V.F., Vinagre, C., Cabral, H., 2006. Growth variability of juvenile sole *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, and comparison with RNA-DNA ratios in the Tagus estuary, Portugal. *Journal Fish Biology* 68, 1551-1562. doi:10.1111/j.0022-1112.2006.001042.x
- Gamito, R., Cabral, H.N., 2003. Mortality of brown-shrimp discards from the beam trawl fishery in the Tagus estuary. *Fisheries Research* 63, 423-427.
- Garthe, S., 1993. Quantifizierung von Abfall und Beifang der Fischerei in der südöstlichen Nordsee und deren Nutzung durch Seevogel. *Hamburger Avifaunistische Beiträge* 25, 125-237.
- Garthe, S., Damm, U., 1997. Discards from beam trawl fisheries in the German Bight (North Sea). *Archive of Fishery & Marine Research* 45 (3), 223-242.
- Groeneveld, S., Fonds, M., 2000. Effects on benthic scavengers of discards and damaged benthos produced by the beam-trawl fishery in the southern North Sea. *ICES journal of Marine Science* 57, 1395-1406.
- Jager, K., Kleef, H.L., Tydeman, P., 1995. Mortality and growth of 0-group flatfish in the brackish Dollard (Ems estuary, Wadden Sea). *Netherlands Journal of Sea Research* 34, 119-129.
- Jennings, S., Kaiser, M.J., Reynolds, J.D., 2001. *Marine Fisheries Ecology*. Blackwell Science Ltd., Oxford, U.K.
- Lopes, J., 2004. Characterization of fishing activities in the Tagus estuary: management propositions. Degree thesis, University of Lisbon, Lisbon.
- Kaiser, M.J., Spencer, B.E., 1995. Survival of by-catch from a beam trawl. *Marine Ecology Progress Series* 126, 31-38.

-
- Kelle, W., 1976. Sterblichkeit untermassiger Plattfische im Beifang der Garnelenfischerei. *Meeresforschung* 25, 77-89.
- Kennelly, S.J., 1995. The issue of bycatch in Australia's demersal trawl fisheries. *Reviews in Fish Biology & Fisheries* 5, 213-234.
- Kuipers, B., 1975. On the efficiency of a two-metre beam trawl for juvenile plaice (*Pleuronectes platessa*). *Netherlands Journal of Sea Research* 19: 69-85.
- Ramsay, K., Kaiser, M.J., Moore, P.J., Hughes, R.N., 1997. Consumption of fisheries discards by benthic scavengers: utilization of energy subsidies in different marine habitats. *Journal of Animal Ecology* 66, 884-896.
- Richards, L.J., Fargo, J., Schnute, J.T., 1995. Factors influencing bycatch mortality of trawl-caught Pacific halibut. *North American Journal of Fisheries Management* 15, 266-276.
- Ross, M.R., Hokenson, S.R., 1997. Short-mortality of discarded finfish bycatch in the Gulf of Maine Fishery for Northern shrimp *Pandalus borealis*. *North American Journal of Fisheries Management* 17, 902-909.
- Van Beek, F.A., van Leeuwen, P.I., Rijnsdorp, A.D., 1990. On the survival of plaice and sole discards in the otter-trawl and beam-trawl fisheries in the North Sea. *Netherlands Journal of Sea Research* 26 (1), 151-160.
- Walter, U., Becker, P.H., 1997. Occurrence and consumption of seabirds scavenging on shrimp trawler discards in the Wadden Sea. *ICES Journal of Marine Science* 54: 684-694.
- Zar, J., 1996: *Biostatistical analysis*. 3rd ed.. Prentice Hall, Engelwood Cliffs.
- Zijlstra, J.J., Dapper, R., Witte, J. IJ., 1982. Settlement, growth and mortality of post-larval plaice (*Pleuronectes platessa*) in the western Wadden Sea. *Netherlands Journal of Sea Research* 15, 250-272.

Conclusions

The investigation on the effect of climate and hydrodynamics upon migrating sole larvae and the estimation of the magnitude of the mortality caused by fishing has put forward important new findings on the factors affecting sole survival during their juvenile period and that will ultimately affect recruitment to the adult stocks.

Analysis of existing data, dating back from 1988, and its relation to climatic and hydrodynamic factors, revealed that there were no significant correlations between peak densities of *S. solea* and *S. senegalensis* and the North Atlantic Oscillation (NAO) index or the prevailing wind direction, over the period when larval sole were assumed to be immigrating towards the estuarine nurseries, in fact only river drainage yielded significant correlations for both species.

The extension of river plumes throughout the coastal areas adjacent to the estuary probably plays a crucial role in the immigration process since it carries chemical clues that larvae use to direct their movement. This means that in rainy years a wider area will be under the influence of such chemicals, thus increasing the probability of detection by fish larvae spawned in the coast.

Climate change will probably have an important effect over larval soles' estuarine colonization. Changes in river drainage magnitude and seasonal pattern are expected due to the alteration of precipitation over the Iberian river basins. A decrease in river drainage occurring during the period of larval migration is expected to have a noticeable impact over both sole species. A more concentrated rainy period will probably affect more *S. senegalensis* because this species spawning extends over a wider period of time than *S. solea*.

The analysis of the impact of fishing upon the juvenile soles of the Tagus estuary allowed for a first estimation of fishing mortality in a multi-cohort population of 0-group juveniles. Fishing mortality estimations suggest that the beam trawl fishery in the Tagus estuary has a considerable impact on *S. solea* and a lower impact on *S. senegalensis* stocks, affecting the year class strength from 28% to 39% and from 4% to 10%, respectively. The main factors that lower *S. senegalensis* fishing mortality are lower catches. The lower catches relative to total numbers are due to the fact that this species colonizes not only nursery A but also nursery B, where fishing effort is much lower. It was thus concluded that nursery B acts as an area where *S. senegalensis* can grow with less human interference.

This chapter revealed the need for close monitoring of river drainage levels and its effects upon densities of sole juveniles within nursery areas, it also highlighted the need for a revision of the unique legislative status of the Tagus estuary, concerning the use of beam trawl in its nursery areas.

CHAPTER 6

- GENERAL CONCLUSIONS AND FINAL REMARKS -

The present work contributed for the narrowing of the knowledge gaps on the ecology of the juveniles of *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, in the Tagus estuary. Analysis of habitat use at different spatial scales revealed highly complex processes and patterns. Experimental work on gastric evacuation and feeding behaviour and its application to wild populations allowed the first estimation of food consumption by these species in the Tagus estuary. The tools applied to the investigation of growth and condition, revealed unknown patterns related to estuarine colonization, allowed the comparison of habitat quality among the two nurseries, and the comparison of growth and spawning in a latitudinal perspective. Investigation on the effect of climate and hydrodynamics upon migrating sole larvae and the estimation of the magnitude of the mortality caused by fishing has put forward important new findings on the factors affecting sole survival during their juvenile period, bringing new insights into the problematic of stock recruitment variability.

The Habitat Suitability models presented were successful in mapping habitat quality for *S. solea* and *S. senegalensis*. The question posed in the introduction, “What variables should be taken into account to model these species habitat use?” was answered. Salinity, temperature, substrate, depth and presence of intertidal mudflats in the distribution of both species were important variables in the definition of broad areas of suitable habitat for these species, yet the inclusion of prey abundance data proved crucial in the definition of high suitability areas and in the prediction of high densities of juveniles.

The stable isotope approach revealed that 0-group *S. senegalensis* present high site fidelity and do not move between nurseries, thus answering the question “Is there connectivity between the two nurseries?”. This study also showed that the food-webs from each of the nursery areas have low connectivity and present different levels of dependence upon freshwater and marine energy pathways. While the Vila Franca de Xira nursery is more dependent on the freshwater energy pathway, the Alcochete nursery has a greater contribution from the marine energy pathway.

The investigation of the effect of the diel and lunar cycles in the activity of *S. senegalensis* intended to answer the question “What factors affect the use of mudflats by these species?”. It was concluded this species use of the intertidal is affected by both the diel and the lunar cycles. The highest densities over the mudflats take place at full-moon during the dusk/dawn period. A semi-lunar activity pattern was detected. While at spring tides abundance

peaks at dusk/dawn, at neap tides abundance peaks during the day. The analysis of the effect of diel and lunar cycles upon its predators along with literature information on that effect upon its prey strongly suggests that *S. senegalensis* activity pattern is closely related to that of its predators and prey.

The experimental studies carried out to determine food consumption, lead to the conclusion that both temperature and salinity have an important effect on gastric evacuation in *S. solea* and *S. senegalensis*. While temperature increased evacuation rates in both species (although not at 26°C, in *S. solea*), the effect of low salinity differed among species, leading to a decrease in gastric evacuation rate of *S. senegalensis* and an increase in *S. solea*.

It was concluded that the observed effect of the 26°C experimental temperature upon *S. solea* was probably due to thermal stress and that this species may be at a disadvantage during the summer months when juveniles of both sole species concentrate in shallow waters, rich in prey but where temperature warms up well above its metabolic optimum. It was also concluded that a different level of adaptation to low salinity is probably the most important factor determining these species partition of space within the nursery area.

The behaviour experiment revealed that the presence of a predator strongly impacts the foraging activity of sole in the presence of prey with a 10% decrease in overall activity.

Temperature, salinity and predation pressure are thus important factors affecting prey consumption by juvenile soles, answering the question posed in the introduction "What affects prey consumption by these species?". The questions "How much prey do soles consume?" and "Is soles' abundance limited by the amount of prey available at the nurseries?" were also answered, yet it was concluded that variability in the abundance of soles and prey may result in different scenarios depending on the temporal period studied.

The estimated daily food consumption was considerably higher for *S. senegalensis* than for *S. solea*. Two distinct peaks of feeding activity were observed, albeit more pronounced for *S. senegalensis* than for *S. solea*. Since studies on *S. solea* food consumption at higher latitudes found pronounced peaks of feeding activity, it was concluded that consumption of *S. solea* in the summer months in the Tagus estuary may be hindered, possibly by thermal stress, like observed in the gastric evacuation experiments.

Food was found not to be a limiting factor for soles, however, more studies concerning variability in predators and prey densities are needed in order to accurately determine food availability and partitioning in the Tagus estuary.

The assessment of growth and condition variability revealed patterns related to the estuarine colonization process, thus answering the question "Are there growth and condition patterns related to the estuarine colonization undertaken by these juveniles?". When young juvenile soles enter the estuary they present fast growth rates and high RNA-DNA ratios that decrease over time. The first cohort to colonize the estuary presents higher growth and condition than subsequent cohorts, possibly due to higher availability of food and less competition.

Differences in habitat specific growth rates were found among the two nursery areas of the Tagus estuary. Results indicate that in 2005 the Alcochete nursery provided higher habitat quality for *S. senegalensis* than the Vila Franca de Xira nursery. No significant differences were found using RNA-DNA ratios, yet it was concluded that soles from the Tagus estuary are in good overall condition. In order to answer the question “Which of the nurseries offers better conditions to these juveniles?” habitat quality assessment will have to be carried out in a broader period of time, since nurseries are very dynamic areas. It was concluded that the simultaneous use of habitat specific growth rates, that integrate the whole life of the fish, and RNA-DNA ratios that only reflect recent conditions, would be interesting for environmental monitoring purposes since the information provided by the two methods is complementary, thus answering the question “Can growth rates based on otolith daily increments and condition based on RNA-DNA ratio be used for habitat quality monitoring of soles’ nurseries?”.

The analysis of growth in a wide geographical perspective revealed a latitudinal variation affecting *S. solea*, in that growth rates are higher and spawning takes place earlier at lower latitudes. The Tagus estuary was slightly off trend in a local context, although growth was higher than in the French estuaries studied, it was lower than in the Douro estuary. Temperature is possibly a key factor hindering *S. solea* growth rates in the Tagus estuary, since water temperatures in the Tagus over the juvenile period of this species are higher than its optimum metabolic temperature. This answered the question “Does *S. solea* grow faster in the Tagus estuary than at higher latitudes?”.

Spawning followed the latitudinal trend, taking place earlier in the Tagus and Douro coastal areas than in northern Europe, meaning that the answer to the question “Are there latitudinal trends in the spawning time of *S. solea*?” is positive. Yet, spawning took place earlier in the French estuaries than in the Portuguese estuaries, supporting recent theories that state that local conditions, oceanographic conditions in particular, may overrule general latitudinal trends. The Portuguese coast is located in a very complex upwelling system which may interfere with larval immigration towards nursery areas and thus confound the back-calculation of spawning based on the survivors that reach the nursery grounds.

Analysis of existing data on soles densities, dating back from 1988, and its relation to climatic and hydrodynamic factors, revealed that only river drainage yielded significant correlations for both species, answering the question “What is the impact of climate and hydrodynamics on the larval immigration of sole towards the Tagus estuary?”. It was concluded that chemical cues carried by river plumes probably play a crucial role in the larval immigration process.

The expected decrease in river drainage due to climate change should have a noticeable impact over both sole species. A more concentrated rainy period will probably affect *S. senegalensis* to a higher degree because this species spawning extends over a wider period of time than *S. solea*.

Fishing mortality in a multi-cohort population of 0-group juveniles was determined for the first time in the present work. It was concluded that the beam trawl fishery in the Tagus

estuary has a considerable impact on *S. solea* and a lower impact on *S. senegalensis* stocks, affecting the year class strength from 28% to 39% and from 4% to 10%, respectively, this way answering the question “What is the impact of fishing mortality upon soles’ juveniles of the Tagus estuary?”.

Fishing mortality of *S. senegalensis* is lower because it colonizes not only the Vila Franca de Xira nursery but also the Alcochete nursery, where fishing effort is much lower. It was thus concluded that the Alcochete nursery acts as protected area where *S. senegalensis* can grow with less fishing pressure.

Alterations concerning the estuarine environment will most probably have an effect on species stocks, meaning that estuarine management and stock management are naturally intertwined. The present study provides new information that should be incorporated into future stock and estuarine management.

Knowledge on the most important variables defining highly suitable areas for sole juveniles, on the high site fidelity displayed by juveniles, on the low connectivity of the food webs of the two nurseries and on their differential dependence on the freshwater energy pathways will be important when considering activities or new infrastructures that may disturb these areas.

Information on sole juveniles’ feeding ecology provided here can be incorporated into future multi-species food-web models for stock and estuarine management. It is important to assess the carrying capacity of the system in order to predict the effect of any activities that may potentially change it.

Monitoring of habitat quality using integrative indexes such as fish growth will be very important for the early detection of any threats to the populations’ health and ultimately to the commercial stock status.

Management of fish stocks under a background of climate change is one of the biggest challenges of current and future times. The identification of the effects of climate upon fish populations gives us the opportunity to predict and plan ahead, which will be crucial for the adaptation of fisheries all around the world. In the case of the Tagus estuary soles, close monitoring of the effect of river flow will be important in the future. The optimal range of flow for larval immigration should be determined, and the hypothesis of reaching that range through the synchronization of dam discharges with spawning periods should be considered, providing that more knowledge and monitoring of spawning stocks are also achieved.

Water management will be one of the most important issues at a national level. Optimization of ecological river flows, maintained through management of dam discharges in each river basin, should take into account freshwater and coastal communities and their different needs over the year.

Knowledge on the magnitude of the mortality caused by fishing upon the two sole species will lead to a better understanding of its effect upon recruitment to the adult stocks. The high level of mortality caused by the beam trawl fisheries should be taken into account and the unique legislative status of the Tagus estuary revised. The reason for the exception regime, the

traditional brown shrimp fishery no longer exists, since the drop in commercial value of this species rendered its capture non-profitable, instead this exception regime is being used to target juvenile sole well below the 24 cm minimum length at capture, as well as other fish juveniles, that are unreported and bypass any health control.

The current species management approach is inadequate. Its major flaw is considering that both species are only one item to be managed. Scientific work has consistently showed that these species have important differences in life-cycle and habitat use patterns and need different protective measures.

Management of these two species as one item may lead to several misconceptions. The Alcochete nursery may be regarded as secondary habitat or alternative habitat for sole. It may be considered that impacts in one of the nursery areas are minimized by the existence of another nursery, yet *S. solea* is only present at one of the nurseries and the connectivity level between *S. senegalensis* populations using both nurseries is very low, if not non-existent for 0-group individuals. Legislation concerning the defence period protects mainly *S. solea* juveniles. In a national context, the non-identification of soles to the species level by the official entities makes a thorough scientific analysis impossible.

An important issue concerning the management of estuarine areas in Portugal will be the need for close articulation between the existing coastal areas management plans (Planos de Ordenamento da Orla costeira - POOCs) and the plans that will be put into practice in the near future for the management of estuaries (Planos de Ordenamento de Estuários - POEs) and protected areas (Planos de Ordenamento de Áreas Protegidas - POAPs). Since these plans clearly overlap in the Tagus nursery areas, they will have to be coherent and clearly state which entities will be responsible for the implementation of management measures.

The future will certainly bring new challenges to the management of sole stocks, as well as, to the Tagus estuary nursery areas. As new information is gathered, and the environmental context changes, new scientific questions arise.

Several studies should be carried out in the near future: The effect of several possible climate change scenarios and the consequences of different levels of local climate warming, disruption of rainfall patterns and changes in coastal hydrodynamics, upon sole populations should be investigated. Possible changes in the estuarine hydrology and on the freshwater input upon the nursery areas should be assessed, as well as, its impact on the estuarine food-webs. For this purpose it would be useful to construct carbon and nitrogen balance models, which could be manipulated in order to simulate different scenarios.

Another important consequence of climate warming is the predicted sea-level rise. The impact of intertidal area loss on the carrying capacity of the Tagus estuary should be an important target of future studies, since it will possibly impact soles recruitment, as well as that of other estuarine fishes.

Assessment of soles metabolism at temperatures higher than those found currently in European habitats, will bring important information on the magnitude of the impact that should be expected due to an increase in water temperature.

The ability to predict change will enable the implementation of measures that may compensate negative effects acting upon these species populations. Among such measures would certainly be a higher level of protection of nursery and spawning habitats in order to enhance survival at early stages and a rigorous control of overexploitation of the commercial stocks.

However, effective measures rely on sound scientific knowledge that has not yet been achieved for the whole life-cycles of either sole species in the Portuguese coast. Monitoring programs focusing on the various life stages of these species are urgently needed. The absence of continuous datasets concerning juvenile and adult abundance hinders the early detection of trends in these species populations, as well as, the investigation of the factors affecting recruitment. The implementation of a continuous sampling program in the Tagus estuary and the rigorous identification of sole landings by the official entities would enable the initiation of valuable datasets for future use.

Another important issue is the effective contribution of each nursery area to the coastal stock, as well as, the relative importance of the Tagus estuary nurseries in a national perspective. Interesting results have been achieved in other coastal areas through the analysis of otolith microchemistry, which functions as a natural tag acquired by the fish throughout its lifetime. Trace element uptake by the otolith is influenced by environmental and physiological factors that might be different among habitats. If so, the environmental history of a fish can be determined by analyzing the chemical composition of the portions of the otolith corresponding to specific time periods. In species that show habitat segregation for juveniles and adults, such as the soles, juvenile otoliths record the environmental conditions experienced in the nursery area, which will correspond to the otoliths' core in adults, thus enabling the estimation of the quantitative contribution of each nursery. Such studies are underway in the Portuguese coast, nonetheless, the assessment of the consistency of the estimations needs to be carried on for a considerable period of time, in order to account for inter-annual variation.

The determination of soles' spawning areas along the Portuguese coast will be an important step for the understanding of the main factors controlling recruitment, since it is at the eggs and larval stages that mortality is higher. It would therefore be important to conduct ichthyoplankton intensive sampling surveys along the Portuguese coast targeting these species eggs and larvae.

Knowledge on the early stages of soles life is very important for the development of three-dimensional models of eggs and larvae movement which coupled with hydrodynamic circulation and temperature models will be an important tool for the analysis of migration towards nursery areas and the factors that may disrupt it.

Finally, the development of multi-species food-web models and coastal transport models for eggs and larvae will certainly provide new insights into the importance of the Tagus estuary nursery grounds for soles and into the most appropriate management strategies.