Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Variability in growth and condition of juvenile common two-banded sea bream (*Diplodus vulgaris*)

Patrícia Nunes Vicente

Dissertação

Mestrado em Ecologia marinha

2015

Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Variability in growth and condition of juvenile common two-banded sea bream (*Diplodus vulgaris*)

Patrícia Nunes Vicente

Dissertação

Mestrado em Ecologia Marinha

Orientadores:

Professor Doutor Henrique Cabral

Doutora Rita Vasconcelos

2015

To my parents

“The love for all living creatures is the most noble attribute of man.”

Charles Darwin

Acknowledgements

It is with joy that I thank all the people that helped me to become possible this dissertation thesis.

Particularly, I would like to thank to:

Professor Doutor Henrique Cabral for accepting me as master student, for the trust deposited in me and for all the useful advices.

Doutora Rita Vasconcelos for accepting me as master student, for the opportunity to work in such interesting theme, for the trust in the work with the otoliths and for the help and advices during this work.

All the Professors who taught and inspired me on this world of science.

All the team of Centro de Oceanografia, especially the group of Marine Zoology.

Doutor Filipe Martinho for the help, teaching and advices in the analysis of microstructure of otoliths.

Professor Doutor Carlos Assis for the help, teaching and advices in the extraction of the three pairs of otoliths of fish: *sagittae*, *lapilli* and *asterisci*.

Doutora Maria Paula Serafim and Mestre Rui Cereja for the help in the fishing of sparids juveniles.

All the fishermen of one of the beaches of Costa da Caparica for the help to get some sparids juveniles.

My parents, my brother and Inês for all the support during my student years.

My friends and colleagues for all the support and companionship.

And the last but not the least, I want to thank to my little dog Snowy, who has been always with me during this years of study, who could not go out sometimes due to my work in this thesis and for always give me his support when something was not going well.

Resumo alargado

Este estudo pretendeu avaliar a condição dos juvenis de *Diplodus vulgaris*, sargo-safia, em áreas de viveiro localizadas nos principais estuários da costa Portuguesa. Os principais objetivos foram: comparar a condição da espécie nos principais estuários da costa Portuguesa (Ria de Aveiro, Mondego, Sado, Mira, Ria Formosa e Guadiana) em Maio e em Julho, com base em diferentes índices de condição: o índice de crescimento recente e o índice de crescimento inicial (estimados a partir da microestrutura dos otólitos), o índice bioquímico ARN:ADN e o índice morfométrico K de Fulton,). Pretendeu-se, ainda, comparar os quatro índices entre si e relacionar os referidos índices com as variáveis ambientais, de modo a aprofundar o conhecimento sobre as suas relações.

Desde o último século que se assiste a um declínio nas populações de peixes marinhos importantes comercialmente. Deste modo, torna-se fundamental perceber a dinâmica populacional destas espécies. Muitas espécies de peixes marinhos utilizam os estuários e zonas costeiras durante uma parte da sua vida, nomeadamente na fase juvenil. Assim, determinados locais dos estuários e de outras zonas costeiras pouco profundas funcionam como áreas de viveiro para os juvenis destas espécies, que se encontram particularmente dependentes dos seus fatores abióticos e bióticos. Estas áreas de viveiro têm caraterísticas especiais que permitem que os peixes cresçam mais rapidamente e em maior segurança até atingirem tamanho suficiente para regressarem ao oceano e integrarem as populações adultas. As áreas de viveiro caraterizam-se por uma elevada disponibilidade de alimento, temperaturas da água favoráveis e proteção de predadores.

O estudo da qualidade do habitat dos estuários, principalmente para as espécies de interesse económico, torna-se crucial uma vez que uma deterioração da qualidade do habitat pode ter consequências para a dinâmica populacional, e deste modo resultar numa diminuição dos mananciais destas espécies disponíveis para a pesca comercial. A necessidade da avaliação da qualidade do habitat para os organismos marinhos é cada vez mais reconhecida, uma vez que cada vez mais se assiste a um declínio das suas populações. A elevada pressão antropogénica nas zonas costeiras é também um fator de risco para estas espécies, contribuindo para a degradação dos seus habitats. Uma correta identificação das áreas de viveiro para as espécies de peixes marinhos, suas condições ambientais e qualidade do habitat, possibilitará também uma eficiente gestão e conservação das suas populações naturais.

A qualidade do habitat de espécies marinhas pode ser avaliada com base na condição individual apresentada pelos peixes que vivem nesse habitat. Existe um grande número de índices de condição individual que podem ser utilizados nos peixes marinhos. Um índice para avaliar a condição individual dos peixes marinhos tem como base a análise da microestrutura dos otólitos dos indivíduos. Os otólitos são concreções calcificadas no interior do ouvido interno produzidas pela maioria dos organismos vertebrados. Nos peixes o seu estudo tem diversas finalidades, entre as quais a de estimar a sua taxa de crescimento. Os incrementos diários dos otólitos caraterizam-se por uma alternância entre uma banda mais larga e hialina (zona incremental) e uma banda mais estreita e opaca (zona descontínua). A largura dos incrementos diários é considerada um indicador da condição do indivíduo na medida em que incrementos mais largos (maiores) correspondem a períodos de mais alimento, e maior crescimento somático, e portanto deverá indicar uma melhor condição do organismo, e consequentemente uma melhor qualidade do habitat. Neste estudo mediu-se as larguras dos 10 últimos e também dos 60 primeiros incrementos diários dos otólitos dos juvenis, denominadas por índice de crescimento recente e índice de crescimento inicial, respetivamente. O índice bioquímico ARN:ADN é vulgarmente usado e consiste no rácio entre o ARN e o ADN do organismo. Considera-se que quanto maior for a quantidade de ARN relativamente ao ADN de cada indivíduo maior a síntese proteica que o organismo efetua, e consequentemente melhor é a sua condição. Outro índice também muito utilizado para avaliar a condição individual de um peixe é o índice morfométrico K de Fulton que se baseia na relação entre o peso e o comprimento do indivíduo. A relação entre o peso e o comprimento está intrinsecamente ligada à morfologia da espécie, e para um mesmo comprimento considera-se que quanto maior for o peso do indivíduo, e consequentemente quanto maior for este índice, melhor é a sua condição.

As variáveis ambientais têm um papel preponderante na condição individual dos peixes marinhos e consequentemente na qualidade de habitat dos locais onde vivem. Em geral, considera-se que a disponibilidade de alimento, temperatura da água favorável e um número reduzido de fatores de stress (como por exemplo, poluição da água ou existência de um grande número de predadores) representam as variáveis ambientais mais determinantes para a sobrevivência e fitness das espécies de peixes marinhos. Deste modo, foi considerada a densidade de macroinvertebrados bentónicos das áreas de viveiro em estudo, que reflete a disponibilidade de alimento. Foram também consideradas a temperatura da água e a salinidade, que poderão ter influência na fisiologia dos organismos, e a profundidade da coluna de água, a percentagem de vasa no sedimento e a latitude de cada um dos locais amostrados como variáveis ambientais passíveis de determinar a qualidade do habitat.

Uma vez que o índice de crescimento recente e o rácio ARN:ADN mostraram estar significativamente relacionados com o comprimento total dos indivíduos foi efetuada uma correção dos referidos índices de forma a eliminar o efeito do comprimento, considerando-se no subsequente estudo os índices de condição corrigidos (índice de crescimento recente' e rácio ARN:ADN'). Também o K de Fulton apresentou-se significativamente relacionado com o comprimento total dos peixes sendo substituído pelo fator de condição K', um índice de condição morfométrico mais adequado para peixes que apresentam um crescimento alométrico. O fator de condição K' foi considerado ao longo do estudo.

Os resultados obtidos indicam diferenças significativas entre os principais estuários da costa Portuguesa para três índices de condição (índice de crescimento recente’, índice de crescimento inicial e fator de condição K'). Não houve diferenças significativas entre estuários para o índice bioquímico ARN:ADN’. Foi também observada a existência de diferenças significativas entre Maio e Julho para três índices de condição (índice de crescimento recente’, índice de crescimento inicial e rácio ARN:ADN’). Não foi observada diferença significativa entre os dois meses relativamente ao fator de condição K’. De um modo geral o Sado, a Ria Formosa e o Guadiana obtiveram os valores mais altos para os índices de condição considerados.

Quando estudadas as correlações entre os quatro índices de condição obteve-se uma correlação positiva significativa entre os índices de crescimento recente’ e de crescimento inicial.

A análise de regressão múltipla efetuada parece indicar uma discrepância de sensibilidade e tempo de resposta inerente aos diferentes índices. De entre estes, o rácio ARN:ADN’ pareceu fornecer informação recente, refletindo fortemente as condições ambientais no momento de captura dos indivíduos. No extremo oposto, o índice de crescimento inicial forneceu uma informação mais antiga e só se relacionou com a latitude no momento de captura dos juvenis. Com uma resposta intermédia, o índice morfométrico fator de condição K' e o índice de crescimento recente’ relacionaram-se com três das cinco variáveis ambientais no momento de captura (temperatura da água, percentagem de vasa no sedimento e densidade de macro invertebrados bentónicos). É possível que o tempo de resposta dos indivíduos à qualidade do habitat varie consoante o índice de condição utilizado. Assim, o rácio ARN:ADN' parece avaliar a condição individual do peixe quase imediatamente após a exposição aos fatores ambientais. Por outro lado, a condição individual estimada pelo índice de fator de condição K' e pelos índices de crescimento estimados pela análise da microestrutura dos otólitos (índice de crescimento recente' e índice de crescimento inicial) parecem estimar a condição do peixe num período de tempo mais alargado. A informação fornecida pelos diferentes índices é complementar, enquanto que o rácio ARN:ADN' permite uma relação mais imediata entre os fatores ambientais das áreas de viveiro e a condição individual do peixe, os índices fator de

condição K' (ou K de Fulton) e de crescimento estimado a partir da análise da microestrutura dos otólitos deverão ser índices de condição mais integradores, uma vez que estimam a condição dos indivíduos a um nível de organização biológica superior relativamente ao estimado pelo rácio ARN:ADN' e com um tempo de resposta mais longo.

Este estudo permitiu investigar detalhadamente as relações entre os diferentes índices de condição numa espécie de peixe marinho. No futuro, mais estudos de comparação entre os diferentes índices deverão ser efetuados de forma a obter relações mais fiáveis. Também mais estudos deverão incidir nas relações entre os índices de condição e as variáveis ambientais, assim como nos mecanismos responsáveis pela variação no tempo de resposta de diferentes índices. Por outro lado, também será importante perceber melhor até que ponto a condição inicial dos indivíduos influenciará a condição individual nas subsequentes fases da vida dos peixes marinhos.

Mais estudos focando a qualidade do habitat e os padrões de distribuição dos juvenis levarão a um conhecimento mais profundo sobre a importância e função destas áreas para as suas populações.

Dada a crescente necessidade de perceber a dinâmica populacional das espécies de peixes marinhos, e também para uma eficiente gestão e conservação das suas populações naturais, deverá ser dada atenção à relação entre a condição individual e a qualidade do habitat das áreas de viveiro.

Palavras-chave: peixes marinhos; estuários e lagoas costeiras; áreas de viveiro; qualidade do habitat; microestrutura dos otólitos

Abstract

The objective of this study was to assess the variability in condition for juvenile common two-banded sea bream *Diplodus vulgaris* in nursery areas of the main Portuguese estuaries using several individual condition indices.

Estuaries and coastal lagoons play an important role for juveniles of marine fish because they offer areas with high availability of food, high water temperature and lower predation. These characteristics permit a fast and safe growth of fish until they reach adequate size to return to the ocean and to join adult populations.

In this study, two indices estimated by analysis of otolith microstructure were used: widths of the 10 last and of the 60 first daily increments were considered as recent growth index and initial growth index, respectively. Furthermore, two common condition indices were also used: biochemical RNA:DNA ratio, that assesses nutritional status of individuals, and morphometric Fulton's K, that gives an estimate of general well-being of individuals.

Since recent growth index and RNA:DNA ratio were significantly related with total length of individuals, a correction was performed and corrected condition indices recent growth' index and RNA:DNA' ratio were considered. Fulton's K was also significantly related with total length of juveniles and for this reason was replaced by condition factor K', which considers allometry in the growth of fish. Condition factor K' was considered in the study. A significant positive correlation between recent growth' and initial growth indices was obtained. Results of the multiple regression analyses seem to reflect the discrepancy in the response times of the different condition indices used. In general, Sado, Ria Formosa and Guadiana provided juveniles in better individual condition.

Future studies should be done to better investigate relationships between condition indices and between condition indices and environmental variables, focusing on the link between individual condition and habitat quality of nursery areas.

Keywords: marine fish; estuaries and coastal lagoons; nurseries; habitat quality; otolith microstructure

Resumo

O objetivo deste estudo foi avaliar a condição dos juvenis de sargo-safia, *Diplodus vulgaris,* em áreas de viveiro dos principais estuários da costa Portuguesa, utilizando vários índices de condição individual.

Os estuários e lagoas costeiras desempenham uma função importante para os juvenis de muitas espécies de peixes marinhos porque oferecem elevada disponibilidade de alimento, águas quentes e menor pressão predatória. Estas caraterísticas permitem que os juvenis cresçam rapidamente e em segurança até atingirem dimensões suficientes para regressar ao mar e recrutar para as populações adultas.

Neste estudo, foram usados dois índices baseados na análise da microestrutura dos otólitos: as larguras dos 10 últimos e dos 60 primeiros incrementos diários foram considerados como um índice de crescimento recente e um índice de crescimento inicial, respetivamente. Além disso, foram considerados o rácio bioquímico ARN:ADN, que estima o estado nutricional dos indivíduos, e o morfométrico K de Fulton, que avalia o bem-estar geral dos indivíduos.

Uma vez que o índice de crescimento recente e o rácio ARN:ADN apresentaram uma relação significativa com o comprimento total dos indivíduos, foi efetuada uma correção e os índices corrigidos índice de crescimento recente' e rácio ARN:ADN’ foram considerados. Também o K de Fulton mostrou-se significativamente relacionado com o comprimento total dos juvenis e foi substituído pelo fator de condição K', o qual considera o crescimento do peixe alométrico. Os resultados mostraram uma correlação positiva significativa entre os índices de crescimento recente’ e de crescimento inicial. Os resultados das regressões múltiplas parecem mostrar discrepância nos tempos de resposta dos diferentes índices de condição usados. Em geral, Sado, Ria Formosa e Guadiana apresentaram juvenis em melhor condição.

No futuro, deve-se investigar melhor as relações entre os índices de condição e entre os índices de condição e as variáveis ambientais, focando a ligação entre condição individual e qualidade do habitat das áreas de viveiro.

Palavras-chave: peixes marinhos; estuários e lagoas costeiras; áreas de viveiro; qualidade do habitat; microestrutura dos otólitos

Index

CHAPTER 1

General introduction

1. Marine fish populations

During the last century, stocks of commercially important fish have diminished, mainly due to industrial advances (Pauly *et al*., 2003). For this reason, the need to understand variable and uncertain replenishment of natural populations of marine fish (Sissenwine *et al*., 2014) has become even more imperious.

Marine fish have a complex life cycle (Biagi *et al*., 1988; Booth, 1995) and characteristically present highly variable recruitment dynamics (Houde and Rutherford, 1993), spatially structured populations (Schunter *et al*., 2014), high fecundity and variable mortality rates in larvae and juveniles (Ciannelli *et al*., 2013). As a consequence, it becomes more difficult to understand the processes leading to success or failure of these populations (Houde and Rutherford, 1993).

The life cycle of marine fish is usually constituted by two distinct phases: planktonic and sedentary (Gunn and Thresher, 1991; Di Franco and Guidetti, 2011). Spawning of eggs, usually triggered by environmental factors (Di Franco and Guidetti, 2011), leads to the planktonic phase. During this phase, eggs and larvae disperse (Di Franco and Guidetti, 2011; González-Wanguemert and Pérez-Ruzafa, 2011) and are particularly dependent on external characteristics, such as water circulation and climate (Overland *et al*., 2010). Settlement of individuals permits the beginning of sedentary phase, where benthic juveniles and adults can swim actively (Searcy and Sponaugle, 2000; Di Franco and Guidetti, 2011). The first year (0) is determinant for survival and success of marine fish. During this period, larvae and juveniles are greatly dependent of abiotic and biotic factors which will influence their development, growth and condition (Matic-Skoko *et al*., 2004; Saenz-Agudelo *et al*., 2011). Furthermore, marine fish of temperate regions are strictly dependent of seasonal changes (Matic-Skoko *et al*., 2004), and the duration of the planktonic phase and of the period spent in nursery areas are also influenced by seasonality (Macpherson, 1998; Matic-Skoko *et al*., 2004).

2. Estuaries and coastal lagoons as nursery areas

Larvae and juveniles of many species of marine fish commonly use estuaries, coastal lagoons and other shallow coastal waters as nursery areas (Beck *et al*., 2001; Able, 2005).

Many studies have addressed the role of key habitats and associated communities in these areas, like seagrass beds or rocky-algal reefs, as essential spawning and nursery areas for important commercial and recreational species (Lloret and Planes, 2003; Able, 2005; Serra-Pereira *et al*., 2014). These areas characteristically present high availability of food, lower predation and also warm waters, comparatively to open sea areas (Beck *et al*., 2001). These environmental factors permit individuals to grow faster and safer until reach adequate size to join adult populations and return to ocean (Biagi *et al*., 1988; Beck *et al*., 2001). Nevertheless, environmental variables of these areas can vary notably (Able, 2005). For this reason it is important to assess which nursery areas and environmental conditions are enabling higher individual condition of fish (Able, 2005), since fish in better condition have higher probability of survival and also higher fitness (Amara *et al*., 2007). Thus, identification of essential fish habitats (EFH's), i.e. habitats of high quality for marine fish, is fundamental (Lloret and Planes, 2003; Bergmann *et al*., 2004). In this way, link between individual condition and habitat quality is crucial for adequate management and conservation purposes (Amara *et al*., 2007; Vasconcelos *et al*., 2007; Neahr *et al*., 2010).

3. Assessing growth and condition of fish

Condition of marine fish can be affected by interactions between abiotic and biotic factors of habitat (e.g. availability of food, available area, intraspecific or interspecific competition, physical and chemical factors of water and sediment, parasitic infections and pollution) (Amara *et al*., 2007) and physiology of individuals (Lloret and Planes, 2003).

As a measurement of energy reserves, condition of individuals can have major consequences for survival and success of populations (Lloret and Planes, 2003; Amara *et al*., 2007). Inadequate energy reserves showed to have consequences for the reproduction of several species of fish by reducing fecundity and/ or quality of eggs and larvae (Lloret and Planes, 2003).

Lower condition (i.e. lower availability of energy reserves) also can decrease capacity of survival of individuals by deficient physiological, development and growth processes, thus enhancing natural mortality (Clemmesen and Doan, 1996; Lloret and Planes, 2003). Exhaustion of energy resources leads to starvation which, particularly in small fishes or in non-feeding periods, causes more vulnerability to predators or stress factors like thermal changes, parasites and contaminants (Lloret and Planes, 2003). It is not yet clear if individual success is more dependent of high density of individuals in same cohort, of food or water temperature during growth of larvae (Meekan *et al*., 2003), of maternal effects (like size of eggs or nutritional reserves of larvae) or of genotype (that will conditioned physiological state of individuals) (Vigliola *et al*., 2007). However, individual condition is a determinant factor of fitness (Amara *et al*., 2007). Individuals in better condition will have a better performance, comparatively to individuals in lower condition (Gilliers *et al*., 2004). So, assessing condition of individuals should provide information about the survival and fitness of marine fish (Amara *et al*., 2009).

Fish condition can be assessed by a variety of indices such as growth indices, biochemical and morphometric (Lloret and Planes, 2003; Gilliers *et al*., 2004).

3.1. Recent growth and initial growth indices

One possible way to estimate fish condition is using the width of the daily increments of otoliths of fish (Campana, 1984; Campana and Thorrold, 2001).

Otoliths are small acellular concretions of calcium carbonate (Victor and Brothers, 1982; Assis, 2000; Lopes, 2004) and other inorganic salts that develop in a matrix of protein, named otholin (Victor and Brothers, 1982; Assis, 2000; Lopes, 2004). These structures are located in the inner ear of vertebrates (Campana, 1999; Assis, 2000).

Teleosts have three pairs of otoliths that differ in size and form: *sagitta* located in *sacculus*; *asterisci* in *lagena*; and *lapilli* in *utriculus* (Radtke, 1984; Assis, 2000; De Rinaldis, 2009). Due to some characteristics of otoliths (dimension, morphologic specificity, accessibility, chemistry composition, microstructure, ontogenetic phase of formation and growth pattern) and to dependence of these properties of otoliths from environmental variables, otoliths became one of the anatomic pieces of fish with bigger utility and practical applications (Victor and Brothers, 1982; Assis, 2000; Lopes, 2004). In fact, otoliths are already present in the developmental phase of embryo (De Rinaldis, 2009), and they grow through all fish life by apposition of daily increments (Victor and Brothers, 1982; Di Franco *et al*., 2011). In larval stage they are particularly small and uniform, but with growth of individual they acquire specific conformation, according to species (Radtke, 1984; Stewart, n.d.).

Daily increments are formed from *primordium* to the external margin of otolith (Campana, 1989; Di Franco *et al*., 2011). Count of the daily increments, when formation of daily increments is validated for the species, allows to determinate the age of fish in days (Vigliola, 1997; De Rinaldis, 2009). Moreover, in otolith microstructure of some species of fish some marks can be detected that represent a transitional stage of life cycle, like hatching or settlement marks (Di Franco *et al*., 2011). Hatching mark corresponds to period of hatch and release of larva from egg (Campana, 1990; Di Franco *et al*., 2011), and settlement mark corresponds to period of settlement of planktonic larvae metamorphosing into benthic juveniles (Campana and Neilson, 1985; Di Franco *et al*., 2011). In otoliths of juveniles three regions can be identified: the core, the center part of otoliths prior to first increment; the larval region, between first increment and settlement mark; and the juvenile region, between settlement mark and the outer margin of otoliths (Di Franco *et al*., 2011).

Advances of technology in analysis of image also facilitated the identification of increments, counts of annular and daily increments and measurement of widths of increments, which now can be done faster and with more precision (Lopes, 2004; Campana, 2005).

Through a light microscope, a daily increment is constituted by two adjacent zones, one bigger and hyaline (incremental zone) and other smaller and opaque (discontinuous zone), produced by deposition of calcium carbonate, in aragonite form, and of otholin protein (Campana and Neilson, 1985; Lopes, 2004; Stewart, n.d.).

Knowledge of the factors responsible for formation and growth of daily increments is important for validation and interpretation of otolith microstructure (Campana and Neilson, 1985; Lopes, 2004). In individuals older than one year, periodicity of the increments is formed by a photoperiod mechanism, acting like a “zeitgeber” (Campana, 1984; Lopes, 2004; Stewart, n.d.), and by environmental factors, such as temperature and availability of food (Campana and Neilson, 1985; Lopes, 2004; Stewart, n.d.). A correct evaluation of influence of abiotic and biotic environmental factors in micro-growth of otoliths can contribute for a better knowledge of the physiology of individuals (Aguilera *et al*., 2009). As a consequence, this knowledge could have applications to ecological level with identification of stress inductors, like absence of food or changes in water temperature (Campana, 1999; Lopes, 2004; Aguilera *et al*., 2009). It could be also used in aquaculture by monitoring growth of individuals (Lopes, 2004).

It is currently accepted that formation of daily increments is result of endogenous circadian rhythms, synchronized in early stages of life of organisms by photoperiod, and of external factors (Campana, 1999; Lopes, 2004). However, daily increments can be mistaken with sub-daily increments, with a similar pattern, formed as consequence of environmental effects and/ or influenced by developmental phases of organisms (Morales-Nin, 2001; Lopes, 2004). Thickness of daily increments in nuclear zones of otoliths are smaller than in zones that correspond to juvenile growth (Campana and Moksness, 1991; Lopes, 2004). After juvenile stage, and with increasing age of individuals, thickness of increments became smaller and could even disappear or became irregular (Campana and Moksness, 1991; Lopes, 2004). Knowledge of these aspects of the growth patterns helps the investigators in the reading of a sequence of increments (Campana and Moksness, 1991; Lopes, 2004; Aguilera *et al*., 2009).

Occurrence of some biological phenomena and variation of environmental factors lead to changes in metabolic processes and consequently in growth of fish (Campana, 1999; Lopes, 2004). Such variations cause changes in otolith growth and in composition of increments of otoliths (Campana, 1999; Lopes, 2004; Aguilera *et al*., 2009). Daily periodicity of increments of otoliths can be altered by variations in water temperature (Vigliola, 1997; Aguilera *et al*., 2009) or in availability of food (Vigliola, 1997; Aguilera *et al*., 2009), and also by stress (Vigliola, 1997; Aguilera *et al*., 2009). Furthermore, daily periodicity in growth of otoliths is not showed in all fish species (Vigliola, 1997). So, validation of daily frequency in growth of otoliths is essential for all species, both in artificial and natural environments (Campana and Neilson, 1985; Vigliola, 1997).

Analysis of otolith microstruture of fish has showed correlations between: larval growth rates and cohort strength at settlement, larval and juvenile growth rates, and juvenile growth rates and post-settlement mortality (Vigliola *et al*., 2007). Width of the peripheral daily increments of otolith is commonly used as a recent growth index (Gilliers *et al*., 2004; Campana, 2005). Namely, width of distance between margin of otoliths back to increment 10 is used to assess fish condition during the previous 10 days before capture (Gilliers *et al*., 2004).

3.2. RNA:DNA ratio

A biochemical index commonly used to assess individual condition of fish is RNA:DNA ratio

(Buckley, 1984). This index gives information about the variation of protein synthesis of individuals (Clemmesen, 1988).

Concentration of RNA varies according to food availability and to protein demand (Clemmesen, 1994), while concentration of DNA remains relatively constant in organism (Clemmesen, 1988). Therefore, RNA:DNA ratio reflects recent growth of individuals since growth in fish is mainly accomplished by proteins synthesis (Buckley *et al*., 2008). The amount of ribosomal RNA sets the capacity of protein synthesis, thus RNA:DNA ratio is also considered an index of nutritional condition (Clemmesen, 1994; Buckley *et al*., 2008).

It has been observed that RNA:DNA ratio reflects changes on nutrition and growth in a very short term, from few days before capture of fish (Buckley, 1984). In this way, RNA:DNA ratio, apparently, is one of the most valuable condition indices because it provides a link between individual condition and environmental variables, since RNA:DNA gives a short term perspective of fish condition at time of capture, when the measurement of environmental variables is also done (Buckley, 1984).

RNA:DNA ratio is considered a valid index for assessing growth and condition of marine fish (Clemmesen, 1988). However, the rate of translation of synthesis of protein per unit of RNA is dependent of water temperature (Buckley *et al*. 2008). Also, the concentration of RNA varies according to the phases of fish life (Buckley, 1984), being fairly high in the first stages of fish life (Buckley *et al*., 2008). So, comparison of RNA:DNA ratio for assessing individual condition should take into consideration fish age and water temperature (Buckley *et al*., 2008).

3.3. Fulton's K and condition factor K'

The morphometric Fulton's K index is also a commonly used measurement for assessing condition of marine fish, mainly due to simplicity (Nash *et al*., 2006; Caldarone *et al*., 2012).

Fulton's K is calculated using the formula K= W/ L^3, where W is the weight and L is the length of the individuals (Nash *et al*., 2006; Caldarone *et al*., 2012). A scaling factor is also commonly applied to turn Fulton's K index close to 1 (Nash *et al*., 2006). Based on the relation between the weight and the length of individuals, this index considers that for the same length a heavier fish is in better condition, comparatively to a more slim one (Nash *et al*., 2006; Caldarone *et al*., 2012).

However, Fulton's K assumes isometric growth (Richter *et al*., 2000; Stevenson and Woods, 2006). So, in order to reduce or eliminate the effect of allometric growth in this condition index, condition factor K' is used instead (Richter *et al*., 2000).

Condition factor K' is calculated by the equation K'= W/L^b, where W is the weight and L is the length of the individuals, and b is a constant calculated from the linear regression between weight (W) and length (L) of fish given by the formula W = a x L^b (Richter *et al*., 2000). As for Fulton's K, a scaling factor is applied to turn this index close to 1 (Nash *et al*., 2006).

In fact, these morphometric indices are known to give a good estimate of the well being of individuals (Richter *et al*., 2000; Nash *et al*., 2006). However, ideally these indices should only compare individuals of the same species and of the same age, especially Fulton's K index, since they are dependent on the relation between weight and length, and consequently on the morphology and on the ontogenetic phase of fish (Suthers, 1998).

4. *Diplodus vulgaris*

General introduction

Common two-banded sea bream, *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), is an important marine fish with a wide distribution in east of Atlantic Ocean, from France to Senegal, including Madeira, Azores and Canary islands, in the Mediterranean Sea (Loy *et al*., 1998; Gonçalves *et al*., 2003; Correia *et al*., 2011) and in the Black Sea (Taieb *et al*., 2012).

It is a teleost fish and classified as a demersal species, living in coastal rocky and sandy bottoms, from shallow waters to a maximum depth of 160 m (Gonçalves *et al*., 2003; Correia *et al*., 2011; Taieb *et al*., 2012). *D. vulgaris* is also considered a resident fish in artificial reefs (Gonçalves *et al*., 2003; Correia *et al*., 2011; Taieb *et al*., 2012).

This sparid has a high commercial value in Portugal, it was the tenth most important species in landings in 2000 (Gonçalves *et al*., 2003). In the southwest coast of Portugal, *D. vulgaris* is an even more important fish (Gonçalves *et al*. 1997; Correia *et al*., 2011), occupying the sixth place in landings in 2000 (Gonçalves *et al*., 2003). In the south coast, it is a primary target species for artisanal fishery with gill nets and longlines (Gonçalves *et al*., 2003).

Usually, adults spawn their eggs in littoral waters that will then be transported by hydrographic processes to shallow coastal waters, including estuaries and coastal lagoons (Correia *et al*., 2011). After a short period of time spent in water column, between one and two months (Correia *et al*., 2011), individuals settle on sandy or boulder substrates (Macpherson *et al*., 1997; Loy *et al*., 1998). This transition from planktonic to sedentary phase, lasting from one to three weeks (Vigliola *et al*., 1998; Correia *et al*., 2011), is considered critical and disturbances during this period can have a severe impact in recruitment (Loy *et al*., 1998). In the metamorphose of larvae into juveniles, several modifications in the organism take place, such as in dentition, in feeding behavior and in swimming capacity (Loy *et al*., 1998). Changes in period of spawning and settlement can occur along their distribution range (Gonçalves *et al*., 2003). However, spawning is expected to occur between September and April (Gonçalves and Erzini, 2000; Correia *et al*., 2011) and settlement between October and February (Macpherson *et al*., 1997). Also, two different pulses can exist (Macpherson *et al*., 1997; Gonçalves and Erzini, 2000). Juveniles form small numbers of monospecifics shoals and never mix with adult shoals present in same nursery area (Macpherson *et al*., 1997). When juveniles become larger, shoals split in a clumped distribution over the nursery area (Macpherson *et al*., 1997). Individuals stay near the settlement area for a period between five and eight months, until they reach a size between 4.5 and 5.5 cm in length (Macpherson *et al*., 1997). After that juveniles disperse out of nursery areas and join shoals of conspecific adults of sizes between 10 and 15 cm of length (Macpherson *et al*., 1997).

5. Objectives

The main objective of this dissertation was to assess growth and condition of common two-banded sea bream juvenile, *Diplodus vulgaris*, in nursery areas along the main estuaries of the Portuguese coast, using different indices. Namely, the condition indices used were recent and initial growth indices based in width of daily increments of otoliths, biochemical RNA:DNA ratio and morphometric Fulton's K. Also, relationships between individual condition indices and between individual condition indices and environmental variables were explored to unravel patterns of habitat quality for *Diplodus vulgaris* along the Portuguese coast.

Literature cited

Able, K.W., 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. Estuar. Coast. Shelf Sci. 64, 5-17.

Aguilera, B., Catalán, I.A., Palomera, I., Olivar, M.P., 2009. Otolith growth of European sea bass (*Dicentrarchus labrax* L.) larvae fed with constant or varying food levels. Scient. Mar., 73(1), 173-182.

Amara, R., Meziane, T., Gilliers, C., Hermel, G., Laffargue, P., 2007. Growth and condition indices in juvenile sole *Solea solea* measured to assess the quality of essential fish habitat. Mar. Ecol. Prog. Ser. 351, 201-208.

Amara, R., Selleslagh, J., Billon, G., Minier, C., 2009. Growth and condition of 0-group European flounder, *Platichthys flesus* as indicator of estuarine habitat quality. Hydrobiologia 627, 87-98.

Assis, C.A.S., 2000. Estudo morfológico dos otólitos *sagitta*, *asteriscus* e *lapillus* de teleósteos (Actinopterygii, Teleostei) de Portugal continental. Sua aplicação em estudos de filogenia, sistemática e ecologia. Dissertação apresentada à Faculdade de Ciências da Universidade de Lisboa para obtenção do grau de Doutor em Biologia, na especialidade de Ecologia e Biossistemática.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51(8), 633-641.

Bergmann, M., Hinz, H., Blyth, R.E., Kaiser, M.J., Rogers, S.I., Armstrong, M., 2004. Using knowledge from fishers and fisheries scientists to identify possible groundfish "Essential Fish Habitats". Fish. Res. 66, 373-379.

Biagi, F., Gambaccini, S., Zazzetta, M., 1998. Settlement and recruitment in fishes: The role of coastal areas. Ital. J. Zool. 65(1), 269-274.

Booth, D.J., 1995. Juvenile groups in a coral-reef damselfish: Density-dependent effects on individual fitness and population demography. Ecol. 76(1), 91-106.

Buckley, L.J., 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80, 291-298.

Buckley, L.J., Caldarone, E.M., Clemmesen, C., 2008. Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis. Mar. Ecol. Prog. Ser. 371, 221-232.

Calderone, E.M., MacLean, S.A., Sharack, B. 2012. Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (*Salmo salar*) to food availability. Fish. Bull. 110, 257-270.

Campana, S.E., 1984. Lunar cycles of otolith growth in the juvenile starry flounder *Platichthys stellatus*. Mar. Biol. 80, 239-246.

Campana, S.E., 1989. Otolith microstructure of three larval gadids in the Gulf of Maine, with inferences on early life history. Can. J. Zool. 67, 1401-1410.

Campana, S.E., 1990. How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci. 47, 2219-2227.

Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Eco. Prog. Ser. 188, 263-297.

Campana, S.E., 2005. Otolith science entering the 21st century. Mar. Fresh. Resear. 56, 485-495.

Campana, S.E., Moksness, E., 1991. Accuracy and precision of age and hatch date estimates from otolith microstructure examination. ICES J. Mar. Sci. 48, 303-316.

Campana, S.E., Neilson, J.D., 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42, 1014-1032.

Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. 58, 30-38.

Ciannelli, L., Fisher, J.A.D., Skern-Mauritzen, M., Hunsicker, M.E., Hidalgo, M., Frank, K.T., Bailey, K.M., 2013. Theory, consequences and evidence of eroding population spatial structure in harvested marine fishes: a review. Mar. Ecol. Prog. Ser. 480, 227-243.

Clemmesen, C.M., 1988. A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. Meeresforsch. 32, 134-143.

Clemmesen, C., 1994. The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Mar. Biol. 118, 377-382.

Clemmesen, C., Doan, T., 1996. Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. Mar. Ecol. Prog. Ser. 138, 33-39.

Correia, A.T., Pipa, T., Gonçalves, J.M.S., Erzini, K., Hamer, P.A., 2011. Insights into population structure of *Diplodus vulgaris* along the SW Portuguese coast from otolith elemental signatures. Fish. Res. 111, 82-91.

De Rinaldis, G., 2009. Durata larvale pelagica del sarago maggiore *Diplodus sargus sargus* (Linnaeus, 1758). Tesi di Laurea Sperimentale in Zoologia e Biologia Marina. Corso di Laurea in Scienze e Tecnologie per l'Ambiente. Universita' del Salento. Facoltà di Scienze Matematiche Fisiche e Naturali.

Di Franco, A., Guidetti, P., 2011. Patterns of variability in early-life traits of fishes depend on spatial scale of analysis. Biol. Lett. 7, 454-456.

Di Franco, A., De Benedetto, G., De Rinaldis, G., Raventos, N., Sahyoun, R., Guidetti, P., 2011. Large scale-variability in otolith microstructure and microchemistry: The case study of *Diplodus sargus sargus* (Pisces: Sparidae) in the Mediterranean Sea. Ital. J. Zool. 78(2), 182-192.

Gilliers, C., Amara, R., Bergeron, J., Le Pape, O., 2004. Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. Env. Biol. Fishes 71, 189-

198.

Gonçalves, J.M.S., Erzini, K., 2000. The reproductive biology of the two-banded sea bream (*Diplodus vulgaris*) from the southwest coast of Portugal. J. Appl. Ichthyol. 16, 110-116.

Gonçalves, J.M.S., Bentes, L., Lino, P.G., Ribeiro, J., Canário, A.V.M., Erzini, K., 1997. Weight-length relationships for selected fish species of the small-scale demersal fisheries of the south and south-west coast of Portugal. Fish. Res. 30, 253-256.

Gonçalves, J.M.S., Bentes, L., Coelho, R., Correia, C., Lino, P.G., Monteiro, C.C., Ribeiro, J., Erzini, K., 2003. Age and growth, maturity, mortality and yield-per-recruit for two-banded bream (*Diplodus vulgaris* Geoffr.) from the south coast of Portugal. Fish. Res. 62, 349-359.

González-Wangüemert, M., Pérez-Ruzafa, Á., 2012. In two waters: contemporary evolution of lagoonal and marine white seabream (*Diplodus sargus*) populations. Mar. Ecol. 33, 337-349.

Gunn, J.S., Thresher, R.E., 1991. Viviparity and the reproductive ecology of clinid fishes (Clinidae) from temperate Australian waters. Env. Biol. Fishes 31, 323-344.

Houde, E.D., Rutherford, E.S., 1993. Recent Trends in Estuarine Fisheries: Predictions of Fish Production and Yield. Est. 16(2), 161-176.

Lloret, J., Planes, S., 2003. Condition, feeding and reproductive potential of white seabream *Diplodus sargus* as indicators of habitat quality and the effect of reserve protection in the northwestern Mediterranean. Mar. Ecol. Prog. Ser. 248, 197-208.

Lopes, C.A.M.V., 2004. Estudo dos Otólitos de juvenis de Robalo, *Dicentrarchus labrax* (LINNAEUS, 1758) da Ria de Aveiro: relações alométricas, microincrementos e relação com variações ambientais. Dissertação apresentada ao Instituto de Ciências Biomédicas de Abel Salazar para obtenção do grau de Mestre em Ciências do Mar-Recursos Marinhos, Biologia Marinha.

Loy, A., Mariani, L., Bertelletti, M., Tunesi, L., 1998. Visualizing allometry: Geometric morphometrics in the study of shape changes in the early stages of the two-banded sea bream, *Diplodus vulgaris* (Perciformes, Sparidae). J. Morphol. 237, 137-146.

Macpherson, E., 1998. Ontogenetic shifts in habitat use and aggregation in juvenile sparid fishes. J. Exp. Mar. Biol. Ecol. 220, 127-150.

Macpherson, E., Biagi, F., Francour, P., García-Rubies, A., Harmelin, J., Harmelin-Vivien, M., Jouvenel, J.Y., Planes, S., Vigliola, L., Tunesi, L., 1997. Mortality of juvenile fishes of the genus *Diplodus* in protected and unprotected areas in the western Mediterranean Sea. Mar. Ecol. Prog. Ser. 160, 135-147.

Matic-Skoko, S., Kraljevic, M., Dulcic, J., Pallaoro, A., 2004. Growth of juvenile salema, *Sarpa salpa* (Teleostei: Sparidae), in the Kornati Archipelago, eastern Adriatic Sea. Sci. Mar. 68(3), 411-417.

Meekan, M.G., Carleton, J.H., McKinnon, A.D., Flynn, K., Furnas, M., 2003. What determines the growth of tropical reef fish larvae in the plankton: food or temperature? Mar. Ecol.

Prog. Ser. 256, 193-204.

Morales-Nin, B., 2001. Mediterranean deep-water fish age determination and age validation: the state of the art. Fish. Res. 51, 377-383.

Nash, R.D.M., Valencia, A.H., Geffen, A.J., 2006. The Origin of Fulton's Condition Factor - Setting the Record Straight. Fish. 31(5), 236-238.

Neahr, T.A., Stunz, G.W., Minello, T.J., 2010. Habitat use patterns of newly settled spotted seatrout in estuaries of the north-western Gulf of Mexico. Fish. Manag. Ecol. 17, 404-413.

Overland, J.E., Alheit, J., Bakun, A., Hurrell, J.W., Mackas, D.L., Miller, A.J., 2010. Climate controls on marine ecosystems and fish populations. J. Mar. Syst. 79, 305-315.Pauly, D., Alder, J., Bennett, E., Christensen, V., Tyedmers, P., Watson, R., 2003. The Future for Fisheries. Sci. 302, 1359-1361.

Radtke, R.L., 1984. Cod fish otoliths: information storage structures. Flødevigen rapportser 1, 273-298.

Richter, H., Lückstädt, C., Focken, U.L., Becker, K., 2000. An improved procedure to assess fish condition on the basis of length-weight relationships. Arch. Fish. Mar. Res. 48(3), 226-235.

Saenz-Agudelo, P., Jones, G.P., Thorrold, S.R., Planes, S., 2011. Connectivity dominates larval replenishment in a coastal reef fish metapopulation. Proc. R. Soc. B 278, 2954-2961.

Schunter, C., Pascual, M., Garza, J.C., Raventos, N., Macpherson, E., 2014. Kinship analyses identify fish dispersal events on a temperate coastline. Proc. R. Soc. B 281, 20140556.

Searcy, S.P., Sponaugle, S., 2000. Variable larval growth in a coral reef fish. Mar. Ecol. Prog. Ser. 206, 213-226.

Serra-Pereira, B., Erzini, K., Maia, C., Figueiredo, I., 2014. Identification of potential essential fish habitats for skates based on fishers' knowledge. Envir. Manag. 53, 985-998.

Sissenwine, M.M., Mace, P.M., Lassen, H.J., 2014. Preventing overfishing: evolving approaches and emerging challenges. ICES J. Mar. Sci. 71(2), 153-156.

Stevenson, R.D., Woods Jr., W.A., 2006. Condition indices for conservation: new uses for evolving tools. Integ. Comp. Biol. 46(6), 1169-1190.

Stewart, W.T., n.d. Otolith aging and analysis. Computational Bioscience Program. Arizona State University, 1-31.

Suthers, I.M., 1998. Bigger? Fatter? Or is faster growth better? Considerations on condition in larval and juvenile coral-reef fish. Aust. J. Ecol. 23, 265-273.

Taieb, A.H., Ghorbel, M., Hamida, N.B.H., Jarboui, O., 2012. Reproductive biology, age and growth of the two-banded seabream *Diplodus vulgaris* (Pisces: Sparidae) in the Gulf of Gabès, Tunisia. J. Mar. Biol. Assoc. U.K. 1-7.

Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Fonseca, V., Latkoczy, C., Günther, D., Costa, M.J., Cabral, H., 2007. Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Mar. Ecol. Prog. Ser. 350, 117-126.

Victor, B.C., Brothers, E.B., 1982. Age and growth of the fallfish *Semotilus corporalis* with daily otolith increments as a method of annulus verification. Can. J. Zool. 60, 2543-2550.

Vigliola, L., 1997. Validation of daily increment formation in otoliths for three *Diplodus* species in the Mediterranean sea. J. Fish Biol. 51, 349-360.

Vigliola, L., Harmelin-Vivien, M.L., Biagi, F., Galzin, R., Garcia-Rubies, A., Harmelin, JG., Jouvenel, JY., Le Direach-Boursier, L., Macpherson, E., Tunesi, L., 1998. Spatial and temporal patterns of settlement among sparid fishes of the genus *Diplodus* in the northwestern Mediterranean. Mar. Ecol. Prog. Ser. 168, 45-56.

Vigliola, L., Doherty, P.J., Meekan, M.G., Drown, D.M., Jones, M.E., Barber, P.H., 2007. Geneticidentity determines risk of post-settlement mortality of a marine fish. Ecol. 88(5), 1263-1277.

CHAPTER 2

CHAPTER 2

Variability in growth and condition of juvenile common two-banded sea bream (*Diplodus vulgaris*) (to be submitted to Journal of Fish Biology)

Vicente, P.[1], Martinho, F.[2], Cabral, H.N.[1], Vasconcelos, R.P.[1]

[1] MARE - Marine and Environmental Science Centre, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;

[2] Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal.

Abstract

This study aimed to assess condition of juvenile *Diplodus vulgaris* along the Portuguese coast based on different individual condition indices: recent and initial growth indices estimated from otolith microstructure, biochemical RNA:DNA ratio and morphometric Fulton's K. Juveniles were collected in nursery areas of estuaries and coastal lagoons along the Portuguese coast in May and July 2006. Environmental variables (latitude, temperature, salinity, depth, percentage of mud in the sediment and density of benthic macroinvertebrates) were also determined. Since three condition indices (recent growth index, RNA:DNA ratio and Fulton's K) showed a significant relationship with total length of individuals, a correction of condition indices (recent growth' index, RNA:DNA' ratio and condition factor K') was performed. A significant positive correlation was found between recent growth' index and initial growth index, but no more significant correlations were found between condition indices. Multiple regression analysis showed that individual condition indices were significantly related with environmental variables but reflected the response times of the different indices. In general, Ria Formosa, Guadiana and Sado presented higher habitat quality for juveniles of *Diplodus vulgaris*.

More studies should be carried out to better understand relationships between individual condition indices and with environmental variables. The mechanisms responsible for the different response times and sensitivity of the different condition indices should also been further investigated. In addition, studies focusing on the relationship between individual condition in early stages of fish life and their subsequent phases should also been done.
In sum, the importance of the link between individual condition of fish and habitat quality should be emphasized, particularly in earlier stages of their life cycle. This link is particularly important in the context of management and conservation of natural populations of marine fish.

Keywords: marine fish, estuaries and coastal lagoons, nursery areas, habitat quality, otolith microstructure, RNA:DNA ratio, Fulton's K index

Introduction

A large number of marine fish species present a complex life cycle (Biagi *et al*., 1988; Booth, 1995; Able, 2005) with two distinct phases (Gunn and Thresher, 1991; Loy *et al*., 1998; González-Wanguemert, 2010; Di Franco and Guidetti, 2011). Eggs and larvae comprehend the planktonic phase of life cycle of fish (González-Wanguemert, 2010) and juveniles and adults comprehend the benthonic phase (Loy *et al*., 1998; Di Franco and Guidetti, 2011). Due to these particularities, population parameters like growth, mortality and reproduction can be highly variable and difficult to estimate (Vigliola *et al*., 1998).

Shallow coastal waters, such as estuaries and coastal lagoons, present well known important nursery areas for early phases of the life cycle of marine fish, namely the juvenile stage, and become of significant importance for these species (Beck *et al*., 2001; Able, 2005). Due to intrinsic characteristics of these nursery areas, mostly high water temperature, high availability of food, low predation, comparatively to areas of open ocean, juveniles can grow faster and safely until reaching an ideal size to join adult populations of conspecifics (Biagi *et al*., 1988; Beck *et al*., 2001). Thus, environmental variables of nursery areas, which determine habitat quality, become fundamental for individuals welfare and condition (Able, 2005). Assessing which nursery areas provide higher individual condition of juveniles will identify which of those present higher habitat quality, i. e. essential fish habitats (Lloret and Planes, 2003; Bergmann *et al*., 2004), and therefore will permit an efficient management and conservation of natural populations of marine fish (Amara *et al*., 2007; Vasconcelos *et al*., 2007b; Neahr *et al*., 2010).

Individual condition indices are valuable indicators of the health of fish (Suthers, 1998; Lloret and Planes, 2003; Amara *et al*., 2007; Vasconcelos *et al*., 2009). Nowadays, a considerable number of condition indices, from morphometric to biochemical can be used to assess individual condition of marine fish in all of the phases of their life cycle (Lloret and Planes, 2003). Analysis of otolith microstructure has been useful to estimate age, in days or years, and growth of fish (Campana, 1984; Campana and Thorrold, 2001). Otoliths are three pairs of structures (*sagittae*, *lapilli* and *asterisci*) of carbonate of calcium and other elements (Radtke, 1984; Victor and Brothers, 1982; Assis, 2000; Lopes, 2004) and are formed before development of fish (De Rinaldis, 2009). Otoliths grow through the entire life of fish by apposition of daily increments (Victor and Brothers, 1982; Campana and Thorrold, 2001). Each daily increment is constituted by two adjacent zones: one larger and hyaline (incremental zone) and one smaller and opaque (discontinuous zone) (Campana and Neilson, 1985; Lopes, 2004; De Rinaldis, 2009; Stewart, n.d.). Larger increment widths can be associated with better individual condition since fishes were capable to aggregate more carbonate of calcium and otolin protein in their inner ear structures (Campana, 1999; Lopes, 2004). Furthermore, otolith growth is strongly related with somatic fish growth, and it is assumed that fishes with higher growth rates are in better condition (Campana, 1999; Lopes, 2004). In this way, recent growth index is a commonly individual condition index used that consists in width of the last daily increments of otoliths (Gilliers *et al*., 2004; Campana, 2005). Width of the last daily increments of otoliths estimate individual condition of fish in the last days before capture (Gilliers *et al*., 2004; Campana, 2005). However, caution must be taken in comparison of this recent growth index between individuals, since otolith and somatic growth are dependent on ontogenetic phase of fish (Morales-Nin, 2001; Lopes, 2004). In the other hand, width of the first daily increments of otoliths estimate individual condition in initial life stage of individuals (Campana, 1989; Aguilera *et al*., 2009). RNA:DNA ratio is a commonly used individual condition index (Buckley, 1984). An individual with a higher RNA:DNA ratio is considered to be in better condition, since RNA is related with synthesis of proteins and DNA is relatively constant in the organism (Clemmesen, 1988). Individuals with higher synthesis of protein are in better nutritional state and thus have better individual condition (Clemmesen, 1988). Morphometric Fulton's K index is widely used to assess individual condition of fish, mainly due to the simplicity of the method (Nash *et al*., 2006; Caldarone *et al*., 2012). This index estimates general well-being of individuals (Richter *et al*., 2000; Nash *et al*., 2006). Fulton's K is calculated using the formula $K = W/L^3$, where W is the weight of individual in milligrams and L is the length of individual in millimeters, and assumes that higher values correspond to individuals in better condition since they present higher weight for a same given length (Suthers, 1998; Richter *et* *al*., 2000; Nash *et al*., 2006). In addition, a scaling factor is used to approach Fulton's K to 1 (Nash *et al*., 2006). Nevertheless Fulton's K considers isometry in growth of fish and this not always occurs, so condition factor K', that considers allometry in growth of fish, can be considered instead (Richter *et al*., 2000; Stevenson and Woods, 2006). Condition factor K' is calculated using the formula $K' = W/L^b$, where W is the weight, L is the length of individuals and b is a constant obtained by the linear regression between weight and length of fish, according with the equation $W = a \times L^b$, where W is the weight and L is the length of individuals, and b is a constant obtained by the linear regression between weight and length of fish, according with the equation $W = a \times L^b$, where W and L are the weight and length of individuals, respectively, and a and b are constants of the linear regression (Richter *et al*., 2000). As in Fulton's K, a scaling factor is used to bring condition factor K' near to 1 (Nash *et al*., 2006).

Diplodus vulgaris, usually known as common two-banded sea bream, is widespread in temperate and sub-tropical waters (Loy *et al*., 1998; Gonçalves *et al*., 2003; Correia *et al*., 2011; Taieb *et al*., 2012). This sparid has a distribution range from north of France to Senegal in east Atlantic ocean, including Madeira, Azores and Canary islands, is present in Mediterranean Sea and also in Black Sea (Loy *et al*., 1998; Gonçalves *et al*., 2003; Correia *et al*., 2011; Taieb *et al*., 2012). The species is abundant in Portuguese coast where it has great economic interest, particularly in the south coast (Loy *et al*., 1998; Vasconcelos *et al*., 2008, Abecasis *et al*., 2009).

This study aimed to assess condition of juvenile common two-banded sea bream *Diplodus vulgaris* in nursery areas along the Portuguese coast through different individual condition indices, namely recent growth index (estimated by width of the 10 last daily increments of otoliths), initial growth index (estimated by width of the 60 first daily increments of otoliths), biochemical index RNA:DNA ratio and morphometric index Fulton's K. Relationships between individual condition indices and between individual condition indices and environmental variables were also investigated.

Materials and Methods

Study area

Sites selected correspond to known nursery areas of main estuaries (Ria de Aveiro, Mondego, Sado, Mira, Ria Formosa and Guadiana) (Fig. 1) along the Portuguese coast (Vasconcelos *et al*., 2007b; Vasconcelos *et al*., 2009), with the exception of Tejo estuary where the number of sampled individuals was not sufficient to be included in the study.

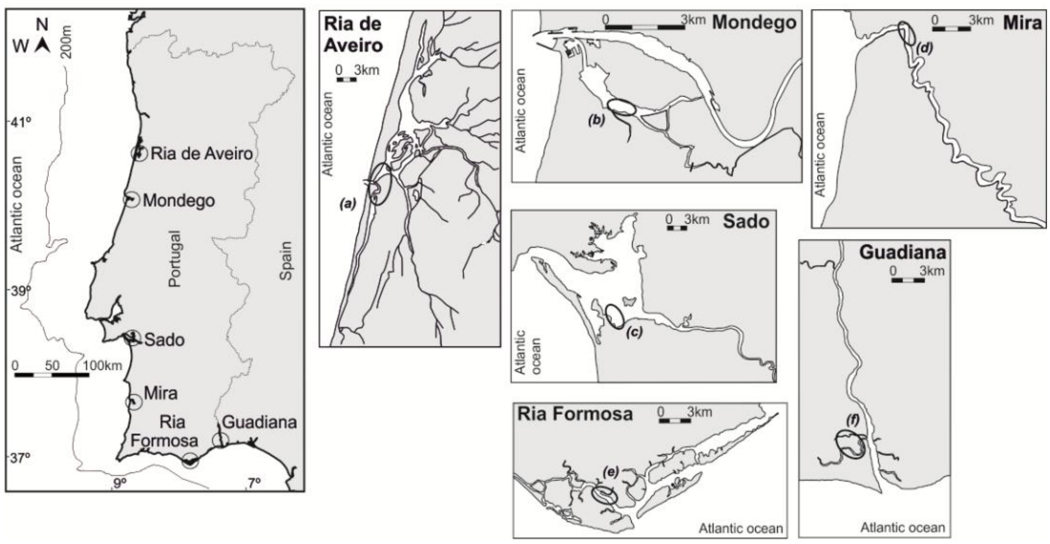


Fig. 1. Map of Portuguese coast with localization of sampled nursery areas in six estuaries: (a) Barra Sul in Ria de Aveiro, (b) Sul in Mondego, (c) Carrasqueira in Sado, (d) Jusante in Mira, (e) Barra in Ria Formosa and (f) Esteiro Norte in Guadiana.

Sampling

Juvenile *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), common two-banded sea bream, were captured using a beam trawl in selected sites of six estuaries (Fig. 1) in May and July 2006. A total of 146 individuals were selected.

In each season, all individuals were captured within the shortest possible time elapsed between all estuaries (a fortnight) to minimize temporal variation. Individuals were preserved in ice and frozen until measurement and dissection. Total length of juveniles was measured to the nearest millimeter and total weight was measured to the nearest milligram. Environmental variables (latitude, temperature, salinity, depth, percentage of mud in the sediment and density of benthic macro invertebrates) were measured in each site and month (see details in Vasconcelos *et al*., 2009).

Individual condition indices

Due to the difficulty in obtaining readable *sagittae* otoliths because of their large dimensions (for lengths in present study), *lapilli* otoliths were chosen for the analysis of daily increments, as was also performed by Di Franco *et al*. (2011). Validation of daily increments in otoliths of *Diplodus vulgaris* was described for *sagittae* by Vigliola (1997). For this species both *sagittae* and *lapilli* otoliths can be used for analysis of microstructure (Di Franco, personal communication; Di Franco *et al*., 2011), although *sagittae* are more commonly used (Vigliola, 1997; Di Franco and Guidetti, 2011). Right *lapilli* were extracted from selected individuals, cleaned and mounted, using mounting adhesive Crystalbond 509, with sulcus up on microscope slides, using a Leica EZ4 loupe. Right *lapilli* were chosen by convention, since right *lapilli* and left *lapilli* of two-banded sea bream are identical, when necessary right *lapilli* were replaced by left *lapilli*. All otoliths were polished in sagittal plane using 0.1 µm sandpaper until daily increments of the whole otolith were visible (as in Martinho *et al*., 2013). Diameter, radius, widths *a* and *b* of otoliths were measured using a Leica DM2000 light microscope at 40X or 100X magnification coupled with a LASv4.1.0 software for image analysis (as in Martinho *et al*., 2013) (Fig. 2). Widths of the 10 last daily increments and of the 60 first daily increments were measured at 400X magnification (as in Martinho *et al*., 2013).

Biochemical index RNA:DNA and morphometric Fulton's K indices were used to assess fish condition (as described in Vasconcelos *et al*., 2009). Briefly, for RNA:DNA determination a sample of a dorsal anterior area (preferably) of the muscle of each juvenile was extracted and stored in a sterilized microcentrifuge tube at -80 °C. After lyophilization, a subsample was stored in a microcentrifuge tube at -20 °C (between 0.01 g and 0.04 g of lyophilized weight, measured to the nearest 0.1 mg). All plastic ware and implements used were sterilized. Nucleic acid concentration was determined following the fluorometric method described by Caldarone *et al*. (2001) that consists on using ethidium bromide (EB) to measure total nucleic acid fluorescence and then using a RNase (ribonuclease) to enzymatically digest the RNA (ribonucleic acid), being the remaining fluorescence after digestion attributed to DNA (deoxyribonucleic acid) as adapted by Fonseca *et al*. (2006).

Fulton's K index values were initially obtained following the equation $K = 100 \times (1000 \times TW) / (TL)^3$ using the total wet weight (TW) in milligrams and the total length (TL) in millimeters. Also condition factor K' was calculated using the formula $K' = 100 \times 1000 \times (TW/ TL^b) \times 10$, where TW and TL are total weight in milligrams and total length in millimeters, respectively, of individuals and b = 3.5156 is the constant of linear regression between total weight and total length given by the equation $TW = a \times TL^b$, where TW is total weight and TL is total length (Richter *et al*., 2000).

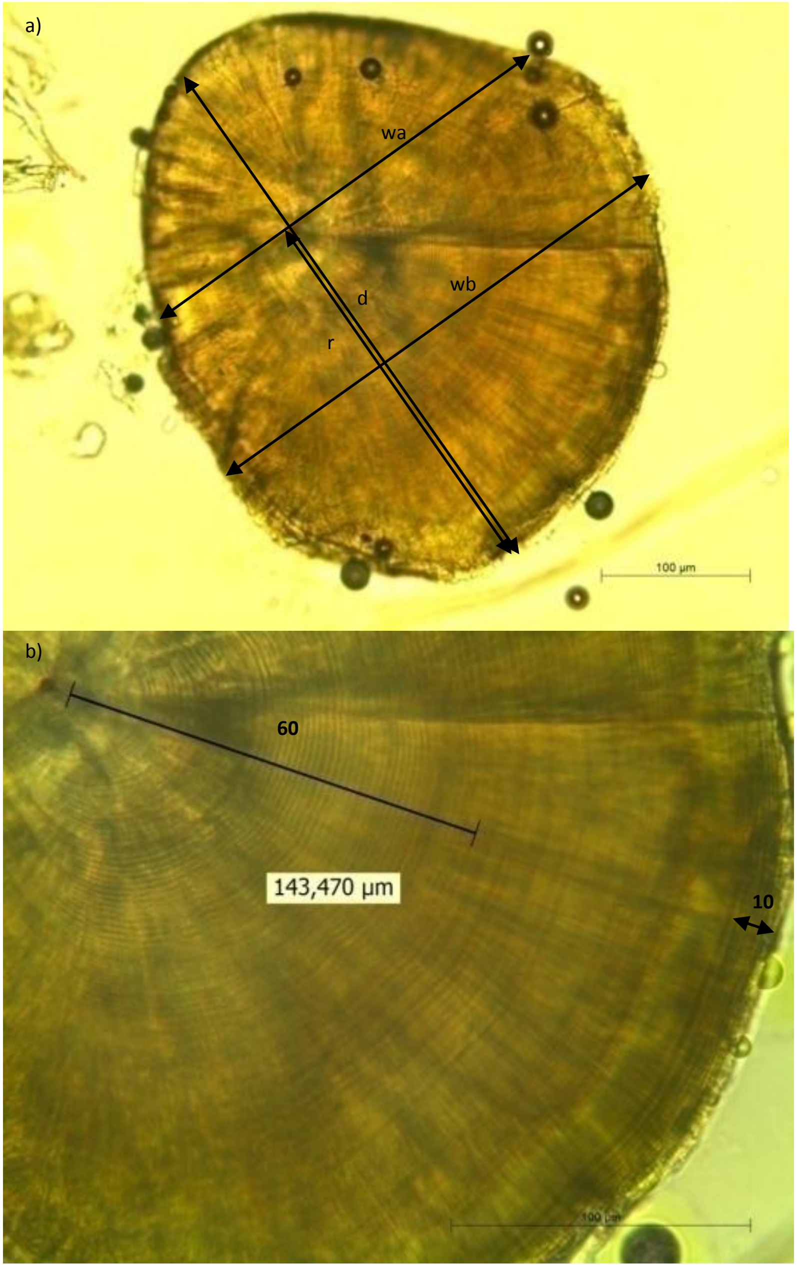


Fig. 2. Photograph of *lapillus* of a juvenile of *Diplodus vulgaris* (Total Length: 24 mm) viewed under light microscope. a) Segments represent diameter (d), radius (r), width **a** (wa) and width **b** (wb) in micrometers, at 100X magnification. b) Segments represent width of the 60 first daily increments (60), in this case 143,5 µm, and width of the 10 last daily increments (10) in micrometers, at 400X magnification.

Data analyses

Exploratory data analysis was performed using Pearson correlation to investigate existence of significant pairwise correlations between different biological variables and also between environmental variables.

Since significant relationships of recent growth index (width of the 10 last increments) and also RNA:DNA ratio with total length of individuals were found, these indices were corrected to eliminate or reduce length bias. Corrected condition indices (recent growth' index and RNA:DNA' ratio) were generated as residuals of linear regression between condition indices (recent growth index and RNA:DNA ratio, respectively) and total length (in millimeters). Fulton's K was replaced by condition factor K' since it deals with the observed allometry in growth of fish (Richter *et al.*, 2000). In subsequent study, corrected condition indices (recent growth' index, RNA:DNA' ratio and condition factor K') and initial growth index were used.

In addition, recent daily growth' and initial daily growth rates of the otoliths (in $mm.day^{-1}$) were also calculated.

Multiple regression analysis was used to investigate relationships of each biological variable (recent growth' index, initial growth index, RNA:DNA ratio' and condition factor K') with the set of measured environmental variables (latitude, temperature, salinity, percentage of mud in the sediment and density of benthic macro invertebrates).

Since the sample design was not fully orthogonal, one-way analyses of variance (ANOVA) of each biological variable were performed to assess significant variation in variables recent growth' index, initial growth index, RNA:DNA' ratio and condition factor K' between estuaries (Ria de Aveiro, Mondego, Sado, Mira, Ria Formosa and Guadiana) and also between months (May and July).

All statistical analyses were performed using Statistica 11 and a 0.05 significance level were considered in all statistical tests.

Results

A total of 146 juveniles of *Diplodus vulgaris* from six estuaries and two months of 2006 (May and July) were analyzed. In May individuals were only obtained in estuaries located in the south Portuguese coast: Sado, Mira, Ria Formosa and Guadiana, due to an earlier recruitment of *D. vulgaris* in southern estuaries comparatively to northern estuaries. Distribution of individuals analyzed by class of total length (10 mm interval), in millimeters, ranged from 11-20 to 111-120 (Table 1).

Table 1 Sample size of *Diplodus vulgaris* juveniles by Estuary, Month and Class of Total Length (mm) used in this study.

		Class of Total Length (mm)											
Estuary	Month	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	101-110	111-120	Total
Ria de Aveiro	July	0	0	0	2	5	4	2	1	0	0	0	14
Mondego	July	0	0	4	5	5	5	0	0	0	0	0	19
Sado	May	0	0	0	0	5	5	5	0	0	0	0	15
	July	0	0	0	0	1	5	5	5	1	2	0	19
Mira	May	3	5	0	0	0	0	0	1	2	3	2	16
	July	0	0	1	2	0	3	2	2	0	0	0	10
Ria Formosa	May	0	5	5	5	5	0	0	0	0	0	0	20
	July	0	0	0	0	4	5	3	1	0	0	0	13
Guadiana	May	0	4	3	1	0	0	0	0	0	0	0	8
	July	0	0	0	0	0	1	5	5	0	1	0	12
Total	-	3	14	13	15	25	28	22	15	3	6	2	146

Total wet weight (TW) was exponentially related with total length (TL) (Fig. 3).

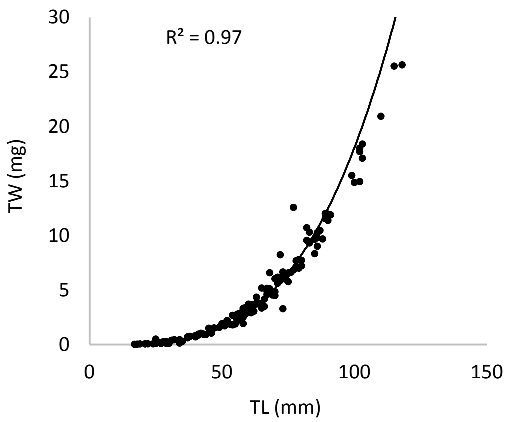


Fig. 3. Regression between total wet weight (TW), in milligrams, and total length (TL), in millimeters, of juvenile *Diplodus vulgaris*.

Diameter, radius, widths *a* and *b* of *lapilli* of juveniles of *Diplodus vulgaris* showed strong positive linear relationships with total length (TL) (Fig. 4).

Based on linear regression analysis, recent growth index (10 last increments) showed significant negative linear relationship with total length (TL) (R^2= 0.11, b= -0.33, p< 0.05) (Fig. 5. a)). Recent growth' index (10 last increments'), which is recent growth index (10 last increments) corrected for length bias, was not significant related with total length (TL) (Fig. 5. b)). In contrast, initial growth index (60 first increments) was not significantly related with total length (TL) (R^2= 0.01, b= -0.08 at p< 0.05) (Fig. 5. c)). In subsequent analysis, recent growth' index (10 last increments') and initial growth index (60 first increments) were considered.

On the other hand, RNA:DNA ratio showed a significant negative linear relationship with total length (TL) (R^2= 0.51, b= -0.71, p< 0.05) (Fig. 6. a)), RNA:DNA' ratio, which is RNA:DNA ratio corrected for length bias, was not significantly related with total length (TL) (Fig. 6. b)), and was used in subsequent analysis.

Condition factor K', which deals with allometry, was not significantly related with total length (TL) (R^2= 0.02, b= -0.15, at p< 0.05) (Fig. 6. c)).

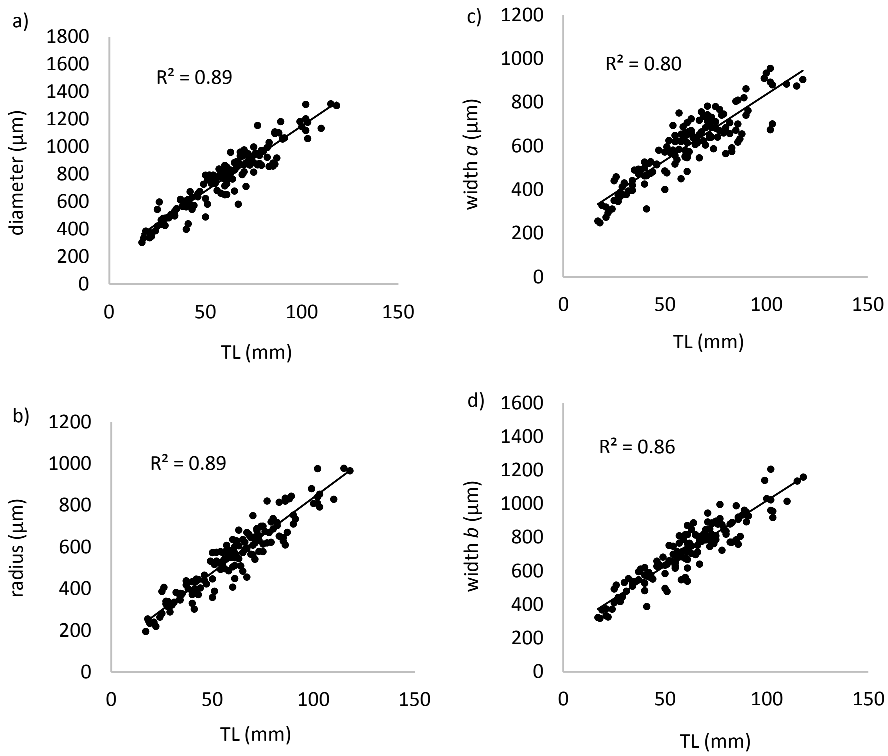


Fig. 4. Relationships between diameter (in micrometers) (a), radius (in micrometers) (b), width *a* (in micrometers) (c) and width *b* (in micrometers) (d) of otoliths with total length (TL) (in millimeters) in juveniles of *Diplodus vulgaris* sampled in nursery areas of estuaries along Portuguese coast.

Pairwise Pearson correlation of the four biological condition indices only showed a significant positive correlation between recent growth' index and initial growth index (Table 3).

In May, biochemical condition index was only assessed for Sado and Ria Formosa estuaries and the other three indices were only determined for Sado, Mira, Ria Formosa and Guadiana.

In this month, all four individual condition indices presented higher values for individuals of Sado and Ria Formosa (Fig. 7).

In July, individual condition indices showed higher values in juveniles of Ria Formosa and Guadiana, with exception of RNA:DNA’ ratio that showed higher values for fish of Ria de Aveiro and Mondego (Fig. 7).

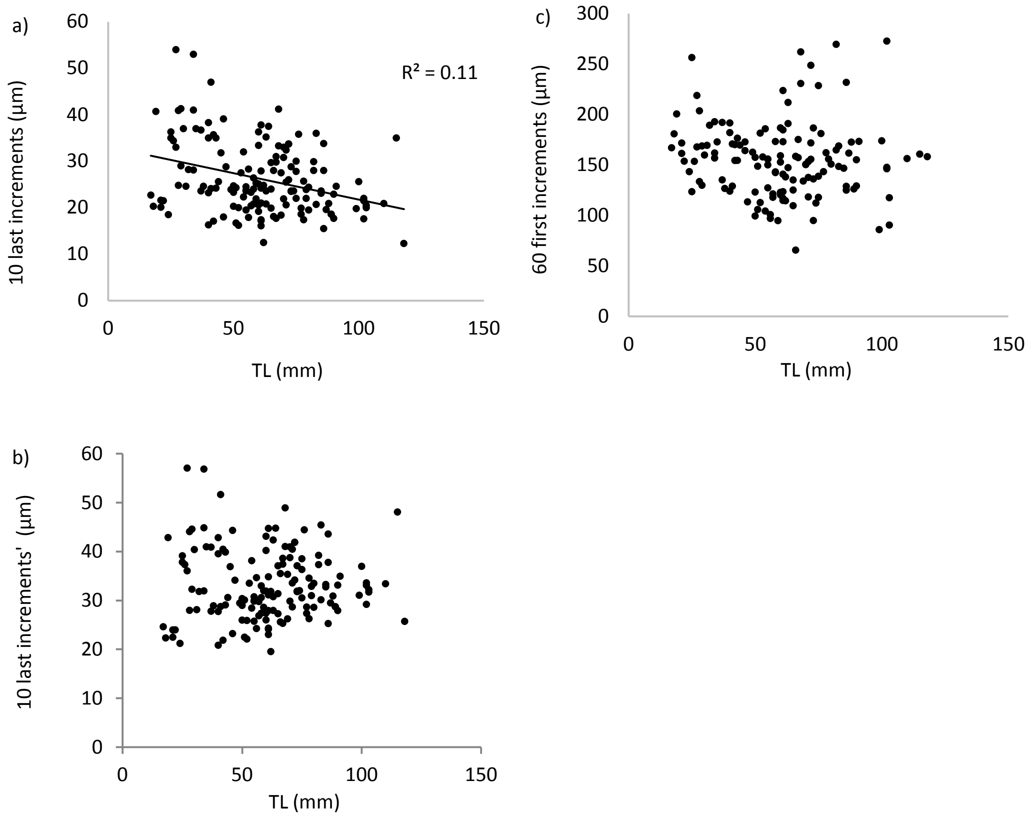


Fig. 5. Relationships between width of the 10 last daily increments (in micrometers) (a), width of the 10 last daily increments' (in micrometers), which is width of the 10 last daily increments corrected for length (b) and width of the 60 first daily increments (in micrometers) (c) of otoliths with total length (TL) (in millimeters) in juveniles of *Diplodus vulgaris* sampled in nursery areas of estuaries along Portuguese coast.

All individual condition indices presented higher values in juveniles captured in May, with the exception of RNA:DNA’ ratio that showed higher values for individuals collected in July (Fig. 7). Recent otolith growth' rates obtained for juveniles of *Diplodus vulgaris* sampled in nursery areas along the Portuguese coast were between 0.0028 mm.day^{-1} (in Mondego and Mira, July) and 0.0045 mm.day^{-1} (in Guadiana, July) and initial otolith growth rates were between 0.0021 mm.day^{-1} (in Mira, July) and 0.0030 mm.day^{-1} (in Guadiana, July).

Recent otolith growth' rates were always superior than initial otolith growth rates of individuals sampled.

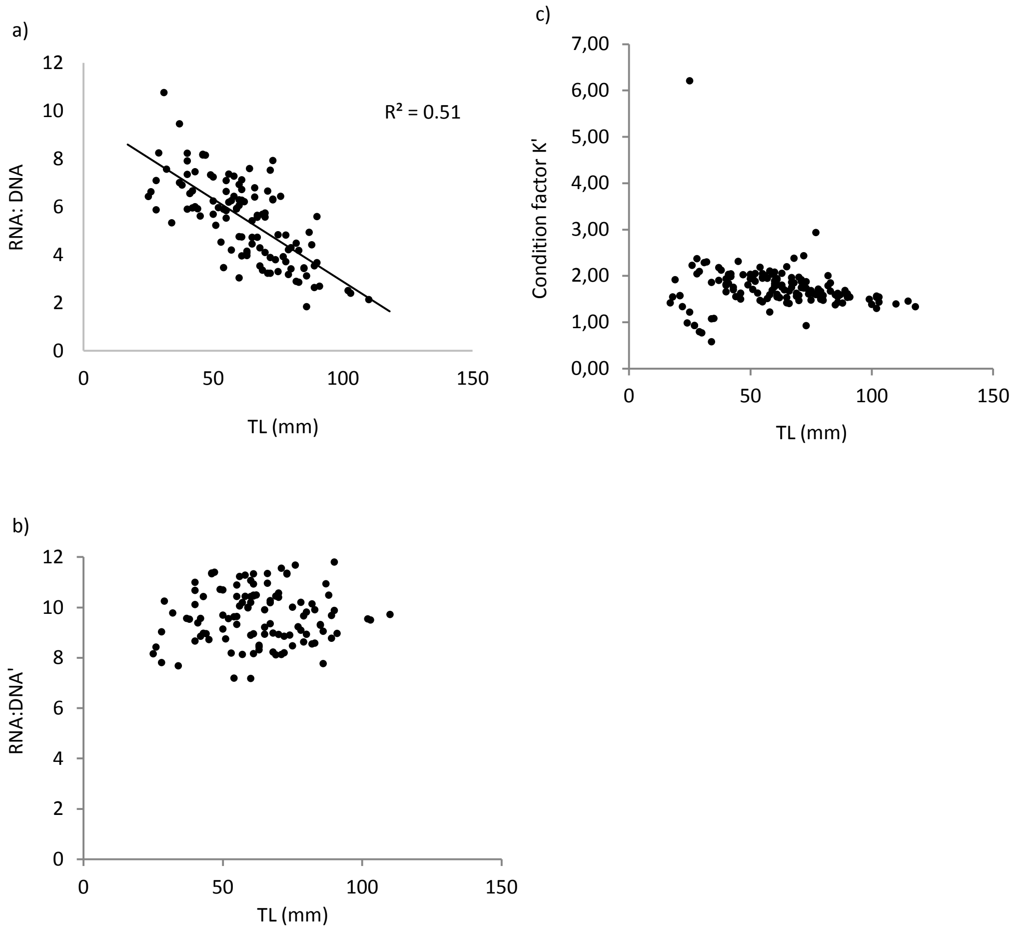


Fig. 6. Relationships between biochemical and morphometric condition indices, RNA:DNA (a), RNA:DNA' (b), which is RNA:DNA ratio corrected for length bias, and condition factor K' (c), with total length (TL) (in millimeters) in juveniles of *Diplodus vulgaris* sampled in nursery areas of estuaries along Portuguese coast.

In May, Sado and Ria Formosa showed higher water temperature and higher salinity (Table 5). Sado presented higher depth of the water column and Mira presented higher percentage of mud in the sediment and higher density of benthic macro invertebrates (Table 5). Guadiana also showed higher percentage of mud in sediment (Table 5).

In July, Ria Formosa and Guadiana presented higher water temperature (Table 5). Sado and Ria Formosa showed higher salinity (Table 5). Ria Formosa showed higher depth of water column (Table 5). Mira and Guadiana showed higher percentage of mud in the sediment and Ria de

Table 3 Pairwise correlation matrix for four variables: recent growth' index (10 last increments'), initial growth index (60 first increments), RNA:DNA' and condition factor K'. *p < 0.05; (ns) not significant

Variable	10 last increments'	60 first increments	RNA:DNA'	Condition factor K'
10 last increments'				
60 first increments	0.39*			
RNA:DNA'	0.02 (ns)	-0.18 (ns)		
Condition factor K'	0.11 (ns)	-0.05 (ns)	-0.15 (ns)	

Aveiro and Mira higher density of benthic macro invertebrates (Table 5).

Significant pairwise correlations were found between some of environmental variables, chiefly depth which was, therefore, excluded from subsequent analysis (results not shown).

Recent growth' index, initial growth index and condition factor K' varied significantly between estuaries (Ria de Aveiro, Mondego, Sado, Mira, Ria Formosa and Guadiana), but RNA:DNA' ratio presented no significant variation (Table 6).

Recent growth' index, initial growth index and RNA:DNA' varied significantly between months (May and July), but for condition factor K' there was no significant difference (Table 6).

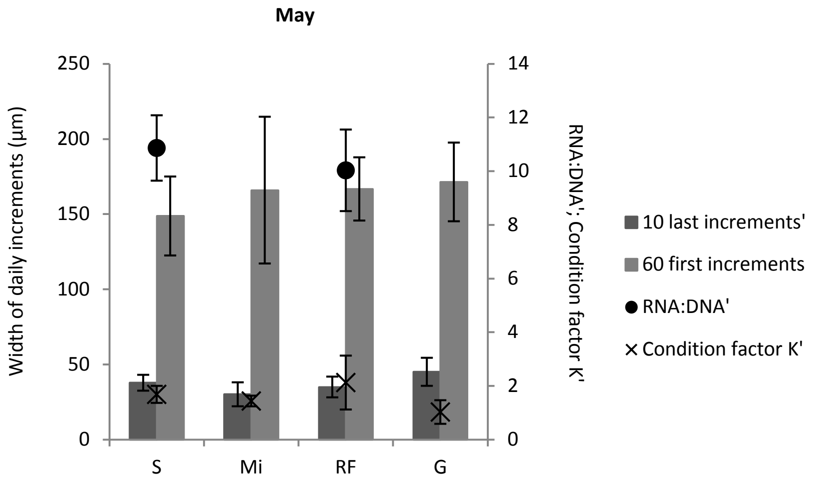
Multiple regression analysis for RNA:DNA' showed a significant relationship with all the environmental variables considered (latitude, temperature, percentage of mud in the sediment and density of benthic macro invertebrates) with exception of salinity (Table 7).

Both condition factor K' and recent growth' index were significantly related with temperature, percentage of mud in the sediment and density of benthic macro invertebrates (Table 7), although not in the same manner.

Finally, initial growth index was only related with latitude (Table 7).

Discussion

a)



b)

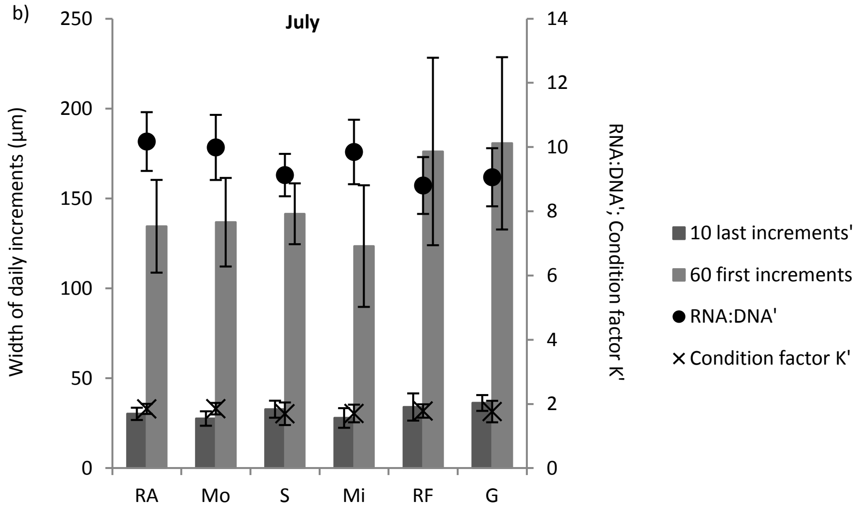


Fig. 7. Recent growth' index (10 last increments'), initial growth index (60 first increments), RNA:DNA' and condition factor K' in juveniles of *Diplodus vulgaris* sampled in nursery areas of estuaries along Portuguese coast. Principal vertical axis (at left) for recent growth' index (10 last increments') and initial growth index (60 first increments), in micrometers. Secondary vertical axis (at right) for RNA:DNA' ratio and condition factor K'. a) May 2006 in four estuaries: Sado (S), Mira (Mi), Ria Formosa (RF) and Guadiana; b) July 2006 in six estuaries: Ria de Aveiro (RA), Mondego (Mo), Sado (S), Mira (Mi), Ria Formosa (RF) and Guadiana (G).

Table 4 Recent otolith growth' rate and initial otolith growth rate (mm.day^{-1}) obtained by analysis of otolith microstructure of sampled juveniles of *Diplodus vulgaris* in nursery areas of estuaries along Portuguese coast and in each month.

Estuary	Month	Recent otolith growth' rate (mm.day^{-1})	Initial otolith growth rate (mm.day^{-1})
Ria de Aveiro	July	0.0030	0.0022
Mondego	July	0.0028	0.0023
Sado	May	0.0038	0.0025
Sado	July	0.0033	0.0024
Mira	May	0.0030	0.0028
Mira	July	0.0028	0.0021
Ria Formosa	May	0.0035	0.0028
Ria Formosa	July	0.0034	0.0029
Guadiana	May	0.0045	0.0029
Guadiana	July	0.0036	0.0030

Relationship of individual condition indices and fish size

Our data showed a strongly exponentially relationship between total weight and total length of juveniles of *Diplodus vulgaris* sampled (Fig. 3).

Individuals of *D. vulgaris* suffer an abrupt growth especially in larval and juvenile stages that then diminishes with age in adults. In advanced age, fish growth is almost null. This corresponds to the general pattern of growth of fish (Bolger and Connolly, 1989).

The diameter, radius, width *a* and width *b* of otoliths of *Diplodus vulgaris* were strongly positively related with total length (Fig. 4). These results would be expected if otolith growth accompanied fish somatic growth (Campana, 1999; Lopes, 2004; Pajuelo and Lorenzo, 2002; Fey *et al*., 2005). Our results showed that, in Diplodus *vulgaris*, otolith growth accompanies somatic fish growth. Therefore, in this species, otolith and fish somatic growth seem to be controlled by same metabolic processes (Labropoulou and Papaconstantinou, 2000). For this reason, it is acceptable to use analysis of otolith microstructure to estimate individual

Table 5 Environmental variables, namely mean (and standard deviation in brackets) in sampled sites: latitude in °N; temperature in °C; salinity; percentage of mud in the sediment in percentage of dry weight (% of Mud) and density of benthic macro invertebrates in individuals.m^{-2} in nursery areas of six estuaries: Ria de Aveiro; Mondego; Sado; Mira; Ria Formosa and Guadiana; and in two months: May and July.

		Latitude (°N)		Temperature (°C)		Salinity		Depth (m)		% of Mud (% dw)		Density of Macro invertebrates (individuals.m^{-2})	
Estuary	Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ria de Aveiro	July	40.6	(0.0)	24.1	(0.6)	29.3	(1.3)	1.1	(0.7)	49.5	(0.0)	6724.0	(0.0)
Mondego	July	40.1	(0.0)	20.5	(0.9)	28.5	(5.1)	0.5	(0.0)	3.0	(0.0)	2080.0	(0.0)
Sado	May	38.5	(0.0)	23.4	(0.0)	34.7	(0.0)	2.9	(0.0)	30.5	(0.0)	1260.0	(0.0)
	July	38.5	(0.0)	23.0	(0.0)	36.7	(0.0)	2.2	(0.0)	30.5	(0.0)	1260.0	(0.0)
Mira	May	37.7	(0.0)	18.0	(0.6)	32.5	(0.8)	2.2	(1.1)	63.4	(0.0)	17634.0	(0.0)
	July	37.7	(0.0)	20.3	(1.2)	35.0	(0.6)	2.3	(1.9)	63.4	(0.0)	17634.0	(0.0)
Ria Formosa	May	37.0	(0.0)	22.1	(0.0)	36.5	(0.0)	1.6	(0.0)	30.0	(0.0)	-	-
	July	37.0	(0.0)	25.7	(0.6)	36.2	(0.3)	2.7	(0.6)	37.7	(45.2)	-	-
Guadiana	May	37.2	(0.0)	23.0	(0.0)	29.5	(0.0)	1.9	(0.0)	74.5	(0.0)	3130.0	(0.0)
	July	37.2	(0.0)	26.3	(0.0)	28.2	(0.0)	2.3	(0.0)	74.5	(0.0)	3130.0	(0.0)

Table 6 One-way ANOVA for biological variables in study recent growth' index (10 last increments'), initial growth index (60 first increments), RNA:DNA' ratio and condition factor K' and for independent factors (Estuary and Month). *p< 0.05; (ns) not significant

	10 last increments'		60 first increments		RNA:DNA'		Condition factor K’	
	F	p	F	P	F	P	F	p
Estuary	11.02	0.00*	5.11	0.00*	1.58	0.17 (ns)	4.42	0.00*
Month	13.40	0.00*	3.94	0.05*	14.36	0.00*	1.55	0.22 (ns)

condition of fish (Campana, 1984; Campana and Thorrold, 2001).

Usually, daily increments are thinner in larval stage, becoming wider in early juvenile stage and then again turning more narrow (Campana and Moksness, 1991; Lopes, 2004). In this way, recent growth index estimated by analysis of otolith microstructure can vary along the life cycle of individuals and diminish with age (Morales-Nin, 2001; Pajuelo and Lorenzo, 2002;

Table 7 Multiple regression analysis for each dependent biological variable recent growth' index (10 last increments'), initial growth index (60 first increments), RNA:DNA' and condition factor K' with set of independent environmental variables (latitude, temperature, salinity, percentage of mud in the sediment and density of benthic macro invertebrates). *p< 0.05; (ns) not significant

	10 last increments' Adjusted R^2 = 0.36 P < 0.00		60 first increments Adjusted R^2 = 0.14 P < 0.00		RNA:DNA' Adjusted R^2 = 0.28 P < 0.00		Condition factor K' Adjusted R^2 = 0.35 P < 0.00	
	b*	p-value	b*	p-value	b*	p-value	b*	p-value
Latitude	-0.16	0.23 (ns)	-0.35	0.04*	-0.49	0.01*	0.00	0.99 (ns)
Temperature	-0.57	0.01*	-0.21	0.42 (ns)	7.48	0.00*	1.27	0.00*
Salinity	0.10	0.26 (ns)	-0.20	0.08 (ns)	-0.01	0.93 (ns)	-0.14	0.15 (ns)
% of Mud	0.86	0.00*	0.24	0.41 (ns)	-8.52	0.00*	-1.25	0.00*
Density of Macro invertebrates	-1.18	0.00*	-0.32	0.30 (ns)	6.35	0.00*	1.35	0.00*

Francis and Campana, 2004; Lopes, 2004). So, it is expected that recent growth index estimated by width of the 10 last daily increments of otoliths varies with age and size of individuals (Morales-Nin, 2001; Pajuelo and Lorenzo, 2002; Lopes, 2004). In this study, recent growth index showed to be significantly negatively related with total length of individuals (Fig. 5 a)). Therefore, comparison of recent growth index should be done between individuals of same life stage at least, and ideally of same age or size (Francis and Campana, 2004). For this reason, recent growth index was corrected for length bias and the corrected recent growth' index was considered in subsequent study.

Initial growth index was not significantly related with length of individuals sampled. Since initial growth index was estimated by width of the 60 first daily increments of otoliths, it represents the same period of age in all individuals. So, it is expected that significant variation assessed by this index would be only due to different individual condition of fish in the two first months of life.

RNA:DNA ratio varies ontogenetically, diminishing with fish age, since protein synthesis for physiological and growth processes is higher in early stages of life (Buckley *et al*, 2008).

In the present study, since a significant relationship was found between RNA:DNA ratio and total length (Fig. 6 a)), a correction for length bias was performed to allow the comparison of individuals from different estuaries and months without the possible confounding effect of length, and the corrected biochemical index RNA:DNA' ratio was considered in subsequent analysis (Fig. 6 b)). Significant relationship between RNA:DNA ratio and length of fish has been detected in other studies (Gilliers *et al*., 2004; Amara *et al*., 2009; Vasconcelos *et al*., 2009).

Fulton's K is based on the relationship between total weight and total length of individuals and considers that for same length a heavier individual is in better condition (Nash *et al*., 2006). In the early life stages of fish, weight and length tend to increase more quickly comparatively to late life stages (Gordoa and Molí, 1997; Loy *et al*., 2001). Moreover, weight increases exponentially comparatively to length (Bolger and Connolly, 1989), as also verified in this study (Fig. 3). A significant relationship between Fulton's K and total length was found in this study, which is in accordance with other studies (Amara *et al*., 2009; Vasconcelos *et al*., 2009; McPherson *et al*., 2011). This result indicates that Fulton's K was dependent of the size of individuals. Furthermore, our results are in concordance with the dependence of this morphometric index on size and age of fish (Suthers, 1998; Barnham and Baxter, 2003). Moreover, the observed significant linear relationship between Fulton's K and total length indicates allometry (Richter *et al*., 2000; Stevenson and Woods, 2006). This would introduce a length bias in the analysis, since the formula of Fulton's K considers isometry in growth of fish (Richter *et al*., 2000; Stevenson and Woods, 2006). For this reason, Fulton's K was substituted by condition factor K', which already considers allometry in growth of individuals (Richter *et al*, 2000).

Relationship between individual condition indices

A significant positive correlation was found between recent growth' index and initial growth index. Initial growth index is related with initial condition of individuals, namely the two first months of life, and recent growth' index estimates individual condition of the 10 last days before capture. So, it seems that individuals in better condition in initial life also presented better condition in subsequent periods of life, particularly in the 10 last days before capture. This relationship between growth of otoliths in larval and juvenile stages has been previously reported (Vigliola *et al*., 2007).

One hypothesis for this significant correlation between recent growth' index and initial growth index could be that some living areas provide better condition for these organisms relatively to others, which is reflected in both larval and juveniles stages (Abecasis *et al*., 2009), and individuals spent larval and juvenile stages in same nursery areas. It is thought that nursery areas are mainly used by larvae, between two and three weeks before settlement phase, and juveniles until reaching adequate size to join adult populations offshore, normally between five and eight months after settlement, usually, individuals of *Diplodus vulgaris* leave nursery areas when they complete one year of life, around autumn, with total length between 80-150 mm (Macpherson *et al*, 1997; Macpherson, 1998; Vigliola *et al*., 2000; Ribeiro et al., 2006).

One second hypothesis, could be that individuals that are in better individual condition during the first life stages have higher probability of being in better individual condition in the following life stages. This could happen because fish in better individual condition can look for better preys, escape from predators easily and fight for more suitable living areas (Searcy and Spounagle, 2000; Macpherson and Raventos, 2005). So, individuals that during their larval stage presented better individual condition due to environmental factors (higher habitat quality of areas where they live) or due maternal effects (larger eggs, more reserves in yolk sac, e.g.) had higher probability to maintain higher individual condition. Several authors have already referred that individuals in better condition at hatching and in early life stages have better fitness in subsequent stages of life and higher probability to reach older age (Searcy and

Spounagle, 2000; Macpherson and Raventos, 2005). In this way, individuals in better condition at hatching time and during early life stages should display bigger sizes and faster growth rates. This last hypothesis is in accordance with the "growth-mortality hypothesis" which refers that smaller fish have lower probability of surviving, comparatively to bigger fish of the same cohort (Macpherson and Raventos, 2005). And this also reflects the "bigger is better" hypothesis, according to which bigger fish, and thus in better individual condition, have lower probability to be caught by predators, could prey individuals of smaller size, and consequently have higher availability of food, could obtain better nutritional food, could defend a better habitat and could cope easily with factors of stress, comparatively to smaller conspecifics (Macpherson and Raventos, 2005). Furthermore, if individuals at hatching were in better individual condition due to characteristics inherited by parents, they would have higher probability of success in subsequent life phases since they would always be in advantage comparatively to smaller and in worst condition fish of same age (Bobko, 2003; Green and McCormick, 2005). However, this relationship between larval and juvenile condition estimated by analysis of otolith microstructure is not always found, as was the case in a study with a reef species performed by Searcy and Speanougle (2000).

Our results showed no significant relationship between condition estimated by analysis of otolith microstructure (recent growth' and initial growth indices), RNA:DNA' ratio and condition factor K'. One possible explanation for the absence of relationship between these different condition indices could be due to their different response times. RNA:DNA' has been shown to assess nutritional status of individuals during only the last three days (at maximum) before capture (Buckley, 1984). Condition factor K' (or Fulton's K) has been shown to assess general well-being of fish and, therefore, individual condition of a longer temporal window, from weeks to months, before capture (Caldarone *et al*., 2012; De Raedemaecker *et al*., 2012).

Recent growth' index corresponds to individual condition of the 10 last days before capture. But environmental factors that contribute for this condition index could have occurred even before these 10 days since it probably takes some time between environmental factors and response of organism in a higher level of organization (Campana, 1999). And the same can apply relatively to condition factor K' that also assess a higher level of animal organization (Caldarone *et al*., 2012; De Raedemaecker *et al*., 2012). Finally, initial growth index corresponds to individual condition of the 60 first days of life of individuals and, like recent growth' index, there is probably a delay between environmental factors and individual condition assessed by analysis of otolith microstructure (Campana, 1999). Moreover, initial growth index could even estimate individual condition given by maternal effects, like size of egg or amount of reserves in yolk sac (Clemmesen and Doan, 1996). Other hypothesis for this absence of relationships between different condition indices (condition estimated by analysis of otolith microstructure, RNA:DNA' ratio and condition factor K') could be that they assess individual condition given by different levels of organization, and therefore given by different physiological processes (Campana, 1999; Rossi-Wongtschowski *et al*., 2003). Moreover, the lack of correlation between different condition indices has already been detected in other studies (Suthers, 1998; Gilliers *et al*., 2004, Gilliers *et al*., 2006; Vasconcelos *et al*., 2009; De Raedemaecker *et al*., 2012). This could also help explain the positive correlation between recent growth' and initial growth indices that, despite assessing individual condition at different times (initial life stage and juvenile stage), are estimated by analysis of same structure (otoliths) and thus by same physiological processes and same level of organization. Particularly there was no significant correlation between RNA:DNA' ratio and recent growth' index, although there is an overlap in the period that the two individual condition indices refer to. For example, Clemmesen and Doan (1996) and Suthers (1998) have described a significant positive relationship between RNA:DNA ratio and recent growth index estimated by otolith microstructure. However, many studies have also not found significant correlation between these two indices (Hovenkamp and Witte, 1991; Morales-Nin *et al*., 2002; Amara *et al*., 2009).

Variation of individual condition indices and relationship with environmental variables

Our results indicated latitude, water temperature, percentage of mud in the sediment and density of benthic macro invertebrates significantly related with RNA:DNA' ratio (Table 7). The analyzed individual condition indices varied significantly between estuaries (except for RNA:DNA' ratio) and generally indicated higher condition in the southern estuaries of the Portuguese coast (Sado, Ria Formosa and Guadiana). The studied nursery areas have distinct environmental characteristics (see Vasconcelos *et al*., 2007a; Vasconcelos *et al*., 2010), particularly in terms of water temperature, salinity, percentage of mud in the sediment and density of benthic macro invertebrates (Vasconcelos *et al*., 2010). In general, southern estuaries of the Portuguese coast (Sado and Ria Formosa) presented warmer waters and all nurseries presented high salinities (Table 5). Also, Mira and Guadiana showed higher percentage of mud in the sediment and Mira presented the highest density of benthic macro invertebrates (Table 5). Generally, it seems reasonable to consider that higher habitat quality for juvenile *Diplodus vulgaris* is presented in southern estuaries of the Portuguese coast: Sado, Ria Formosa and Guadiana (Fig. 7). Ria Formosa has already been described as an important nursery area for juvenile *Diplodus vulgaris* (Ribeiro *et al*., 2006; Ribeiro *et al*., 2008; Vasconcelos *et al*., 2010) that use this shallow coastal lagoon for at least more than one month (Abecasis *et al*., 2008).

RNA:DNA' ratios obtained were considerably high, between 8.81 (in Ria Formosa, July) and 10.86 (in Sado, May) (Fig. 7), generally indicating a good nutritional status of juveniles (Clemmensen, 1988) in all estuaries and in both months, although indicative values depend on the species. For example, according with a study by Clemmensen (1988), RNA:DNA ratio in larvae of turbot varied between 2 and 8, which is lower than results obtained in this study. Furthermore, Clemmensen (1988) described in 18 days old individuals of turbot RNA:DNA ratios between 2 and 3 for larvae with no food available during the last 5 days, values between 3.5 and 6.5 for individuals with medium density of food available and finally values between 6.8 and 8.1 for individuals with a high food density available. Many studies indicated that RNA:DNA ratio is influenced by water temperature and availability of food. For example, Kerambrun (2012) observed a difference in RNA:DNA ratio from 5.0 to 2.8 in individuals of turbot starved for 7 days and starved for 21 days, respectively. Moreover, a study performed by Ozório *et al*. (2009) indicated that concentration of RNA, and thus transcription rate and capacity of protein synthesis in muscle cells, is influenced by dietary protein level. In the other hand, individuals fed with a lower dietary protein level presented higher DNA concentration indicating that higher DNA concentration is an effect of reduction of size of cells, in accordance with it is observed in starved fishes (Ozório *et al*., 2009). Moreover, in a study with farmed juveniles of *D. vulgaris* RNA:DNA ratio varied from 0.98 to 2.27 (Ozório *et al*., 2009). So, considering higher RNA:DNA ratio obtained in juveniles of *D. vulgaris*, we can assume that a good availability of prey for juveniles of this species likely exists along the Portuguese coast.

Condition factor K’ was significantly related with water temperature, percentage of mud in the sediment and density of benthic macro invertebrates (Table 7). Condition factor K' varied between 1.02 (in Guadiana, May) and 2.12 (in Ria Formosa, May) (Fig. 7). In a study by Aydin (2011), Fulton's K of older juveniles and adults of angled species (*Diplodus annularis*, *Diplodus vulgaris*, *Spicara flexuosa* and *Boops boops*) in Mediterranean Sea varied between 0.85 and 2.08, with 1.50 for *Diplodus vulgaris*. Also, in juveniles of *Solea solea* in French estuaries had Fulton’s K values between 1.0 and 1.2 (Amara *et al*., 2007). And, Lloret and Planes (2003) observed adults of white sea breams (*Diplodus sargus*) with Fulton's K between 1.43 and 2.43. Moreover, Caldarone *et al*. (2012) observed in juveniles of Atlantic salmon (*Salmo salar*) reared in laboratory that Fulton's K varied between 0.91 and 1.05 in fed individuals, between 0.85 and 1.01 in fasted individuals and between 0.89 and 0.98 in fasted for 11 days and then refed individuals. Furthermore, Kerambrun *et al*. (2012) observed that in individuals of turbot exposed in a sediment of reference Fulton's K varied between 1.53 and 1.62, in a contaminated sediment between 1.40 and 1.58, and in starved individuals varied between 1.44 and 1.57. Along the Portuguese coast, Tanner *et al*. (2009) described that in juveniles and adults of *Trisopterus luscus* Fulton's K varied between 0.91 and 0.98, and 1.17 and 1.37, respectively. Also, in a study performed by Ozório *et al*. (2009) in juveniles of *Diplodus vulgaris* reared in laboratory and under different dietary levels of protein, Fulton's K varied between 1.48 and 2.33, being 1.48 the mean value for individuals fed with only 5% of protein level in diet. In this way, range of condition factor K' assessed in juveniles along the Portuguese coast was in accordance with values in other studies, and the relative importance of contributing environmental factors (temperature and food availability) could not be fully clarified, as for RNA:DNA ratio. In sum, we can say that juveniles of *D. vulgaris* presented a relatively lower condition factor K' and that could correspond to a lower level of protein in their diet.

Recent growth' index was also significantly related with water temperature, percentage of mud in the sediment and density of benthic macro invertebrates but in a different manner than RNA:DNA' or condition factor K' (Table 7). Recent growth' index obtained ranged between 27.67 µm (in Mondego, July) and 45.03 µm (in Guadiana, May) (Fig. 7). Recent otolith daily growth' rates varied between 0.0028 $mm.day^{-1}$ (in Mondego and Mira, July) and 0.0045 $mm.day^{-1}$ (in Guadiana, May) (Table 4). In a study by Brothers *et al*. (1976) in otoliths of adult hake, average of growth rate of daily increments were between 0.0030 $mm.day^{-1}$ and 0.0040 mm.day^{-1}. Also Kerambrun *et al*. (2012) described a recent growth rate between 0.0015 mm.day^{-1} and 0.0016 mm.day^{-1} in juveniles of turbot exposed to a contaminated sediment, in individuals starved for 21 days, and in individuals in a sediment of reference.

Initial growth index was only significantly related with latitude (Table 7). Initial growth index varied between 123.57 µm (in Mira, July) and 180.73 µm (in Guadiana, July) (Fig. 7) and initial growth rates ranged from 0.0021 mm.day^{-1} (in Mira, July) and 0.0030 mm.day^{-1} (in Guadiana, July) (Table 4). Initial growth index assessed individual condition of individuals in the first 60 days of life which seems to justify that none of the local environmental variables (water temperature, salinity, percentage of mud in the sediment and density of benthic macro invertebrates) appeared significantly related at time of sampling. On the other hand, the fact that latitude was significantly negatively related with individual condition of individuals in the first 60 days of life likely relate with the delayed recruitment to nursery areas with increasing latitude could indicate that individuals were already in that nursery areas or that were at least at same latitude of the nursery areas.

Mean recent otolith growth' rates were larger than initial otolith growth rates in all estuaries/ months (Table 4). Recent otolith growth' refer to the 10 last days of fish life before capture and estimated daily growth of otoliths during juvenile stage of *D. vulgaris*. On the other hand, initial otolith growth rates refer to the 60 first days of fish life and estimated growth rate per day of otoliths in larval stage of this species. Searcy and Sponaugle (2000) described in larvae of common coral reef fish *Thalassoma bifasciatum* growth rates between 0.0016 mm.day^{-1} and 0.0073 mm.day^{-1} in a post hatch phase, between 0.0030 mm.day^{-1} and 0.0064 mm.day^{-1} in a post settlement phase and between 0.0069 mm.day^{-1} and 0.0082 mm.day^{-1} in juveniles, indicating that growth rates during juvenile stage are generally higher than growth rates during larval stage as has been shown for many fish species. Our results were in accordance with these previous studies.

Recent growth' index, initial growth index and RNA:DNA' ratio varied significantly between months, but condition factor K' did not (Table 6). Recent growth' index presented higher values in May (Fig. 7). The most plausible hypothesis for this result could be the first hypothesis presented above to explain higher RNA:DNA' ratio in May.

The initial growth index presented higher values for individuals captured in July comparatively to individuals captured in May (Fig. 7). This indicates that individuals captured in July were in better individual condition in early larval stage than individuals captured in May. One first hypothesis could be that individuals captured in July (total length range between 31 and 120 mm) had more favorable environmental variables during first two months of life than individuals captured in May. Perhaps, before May water temperature and availability of food were not yet adequate for early larvae reach higher individual condition. In contrast, for individuals captured in July, early larvae already faced warmer water and more availability of food. In concordance, Saoud *et al*. (2008) have shown a variation in fish condition along the different seasons of year in the Mediterranean Sea, with higher condition when environmental variables were more favorable, usually during the months of Spring and Summer. Also, Searcy and Sponaugle (2000) have referred variation in growth of larvae of a coral reef fish between cohorts, with higher growth of individuals during the months of more favorable environmental variables (availability of food, more favorable water temperature, e.g.). Furthermore, the spawning season of *Diplodus vulgaris* along the Portuguese coast goes from December to March (Gonçalves and Erzini, 2000) and variation in early life conditions among cohorts due to seasonality could be expected. A second hypothesis could be that individuals captured in July presented higher individual condition at time of hatching due to maternal effects, and therefore had higher probability to present higher individual condition in early larvae stage

(Green and McCormick, 2005). In this way, individuals in better individual condition at hatching could have higher probability of being in better individual condition in their larvae stage (Green and McCormick, 2005; Macpherson and Raventos, 2005).For RNA:DNA' ratio, higher values were found in May (although only two estuaries were assessed in May) (Fig. 7). One hypothesis to explain this result could be existence of higher availability of food and lower competition/ predation, since it has been shown that earlier cohorts can have higher condition/ growth rates than subsequent cohorts (Fonseca *et al*., 2006). A second hypothesis could be that the difference obtained between months is related with size or age of individuals (although a correction in this index was performed to reduce length bias). Individuals in May ranged from 11 to 120 mm and in July from 31 to 110 mm and the presence of these younger individuals, between 11 and 31 mm, likely enhanced mean RNA:DNA' ratio since RNA concentration in organism is higher in younger fish (Buckley, 1984; Buckley *et al*., 2008). One third hypothesis, could be that higher water temperature was responsible for the decrease in RNA concentration since it has been referred that RNA:DNA ratio could be inhibited by higher temperatures (Malloy and Targett, 1994; De Raedemaecker *et al*., 2012). Water temperature ranged between 18.0 °C and 23.4 °C, and between 20.3 °C and 26.3 °C in May and July, respectively (Table 5).

Results also seem to reflect the different response times of the four variables studied (recent growth' index, initial growth index, RNA:DNA' ratio and condition factor K') (Table 7). RNA:DNA' was related with many environmental variables at time of capture (latitude, water temperature, percentage of mud in the sediment and density of benthic macro invertebrates) which is in agreement with the fact that RNA:DNA' assesses the most recent individual condition of fish, just before capture. RNA:DNA ratio is referred as assessing individual condition of fish to 1 to 3 days before capture (Buckley, 1984; Gilliers *et al*., 2004; Vasconcelos *et al*., 2009). On the other hand, condition factor K' and recent growth' index were related with three environmental variables (water temperature, percentage of mud in the sediment and density of benthic macro invertebrates) and seem to give an estimate of individual condition in a prior period comparatively to RNA:DNA' ratio. In this study, recent growth' index assessed condition of fish in the 10 last days before capture. Condition factor K' is considered an even more long term indicator of individual condition (Gilliers *et al*., 2004; Caldarone *et al*., 2012), comparatively to recent growth index assessed by analysis of otolith microstructure. Finally, initial growth index was only related with latitude, and was not related with environmental characteristics of estuarine areas just before capture. This temporal variation between RNA:DNA' ratio, condition factor K' and recent growth' index has already been detected in other studies (Rossi-Wongtschowski *et al*., 2003; Gilliers *et al*., 2004; De Raedemaecker *et al*., 2012).

Future studies should better investigate relationships between fish condition indices and between condition indices and environmental variables in both spatial and temporal scales. Further study on the mechanisms responsible for the response time of different indices should also be developed. Moreover, it would be also important to better investigate relationship between individual condition in early stages and subsequent phases of fish life cycle.

In sum, assessing habitat quality is fundamental to understand the dynamics of marine fish populations, and the link between habitat quality and fish condition proved to be an excellent way to accomplish this goal.

Acknowledgements

Research was funded with project PTDC/MAR/117119/2010, Centro de Oceanografia with strategic project PEst-439 OE/MAR/UI0199/2011, F. Martinho and R.P. Vasconcelos were funded with Investigador FCT Programme (respectively IF/01410/2012 and IF/00058/2013), all from Fundação para a Ciência e a Tecnologia.

Literature cited

Abecasis, D., Bentes, L., Coelho, R., Correia, C., Lino, P.G., Monteiro, P., Gonçalves, J.M.S., Ribeiro, J., Erzini, K., 2008. Ageing seabreams: A comparative study between scales and otoliths. Fish. Res. 89, 37–48.

Abecasis, D., Bentes, L., Erzini, K., 2009. Home range, residency and movements of *Diplodus sargus* and *Diplodus vulgaris* in a coastal lagoon: Connectivity between nursery and adult habitats. Estuar. Coast. Shelf Sci. 85, 525–529.

Able, K.W., 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. Estuar. Coast. Shelf Sci. 64, 5-17.

Aguilera, B., Catalán, I.A., Palomera, I., Olivar, M.P., 2009. Otolith growth of European sea bass (*Dicentrarchus labrax* L.) larvae fed with constant or varying food levels. Scient. Mar., 73(1), 173-182.

Amara, R., Meziane, T., Gilliers, C., Hermel, G., Laffargue, P., 2007. Growth and condition indices in juvenile sole *Solea solea* measured to assess the quality of essential fish habitat. Mar. Ecol. Prog. Ser. 351, 201-208.

Amara, R., Selleslagh, J., Billon, G., Minier, C., 2009. Growth and condition of 0-group

European flounder, *Platichthys flesus* as indicator of estuarine habitat quality. Hydrobiologia 627, 87-98.

Assis, C.A.S., 2000. Estudo morfológico dos otólitos *sagitta*, *asteriscus* e *lapillus* de teleósteos (Actinopterygii, Teleostei) de Portugal continental. Sua aplicação em estudos de filogenia, sistemática e ecologia. Dissertação apresentada à Faculdade de Ciências da Universidade de Lisboa para obtenção do grau de Doutor em Biologia, na especialidade de Ecologia e Biossistemática.

Aydin, I., 2011. Is natural bait type a stochastic process for size and condition of fishes in the recreational fishery of Izmir Bay? Medit. Mar. Sci. 12(2), 390-400.

Barnham, C., Baxter, A., 2003. Condition factor, K, for salmonid fish. Fish. Notes 1-3.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51(8), 633-641.

Bergmann, M., Hinz, H., Blyth, R.E., Kaiser, M.J., Rogers, S.I., Armstrong, M., 2004. Using knowledge from fishers and fisheries scientists to identify possible groundfish "Essential Fish Habitats". Fish. Res. 66, 373-379.

Biagi, F., Gambaccini, S., Zazzetta, M., 1998. Settlement and recruitment in fishes: The role of coastal areas. Ital. J. Zool. 65(1), 269-274.

Bobko, S.J., 2003. Effects of maternal age on reproductive success in black rockfish, *Sebastes* *melanops*. Dissertation Thesis for the degree of Doctor of Philosophy in Fisheries Science, Oregon State University.

Bolger, T., Connolly, P.L., 1989. The selection of suitable indices for the measurement and analysis of fish condition. J. Fish Biol. 34, 171-182.

Booth, D.J., 1995. Juvenile groups in a coral-reef damselfish: Density-dependent effects on individual fitness and population demography. Ecol. 76(1), 91-106.

Brothers, E.B., Mathews, C.P., Lasker, R., 1976. Daily growth increments in otoliths from larval and adult fishes. Fish. Bull. 74(1), 1-8.

Buckley, L.J., 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80, 291-298.

Buckley, L.J., Caldarone, E.M., Clemmesen, C., 2008. Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis. Mar. Ecol. Prog. Ser. 371, 221-232.

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 (Reference document 01-11). Northeast Fisheries Science Center, USA, 1-22.

Calderone, E.M., MacLean, S.A., Sharack, B. 2012. Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (*Salmo salar*) to food availability. Fish. Bull. 110, 257-270.

Campana, S.E., 1984. Lunar cycles of otolith growth in the juvenile starry flounder *Platichthys stellatus*. Mar. Biol. 80, 239-246.

Campana, S.E., 1989. Otolith microstructure of three larval gadids in the Gulf of Maine, with inferences on early life history. Can. J. Zool. 67, 1401-1410.

Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Eco. Prog. Ser. 188, 263-297.

Campana, S.E., 2005. Otolith science entering the 21st century. Mar. Fresh. Resear. 56, 485-495.

Campana, S.E., Moksness, E., 1991. Accuracy and precision of age and hatch date estimates from otolith microstructure examination. ICES J. Mar. Sci. 48, 303-316.

Campana, S.E., Neilson, J.D., 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42, 1014-1032.

Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. 58, 30-38.

Clemmesen, C.M., 1988. A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. Meeresforsch. 32, 134-143.

Clemmesen, C., Doan, T., 1996. Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. Mar. Ecol. Prog. Ser. 138, 33-39.

Correia, A.T., Pipa, T., Gonçalves, J.M.S., Erzini, K., Hamer, P.A., 2011. Insights into population structure of *Diplodus vulgaris* along the SW Portuguese coast from otolith elemental signatures. Fish. Res. 111, 82-91.

De Raedemaecker, F., Brophy, D., O'Connor, I., O'Neill, B., 2012. Dependence of RNA:DNA ratios and Fulton's K condition indices on environmental characteristics of plaice and dab nursery grounds. Estuar. Coast. Shelf Sci. 98, 60–70.

De Rinaldis, G., 2009. Durata larvale pelagica del sarago maggiore *Diplodus sargus sargus* (Linnaeus, 1758). Tesi di Laurea Sperimentale in Zoologia e Biologia Marina. Corso di Laurea in Scienze e Tecnologie per l'Ambiente. Universita' del Salento. Facoltà di Scienze Matematiche Fisiche e Naturali.

Di Franco, A., Guidetti, P., 2011. Patterns of variability in early-life traits of fishes depend on spatial scale of analysis. Biol. Lett. 7, 454-456.

Di Franco, A., De Benedetto, G., De Rinaldis, G., Raventos, N., Sahyoun, R., Guidetti, P., 2011. Large scale-variability in otolith microstructure and microchemistry: The case study of *Diplodus sargus sargus* (Pisces: Sparidae) in the Mediterranean Sea. Ital. J. Zool. 78(2), 182-192.

Fey, D.P., Martin, G.E.B., Morris, J.A., Hare, J.A., 2005. Effect of type of otolith and preparation technique on age estimation of larval and juvenile spot (*Leiostomus xanthurus*). Fish. Bull. 103, 544-552.

Fonseca, V., Vinagre, C., Cabral, H., 2006. Growth variability of juvenile soles *Solea solea*

(Linnaeus, 1758) and *Solea senegalensis* (Kaup, 1858), based on condition indices in the Tagus estuary, Portugal. J. Fish Biol. 68, 1551-1562.

Francis, R.IC.C., Campana, S.E., 2004. Inferring age from otolith measurements: a review and a new approach. Can. J. Fish. Aquat. Sci. 61, 1269-1284.

Gilliers, C., Amara, R., Bergeron, J., Le Pape, O., 2004. Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. Env. Biol. Fishes 71, 189-198.

Gilliers, C., Le Pape, O., Désaunay, Y., Bergeron, J., Schreiber, N., Guerault, D., Amara, R., 2006.

Growth and condition of juvenile sole (*Solea solea* L.) as indicators of habitat quality in coastal and estuarine nurseries in the Bay of Biscay with a focus on sites exposed to the Erika oil spill. Sci.Mar. 70(1), 183-192.

Gonçalves, J.M.S., Erzini, K., 2000. The reproductive biology of the two-banded sea bream (*Diplodus vulgaris*) from the southwest coast of Portugal. J. Appl. Ichthyol. 16, 110-116.

Gonçalves, J.M.S., Bentes, L., Coelho, R., Correia, C., Lino, P.G., Monteiro, C.C., Ribeiro, J., Erzini, K., 2003. Age and growth, maturity, mortality and yield-per-recruit for two-banded bream (*Diplodus vulgaris* Geoffr.) from the south coast of Portugal. Fish. Res. 62, 349-359.

González-Wangüemert, M., Cánovas, F., Pérez-Ruzafa, A., Marcos, C., Alexandrino, P., 2010. Connectivity patterns inferred from the genetic structure of white seabream (*Diplodus sargus* L.). J. Exp. Mar. Bio. Ecol. 383, 23–31.

Gordoa, A., Molí, B., 1997. Age and growth of the sparids *Diplodus vulgaris*, *D. sargus* and *D. annularis* in adult populations and the differences in their juvenile growth patterns in the north-western Mediterranean Sea. Fish. Res. 33, 123–129.

Green, B.S., McCormick, M.I., 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Mar. Ecol. Prog. Ser. 289, 263–272.

Gunn, J.S., Thresher, R.E., 1991. Viviparity and the reproductive ecology of clinid fishes (Clinidae) from temperate Australian waters. Env. Biol. Fishes 31, 323-344.

Hovenkamp, F., Witte, J.IJ., 1991. Growth, otolith growth and RNA/DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987 to 1989. Mar. Ecol. Prog. Ser. 70, 105-116.

Kerambrun, E., Henry, F., Perrichon, P., Courcot, L., Meziane, T., Spilmont, N., Amara, R., 2012. Growth and condition indices of juvenile turbot, *Scophthalmus maximus*, exposed to contaminated sediments: Effects of metallic and organic compounds. Aquat. Toxicol. 108, 130–40.

Labropoulou, M., Papaconstantinou, C., 2000. Comparison of otolith growth and somatic growth in two macrourid fishes. Fish. Res. 46, 177–188.

Lloret, J., Planes, S., 2003. Condition, feeding and reproductive potential of white seabream *Diplodus sargus* as indicators of habitat quality and the effect of reserve

protection in the northwestern Mediterranean. Mar. Ecol. Prog. Ser. 248, 197-208.

Lopes, C.A.M.V., 2004. Estudo dos Otólitos de juvenis de Robalo, *Dicentrarchus labrax* (LINNAEUS, 1758) da Ria de Aveiro: relações alométricas, microincrementos e relação com variações ambientais. Dissertação apresentada ao Instituto de Ciências Biomédicas de Abel Salazar para obtenção do grau de Mestre em Ciências do Mar-Recursos Marinhos, Biologia Marinha.

Loy, A., Mariani, L., Bertelletti, M., Tunesi, L., 1998. Visualizing allometry: Geometric morphometrics in the study of shape changes in the early stages of the two-banded sea bream, *Diplodus vulgaris* (Perciformes, Sparidae). J. Morphol. 237, 137-146.

Loy, A., Bertelletti, M., Costa, C., Ferlin, L., Cataudella, S., 2001. Shape changes and growth trajectories in the early stages of three species of the genus *Diplodus* (Perciformes, Sparidae). J. Morphol. 250, 24-33.

Macpherson, E., Raventos, N., 2005. Settlement patterns and post-settlement survival in two Mediterranean littoral fishes: influences of early-life traits and environmental variables. Mar. Biol. 148, 167-177.

Macpherson, E., Biagi, F., Francour, P., García-Rubies, A., Harmelin, J., Harmelin-Vivien, M., Jouvenel, J.Y., Planes, S., Vigliola, L., Tunesi, L., 1997. Mortality of juvenile fishes of the genus *Diplodus* in protected and unprotected areas in the western Mediterranean Sea. Mar. Ecol. Prog. Ser. 160, 135-147.

Malloy, K.D., Targett, T.E., 1994. The use of RNA:DNA ratios to predict growth limitation of juvenile summer flounder (*Paralichthys dentatus*) from Delaware and North Carolina estuaries. Mar. Biol. 118, 367-375.

Martinho, F., van der Veer, H.W., Cabral, H.N., Pardal, M.A., 2013. Juvenile nursery colonization patterns for the European flounder (*Platichthys flesus*): A latitudinal approach. J. Sea Res. 84, 61–69.

Macpherson, E., 1998. Ontogenetic shifts in habitat use and aggregation in juvenile sparid fishes. J. Exp. Mar. Biol. Ecol. 220, 127-150.

McPherson, L.R., Slotte, A., Kvamme, C., Meier, S., Marshall, C.T., 2011. Inconsistencies in measurement of fish condition: a comparison of four indices of fat reserves for Atlantic herring (*Clupea harengus*). ICES J. Mar. Sci. 68(1), 52-60.

Morales-Nin, B., 2001. Mediterranean deep-water fish age determination and age validation: the state of the art. Fish. Res. 51, 377-383.

Morales-Nin, B., Palomera, I., Busquets, X., 2002. A first attempt at determining larval growth in three Antarctic fish from otoliths and RNA/DNA ratios. Polar Biol. 25, 360-365.

Nash, R.D.M., Valencia, A.H., Geffen, A.J., 2006. The Origin of Fulton's Condition Factor - Setting the Record Straight. Fish. 31(5), 236-238.

Neahr, T.A., Stunz, G.W., Minello, T.J., 2010. Habitat use patterns of newly settled spotted seatrout in estuaries of the north-western Gulf of Mexico. Fish. Manag. Ecol. 17, 404-413.

Ozório, R.O.A., Valente, L.M.P., Correia, S., Pousão-Ferreira, P., Damasceno-Oliveira, A., Escórcio, C., Oliva-Teles, A., 2009. Protein requirement for maintenance and maximum growth of two-banded seabream (*Diplodus vulgaris*) juveniles. Aquac. Nut. 15, 85-93.

Pajuelo, J.G., Lorenzo, J.M., 2002. Growth and age estimation of *Diplodus sargus cadenati*

(Sparidae) off the Canary Islands. Fish. Res. 59, 93-100.

Pauly, D., Alder, J., Bennett, E., Christensen, V., Tyedmers, P., Watson, R., 2003. The Future for Fisheries. Sci. 302, 1359-1361.

Radtke, R.L., 1984. Cod fish otoliths: information storage structures. Flødevigen rapportser 1, 273-298.

Ribeiro, J., Bentes, L., Coelho, R., Gonçalves, J.M.S., Lino, P.G., Monteiro, P., Erzini, K., 2006. Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). Estuar. Coast. Shelf Sci. 67, 461-474.

Ribeiro, J., Monteiro, C.C., Monteiro, P., Bentes, L., Coelho, R., Gonçalves, J.M.S., Lino, P.G., Erzini, K., 2008. Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. Est. Coast. Shelf Sci. 76, 57-68.

Richter, H., Lückstädt, C., Focken, U.L., Becker, K., 2000. An improved procedure to assess fish condition on the basis of length-weight relationships. Arch. Fish. Mar. Res. 48(3), 226-235.

Rossi-Wongtschowski, C.L.D.B., Clemmesen, C., Ueberschär, B., Dias, J.F., 2003. Larval condition and growth of *Sardinella brasiliensis* (Steindachner, 1879): preliminary results from laboratory studies. Sci. Mar. 67(1), 13-23.

Saoud, I.P., Batal, M., Ghanawi, J., Lebbos, N., 2008. Seasonal evaluation of nutritional benefits of two fish species in the eastern Mediterranean Sea. Int. J. Food Sci. Tech. 43, 538-542.

Searcy, S.P., Sponaugle, S., 2000. Variable larval growth in a coral reef fish. Mar. Ecol. Prog. Ser. 206, 213-226.

Sissenwine, M.M., Mace, P.M., Lassen, H.J., 2014. Preventing overfishing: evolving approaches and emerging challenges. ICES J. Mar. Sci. 71(2), 153-156.

Stevenson, R.D., Woods Jr., W.A., 2006. Condition indices for conservation: new uses for evolving tools. Integ. Comp. Biol. 46(6), 1169-1190.

Stewart, W.T., n.d. Otolith aging and analysis. Computational Bioscience Program. Arizona State University, 1-31.

Suthers, I.M., 1998. Bigger? Fatter? Or is faster growth better? Considerations on condition in larval and juvenile coral-reef fish. Aust. J. Ecol. 23, 265-273.

Taieb, A.H., Ghorbel, M., Hamida, N.B.H., Jarboui, O., 2012. Reproductive biology, age and growth of the two-banded seabream *Diplodus vulgaris* (Pisces: Sparidae) in the Gulf of Gabès, Tunisia. J. Mar. Biol. Assoc. U.K. 1-7.

Vasconcelos, R.P., Reis-Santos, P., Fonseca, V., Maia, A., Ruano, M., França, S., Vinagre, C., Costa, M.J., Cabral, H.N., 2007a. Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: A multi-metric index and conceptual approach. Sci. Tot. Env. 374(2-3), 199-215.

Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Fonseca, V., Latkoczy, C., Günther, D., Costa, M.J., Cabral, H., 2007b. Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Mar. Ecol. Prog. Ser. 350, 117-126.

Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Maia, A., Latkoczy, C., Günther, D., Costa, M.J., Cabral, H., 2008. Evidence of estuarine nursery origin of five coastal fish species along the Portuguese coast through otolith elemental fingerprints. Estuar. Coast. Shelf Sci. 79, 317-327.

Vasconcelos, R.P., Reis-Santos, P., Fonseca, V., Ruano, M., Tanner, S., Costa, M.J., Cabral, H.N., 2009. Juvenile fish condition in estuarine nurseries along the Portuguese coast. Estuar. Coast. Shelf Sci. 82(1), 128-138.

Vasconcelos, R.P., Reis-Santos, P., Maia, A., Fonseca, V., França, S., Wouters, N., Costa, M.J., Cabral, H.N., 2010. Nursery use patterns of commercially important marine fish species in estuarine systems along the Portuguese coast. Estuar. Coast. Shelf Sci. 86, 613-624.

Victor, B.C., Brothers, E.B., 1982. Age and growth of the fallfish *Semotilus corporalis* with daily otolith increments as a method of annulus verification. Can. J. Zool. 60, 2543-2550.

Vigliola, L., 1997. Validation of daily increment formation in otoliths for three *Diplodus* species in the Mediterranean sea. J. Fish Biol. 51, 349-360.

Vigliola, L., Harmelin-Vivien, M.L., Biagi, F., Galzin, R., Garcia-Rubies, A., Harmelin, JG., Jouvenel, JY., Le Direach-Boursier, L., Macpherson, E., Tunesi, L., 1998. Spatial and temporal patterns of settlement among sparid fishes of the genus *Diplodus* in the northwestern Mediterranean. Mar. Ecol. Prog. Ser. 168, 45-56.

Vigliola, L., Harmelin-Vivien, M., Meekan, M.G., 2000. Comparison of techniques of back-calculation of growth and settlement marks from the otoliths of three species of *Diplodus* from the Mediterranean Sea. Can. J. Fish. Aquat. Sci. 57, 1291-1299.

Vigliola, L., Doherty, P.J., Meekan, M.G., Drown, D.M., Jones, M.E., Barber, P.H., 2007. Genetic identity determines risk of post-settlement mortality of a marine fish. Ecol. 88(5), 1263-1277.

CHAPTER 3

Final remarks

The objective of this dissertation was to assess and compare the condition of juvenile common two-banded sea bream *Diplodus vulgaris* in nursery areas along the Portuguese coast, using different individual condition indices.

Estuaries and coastal lagoons are known to be used by several species of marine fish, particularly as juveniles (Beck *et al*., 2001; Able, 2005). In these areas, individuals find high food availability, suitable water temperature and lower number of predators, which permit them to grow more safely and faster until reaching adequate size to join adult populations (Biagi *et al*., 1988; Beck *et al*., 2001). Understanding which areas provide better habitat quality and which factors allow better individual condition of fish has become fundamental for an efficient management and conservation of marine fish populations (Amara *et al*., 2007; Vasconcelos *et al*., 2007; Neahr *et al*., 2010).

In this study, different individual condition indices were used to assess and compare fish condition and also to better understand relationships between them. In this way, recent growth index (width of the 10 last daily increments of otoliths), initial growth index (width of the 60 first daily increments of otoliths), biochemical RNA:DNA ratio and morphometric Fulton's K were determined in juveniles.

Diplodus vulgaris have a distribution in east of Atlantic ocean from Bay of Biscay to Senegal (Loy *et al*., 1998; Gonçalves *et al*., 2003; Correia *et al*., 2011). This species prefers warmer and higher salinity waters (see Reis-Santos *et al*., 2008; Vasconcelos *et al*., 2010) and live in rocky and sandy substrata (Gonçalves *et al*., 2003; Correia *et al*., 2011; Taieb *et al*., 2012). Individuals feed mainly on algae, particularly in seagrass beds, although benthic macro invertebrates are also prey for these species (Ribeiro *et al*., 2006; see Reis-Santos *et al*., 2008; Ribeiro *et al*., 2008). In agreement, results showed that southern estuaries of Portuguese coast, namely Sado, Ria Formosa and Guadiana, presented higher habitat quality for this species since, in a general way, better individual condition assessed by recent growth' index, initial growth index and condition factor K' were observed in these systems in both months (May and July). However, in contrast with these three indices, although RNA:DNA' ratio is a short term condition index that reflects immediate individual condition status according with environmental variables (Buckley, 1984; Gilliers *et al*., 2004; Vasconcelos *et al*., 2009), it can be less indicated to provide a more integrative view of habitat quality of nursery areas where marine fish live (Gilliers *et al*., 2004; De Raedemaecker *et al*., 2012).

As revealed with multiple regression analysis, three of the analysed condition indices (recent growth' index, RNA:DNA' ratio and condition factor K') were significantly related with water temperature, percentage of mud in the sediment and density of benthic macro invertebrates. So, it seems that percentage of mud in the sediment also plays an important role in growth and condition of juvenile *Diplodus vulgaris*. One explication for negative influence of percentage of mud in the sediment on individual condition of *D. vulgaris* assessed by RNA:DNA' ratio and condition factor K' is that juveniles of *D. vulgaris* prefer rocky and sandy bottoms to settle and live (Gonçalves *et al*., 2003), and therefore this species could have more difficulty to find suitable food in sediment with higher percentage of mud. Consequently, individual condition assessed by indices that are more significantly positively related with availability of food, like RNA:DNA' ratio and condition factor K', would be lower. However, it is also possible that food availability was not perfect assessed, since there were missing data for one of the sites and food availability was only characterized in terms of benthic macro invertebrates, and availability of algae was not considered in study although they are important prey. In this study, a temporal discrepancy between condition indices considered was also shown which was already documented in other studies (Rossi-Wongtschowski *et al*., 2003; Gilliers *et al*., 2004; De Raedemaecker *et al*., 2012). This temporal discrepancy was evidenced by distinct patterns between condition indices and environmental variables. RNA:DNA' ratio showed to be related with four environmental variables of study (latitude, water temperature, percentage of mud in the sediment and density of benthic macro invertebrates) and consequently gave the most recent condition of fish before capture. For this reason, RNA:DNA' ratio could be considered a suitable condition index to immediately link individual condition with environmental variables that will provide higher habitat quality for individuals. Both recent growth' index and condition factor K' were related with three environmental variables (water temperature, percentage of mud in the sediment and density of benthic macro invertebrates) which indicates that these indices assess condition of individuals in a previous period that the one assessed by RNA:DNA' ratio, although their responses are not equivalent. Finally, initial growth index showed to be related with only one of the environmental variables (latitude) and thus estimate an individual condition prior to that given by the other condition indices mentioned above. The link between the environment and condition of individuals depends on the condition index considered.

Furthermore, lack of correlation between different condition indices (condition estimated by analysis of otolith microstructure, RNA:DNA' ratio and condition factor K') in addition to the temporal discrepancy showed could indicate a temporal difference between environmental factors and response given by individual condition of fish (Campana, 1999; Rossi-Wongtschowski *et al*., 2003). In this way, biochemical RNA:DNA' ratio presents the shortest time between influence of external factors of fish habitat and their individual condition (Buckley, 1984; Gilliers *et al*., 2004). Condition factor K' and recent growth' index show a longer delay between effect of environmental variables and condition of individuals (Gilliers *et al.*, 2004; Caldarone *et al.*, 2012; De Raedemaecker *et al.*, 2012). And initial growth index shows condition of fish as influenced by environmental factors at their early stage of life and probably also by individual condition descendant from maternal effects (size of egg and energy reserves) (Clemmesen and Doan, 1996; Campana, 1999). It is known that growth of otolith is influenced by both internal and external factors (Campana, 1984; Campana and Neilson, 1985; Lopes, 2004), and the time lag between effect of environmental variables and condition of individuals should be longer than just a few days (Campana, 1999). Condition factor K' (or Fulton's K) index should also show a delay bigger than a few days (Caldarone *et al.*, 2012), since is based on the relationship between weight and length of fish. In accordance with this, it is possible that condition factor K' and condition estimated by analysis of otolith microstructure give a longer-term view of individual condition, since it takes more time to reflect the effects of environmental factors on individual condition, comparatively to RNA:DNA ratio index that is a biochemical index that more rapidly changes with environmental conditions (Campana, 1999; Rossi-Wongtschowski *et al.*, 2003). In this way, caution should also been taken in choosing indices of fish condition and at which biological level (biochemical level, cellular level, organ level, system level, or of all of organism level), considering that the effects of environmental variables are more rapidly reflected in individual condition assessed by condition indices that assess lower levels of organization of animals.

In future, more studies should be carried out to better understand relationships between different condition indices, from biochemical to morphometric, including condition indices based in analysis of otolith microstructure, as recent growth and initial growth indices. It would be also important to better study mechanisms underlying different response times of individual condition indices. Temporal discrepancy between environmental variables and organism response given by individual condition indices could be the reason why different condition indices were not significantly related in this study, with exception for the two indices estimated by otolith growth. On the other hand, it would be also important to further study the extent to which individual condition in initial stage of life influence subsequent life stages of marine fish.

Further studies on habitat quality and distribution patterns of juveniles should allow a deeper knowledge of importance and function of nursery areas for marine fish populations. Given the increasing need to understand population dynamic of marine fish, and also aiming at an efficient management and conservation of natural populations, focus should be given to the link between individual condition of marine fish and habitat quality of their nursery areas.

Literature cited

Able, K.W., 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. Estuar. Coast. Shelf Sci. 64, 5-17.

Amara, R., Meziane, T., Gilliers, C., Hermel, G., Laffargue, P., 2007. Growth and condition indices in juvenile sole *Solea solea* measured to assess the quality of essential fish habitat. Mar. Ecol. Prog. Ser. 351, 201-208.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51(8), 633-641.

Biagi, F., Gambaccini, S., Zazzetta, M., 1998. Settlement and recruitment in fishes: The role of coastal areas. Ital. J. Zool. 65(1), 269-274.

Buckley, L.J., 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80, 291-298.

Calderone, E.M., MacLean, S.A., Sharack, B. 2012. Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (*Salmo salar*) to food availability. Fish. Bull. 110, 257-270.

Campana, S.E., 1984. Lunar cycles of otolith growth in the juvenile starry flounder *Platichthys stellatus*. Mar. Biol. 80, 239-246.

Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Eco. Prog. Ser. 188, 263-297.

Campana, S.E., Neilson, J.D., 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42, 1014-1032.

Clemmesen, C., Doan, T., 1996. Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. Mar. Ecol. Prog. Ser. 138, 33-39.

Correia, A.T., Pipa, T., Gonçalves, J.M.S., Erzini, K., Hamer, P.A., 2011. Insights into population structure of *Diplodus vulgaris* along the SW Portuguese coast from otolith elemental signatures. Fish. Res. 111, 82-91.

De Raedemaecker, F., Brophy, D., O'Connor, I., O'Neill, B., 2012. Dependence of RNA:DNA ratios and Fulton's K condition indices on environmental characteristics of plaice and dab nursery grounds. Estuar. Coast. Shelf Sci. 98, 60–70.

Gilliers, C., Amara, R., Bergeron, J., Le Pape, O., 2004. Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. Env. Biol. Fishes 71, 189-198.

Gonçalves, J.M.S., Bentes, L., Coelho, R., Correia, C., Lino, P.G., Monteiro, C.C., Ribeiro, J., Erzini, K., 2003. Age and growth, maturity, mortality and yield-per-recruit for two-banded bream (*Diplodus vulgaris* Geoffr.) from the south coast of Portugal. Fish. Res. 62, 349-359.

Lopes, C.A.M.V., 2004. Estudo dos Otólitos de juvenis de Robalo, *Dicentrarchus labrax* (LINNAEUS, 1758) da Ria de Aveiro: relações alométricas, microincrementos e relação com variações ambientais. Dissertação apresentada ao Instituto de Ciências Biomédicas de Abel Salazar para obtenção do grau de Mestre em Ciências do Mar-Recursos Marinhos, Biologia Marinha.

Loy, A., Mariani, L., Bertelletti, M., Tunesi, L., 1998. Visualizing allometry: Geometric morphometrics in the study of shape changes in the early stages of the two-banded sea bream, *Diplodus vulgaris* (Perciformes, Sparidae). J. Morphol. 237, 137-146.

Neahr, T.A., Stunz, G.W., Minello, T.J., 2010. Habitat use patterns of newly settled spotted seatrout in estuaries of the north-western Gulf of Mexico. Fish. Manag. Ecol. 17, 404-413.

Reis-Santos, P., Vasconcelos, R.P., Ruano, M., Latkoczy, C., Günther, D., Costa, M.J., Cabral, H.N., 2008. Inter-specific variations of otolith chemistry in estuarine fish nurseries. J. Fish Biol. 72(10), 2595-2614.

Ribeiro, J., Bentes, L., Coelho, R., Gonçalves, J.M.S., Lino, P.G., Monteiro, P., Erzini, K., 2006. Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). Estuar. Coast. Shelf Sci. 67, 461-474.

Ribeiro, J., Monteiro, C.C., Monteiro, P., Bentes, L., Coelho, R., Gonçalves, J.M.S., Lino, P.G., Erzini, K., 2008. Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. Est. Coast. Shelf Sci. 76, 57-68.

Rossi-Wongtschowski, C.L.D.B., Clemmesen, C., Ueberschär, B., Dias, J.F., 2003. Larval condition and growth of *Sardinella brasiliensis* (Steindachner, 1879): preliminary results from laboratory studies. Sci. Mar. 67(1), 13-23.

Taieb, A.H., Ghorbel, M., Hamida, N.B.H., Jarboui, O., 2012. Reproductive biology, age and growth of the two-banded seabream *Diplodus vulgaris* (Pisces: Sparidae) in the Gulf of Gabès, Tunisia. J. Mar. Biol. Assoc. U.K. 1-7.

Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Fonseca, V., Latkoczy, C., Günther, D., Costa, M.J., Cabral, H., 2007. Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Mar. Ecol. Prog. Ser. 350, 117-126.

Vasconcelos, R.P., Reis-Santos, P., Fonseca, V., Ruano, M., Tanner, S., Costa, M.J., Cabral, H.N., 2009. Juvenile fish condition in estuarine nurseries along the Portuguese coast. Estuar. Coast. Shelf Sci. 82(1), 128-138.

CHAPTER 3

Vasconcelos, R.P., Reis-Santos, P., Maia, A., Fonseca, V., França, S., Wouters, N., Costa, M.J., Cabral, H.N., 2010. Nursery use patterns of commercially important marine fish species in estuarine systems along the Portuguese coast. Estuar. Coast. Shelf Sci. 86, 613-624.