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Doutoramento em Biologia
Especialidade Biologia Marinha e Aquacultura
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Tese orientada pela Professora Doutora Maria José Rosado Costa
e pela Professora Doutora Cynthia M. Jones

Tese especialmente elaborada para obtenção do grau de doutor

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Lisboa, 11 de Dezembro de 2013,

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Por decisão do autor, esta tese não segue as regras do novo acordo ortográfico.

Abstract

Life history parameters of valuable marine fish remain poorly studied worldwide. The meagre (*Argyrosomus regius*) is a large sciaenid from European and North African waters that, on its European range, supports regional small-scale and recreational fisheries and is considered a promising candidate species for aquaculture. However, its fisheries and ecology have remained poorly documented. The present work describes (1) progress made on raising scientific and societal awareness on this fish resource, (2) research carried out on the Portuguese fisheries targeting meagre and on the main biological parameters of the species in Portuguese waters. The study of fish remains from archaeological sites showed that both adult and juvenile meagre have been fished in Portuguese estuarine and coastal environments since, at least, the Mesolithic period. Today, two main commercial fisheries on meagre exist: one that targets meagre on the Western coast (within the Tagus estuary and off Peniche); and the other that captures the fish as by-catch in the Southeastern coast of Portugal. Using a new sampling methodology (commercial mark-recapture) a comprehensive set of otoliths and gonads was collected. Analyses of these samples showed that meagre is long-lived (up to 43 years old), displays fast juvenile growth and is reproductively active in spring and summer both in estuaries (Tagus and Guadiana) and adjoining coasts. Furthermore, it shows that both meagre males and meagre females display some signs of precocious maturity and that the meagre females are asynchronous batch spawners that likely have indeterminate fecundity. Moreover, microsatellite work showed that meagre populations in Europe and North Africa are highly fragmented. Finally, a statistical time series methodology is presented (SARIMA) that uses landings under a process control perspective to provided baseline monitoring to fisheries resources currently found in data-poor situation.

Keywords: Meagre; *Argyrosomus regius*; fisheries; ecology; life-history; Portugal

Resumo Alargado

A corvina-legítima, *Argyrosomus regius* (Asso, 1801), é um peixe ósseo pertencente à família Sciaenidae. De entre as cinco espécies de Sciaenidae existentes em Portugal, a corvina-legítima é a que atinge maior dimensão, maiores desembarques e valor comercial mais significativo em águas europeias. Por outro lado, a corvina-legítima é uma das espécies consideradas como promissoras para a diversificação e dinamização da aquicultura europeia. Apesar da sua dimensão física e importância económica, a biologia, ecologia e pesca da corvina-legítima em águas europeias encontravam-se à data de início dos trabalhos conducentes à presente tese pouco estudadas, conduzindo à categorização da corvina como espécie "pobre(s) em dados". Esta situação levantava dificuldades consideráveis na gestão pesqueira do recurso, na exploração do seu potencial aquícola, e sobretudo impedia uma apreciação correcta da importância pesqueira e ecológica do recurso em águas nacionais e europeias.

A presente tese, composta por 7 capítulos, pretende dar resposta a algumas das deficiências de informação científica anteriormente mencionadas. Cada capítulo integra uma ou mais publicações científicas. Os capítulos são precedidos por uma introdução geral e sucedidos por uma discussão geral que integram, respectivamente, o trabalho a realizar e realizado, no conhecimento científico existente. Na escrita da tese optou-se pela língua inglesa como forma de operacionalizar a comunicação com uma das orientadoras dos trabalhos e de permitir mais ampla divulgação dos seus resultados junto da comunidade científica internacional bem como de potenciais leitores da área geográfica abrangida pela espécie. Tendo isto em conta, foram objectivos dos trabalhos constantes nesta tese: (1) contribuir para uma elevação da percepção da importância social e científica assumida pela corvina-legítima em águas nacionais e europeias; (2) contribuir para a elevação do recurso e suas pescarias da sua situação de "pobre(s) em dados" para uma situação mais consentânea com a importância pesqueira e ecológica que possui e o potencial económico que a sua produção aquícola exibe; e (3) executar uma primeira apreciação do estado do manancial de corvina-legítima existente em águas nacionais e sugerir algumas medidas para o melhoramento da sua gestão.

A corvina-legítima é um recurso explorado pelas comunidades humanas desde a pré-história. Em alguns depósitos arqueológicos datados do Mesolítico foram encontrados otólitos e vértebras de espécies de várias espécies de peixe, incluindo corvina-legítima. Recorrendo a análises de regressão linear estabelecidas com base em otólitos e vértebras de espécimes contemporâneos, estimou-se o número mínimo de indivíduos (e os seus tamanhos) presentes em quatro sítios arqueológicos localizados na região costeira do centro e sul do país. A análise revelou que a corvina-legítima é uma das espécies mais

representadas nesses sítios sugerindo elevada importância da espécie para subsistência das comunidades humanas do mesolítico. Em paralelo, as análises de regressão evidenciaram que, à semelhança das comunidades piscatórias actuais, as comunidades mesolíticas capturavam tanto indivíduos juvenis como indivíduos adultos.

A identificação e caracterização espacio-temporal das pescarias é um elemento essencial à percepção da importância económica dos recursos e à gestão pesqueira. Espécies como a corvina-legítima são particularmente difíceis de amostrar de forma representativa em cruzeiros científicos o que leva a que a caracterização espacio-temporal das pescarias e a identificação dos meses e locais em que é exercido esforço de pesca dirigido a esta espécie se constituam como fontes de primordiais de informação ecológica. Usando uma combinação de análises de séries temporais e estatística descritiva, analisou-se a distribuição espacial e temporal das pescarias de corvina existentes na costa portuguesa, verificando-se a existência de uma sazonalidade bem marcada e de pesca dirigida sobre adultos e juvenis no Estuário do Tejo (entre Maio e Setembro), sobre juvenis na zona costeira adjacente a este estuário (entre Setembro e Novembro), e a existência de uma pescaria significativa, mas acessória, sobre os adultos desta espécie entre Junho e Novembro na costa sueste do país. Em paralelo, as análises realizadas corroboraram a existência de migrações de adultos e juvenis entre a zona costeira e o Estuário do Tejo no final da Primavera e início do Verão, o retorno dos juvenis às zonas costeiras adjacentes durante o Outono, bem como movimentações ao longo da costa algarvia de adultos em estado pré-reprodutor (no início da Primavera) e reprodutor (no Verão).

Uma das principais dificuldades sentidas no estudo biológico de peixes marinhos de grande dimensão e valor comercial reside na própria obtenção de amostras biológicas. No presente trabalho foi desenvolvida e aplicada uma nova metodologia de amostragem - a marcação e recaptura comercial (MRC) – que permitiu obter amostras de corvina a custos mais comportáveis. A MRC consiste na colocação de marcas em indivíduos desembarcados em lota que são posteriormente recuperadas conjuntamente com vísceras e otólitos junto de intervenientes do circuito comercial. A aplicação desta metodologia à corvina-legítima capturada no Sul de Portugal demonstrou que a MRC é altamente eficaz, permitindo recolher elevado número de amostras biológicas representativas da população capturada comercialmente. O sucesso obtido na aplicação da MRC à corvina-legítima torna expectável que esta metodologia venha a ser aplicada futuramente noutras espécies de elevada dimensão e valor comercial valor contribuindo decisivamente para o melhoramento das informação biológica sobre elas disponível.

Um bom conhecimento da idade e crescimento e da reprodução dos peixes é essencial à correcta gestão pesqueira e ao aprofundamento dos estudos ecológicos. No âmbito desta tese foi elaborado e publicado um protocolo que permite a determinação de idade em corvina-legítima a partir de secções finas de otólitos ou de impressões de escamas em acetato. Este protocolo tem sido utilizado nas leituras de idade realizadas em Portugal, Espanha e França, permitindo a comparação das estimativas de crescimento da espécie e

comprovar, desde já o seu rápido crescimento e a elevada longevidade em águas nacionais (idade máxima registada: 43 anos). Em paralelo, foram desenvolvidas e validadas histologicamente escalas macroscópicas de maturação de machos e fêmeas de corvina-legítima, e realizado o estudo do seu desenvolvimento ovárico e oocitário e do seu padrão de fecundidade. A análise histológica revelou a existência de alguns indícios de maturação precoce nos machos e fêmeas desta espécie que importa ter em conta na análise da sua dinâmica reprodutiva e na estimativa de ogivas de maturação. Como consequência destes estudos é hoje possível afirmar que a corvina-legítima possui desenvolvimento ovárico assíncrono caraterizado por posturas múltiplas e parciais e fecundidade indeterminada e que os Estuários do Tejo e Guadiana e as zonas costeiras adjacentes são zonas de reprodução desta espécie entre Abril e Agosto.

O conhecimento da estrutura espacial dos mananciais pesqueiros é interessante do ponto de vista evolutivo e importante para o correcto aconselhamento pesqueiro. Um estudo genético envolvendo 11 microsatélites e realizado com amostras das zonas Oeste e Sul de Portugal e de 10 outras zonas geográficas de cinco países (França, Mauritânia, Espanha, Egipto e Turquia) indicou a existência de, pelo menos, duas regiões com populações de corvina-legítima distintas: a região Mediterrânica e a região Atlântica. Por outro lado, verificou-se que a corvina-legítima exibe um nível de diferenciação genética consideravelmente elevado quando comparado com o nível de outros peixes marinhos. Estes resultados encontram justificação provável em barreiras geográficas, em pulsos de glaciação, e na existência de um número relativamente limitado de locais favoráveis à reprodução e crescimento da espécie. Em paralelo, foram detectados indícios de separação entre o manancial de corvina-legítima existente da costa ocidental portuguesa e o manancial de corvina da costa sul e Golfo de Cádiz. A possível existência na costa portuguesa de fragmentação populacional ao nível de um recurso pesqueiro com elevado potencial migratório coloca dificuldades consideráveis à gestão do recurso, pelo que é de aconselhar o aprofundamento dos estudos antes de tomadas de decisão de gestão desta espécie.

A larga maioria dos recursos pesqueiros actualmente explorados são, tal como a corvina-legítima, "pobres em dados". Esta situação torna praticamente impossível a sua avaliação analítica com os modelos matemáticos sofisticados usados nas pescarias industriais. O último capítulo da presente tese apresenta uma metodologia de séries temporais (SARIMA) cujas previsões, quando perspectivadas do ponto de vista do controlo estatístico de processos, podem ser utilizadas na monitorização de recursos que actualmente não são alvo de avaliação. A corvina-legítima é utilizada como caso de estudo e os resultados obtidos indicam que os desembarques da espécie no estuário do Tejo se encontram dentro do esperado pela variabilidade natural não evidenciando motivos para alarme.

Palavras chave: Corvina-legítima; *Argyrosomus regius*; pesca; ecologia; ciclo de vida; Portugal

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Introduction

General Introduction

Importance and Aims

Thesis Outline

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1. General Introduction

Fisheries research and management have traditionally focused on resources with the greatest landings and revenues (Scandol, 2005; Vasconcellos and Cochrane, 2005). Such species and stocks are generally termed as “commercially important” and have the available funds and expertise required to obtain a nearly complete understanding of their biology, ecology and fisheries. However, that is not the case for the vast majority of fish species worldwide, which are seriously data-deficient and remain subjected to limited (if any) assessment and management (Mahon, 1997; Vasconcellos and Cochrane, 2005; Costello *et al.*, 2012). The latter have been collectively termed "data-poor fisheries" (or “data-deficient fisheries”) and their target resources as “data-poor species” or "data-limited stocks" (Mahon, 1997; Scandol, 2005). Among the world's data-poorest cases are nearly all fisheries and exploited resources in developing countries but also many industrial fisheries in developed countries (Mahon, 1997; Costello *et al.*, 2012; ICES, 2012). Data deficiencies are, however, more severe for fisheries that are primarily locally or regionally important as and contribute little to national totals of overall capture production and revenues (NMFS, 2011). The meagre and its fisheries in European waters are one such case.

The meagre (*Argyrosomus regius*, Asso 1801) is one of the world’s largest marine teleosts and one of the five species from the Family Sciaenidae present in European waters (Chao, 1986). Its specimens present fast growth, high fecundity and may attain over 180 cm in total length and 50 kg in weight, fetching over 200€ per specimen in ex-vessel price (Quéméner, 2002). These characteristics make the meagre particularly valuable for small-scale commercial fishers and recreational fishers and add potential to its aquaculture development (Quéméner, 2002). However, the life-history characteristics of this species (e.g., longevity, large size and age at maturity, large variability in annual recruitment, formation of spawning aggregations in coastal waters and estuaries) pose significant management and conservation problems for it making the meagre rank high among the world's most vulnerable species (Cheung *et al.*, 2007).

The biology, ecology and fisheries of the meagre are poorly documented, particularly in European waters, and only recently has interest in aquaculture production, management of artisanal fisheries, and the conservation of data-poor fish resources resulted in some direct scientific research on this species (Quéméner, 2002). The meagre is a coastal fish (<80 m deep) whose current distribution extends from the English Channel to Senegal (including the Eastern Mediterranean Sea and Black Sea) (Griffiths and Heemstra, 1995). North and southof its main

distribution range, the meagre is relatively rare (Quéro and Vayne, 1987). However, in the past the species was present and sometimes abundant in other areas such as the Central and Western Mediterranean and the North Sea (Quéro, 1989; Wolff, 2000). At present, the main meagre fisheries are located in Mauritania, Morocco, and Egypt where over 80% of the *ca.* 10 000 t world annual catch is taken by regional large-scale and small-scale fisheries (FAO, 2012a). In Europe, meagre fisheries are mostly small-scale and take place in very localized areas of the Atlantic coasts of France, Spain and Portugal (Biais, 2002; Silva and Sobrino 2002; Prista *et al.*, 2008). Compared to its North African counterparts, the European fisheries are smaller sized (France: ca. 800 t/year; Spain: ca. 150 t/year, Portugal: ca. 400 t/year) and mostly characterized by large inter-annual variability in landings and fish supply to local markets (Quéro and Vayne, 1987; FAO, 2012a; González-Quirós, 2011; Prista *et al.*, 2011). This variability in fish supply and increase in consumer demand for fish products, alongside the fast growth rate and the good properties of the meat in meagre and putatively good biological properties for growth in captivity (Quémener, 2002) led to a spark in aquaculture production in France during the late nineties (Quémener, 2002; Monfort, 2010). At present, aquaculture production of meagre has expanded to seven other southern European countries, including Portugal, reaching 14 000 t/year and being worth nearly 48 million USD/year (FAO, 2012b).

Until the early 2000s, most studies addressing the meagre life-cycle and fisheries took place in Northern Africa and the Southern Mediterranean (Rafail 1971; Tixerant, 1974; Chakroun and Kthari, 1981; Rizk and Hashem 1981; Chakroun *et al.*, 1982; Garcia, 1982; Chakroun-Marzouk and Kthari, 1989; Hermas, 1995; Bebars *et al.*, 1997). Additional research had been carried out in France but it consisted mostly of landing analysis or synthesis of available knowledge published in grey literature (Oliver and Lafon, 1981; Quéro and Vayne, 1987; Quéro, 1989; Quéro and Vayne, 1993). Consequently, existing biological knowledge on meagre was mostly composed of older data, limited to North African waters, using mostly outdated methodologies (Tixérant, 1974; Quéro and Vayne, 1987; Hermas, 1995). Such data-deficiencies greatly contrasted the situation of other similarly sized sciaenids which fisheries were being increasingly managed and for many of which aquaculture had already developed. That was the case of *A. coronus* and *A. japonicus* in Southern Africa and Australia (Griffiths and Hecht, 1995; Griffiths 1996, 1997a,b; van der Bank and Kirchner, 1997) and *Pogonias cromis*, *Sciaenops ocellatus* and *Atractoscyon nobilis* in North America (e.g., Murphy and Taylor, 1989, 1990; Niedland and Wilson, 1993; Wilson and Niedland, 1994; Donohoe, 1997; Campana and Jones 1998; Jones and Wells, 1998, 2001). However, even the limited studies in France and North African waters did highlight the potential of the meagre for biological research and aquaculture production, and indicated that the meagre as fast-growing, fairly fecund and long-lived. Such studies were reviewed by Quéméner (2002) who highlighted the knowledge gaps, pointed out conservation and management risks and stressed the potential of meagre for aquaculture production in European waters.

The situation European research on meagre faced in the beginning of the 21st century becomes clear when reading Quéméner (2002) work as it contains pratically no information on

the presence of the fish in Iberian waters and no reference to spawning areas other than the Gironde estuary, the Banc D'Arguin and the Nile Delta. However, Quéméner (2002) review higlighted the research gaps that management and aquaculture production faced and drew research attention to the species. As a consequence, an increasing amount of indirect references and directed studies appeared in recent years that have significantly increased the body of knowledge available on meagre. This research, to which the works presented in this thesis have significantly contributed, now includes all southern European coasts, including the Iberian Peninsula, and has addressed both captive stocks (Angelini *et al.*, 2002; Jiménez *et al.*, 2005; El-Shebly *et al.*, 2007; Jiménez *et al.*, 2007; Grigorakis *et al.*, 2011; Duncan *et al.*, 2012; Schiavone *et al.*, 2012; Mylonas *et al.*, 2013) and wild populations (Catalán *et al.*, 2006; Morales-Nin *et al.*, 2010; González-Quirós *et al.*, 2011, Morales-Nin *et al.*, 2012, *Gil et al.*, 2013) covering all the major aspects of the species' life-history.

2. Importance and Aims

At the start of the field work that led to this thesis no directed study on the meagre biology and life-cycle had been carried out in European waters. This situation restricted the appraisal of this species for aquaculture production and did not allow a full appreciation of its conservation risks and the need to manage its fisheries. A literature review carried out at the time indicated that in Portugal evidence on meagre abundance and distribution was largely restricted to historical literature (e.g., Baldaque da Silva, 1891), presence/absence records in species lists (e.g., Costa and Bruxelas, 1989; Bexiga 2002; Chícharo *et al.*, 2006; Gonçalves *et al.*, 2007) and very fragmented ecologic and fisheries research (e.g., Cabral and Ohmert, 2001; Cabral *et al.*, 2001; Dias *et al.*, 2001; Santos, Saldanha, and Garcia, 2002; Santos, Gaspar *et al.*, 2002). This literature provided only minimal information on meagre fisheries, ecology and biology. Simple manifestations of the scientific, ecologic and economic impact of such data-limited situation are exemplified in three situations that took place during the early 2000's. For example, there was considerable scientific surprise when in 2000–2002 abnormally high biomass of meagre juveniles was detected in research surveys taking place in the Tagus estuary (Costa and Bruxelas, 1989; Costa *et al.*, 2005) because at the time it was not known that the species spawned in Portuguese waters and the reproduction of marine species in estuarine was in general deemed unlikely. Concurrently, considerable legislative changes took place in 2001 and 2002 (Portaria 27/2001 de 15 de Janeiro; Portaria 402/2002, de 18 de Abril) that resulted in a 18 cm decrease in the minimum landing size of meagre without any known record of reproduction or growth studies of this fish. Finally, there was a generalized lack of interest for aquaculture production of this fast native species despite the fact that production was already taking place and proving profitable in other southern European regions (Quéméner, 2002; Costa *et al.*, 2008).

Within this context, the objectives of the PhD research portrayed in this thesis were to: (1) contribute to scientific and society awareness of the historical and present significance of this

species in Portuguese waters; (2) contribute to a sustained elevation of this European species from a data-poor situation to a more data-rich situation; and (3) carry out a first appraisal on the life-history of the Portuguese meagre and, if necessary, recommend fisheries management actions. Meeting the objective (2) involved: (a) analyzing the available fishery dependent data and extracting the best information possible from it; (b) developing methodologies that could make biological sampling of this species financially possible; and (c) re-evaluating procedures previously used in the analyses of biological data to ensure steps forward were done on solid grounds.

3. Thesis Outline

This thesis narrates scientific progress made on the ecology and fisheries of meagre in Portuguese waters. Overall, the thesis comprises 7 chapters that include 11 scientific publications. These publications are published in international peer-reviewed journals (n=4), in in conference proceedings (n=3), or in national peer-reviewed journals (n=1). One publication has recently been accepted and two are currently being prepared for future publication.

Chapter 1 examines the significance of meagre fishery in pre-historic times by analyzing meagre otoliths collected from Mesolithic sites and estimating the size of fish caught during that period. Chapter 2 analyses modern day meagre fisheries by describing the seasonal and spatial variability of landings made by the small-scale fleet along the Portuguese coast and the size and seasonal structure of the catch from a tuna-trap located in the southeastern coast. The two publications in this chapter explore how fishery dependent data helped to establish the main migratory patterns of the species in Portuguese waters. Chapter 3 deals with the sampling difficulties that have constrained the study of meagre and other large valuable fish and describes a new sampling methodology that allowed the collection of a comprehensive set of otoliths and gonads from the Portuguese fisheries. Chapters 4 and 5 explore these biological samples, establishing guidelines for their correct processing and interpretation and providing new information on the growth and reproduction of the meagre. Chapter 6 analyses the population structure of meagre in European, Mediterranean and North African Waters, showing its high genetic fragmentation and discussing its causes and consequences. Finally, Chapter 7 addresses the worldwide problem of providing data-poor species and fisheries with a minimal degree of monitoring by exploring the use of statistical time-series methodologies to analyze fisheries landings.

This thesis ends with a general discussion that pools together the main conclusions of each chapter and highlights their contribution to science. A final set of remarks includes suggestions for future research on this species and data-poor fisheries in general.

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Chapter 1

Historical Meagre Fisheries

Chapter 1

Estimating meagre (*Argyrosomus regius*) size from otoliths and vertebrae

Sónia Gabriel[a], Nuno Prista[b,c,d], Maria José Costa[b,c]

[a]Laboratório de Arqueociências, IGESPAR, IP. and LARC/CIBIO/InBIO, Rua da Bica do Marquês 2, 1300-087 Lisboa, Portugal
[b]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[d]Present address: Instituto Nacional de Recursos Biológicos (INRB/IPIMAR). Avenida de Brasília, 1449-006 Lisboa (Portugal)

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Estimating meagre (*Argyrosomus regius*) size from otoliths and vertebrae

Abstract: This study presents a method for predicting meagre (*Argyrosomus regius*) body size (total length) from otoliths and vertebrae recovered from archaeological sites. The method involves regression equations calculated from a reference collection of 36 meagre skeletons and 113 meagre otoliths (sagitta) and allows the simultaneous estimation of original body size and minimum number of individuals (MNI) from archaeological bone structures. We selected the following measurements to predict meagre body size: greatest dorso-ventral height, greatest mediolateral breadth, and greatest anteroposterior length of the vertebrae centra; maximum anteroposterior length, medial anteroposterior length, and dorso-ventral height of the sagitta. Our results show that the original body size of meagre can be accurately predicted from many bone measurements (r^2 range: 0.921-0.992). We exemplify the use of the regressions in the assessment of size variation and MNI of meagre from four Portuguese Mesolithic sites. We show that regression results provide additional insight into the significant role that this fish played in the subsistence of coastal fisher-hunter-gatherers, who targeted medium-sized animals but were also capable of acquiring rather larger specimens.

Keywords: Meagre, size estimates, regression analysis, otoliths and vertebrae, *Argyrosomus regius*, Portugal, Mesolithic, aquatic resources

1. Introduction

Aquatic resources played an important role in Holocene cultures. Despite their importance to the economy of past communities, few detailed studies of fish have been undertaken. Because species lists are not informative about animal size and size can vary widely between different fish of the same species (Casteel, 1976; Enghoff, 1989, 1991), studies generally resort to estimates of fish size obtained from present-day fish-size vs. bone-size relationships (Zohar *et al.*, 1997; Smith,1995; Desse and Desse-Berset, 1994; Johnsson, 1994; Enghoff,1983; Bèarez, 2000, Orchard 2003, 2005).

The meagre (*Argyrosomus regius* Asso, 1801) is one of the world's largest sciaenids, attaining over 180 cm in total length and 50 kg in weight (Quéméner, 2002; Costa *et al.* 2008). It is a coastal fish (< 80 m deep) whose distribution extends from the English Channel to Senegal (including the Mediterranean and Black Seas). Today, its largest fisheries are in Mauritania, Morocco, and Egypt (Quéméner, 2002; FAO, 2009) but due to its large size, high ex-vessel price, and high seasonal availability, the meagre still constitutes an important target species for many local small-scale commercial and recreational fleets, particularly those operating in (or near to) large European estuaries (Quéro & Vayne, 1987; Prista *et al.*, 2008).

Meagre remains have been found in many archaeological sites in Portugal (Lentacker, 1986), Spain (Izquierdo & Muñiz, 1990), Greece (Reese *et al*., 2000), eastern Mediterranean (Van Neer *et al*., 2004), Mauritania (Vernet & Tous, 2004), and the North Sea (Wolff, 2000). In some cases, meagre is the most abundant species, dominating fish bone lists. However,

ecological and archaeological interpretations of these data have been hampered by the inexistence of concurrent body size information.

Here, we present a set of relationships between meagre bones (vertebrae), otoliths (sagittae), and fish size (total length = TL) that will allow researchers to overcome these shortcomings. As an example, we use them to carry out a first evaluation of meagre size distribution at four Portuguese Mesolithic sites from the Atlantic period: Cabeço dos Morros (CMRR. Rolão, 1999); Arapouco (ARA. Arnaud, 1987); Poças de São Bento (PSB. Arnaud, 1987; 2000), and Samouqueira-I (SAM-I. Soares, 1995; Lubell, 2007).

2. Materials and methods

2.1. Reference collection

We assembled a reference collection of meagre skeletons and otoliths. The bones were collected between 2002 and 2007 and included vertebrae from 36 fish and otoliths from 113 fish. The entire size range generally found in Portuguese and other European waters ($range_{vert}$ = 14-136 cm; $range_{oto}$ = 12-160 cm) was sampled. Skeletons and otoliths came from fishermen's donations, fish acquired at local markets and concurrent studies on the biology and fishery of present-day meagre in Portuguese waters (Costa *et al*., 2008).

All fish were measured (TL to the lowest cm) and weighed (total weight, 0.001-0.1kg precision). Sagittae were removed and cleaned according to Prista *et al*. (2009). Skeletons were manually defleshed according to Lepiksaar (1989), cleaned in a solution of water and *Neutrase 80L* following (Davis and Payne, 1992), and vertebrae set apart for analysis. Other bones were prepared but we restricted our analysis to vertebrae and sagittae because these are the most frequent meagre remains found in our archaeological sites. Meagre skeletons are available at the IGESPAR's reference collection.

Otoliths: The meagre has a pair of robust and bilaterally symmetric sagittae (Prista *et al.*, 2009). A preliminary analysis of otoliths from *n*=30 fish indicated no significant differences between left and right sagittae so measurements were taken on a randomly chosen *sagitta* from each fish. On each otolith, three measurements were taken to nearest 0.1 mm: maximum anteroposterior length (MAX_OL), medial anteroposterior length (MED_OL), and maximum dorsoventral height (MAX_OH) (Figure 1) (Assis, 2000).

Vertebrae: Similarly, the meagre vertebral column is composed of 25 vertebrae. On each vertebra, three measurements were taken to the nearest 0.01 mm - greatest dorso-ventral height of the centrum (M_1), greatest mediolateral breath of the centrum (M_2), and greatest craniocaudal length of the centrum (M_3) (Figure2) - except on the urostyle where only M_1 and M_2 were measured (Morales & Rosenlund, 1979).

Figure 1. *Argyrosomus regius*' otolith (*sagitta*). Figure indicates otolith measurements: maximum anteroposterior length (OL), and maximum dorso-ventral height (OH).

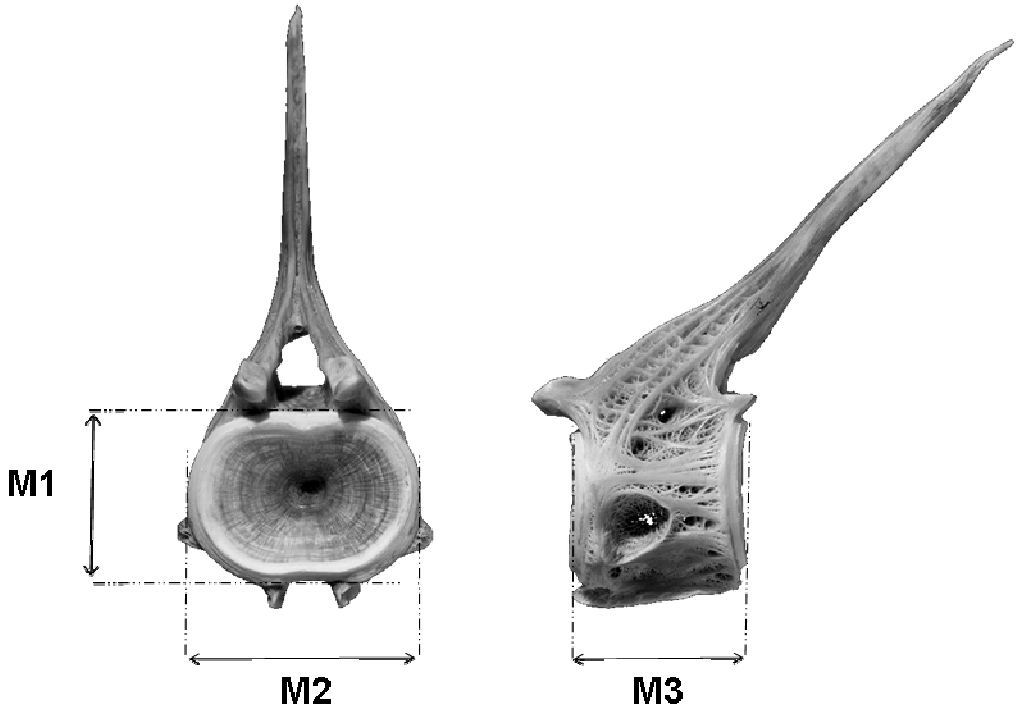

Figure 2. *Argyrosomus regius* vertebrae. Figure indicates vertebral measurements: greatest dorso-ventral height of the centrum (M1), greatest mediolateral breath of the centrum (M2), and greatest craniocaudal length of the centrum (M3).

Since identification of vertebrae position along the spine is a necessary prerequisite for applying the regression equations to archaeological material, we describe the differences between individual vertebrae of meagre.

Identifying individual meagre vertebrae to their position along the spine

The criteria used to identify individual meagre vertebrae include: body size proportions; position, shape and orientation of fossae for the pleural ribs; as well as position, shape and orientation of the hemal arch (Table 1). Additionally, attention was paid to individual variation and (if relevant), size-related morphological changes.

Meagre vertebrae vary anatomically within the species, depending on their position in the vertebral column. Precaudal vertebrae are characterized by the presence of ribs and by the absence of a hemal arch. These vertebrae bear neural zygapophyses for articulation with adjacent elements. The 1st vertebra is smooth on the ventral surface. The 2nd through 5th provide ventral attachment for internal organs (Figure 3).

Table 1. Distinction between individual vertebrae of meagre (*Argyrosomus regius*) based on the examination of 39 modern meagre vertebral spines ($TL_{specimens\ range}$ = 14-136 cm)

V1	Exhibits a short, thick centrum, with a solid neural spine of autogenous origin. Neural spine's bifurcated base articulates with two depressions on the dorsal surface of the centrum. Anterodorsally, the centrum broadens into two condylar surfaces which (with the concave face of the centrum below) form the usual tricondylar attachment to the skull. Its height is larger than its length.
V2	Is marked by an extended pair of prezygapophyses which expand above the posterior end of V1. Laminae bridging these processes to the erect spine give the neural element a sail-like appearance in profile. A pair of fossae accommodates the heads of the first pair of pleural ribs (F). These are placed anterodorsally and about ⅓ of their area reach the laminae that bridge the prezygapophyses and the erect spine. They are oval in shape (Figure 3).
V3	The prezygapophyses expand above the posterior end of V2. Paired dorsolateral fossae (F) of rather large dimension extend to the heads of pleural ribs. These are positioned anterodorsally, the upper outer area reaches the laminae that bridge the prezygapophyses and the erect spine. Fossae show oval shape (Figure 3).
V4	The prezygapophyses expand above the posterior end of V3. Paired dorsolateral fossae of rather large dimension extend to the heads of the pleural ribs (F). These are placed anterodorsally. Fossae are oval-round, occupying about ½ of the centra: the lower edges of the fossae touch the middle of the vertebral boddy (Figure 3).
V5	The prezygapophyses expand above the posterior end of V4. Paired fossae of rather large dimension extend for the heads of pleural ribs (F). In V5 these are placed anteroventrally. Fossae have a 'D' shape, and occupy about ½ of the centra: the upper edge of the fossae touch the middle of the vertebral body (Figure 3).
V6-10	Lack fossae for the heads of pleural ribs. Parapophyses first appear in V6. V9 shows an osseous bridge between the parapophyses (not shown in the picture). By V7, a lateral strengthening ridge appears (R). The strengthening ridge divides the lateral aspect into dorso- and ventrolateral depressions. (Figure 3).
V11-20	The hemapophyses on which the haemal spine is based, originate well ahead of the origin of the neurapophyses which form the base of the neural spine (Figure 3).
V15-19	Vertebrae are ventrally horizontal (h), and dorsally oblique (ho) (Figure 3).
V20	Vertebra is dorsally oblique (ho), and ventrally curved (c) (Figure 3).
V21-23	Vertebrae are dorsally and ventrally curved (c) (Figure 3). The neurapophyses on which the hemal spine is based, originate next to the posterior edge of the centra.
V22-23	The hemapophyses on which the haemal spine is based are placed closer to the posterior end of the centra. The hemapophyses and the haemal spine are adjacent to the centra, narrowing the angle. The angle is wider in V22 (Figure 3).
V23	The hemal spine is autogenous.
V24	The hemal spine is autogenous and compressed.
V25	Centrum is abbreviated and contains the upturned unipartite urostyle.

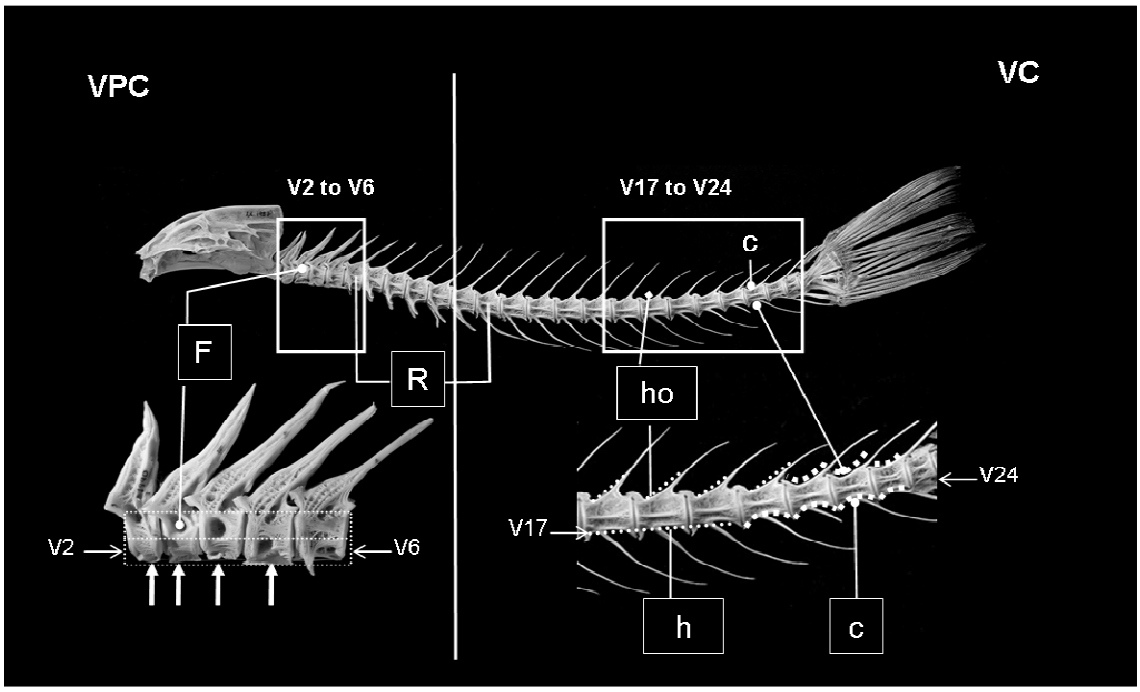

Figure 3. *Argyrosomus regius* brain case (neurocranium) and vertebral column (top). The bottom figures represent the precaudal and caudal zones, homologous to the identified archaeological material. The arrows point the ventral attachment for internal organs. Key: F - fossae for insertion of the heads of the pleural ribs; PRZ – prezygapophyses; R – strenghthening ridge; V – vertebrae; VPC – precaudal vertebrae; VC – caudal vertebrae, c – curved; h – horizontal; ho – oblique.

Except for the first vertebra, the prezygapophyses (PRZ) are strongly developed, showing their maximum size in the 2nd vertebra. The 2nd through 5th provide paired dorsolateral fossae of rather large extension, to articulate with the heads of pleural ribs (F) (Figure 3). If preservation is good, prezygapophyses and fossae can be observed in archaeological material.

Vertebrae are higher than long (M1>M3), the difference between these two measurements becomes smaller in V4.

Caudal vertebrae are characterized by the presence of neural and complete hemal arches and spines, formed by the convergence of the paired parapophyses. A strenghthening ridge (R) divides the lateral aspect into dorso- and ventrolateral depressions. The last three vertebrae are involved in caudal support (Figure 3).

The height of these vertebrae is smaller than their length (M1<M3) and the difference between these two measurements becomes smaller from V21 onwards.

Hemapophyses and variable portions of the haemal spine may preserve in archaeological material.

2.2. Prediction models

General linear models can be applied to reference collection data to generate prediction models and estimate the original length of fish from vertebra and *sagitta* remains (Quinn & Keough, 2002, Orchard, 2005) (the reference data for otolith and vertebrae are available in a supplementary file provided online – see Appendix A).

In meagre, we modelled the relationship between fish length and vertebrae measurements as

$$y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$$

where y_i is the total length of fish i, x_i is bone measurement of fish i ($V_jM_{k,i}$, j=1,…,25, k=1,…,3), β_0 and β_1 are regression coefficients and ε_i is an error term. We fitted this model to the raw (i.e., untransformed) data because variance of fish length was homogeneous along the range of the bone measurements. *Post-hoc* residuals analysis indicated that the linear model adequately described the relationships.

In contrast, we found fish size to be nonlinearly related to otolith measurements and the variance of the relationships to increase with otolith size. Such non-linearity and heterogeneity of variance is common in fish data (e.g., Hayes *et al*., 1995) and can be handled by fitting the linear model to the log-transformed data (as opposed to raw data). Accordingly, our fish length vs. otolith measurements model was

$$Ln(y_i) = Ln(\beta_0) + \beta_1 Ln(x_i) + \varepsilon_i$$

where $Ln(y_i)$ is the natural logarithm of the total length of fish i, $Ln(x_i)$ is the natural logarithm of otolith measurement of fish i (MAX_OL_i , MED_OL_i, or MAX_OH_i) and $Ln(\beta_0)$ and β_1 are regression coefficients and ε_i is an error term. This model corresponds to the usual $y_i = a\ x^b$ nonlinear allometric equation with multiplicative lognormal error, where $a = \ln(\beta_0)_i$ and $b = \beta_1$ (Hayes *et al*., 1995). *Post-hoc* residuals analysis indicated adequate fit of this model as well as a normal distribution of residuals.

2.3. Analysis of archaeological data

We used the prediction models to estimate the size and number of *A. regius* individuals present at four Portuguese Mesolithic sites (ca. 6000 BP to 4500 BP). These sites are located in the surroundings of two large estuaries - the Tejo [Cabeço dos Morros] and the Sado [Arapouco and Poças de São Bento] – and in the adjacent Atlantic coast - Sines [Samouqueira] (Figure 4) - and were coded as ARA, PSB, CMORR and SAM-I Substantial *A. regius* evidence has been found at these sites (n=6 vertebrae, n=153 otoliths) but the number of specimens and their size distribution was never estimated.

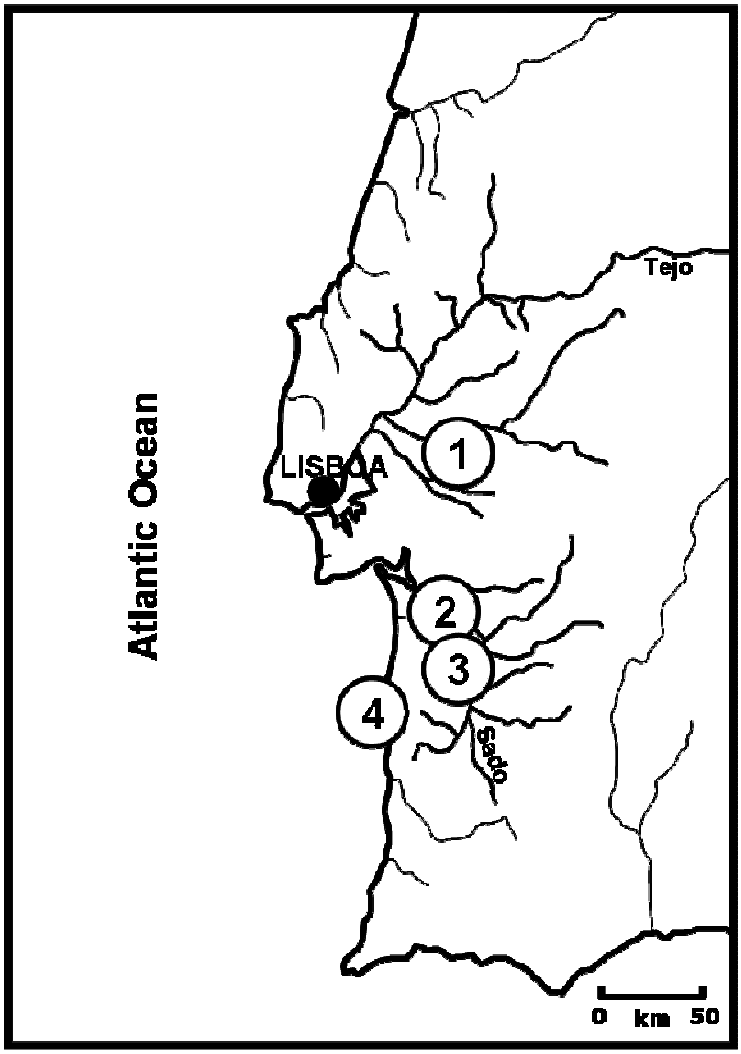

Figure 4. Map of Portugal (southern regions) showing the sites mentioned in the text: 1) Cabeço dos Morros; 2) Arapouco; 3) Poças de São Bento; and 4) Samouqueira-I.

To determine the number of specimens and the size distribution of meagre present at the Portuguese sites we relied on the regression method proposed by Orchard (2005). Briefly, we first estimated the mean length (and its 95% prediction interval) of each archaeological specimen by applying the prediction models to the archaeological measurements data of each site (Tables 2 and 3, and Appendix A). Then we separated otolith-derived fish length into two distinct groups (left and right), sorted them in ascending order, and performed pairwise comparisons among them, starting in the smaller fish. When the prediction intervals obtained from otoliths of left and right groups were found to overlap, the otoliths were judged to belong to the same fish, the fish with the smaller size being retained and its "opponent" removed and replaced with the next fish of that group. In contrast, when the prediction intervals of left and right groups did not overlap, the otoliths were judged to belong to different fish, both fish being retained and comparisons advancing in both groups. The comparisons continued until all otoliths were compared and a final set of otolith-derived fish lengths was found. Then, when vertebrae were present, the set of otolith-derived lengths was compared to vertebrae-derived

Table 2. M1, M2, & M3 measurements for the 6 archaeological vertebrae by site

Site	Layer	Bone	Measur.	Total
ARA	2	V3	M1	14,4
			M2	17,7
			M3	10,8
PSB	B/220-230	V4	Mean_M3	19,9
	C/240-250	V2	M1	23,2
			M2	32,2
			M3	15,9
SAM1	C3a	V2	M1	28,9
			M2	30,0
			M3	15,4
		V5	M1	26,1
			M2	35,1
			M3	24,4
		V22	M1	16,6
			M2	19,2
			M3	24,4

Table 3. Average otolith measurements (MAX_OL, MED_OL, MAX_OH) by site. Side: L= left; R= right

Site	Layer	Bone	Side	Measur._mean	Total
ARA	1	*Sagitta*	L		37
				Mean OL_MAX	16,1
				Mean OL_MED	16,0
				Mean OH_MAX	9,0
		Sagitta	R		33
				Mean OL_MAX	14,8
				Mean OL_MED	19,5
				Mean OH_MAX	9,2
	2	*Sagitta*	L		39
				Mean OL_MAX	14,1
				Mean OL_MED	14,8
				Mean OH_MAX	8,2
		Sagitta	R		40
				Mean OL_MAX	15,0
				Mean OL_MED	15,2
				Mean OH_MAX	8,1
	3	*Sagitta*	L		1
				Mean OL_MAX	20,7
				Mean OL_MED	20,3
				Mean OH_MAX	11,3
CMORR	50-60	*Sagitta*	L		1
				Mean OH_MAX	16,1
PSB	B/80-90	*Sagitta*	L		1
				Mean OL_MAX	16,2
	C1/90-100	*Sagitta*	L		1
				Mean OL_MAX	13,9

lengths in similar fashion to obtain a final Minimum Number of Individuals (MNI) and length distribution at each site. This pairwise comparison strategy bears the advantage of objectively evaluating the likelihood of different fish remains belonging to the same fish, providing a conservative MNI estimate that is free of the major redundancies in the archaeological data while avoiding being excessively conservative (Orchard, 2005). In fact, only when broken (unmeasured) otolith and vertebra remains were present, did they have to be carefully evaluated for side, type of vertebra, and size and a more subjective judgment made as to whether (or not) to include them on the MNI estimate. In the meagre case, this happened in ARA and we made no attempt to extrapolate on the size of the original fish based on such incomplete data.

The total weight (TW) of meagre specimens was determined using a length-weight relationship derived for the Portuguese coast (n=1459, $BM=1.5x10^{-5}TL^{2.869}$, r^2=0.99) (Costa *et al.*, 2008). This relationship was applied to the estimated lengths of archaeological specimens in order to obtain individual body mass estimates. Length distributions across sites were compared using two-sample Kolmogorov-Smirnov tests.

All statistical analyses were performed in R 2.8.1. (R Development Core Team, 2008). The determination of MNI and final fish lengths using Orchard (2005) methodology is cumbersome and time consuming in large samples because a large number of pairwise comparisons are involved. Accordingly, an automated R script was developed for this purpose. This script is available from the authors upon request.

3. Results

The fits of the general linear models to the bone measurement and fish length data are presented in Table 4. All relationships between vertebra and fish size were linear (Figure 5). On the contrary, the otolith size vs. fish size relationships were better described by power curves (Figure 6 and 7). Irrespective of these differences, all β_1 coefficients were significantly higher than 1 ($P<0.001$) and large r^2 values were registered in all relationships (range: 0.921-0.992). These results indicate a good fit of the general linear models to the meagre data and, most importantly, that meagre size can be accurately predicted from vertebra or otolith measurements using the equations in Table 4.

Table 4. Descriptive statistics and fish length (cm) vs. bone relationships (mm) of *Argyrosomus regius*. All models $P<0.05$

Bone	Measur.	*n*	Mean X ± S.D. (Min-Max)	Mean Y ± S.D. (Min-Max)	Equation	s.e. (β_0)	s.e. (β_1)	σ	r^2	*F*
V1	M1	35	10.877 ± 4.79 (2.30-21.81)	68.5 ± 29.2 (14-136)	Y = 2.506+6.066X	1,401	0,118	3,300	0,988	2636,36
V1	M2	35	11.444 ± 5.264 (2.28-24.40)	68.5 ± 29.2 (14-136)	Y = 5.812+5.476X	2,034	0,162	4,968	0,972	1144,39
V1	M3	35	8.901 ± 3.635 (2.48-17.42)	68.5 ± 29.2 (14-136)	Y = -0.693+7.772X	3,460	0,361	7,644	0,934	464,39
V2	M1	36	12.090 ± 5.685 (2.38-25.47)	67.4 ± 29.6 (14-136)	Y = 5.062+5.154X	1,631	0,122	4,116	0,981	1773,1
V2	M2	36	12.189 ± 5.856 (2.30-27.48)	67.4 ± 29.6 (14-136)	Y = 7.825+4.886X	2,964	0,220	7,614	0,936	494,12
V2	M3	36	7.996 ± 3.462 (1.68-15.75)	67.4 ± 29.6 (14-136)	Y = 0.255+8.394X	2,363	0,272	5,568	0,966	953,6
V3	M1	36	11.087 ± 4.663 (2.28-22.21)	67.4 ± 29.6 (14-136)	Y = -0.735+6.143X	3,247	0,270	7,463	0,938	515,8
V3	M2	36	13.953 ± 6.987 (2.80-29.43)	67.4 ± 29.6 (14-136)	Y = 10.074+4.107X	2,737	0,176	7,272	0,941	544,98
V3	M3	36	8.870 ± 3.736 (2.05-17.89)	67.4 ± 29.6 (14-136)	Y = -1.326+7.746X	2,697	0,281	6,207	0,957	760,78
V4	M1	36	10.797 ± 4.484 (2.22-22.13)	67.4 ± 29.6 (14-136)	Y = -1.563+6.385X	3,313	0,284	7,534	0,937	505,49
V4	M2	36	14.206 ± 7.178 (2.82-29.53)	67.4 ± 29.6 (14-136)	Y = 9.851+4.049X	2,076	0,131	5,555	0,966	958,3
V4	M3	35	10.214 ± 3.922 (2.42-19.13)	67.4 ± 30.0 (14-136)	Y = -7.749+7.353X	4,024	0,368	8,426	0,923	398,24
V5	M1	35	11.350 ± 4.907 (2.34-22.37)	67.4 ± 30.0 (14-136)	Y = -0.824+6.007X	2,457	0,199	5,699	0,965	909,86
V5	M2	35	14.339 ± 7.054 (2.77-29.83)	67.4 ± 30.0 (14-136)	Y = 7.213+4.195X	1,968	0,123	5,079	0,972	1153,85
V5	M3	35	11.657 ± 5.055 (2.87-23.02)	67.4 ± 30.0 (14-136)	Y = -0.591+5.829X	2,475	0,195	5,756	0,964	891,22
V6	M1	35	11.497 ± 5.175 (2.36-23.71)	67.4 ± 30.0 (14-136)	Y = 2.378+5.652X	2,838	0,226	6,810	0,950	627,32
V6	M2	35	13.784 ± 7.091 (2.73-29.45)	67.4 ± 30.0 (14-136)	Y = 10.26+4.142X	2,332	0,151	6,238	0,958	753,8
V6	M3	35	12.603 ± 5.574 (3.10-25.10)	67.4 ± 30.0 (14-136)	Y = 0.735+5.286X	2,440	0,177	5,767	0,964	887,5
V7	M1	35	11.910 ± 5.277 (2.57-24.24)	67.4 ± 30.0 (14-136)	Y = 1.188+5.556X	2,746	0,211	6,501	0,954	691,44
V7	M2	35	13.700 ± 7.672 (2.59-31.05)	67.4 ± 30.0 (14-136)	Y = 14.751+3.84X	2,031	0,130	5,806	0,964	875,19
V7	M3	35	13.237 ± 5.58 (3.14-25.36)	67.4 ± 30.0 (14-136)	Y = -2.006+5.24X	3,015	0,210	6,844	0,950	620,6
V8	M1	36	12.144 ± 5.452 (2.57-24.83)	67.4 ± 29.6 (14-136)	Y = 2.783+5.319X	2,436	0,183	5,916	0,961	840,97
V8	M2	36	13.786 ± 7.615 (2.57-31.35)	67.4 ± 29.6 (14-136)	Y = 15.012+3.798X	2,188	0,139	6,279	0,956	742,62
V8	M3	36	14.387 ± 6.306 (3.46-28.31)	67.4 ± 29.6 (14-136)	Y = 0.915+4.619X	2,186	0,139	5,204	0,970	1096,61
V9	M1	36	12.363 ± 5.67 (2.62-25.93)	67.4 ± 29.6 (14-136)	Y = 4.54+5.082X	2,739	0,202	6,772	0,949	633,64
V9	M2	36	14.040 ± 7.845 (2.59-33.45)	67.4 ± 29.6 (14-136)	Y = 15.531+3.693X	2,090	0,130	6,051	0,959	802,15
V9	M3	34	15.552 ± 7.18 (3.59-31.42)	67.7 ± 30.4 (14-136)	Y = 2.641+4.183X	2,059	0,121	4,972	0,974	1204,07

Table 4 (Cont.). Descriptive statistics and fish length (cm) vs. bone relationships (mm) of *Argyrosomus regius*. All models $P<0.05$

Bone	Measur.	*n*	Mean X ± S.D. (Min-Max)	Mean Y ± S.D. (Min-Max)	Equation	s.e. (β_0)	s.e. (β_1)	σ	r^2	*F*
V10	M1	35	12.366 ± 6.059 (2.64-26.69)	67.6 ± 30.0 (14-136)	Y = 7.968+4.819X	2,701	0,197	6,948	0,948	600,41
V10	M2	35	14.740 ± 8.339 (2.60-34.56)	67.6 ± 30.0 (14-136)	Y = 15.383+3.54X	1,871	0,111	5,389	0,969	1019,86
V10	M3	34	16.448 ± 7.605 (3.68-34.24)	67.7 ± 30.4 (14-136)	Y = 2.64+3.955X	1,936	0,107	4,679	0,977	1363,95
V11	M1	35	12.985 ± 6.864 (2.61-29.43)	67.6 ± 30.0 (14-136)	Y = 11.787+4.295X	2,041	0,139	5,578	0,966	949,6
V11	M2	35	14.745 ± 8.598 (2.65-35.46)	67.6 ± 30.0 (14-136)	Y = 17.144+3.419X	2,044	0,120	6,024	0,961	809,58
V11	M3	35	17.459 ± 8.623 (3.84-38.01)	67.6 ± 30.0 (14-136)	Y = 7.915+3.416X	2,204	0,114	5,708	0,965	905,48
V12	M1	36	12.835 ± 6.525 (2.65-28.71)	67.4 ± 29.6 (14-136)	Y = 10.192+4.455X	2,059	0,143	5,535	0,966	965,47
V12	M2	36	15.184 ± 8.357 (2.62-34.28)	67.4 ± 29.6 (14-136)	Y = 14.28+3.497X	1,621	0,094	4,638	0,976	1389,19
V12	M3	35	17.985 ± 8.547 (4.15-39.84)	65.9 ± 28.6 (14-136)	Y = 6.51+3.302X	1,951	0,098	4,895	0,972	1130,39
V13	M1	35	12.485 ± 6.18 (2.57-28.58)	65.9 ± 28.6 (14-136)	Y = 8.915+4.564X	1,933	0,139	5,013	0,970	1076,23
V13	M2	35	14.086 ± 7.861 (2.68-34.38)	65.9 ± 28.6 (14-136)	Y = 15.535+3.575X	1,949	0,121	5,557	0,963	869,73
V13	M3	35	18.790 ± 9.331 (4.29-41.53)	65.9 ± 28.6 (14-136)	Y = 9.035+3.026X	1,854	0,089	4,822	0,972	1165,77
V14	M1	35	12.300 ± 6.163 (2.54-28.31)	65.9 ± 28.6 (14-136)	Y = 9.696+4.57X	2,004	0,146	5,251	0,967	978,18
V14	M2	35	14.258 ± 7.983 (2.63-34.63)	65.9 ± 28.6 (14-136)	Y = 15.68+3.522X	1,922	0,118	5,495	0,964	890,41
V14	M3	35	19.163 ± 9.48 (4.34-44.23)	65.9 ± 28.6 (14-136)	Y = 9.166+2.961X	2,220	0,104	5,757	0,961	808,23
V15	M1	35	12.293 ± 6.294 (2.56-28.67)	65.9 ± 28.6 (14-136)	Y = 10.984+4.467X	2,062	0,150	5,496	0,964	889,8
V15	M2	35	13.889 ± 8.017 (2.60-35.77)	65.9 ± 28.6 (14-136)	Y = 17.38+3.493X	2,073	0,130	6,065	0,956	724,92
V15	M3	35	19.518 ± 9.906 (4.44-43.51)	65.9 ± 28.6 (14-136)	Y = 10.892+2.818X	2,443	0,112	6,467	0,950	633,64
V16	M1	35	12.196 ± 6.21 (2.74-28.42)	65.9 ± 28.6 (14-136)	Y = 10.621+4.533X	2,013	0,148	5,342	0,966	943,92
V16	M2	35	13.824 ± 7.887 (2.55-35.48)	65.9 ± 28.6 (14-136)	Y = 16.854+3.548X	2,128	0,134	6,171	0,955	699,07
V16	M3	35	19.503 ± 10.108 (4.49-44.04)	65.9 ± 28.6 (14-136)	Y = 12.207+2.753X	2,543	0,116	6,843	0,945	562,3
V17	M1	36	12.671 ± 6.555 (2.46-28.18)	67.4 ± 29.6 (14-136)	Y = 10.735+4.47X	1,496	0,105	4,078	0,982	1807,61
V17	M2	36	14.189 ± 8.007 (2.52-34.07)	67.4 ± 29.6 (14-136)	Y = 15.906+3.627X	1,942	0,120	5,664	0,964	920,29
V17	M3	36	20.009 ± 10.616 (4.40-45.45)	67.4 ± 29.6 (14-136)	Y = 13.247+2.705X	2,582	0,114	7,181	0,943	559,75
V18	M1	36	12.390 ± 6.418 (2.38-27.35)	67.4 ± 29.6 (14-136)	Y = 10.867+4.561X	1,578	0,113	4,306	0,979	1617,28
V18	M2	36	13.974 ± 7.742 (2.45-33.70)	67.4 ± 29.6 (14-136)	Y = 15.088+3.742X	2,102	0,132	6,046	0,959	803,51
V18	M3	36	20.060 ± 10.639 (4.46-44.44)	67.4 ± 29.6 (14-136)	Y = 13.059+2.708X	2,449	0,108	6,810	0,949	626,32

Table 4 (Cont.). Descriptive statistics and fish length (cm) vs. bone relationships (mm) of *Argyrosomus regius*. All models $P<0.05$

Bone	Measur.	*n*	Mean X ± S.D. (Min-Max)	Mean Y ± S.D. (Min-Max)	Equation	s.e. (β_0)	s.e. (β_1)	σ	r^2	*F*
V19	M1	36	12.316 ± 6.45 (2.38-27.76)	67.4 ± 29.6 (14-136)	Y = 11.593+4.529X	1,703	0,123	4,688	0,976	1359,27
V19	M2	36	13.754 ± 7.776 (2.40-31.88)	67.4 ± 29.6 (14-136)	Y = 16.005+3.735X	1,946	0,124	5,685	0,964	913,48
V19	M3	36	19.874 ± 10.321 (4.41-44.21)	67.4 ± 29.6 (14-136)	Y = 12.102+2.781X	2,648	0,119	7,242	0,942	549,74
V20	M1	36	11.807 ± 5.701 (2.40-25.06)	67.4 ± 29.6 (14-136)	Y = 6.984+5.115X	1,945	0,149	5,016	0,972	1182,88
V20	M2	36	12.788 ± 6.724 (2.32-29.90)	67.4 ± 29.6 (14-136)	Y = 12.169+4.317X	2,087	0,145	5,763	0,963	887,99
V20	M3	36	18.104 ± 8.173 (4.29-37.46)	67.4 ± 29.6 (14-136)	Y = 2.937+3.559X	2,222	0,112	5,422	0,967	1007,72
V21	M1	36	11.390 ± 5.354 (2.36-23.74)	67.4 ± 29.6 (14-136)	Y = 4.78+5.495X	1,222	0,097	3,083	0,989	3188,08
V21	M2	36	11.779 ± 5.401 (2.27-25.95)	67.4 ± 29.6 (14-136)	Y = 5.375+5.264X	3,352	0,259	8,284	0,924	412,14
V21	M3	36	16.213 ± 6.82 (4.02-31.45)	67.4 ± 29.6 (14-136)	Y = -1.75+4.264X	2,394	0,136	5,503	0,966	977,07
V22	M1	36	10.602 ± 4.74 (2.35-22.32)	67.4 ± 29.6 (14-136)	Y = 2.003+6.166X	1,901	0,164	4,601	0,976	1412,57
V22	M2	36	11.406 ± 5.279 (2.20-24.17)	67.4 ± 29.6 (14-136)	Y = 4.839+5.483X	2,485	0,198	6,191	0,957	764,91
V22	M3	36	14.030 ± 6.16 (3.86-28.46)	67.4 ± 29.6 (14-136)	Y = 1.332+4.707X	2,483	0,162	5,918	0,961	840,17
V23	M1	35	9.961 ± 3.945 (2.22-20.32)	67.6 ± 30.0 (14-136)	Y = -5.079+7.295X	3,971	0,371	8,543	0,921	385,77
V23	M2	35	11.181 ± 5.284 (2.14-23.03)	67.6 ± 30.0 (14-136)	Y = 4.405+5.651X	1,115	0,090	2,784	0,992	3909,46
V23	M3	35	12.182 ± 5.288 (3.3-25.49)	67.6 ± 30 (14-136)	Y = 0.726+5.488X	3,279	0,247	7,632	0,937	491,77
V24	M1	35	9.551 ± 3.869 (2.06-20.02)	67.5 ± 30.0 (14-136)	Y = -4.258+7.51X	3,464	0,337	7,599	0,938	497,06
V24	M2	35	10.276 ± 4.652 (2.07-22.43)	67.5 ± 30.0 (14-136)	Y = 2.838+6.29X	2,801	0,249	6,752	0,951	638,44
V24	M3	35	10.221 ± 4.273 (2.58-21.58)	67.5 ± 30.0 (14-136)	Y = -3.319+6.926X	2,215	0,200	4,992	0,973	1195,21
V25	M1	36	9.329 ± 4.018 (1.97-18.69)	67.4 ± 29.6 (14-136)	Y = 0.373+7.182X	2,803	0,277	6,575	0,952	674,3
V25	M2	36	9.578 ± 4.041 (2.01-19.17)	67.4 ± 29.6 (14-136)	Y = -1.194+7.159X	2,710	0,261	6,244	0,957	751,27
Sagitta	MAX_OL	113	18.95 ± 5.89 (5.2-31.8)	77.6 ± 35.0(12-160)	Ln (Y) = -0.150+1.516Ln(X)	0,051	0,018	0,069	0,985	7353,58
Sagitta	MED_OL	113	18.73 ± 5.74 (5.2-31.4)	77.6 ± 35.0(12-160)	Ln (Y) = -0.178+1.531Ln(X)	0,050	0,017	0,067	0,986	7917,30
Sagitta	MAX_OH	112	10.97 ± 2.98 (3.9-17.7)	78.2 ± 34.7(12-160)	Ln (Y) = 0.116+1.751Ln(X)	0,067	0,028	0,091	0,972	3855,04

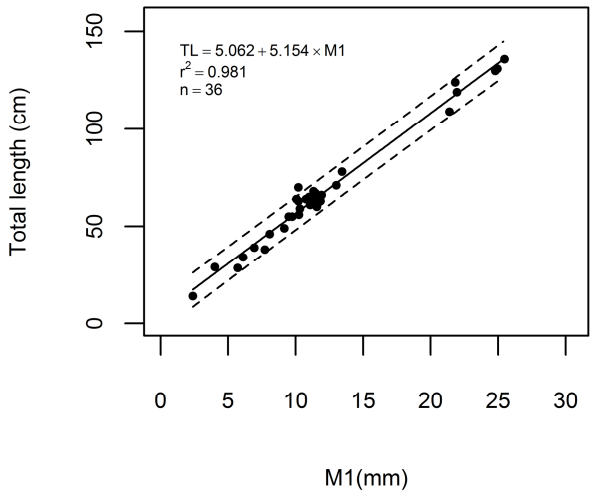

Figure 5. Relationship between fish total length (TL) and the greatest dorso-ventral height of the *A. regius* V-2 centrum (M1). The dashed lines represent 95% prediction bands of the prediction model.

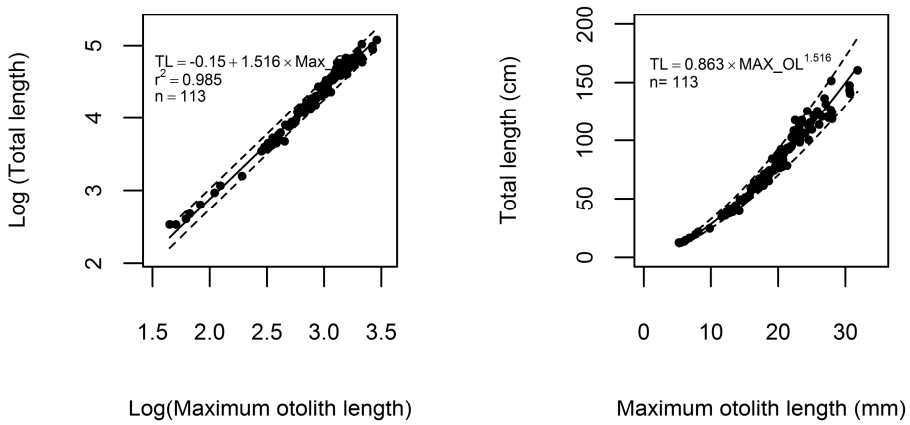

Figure 6. Relationship between fish total length (TL) and the maximum length of the *A. regius* sagitta (MAX_OL). Left panel: fitted data. Right panel: back-transformed data. The dashed lines represent 95% prediction bands of the prediction model.

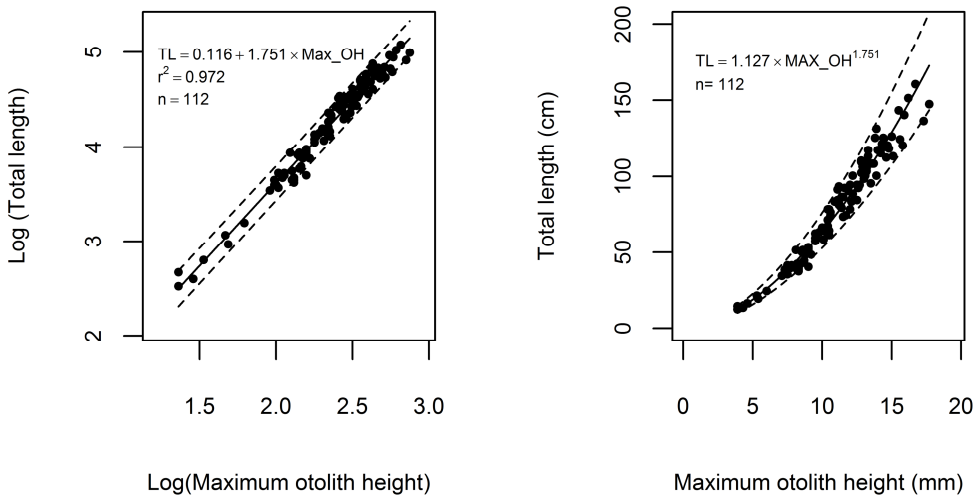

Figure 7. Relationship between fish total length (TL) and the maximum height of the *A. regius* sagitta (MAX_OH). Left panel: fitted data. Right panel: back-transformed data. The dashed lines represent 95% prediction bands of the prediction model.

The length details of meagres found at the four Mesolithic sites are summarized in Table 5 and the length distribution of the most represented site shown in Figure 8. Fish found at ARA and in the upper layers of PSB were less than 1 m TL (~8 kg) while fish at other sites and layers were essentially over 1.20 m (~14 kg) (Table 5). Layers 1 and 2 of site ARA presented fish of overlapping length ranges and similar length compositions (Kolmogorov-Smirnov D: 0.2194, P>0.05) (Table 5). Specifically, ARA layer 1 registered 83% of fish between 35 and 80 cm long and layer 2 registered 92% of fish between 30 and 70 cm long. Remains belonging to fish in the 35-40 cm size class (~400–600 g) being the most abundant in both layers (Figure 8). The largest individual was found at CMRR, measuring 146 cm (~24 kg).

Table 5. Estimated Minimum Number of Individuals (MNI) and total length (TL) of *Argyrosomus regius* found at archaeological sites

Site	Remains (*n*)	MNI	Estimated TL ± S.D. (Min-Max)
ARA1	sagitta (67)	37	55.5 ± 19.8 (29.3-102.2)
ARA2	sagitta (79), V-22 (1)	40	46.6 ± 14.1 (18.8-84.6)
ARA3	sagitta (1)	1	78.5 (---)
CMORR50-60	sagitta (1)	1	146.0 (---)
PSB B/80-90	sagitta (1)	1	58.9 (---)
PSB C1/90-100	sagitta (1)	1	46.7 (---)
PSB220-230	V-4 (1)	1	138.3(---)
PSB240-250	V-2 (1)	1	124.8 (---)
SAM1 C3a	V-2(1), V-5 (1), V-22 (1)	2	122.9 ± 26.4 (104.2-141.6)

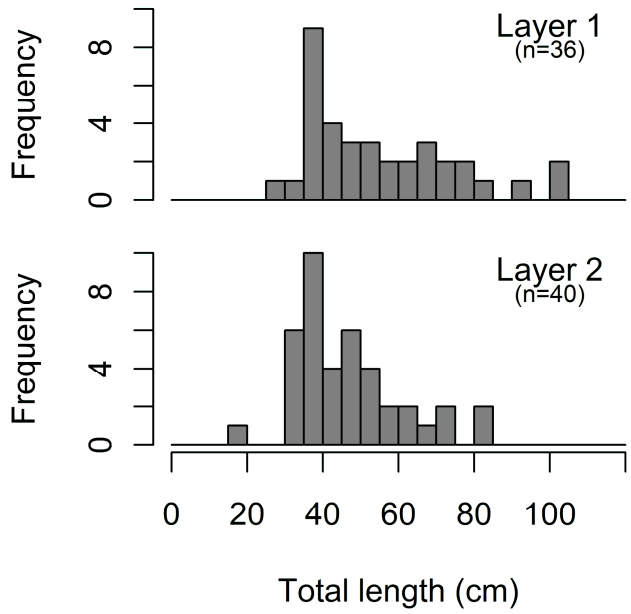

Figure 8. Size distribution of individual *A. regius* found in Arapouco: layer 1 (upper panel); and layer 2 (lower panel). Each size-class is 5 cm wide.

4. Discussion and final considerations

The importance of reconstructing fish body size from bones recovered in archaeological sites has been largely discussed in the literature (Enghoff, 1983, 1989, 1991; Johnsson, 1994; Desse and Desse-Berset, 1994, s/d; Smith,1995; Zohar *et al.*, 1997; Bèarez, 2000; Orchard 2003, 2005). To date, several authors have referenced the presence of meagre bones among archaeological remains (Arnaud, 2000; Roselló, 1989, Le Gall *et al.* 1994; to name a few) but few have indicated length composition of meagre specimens captured by prehistoric communities (Lentacker, 1986). As shown in Figures 6 and 7 there is substantial variability in the relationship between otolith size and fish size. This makes it difficult to determine fish size from otolith size in the absence of representative fish collections and mathematical models that explicitly account for such variability. It also cautions against drawing conclusions derived from earlier evaluations based on small reference collections (e.g., n=3 specimens, Lentacker, 1986).

The use of fish bone vs. fish size relationships to predict the size of fish captured by prehistoric communities requires good bone preservation. In general, poor preservation of fish bones will result in partial disappearance of bone from specimens and, thus, in biased, under-estimates of fish. Potential sources of differential preservation of fish bone before and after deposition include bone structure, processing, ingestion and subaerial weathering, chemistry of the disposal context and other mechanisms of fish bone destruction (Butler and Charters, 1994; Lubinsky, 1996; Nicholson, 1992). The material from the four sites is reasonably well-preserved, allowing the determination of at least one measurement on each otolith and vertebra. This leads us to believe that it should approximate well the original meagre size composition.

Our results for the Portuguese case-study indicate that Mesolithic communities not only consumed small meagre (ARA), probably captured in large schools, but were also able to

capture large fish weighing over 20 kg (CMORR, PSB, SAM-I). In the wider array of fish identified in ARA (including the Tope shark *Galeorhinus galeus*, the European seabass *Dicentrarchus labrax*, the Gilthead seabream *Sparus aurata*, to name a few) meagre minimum number of individuals (MNI) represented 55% of fish found in layer 1 and 33% of fish found in layer 2 (S. Gabriel, unpub. data). This evidence suggests that meagre may have made a significant contribution to the subsistence of the Mesolithic communities of the Portuguese coast.

Bone sizes vs. fish size relationships greatly increase the range of interpretations of zooarchaeology studies. In the case of the meagre, it is known that juvenile and adult fish aggregate in Portuguese and French estuaries in spring and summer (Quéméner, 2002; Prista *et al.*, 2008). Assuming meagre behaviour has not changed since Mesolithic times, it is likely that many of the Portuguese meagre remains belong to specimens caught in estuarine environments during spring-summer. In the ARA case, the most likely place is the nearby Sado estuary. In the Sado valley, several shell-middens are ground in a 30 km area surrounding the inner estuary. These sites are highly variable in composition. Those situated closer to the river mouth, such as ARA, are rich in fish remains and have few mammal bones; those further inland are very rich in mammal bones. Following Arnaud (1987), this pattern can be interpreted as the archaeological image of a collector settlement-subsistence system, where sites like ARA act as specialized camps occupied in the framework of the procurement of resources (specifically fish). Interestingly, today meagre is uncommonly caught in that estuary (N. Prista, *pers. obs.*) and much more abundant in the Tagus estuary, ~40 km to the north. Further investigation (e.g., age distributions and validation through isotopic analysis) may provide additional insights into the seasonality of the human occupation of ARA and the reasons for possible temporal shifts in the nursery and spawning grounds of meagre in the course of time.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jas.2012.04.046.

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Chapter 2

Present-day meagre fisheries

Chapter 2A

Inferring fish movements from small-scale fisheries data: the case of *Argyrosomus regius* (Sciaenidae) in Portugal

Nuno Prista[a,b], Cynthia Jones[c], José Lino Costa[a], Maria José Costa[a,b]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Center for Quantitative Fisheries Ecology, Old Dominium University, 800W 46th St., Norfolk, VA 23508, USA

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Inferring fish movements from small-scale fisheries data: the case of *Argyrosomus regius* (Sciaenidae) in Portugal

Abstract: The life history of several marine resources remains scarcely known worldwide. This is particularly the case of many marine fish exploited by small-scale artisanal fisheries for which current fishery independent and fishery dependent sampling is limited. In some such data-poor situations time series of regional landings may be available that may be used to infer fish life history. The meagre *Argyrosomus regius* is one example of a valuable data-limited species, exploited by small-scale local fleets off the European Atlantic coasts, for which distribution and migration patterns are still scarcely known. We used time series techniques (periodogram analysis, generalized non-linear harmonic regression and seasonal-trend decomposition based on loess) to analyze 5-year datasets of monthly landings of meagre across 6 regions of Portugal. We then correlated the time series results on the spatio-temporal distribution of the landings with several descriptors of the fishery, including an analysis of the temporal evolution of the fishing effort and the determination of the regions and months when target effort on the species takes place. Our results indicate that the meagre fishery presents annual periodicity in all regions of the Portuguese coast and that the landings periodicity is likely to be generated by a determinist annual cycle associated with migrations of meagre juveniles from the estuary to the coast. Meagre juveniles concentrate in the Tagus estuary from May to September and migrate northwards and southwards along the Western coast in Autumn-Winter months. These results demonstrate that if adequate time series analysis are used and some effort patterns are considered, preliminary hypothesis testing on the spatial distribution and migratory behaviour of scarcely known fish resources may be performed from relatively short and noisy time series of landings such as the ones generated by small-scale artisanal fleets.

Keywords: small-scale artisanal fisheries, fisheries landings, time-series analysis, fishing effort, target effort, meagre

1. Introduction

Fisheries research and assessment have traditionally focused on large stocks, exploited by relatively well-characterized industrial fleets. In comparison, most small scale fisheries are poorly documented and very data limited (Mahon, 1997; Vasconcellos and Cochrane, 2005; ICES, 2007). When investigating the life history of fish exploited by small scale fisheries, researchers frequently find that landings (eventually coupled to rough indicators of fishing effort) are the only readily available data to conduct research and test hypothesis on life history patterns. In such situations the use of statistical time series methods assumes relevance since monthly landings data from small-scale fisheries typically present low mean values and the identification in them of fish and fisheries cycles is confounded by substantial biologically-induced, fisheries-induced and/or measurement induced correlated noise.

The meagre (*Argyrosomus regius*) fishery in Portugal is an example of small-scale fishery in a data-poor situation. The meagre is one of the largest sciaenids in the world and presents economic significance to local small-scale commercial fishing communities along the Atlantic coasts of France, Spain and Portugal (Quéméner 2002; Silva *et al.*, 2002; Prista *et al.*, 2007). Current knowledge on the distribution and migratory behaviour of the fish relies essentially on

non-statistical analysis of fisheries patterns landings performed in the Bay of Biscay that predict that both juveniles and adults migrate seasonally between the coastal and estuarine waters (Quéro and Vayne, 1987; Quéro, 1989). However, this evidence is still unconfirmed and could benefit from the application of statistical time series analysis and a more thorough consideration of the possibility that fishing effort patterns might generate such patterns.

In this study we use time-series methods to estimate the periodicity of the small-scale fishery on meagre in Portugal. We complement these results with temporal analysis of fishing effort evaluating the likelihood of the patterns in fisheries landings being fisheries induced and/or life cycle induced. In doing this, we provide the first statistical description of the spatio-temporal dynamics of meagre in European waters and most importantly, provide a case study of how statistical time-series methods and effort analyses can be combined to infer fish life history from time series of fisheries landings.

2. Materials and methods

To study the seasonality of the meagre in Portugal we obtained various landings and fleet data from the Portuguese General-Directorate for Fisheries and Aquaculture (DGPA): a) data on total monthly landings (kg) of meagre per port and fleet segment (period: January-1991 through December-2007), b) data on the physical and gear characteristics of the vessels that landed meagre (period: 2005), c) data on monthly species landings (kg) of the vessels that landed meagre per port (period: 2005) and d) data on total monthly sales (number) of individual vessels per port (period: January-2002 through December-2007). All data referred to landings made by Portuguese vessels operating in ICES Subarea IXa but our analysis focused on the "*polivalente*" segment of the fleet (90-98% of annual meagre landings), excluding the trawl and seine fleets and a pound-net that operates in Southern coast (Prista *et al.*, 2007). We restricted our temporal analysis of landings to the period May-2002 to April-2007 (60 months) and subdivided our spatial analysis into 6 non-overlapping regions: Northwest (NW), Central West (CW), Lisbon-marine (Lm), Lisbon-estuarine (Le), Southwest (SW) and South (S) (Figure 1).

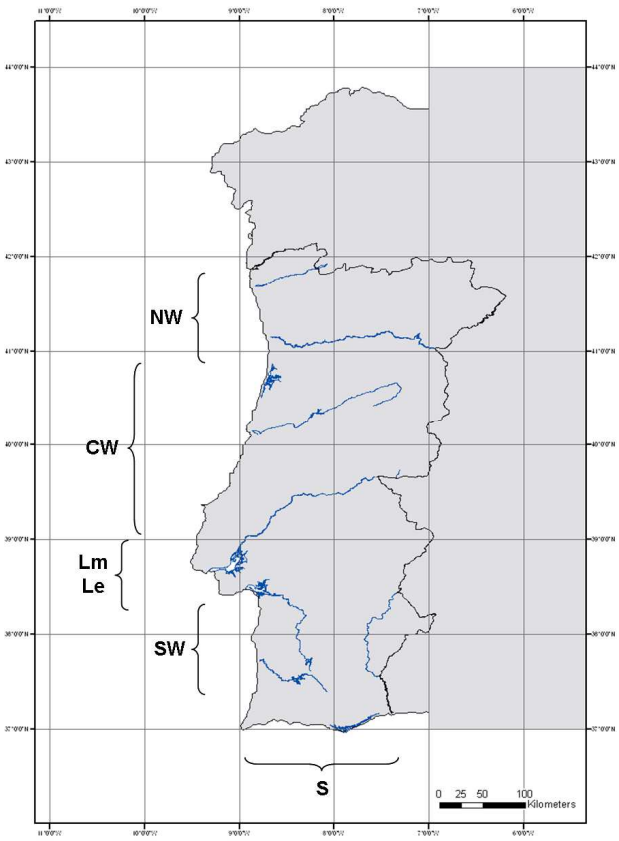

Figure 1. Regions considered in the analysis of the meagre fishery in the Portuguese coast. NW – Northwest region (ports from the Caminha maritime jurisdiction to the Douro maritime jurisdiction); CW – Central West (ports from the Aveiro maritime jurisdiction to the Peniche maritime jurisdiction), Lm – Lisbon-marine (selected marine ports from the Cascais, Sesimbra, Lisboa and Setúbal maritime jurisdictions); Le – Lisbon-estuarine (selected estuarine ports from the Cascais, Sesimbra, Lisboa and Setúbal maritime jurisdictions); SW– Southwest (ports in the Sines maritime jurisdiction); S – South (from the Lagos maritime jurisdiction to the Vila Real de Santo António maritime jurisdiction). Map credits: José Loff.

Spatio-temporal analysis of meagre landings was based on statistical analysis of time series of landings from the different regions of the coast (Figure 2). We transformed our data to meet the stationary assumption of time series analysis (Figure 3) and used periodograms to test the hypothesis that a deterministic cycle, of near annual periodicity, could be present in the data (Wilks, 2006). We then used non-linear harmonic regression to test if this periodicity differed from a purely annual periodicity and to compare the regional amplitudes and phase of the main fisheries cycle (Bloomfield, 2000). Next we used seasonal decomposition based on lowess (STL) to analyze the shape of the periodicity and better delimit the specific months of meagre landings (Cleveland *et al.*, 1990). Finally, to explore the possibility that the temporal patterns in landings could be fisheries-induced we a) examined the correlation between meagre landings and a rough fishing effort indicator (monthly number of daily fish sales), b) investigated cumulative plots of landings to detect months of target/by-catch effort (Biseau, 1998), and c) compared the composition of the landings of the vessels that landed meagre during the meagre season and out of meagre season to evaluate if drastic differences in the fishing techniques and spatial allocation of fishing effort could be generating the observed patterns in the landings data.

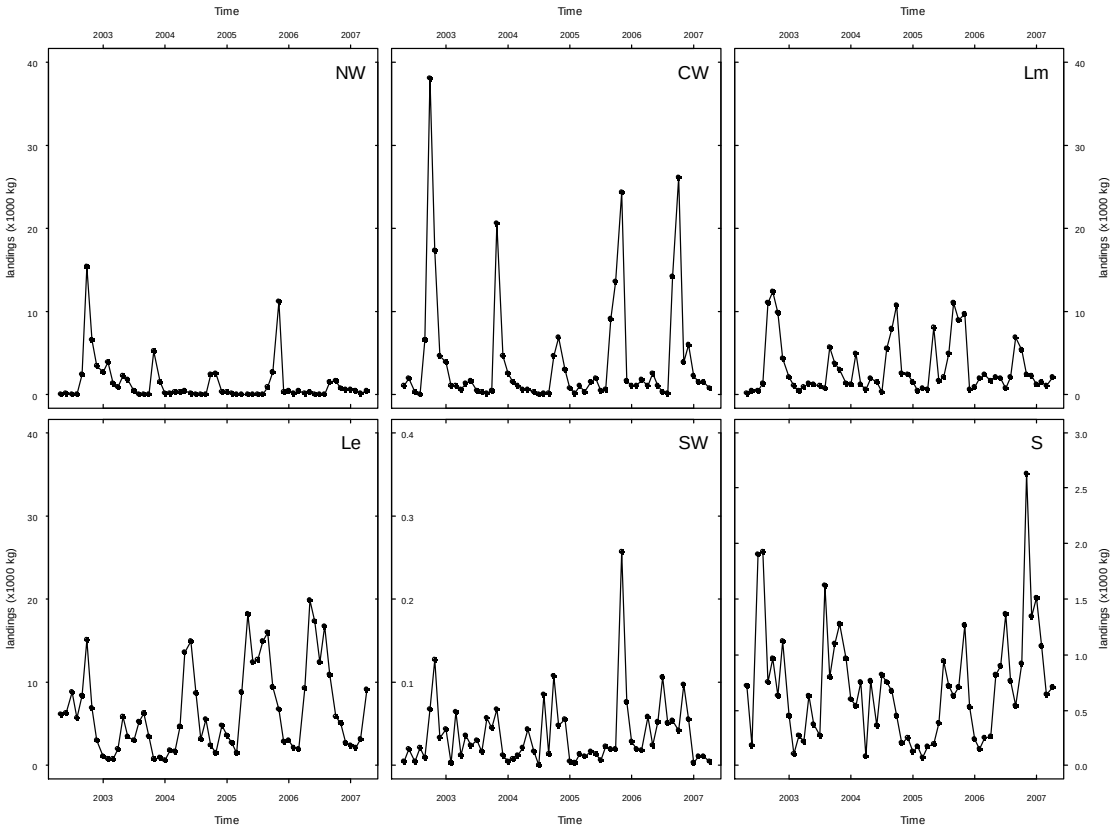

Figure 2. Regional landings of meagre from the Portuguese *polivalente* fleet operating in Portuguese waters (period: May-2002 through Apr-2007) (Source: DGPA). In each plot the *y*-axis presents the raw landings (x1000 kg) and the *x*-axis presents time. Note the different scale used in the *y*-axis of region SW and S. Regional acronyms as in Figure 1.

3. Results and discussion

The log transformed regional time series showed no evidence of increased variance with series mean or of trends in the landings, thus meeting the stationarity assumption of time series analysis. Periodogram analysis revealed that frequencies (ω) near the annual cycle (ω=1/12≈0.0833 equivalent to a 12-months period) account for most of series variance in all regional data (Figure 4). The proportion of variance explained by this cycle and its first harmonic was higher in the Northern Western and Central Western regions (NW to Le) than in the Southern regions (SW and S). The hypothesis that the ordinate value of ω=1/12 might have been generated stochastically (from a white-noise (WN), autoregressive (AR) or a moving average (MA) process) was rejected with a level of significance α=0.05 in all regional series. Accordingly, periodogram analysis indicated that a deterministic model involving a harmonic component with frequency approximately 1/12 month^{-1} (i.e. periodicity *ca.* annual) and an eventually correlated noise structure might be a good model for meagre landings in all regions.

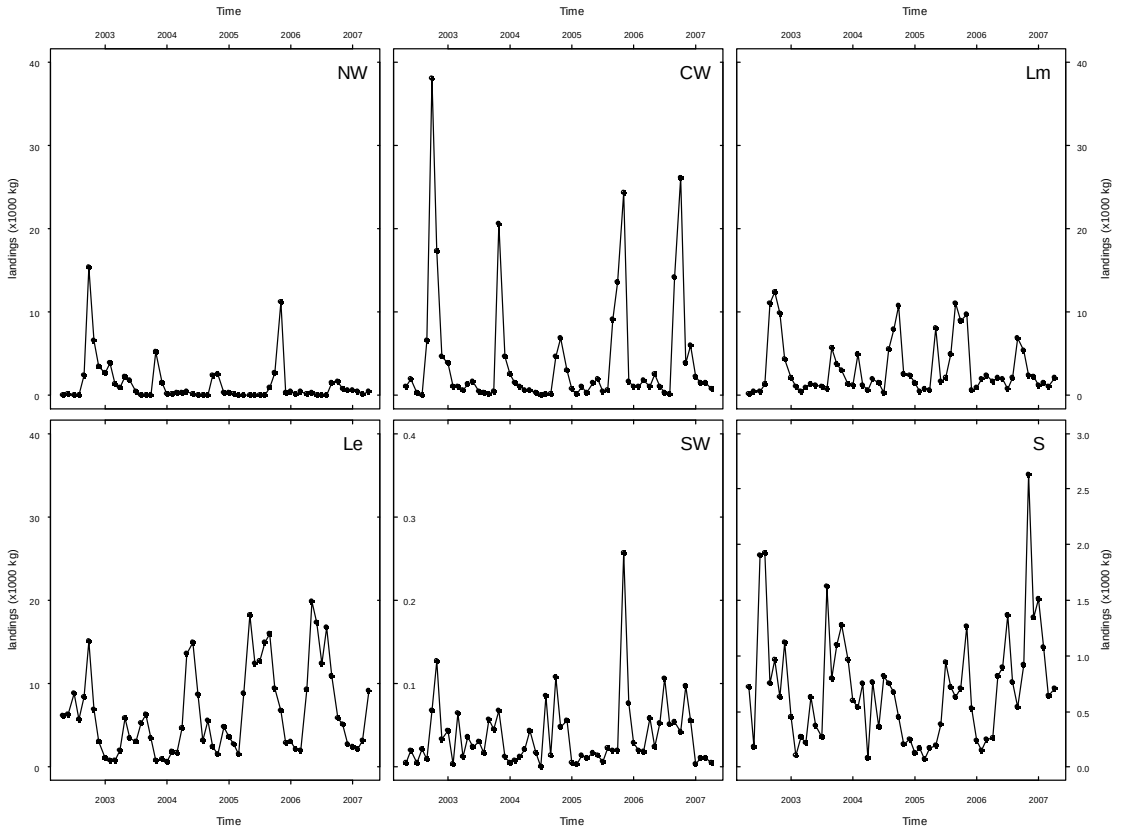

Figure 3. Transformed centered regional landings of meagre (period: May-2002 through Apr-2007). In each plot the *y*-axis presents the transformed [X'=$\log_{10}$ (landings+1)] centered [X'- mean (X')] landings (kg) and the *x*-axis presents time. Mean of logarithmic data: NW – 2.42; CW – 3.15; Lm – 3.30; Le – 3.66; SW – 1.37; S – 2.73. Regional acronyms as in Figure 1.

The generalized non-linear harmonic model with a variable single frequency fitted well the regional landing time series (Table 1). The near annual periodicity detected in the periodograms proved to be embedded in low order moving-average or autogressive noise in most regions and did not differ significantly from 12 months (Table 1). These results refine the evidence obtained from the periodogram analysis by improving the initially relatively low resolution of this analysis (frequency intervals of 1/60 month^{-1}) and establishing the meagre fishery as presenting a true annual periodicity in all regions of Portugal. Final amplitude and phase measurements obtained from fitting the regional data with a harmonic model with single fixed frequency (ω=1/12) indicate substantial differences in the timing and power of the seasonality in the different regions (Table 2, Figure 5). Amplitude estimates were higher but also more variable in NW and CW evidencing large intra-annual and inter-annual variations in the landings of these two regions (Table 2, Figure 5). On the other hand, the estimates of model phase indicated that the annual cycle is centered in July in one region (Le), September-October in three regions (Lm, SW and S) and in November-January in the two northern regions (CW and NW). These results indicate that the meagre fishery takes place essentially in the second half of the year, and landings occur essentially in the summer in the estuarine area (Le), early autumn in the

adjoining coastal areas (Lm and SW) and in late autumn and winter in the Northern regions (CW, NW).

Table 1. Estimates of the fit of an harmonic regression with a single variable frequency[1] to the regional time series of meagre landings (period: May-2002 through Apr-2007). The model was fitted independently to each region using generalized non-linear least-squares (Pinheiro and Bates, 2000) implemented in R (R Development Core Team, 2007). RSE is the residual standard error of the fit. The 95% confidence intervals (CI 95%) were computed using normal approximation to the distribution of the maximum likelihood estimator of the parameters. The normalized residuals of all models were uncorrelated (Ljung-Box tests) and normal (Shapiro-Wilks tests). Regional acronyms as in Figure 1

i	$\hat{C}_i$	$\hat{\omega}_i$	$\hat{\Phi}_i$	$\hat{\theta}_{1,i}$	$\hat{\theta}_{2,i}$	$\hat{\phi}_{1,i}$	$\hat{\phi}_{2,i}$	RSE	CI 95% ($\hat{\omega}_i$)
NW	0.702 (0.197)	0.0845 (0.0025)	4.670 (0.562)	0.486	-----	-----	-----	0.834	[0.0795; 0.0895]
CW	0.508 (0.129)	0.0857 (0.0024)	4.432 (0.525)	0.393	-----	-----	-----	0.567	[0.0809; 0.0904]
Lm	0.391 (0.071)	0.0870 (0.0017)	3.653 (0.377)	0.269	-----	-----	-----	0.328	[0.0835; 0.0904]
Le	0.402 (0.072)	0.0850 (0.0015)	1.972 (0.340)	-----	-----	0.591	-----	0.279	[0.0820; 0.0881]
SW	0.278 (0.076)	0.0860 (0.0026)	3.673 (0.572)	-----	-----	-----	-----	0.418	[0.0807; 0.0912]
S	0.271 (0.068)	0.0831 (0.0023)	2.623 (0.503)	-----	-----	0.235	0.288	0.311	[0.0784; 0.0877]

[1] Regression model given by $\log_{10}(L_{i,t}+1) = C_i \cos(2\pi\,\omega_i\,t - \Phi_i) + \varepsilon_{i,t}$, where $L_{i,t}$ are the landings in region i at time t , and C_i the amplitude, ω_i the frequency and Φ_i the phase of the harmonic term. $\varepsilon_{i,t}$ represents an error term with AR structure ($\phi_{1,i}$ and $\phi_{2,i}$) or MA structure ($\theta_{1,i}$ and $\theta_{2,i}$).

The STL application to regional landings data confirmed in a non-parametric way the main results of harmonic analysis. STL did not detect consistent temporal trends in landings data and similarly to the harmonic models it evidenced the higher amplitude of the seasonality in NW and CW. Monthly cycle subseries plots indicated that the meagre season is essentially unimodal in all regions and takes place every year between May and September in region Le, September and November in region Lm and SW, October and December in CW and between October and February in region NW (Figure 6). In region S the meagre season was considerably wider taking place between July and December. On the other hand, strictly negative seasonal coefficients took place between June and August in region NW and CW and in December-March in Le establishing these months as having comparatively reduced meagre available to the fishery.

Table 2. Estimates of the fit of an harmonic regression with a single fixed frequency[1] to the regional time series of meagre landings (period: May-2002 through Apr-2007). The model was fitted independently to each region using generalized non-linear least-squares (Pinheiro and Bates, 2000) implemented in R (R Development Core Team, 2007). RSE is the residual standard error of the fit. The 95% confidence intervals (CI 95%) were computed using normal approximation to the distribution of the maximum likelihood estimator of the parameters. The normalized residuals of all models were uncorrelated (Ljung-Box tests) and normal (Shapiro-Wilks tests). Regional acronyms as in Figure 1

i	$\hat{C}_i$	$\hat{\Phi}_i$	$\hat{\theta}_{1,i}$	$\hat{\theta}_{2,i}$	$\hat{\phi}_{1,i}$	$\hat{\phi}_{2,i}$	RSE	CI 95% ($\hat{C}_i$)	CI 95% ($\hat{\Phi}_i$)
NW	0.695 (0.196)	4.445 (0.280)	0.488	-----	-----	-----	0.829	[0.303; 1.087]	[3.884; 5.006]
CW	0.494 (0.130)	3.984 (0.265)	0.402	-----	-----	-----	0.568	[0.233; 0.755]	[3.454; 4.515]
Lm	0.368 (0.075)	3.126 (0.205)	0.309	-----	-----	-----	0.339	[0.217; 0.518]	[2.715; 3.538]
Le	0.397 (0.073)	1.631 (0.179)	-----	-----	0.603	-----	0.282	[0.251; 0.544]	[1.272; 1.990]
SW	0.267 (0.077)	3.136 (0.286)	-----	-----	-----	-----	0.419	[0.114; 0.420]	[2.562; 3.709]
S	0.271 (0.068)	2.666 (0.251)	-----	-----	0.236	0.289	0.308	[0.135; 0.406]	[2.164; 3.167]

[1] Regression model given by $\log_{10}(L_{i,t}+1) = C_i \cos(2\pi t/12 - \Phi_i) + \varepsilon_{i,t}$, where $L_{i,t}$ are the landings in region i at time t, and C_i the amplitude and Φ_i the phase of the harmonic term. $\varepsilon_{i,t}$ represents an error term with AR structure ($\phi_{1,i}$ and $\phi_{2,i}$) or MA structure ($\theta_{1,i}$ and $\theta_{2,i}$).

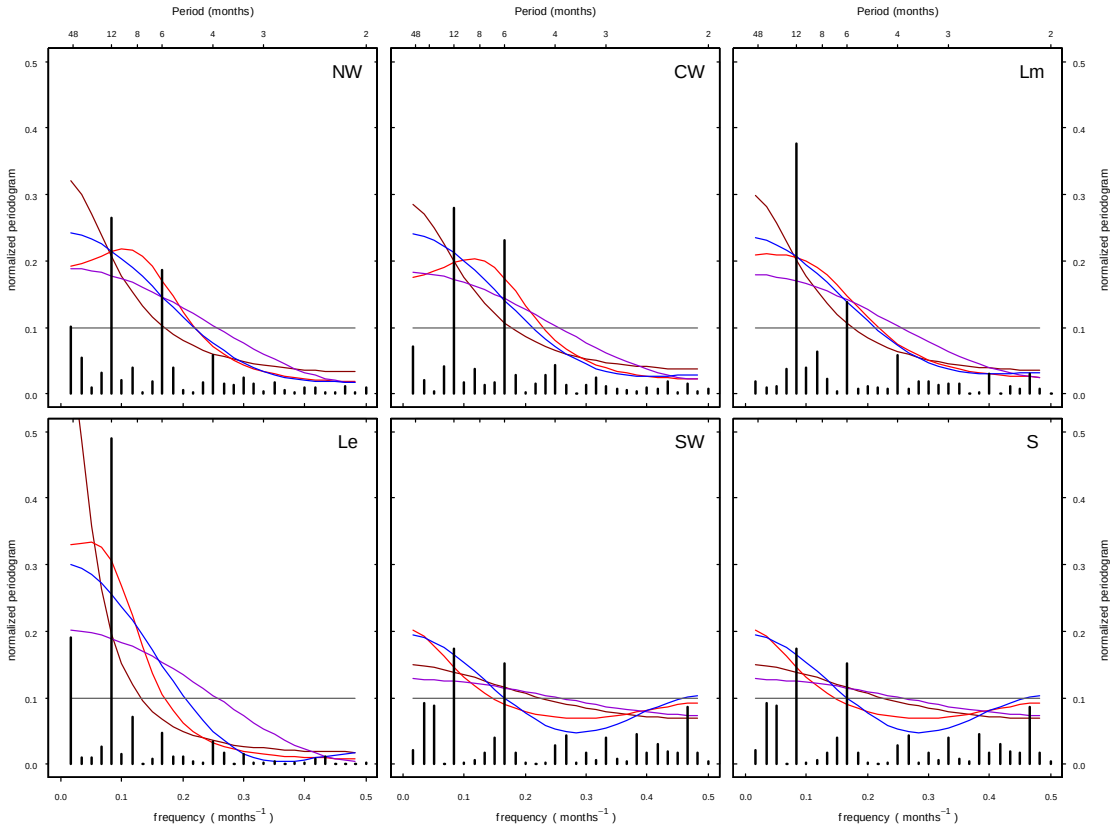

Figure 4. Normalized periodograms of the regional meagre landings (period: May-2002 through Apr-2007). In each plot the *y*-axis presents the normalized periodogram values and the lower *x*-axis presents frequency (ω). The upper *x*-axis presents frequency converted to period. Black vertical lines indicate the value of the periodogram at each frequency. The upper limit of the 95% confidence interval of the stochastic models used to test the significance of ω=1/12 were calculated according to Box *et al.* (1994) and Wilks (2006), and are displayed in colored lines: Grey line – White noise; Brown line – AR(1); Red line – AR(2); Violet line – MA(1); Blue line – MA(2). All confidence intervals were calculated assuming a χ^2 distribution with 2 df. Regional acronyms as in Figure 1.

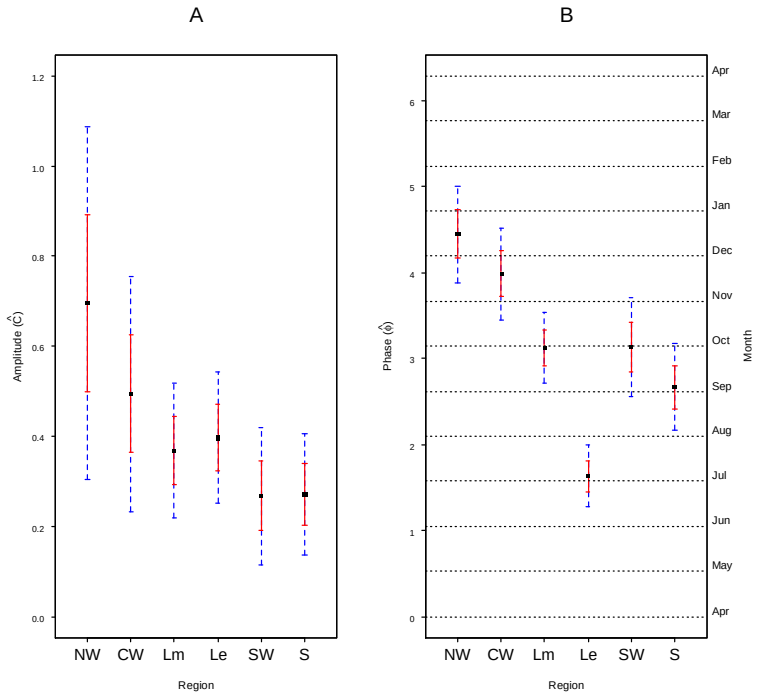

Figure 5. Estimates of the amplitude (A) and phase (B) of the annual period of regional meagre landings. These graphs represent the results of the fit of the fixed frequency harmonic regression to the regional data (see Table 2). In plot A the *y*-axis presents amplitude. In plot B the left *y*-axis displays phase values and the right *y*-axis the same phase values converted to months. The *x*-axis of both plots presents regions. Black squares present the point estimates; Red whiskers indicate the standard error of the estimate; Blue whiskers indicate the 95% confidence interval of the estimates. Regional acronyms as in Figure 1.

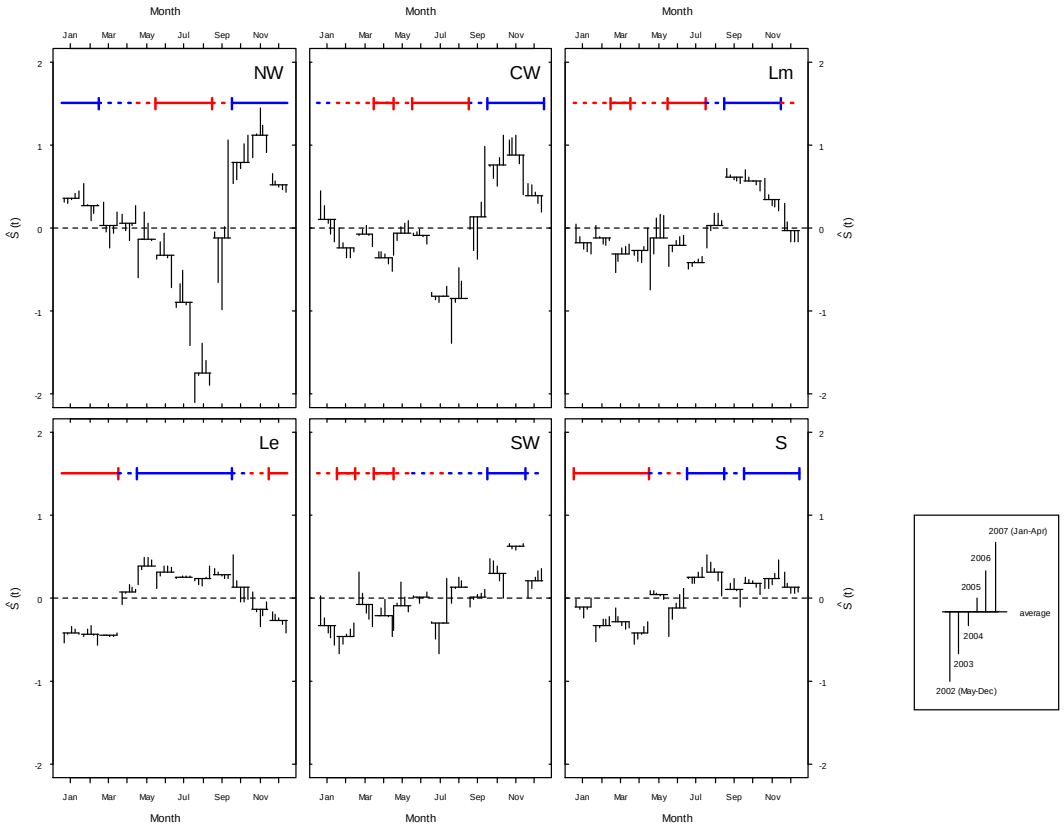

Figure 6. Regional seasonal cycle subseries plots ("monthplots") of the meagre fishery (period: May-2002 through Apr-2007). These results were derived from STL application on the transformed regional time series of landings and present details on intra-annual and inter-annual variations in the seasonality of the each time series. In each plot the *y*-axis presents the estimate of the seasonal component of the STL model and the *x*-axis the time (months). Horizontal black lines –inter-annual average of the monthly seasonal coefficients; Vertical black lines – values of the annual monthly seasonal coefficients in sequential order (also detailed in graph legend); Blue (red) full segments - months that registered positive (negative) seasonal coefficients in all years; Blue (red) dotted segments - months that registered positive (negative) average of seasonal coefficients but these were not positive (negative) in all years. Regional acronyms as in Figure 1.

To infer on migratory behavior of meagre using the observed seasonality in fisheries landings, the possibility that seasonality in fishing effort may be driving the annual seasonality of the landings must be excluded. For this, we examined sequentially three hypotheses: I) a strong seasonality in total *polivalente* fishing effort is inducing seasonality in the landings, II) the development of target effort by some vessels of the fleet in very specific months generates the observed patterns even under no effort-landings correlation of the overall fleet and III) seasonal changes in the fishing techniques or spatial location of the *polivalente* fishery take place that induce patterns on the fishery even under constant effort and no target effort. To evaluate hypothesis I, we examined the correlation between an effort indicator of the *polivalente* segment (monthly number of fish sales) and the meagre landings. Low correlation or even inverse correlation between effort and meagre landings was observed in all regions, except region Le (Figure 7). Accordingly, hypothesis I is likely to be rejected for all regions of the Portuguese coast with the exception of the Lisboa estuarine area.

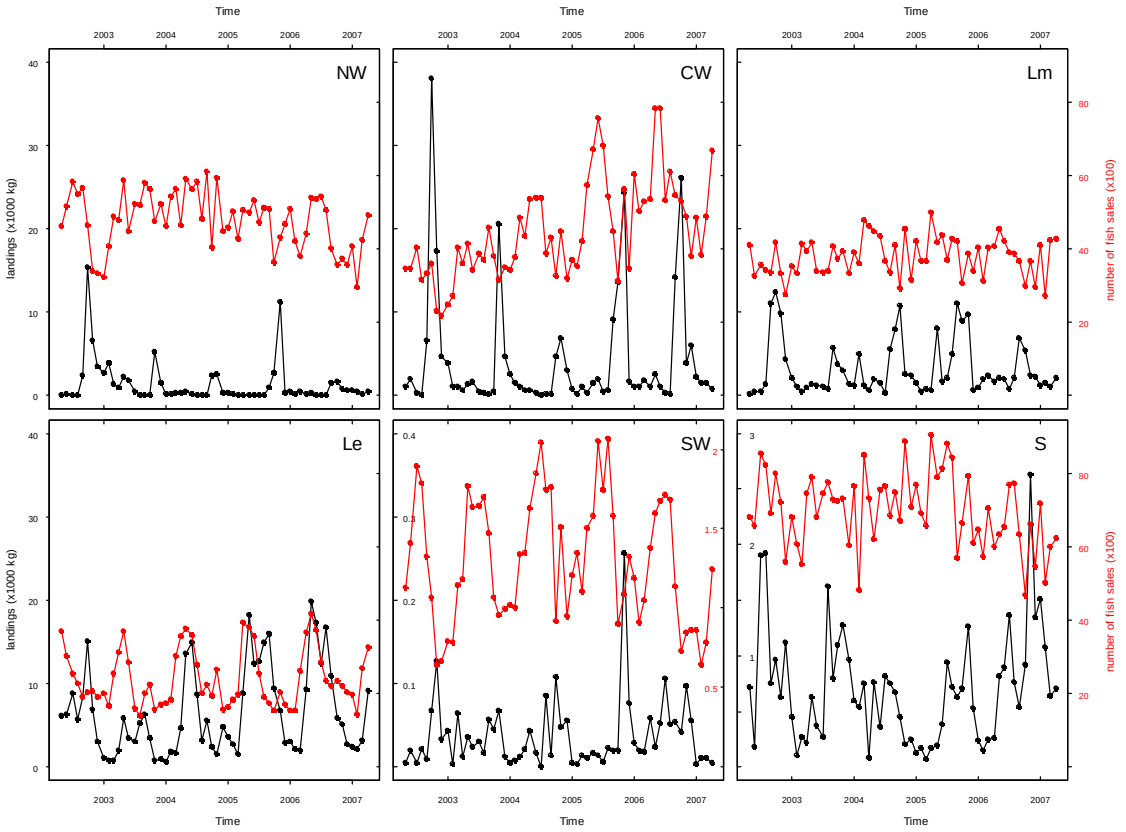

Figure 7. Relationship between regional landings of meagre and the fishing effort of the *polivalente* fleet (period: May-2002 through Apr-2007). In each plot, the left *y*-axis (and black line) present meagre landings (x1000 kg) and the right *y*-axis (and red line) present a monthly fishing effort measure of the fleet (defined as the sum across vessels of the number of fish sales registered each month by each *polivalente* vessel, red line). The *x*-axis presents time (in months). Pearson's correlation (r) and Spearman's rank correlation (r_S) - NW: r=-0.25, r_S=-0.43; CW: r=-0.14, r_S=-0.26; Lm: r=-0.15, r_S=-0.12; Le: r=0.50, r_S=0.46; SW: r=-0.17, r_S=-0.09; S: r=0.08, r_S=0.07. Regional acronyms as in Figure 1.

The regional plots of the proportion of meagre in vessels monthly landings against monthly cumulative meagre landings indicate that in Le the meagre landing season is established by targeted effort exerted by significant part of the fleet (Figure 8). On the contrary, in NW, CW and SW meagre landings should result from by-catch of other fisheries. In region Lm and S an intermediate graphical pattern indicates active targeting by only a reduced proportion of the *polivalente* vessels. Accordingly, hypothesis II should be rejected in regions SW, CW and NW.

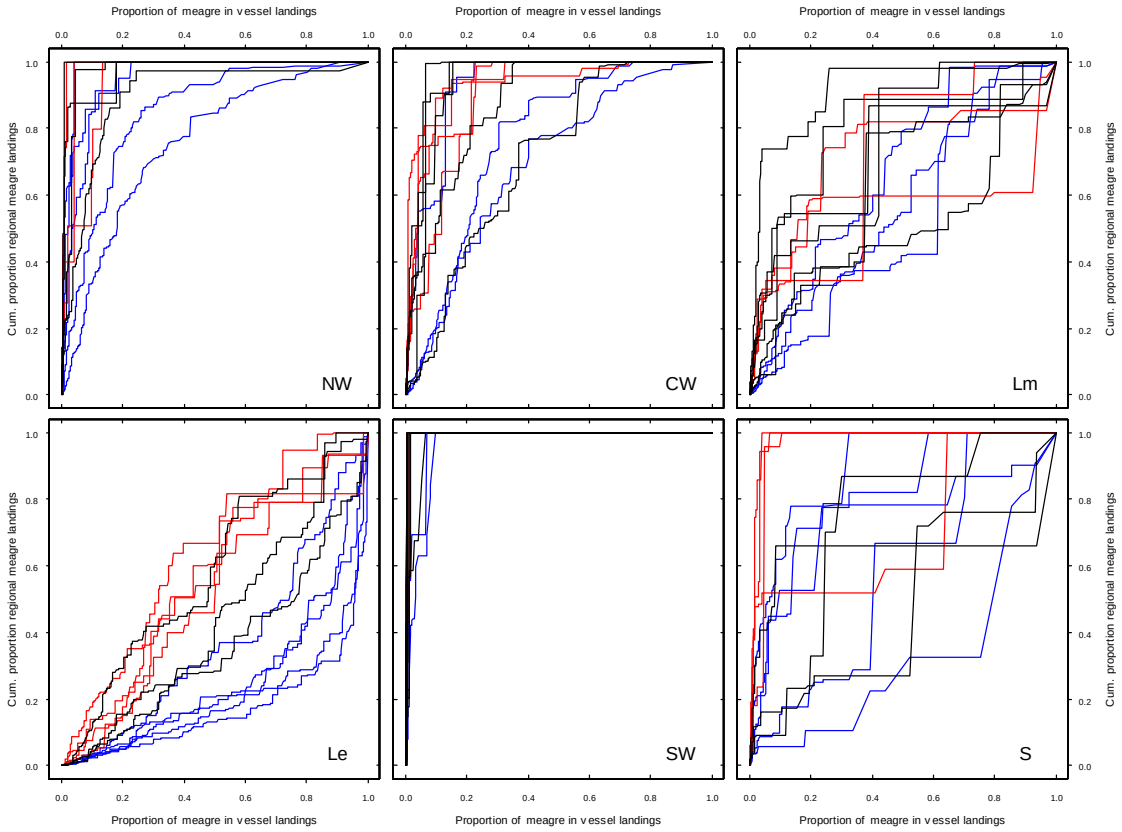

Figure 8. Monthly target and by-catch plots of the *polivalente* fleet that landed meagre (period: Jan-2005 through Dec-2005). In each plot, the *y*-axis presents the contribution of each vessel to the regional meagre landings (kg) and the *x*-axis presents the proportion of meagre in the overall monthly landings performed by each vessel. Color of the lines is related to the results of STL analysis (Figure 6): Blue lines correspond to months of the meagre landing season (blue segments in Figure 6); Red lines correspond to months of reduced meagre landings (red segments in Figure 6); Black lines correspond to months with intermediate landings (dotted segments in Figure 6). A sigmoidal or concave curve indicates target effort by a substantial part of the fleet while a convex pattern evidences the fishery as by-catch. See Biseau (1998) for more details on this type of plot. Regional acronyms as in Figure 1.

The main fish species landed by the vessels landing meagre in the different regions differed but did not vary markedly between the annual period of higher meagre landings and the annual period of lower meagre landings along the major landing areas of the Western coast of Portugal (Table 3). In fact, the meagre fishery seems to overlap the pouting fishery in NW, the pouting and ray fishery in CW and the hake and ray fishery in Lm. These resources constitute the major fisheries of the vessels landing meagre in these areas both during and outside the meagre season. These results give evidence for rejecting hypothesis III for the CW and NW area.

Table 3. Main species landed by the vessels *polivalente* fleet that landed meagre in the different regions (period: Jan-2005 through Dec-2005). Values present the proportion of species (or species group) in regional landings during meagre landing season (s) and outside the meagre landing season (ns). Seasonality was defined based on STL analysis (see Figure 6). Months included in s registered positive seasonal coefficients in all years (blue segments in Figure 6) and months included in ns registered negative seasonal coefficients in all years (red segments in Figure 6). Highlighted values are >10%. Species not representing >10% of any region's landings during the meagre landing season were grouped into the "other" category. Regional acronyms as in Figure 1

Species or group	NW		CW		Lm		Le		SW		S	
	ns	s	ns	s	ns	s	ns	s	ns	s	ns	s
conger eel	9.6	4.2	5.6	4.5	0.9	0.8	5.8	5.8	1.0	0.4	0.8	1.1
cuttlefish	0.4	1.0	2.9	2.1	3.2	2.7	**11.0**	1.7	4.9	**11.2**	**13.6**	4.3
gurnards	1.9	4.6	0.9	2.4	0.3	0.3	1.4	0.2	1.5	0.7	1.3	0.7
hake	8.0	4.3	1.4	0.9	8.6	8.0	3.5	5.1	1.0	1.0	2.0	4.0
horse mackerel	3.4	2.9	8.3	2.2	3.7	5.0	1.0	4.8	4.3	4.6	2.1	**12.1**
mackerels	**11.2**	1.6	**19.4**	7.2	4.0	2.8	0.1	3.6	8.9	**21.0**	**21.5**	**14.7**
meagre	0.0	1.6	0.2	5.1	0.8	4.1	5.4	**25.8**	0.0	0.4	0.1	0.4
monkfish	2.0	1.4	2.5	0.0	1.6	0.0	0.1	0.1	8.3	0.0	2.2	0.2
octopus	9.7	**21.6**	**10.5**	**26.5**	**13.8**	**10.7**	**35.0**	8.9	**21.1**	**16.0**	**16.2**	**15.6**
plaice	0.6	6.1	0.3	0.8	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.1
pouting	**22.4**	**21.2**	9.1	**11.6**	0.9	1.2	1.8	1.8	2.1	2.6	1.7	1.0
rays	4.4	3.6	9.6	**10.6**	6.4	4.5	7.3	3.7	9.5	8.5	4.9	4.2
sardine	7.9	3.3	4.4	2.9	5.3	2.4	0.3	8.1	1.2	1.1	4.3	**12.3**
seabass	1.1	4.4	1.1	3.9	0.3	0.4	4.7	2.9	1.5	4.0	1.0	0.8
soles	2.3	4.8	3.1	3.5	5.6	7.1	9.6	9.4	**13.6**	3.6	7.5	3.5
sparids	1.6	2.7	3.5	4.4	2.6	4.1	1.2	3.3	6.7	**16.2**	**12.8**	**13.6**
other	**13.4**	**10.7**	**17.1**	**11.5**	**42.0**	**45.8**	**11.6**	**14.6**	**14.3**	8.9	7.7	**11.2**

Taking into consideration the results of the analyses on hypotheses I, II and III and the fact that a) in region Le there is a plentiful array of observations attesting that vessels have adapted their fishing practices to the exploitation of the resource, b) the meagre fishery in Le targets adults and smaller sizes but mostly small sizes are landed during the main landing seasons in CW and NW, and c) meagre juveniles are absent from estuaries north of the Tagus estuary, we conclude that it is likely that the meagre juveniles present significant seasonal movements along the Portuguese coast. According to our data these movements involve a concentration in the Lisboa estuarine area from May to September followed in Autumn-Winter months by a significant reduction of abundance in this area. This reduction is accompanied by a spreading northwards (and eventually southwards) along the Western coast of Portugal during autumn and winter. These results give extra statistical credibility to the overall juvenile distribution pattern proposed by Quéro (1987) and Quéro and Vayne (1989) in the Bay of Biscay confirming the generalization of the proposed juvenile migration to the western coast of Portugal. The Southern coast evidences a somewhat distinct cycle that may be associated with larger equitability of adult and juveniles landings of the species and does not seem to be related to the western coast movements.

4. Concluding remarks

Our results shed new light on the practical uses of readily available landings data in the context of data-poor fish and fisheries. The combination of periodogram, harmonic regression and STL can successfully analyze periodical patterns in time series of landings even when landings are low in value and noisy in appearance as is the case of small-scale fisheries. Additionally, data on fisheries landings also provides significant insight into the structure of the fishing effort even when this is exerted by multi gear and multi species fleets. In the meagre case, the combination of these two types of analyses presented sufficient power to evaluate major hypotheses on its life cycle. The narrowing of the vast array of possible life history patterns to the most relevant ones and the ability to identify the major patterns of small-scale artisanal fisheries is of particular significance in both developed and undeveloped countries since resources allocated to research on these fish and fisheries are generally reduced (Mahon, 1997). Given this, the set of techniques and analyses used in the study of the Portuguese meagre may well contribute to the understanding of fish life history and fisheries dynamics in the wider array of data-poor situations still existent worldwide.

Acknowledgments

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Chapter 2B

What can a Japanese tuna trap set off the coast of southern Portugal tell of the meagre *Argyrosomus regius* life-history?

Nuno Prista[a,b], Miguel Neves dos Santos[c], Hirofumi Morikawa[d], Pedro Gil Lino[c], José Lino Costa[a], Cynthia Jones[e] and Maria José Costa[a,b]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Unidade de Recursos Marinhos e Sustentabilidade, Instituto Português do Mar e da Atmosfera, Avenida 5 de Outubro s/n, 8700-305 Olhão, Portugal
[d]Tunipex – Empresa de Pesca de Tunídeos S.A., Armazém 2, PO Box 456, 8700-914 Olhão Portugal
[e]Center for Quantitative Fisheries Ecology, Old Dominium University, 800W 46th St., Norfolk, VA 23508, USA

Proceedings of the 5th World Fisheries Congress, Yokohama, Japan (2008).

What can a Japanese tuna trap set off the coast of southern Portugal tell of the meagre *Argyrosomus regius* life-history?

Abstract: *Argyrosomus regius* is a large valuable sciaenid with scarcely known life history in European waters. In Southern Portugal individual fish may be worth 100-300**€** but even so are not significantly targeted by the local fishing fleets that remain largely unaware of its temporal and spatial distribution. Off the Southern coast of Portugal a Japanese model stationary uncovered pound net (*Teichi-ami*) regularly catches *A. regius* and provides a unique opportunity to study the species life-history in European coasts. We compiled 10 years (1995-2004) worth of daily data on *A. regius* landings, capture dates, number of individuals and individual size from this fishing gear and use this information to analyze the fish temporal patterns and size structure of its schools. *A. regius* was caught year-round being most frequent and abundant between June and October. Most individuals (99%) were adults. Solitary adults were common throughout the year but exceptionally large schools were caught between June and November. These schools included a wide range of fish sizes. These data constitute the first evidence of systematic schooling of *A. regius* in European waters from early summer to autumn. We discuss these and other results within the context of worldwide sciaenid life-histories.

Keywords: fisheries landings, set net, size distribution, migration, meagre

1. Introduction

The meagre (*Argyrosomus regius*) is one of the largest sciaenids in the world. Its distribution ranges from the English Channel to Senegal (including the Mediterranean Sea). In Europe it attains large individual size and commercial value (up to 2 m and 50 kg; > 12 €.kg^{-1}) and presents economic significance to recreational and small-scale commercial fishers along the Atlantic coasts of France, Spain and Portugal (Quéméner, 2002; Prista *et al.*, 2008). However, the meagre life-history in European waters including its seasonal distribution and migratory patterns are still scarcely known (Quéméner 2002; Prista *et al.*, 2008). Current knowledge on the distribution and migratory behavior of the fish in Europe indicates that adult and juvenile meagres form schools that migrate seasonally from coastal waters to estuarine spawning and nursery grounds where the major fisheries take place (Quéro and Vayne, 1987; Prista *et al.*, 2008).

In comparison to estuaries, much less information exists on the distribution of meagres in the marine environment (Quéro and Vayne, 1987; Quéro and Vayne, 1993). This is particularly the case of Portugal where there is little commercial targeting of the meagre outside the Tagus estuary (Prista *et al.*, 2008). In this context, the operation off southern Portugal of a particular gear (a tuna trap) where meagre is a frequent by-catch (Santos *et al.*, 2002) provides an opportunity to further investigate the marine part of the species life-cycle. In this study we analyze the meagre seasonality and schooling behavior in coastal waters using 10-yr of detailed

landings information from the tuna trap. We interpret the observed patterns in light of what is currently known about meagre seasonal distribution and migratory behavior in European waters.

2. Materials and methods

The Portuguese tuna trap is a Japanese-design stationary uncovered pound net (*teichi-ami*) operating off the port of Fuzeta in southeastern Portugal (Prista *et al.*, 2007). It is located over sandy bottoms at about 30 m deep. The trap is composed of two leader-nets (a full-time inshore net and a seasonally placed offshore-net) that extend perpendicular to the coastline (from 20 to 60 m depth) and intercept fish moving along the coast. The leader-nets convey the fish to a main-frame composed of playground, a slope-net and a bag-net (80-120 m long). Associated to the bag-net are one to two sea-cages which are used for fish husbandry. The mesh size is relatively large, varying between 60 and 90 cm in leader-nets and 9 and 15 cm in the bag-net. Daily hauling of the bag-net provides the landings from this gear but, on some occasions, part of the catch (including meagre) is redirected to the adjoining cages and the fish are landed a later occasion (Prista *et al.*, 2007). The overall geometry of the tuna trap has been suffered changes throughout the years (e.g. the inshore leader-net has varied between 650 m and 1000 m in length).

Date, catch in numbers and individual weights of all meagres landed daily between November-1995 and December-2004 were retrieved from fish sales tickets and checked against the company logbooks on a day-by-day basis. This allowed the identification of the accurate dates when caged individuals were landed as-well as the determination of the gear soaking time between hauls. The data (n = 10174 individual fish weights, dates and soaking times of n = 1755 hauls) was logged into a databases and analyzed in terms of catch per unit effort (CPUE), monthly frequency of occurrence (%FO) and size of schools caught. CPUE was defined as number of fish per haul. Monthly %FO was defined as the number of hauls with meagre over the total number of hauls carried out in each month. School type was defined as a categorical variable with the following categories: isolated (1 fish in haul), small school (2–10 fish in haul); medium school (11–50 fish in haul), large school (>51 fish in haul). To investigate the monthly size distribution of the catch, weight frequency analyses were carried out. In this analysis 1 kg size classes were considered. Since preliminary data analysis indicated that soak time had a small but positive effect on the daily catch, hauls with soak time >1 day were excluded from the analyses involving school type. Similarly, to avoid probable effects of husbandry on the weight of individual fish only non-caged individuals (i.e. of the fish landed immediately after capture) were considered in weight frequency analyses.

3. Results

The meagre was present in the tuna trap catches throughout the year (Figure 1). From the 1755 fishing days recorded, in 1451 the bag-net had been checked the day before. These 1-day

soak time hauls, registered 7515 of the 10174 meagres registered in the period. In seven fishing dates more than 150 meagres were landed, six of them registering a soak time of only 1day.

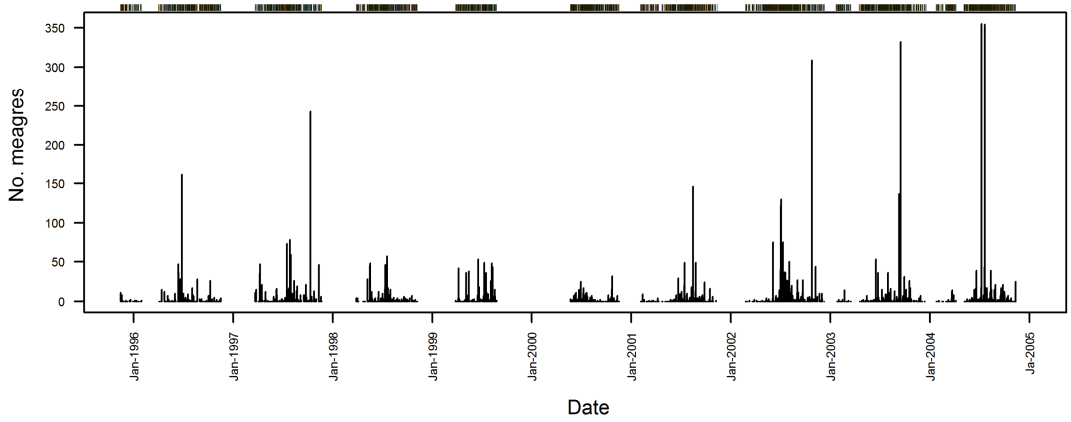

Figure 1. Number of meagres landed from the tuna trap per hauling date. Total hauling dates (with or without meagre) are represented by a "I" on the top of the graph.

The fishing effort and catch per unit effort varied throughout the year as did the meagre occurrence in tuna trap landings (Figure 2). Fishing effort was lower in winter months when the nets are frequently brought back for clean up and prevent damage from winter storms. Maximal fishing effort took place in summer when the bag-net is hauled nearly everyday (Figure 2, left panel). CPUE varied similarly with low catches in winter and high catches throughout the summer and autumn. However, in spring CPUE remained low, registering a local minimum in May (Figure 2, right panel).

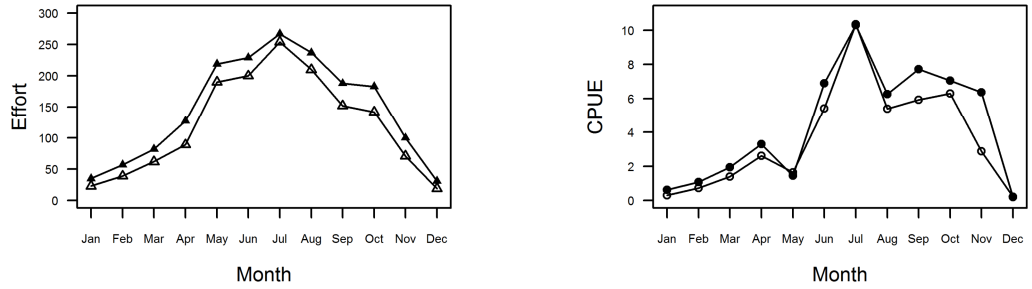

Figure 2. Monthly effort (number of hauls, left panel) and catch per unit effort (meagre/haul, right panel) of the tuna-trap during the 1995-2004 period. Full symbols represent all hauls. Open symbols represent hauls with 1-day soak time.

Minimum frequencies of occurrence were registered in winter and spring (November to May) and maximum frequencies of occurrence in summer and early autumn (June to October) (Figure 3). In all months, the vast majority (>80%) of hauls yielded no meagre or yielded it in

small quantities (<10 individuals per haul). Large schools (max. 355 fish) were only captured from June to October and in a very low number of hauls (1% of total hauls, <4% of monthly hauls). Despite their scarcity, moderate and large schools accounted for 77% of the meagre landings (Figure 3, right panel).

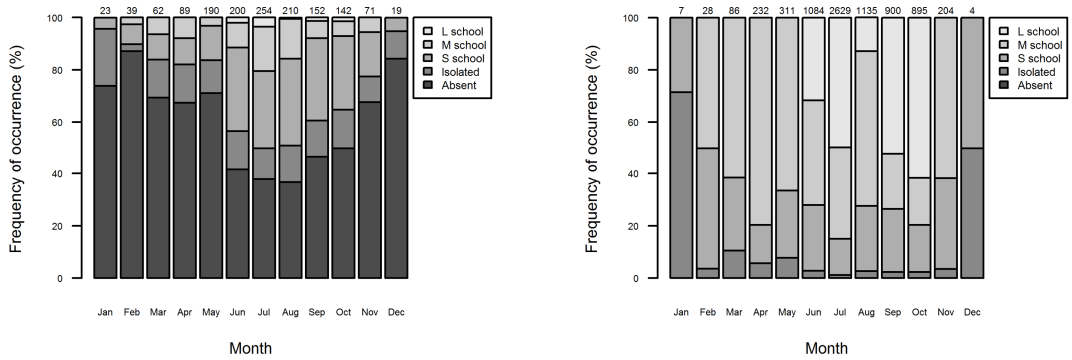

Figure 3. Frequency of occurrence (left panel) and number (right panel) of meagre in tuna trap hauls with 1-day soak time. “Isolated” = 1 meagre in haul; “S school” = Small school (2–10 meagres); “M school” = Medium size school (11–50 meagres); “L school = Large size school” (>50 meagres). Numbers above bars represent number of hauls (left panel) and number of meagres (right panel).

The fish landed from the tuna trap ranged 1–57 kg (average: 19 kg, median: 17 kg), over 80% of them being over 10 kg. Under-representation of fish smaller than 10 kg suggests these that fish may not be fully recruited to the gear or that smaller sized meagres were not significantly present in the fishing grounds. In all months, a relatively wide size range was observed in all types of schools suggesting that school formation in meagre is not size specific. Seasonal variations in median weight were observed across months that were relatively consistent across school types and capture years. These variations involved higher median weight in March-April (23 kg and 22 kg, respectively), followed by a sharp decrease in weight during May (12 kg), a new rise in June, July and August (21 kg, 20 kg and 21 kg, respectively) and a new sharp decrease in September, October and November (15 kg, 13 kg and 12 kg, respectively). In winter (December–February) the tuna trap catches were increasingly dominated by larger individuals (13 kg, 16 kg and 24 kg, respectively).

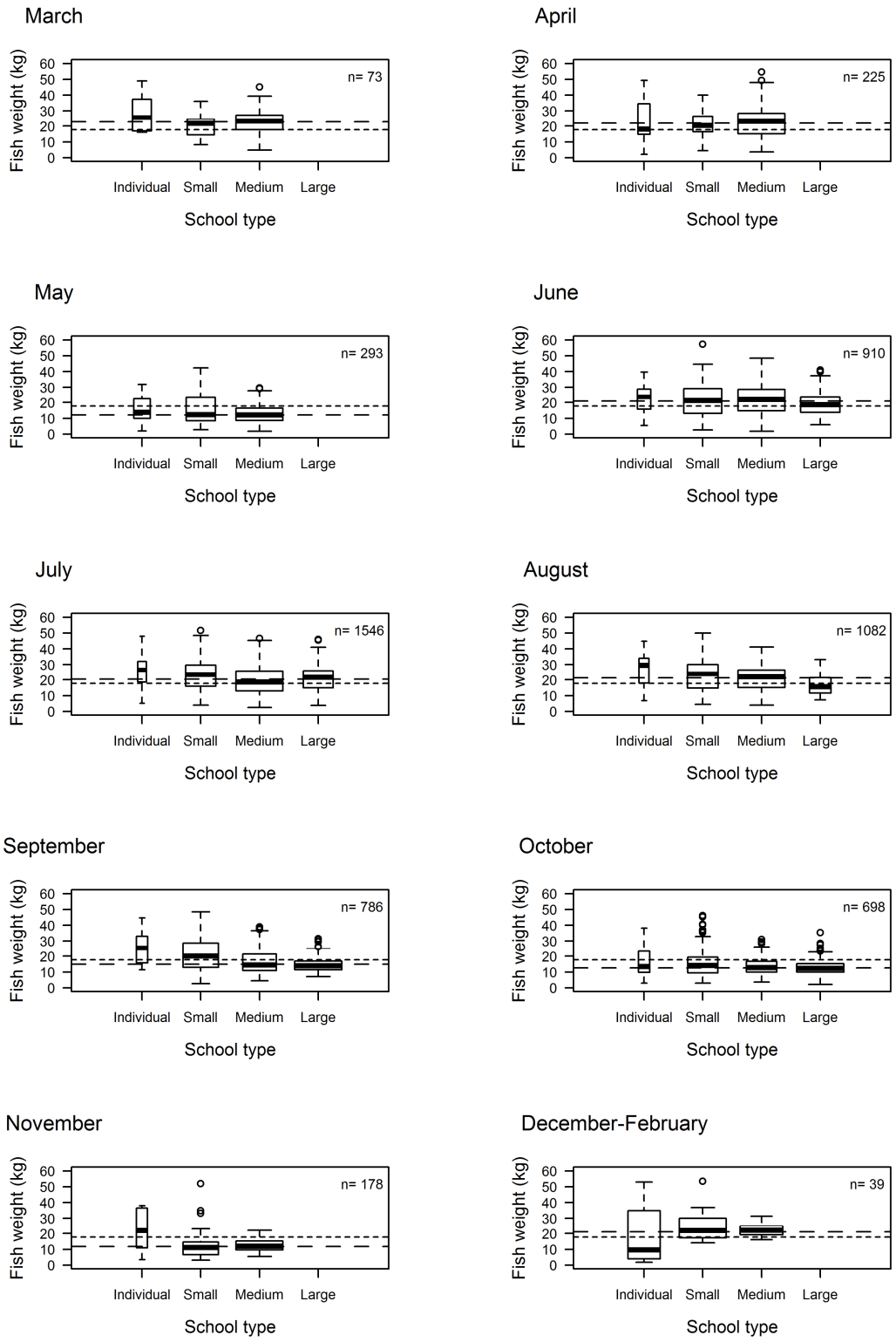

Figure 4. Monthly size distribution of meagre schools caught by the tuna trap in hauls with soak time of 1day. The width of the boxes is proportional to sample size. Dotted line indicates the overall median size of the 1995-2004 period (17.7 kg). The dashed line represents monthly median size across the period. School sizes as in Figure 3.

4. Discussion

The analysis of the tuna trap landings provides first evidence that meagre adults form large schools during summer in the shallow coastal waters off southern coast of Portugal. These schools seem to remain largely unnoticed by most local fishermen other than the tuna trap (Prista *et al.*, 2008). This might be due to lack of awareness by some fishers on the species abundance in the area but is most likely a result of the daily unpredictability in the spatio-temporal distribution of large schools. Even under the high commercial value of the meagre (a 20 kg fish may be worth over 250 €) this unpredictability probably rends unprofitable the use of the specialized gears required to target it (e.g. large mesh gill nets) since they also imply a lower chance of capturing smaller, but more predictable, species (e.g., sole, sea breams). Furthermore, the analyses showed that meagre catches are seasonal in the southern coast of Portugal. This seasonality may be related to seasonal variations in fish abundance in the area or to seasonal variations in fish activity, both of which may condition the catches from the tuna trap. The observed seasonality largely coincides with the distribution patterns and migratory behavior previously described for the French Atlantic coast (Quéro and Vayne, 1987; Quéméner, 2002) that indicate adult movement towards estuarine spawning habitats in late spring. They also strengthen the idea that the meagre adults are migratory and that migration patterns of both juveniles and adults may be similar along the European coast (Prista *et al.*, 2008). These patterns should involve schooling of pre-spawning adults and late spring movement of schools along the coast towards estuarine reproduction areas (Quéro and Vayne, 1987). Under this hypothesis, a temporally distinct movement pattern of larger (earlier) and smaller (later) adults may explain the drop in fish weight observed in May that largely exceeds weight losses resulting from fish reproduction. Also assuming migration, the mass schools detected in June and July may correspond to larger probably post-spawning fish migrating through the area and/or feeding in the area. The lack of large schools in spring probably relates to different routes of pre-spawning and post-spawning fish. More difficult to explain is the reduction in fish weight observed during late autumn. One possible explanation is that smaller fish may lag behind the bulk of larger adults in their movements towards deeper water overwintering grounds (Quéro and Vayne, 1993). The tuna trap data supports this explanation by evidencing a decrease in CPUE in late autumn and winter. However, this last result should be interpreted cautiously since fishing effort was reduced in the winter.

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Chapter 3

Meagre Sampling

Chapter 3

New methodology for studying large valuable fish in data poor situations: commercial mark-recapture of meagre *Argyrosomus regius* in the southern coast of Portugal

Nuno Prista[a,b], José Lino Costa[a], Maria José Costa[a,b], Cynthia Jones[c]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Center for Quantitative Fisheries Ecology, Old Dominium University, 800W 46th St., Norfolk, VA 23508, USA

ICES CM 2007/O:43, Copenhagen, Denmark, 18 pp.

New methodology for studying large valuable fish in data poor situations: commercial mark-recapture of meagre *Argyrosomus regius* in the southern coast of Portugal

Abstract: Life history parameters of several valuable marine fish remain unstudied in European waters. Such situations frequently arise because fishery-independent sampling methods are logistically difficult and/or costly to undertake initially given the scarcity of available biological knowledge, and so adequate and cost-effective surveys cannot be designed. In such cases, fishery-dependent sampling can provide representative samples while simultaneously keeping the costs per sample low. We developed a new fishery-dependent sampling methodology that combines representative sampling of fishery landings with economically feasible market sampling, and thus is capable of providing life history information on previously unstudied marine resources. This new methodology, termed "Commercial Mark-Recapture", involves tagging landed fish and the subsequent recapture of their body parts in the marketplace. As a case study, we applied this method to meagre (*Argyrosomus regius*), the largest and most expensive sciaenid landed from European waters. Little is known about its age, growth and reproduction even though it is economically important to local fleets of the Portuguese, Spanish, and French Atlantic coasts. Our results show that "Commercial mark-recapture" is highly effective, allowing a significant number of samples to be obtained, at low cost, with quantifiable spatial, temporal, size and gear coverage of landings. The conditions and assumptions required for the successful application of this methodology are discussed, as well as its applicability to the study of life history and fishery details of other European marine fish.

Keywords: life history, fishery-dependent sampling, market sampling, *Argyrosomus regius*

1. Introduction

Surveys provide data on fish abundance indexes, population structure and fish life history parameters that are used directly and indirectly in most fish stock assessments. Two types of fisheries surveys are generally considered: fishery-dependent (sampling of commercial and recreational fisheries) and fishery-independent (sampling during research surveys) (Hilborn and Walters 1992; NRC, 2000).

Fishery-independent surveys rely mostly on expensive research cruises performed during short periods of time. Survey data is considered of superior quality because they are independent of management measures, standardized fishing procedures are used, and both sampling statistics and biological information of target species are taken into consideration during survey design (NRC, 2000). However, they generally present a high cost-per-sample and a limited temporal duration that may compromise representative coverage of fish populations yielding less precise and potentially biased estimates of biological parameters. This is particularly the case of commercially significant stocks with high spatial and temporal variability (migratory stocks) (NRC, 2000) and stocks where research survey catches are so scarce that an adequate number of specimens cannot be easily obtained (ICES, 2007a).

Fishery-dependent surveys are useful in such cases. Along with providing age-length composition of landings for incorporation in assessment models, they can also be the source of

a significant number of biological samples destined to other studies. That is the case of market/port sampling, the most frequently used fishery-dependent method (Hilborn and Walters, 1992). Market sampling for age and reproduction is generally considered a cost-effective technique capable of comprehensive monthly coverage of fish populations despite several recognized limitations (*e.g.* Hilborn and Walters, 2002; ICES, 2007a). These surveys generally involve buying boxes of fish, either from the fishers or retail intermediaries. However, this is only possible if fish are landed round and only cost efficient if individual boxes comprise reasonable numbers of specimens and/or their acquisition costs are low. Where specimens attain large weight and/or high prices at local markets, biological sampling, even at the market place, also becomes sample-limited particularly if, under the need to maintain fish appearance and value, extraction of body parts cannot take place on specimens to be sold (Fritsch, 2005; Pilling *et al.*, 2007).

We developed a market-based sampling methodology that allows biological samples from large valuable fish to be obtained at low cost. The methodology, termed "Commercial Mark-Recapture" (CMR), is based on models of capture-recapture studies used for closed populations (Williams *et al.*, 2001) and tackles the above mentioned sampling difficulties without requiring specimen acquisition or direct manipulation of the fish being sold. CMR methodology relies instead on obtaining the samples at the point of the fish commercial circuit where dressing generally takes place and afterwards matching those samples to the original fish characteristics collected at landing ports. In the present note the CMR methodology is described and exemplified by application to sampling of otoliths and gonads of meagre (*Argyrosomus regius*), the largest and most expensive sciaenid in the European coasts.

2. Materials and methods

2.1. Commercial mark-recapture (CMR) definition

Commercial mark-recapture is the individual tagging of fish specimens before 1st sale followed by the recapture of body parts (biological samples) after being sold. Fish measurement, weighing and tagging takes place as the fish are landed. Fish are tagged with visible individually-coded tags attached to its body in a non-damaging way. After the sale, the persons responsible for fish dressing are asked to return both tags and biological samples to researchers that can then match samples to body measurements taken previously.

2.2. Case study: Meagre *Argyrosomus regius*

Fishery and landings

The meagre (*Argyrosomus regius*) is a large sciaenid that may attain 2 m total length and 50 kg weight (Quéméner, 2002). Age, growth and reproductive patterns of adult specimens (>70 cm) (Tixerant, 1974) are scarcely known in European coasts. In 2004 and 2005 the European commercial fishery along ICES Subareas VIIIa, VIIIb and IXa (landings taken place in France,

Spain and Portugal) registered ca. 800 t to 1700 t, respectively. Being a trophy fish adult meagres are also subjected to significant recreational fishery along its distribution range although no quantification of this component has ever been made. Along the Portuguese coast a minimum landing size of 42 cm is enforced. All landings consist of round fish with ca. 68% of them taking place in the Sesimbra-Peniche area (Western coast) and ca. 18% throughout Algarve (South Coast) (Portuguese Fisheries General Directorate: 2005 data). Landings in the Western Coast are composed of a mixture of juvenile and adult specimens, while in Algarve larger specimens are frequently landed (Santos *et al.*, 2002). Meagre supply to Southern markets is mainly conducted by a single fishing gear – a setnet - operating near Olhão. This gear lands fish between 60 cm and 180 cm (Santos *et al.*, 2002) that averaged ca. 15 kg in 2004 (N. Prista, unpub. data). Husbandry of adult meagres in nearby cages is performed when large schools are caught. At port, the setnet landings are frequently subjected to some degree of size selection and distributed throughout several Algarve 1st sale (=ex-vessel) auctions, namely Lagos, Quarteira, Olhão and Vila Real de Santo António (Figure 1). At the Olhão 1st sale auction meagre prices averaged 10 € kg^{-1} in 2005 (Portuguese Fisheries General Directorate: 2005 data) which caused most round specimens landed to cost over 100 € and specimens >40 kg over 400 €. Southern Portugal is a tourist area and meagre prices remain relatively stable year-round with minor fluctuations during the main landing period of meagre adults of the western coast (May and June). After 1st sale, retail prices escalade with meagre steaks found frequently at 16-24 € kg^{-1} in local fresh-fish markets.

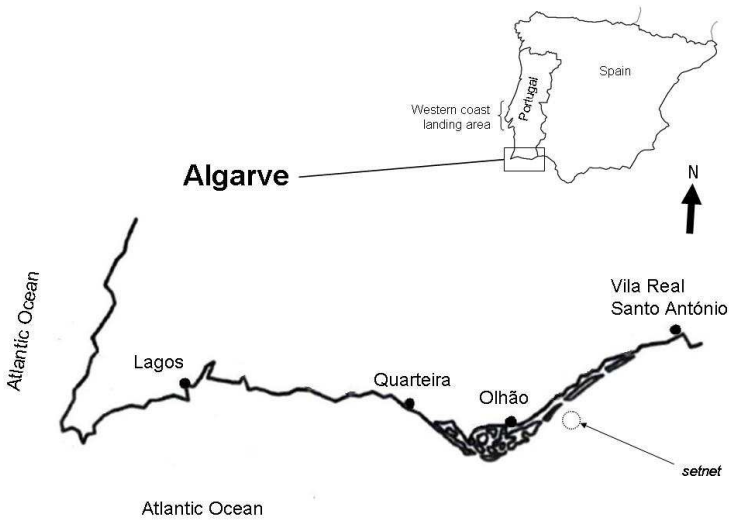

Figure 1. Geographical position of the setnet and main *A. regius* 1st sale auctions in Algarve region (South Portugal).

A. regius *commercial circuit*

Meagres landed from the setnet are generally auctioned in early morning (ca. 6:00 to 7:00). Meagre specimens are shown to buyers before being auctioned and afterwards removed by individual buyers to several Algarve fresh-fish markets. In some auctions, namely Vila Real de Santo António, some fish are bought by Spanish dealers and exported to the Spanish market. In

the remaining auctions meagres are generally bought individually (one fish = 1 box per buyer) and sold to final consumers over the next 1-2 days in the buyers' own market benches. In Southern Portugal, fresh-fish markets are open from ca. 8:00 to 13:00. There is no dealer specialization in meagre, with each dealer making available several fish species at his market bench. The meagre marketing strategy of individual dealers adapts depending, amongst other things, on specific orders, other fish species available, fish sold so far, or weekly planning based on evaluation of current and future demand (weekends). Some buyers sell meagre to local restaurants and hotels (round, gutted or dressed) but most are dressed at the markets and sold as fish steaks. Large fish heads present variable commercial value and are generally sold halved with the larger otoliths being kept as good-luck charms. During spring and early summer, ripe female gonads also present commercial value, being sold at around 10 € kg^{-1} and weighing between 1 kg and 4 kg. No commercial value is attributed to male gonads or less ripe female gonads.

CMR adaptations and procedures under the A. regius *commercial circuit*

We obtained the cooperation of the setnet fishers and of company that runs the 1st sale fish auctions prior to CMR application. This was necessary so that landings could be anticipated in a few hours (allowing one or two staff members to travel to the Olhão port) and, so that caged and catch-of-the-day fish would be kept separate once captured. After unloading, fish were measured (total length, to lowest 0.01 m), weighed (round weight, to nearest 0.1 kg) and tagged. The tags consisted of large bovine ear tags (PVC, 63x74x4 mm, 11 g, orange colour) imprinted with an individual fish code on one side and contact information on the other (Figure 2A). A loose cable tie was used to attach the tag around the 1st gill arch thus avoiding damage to the fish skin and the fish's fresh appearance. The tag was left visible, appearing slightly outside the fish operculum (Figure 2B). After fish auctions, fish buyers are generally hurrying to return to their markets to sell that morning. So immediately after the auction, we explained the purpose of the tags to fish buyers and briefed them on the study objectives. If the fish was to be gutted and/or beheaded they were asked to store the tag, along with viscera, gonads and one of the otoliths in a given plastic bag. Sample recapture took place during the following hours at the local markets after obtaining phone confirmation of fish dressing. Rewards for sample return were non-monetary and study-related (sectioned otolith pictures, fish recipes, certificates of participation in the study, information of fish names in foreign languages) with a minor component of small but useful gifts being distributed at random occasions (lanyards, ball-point pens). Occasionally we purchased fish gonads and tipped dealers' employees (1-2 €) for setting apart the samples during daily dressing procedures.

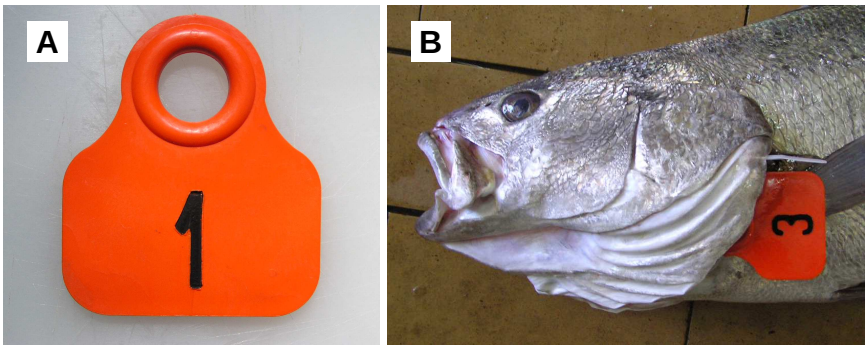

Figure 2. Tag (material: PVC; dimensions: 63x74x4 mm; weight: 11 g) used for commercial mark-recapture operations (A) and *A. regius* (L_T: 94 cm, W_T: 7.3 kg) with tag placed attached to the 1st gill arch (B).

2.3. Statistical design and analyses

External sexual dimorphism is not present in meagres, so sex identification was rarely possible previous to gonad recaptures. Taking this into consideration, our main objective was to recapture at least 10 males and 10 females per 10 cm size class (100 cm to 180 cm). Accordingly, we intensified tagging operations during the main period of setnet adult landings (June to September). In parallel, to study the adult seasonal reproductive cycle (and obtain samples for age validation studies) we maintained CMR operations even in months of lower setnet landings. In periods of higher adult landings (June to September) 10-12 fish per day were generally landed at each auction. The selection of the auction(s) where fish were to be tagged was made considering the personnel available (minimum 1 person per auction), the analysis of size distribution destined to each auction and the length classes that remained below our objectives. We tagged indiscriminately fish landed from cage and catch-of-the-day so that our study would not influence their usually equal price. Generally a decision was made to tag at least at Olhão auction and, if logistically possible and fish from specific size classes were present, at others auctions (Lagos, Quarteira or Vila Real de Santo Antonio). Frequently, in periods of lower captures when few fish are landed, these few are generally sold at Olhão auction and were tagged.

In CMR statistical analyses, we considered a tagging operation the daily tagging of *n* fish destined to a local auction. We modelled the probability of recapturing a tag (and sample) as the parameter *p* of a binomial model with *n* being the number of fish (and samples) tagged. We named p_{tag} this probability. In this type of CMR model, the binomial distribution describes the probability p_{tag} of obtaining X number of tags (and samples) out of the *n* fish tagged. The daily sampling is a census of tags where note is made on which ones are recaptured. Therefore, "recapture" (or binomial success) is defined as the recovery of both tag and sample and "lost" (or binomial failure) as the non-recapture of tag and sample. As a "sample" we define recapture of at least *i*) one sagitta "intact-enough" for ageing, or *ii*) gonads "intact-enough" for sex identification and weighing, or *iii*) gut "intact-enough" for stomach analysis. This last possibility was rare in our study (3% of recaptures) being included as success for sake of overall model simplicity. Our main interest was determining an estimate $\hat{p}_{tag}$, which we did for Olhão auction

data, that included the majority of tagging operations and tags placed. We used the additive properties of binomials to pool tag results from distinct tagging operations at this auction and estimated the 95% confidence intervals for $\hat{p}_{tag}$ using the normal approximation (Lindgren, 1993). Because under our sampling design *i*) no untagged fish is ever recaptured, *ii*) no distinction between failure reasons is made (all eventual reasons are pooled within the failure probability) and *iii*) no tag shedding occurs (*i.e.* recapture of samples with no tag code attributed to them) the daily tagging operations can be considered as having a fixed size (*i.e.* being closed) like the binomial model implicitly assumes. Additionally, by using the binomial model and then pooling daily tagging operations it is also assumed that tags results are independent and that their probability of recapture remains constant in each tagging operation (Lindgren, 1993).

The goodness of fit of the Olhão CMR binomial model was evaluated by applying our estimate $\hat{p}_{tag}$ to the number of tags placed at each tagging operation and subsequently comparing observed and expected recaptures with a Kolmogorov-Smirnov test. Finally, Chi-square tests were used to test independence between otolith and gonad recapture frequencies and assess the independence of each type of sample to fish size. We pooled size categories into [80, 110[cm, [110, 150[cm and [150-180[cm to increase sample size within each contingency table cell (Quinn and Keough, 2002). Given the practical interest of providing separate estimates for the probability of recapturing otoliths ($\hat{p}_{oto}$), gonads ($\hat{p}_{gon}$) and otoliths and gonads ($\hat{p}_{mix}$), we estimated these quantities using a similar procedure. A value of $\alpha = 0.05$ was used in all statistical tests and results for $\alpha = 0.1$ also presented where relevant.

3. Results

A total of 378 fish were tagged in 69 tagging operations. Tagging operations took place between June 2004 and May 2006, and accompanied the setnet landings with 84% of tagging operations (85% of tags) taking place in June-September. Overall, 72% of tagging operations (68% of tags) took place at Olhão auction and 20% (28% of tags) at Lagos auction. A mean of 5.5 fish (s.d.=3.8, min=1, max=18) was tagged per tagging operation and either one or two persons participated in CMR operations depending on number of auctions and number of fish tagged. The average length and weight of individuals tagged was 127 cm (s.d.=20, min=80, max=178) and 16.2 kg (s.d.=8.0, min=3.7, max=45.0), respectively.

The result from all tagging operations was 259 tags (and samples) returned (69% of tagged samples). The tags recaptured represented 239 gonads samples, 177 otolith samples and 166 samples comprising both otoliths and gonads. Only nine tags were returned with just guts present. Recapture numbers of otolith and gonads followed the size (Figure 3A) and monthly distribution of tagged fish (Figure 3B). Our aims of obtaining at least 10 otoliths of males and females per 10 cm size class were fulfilled between 110 cm and 150 cm in females, and between 100 cm and 140 cm in males, but we obtained at least two otoliths samples on all size classes above 90 cm. In what concerns reproductive samples (excluding caged individuals),

over 8 female gonads were collected monthly between June and September (6 in March and November) and at least 6 male gonads were recaptured in June, August, September and November (4 in March). Accordingly we obtained at least 2 male and female gonads in every month we tagged except for April and October (1 male each).

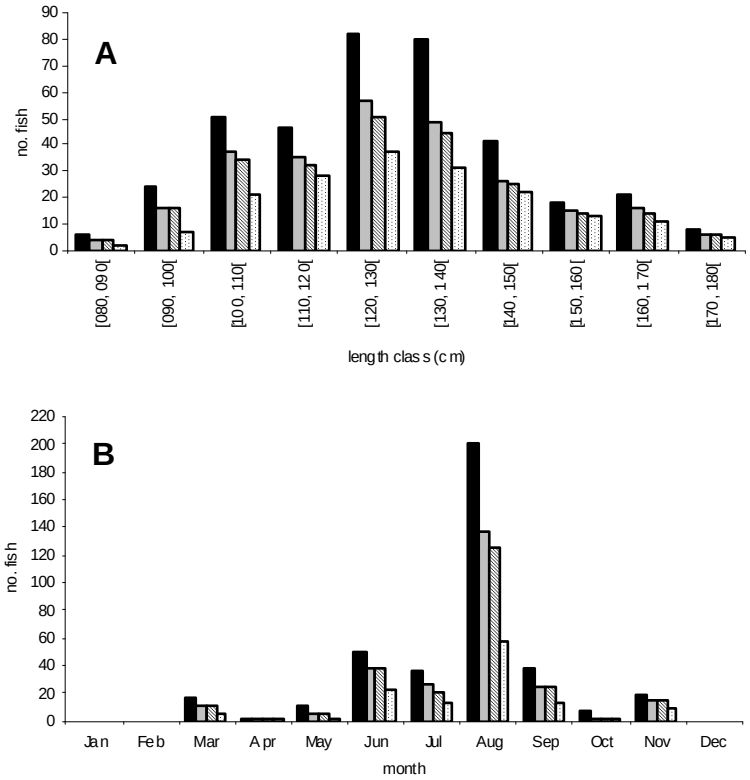

Figure 3. Distribution of fish tagged (black), fish recaptured (grey), gonad (striped) and otolith (dotted) samples obtained during 2004-2006 commercial mark-recapture operations according to size class (A) and landing month (B).

The binomial model estimates for the Olhão auction reveal a recapture probability $\hat{p}_{tag}$ =0.75 (s.e.=0.03, C.I. (95%): 0.69 to 0.80) and a good fit of the binomial model (n, 0.75) in predicting the number of tags obtained at each day (K-S test: D=0.18, p>0.05, n=50). Contingency table analyses to recaptured and non-recaptured tags at Olhão did not present significant variation with fish size (Chi-sq=0.91, d.f.=2, p>0. 05 n=378) (Figure 4).

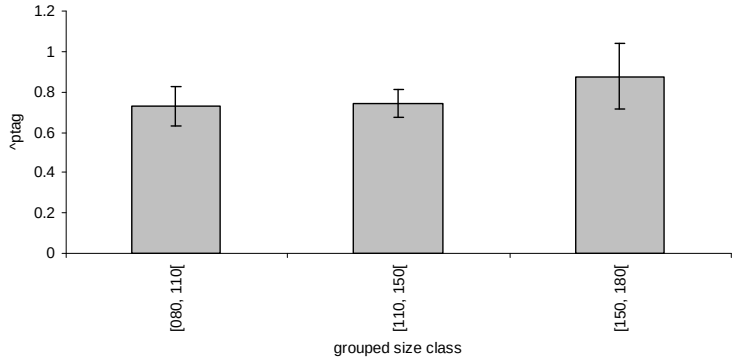

Figure 4. Estimates of probabilities of recapture ($\hat{p}_{tag}$) (and 95% C.I.) per grouped size class (data: Olhão auction house, 2004-2006 tagging operations; $n_{[080\text{-}110[}$=77; $n_{[100\text{-}150[}$=163; $n_{[150\text{-}180[}$=16).

The recapture frequencies of gonads and otoliths were both independent from fish size (Chi-sq_{gon}=1.07, d.f.=2, p>0.05; Chi-sq_{oto}=4.95, d.f.=2, 0.05<p<0.1; n=256) (Figure 5). However there seems to be a slight trend towards lower frequencies of otolith recaptures in fish of smaller sizes (Figure 5) that may be affected by the slight non-independence of gonad and otolith recapture frequencies (Chi-sq=3.63, d.f.=1, 0.05<p<0.1) and the low sample size at the largest class ($n_{[80\text{-}110[}$=56; $n_{[110\text{-}150[}$=121; $n_{[150\text{-}180[}$=14).

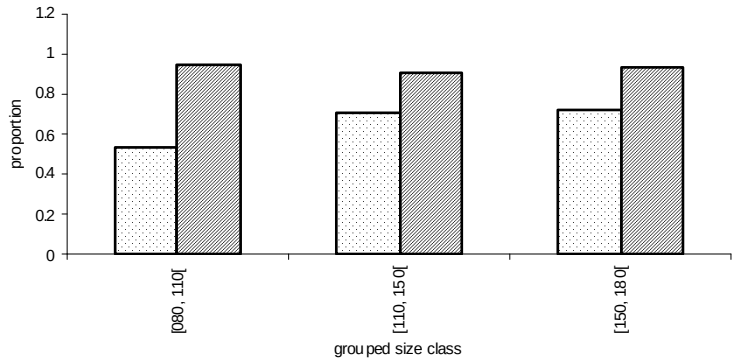

Figure 5. Proportion of recaptured tags presenting otoliths (dotted) and gonads (striped) per grouped size class (data: Olhão auction house, 2004-2006 tagging operations) ($n_{[080\text{-}110[}$=56; $n_{[100\text{-}150[}$=121; $n_{[150\text{-}180[}$=14).

The Olhão estimates of sample recapture probabilities were $\hat{p}_{oto}$ =0.49, $\hat{p}_{gon}$ =0.68 and $\hat{p}_{mix}$ =0.46. However since there was some evidence for dependence of otolith recaptures from fish size and for non-independence of otolith and gonads recaptures, estimates $\hat{p}_{oto}$, $\hat{p}_{gon}$ and $\hat{p}_{mix}$ should be viewed as merely indicative under the currently applied CMR design.

4. Discussion

In the present study a new market-sampling based methodology, the "commercial mark-recapture" (CMR) was tested on age and reproductive sampling of the meagre (*Argyrosomus regius*), a large valuable fish of Northeast Atlantic waters. Like several other large valuable fish, adult meagres are: a) long-lived and fecund apex predators, b) an important resource for regional recreational and commercial fisheries and, c) present significant sampling difficulties related to scarcity in surveys and high commercial price of biological samples (Quéro and Vayne, 1987; Hermas, 1995; Quéméner, 2002). In the present study, with the application of CMR, a first comprehensive set of adult *A. regius* otoliths and gonads was obtained with associated costs being restricted to minor advertising, crew wages, and local travel expenses related to recapturing the samples. The recaptured *A. regius* samples consisted of 259 fish samples from 80 cm to 180 cm total length, amounting to 4.2 ton round fish weight. Similar fish samples, from such a wide size spectrum, would be achieved with great difficulty using conventional fishery-independent surveys that generally yield limited number of adult specimens (Quéro and Vayne, 1987). On the other hand, assuming that all these samples had to be bought at 1st sale, an investment of about 42 000 € would be necessary for regular market-sampling these ≈250 fish. Such amount would most likely be unavailable to initial studies on this species unless regional management problems and/or international economic relevance were identified. The CMR methodology provides an additional possibility of circumventing the problem of "no funding→ no research→ no funding" and so may prove beneficial to other preliminary studies of large valuable species such as those on *A. regius* in other geographical areas.

The results obtained while applying CMR to the commercial circuit of *A. regius* in Algarve provide insight into factors that may affect its application to other large valuable fish life history studies. Underlining the efficiency of CMR in the presented case study was the assurance of relatively stable recapture rates from fish dressing locations. In the case study presented, the commercial circuit was simple - mostly developing locally within a ca. 50 km radius from each auction house - and the fish auction buyers were both the final retailers and the ones responsible for the fish dressing procedures. CMR can however be adapted to commercial circuits involving multiple intermediaries, wider spatial distribution of tagged fish, and distinct fish processing schemes as long as the fish dressing points in the commercial circuit are identified and agree to cooperate in sample recapture. On the other hand, in the case study presented, approximately two months of field work were carried out sometimes under high variability in weekly landings. However, it is possible that, when applied to species with more regular landings, CMR tagging procedures may become well-integrated within already existent port-sampling plans of significant fish resources (Commission Regulations CR 1639/2001 and CR 1581/2004). Several large valuable fish species whose regional studies are currently plagued by low sample sizes and few resources allocated might benefit from these kinds of CMR adaptations. Included in such resources are some that have recently been considered within ICES working groups and whose life history parameters are still scarcely known in some parts of their distribution range (ICES 2007b, ICES 2007c).

In the present study, CMR estimates of proportion of tags recaptured (and their standard errors) were obtained based on a binomial model. The Olhão binomial model presented a good model fit so the estimated probability of recapture (p_{tag}) seems to be reliable predictor of recaptures obtained. However, the validity of this model in the CMR context relies on defined assumptions: a) there are only two possible outcomes to each tag (success or failure); b) the tags are independent; and c) the tags share the probability of success/failure. Of these, a) is guaranteed by our case study design. However, a more detailed analysis of b) and c) may prove beneficial in the context of broader CMR applications more subjected to the need of obtaining truly representative samples from the landed population:

b) Independence of tags: in our study of Olhão auction, over 25 different dealers were involved in *A. regius* buying but it was common practice that a core group of dealers would make more systematic acquisitions then others. Additionally, some dealers would buy up to 3 fish in a single auction when higher numbers of fish were available and their individual price was lower. These incidents of clustered tags generate lack of true independence. In a situation where true independence is required, a more complex model accounting for such buying behaviour stratification would be necessary to obtain a true random sample of the population, particularly if dealers were found to actively select for fish size or other fish characteristics.

c) Unequal tag recapture probabilities: unequal probabilities issues in CMR can be divided into two categories: *i*) those related to fish and *ii*) those related to study design. In what concerns *i*) fish related probabilities it is known that wild mark-recapture or band-recovery studies are frequently affected by size or sex-related biases (Williams *et al.*, 2001). Our case study results indicated that a slight size-related reduction in otolith recapture frequencies may be present in lower size classes (Figure 5). One explanation for this is that smaller fish heads are less frequently sliced (the fish being sold whole but gutted) thus inducing lower otolith recaptures at smaller sizes. Carrying out a study to evaluate this hypothesis would implicate a study-design that assured independence between otolith and gonad recaptures so that the effective reduction on otolith recapture probability could be accurately estimated. In what relates to sex-related biases, they were not tested for because there was no previous knowledge of sex distribution. As most of the tags were placed out of the reproductive season and *A. regius* is sexually monomorphic, sex-related biases in our recapture probabilities estimates should be minor. However, a more comprehensive analysis of these effects would be necessary if we were interested in estimating, for example, the sex-ratio of the population (thus requiring extra certainty on the statistical coverage of our recaptures in relation to the tagged population). In what concerns *ii*) study design, CMR p_{tag} estimates may be vulnerable to differences in both recapture effort and number of tags placed on each tagging operation. In Olhão auction the maximum number of tags placed per day was 12 (in 4 tagging operations). However, it seems reasonable that under a higher number of tags the probability of recapture of the last tags of a tagging operation might change. Amongst other possibilities, this change in probability may occur due to recapture effort limitations (e.g. with the same personnel sampling not all tags may be recaptured), due to higher clustering when higher numbers of fish are present at auction (see

above) or due to higher dealer unwillingness to cooperate (e.g. if tag return demands are considered excessive by dealers). Again, as long as the un-recaptured group of each tagging operation is a random sample in terms of the characteristics of interest (e.g. length or sex) this reduction in individual probabilities of recapture should not implicate significant bias in the sample obtained.

The major potential of CMR relies on the fact that a fish sample can be accurately matched to original fish data (length, weight, scales, genetics, etc.) even if recaptured in a distinct temporal and spatial framework. By doing so, CMR allows larger (and hence more precise) biological samples to be obtained that could be difficult to obtain otherwise. As seen above, besides eventual contribution to the knowledge of large fish species whose biology is still scarcely known, the CMR may be applicable on a wider basis to the study of stocks traditionally difficult to assess like tuna and billfish (Pilling *et al.*, 2007). If the above mentioned assumptions of the CMR model can be fulfilled (or their eventual biases quantified) and tagging is done randomly, the recaptured samples matched to specific areas, gears, or months, will be representative of the tagged population and hence of landings. If such can be guaranteed, applicability of the CMR to a broader array of fisheries studies including sex-ratio, age-length key, and maturity ogive determinations may become feasible, particularly on highly migratory species whose market-sampling design is, due to necessity, less constrained to the significant bias introduced by sole landing analysis (Pilling *et al.*, 2007)

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Chapter 4

Age and Growth

Chapter 4

Age determination in meagre *Argyrosomus regius*

Nuno Prista[a,b], José Lino Costa[a], Maria José Costa[a,b] and Cynthia Jones[c]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Center for Quantitative Fisheries Ecology, Old Dominium University, 800W 46th St., Norfolk, VA 23508, USA

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Age determination in meagre *Argyrosomus regius*

Abstract: The meagre is one of the world's largest sciaenids but its ecology, fishery, and population parameters are scarcely known. In the project "Meagre, *Argyrosomus regius* – biological data towards management and production of a finfish resource" (DGPA-MARE: 22-05-01-FDR-00036), the Centro de Oceanografia of FCUL (Portugal) and the Center for Quantitative Fisheries Ecology of ODU (VA, USA) investigated a set of methodologies to improve meagre age determination along its distribution range. In this study, we provide detailed protocols on the use of otolith thin sections and scale acetate imprints in determining meagre age. For each hard part, we present textual and photographic descriptions of the collection, preparation, and interpretation procedures, and report on the main difficulties met by age readers during age interpretations. We also provide details on the calculations involved in final age assignment to meagre specimens captured on the Portuguese coast. Finally, we discuss the relative importance of scales and otoliths, and their different preparation methods in routine meagre age determination and integrate the procedures into existing knowledge on age determination of other sciaenid species.

Keywords: age determination, growth, meagre, *Argyrosomus regius*, otoliths, scales.

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1. Introduction

The meagre (*Argyrosomus regius*, Asso 1801) is one of the world's largest sciaenids, attaining over 180 cm in total length and 50 kg in weight (Quéméner, 2002; Costa *et al.* 2008). It is a coastal fish (<80 m deep) whose distribution extends from the English Channel to Senegal (including the Mediterranean Sea and Black Sea). Its largest fisheries take place in Mauritania, Morocco, and Egypt, which together comprise over 80% of the *ca.* 10 000 t world annual catch (Quéméner, 2002; FAO, 2009). In European countries, annual meagre landings are generally below 500 t and the fish is of secondary importance in national capture production totals (FAO, 2009). Even so, due to its large size, high ex-vessel prices, and high seasonal availability in inshore and nearshore waters, the meagre constitutes an important target species for many local small-scale multi-gear multi-species commercial fleets and the recreational sector (Quéro and Vayne, 1987; Quéméner, 2002; Silva *et al.*, 2002; Prista *et al.*, 2008). This importance is underscored by the recent development of meagre aquaculture production and by the ecologic value the species presents as a top marine predator in European coastal waters (Quéro and Vayne, 1987; Quéméner, 2002; Jiménez *et al.*, 2005). However, to date the biological characteristics of the meagre have remained scarcely studied worldwide and its fisheries are yet to be routinely monitored or assessed in African and European waters.

Determinations of fish age are an important step of fisheries research and stock assessment because age data is a primary input in the estimation of population vital rates like growth or mortality (e.g., Haddon, 2001). This is particularly so in long lived species where other methods, e.g., length-based approaches, are difficult to apply (Sparre and Venema, 1998). Until recently, the age of meagre had only been studied in North African waters where its long-lived nature was established (maximum age: 15 to 31 years) and its growth first modeled (Tixerant, 1974; Hermas, 1995). However, past research relied on methodologies that were neither detailed nor validated and that are currently considered outdated for sciaenid age determination (namely, break-and-burn of otoliths and analysis of fresh scales). In fact, it is now widely accepted that analysis of otolith thin sections is the most reliable method to determine sciaenid age (Lowerre-Barbieri *et al.*, 1994; Campana and Jones, 1998; VanderKooy and Guindon-Tisdel, 2003; Liao *et al.*, 2008) and that, if scales must be used, they should be imprinted prior to observation to facilitate their interpretation (Matlock *et al.*, 1993; Lowerre-Barbieri *et al.*, 1994; VanderKooy and Guindon-Tisdel, 2003). Furthermore, it is widely recognized that age determinations of any fish species should be based on standardized and validated protocols that assure the validity, replicability and comparability of results across studies and geographical areas (Campana, 2001; Morison *et al.*, 2005).

Recently, Costa *et al.* (2008) made a first evaluation of the main biological characteristics of the meagre captured on the Portuguese coast. Costa *et al*. report was published in Portuguese language and so was of limited availability to the international community; however, it provided the first comprehensive analysis of the meagre growth and age structure in European waters (e.g., new maximum age: 43 years) and involved the development and validation of age determination criteria for meagre otolith thin sections. Nevertheless, because

of the need to focus on the estimation of the biological parameters of the species and discuss fisheries management and aquaculture production, the authors did not provide a full account of the age determination protocols they used nor did they detail specifics of meagre otolith interpretation; they also did not report on subsequent research carried out on the use of scale acetate imprints to determine meagre age, which may be useful to assess meagre fisheries in budget-limited situations (Prista *et al.*, 2007).

In this study we provide detailed protocols on the use of otolith thin sections and scale acetate imprints in meagre age determination. These protocols are the basis of the Costa *et al.* (2008) report and present the methodologies currently used to determine the age of meagre on the Portuguese coast. In the protocols, we provide in-depth detail on the specific procedures required to collect, prepare and interpret each meagre hard part. Additionally, we report on the most common difficulties met during meagre age interpretations and provide details and examples on final age assignment. This work is considered important because it updates and substantiates past literature on meagre age determination, promoting the training of hard part readers across several European and North African countries, and contributing to a standardization of age determination procedures across several fields of research, namely fisheries, ecology and aquaculture.

2. Materials and Methods

The protocols are based on the observation of meagre otoliths (n = 748) and scales (n = 362) collected from the Portuguese coast from 2000 to 2007. The sample comprised fish from both sexes and included at least 10 otoliths and 10 scales from each month. The fish ranged between 5 cm and 182 cm total length, thus spanning the size range of the species. Otolith samples comprised at least 10 fish for each 10-cm size class between 0 cm and 180 cm, fish over 180 cm being less well represented (n = 4). Scale samples comprised at least 10 fish for each 10-cm size class between 20 cm and 180 cm, fish over 180 cm and fish below 20 cm being less well represented (n = 3 and n = 6, respectively). More detailed coverage of the sampling methodologies can be found in Prista *et al.* (2007) and Costa *et al.* (2008).

The terminology, methods and protocol structure were based on Pentilla and Dery (1988), Schwarzhans (1993), Ericksen (1999), Assis (2000), Panfili *et al.* (2002), VanderKooy and Guindon-Tisdel (2003) and Liao *et al.* (2008), with adaptations and additions as required by meagre specifics. Preparation of the hard parts for observation was carried out according to section 3.2 and section 4.2. Otolith thin sections were observed at 8–40x magnification on a Leica MZ-12 stereomicroscope equipped with hand-adjusted light orientation, pointer unit, and dark-field polarizing filter. Scale imprints were observed on a Bell and Howell R-735 microfiche reader equipped with 20 mm and 29 mm lenses (20x and 32x magnification, respectively). The primary criteria established for age interpretation (as well as any references made to the precision of the age determination methods) resulted from randomized observations of hard-part preparations. These observations were carried out with knowledge of month of capture but

without knowledge of any collection detail. Additional interpretation criteria (sections 3.3.5.3 and 4.3.5.3) resulted from observations carried out with knowledge of fish size or after analyzing size-at-age plots. Finally, in agreement with previous work that established the interpretation of otolith thin sections as an accurate means of ageing long-lived sciaenids (Campana and Jones, 1998), a joint analysis of 77 otoliths and matching scales was carried out to check and refine the scale interpretation criteria.

Digital pictures of thin sections (resolution: 150 ppi) were taken at 6.3–25x magnification on a Leica MZ-6 stereomicroscope equipped with a Leica DFC 280 digital camera using Leica Image Manager 500. Digital pictures of scales imprints (resolution: 800 ppi) were taken at 9–50x magnification on a Minolta MS-7000 digital microfilm scanner using IrfanView. Image processing after capture was carried out in Paint.net and was restricted to left–right flipping, resizing and rotation, contrast and brightness adjustments, and minor background clean ups.

3. Otolith Protocol

3.1. Collection

The otoliths used for meagre age determination are the *sagittae*. In meagre, the simplest method to collect these otoliths involves sawing off the top of the fish head (**Figure 1**). This is accomplished by making two cuts on the fish head – one longitudinal and one transverse – that expose the top part of the brain cavity. The cuts may be done with a strong knife (small specimens) or an electric hand saw (large specimens). The longitudinal cut should run parallel to the frontal plane of the fish and pass slightly above the eyes; the transverse cut should run parallel to the transverse plane of the fish and pass near the insertion of the opercula (**Figure 1A**). After this, the top of the head should come off easily and the fish brain should be exposed **(Figure 1B–C).** The *sagittae* are located in the posterior ventrolateral regions of the brain cavity and can be removed with tweezers (**Figure 1D**). The sawing off method is fast and easy to integrate into schemes involving routine sampling of biometric and reproductive variables. However, it severely damages the appearance of the fish, thus reducing its commercial value.

When it is necessary to avoid loss of commercial value, the *sagittae* are better removed using less damaging techniques (**Figure 2** and **Figure 3**). In meagre, the otic capsules are located at the base of the skull, underneath the pharyngeal teeth and near the dorsal insertions of the first gill arches. In smaller meagre, the best way to reach the capsules is through the gill cavity by pulling the operculum open (**Figure 2A**) and making a small anteroposterior incision at the dorsal insertion of the upper limb of the first branchial arch (**Figure 2B**). The incision should be just enough to loosen the arch without detaching it, leaving the capsule's surface exposed (**Figure 2C**). Then, a small lid can be carved out of the capsule using a scalpel or a sharp knife (**Figure 2D, 2E)** and the otoliths extracted. After the extraction, the bone lid, the gill arches, and the operculum can be put back in their original positions, leaving the external appearance of the fish intact for marketing purposes (**Figure 2F**).

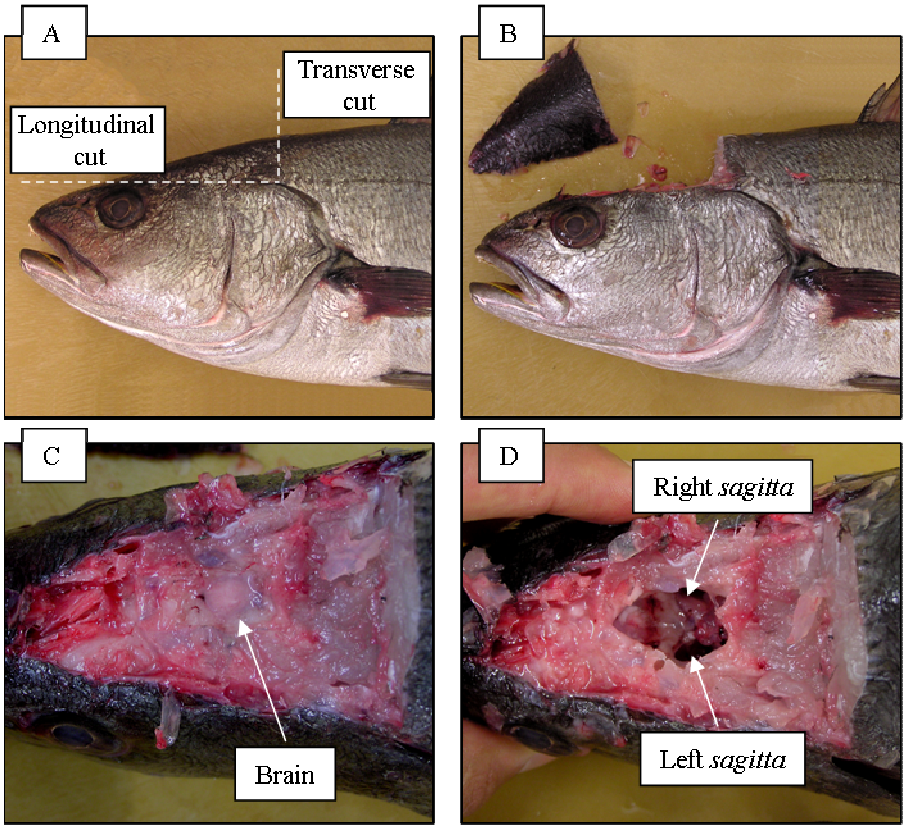

Figure 1 – Otolith extraction by sawing off the top of the fish head. See explanation in text.

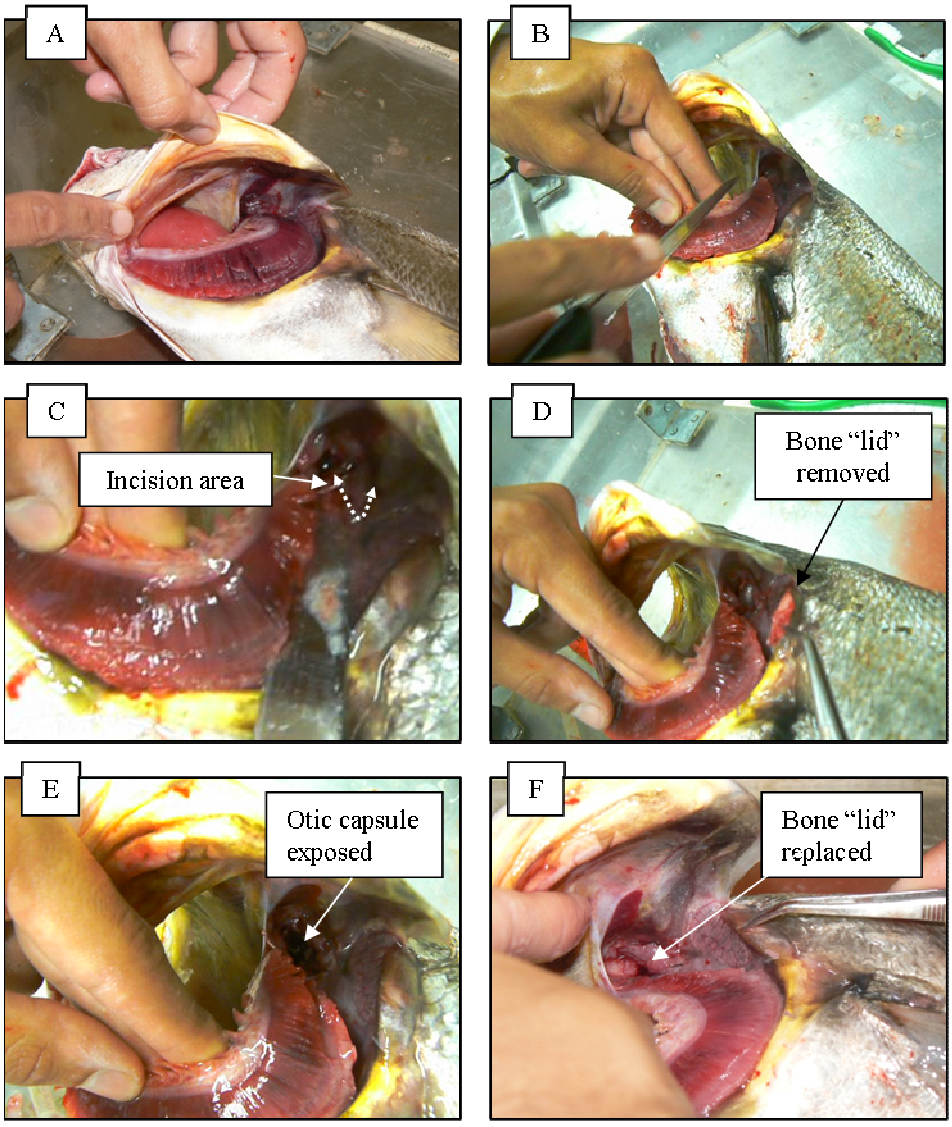

Figure 2 – Otolith extraction through the gills (small fish). See explanation in text.

In larger meagre, the opercula and the gill arches are stiffer, so reaching the otic capsules without damaging the appearance of the fish becomes increasingly difficult. In such cases the otic capsules are best reached through the top of the pharynx **(Figure 3)** than through the top of the first gill arch (**Figure 2**). This is achieved by making a dorsoventral incision just posterior to the fifth gill arch (**Figure 3A**). The incision should extend from the dorsal to the ventral insertions of the gill arches, loosening them without detaching them. After that, the gill arches can be lifted against the operculum and a second cut is made around the upper pharyngeal tooth plates' to expose the otic capsules (**Figure 3B**). An elliptical bone "lid" may then be carved out of the capsule's surface using a strong knife (e.g., an oyster knife) (**Figure 3C–D**) and the otoliths pulled out inside their sacs (**Figure 3E**). After the extraction, the bone lid, gill arches, and the operculum can be put back into position to preserve fish market value (**Figure 3F**).

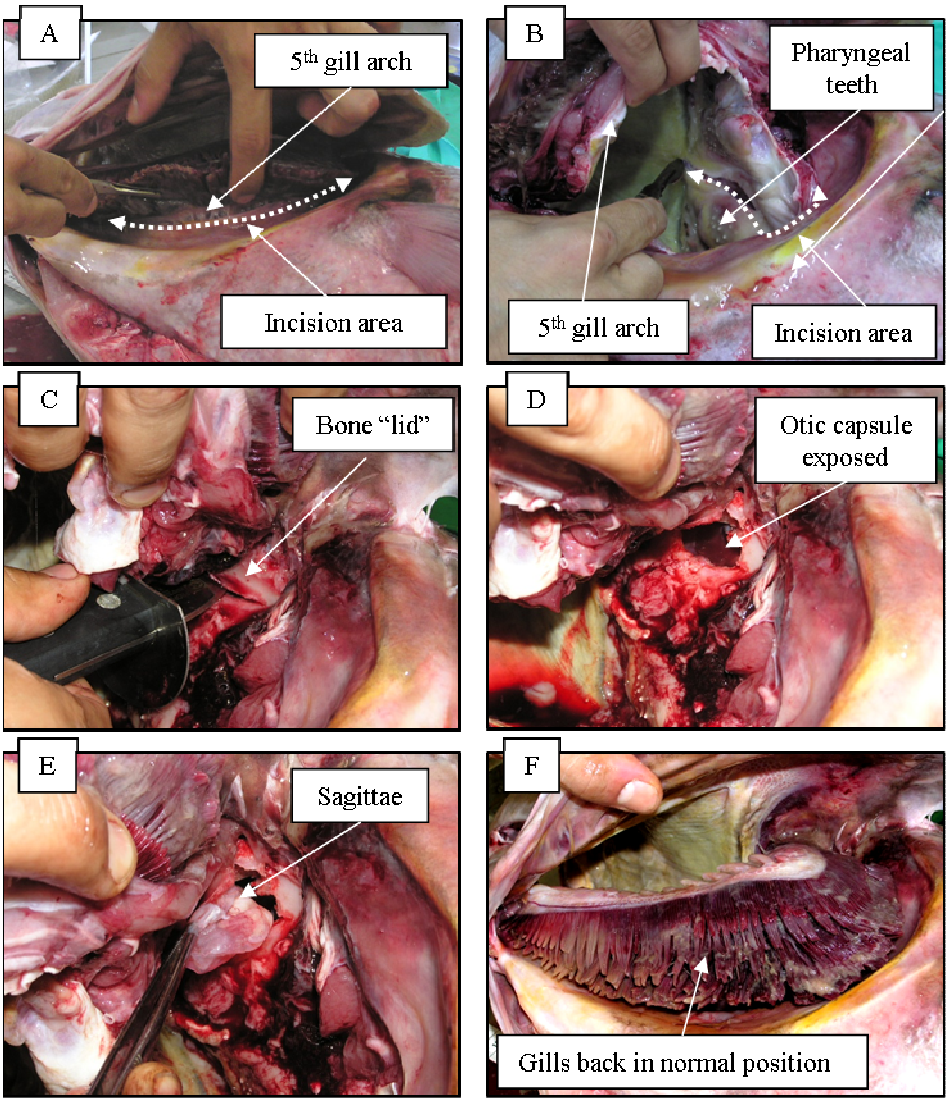

Figure 3 – Otolith extraction through the gills (large fish). See explanation in text

The meagre *sagittae* are large and robust, weighing up to 14 g each. Consequently, they can be freely handled without much risk of breaking. Before storage, any remains of adherent tissue should be removed from the otolith surfaces by scrubbing them with a soft toothbrush

under running tap water. The clean otoliths can then be left to dry at room temperature for a few hours and stored in plastic vials.

3.2. Preparation

The meagre otoliths are too thick for direct use in age determination. Consequently, thin sections have to be obtained before they can be used to determine fish age. In meagre, otolith thin sections are taken along a specific plane of the otolith body so some familiarization with otolith's external morphology is required to carry out the sectioning procedures.

Meagre *sagittae* present distinct morphological features on their proximal (or inner) and distal (or outer) sides (**Figure 4)**. The most conspicuous features are a tadpole shaped *sulcus acusticus* on the proximal side (further divided into an anterior *ostium* and a posterior *cauda*) and a conspicuous protuberance termed "umbo" on the distal side[1]. When observed in proximal view, left and right *sagittae* are easy to distinguish: left *sagittae* present the tip of the *cauda* to the right of the observer, and right *sagittae* present it to his left (**Figure 4)**.

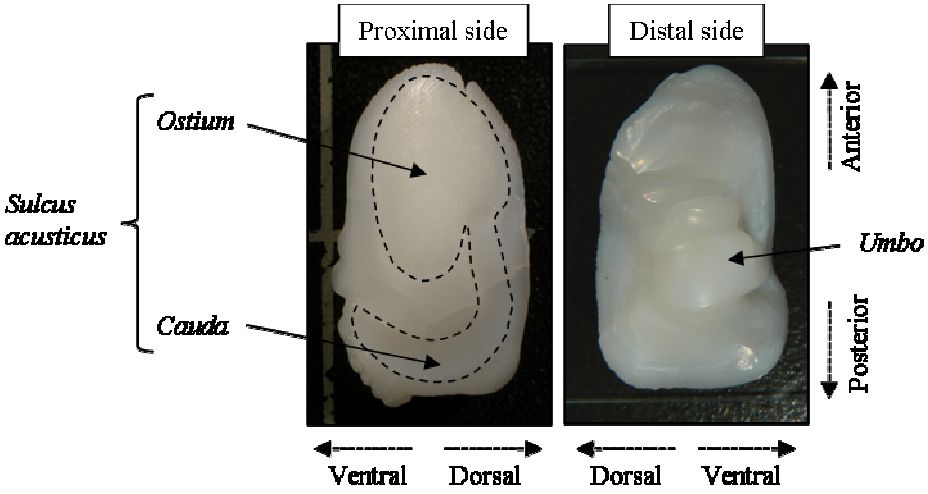

Figure 4 – External morphology of a right meagre *sagitta*. The dashed line circumscribes the *sulcus acusticus*.

To obtain thin sections, meagre otoliths are mounted, partially embedded in a clear adhesive (e.g., Aremco Crystalbond 509), onto standard microscope slides. For age determination, it makes no difference which otolith (left or right) is mounted but abnormally crystallized otoliths should be avoided (**section 3.3.5.3)**. Otolith embedding in Crystalbond adhesive requires previous softening of the originally solid adhesive sticks over a hot plate. In doing this, care should be taken to keep the adhesive temperature just above its softening point (71ºC) because higher temperatures may crack the otolith surface. Then, a bed of soft Crystalbond is laid on the glass slide and the otolith is placed, distal side downwards, embedded into the adhesive. While doing this, it is important to make sure the otolith is in tilted

[1] Note: younger meagre present several protuberances instead of a single umbo. These protuberances represent the internal primordia that in older fish appear fused into a single umbo (see Figure 6).

position, i.e., both its anterior tip and its *umbo* should be in contact with the slide **(Figure 5A)**, because this improves section quality (see **section 3.3.5.1)**. It is also important to make sure that the adhesive bed completely encompasses the distal side of the otolith **(Figure 5A)** because this will confer robustness to the mount and reduce otolith breaking during sectioning. Crystalbond adhesive takes a few seconds to harden and can be reheated if it is necessary to readjust otolith position. After embedding, a dorsoventral pencil mark is drawn on the otolith's outer face. This marking should be located at one-third the distance between the posterior margin of the *ostium* and the anterior margin of the *cauda* and indicates the sectioning plane **(Figure 5B)**.

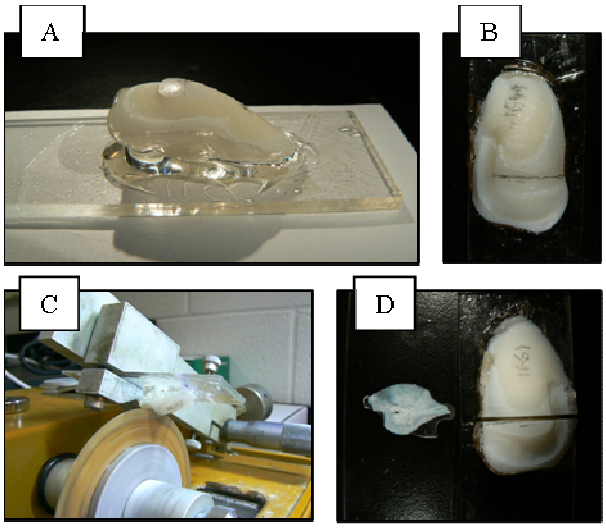

Figure 5 – Aspects of otolith preparation. A – embedded otolith; B – marked otolith; C – low speed sectioning; D – overview of sectioned otolith and otolith thin section.

Meagre otoliths should be sectioned on a low speed saw (e.g., a Buehler IsoMet Low Speed Saw) equipped with a fine-grit diamond-impregnated grinding wheel (e.g., a Norton 3-in diameter 0.006-in thick 1A1 Diamond Grinding Wheel). Given the large size of many otoliths a "one-blade" saw setup is preferable to a "two-blade + spacer" saw setup. However, the latter may still be used to provide faster sections of smaller otoliths. Under a "one-blade" setup, the otolith slide is positioned so that the grinding wheel runs immediately posterior and parallel to the pencil mark. The saw is then turned on and the otolith is slowly rested on the wheel for sectioning **(Figure 5C)**. After a few turns, arm weights (up to 75 g) can be added to speed up the sectioning. The first cut should stop when the wheel hits the adhesive bed. At that time the arm is adjusted approximately 0.5 mm in anterior direction and the second cut is performed. When the second cut finishes the thin section is ready and can be removed from the adhesive after slight reheating of the glass slide **(Figure 5D)**. Overall, the preparation of meagre thin sections may take between 5 and 90 minutes depending on the otolith size and the saw speed and arm loads being used.

Thin sections of meagre otoliths are relatively robust and can be freely handled with tweezers without risk of breaking. Before final mounting, the sections should be cleansed in tap

water and any remnants of Crystalbond adhesive should be removed. In general, no further preparation (e.g., polishing, baking or staining) is required. However, at this stage it is important to check the quality of the sections, making sure it is not necessary to perform additional cuts (see **section 3.3.5.1.**). Final section mounting is carried out on clean microscope slides using, e.g., Lerner Laboratories Flo-Texx mounting medium. Flo-Texx requires no cover slip and improves section's visual appearance while preserving it for long-term use. When Flo-Texx is dry (≈12 hours), the glass slides can be labeled with a diamond scribing pen and stored into their final slide boxes.

3.3. Reading

Fish age determination from otolith thin sections requires the interpretation (or reading) of specific patterns occurring on section's surface. This interpretation requires specific equipment and knowledge of section morphology (**section 3.3.1)** and involves three main steps: annuli interpretation and count (**section 3.3.2**), evaluation of the marginal increment (**section 3.3.3**) and data logging **(section 3.3.4)**. Similar to other fish species, knowledge and training on specific difficulties of the meagre thin sections will improve the quality of final readings (**section 3.3.5**) and ultimately lead to better age determinations.

3.3.1. Equipment and terminology

Meagre otolith thin sections should be read on a stereomicroscope under transmitted light. Under such circumstances, opaque structures will appear dark while translucent structures will appear bright. In general, meagre sections are read under low magnification (8–10x), but higher magnifications (20–40x) may be required to evaluate some specific features. As illumination greatly influences the final perception readers get from a thin section, a microscope base that allows manual control of the intensity and orientation of the light source is to be preferred (see **section 3.3.5.2)**. Additionally, whenever possible, the stereomicroscope should also be equipped with a pointer unit (that eases the interpretation of older sections) and a dark-field polarizing filter (which enhances the contrast and improves overall image appearance).

The meagre otolith sections present several internal morphological features which knowledge is required during the readings. Each section can be broadly divided into two main regions: a distal region (that presents several outgrowths) and a proximal region (that presents a conspicuous opaque / translucent banding). These two regions are separated at a proximodistal interface that runs across the section in dorsoventral direction **(Figure 6).**

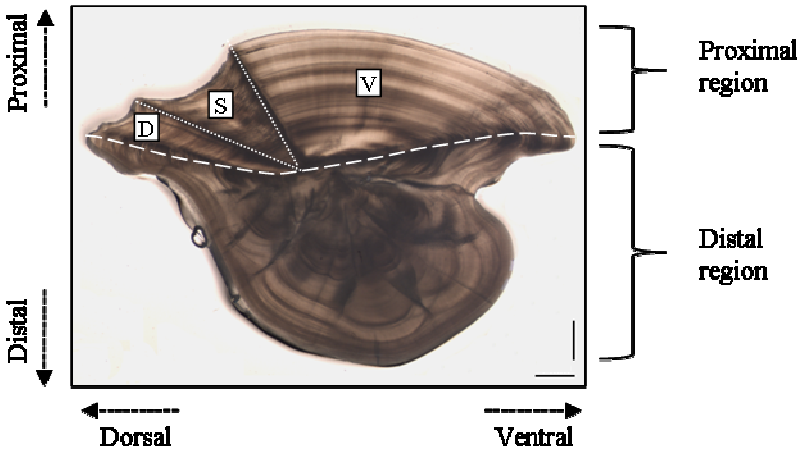

Figure 6 – Regions of the meagre otolith thin section. D – dorsal subregion; S – sulcal subregion; V – ventral subregion. The proximodistal interface is indicated by a white dashed line. Scale bars=1 mm, 8x.

The distal region mainly evidences the internal structure of the umbo (**Figure 4**). Its main feature is a set of accessory primordia that appear as dark outgrowths extending away from the proximodistal interface in distal direction **(Figure 7)**. In younger fish (less than 3 years old), the primordia are well separated so the distal edge of the section appears bumpy. However, at older ages the primordia appear fused and encompassed by a continuous overgrowth that makes the distal edge appear smooth **(Figure 4, Figure 7)**. Overall, the usefulness of the distal region of the section for age determination is low compared to the proximal region. However, at lower magnifications, opaque bands can be observed that span continuously across the primordia and that are related to the banded pattern observed in the proximal region. The most central of these distal bands are sometimes useful to corroborate age interpretations made in the proximal region of the section.

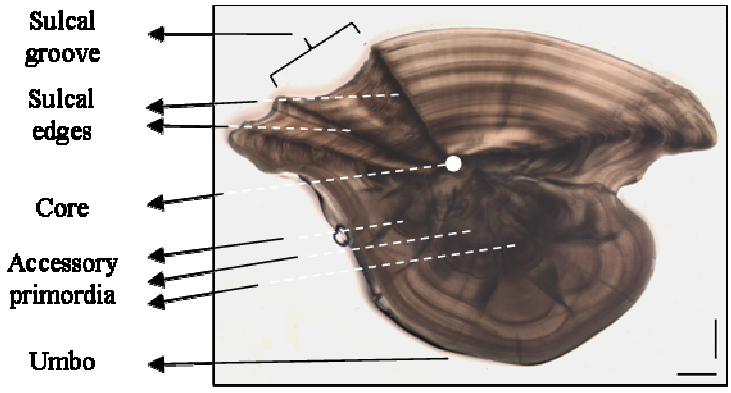

Figure 7 – Internal morphology of the meagre otolith thin section. Scale bars=1 mm, 8x.

The proximal region of the meagre otolith presents three main morphological features: the sulcal groove, the sulcal edges, and the core **(Figure 7)**. The sulcal groove is located in slightly dorsal position along the proximal edge of the section, and shows the concave profile of the

otolith *cauda* **(Figure 4)**. The sulcal edges are two intersecting dark lines that prolong the sulcal groove internally into the proximodistal interface. The core is defined by the intersection of the proximodistal interface and the sulcal edges, and constitutes the region around which the otolith grew. Overall, sulcal groove, sulcal edges, and core constitute the base, legs, and top vertex of an upside-down isosceles triangle that divides the proximal region into three subregions: dorsal, sulcal, and ventral **(Figure 6)**. Contrary to the distal part of the section, very conspicuous opaque / translucent bands can be observed throughout the entire proximal region of the section. It is the interpretation of these bands that constitutes the heart of the meagre age determination process.

3.3.2. Annuli interpretation and count

Determination of fish age from otolith thin sections relies on the interpretation and count of opaque bands that are assumed to form annually at a specific season. These annual opaque bands are termed “annuli” (singular: annulus). The annuli of meagre otolith sections are relatively easy to identify under transmitted light: they are visible in the proximal region, even at low magnification, as continuous concentric opaque (dark) bands that are separated by more translucent (bright) bands. In meagre, annuli exhibit a markedly conspicuous and parallel structure showing up convex in the ventral subregion, concave in the sulcal subregion, and concave to straight in the dorsal subregion. Frequently, central annuli (up to the fifth or sixth from the core) can also be traced across the primordia of the distal region but this becomes increasingly difficult in the peripheral annuli of older specimens.

Adequate interpretation of annuli for purposes of age determination requires the distinction between opaque bands that form annually at a specific season (or “true annuli”) and other opaque bands that may not be laid at annual frequency or that simply should be ignored during age determination (broadly termed “false annuli”). In general, the true annuli of meagre sections are strongly opaque and well-separated by translucent bands throughout the entire section which makes them relatively easy to discriminate. Conversely, false annuli appear as thin inconspicuous opaque bands that either cannot be discriminated throughout the whole proximal area or are suspiciously close to nearby true annuli. In meagre otolith thin sections, false annuli are rare. Consequently precise readings can generally be obtained by any reader that has previously trained with the sections and that is aware of some specifics of their interpretation (see **section 3.3.5**).

In meagre, true annuli (hereafter termed annuli for sake of simplicity) are counted in outward direction from the core to the proximal margin along four predefined axes: the sulcal groove axis (located in the middle of the sulcal subregion), the sulcal edge axis (located along the ventral side of the ventral sulcal edge), the midventral axis (located near the middle of the ventral subregion) and the ventral interface axis (located in the ventral subregion along the proximal side of the proximodistal interface) **(Figure 8)**. Counts are occasionally performed

along the dorsal interface axis (located on the dorsal subregion, along the proximal side of the proximodistal interface) but essentially to corroborate readings obtained on other axes **(Figure 8)**. The sulcal edge axis is generally found the most useful axis to count meagre annuli. However, annuli should be routinely examined on all axes before a final annuli count is assigned to the specimen (see **section 3.3.5**). In doing this, it is useful to have a pointer unit coupled to the stereomicroscope because it eases the tracing of the putative annuli across the different axes and facilitates the counting of the numerous annuli of older meagre. Some examples of final meagre annuli counts are shown in **Figures 9 and 10**.

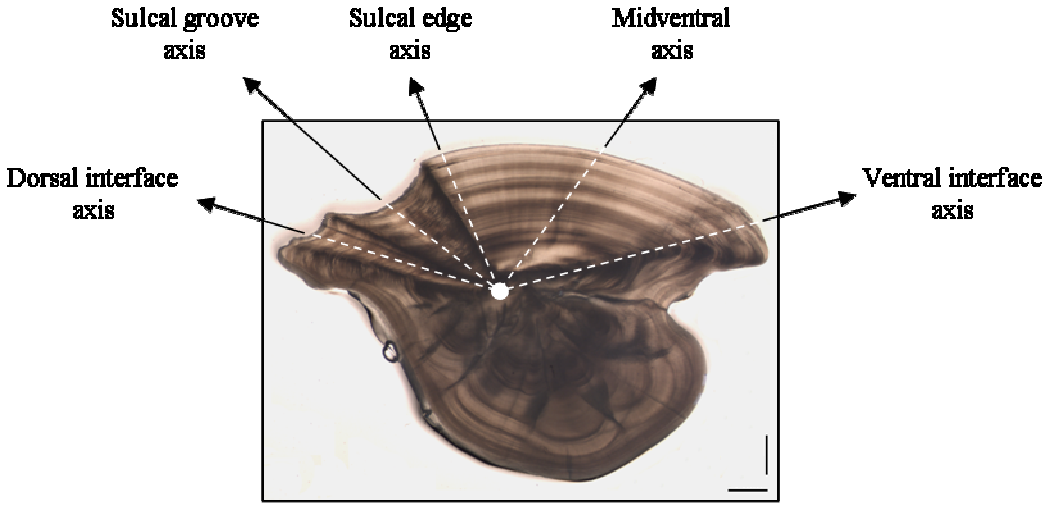

Figure 8 – Axes of the otolith section where the annuli are counted. Scale bars=1 mm, 8x

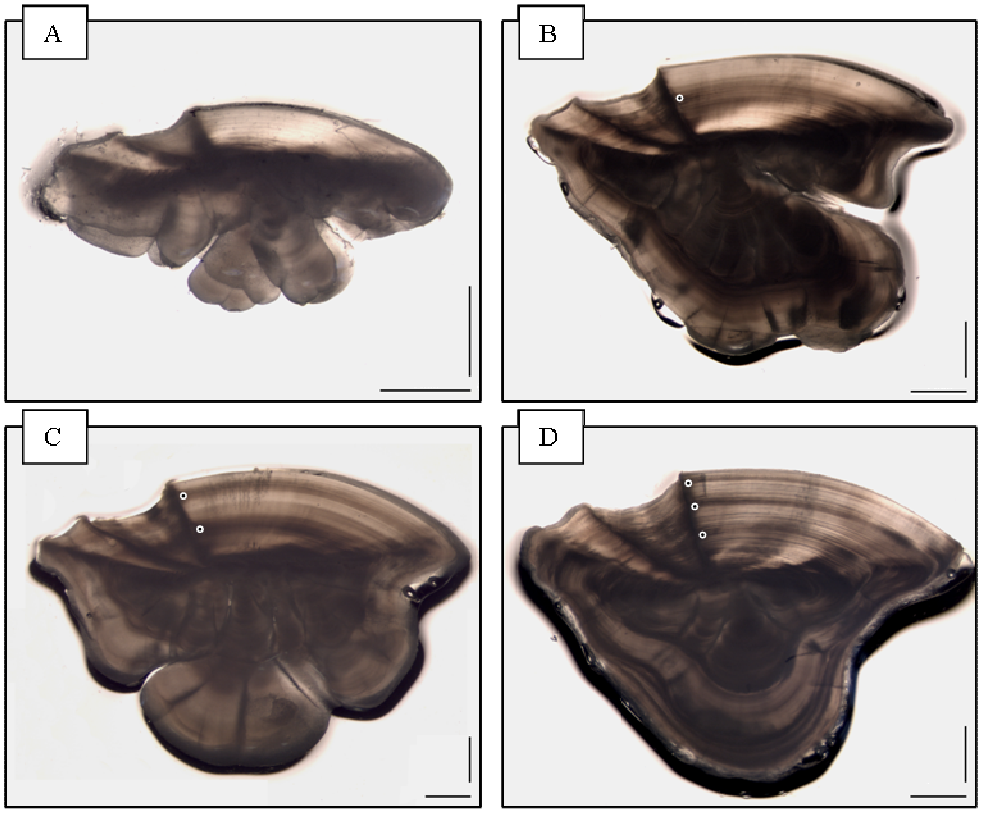

Figure 9 – Annuli counts in younger meagre. A – 0 annulus; B – 1 annulus; C – 2 annuli; D – 3 annuli. The white dots along the sulcal edge axis indicate the annuli. Scale bars=1 mm, 20x (A), 12.5x (B), 10x (C), 12.5x (D).

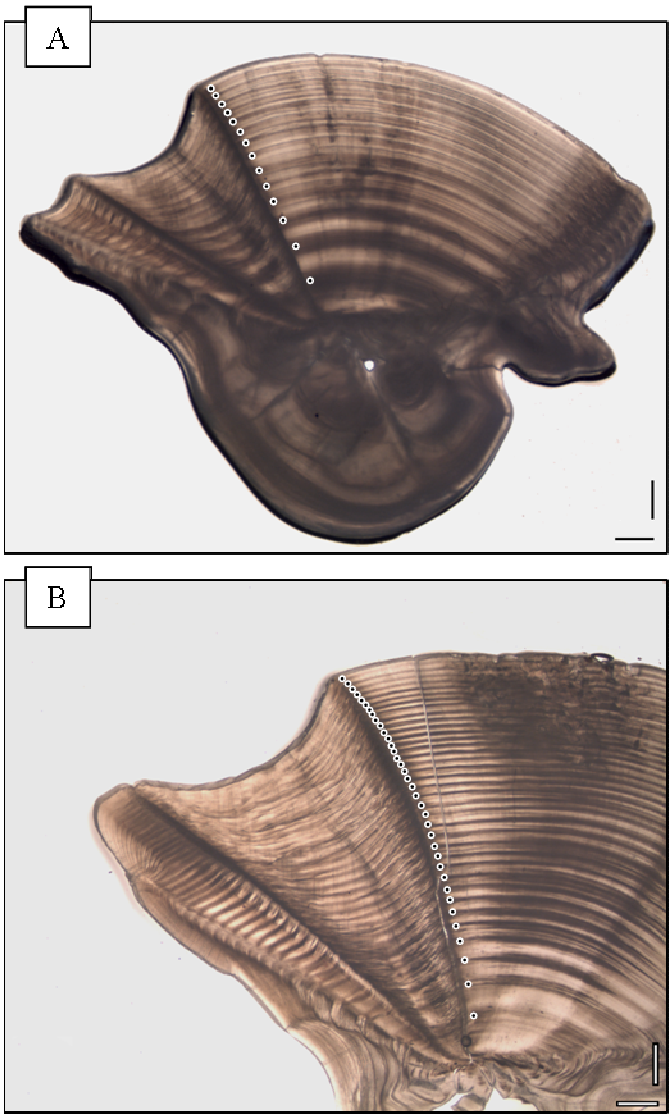

Figure 10 – Annuli counts in older meagre. A – 14 annuli; B – 36 annuli. The white dots along the sulcal edge axis indicate the annuli. Scale bars=1 mm, 6.3x.

3.3.3. Marginal increment analysis

The marginal increment (MI) is the distance between the last annulus and the margin of the otolith. It corresponds to the otolith growth that took place between the time of the deposition of the last annulus and the time of fish capture. In routine age determinations, the marginal increments of the sections are evaluated qualitatively, but if necessary, corroboratory measurements may be taken along the sulcal edge axis. The following categorical scale is suggested for rapid evaluation of the marginal increment of the meagre otolith thin sections **(Figure 11)**:

Type I – An annulus is clearly observable along the margin of all reading axes. No translucent marginal increment is observed or, if so, it is inconspicuous **(Figure 11A)**.

Type II – A narrow translucent marginal increment is observed between the last annulus and the otolith margin **(Figure 11B)**. The width of the marginal increment is generally <50%

the width of the last annual increment (LAI), i.e., <50% the distance between the last annulus and the previous one.

Type III – A wide translucent marginal increment is visible between the last annulus and the margin **(Figure 11C–D)**. It is expected that a new annulus will form soon. The beginnings of this new annulus may be visible along some reading axes but, if so, are inconspicuous. The width of the marginal increment is generally >50% the width of the last annual increment.

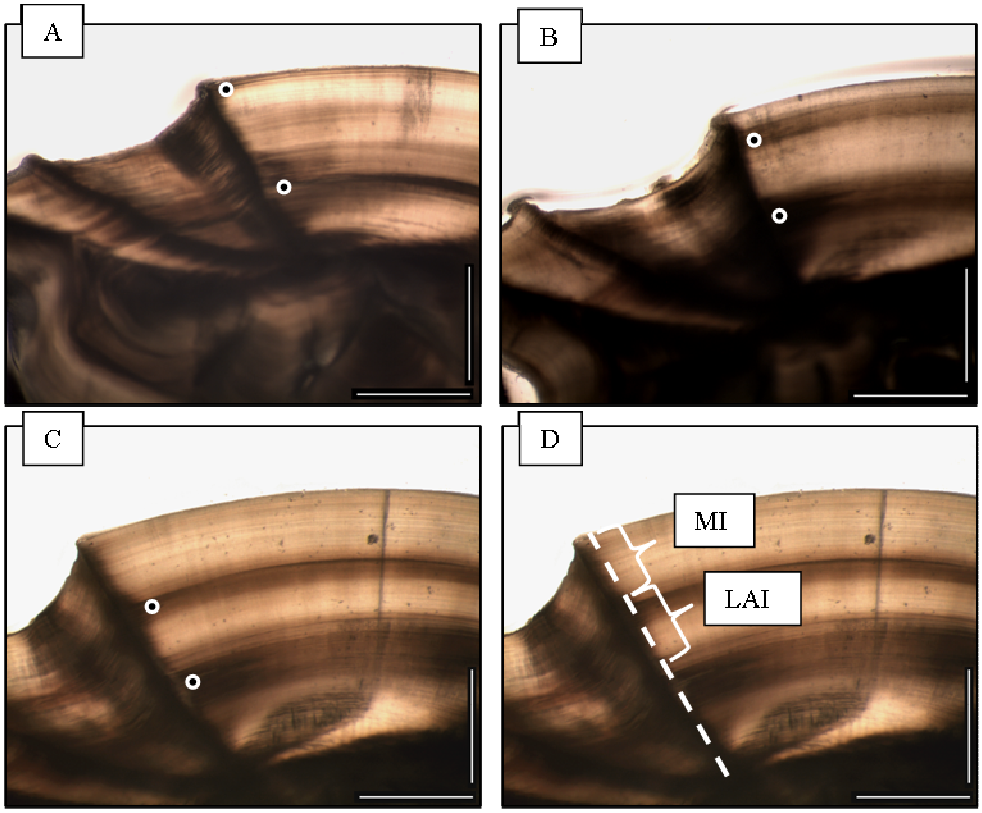

Figure 11 – Marginal increment analysis of meagre otoliths. A – type I margin; B – type II margin; C – type III margin; D – Measurements. The white dots indicate the annuli. Figure D displays the measurement axis (dashed line), the marginal increment (MI), and the last annual increment (LAI). Scale bars = 1 mm, 25x.

3.3.4. Data collection and data logging

During routine age determinations, meagre otolith sections should be read in random order without knowledge of fish size. Providing readers with knowledge of month of capture is optional but will prevent unnecessary mistakes in marginal increment evaluations[2]. Data from otolith readings can be entered into tables similar to **Table 1**. During the readings, the "Age notation" column is commonly filled immediately according to **section 5.1**. Notes should always be kept on doubtful section interpretations.

[2] Note: knowledge of month of capture should not be provided to readers if the periodicity and season of annulus deposition are being established at the same time as the age readings are done.

Table 1 – Example of datasheet for logging otolith readings. Boldface indicates information available to reader. Italics indicate the data entered during hypothetical readings. The "Age notation" column is filled according to **section 5.1**

Specimen	Month of capture	Annuli count	Margin type	Age notation	Notes
036	**8**	*4*	*II*	*4+4*	
198	**2**	*18*	*III*	*18+19*	
⋮		⋮	⋮	⋮	
075	**6**	*9*	*I*	*9 (9)*	

3.3.5. Difficulties in annuli interpretation

Compared to some other fish species, the annuli of well-prepared meagre thin sections are clearly distinguishable against a well-lit background and therefore relatively easy to interpret. Also, false annuli are rare and, when present, they can generally be readily distinguished from true annuli based on aspects such as opacity, width, or continuity (see **section 3.3.2**). Consequently, otolith readings tend to be precise even when older fish are included in the sample. Even so, practice shows that substantial improvements to the accuracy and precision of the final age determinations are be achieved with increased staff awareness and training on specific aspects of the meagre thin sections. Three main aspects should be considered in that training: a) preparation-related issues (**section 3.3.5.1**), b) observation-related issues (**section 3.3.5.2**), and c) more meagre-specific issues (**section 3.3.5.3**).

3.3.5.1 Preparation-related issues

Well-prepared sections are fundamental for accurate and precise readings. Consequently, it is important to check the quality of the thin sections before mounting them into their final glass slides. A well-prepared thin section is interpretable along all reading axes and presents clearly outlined annuli and a sulcal subregion that accurately defines the otolith core (**Figure 9, Figure 10**). Common preparation-related imperfections found in meagre thin sections are a) excessive opacity or brightness, b) the presence of an ill-defined core, and/or c) the presence of an *ostium* blotch:

a) Excessive opacity or brightness: Excessive opacity and excessive brightness impair annuli identification by making it difficult to distinguish between opaque and translucent bands. Excessive opacity occurs when meagre thin sections are cut wider than 0.5 mm and to correct it the section must be polished until a ≈0.5 mm width is attained. Most frequently, the grinding can be done manually over a flat surface using, e.g., 3 µm Buehler Fibrmet discs. Conversely, excessive brightness occurs when meagre thin

sections are cut narrower than 0.5 mm. Excessive brightness is rarer than excessive opacity because narrow sections often break during sectioning. To solve it a new thicker section must be made. In doing it, care should be taken not to obtain a section that presents other ill-preparation issues such as an ill-defined core or a large *ostium* blotch.

b) Ill-defined core: The presence of an ill-defined core usually impairs the identification of more central annuli of the section. This is particularly the case of the first and the second annulus which are located nearer to the core. Two types of core ill-definition may take place: a “blunt sulcal tip” (i.e., the sulcal vertex appears rounded instead of sharp and ends before the proximodistal interface) or a “tornado sulcal tip” (i.e., the sulcal vertex appears twisted in ventral direction and does not directly intercept the proximodistal interface) (**Figure 12)**. In general, only one type of ill-definition will be found in a section and most frequently, it will be detectable only on one of its sides. When so, the section can be mounted with the best-prepared side facing upwards as reliable interpretations can still be drawn from it. However, if that is not the case, core ill-definition is indicative that the sectioning took place at a wrong location of the otolith surface (**Figure 5B**) and a new section must be prepared. In doing this, evidence may be gathered from the ill-prepared section that will help determine the position of the new section: if a “blunt sulcal tip” was present, the new section should be taken further away from the *ostium* (i.e., closer to posterior edge of the otolith); if a “tornado sulcal tip” was present, the new section should be taken closer to the *ostium* (i.e., closer to anterior edge of the otolith).

c) *Ostium* blotch: A common problem found in meagre thin sections is the presence of a broad dark blotch in the ventral subregion (**Figure 12**). The blotch is caused by the section cutting across the internal extension of the *ostium*, a region that presents different light transmission properties from adjoining areas. Most frequently, the presence of this blotch impairs age interpretations along the sulcal edge and midventral axes, but the extent of this impairment generally depends on the effective position and tilt of the sectioning plane. There are two possible causes for the *ostium* blotch: it may be caused by insufficient tilting of the otolith when originally embedded in Crystalbond (**Figure 5A**) or it may result from the pencil marking having been misplaced on the otolith surface (**Figure 5B**). When the former happens, and readings are judged to be severely impaired, it is necessary to prepare the other *sagitta*. When the latter happens, the blotch is generally found associated to a “blunt sulcal tip” (**Figure 12**) and a new section, taken from a slightly posterior position, is generally sufficient to improve readability (see “ill-defined core”).

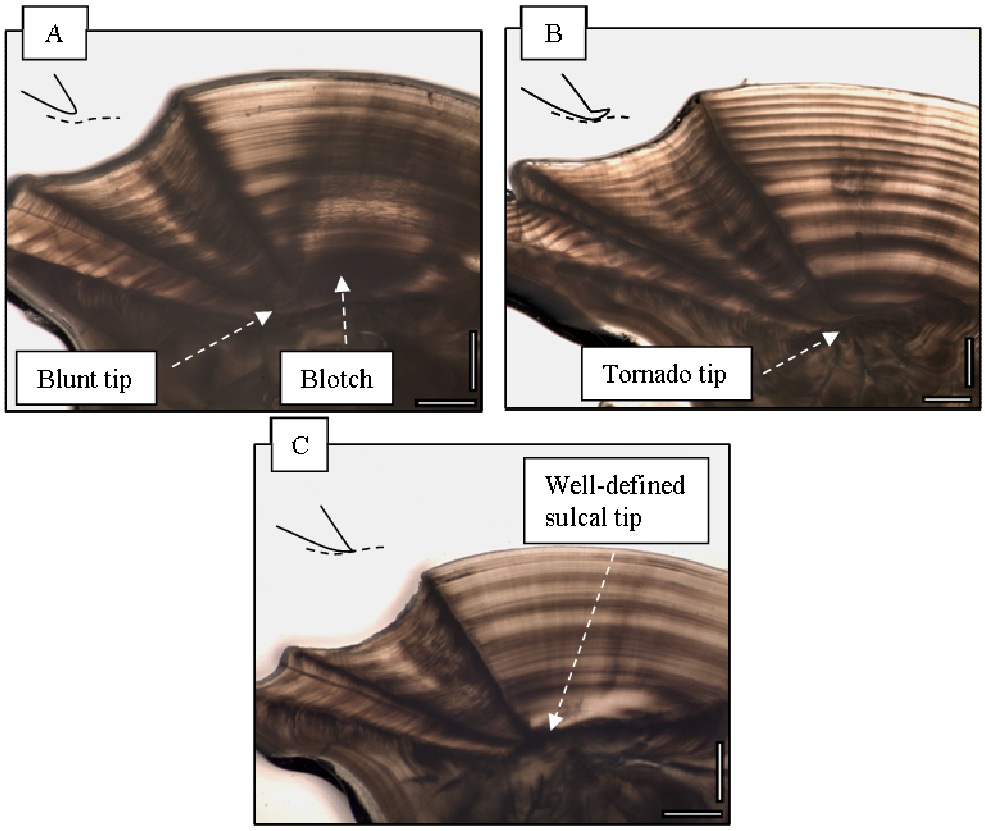

Figure 12 – Quality checking of otolith thin sections. A – blunt sulcal tip and *ostium* blotch; B – tornado sulcal tip; C – well-prepared otolith section. Inset drawings show the position of the tip relative to interface. Scale bars = 1 mm, 12.5x (A), 10x (B), 12.5x (C).

3.3.5.2 Observation-related issues

Two main observation-related issues must be considered when reading meagre otolith thin sections: parallax errors and issues related to the orientation of the transmitted light. Both these issues impact the readability of the sections by changing the final image that readers observe through the stereomicroscope lens. To prevent or ameliorate them it is important to include specific practices into the reading routines.

Parallax errors: The 0.5 mm thickness of meagre otolith sections is important to obtain nicely contrasted annuli but it also makes annuli counts more susceptible to parallax errors. Parallax errors occur because a reader observes a section as combination of three-dimensional details that are present across the section's width and not just the details present on the section's upper surface. As a consequence, the image obtained from the section is highly dependent on the observation angle and so are the annuli counts and the marginal increment evaluations made. In fact, when readings are done at directions not parallel to the width of the annuli, the latter tend to look wider than they really are and may even appear fused to adjoining annuli. Additionally, it is also common that marginal increment evaluations done at directions that are oblique to otolith surface become confounded by the margins' own width, revealing an opaque margin when in fact the margin is translucent. To avoid these types of parallax errors, readers must search for a reading plane that is as parallel as possible to the plane of the annuli

and to the plane of the margin before performing the final annuli counts and marginal evaluations. That plane is section-specific and very dependent on the exact tilt and positioning of the sectioning plane. Consequently, the best way to find it requires readers to observe each section tilted at different angles while looking for the orientation that provides them with the narrower annuli, wider interannuli spaces, and the narrower otolith margin.

Transmitted-light orientation: Transmitted-light microscope bases may provide for a fixed-light orientation or allow for hand-adjusted control of light orientation. Different light orientations provide for different directions from which the light waves interact with the three-dimensional structure of the otolith sections. These different directions can change the reader's perception of the section by, e.g., making annuli less apparent or providing emphasis to false annuli, and consequently interfere with age interpretation. Because of this, it is preferable to read meagre otolith sections on a microscope base that allows for hand-adjusted control of light orientation since this will allow readers to obtain crispier images. Additionally, it is also advantageous to have a dark-field polarizing filter attached to the stereomicroscope objective. Dark-field polarizing filters confer a dark appearance to the bright background of transmitted-light observations, substantially reducing glare and enhancing the image contrast, thus enhancing overall section readability (**Figure 13**).

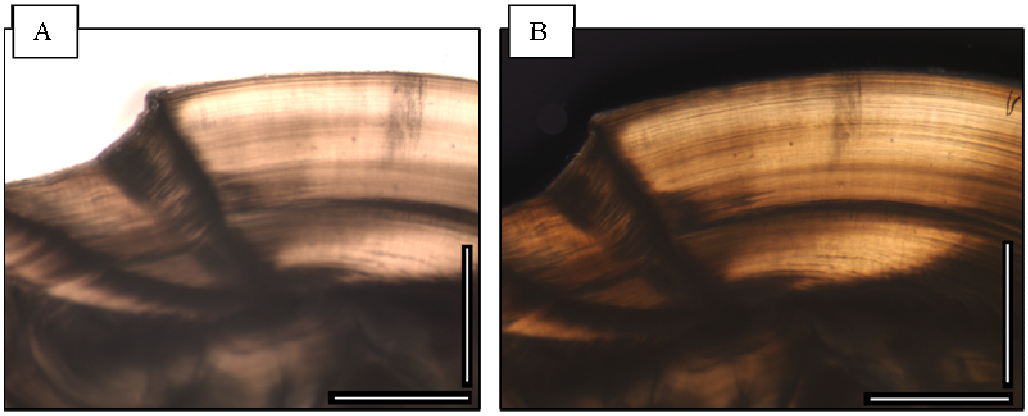

Figure 13 – Effect of dark-field polarization on otolith section readability. A – without filter; B – with filter (a Nikon dark-field polarizing filter was used). Scale bars = 1 mm, 30x.

3.3.5.3 Other issues

Three types of difficulties are generally reported by readers when they are first introduced to meagre otolith thin sections: a) difficulties in the identification of the first annulus, b) difficulties related to annulus splitting, and c) difficulties related to abnormal otolith crystallization:

a) Difficulties in first annulus identification: To the less experienced reader, the identification of the first annulus is the main difficulty met when interpreting meagre sections. In many specimens the first annulus is difficult to discriminate along the sulcal groove axis because it is close to the core and appears masked by the filamentous appearance of the sulcal subregion. Often, this is not a major difficulty because the

annulus will still show up sufficiently opaque and distant from the proximodistal interface along the remaining axes to be clearly outlined **(**e.g., **Figure 9)**. However, cases exist where first annulus identification remains troublesome along the remaining axes. When this happens, three main issues are found to be the cause:

- Annulus "brightness": In some specimens, the first annulus appears brighter than usual and presents little contrast to adjacent translucent bands **(Figure 14)**. Usually, this happens along the sulcal edge axis or midventral axis, and is particularly noticeable when an *ostium* blotch, even of small size, is present near the core **(**see **section 3.3.5.1)**. In these cases, to verify if an annulus effectively exists near the core, the ventral interface axis should be examined: if present, the annulus will show up as a strongly opaque bend backwards that penetrates the distal region; if not, the bend will not be observed and the annulus should be searched for farther away from the otolith core **(Figure 14)**.

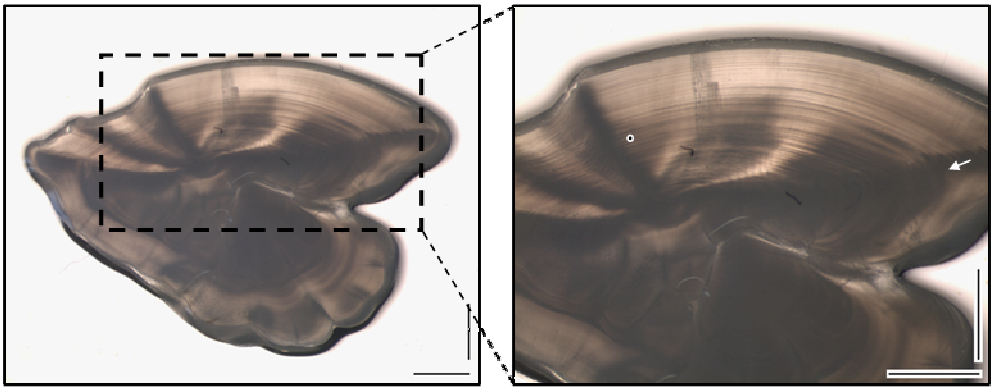

Figure 14 – First annulus "brightness". The white dot indicates the first annulus. The white arrow indicates the bend of the annulus towards the distal region. Scale bars = 1 mm, 12.5x (left), 20x (right).

- Annulus "rippling": In some specimens, a set of concentric opaque "ripples" occurs near the otolith core which causes the first annulus to be mistaken as several distinct annuli **(Figure 15)**. In most such cases, the first annulus will remain clearly identifiable along the ventral interface axis and readings can proceed. However, even if not, practice shows that the first annulus can be confidently assigned to the entire set of ripples and that regular counts should be resumed at the second annulus.

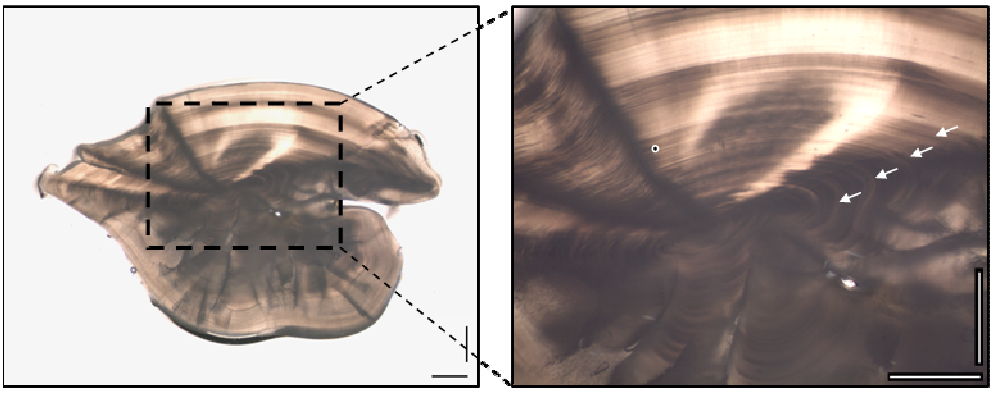

Figure 15 – First annulus rippling. The white dot indicates the first annulus. The white arrows indicate the ripples. Scale bars = 1 mm, 8x (left), 20x (right).

- "Dent": In some specimens, a dark mark similar to a dent occurs near the otolith core. To inexperienced readers this dent resembles a very early first annulus **(Figure 16)**. However, the dent results from sectioning imperfections generated at the interception of the sectioning plane with the internal structure of the *ostium*. Consequently, it should not be counted as the first annulus and readers should look farther away from the core for better evidence of this annulus.

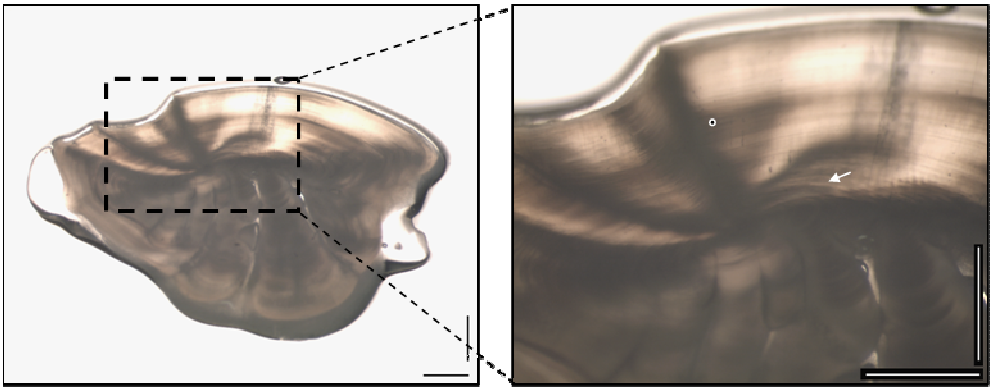

Figure 16 – Dent. The white dot indicates the first annulus. The white arrow indicates the dent. Scale bars = 1 mm, 10x (left), 25x (right).

b) Difficulties due to annulus splitting: In meagre, split annuli are relatively frequent between the third and the ninth annulus (**Figure 17**). Annuli are generally found to split into two distinct branches near the ventral sulcal edge. The two branches then run parallel to each other – separated only by a thin translucent band – throughout the ventral subregion and rejoin only near the ventral interface axis. Because annulus splitting does not usually extend to all reading axes, it is generally detected when annuli counts from different axes are compared. However, to completely resolve the issue, readers should trace down the branches of each putative split annulus to check if they effectively rejoin at the ventral interface axis. In doing this, it is particularly advantageous to have the stereomicroscope equipped with a pointer unit because this will ease the tracking down of the split branches across the large ventral subregion.

Once all split annuli have been identified, readers should obtain the final counts by doing some "jumping around" between the different axes, i.e., by counting each annulus at the axis (or axes) where the annulus was not observed to split.

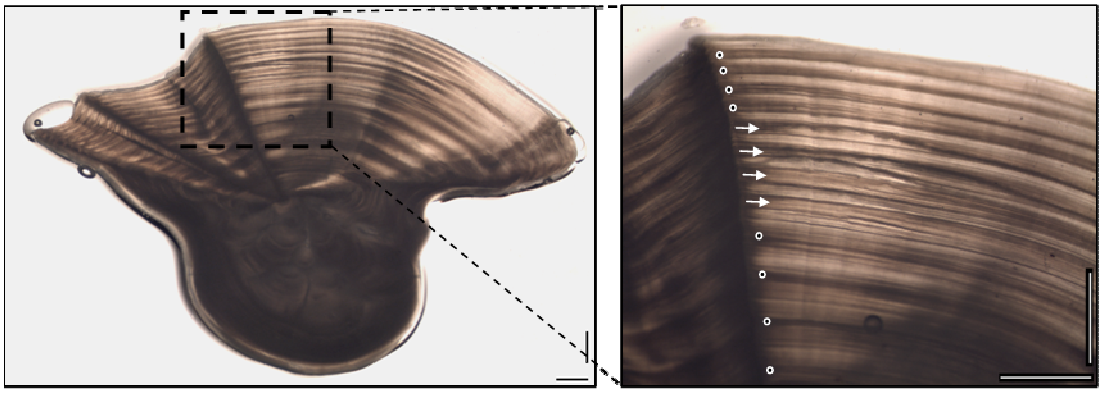

Figure 17 – Annulus splitting. The white dots indicate regular annuli. The white arrows indicate four annuli that split near the ventral sulcal edge. Scale bars = 1 mm, 6.3x (left), 20x (right).

c) Abnormal otolith crystallization: Some meagre otoliths evidence abnormal crystallization in one (or both) of the otoliths. Abnormal crystallization is caused by major crystallization of calcium carbonate as vaterite crystals (instead of the usual aragonite crystals) and results in otoliths that are lighter than usual, externally very irregular, and internally very translucent (**Figure 18**). When abnormal crystallization occurs, it generally extends from a specific point in the interior of the section all the way to its periphery, and it is clearly noticeable on the otolith surface (**Figure 18A**). To circumvent it, it is advisable to select for sectioning the normal (or the less impacted) *sagitta*. However, if necessary, an attempt may still be made at sectioning abnormal otoliths because their annuli are generally still interpretable along some of the reading axes (**Figure 18B**). When sectioning abnormally crystallized otoliths, lower saw speeds and lighter arm loads should be used because the otoliths are brittle and break easily if too much pressure is exerted on them.

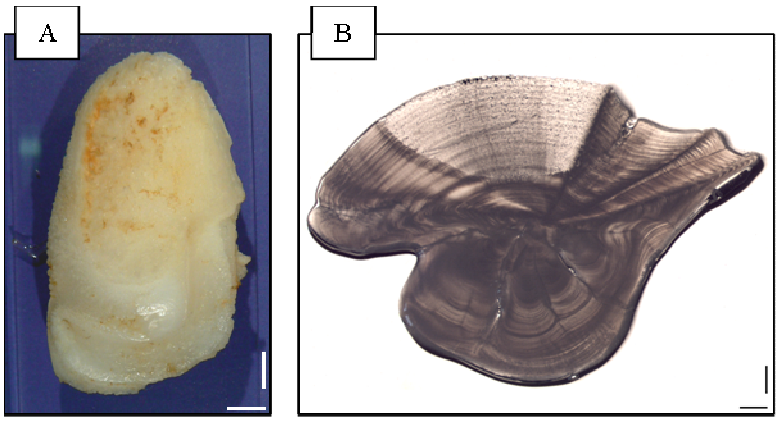

Figure 18 – Abnormal crystallization in meagre otoliths. A – whole otolith;
B – otolith section. A and B were taken from different fish. Scale bars = 5 mm (A), 1 mm (B), 6.3x (B).

4. Scale Protocol

4.1. Collection

Meagre scales are generally collected from the left side of the fish from the region located between the first dorsal fin and the lateral line **(Figure 19)**. In general, 10 to 15 scales are collected from each fish. In meagre, the scales are tightly embedded in the dermis so they are not easy to release from the fish body. The simplest method to collect scales involves rubbing a knife over the skin surface, in successive posterior to anterior movements, while exerting pressure to insert its blade underneath the scales. This method releases many scales, making them “jump” out of the skin, but also damages the external appearance of the fish. In many cases, the latter has to be avoided because specimens will enter the commercial circuit. When so, scales should be collected one-by-one with tweezers **(Figure 19)**. Collecting scales with tweezers takes more time but causes no loss of commercial value because the fish skin can be brought back to its original appearance with a gentle rub in posterior direction.

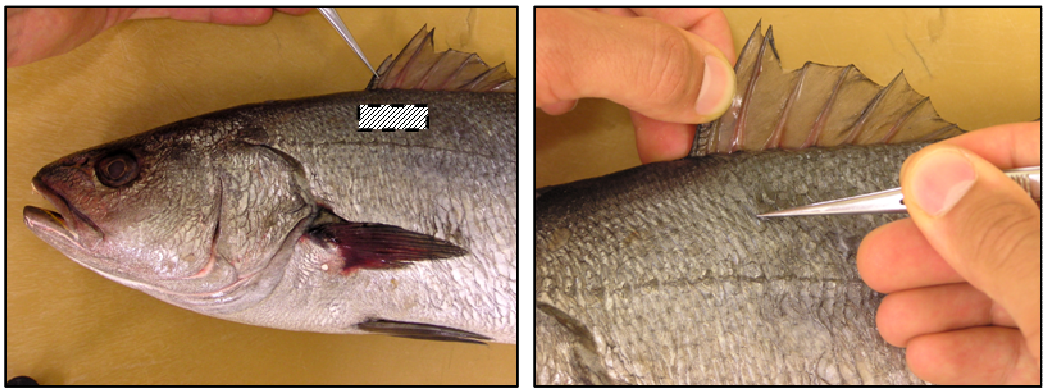

Figure 19 – Aspects of the collection of meagre scales. The shaded rectangle indicates the area of collection.

During field sampling, meagre scales are commonly put inside labeled paper envelopes without much cleaning. Back in the lab, they should be cleaned before being stored. For this, scales are first immersed in water for a few minutes to soften and separate and then rubbed individually between the thumb and the index finger to remove dirt and adherent tissues. If necessary, a soft toothbrush may be used but excessive pressure should be avoided because it will scratch the scale surface. Once clean, meagre scales may be left to dry at room temperature until stored into their final paper envelopes.

4.2. Preparation

Meagre scales are frequently too thick to be directly evaluated under a stereomicroscope or microfiche reader. Consequently, their external surface should be imprinted into acetate slides before readings take place. Before imprinting, some preliminary sorting and selection of scales is generally required. In sorting and selection, preference should be given to scales that

present continuous margins and a roughly similar shape and size. Rectangular scales, slightly wider than longer, are common in the collection area and among the easiest to interpret. However, most importantly, the scales selected for imprinting must not present signs of regeneration **(Figure 20)**. In general, regenerated scales can be distinguished from nonregenerated scales before being pressed on the basis of their extreme flexibility and their inconsistent microstructure when observed under a common lens. Flexibility and microstructure are, however, hard to evaluate in smaller scales or when regenerated portion is small. Consequently, besides careful sorting, it is good practice to always imprint a larger number of scales than the number necessary to determine the fish age (3–5 scales) as this will ensure that enough nonregenerated scales are present on the final slides.

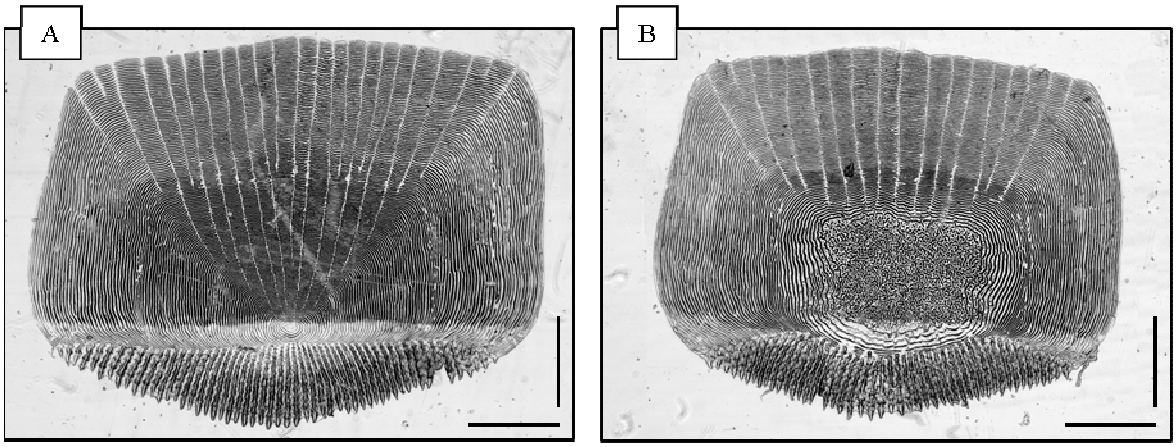

Figure 20 – Scale regeneration. A – nonregenerated scale, B – regenerated scale (same fish). Scales were imprinted into acetate slides. Scale bars = 1 mm, 50x.

Meagre scales may be imprinted onto transparent cellulose acetate slides (25 mm x 75 mm x 0.5 mm) using a heated press (e.g., a Carver Laboratory Heated Press Model C) **(Figure 21A)**. Scales of older fish tend to be large and thick and consequently require larger and thicker acetate slides to be imprinted (e.g., 30 mm x 75 mm x 1 mm). In general, between 2 and 10 scales can be imprinted on each slide and between 1 and 4 acetate slides can be pressed at each press operation. Before pressing, the scales should be aligned with their external side (i.e., the side that appears rougher and less reflective to light) kept in contact with the acetate slide **(Figure 21B)**. Then, the slides are inserted between a pair of portable platens and transparency-film coverslips and put to press (**Figure 21C**). Under such a setup, the standard conditions for pressing meagre scales involve pressing for 7 min., at a pressure of 109.5 MPa, and temperature of 75°C. However, slight adjustments to time, pressure, and temperature may be required to achieve adequate imprints across the entire thickness range of the meagre scales (see **section 4.3.5.1**).

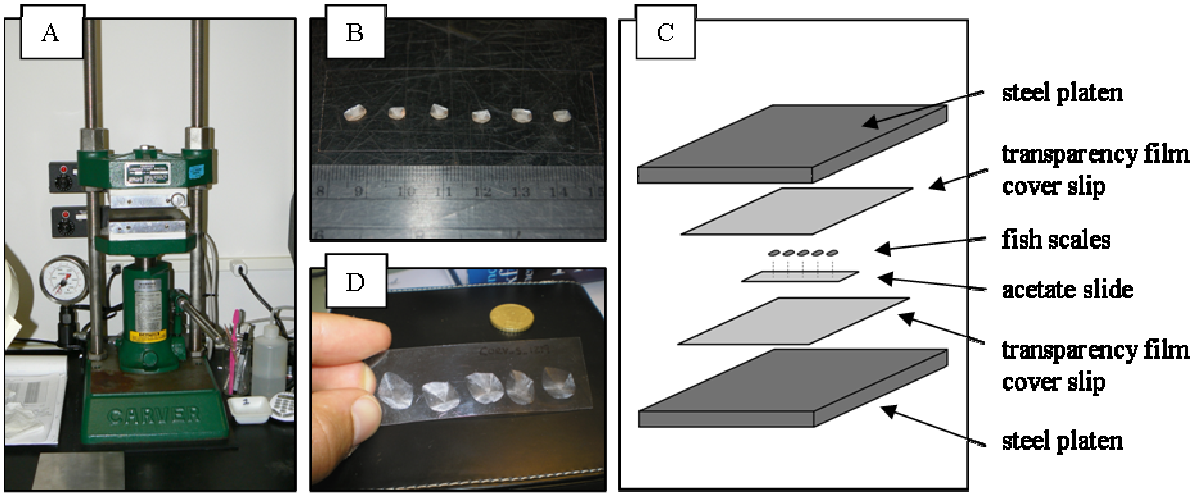

Figure 21 – Aspects of scale preparation. A – Carver Laboratory Heated Press Model C; B – meagre scales ready to be pressed (photo courtesy of Christina Morgan); C – pressing setup; D – meagre scale imprints ready for storage.

After pressing, meagre scales are generally found adhered to the acetate slide. Gentle pulls with tweezers can be used to release them as long as care is taken not to scratch the imprint with the tweezers' tips. Then, before storage, it is good practice to perform a preliminary quality check on the imprints to guarantee that all scales have been adequately pressed (**see section 4.3.5.1**). At this time, if necessary, new scales can be readily imprinted and future delays avoided. However, because previously pressed scales tend to be brittle, curved up, and/or cracked, a new set of scales must be prepared. When imprint quality is found appropriate, final scale slides are labeled with a permanent marker and stored inside microscope slide boxes until readings are done (**Figure 21D**).

4.3. Reading

Age determination from fish scales involves the interpretation (or reading) of a set of markings on the scale surface that are faithfully depicted on the acetate imprints. Interpreting those markings requires specific equipment and knowledge of scale morphology (**section 4.3.1)** and involves three main steps: the interpretation and counting of annuli (**section 4.3.2),** an evaluation of the marginal increment **(section 4.3.3)** and data logging **(section 4.3.4)**. Meagre scales, particularly from older fish, are difficult to interpret even to experienced readers. Consequently, adequate scale collection and preparation, and full awareness and training on specific patterns and details of the meagre scales, are fundamental to the age determination process (**section 4.3.5**).

4.3.1. Equipment and terminology

Acetate imprints of meagre scales are read on a microfiche reader. Common microfiche readers work on transmitted light and the acetate imprints show up inverted on a screen. Because the meagre scales exhibit a large variability in size, the microfiche reader should be

equipped with lenses that provide for somewhat different magnifications in the range of 10x to 30x.

Well-prepared acetate imprints faithfully depict the morphology of the external surface of the scales. When an imprint is observed at low magnification, the thicker areas of the original scale (namely its center portion) appear darker and the thinner areas (namely its periphery and margins) appear brighter. At higher magnification, the crests and ridges of the original scale appear as dark lines, whereas the grooves show up as bright lines. In the body region where samples are collected, the scales are ctenoid and their margins show up undulated (anterior margin), straight (lateral margins), and prickly (posterior margin). Four main regions can be defined on the scale surface – a "posterior field", two "lateral fields" (dorsal and ventral) and an "anterior field" **(Figure 22)**.

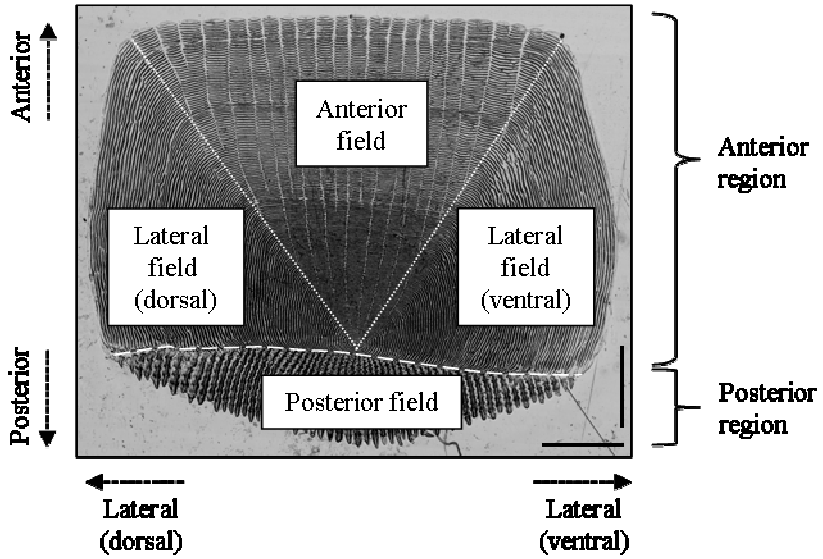

Figure 22 – Morphology of the meagre scale. The anteroposterior interface is indicated by a white dashed line. Scale bars = 1 mm, 40x.

The posterior field presents a spiny appearance and corresponds to the part of the scale that is directly exposed to the environment. This field is separated from the remaining fields of the scale by an interface (the "anteroposterior interface") that broadly divides the scale into a posterior region and anterior region **(Figure 22)**. The spiny appearance of the posterior field results from long segmented tube-like structures (the "*ctenii*") that extend from the interface to the posterior margin of the scale **(Figure 23)**.

The lateral and anterior fields are both located in the anterior region of the scale. Both fields exhibit a markedly parallel appearance and correspond to parts of the scale that, while in the fish body, are largely concealed underneath neighboring scales. The lateral and anterior fields present thin concentric ridges (the "*circuli*") which run from one lateral field to the next across the anterior field. In meagre, all *circuli* are centered in the same region (the "focus") that is located in medial position near the anteroposterior interface **(Figure 23)**. Even so, the

appearance of *circuli* changes drastically from the lateral to the anterior fields: in the lateral fields, *circuli* run in anteroposterior direction and are continuous; in contrast, in the anterior field, *circuli* run in dorsoventral direction and are divided into numerous segments (the "platelets") by a set of radial grooves that stem outwards from the focus towards the anterior margin (the "*radii*") **(Figure 23)**. For simplicity of this protocol, we termed the part of a *circulus* that appears segmented on the anterior field as "segmented *circulus*" and its nonsegmented part, located along each lateral field, as "continuous *circulus*" **(Figure 23)**.

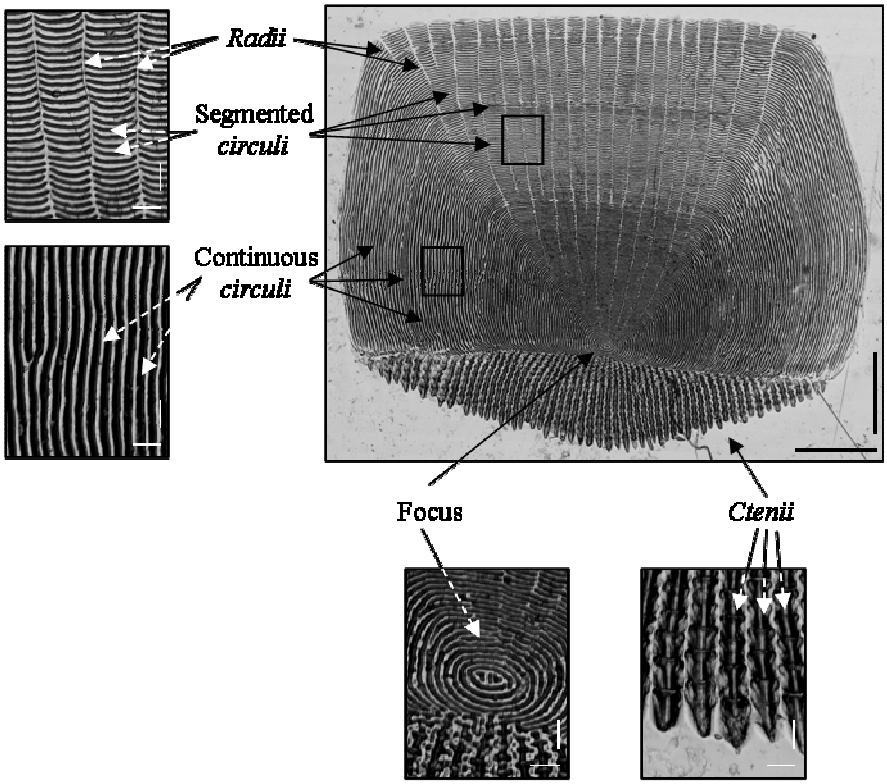

Figure 23 – Morphology of the meagre scale. Each segment of a "segmented *circulus*" is termed a "platelet". Scale bars = 1 mm (main figure), 0.1 mm (details), 40x (all).

4.3.2. Annuli interpretation and count

The use of scales in age determination relies on the interpretation and count of specific scale markings that are assumed to form at annual intervals (termed "annuli"). Ctenoid scale annuli are relatively narrow continuous concentric bands that extend across the lateral and anterior fields of the scale. In meagre, scale annuli encompass small groups of homogenous-looking *circuli* that can be discriminated from adjoining, closely-resembling, nonannulus *circuli* using specific structural criteria (termed "primary criteria"). The primary criteria used to identify

annuli in meagre scales are: a) *circuli* "disruption", b) *circuli* "straightening out", and c) *circuli* "compaction" **(Figure 24)** [3]:

a) *Circuli* "disruption": The vast majority of continuous *circuli* do not suffer any significant interruption. However, the continuous *circuli* that belong to an annulus present disruptions to their continuity which resemble small strings, or aggregations, of white spaces within the continuous parallel pattern of the lateral fields **(Figure 24A)**.

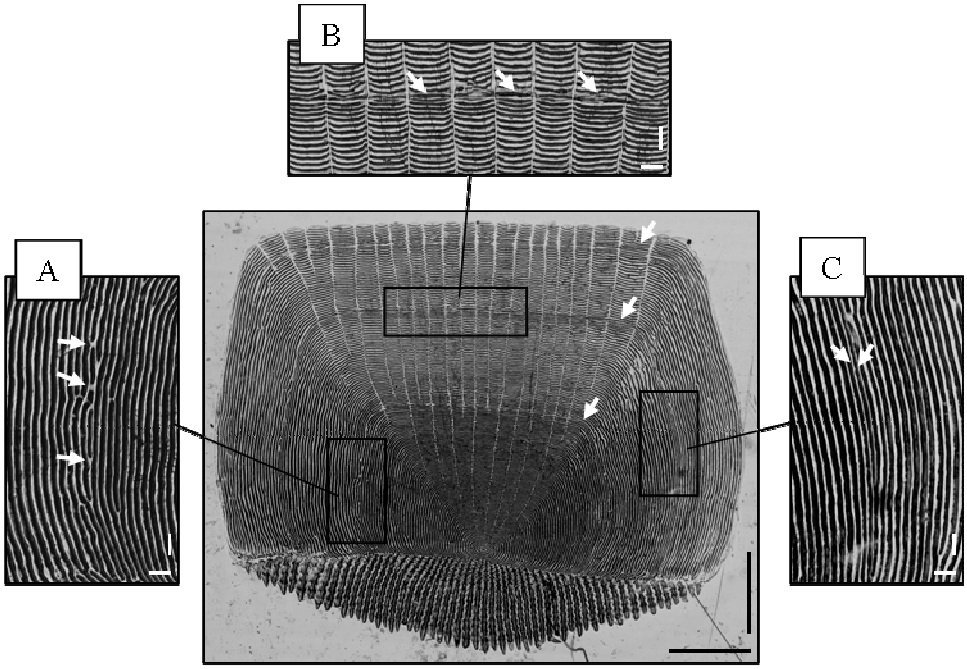

Figure 24 – Aspects of primary criteria used in annuli interpretation. White arrows in central picture indicate annuli position. White arrows in lateral pictures indicate disruption (A), straightening out (B) and compaction (C). Scale bars = 1 mm (main figure), 0.1 mm (details), 40x (all).

b) *Circuli* "straightening out": The vast majority of segmented *circuli* are composed of concave platelets. However, at an annulus, the platelets of one or more *circuli* become straight (or, in older fish, highly irregular) instead of concave, which causes the annulus to resemble a string of whitish nodules extending across the anterior field **(Figure 24B)**.

c) *Circuli* "compaction": At an annulus, both continuous and segmented *circuli* appear more compact than in adjoining areas due to a reduction in inter-*circuli* distances. In the lateral fields, this compaction is generally noticed as a band of continuous *circuli* that looks somewhat darker and more compact than the surroundings areas **(Figure 24C)**. In the anterior field, *circuli* compaction generally takes place immediately before and / or after the straigtening out of segmented *circuli* and also provides a contrasting darker appearance to the annulus region when compared with adjacent regions **(Figure 24B)**.

[3] Note: the meagre "*circuli* disruption" and "*circuli* straightening out" bear some resemblance to the "cutting over" marks (also known as "crossing over" marks) observed in, e.g., summer flounder and striped bass scales (Pentilla and Dery, 1988; Liao *et al.*, 2008).

In meagre scales, a group of *circuli* is considered an annulus when it matches all primary criteria and can be traced throughout the lateral and anterior fields of the scale. If these two characteristics (criteria match and traceability) are not met, then the group of *circuli* belongs to an interannuli region or to some other type of distinct scale feature that should be ignored for effects of age determination (broadly termed “false annulus”). In meagre scales, false annuli are relatively frequent but can generally be distinguished from true annuli because they match only one criterion and they cannot be traced throughout the anterior regions of the scale. However, despite their apparent objectivity, the use of primary criteria and traceability to identify scale annuli is not always clear-cut. The reason for this is that some criteria are easier to observe in some parts of the scale (or in some annuli) than others. That is the case of, e.g., *circuli* compaction (that is sometimes easier to verify in the lateral fields than in the anterior field) and *circuli* disruption (which is more evident in central annuli than in peripheral ones). To circumvent these and other annuli identification difficulties, it is generally acceptable to extend the annulus definition to any group of *circuli* that meets, at least, two primary criteria while remaining traceable throughout the scale. However, should this broader definition be used and annulus identification must be supplemented with a few corroboratory criteria to reduce the increased risks of assigning true annuli to false annuli (**see section 4.3.5.3).**

In meagre scales, annuli are counted from the focus to the periphery along specific axes **(Figure 25)**. Because some criteria are more observable along some reading axes than along other, some “jumping around” between different axes may be necessary as counts proceed, particularly in older scales. In general, the anteroposterior axis and the two anterolateral axes are the most useful to count meagre annuli, but the final count of each scale should be based on a consensus among the counts obtained on the different axes. Finally, the annuli count of each specimen is based on a consensus among the counts attained in at least three scales among the several imprinted for that particular fish. In the selection of the latter set of scales, it is important to exclude regenerated scales (because they may not show all annuli) and scales which counts differ markedly from the remaining (because they may have originated from different fish) (see **section 4.3.5.1)**.

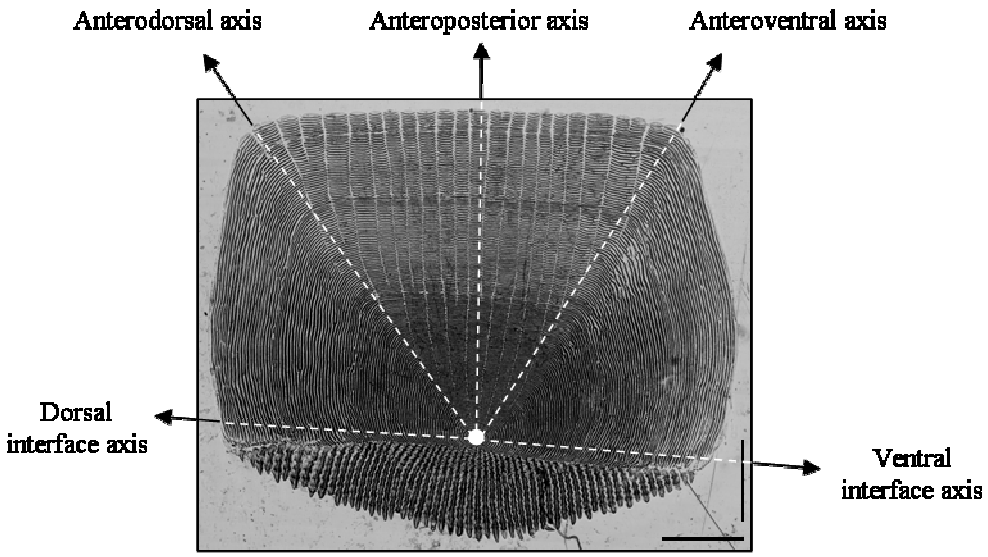

Figure 25 – Axes of the meagre scale where the annuli are counted. Scale bars = 1 mm, 40x.

Some examples of final annuli counts in meagre scales are shown in **Figures 26 and 27**.

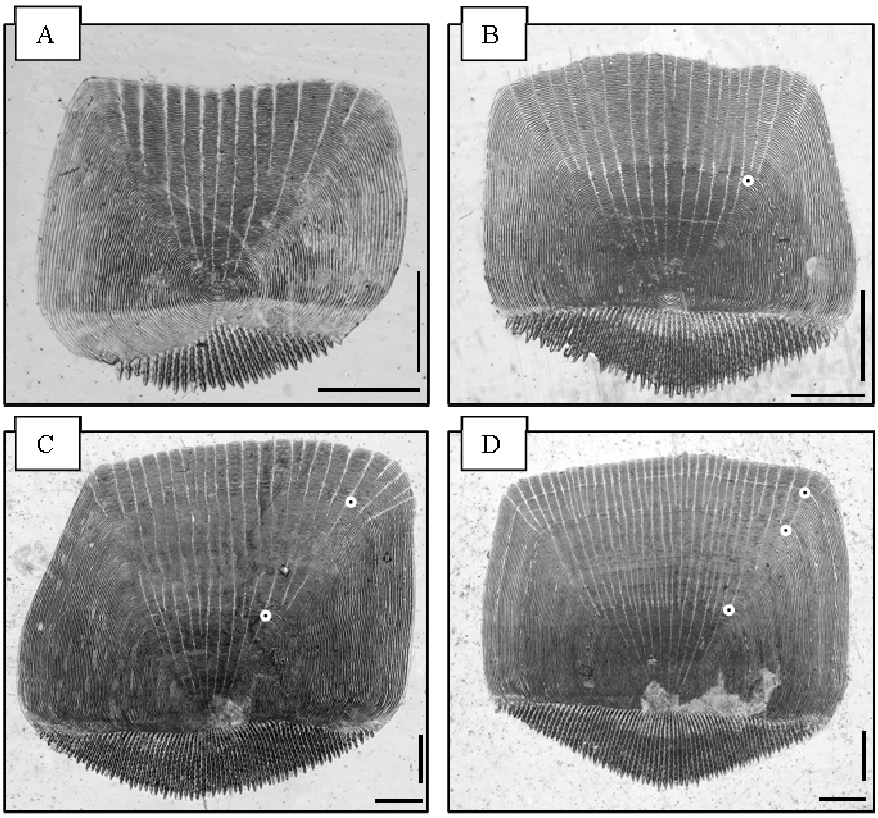

Figure 26 – Annuli counts in younger meagre. A – 0 annulus; B – 1 annulus; C – 2 annuli; D – 3 annuli. The white dots along the anterodorsal axis indicate the annuli. Scale bars=1 mm, 50x (A), 50x (B), 25x (C), 30x (D).

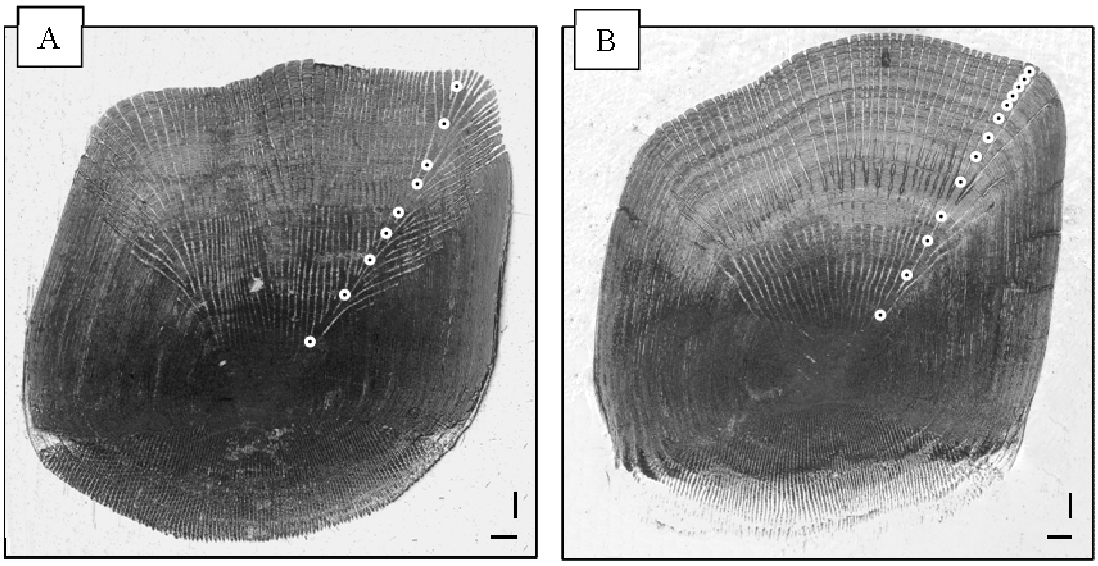

Figure 27 – Annuli counts in older meagre. A – 9 annuli; B – 13 annuli. The white dots along the anterodorsal axis indicate the annuli. Scale bars=1 mm, 11x (A), 9x (B).

4.3.3. Marginal increment analysis

The marginal increment (MI) is the distance between the last annulus and the margin of the scale. It corresponds to the scale growth that took place between the time of the deposition of the last annulus and the time of fish capture. In routine age determination, the marginal increment of meagre scales is evaluated qualitatively, but if necessary corroboratory measurements may be taken along the anteroposterior axis. The following categorical scale is suggested for evaluating the marginal increment of the meagre scales **(Figure 28)** [4]:

Type I – the last annulus is located at the scale margin or very near to it. It is not expected that a new annulus will form soon. The marginal increment is <25% the width of the last annual increment (LAI), i.e., <25% the distance between the last annulus and the previous one.

Type II – the last annulus is located relatively distant to the margin. The marginal increment width is 25%–75% of the last annual increment width.

Type III – the last annulus is located very distant to the margin. It is expected that a new annulus will form soon. The marginal increment width is >50% of the last annual increment width.

[4] Note: in this classification, the overlapping percentages of type II and type III margins reflect some inherent difficulties of meagre scale interpretation in the Portuguese coast (compare with **section 3.3.3** on meagre otoliths). Amongst other, these difficulties are related to a large variability in the interannuli distances and to a long annulus deposition period (**section 5**).

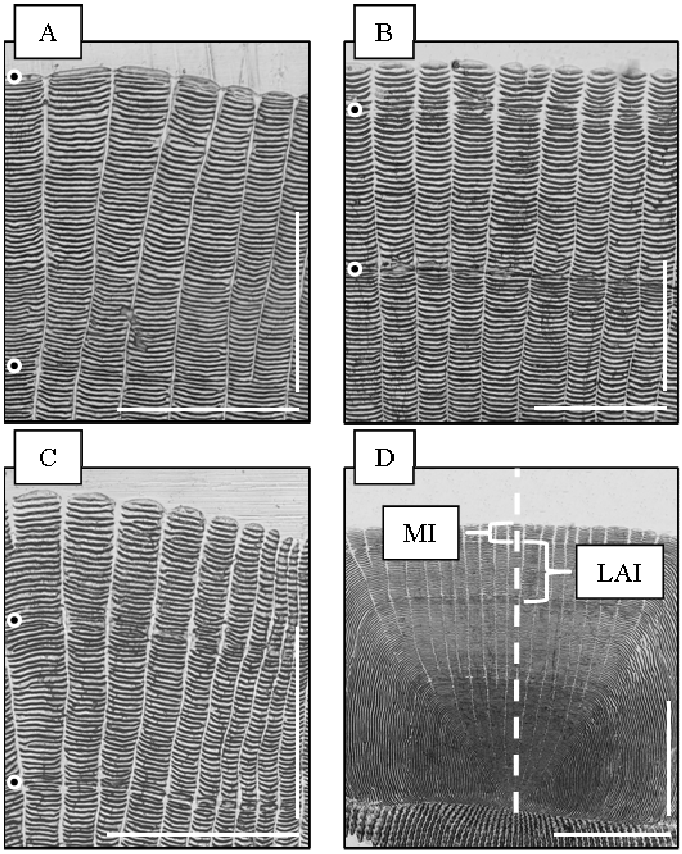

Figure 28 – Marginal increment analysis of meagre scales. A – type I margin; B – type II margin; C- type III margin; D – measurements. The white dots indicate the annuli. Figure D displays the measurement axis (dashed line), the marginal increment (MI) and the last annual increment (LAI). Scale bars=0.1 mm (A-C), 1 mm (D), 50x (A), 40x (B-D).

4.3.4. Data collection and data logging

During routine age determinations, meagre scale imprints should be read in random order without knowledge of fish size. Providing readers with knowledge of month of capture is optional but will prevent unnecessary mistakes in the marginal increment evaluations[5]. The final consensus on annuli counts and margin evaluations may be entered into tables similar to **Table 2**. During the readings, the "Age notation" column is commonly filled immediately according to **section 5.1**. Notes should always be kept on doubtful scale imprint interpretations.

[5] Note: knowledge of month of capture should not be provided to readers if the periodicity and season of annulus deposition are being established at the same time as the age readings are done.

Table 2 – Example of datasheet for logging scale readings. Boldface indicates information available to reader. Italics indicate the data entered during hypothetical readings. The "Age notation" column should be filled according to **section 5.1**

Specimen	Month of capture	Annuli count	Margin type	Age notation	Notes
036	**10**	*4*	*II*	*4+4*	
078	**2**	*18*	*III*	*18+19*	
⋮		⋮	⋮	⋮	
011	**8**	*9*	*I*	*9 (9)*	

4.3.5. Difficulties in annuli interpretation

When interpreting meagre scales it is frequent for disagreements to occur at within-sample level (i.e., between the several scales pressed for a specific fish), at within-reader level (i.e., between readings obtained by a single reader on different occasions), and at between-reader level (i.e., between readings obtained by multiple readers). Additionally, it is not infrequent for annuli counts obtained from scales to be substantially different from annuli counts obtained from the otolith sections of the same fish. This is so, even when experienced readers are involved and results essentially from difficulties in standardizing scale preparation and in objectively applying the primary criteria used in meagre scale annuli identification (**see section 4.3.2**). Even so, practice shows that the final age estimates obtained at all reading levels can be much improved, particularly in younger fish, if the personnel involved in meagre scale collection, preparation and interpretation is given training on specific issues of the meagre scales. This training should cover: collection- and/or preparation-related issues (**section 4.3.5.1**), observation-related issues (**section 4.3.5.2**), and more meagre-specific issues (**section 4.3.5.3**).

4.3.5.1 Collection- and/or preparation-related issues

Contrary to otoliths, scales are external structures that can easily detach from the fish body and "contaminate" nearby fish. Consequently, if scale collection is careless there is a high probability that each paper envelope will contain scales from more than one fish. If that occurs, it will become increasingly difficult to establish a consensus between the readings of the several scales imprinted for each fish and the quality of age determinations will decrease. To avoid sample contamination, field personnel should always clear the fish skin from already detached scales before collecting the samples. Additionally, the scales should always be collected in large numbers as this will reduce the proportion of alien scales in each sample. These two

practices are relatively obvious but should be routinely stressed to field samplers as this will prevent unnecessary errors in the final ages.

Scale interpretations become increasingly difficult when dealing with acetate slides that contain imprints of scales of markedly different size, shape, or thickness. The main reason for this is that imprints on such slides tend to show up unevenly pressed. Additionally, it is generally hard to achieve consensus among differently-shaped scales. Most of these difficulties can be avoided with increased standardization of the collection and preparation procedures. The size and shape of the imprints can be made more homogeneous if large samples are collected and scales are removed from the target area instead of regions too close to the dorsal fin or the lateral line. Additionally, during preparation it is important to take time to carefully select the most similarly-looking scales from each sample. Stressing such simple practices will seem unnecessary to many field and lab technicians and implementing them will generally increase the time spent in collection and preparation of the scales; however, these will be largely rewarded with less reading time and an overall improvement of the age determinations.

The quality of final age determinations is highly dependent on the quality of the acetate imprints. Consequently, before acetate slides are stored into their final slide boxes it is good practice to carry out a preliminary check on the quality of the imprints. A well-prepared scale imprint presents well-resolved *circuli* (both at the center and periphery), clearly defined anterior and lateral margins, and no cracks (e.g., **Figure 26, Figure 27**). If that is not the case, the imprint should be considered unsatisfactory and pressing should be repeated in a new set of scales with readjusted press settings. In doing this, lower temperatures, shorter pressing times, and lower pressures will provide for lighter markings; however, if excessively low, they will also cause insufficient pressing of the margins and lead to imprints with heterogeneous appearance (**Figure 29**). In contrast, higher temperatures, longer pressing times, and higher pressures will provide for stronger markings and clearer marginal contours; however, if excessively high, they will also cause cracks and/or blurred *circuli* thus troubling annuli interpretations (**Figure 30**). In meagre, adequate pressing is particularly hard to achieve in larger and thicker scales, which frequently do not show up well-pressed at the first attempts. Consequently, particularly at the beginning of a study, it is important to collect a larger-than-average number of scales from the bigger fish (e.g., over 20 scales) in order to ensure that enough scales are available to obtain good imprints. Later on, with increased technician expertise, this number can generally be dropped down to the 10 to 15 scales typical of the routine collection protocol **(section 4.1**).

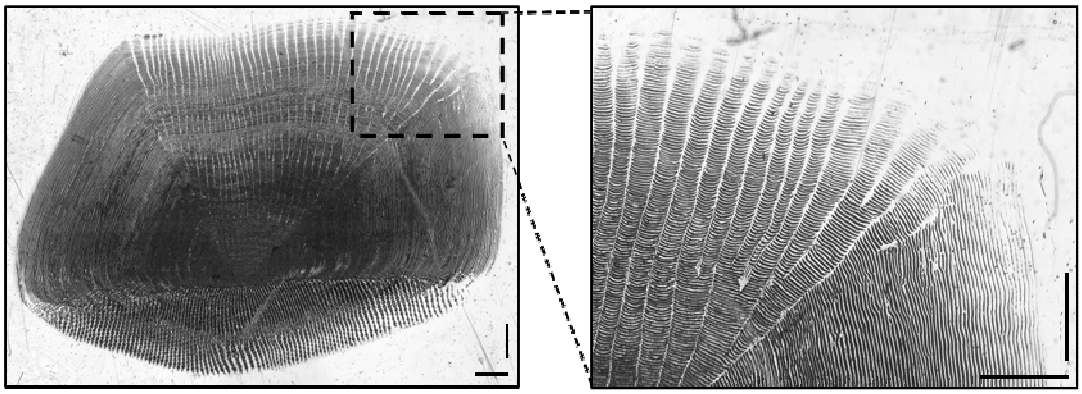

Figure 29 – Aspects of a badly-prepared scale imprint due to insufficient temperature, pressure and time. Scale bars = 1 mm, 16x

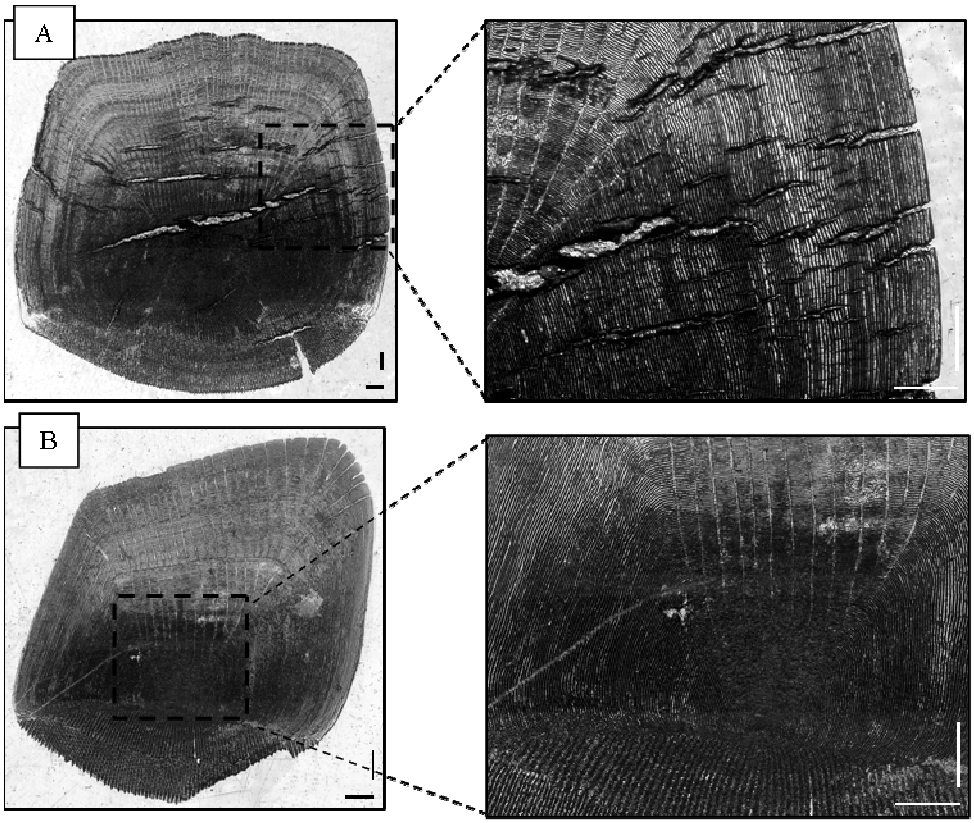

Figure 30 – Aspects of badly-prepared scale imprints due to excessive temperature, pressure and time. A – cracks, B – blurred center. Scale bars = 1 mm, 9x (A), 16x (B).

4.3.5.2 Observation-related issues

The annuli of meagre scale imprints are searched for within a high-resolution grayscale image that bears hundreds of similarly looking concentric *circuli*. Analyses of this type of images are difficult and tiresome to the human eye making scale annuli counts susceptible to optical illusions and eye-weariness biases. Two simple practices may be adopted that reduce these negative impacts by aiding in pattern recognition, reducing eye weariness, and/or helping to maintain reader's motivation during scale readings. The first practice involves readers routinely

alternating between focused and unfocused images, and between close-up observations (e.g., 30 cm from screen) and more distant observations (e.g., 1 m from screen), as doing this will help reveal obscure annuli and reduce effort and time spent in annuli search. The second practice involves readers taking frequent breaks during the reading sessions (e.g., every 1–1.5 hours) because this helps reducing eye weariness and sustaining reader's motivation, improving consistency across the usually long periods of exposure to microfiche reader illumination.

4.3.5.3 Other issues

The major difficulties met in reading meagre scales cannot be directly avoided because they are related to the long life span of the fish, to the slow growth of its scales at older ages, and to the large thickness of the scales collected from older specimens. In fact, the annual scale increments of meagre older than 10 years are small (frequently less than 0.5 mm), which causes the most peripheral annuli to appear very compact ("crowded") near the scale margin. This crowding effect takes place throughout the whole anterior field of the scale and makes primary criteria like *circuli* compaction and *circuli* disruption difficult to evaluate. Additionally, the scales of older meagre tend to be very thick (commonly over 0.30 mm, up to 0.75 mm) making it particularly difficult to obtain good imprints (see **section 4.3.5.1**). Altogether, these aspects lead to increased subjectivity in the discrimination of scale annuli from younger to older fish. In fact, readers commonly report objective scale readings only up to the tenth annulus. Thereafter, scale annuli counts are deemed increasingly subjective and frequently found to underestimate otolith-derived ages up to a factor of 2, even if readers relax the application of the primary criteria and, e.g., begin to count every straightening-out region visible in the anterior field.

Even if the difficulties of reading older meagre scales cannot be avoided, the interpretations of scales of younger fish can be made precise and comparable to otolith-derived ages. However, for this to happen it is important that readers are aware of some specific patterns of the meagre scales. One such pattern is annulus splitting. Annulus splitting involves the branching of single annulus into two (or, more rarely, three) distinct branches and if unaccounted for can lead to an overestimation of the annuli counts of younger fish. Splitting generally takes place in the anterior field of the scale and shows up as a set of distinct straightening-out and compaction areas that are very close to one another and resemble different annuli (**Figure 31**). However, a closer look at these putative annuli generally confirms that they are distinct branches of a single annulus that effectively rejoin in the lateral fields of the scale. Consequently, the only effective way to prevent the errors caused by undetected annulus splitting is through increased practice and training in the analysis of meagre scale patterns. In doing this, it is particularly important to ensure that the habits of systematically tracing annuli across the entire anterior region and systematically comparing the readings obtained from different axes are well-included into the reading routines.

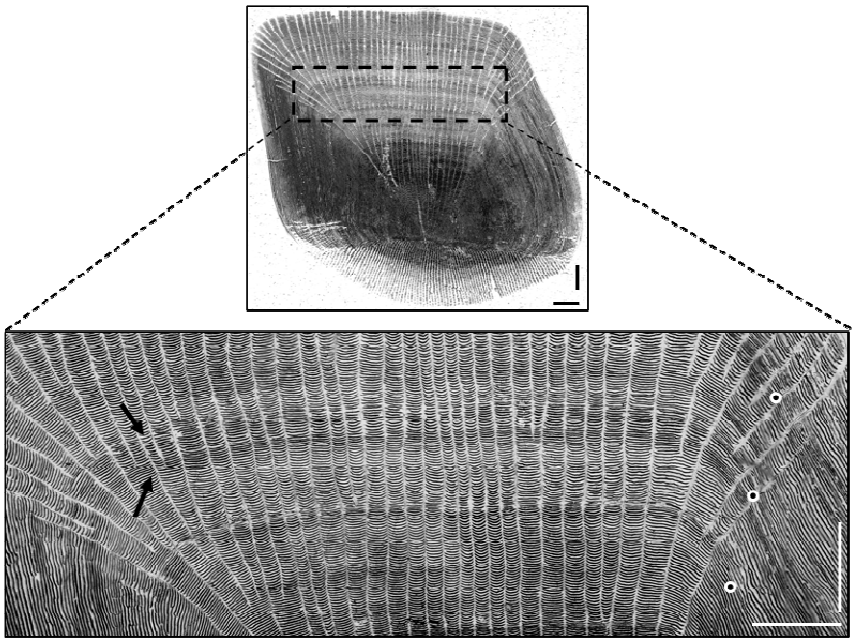

Figure 31 – Annulus splitting. The arrows indicate a doubled annulus. The white dots indicate the actual annuli. Scale bars = 1 mm, 16x (above), 30x (bottom).

To counteract the major difficulties felt in applying the primary criteria of annulus identification, a set of secondary (or corroboratory) criteria exists. These criteria are not to be used singly to assign annuli but along with the primary criteria have been found to improve the reliability of the scale interpretations, particularly when the primary criteria are not met by all annuli or along the entire course of every single annulus. The corroboratory criteria used in meagre readings can be broadly divided into a) criteria used to identify the first annulus and b) other criteria used in annulus identification:

a) Criteria used to identify the first annulus: The segmented *circuli* of the first annulus are frequently too narrow and compact for the straightening-out effect to be clearly observable. Additionally, the center portion of the thicker scales frequently appears dark and blurred, troubling annuli identification in that part of the scale. In such cases, three types of evidence have been found to aid in first annulus identification. The first evidence comes from the observation that the *circuli* compaction tends to be much larger before the first annulus than immediately after it, particularly in the anterior field. This difference in *circuli* compaction creates a contrast between the region located just before the first annulus and the region immediately after it that can be used to corroborate the annulus when primary criteria are not conclusive **(Figure 32)**. The second evidence comes from a similar observation but in the posterior field of the scale, where the first annulus is frequently evidenced by a semicircular band of lighter *ctenii* that contrasts the darker appearance of more peripheral regions **(Figure 32)**. Finally, it has also been observed that the first annulus is found along the anteroposterior axis of the scale at a distance of 1.3–2.6 mm from the focus (average: 1.9 mm). Consequently,

taking some measurements along the anteroposterior axis is frequently useful in narrowing the region where *circuli* are inspected for primary criteria match.

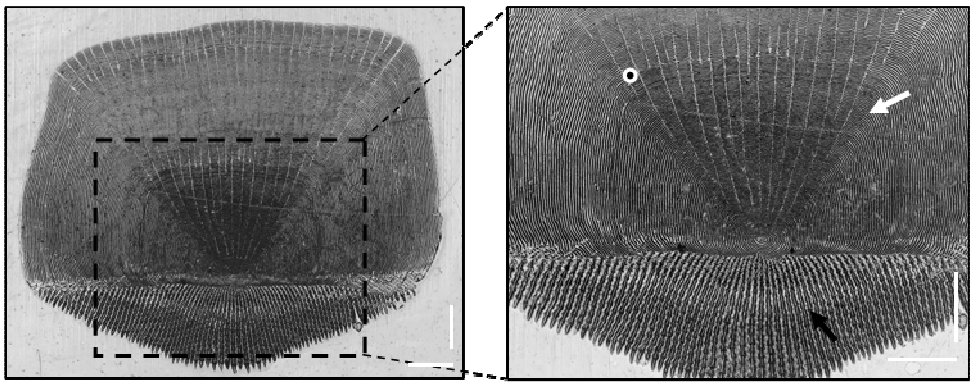

Figure 32 – Aspects of corroboratory evidence for the first annulus. The white dot indicates the first annulus. The white arrow indicates center compaction. The black arrow indicates the lighter posterior band. The first annulus is at a distance of 2.5 mm from the focus. Scale bars = 1 mm, 20x (left), 40x (right).

b) Other criteria used in annulus identification:

- "Dark margin": An annulus forming at the margin of the scale is generally difficult to ascertain because it is rarely observable along the whole anterior and lateral fields of the scale and because primary criteria like *circuli* "straightening out" or "*circuli* disruption" are hard to apply without comparing *circuli* appearance to more peripheral regions. In those cases, practice shows that if a) a large marginal increment is observed beyond the last clearly observed annuli, b) there are some signs of *circuli* straightening out along the anterior margin, and c) a very dark *circulus* can be seen outlining the lateral fields of the scale, then an annulus should be assigned to the scale margin (**Figure 33**).

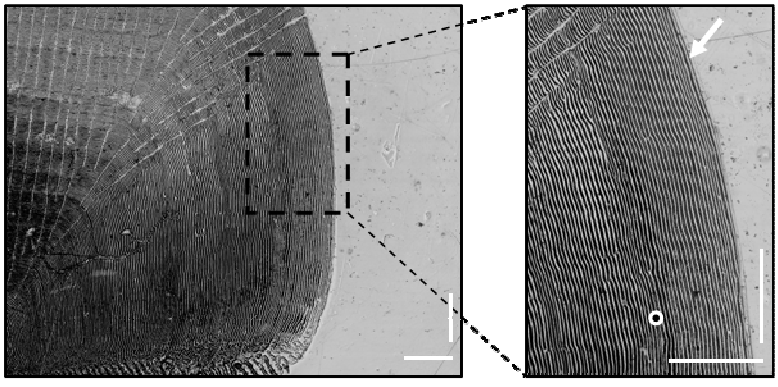

Figure 33 – Aspects of corroboratory evidence for marginal annulus. The white dot indicates the last clearly visible annulus. The white arrow points to the dark margin that evidences that a new annulus is just forming. Scale bars = 1 mm, 20x (left), 50x (right).

- "Annulus protrusion": At the anteroposterior interface, most continuous *circuli* halt their course and do not penetrate the posterior region of the scale. However, at an annulus

they are frequently observed to protrude into the posterior field, traversing the interface as straight dark lines **(Figure 34)**. Practice shows that such "annulus protrusion" into the posterior field is useful for corroborating intermediate annuli when primary criteria do not verify along the full extent of the anterior region of the scale.

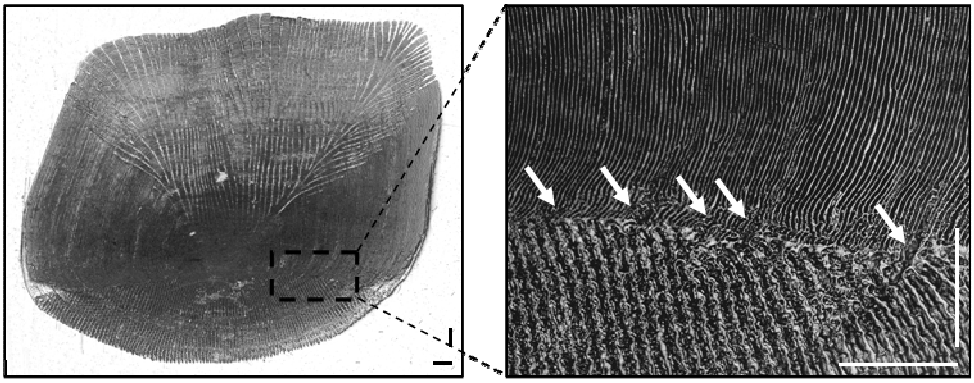

Figure 34 – Aspects of other corroboratory evidence for meagre annuli. The white arrows indicate annulus protrusion areas. Scale bars = 1 mm, 11x (left), 40x (right).

- Within-sample "scale regularities": Several regularities occur in the annuli structure of scale imprints taken from a single meagre specimen. The most important of these is that the imprints – even if presenting slightly different sizes and shapes – tend to have correlated interannuli distances and/or split annuli at similar locations. Consequently, when reading meagre scales it is good practice to start by taking an overall look at the several imprints to identify the main scale patterns before carrying out more detailed analyses on individual scales.

- Between-sample "scale regularities": The absolute annual growth of both meagre and its scales is very variable. Consequently, it is generally incorrect to assume that an annulus (other than the first) should sit at any specific distance from the scale focus. However, similar to other fish, there is an overall trend toward successively shorter interannuli distances from the center to the periphery of the scales. Considering this radial trend may be useful to corroborate some doubtful annuli interpretations: e.g., if a peripheral annuli is thought to sit very far from a previous one, it is probable that one or more annuli may have been missed; conversely, if a central annuli sits very close to a previous one, it is possible that it is a split branch and not a true annuli[6].

[6] Note: it is important to bear in mind that despite the long-term decrease in interannuli distances, large variability in interannuli distances still occurs in the short-term. An example of this is that, in meagre, it is not infrequent for the third annuli to be found very close to the second. For this reason, annuli corroboration based on interannuli distances should be used only in scales that bear at least 10 annuli and, particularly, never as a sole criterion to assign the most marginal annuli.

5. Age Assignment

The information required to determine the final age of meagre is: a) hard part reading data (annuli counts and marginal increment evaluations), b) stock-specific information (annulus deposition periods and spawning season), and c) sample-specific information (date of capture). In Portugal, marginal increment analyses indicate that otolith annuli are laid down from March to June and scale annuli are laid down from April to September (Costa *et al.*, 2008, N. Prista, *unpub. data*). Additionally, reproductive studies indicate that the meagre spawns from March to July with a peak in May and June (Costa *et al.*, 2008). Using this information, three types of age-related results can be calculated for each meagre specimen: age group (**section 5.1**), year class (**section 5.2**), and biological age (**section 5.3**).

5.1. Age group

The age group of a fish is the number of calendar years the fish lived until it was captured. To determine age group, data on hard part readings (annuli counts and marginal increment evaluation), on the month of capture, and on the annulus deposition season of the hard part under analysis are required. The meagre age groups are determined using January 1 as a standard birth date (i.e., a fish born in May 2000 will be assigned to age group 0 if captured until December 2000, to age group 1 if captured during 2001, to age group 2 if captured in 2002, and so on). The following procedure is used to determine age group:

- Fish captured between January 1 and the beginning of the annulus deposition season: The fish are generally assigned an age notation of $x + (x + 1)$, where x is the number of annuli in the otolith or scale. Their age group is $x + 1$.

  Examples (Portuguese coast):

  Otoliths: Any fish captured between January 1st and February 28/29th with three annuli and a translucent margin (type II/III), should be assigned an age notation 3 + (3 + 1), i.e., 3 + 4. The fish is age group 4.

  Scales: Any fish captured between January 1st and March 31st with five annuli and type II/III margin, should be assigned an age notation 5 + (5 + 1), i.e., 5 + 6. The fish is age group 6.

- Fish captured between the end of the annulus deposition season and the end of the year: The fish are generally assigned an age notation of $x + x$, where x is the number of annuli counted in the otolith or scale. Their age group is x.

  Examples (Portuguese coast):

  Otoliths: Any fish captured between July 1st and December 31st with three annuli and a translucent margin (type II/III), should be assigned an age notation of 3 + 3. The fish is age group 3.

Scales: Any fish captured between October 1st and December 31st with five annuli and a type II/III margin, should be assigned an age notation 5 + 5. The fish is age group 5.

- Fish captured during the annulus deposition season: The fish are assigned an age notation *x* (*x*), *x* + *x*, or *x* + (*x* + 1), depending on the development of the hard-part margin: if an annulus is visible at the margin (type I), the notation is *x* (*x*) and the fish age group is *x*; if little growth has taken place beyond it (type II), the notation is *x* + *x* and the fish age group is *x*; if substantial growth is visible beyond the last annulus (type III) the notation is *x* + (*x* + 1) and the fish age group is *x* + 1.

  Examples (Portuguese coast):

  Otoliths: A fish captured between March 1st and June 30th with three annuli: if the last annulus is on the edge (type I) or there is little growth beyond it (type II) the fish should be assigned an age notation 3 (3) or 3 + 3, respectively, and belongs to age group 3; if, however, significant growth occurred beyond the last annulus or a new annulus is anticipated to be forming soon (type III) the fish should be assigned an age notation 3 + 4 and belongs to age group 4.

  Scales: A fish captured between April 1st and September 30th with five annuli: if the last annulus is on the edge (type I) or there is little growth beyond it (type II) the fish should be assigned an age notation 5 (5) or 5 + 5, respectively, and belongs to age group 5; if, however, significant growth occurred beyond the last annulus and a new annulus is anticipated to be forming soon (type III) the fish would be assigned an age notation 5 + 6 and belongs to age group 6.

5.2. Year class

The year class is the year when the fish was born (e.g., 1997 year class). Year class (*YC*) is calculated as *YC* = *CY* – *AG*, where *CY* is the year of capture and *AG* is the age group:

Example: a fish captured in 2004 and age group 3 is from the 2004 – 3 = 2001 year class.

5.3. Biological age

Biological age is the time elapsed from fish birth to fish capture. To determine biological age, information on the fish age group and capture date is required. Furthermore, it is necessary to assume a common birthday for all fish in the stock (June 1 in Portuguese waters). Biological age (*BA*) is generally expressed in months and calculated as *BA* = 12 x *AG* – (*BD* – *CD*), where *AG* is the age group, *BD* is the month of birth and *CD* is the month of capture, with minor corrections being needed only in larval fish:

Example (Portuguese coast):

A fish belonging to age group 4 and captured in February is 12 x 4 – (6 – 2) = 44 months.

5.4. Examples

In **Table 3**, readings and final age assignments are presented for all otolith sections and scales imprints displayed in the current study.

Table 3 – Full set of readings and age assignments of the otolith and scales depicted in sections 3 and 4. Fish total length is provided for indicative purposes.

Figure	Specimen ID	Total length (cm)	Struct.	Date of capture	Annuli	Margin	Age notation	Age group (years)	Year class	Biological age (month)
6–8, 12C	CORV_0194	92	Otolith	01-07-2005	5	II	5+5	5	2000	61
9A	CORV_1846	17	Otolith	03-09-2003	0	III	0+0	0	2003	3
9B	CORV_0913	41	Otolith	10-01-2005	1	III	1+2	2	2003	19
9C	CORV_0123	61	Otolith	23-08-2004	2	II	2+2	2	2002	26
9D	CORV_1769	41	Otolith	20-06-2006	3	II	3+3	3	2003	36
10A	CORV_0334	157	Otolith	01-08-2005	14	II	14+14	14	1991	170
10B	CORV_0216	182	Otolith	13-10-2005	36	II	36+36	36	1969	436
11A, 13	CORV_0072	35	Otolith	07-03-2002	2	I	2 (2)	2	2000	20
11B	CORV_1672	59	Otolith	19-07-2006	2	II	2+2	2	2004	25
11C, 11D	CORV_1401	81	Otolith	16-02-2006	2	III	2+3	3	2003	32
12A	CORV_0257	126	Otolith	17-08-2005	8	II	8+8	8	1997	98
12B	CORV_0188	152	Otolith	13-10-2005	12	III	12+12	12	1993	148
14	CORV_0092	38	Otolith	11-01-2005	1	III	1+2	2	2003	19
15	CORV_1658	86	Otolith	15-05-2006	3	III	3+4	4	2002	47
16	CORV_1657	51	Otolith	15-05-2006	1	III (a)	1+2	2	2004	23
17	CORV_1231	148	Otolith	09-07-2005	13	II	13+13	13	1992	157
18B	CORV_0435	161	Otolith	20-06-2005	13	II	13+13	13	1992	156
20	CORV_0749	39	Scale	18-04-2005	1	III	1+2	2	2003	22
22–25, 28B, 28D	CORV_1764	53	Scale	20-06-2006	3	II	3+3	3	2003	36
26A	CORV_0607	22	Scale	28-10-2000	0	III	0+0	0	2000	4
26B	CORV_0768	42	Scale	13-10-2004	1	III	1+1	1	2003	16
26C	CORV_0599	59	Scale	13-08-2004	2	II	2+2	2	2002	26
26D	CORV_1772	51	Scale	20-06-2006	3	II	3+3	3	2003	36
27A, 34	CORV_0024	144	Scale	19-06-2004	9	II	9+9	9	1995	108
27B	CORV_0334	157	Scale	01-08-2005	13	III	13+14	14	1991	170
28A	CORV_0843	71	Scale	23-08-2004	4	I	4 (4)	4	2000	50
28C	CORV_0064	79	Scale	06-06-2004	3	III	3+4	4	2000	48
29	CORV_1203	100	Scale	25-04-2005	4	III	4+5	5	2000	58
30A	CORV_0004	162	Scale	06-08-2005	12	II	12+12	12	1993	146
30B	CORV_0368	109	Scale	08-11-2005	4	II	4+4	4	2001	53
31	CORV_1446	95	Scale	17-05-2006	5	III	5+6	6	2000	71
32	CORV_0036	61	Scale	22-08-2004	2	II	2+2	2	2002	26
33	CORV_0338	111	Scale	10-08-2005	6	I	6 (6)	6	1999	74

(a) Note: the observation plane does not account for parallax errors making the section look like a margin I.

6. Discussion

The European meagre has recently become the focus of increased scientific attention. On the one hand, the species is considered a promising species for European aquaculture (Angelini *et al.*, 2002; Quéméner *et al.*, 2002) and there has been increased interest in studying the biology of its wild populations to optimize aquaculture production (Quéméner, 2002; Jiménez *et al.*, 2005; Costa *et al.*, 2008); on the other hand, some concerns have been raised on the conservation status of the meagre populations in France, Spain, and Portugal, which have sparked research on the meagre fisheries and population parameters (Quéméner, 2002; Muñoz *et al.*, 2006; Prista *et al.*, 2007; Costa *et al.*, 2008; Prista *et al.*, 2008). Concomitantly, appendix VII of Council Decision 2008/949/EC recently established minimum age-sampling requirements for the European meagre (50 fish per 1000 t landed) which, given the geographic distribution of the species, will prompt routine sampling of commercial meagre landings along ICES Subareas XIa and VIIIa–c. Similar efforts and concerns have also taken place in Northern Africa – namely in Egypt, Mauritania, Senegal, and Morocco – where the species constitutes a more significant resource for local economies and also represents a promising candidate for aquaculture production (Hermas, 1995; Bebars *et al.*, 1997; Quéméner, 2002; El-Shebly, 2007). Altogether, these aspects make relevant the existence of validated standardized protocols for age determination of the species as only these will provide the quality and comparability of results required for progress in research, assessment and management at both national and international levels. The otolith and scale protocols presented in this study constitute a first step towards that faster progress as they detail the procedures involved in the collection and preparation of the meagre hard parts, specify the criteria used in the readings, and highlight many aspects and difficulties that should be addressed in reader's training across the species geographical range.

Sampling meagre hard parts for age and growth determination is a difficult task. In Europe, adult meagres are absent or rare in most marine fishery-independent surveys (Quéro and Vayne, 1987, Fátima Cardador, INRB/IPIMAR, *pers. comm.*) and commercial landings are low, seasonal, and size specific (Quéméner, 2002; Prista *et al.*, 2008). Additionally, the meagre is marketed round at local ports (i.e., neither beheaded nor gilled or gutted) and presents a high commercial value (large specimens may cost over 400 € ex-vessel) which makes otolith collection expensive and scale collection a delicate task (Quéméner, 2002; Prista *et al.*, 2007). Such situations markedly contrast those of other large sciaenids in, e.g., the Eastern United States, whose carcasses (and body parts) can be obtained from commercial and recreational fisheries at relatively low cost (Liao *et al.*, 2008); they also largely justify the comparative scarcity of meagre age and growth research in European waters and the need to consider alternative sampling techniques and alternative hard parts in determining the age of European meagre.

In Portugal, Prista *et al.* (2007) have shown that it is possible to obtain representative samples of meagre otoliths from the fishery, at low cost, by means of commercial mark-

recapture. However, given to the large geographical extent of the Portuguese meagre fisheries (Prista *et al.*, 2008) and the large size range of its landings (42–184 cm), it is unlikely that commercial mark-recapture can solely provide for routine long-term samples unless the meagre fishery becomes a management priority. Scales have long been used in fish age determination for an array of reasons, including the fact that they are easier to collect, can be collected without jeopardizing the fish commercial value, and generally present lower costs and preparation times than otolith thin sections (VanderKooy and Guindon-Tisdel, 2003). However, unlike otoliths, fish scales suffer from regeneration, erosion, or resorption, all of which complicate and bias age interpretations (Ericksen, 1999; VanderKooy and Guindon-Tisdel, 2003). Additionally, scale patterns are in general much harder to interpret (Lowerre-Barbieri *et al.*, 1994; Liao *et al.*, 2008) and much more prone to underage older fish than otolith thin sections (Lowerre-Barbieri *et al.*, 1994; Panfilli *et al.*, 2002; VanderKooy and Guindon-Tisdel, 2003). The latter is the case of scales from meagre and other long-lived species (e.g., striped bass *Morone saxatilis*), where annuli must be searched for within visually complex *circuli* patterns, and where substantial annuli crowding takes place at the periphery of older scales (Lowerre-Barbieri *et al.*, 1994; Liao *et al.*, 2008). However, even if suboptimal, scales may be worth considering in the sampling of, at least, some segments of the fishery and/or size classes.

A detailed comparison between scales and otoliths as hard parts used for meagre age determination was beyond the objectives of the current study and will be addressed elsewhere. Otolith thin sections are indubitably the most valid and effective method of determining sciaenid ages across the entire size range of the species (Beckman *et al.*, 1989; Lowerre-Barbieri *et al.*, 1994; Griffiths and Hecht, 1995; Campana and Jones, 1998; VanderKooy and Guindon-Tisdel, 2003; Liao *et al.*, 2008). However, readings of scale imprints have generally been considered sufficiently reliable to age the younger fish of the stock (Matlock *et al.*, 1993; Lowerre-Barbieri *et al.*, 1994). In meagre, even if more expensive to sample and time consuming to prepare, otolith thin sections should also be preferred to scale acetate imprints on basis of their easier interpretation and better performance in older fish. However, scales may constitute a valid alternative for meagre age determination if the research or assessment context involves samples composed of smaller fish (e.g., recruitment studies). In fact, a reasonable agreement (>90%) between otolith and scale readings is generally observed in fish younger than 4 years and smaller than 60 cm, even if underestimations of over 10 annuli are common in fish older than 10 years and larger than 160 cm (N. Prista, unpub. data).

In the current study, we obtained transverse thin sections of meagre otoliths and observed them under transmitted light without further processing. In doing this, we adapted the standard protocols used to determine sciaenid ages in the Eastern USA (Beckman *et al.*, 1989; Lowerre-Barbieri *et al.*, 1994; Campana and Jones, 1998; VanderKooy and Guindon-Tisdel, 2003; Liao *et al.*, 2008), but departed from other existing studies on meagre (Tixerant, 1974; that used the break-and-burn technique and reflected light) and other *Argyrosomus* (e.g., Griffiths and Hecht, 1995; that used longitudinal thin sections and reflected light). These departures were motivated by preliminary analyses carried out on different preparation procedures, where aspects such as

sectioning speed (low speed vs. high speed), sectioning plane (longitudinal vs. transverse), and postsectioning enhancement procedures (polishing and baking) were examined in terms of the relative improvement they brought to the readability and processing times of the otolith thin sections (N. Prista, unpub. data). The outcomes of these analyses indicated that, even if lower-speed single-otolith setups presented longer sectioning times (e.g., when compared to high-speed multiple-otolith setups), they provided for a better control of the sectioning plane and yielded easier to interpret sections that required no postsectioning enhancement. Additionally, they also indicated that readings obtained from longitudinal and transverse sections were alike and, consequently, that no readability advantage occurred in longitudinal sections that could justify the longer times they take to prepare. Quite on the contrary, it was found that transverse sectioning, when properly carried out, actually minimized the *ostium* blotch which is found limiting the interpretation of longitudinal sections (e.g., Griffiths and Hecht, 1995) thus providing an improvement to the overall readability of the otolith thin sections.

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Chapter 5

Reproduction

Chapter 5A

Reproductive phase determination in male meagre (*Argyrosomus regius*, Sciaenidae): testis development and histologic corroboration of a gross anatomical scale

Nuno Prista[a,b], Leonel Gordo[a,b], José Lino Costa[a], Maria José Costa[a,b] and Cynthia Jones[c]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Center for Quantitative Fisheries Ecology, Old Dominium University, 800W 46th St., Norfolk, VA 23508, USA

Scientia Marina

Reproductive phase determination in male meagre (*Argyrosomus regius*, Sciaenidae): testis development and histologic corroboration of a gross anatomical scale

Abstract: Reproductive stage determination of male gonads has received sparse attention in fish biology literature with few studies detailing the building of gross anatomical- and histologic scales. The meagre (*Argyrosomus regius*) is one of the world's largest sciaenids and supports a significant regional fishery in European and North African waters whose reproductive patterns are yet to be fully investigated. In the present study, we derive a macroscopic grading system for meagre testis using semi-quantitative graphs that feature the testis variability along the species size range and time of the year. We then describe the histological stages and reproductive phases of male testes and determine the extent to which they corroborate the anatomical scale. Our results indicate that gross anatomical analyses are accurate in assessments of the meagre spawning season but may not accurately distinguish the testes of well-mature fish and first spawning virgins. Furthermore, we show that milt expression varies widely with size and misclassifies as immature many smaller fish in spawning-capable condition. We discuss these findings in terms of their contribution to the understanding of testes development and the uncertainties involved in determining the size-at-maturity of male fish using gross anatomical scales.

Keywords: histology, testes, reproduction, males, validation, *Argyrosomus regius*

1. Introduction

The meagre (*Argyrosomus regius*, Asso 1801) is one of the world's largest sciaenids, attaining over 180 cm in total length and 50 kg in weight (Quéméner 2002). Its distribution ranges from the English Channel to Senegal (including the Mediterranean Sea and the Black Sea). Most of the year the meagre occurs in coastal waters (<80 m deep) but in spring and summer it migrates to shallow coastal waters and/or large European estuaries that it uses as spawning and nursery grounds (Quéro and Vayne 1987). The largest meagre fisheries take place in Mauritania, Morocco, and Egypt, which together comprise over 80% of the *ca*. 10000 t world annual catch (Quéméner 2002, FAO 2009). In Europe, national meagre landings are generally below 500 t/year (FAO 2009) but due to its large size, high ex-vessel prices and seasonal availability in inshore and nearshore waters, it is an important target species for local small-scale commercial and recreational fleets (Quéro and Vayne 1987, Quéméner 2002, Prista *et al.* 2008). This importance is underscored by the recent development of meagre aquaculture production and by the ecological significance of the meagre as a top marine predator in European coastal waters (Quéro and Vayne 1987, Quéméner *et al.* 2002, Schiavone *et al.* 2012). However, to date the biological characteristics of the meagre have remained scarcely studied worldwide and its fisheries have yet to be routinely monitored or assessed in both African and European waters (Prista *et al.* 2011).

Studies of gonad morphology at both anatomical and histological levels have long been done by fish biologists to identify reproductive cycles, spawning seasons and breeding areas, or

to determine size-at-maturity (Hunter and Macewicz 2003). In fisheries science, the determination of the reproductive state of a fish population in a specific area, time, and/or size class plays a vital role because these parameters are closely related to stock productivity and are the basis of many regulatory measures (e.g. minimum landing size) (Hunter and Macewicz 2003). Until recently, the reproduction of meagre had only been studied in North African waters on the basis of macroscopic observations of fish gonads and the monthly evolution of gonadosomatic indexes (Tixerant 1974, Hermas 1995). From 2000 onwards increased interest in aquaculture production and concern with data-poor regional fisheries sparked research and led to a new array of studies (e.g. González-Quirós *et al.* 2011, Prista *et al.* 2011, Abou Shabana *et al.* 2012, Morales-Nin *et al.* 2012, Schiavone *et al.* 2012, Gil *et al.* 2013). Among these are descriptions of the meagre life cycle in the Gulf of Cádiz (González-Quirós *et al.* 2011, Morales-Nin *et al.* 2012), two studies of meagre reproduction in aquaculture facilities (Schiavone *et al.* 2012, Gil *et al.* 2013), and one study that analyses the reproduction of males of wild meagre but with a very limited sample size (Abou Shabana *et al.* 2012).

Macroscopic maturity scales (also known as the gross anatomical grading systems) of fish ovaries and testes are among the most frequently used indexes in assessments of fish reproductive condition (West 1990, Hunter and Macewicz 2003). The use of these methods is rooted in historical fish biology literature and their inexpensiveness and fast applicability—along with proven capabilities to detect major reproductive events (namely spawning season)—has built them into routine research protocols of nearly every fisheries survey in the world. However, several authors have warned of their imprecision and biases in assessments of reproductive parameters that require fine resolution (e.g. size-at-maturity). Such flaws can be circumvented through validation studies in which independent histological observations (assumed to accurately reflect the internal development of fish gonads) are used to fine tune the anatomical scales and estimate their biases (West 1990, Hunter and Macewicz 2003, Brown-Peterson *et al.* 2011).

Historically, the vast majority of histological studies concerning fish reproduction have addressed female reproduction, with less information being available on male reproduction (Grier *et al.* 1987, Parenti and Grier 2004, Lowerre-Barbieri *et al.* 2011, Brown-Peterson *et al.* 2011). However, the recent fish reproduction literature in ecology, fisheries and aquaculture has increasingly noted the importance of fully understanding male reproductive patterns (Parenti and Grier 2004, Brown-Peterson *et al.* 2011) because males may have different reproductive parameters from females such as a different span of spawning seasons and a different size-at-maturity (Grau *et al.* 2009, Lowerre-Barbieri *et al.* 2011). Pin-pointing and understanding such differences is biologically significant and important for fisheries because they constitute the supporting evidence underlying frequently adopted management measures (e.g. minimum landing size).

In this study we provide the first detailed description of meagre testis development at macroscopic (anatomical) and microscopic (histologic) level. In doing this, we describe the size-related and seasonal variability of macroscopic and microscopic characteristics and use these

to build an anatomical scale and a histological scale. Then, we the compare histological and macroscopic information and use these results to corroborate the anatomical scale, identify its main biases and discuss how they can be minimized. To our knowledge, these results constitute the first comprehensive reproductive study of testes from wild meagre in European waters and also one of the first attempts at the step-by-step construction and histological corroboration of an anatomical grading system for fish testes.

2. Materials and methods

2.1. Sampling methodology

A total of 2418 meagres were sampled from 2003 to 2007 during a large-scale study that targeted the meagre fishery and biology on the Portuguese coast. Detailed coverage of the sampling methodologies, the fisheries and areas covered can be found in Prista *et al.* (2007, 2008) and Costa *et al.* (2008). Biological sampling was carried out monthly in two geographical areas that encompass the main meagre fisheries in Portugal: the Tagus estuary (central-western coast) and the coast of Olhão (southern coast). Monthly sampling goals were set at 10 males per 10-cm size class following the requirements of a concurrent age and growth study with effectively achieved goals largely dependent on the seasonal availability of the fish in the two geographical areas. In general, all meagre specimens were measured (total length) and weighed (total weight), and had their abdomen slightly squeezed for expressible milt or roe. Whenever possible, the individuals were gutted and sexed, and their gonads were weighed (to the nearest 1 g), checked for the presence of the large-bodied female nematodes of the genus *Philometra* (Moravec *et al.* 2007) and analysed for macroscopic characteristics and/or preserved for histology. In general, otoliths were removed and later processed for data determination following Prista *et al.* (2009).

2.2. Macroscopic analysis

Meagre testes were subjected to macroscopic classification with respect to a set of pre-defined characteristics (Table 1). The set included characteristics reported in previous meagre studies (Tixerant 1974) and in studies of other large sciaenids (Murphy and Taylor 1990, Griffiths 1996, Farmer 2003), and two characteristics that were found varying with fish size and/or season: a) the presence/absence of a groove running longitudinally along the proximal side of each testicle which makes this side appear concave (hereafter termed the *proximal groove*) and b) the presence/absence of striae, generally over two centimetres long, running longitudinally on the ventral surface of spent testicles (hereafter termed *ventral striae*). Objectively defined levels were set for each morphological feature with no a priori judgment of gonad maturity being made. To make the observation of each morphological feature more objective and reduce observation biases, the laboratory protocol involved the observation of the several features, one at a time, across the several fish from each sample, and only in the end

were the gonads photographed and coarsely graded as to their overall macroscopic appearance. A final anatomical grading system was later obtained from analysis of variations in the frequency of occurrence of each macroscopic characteristic across size (within the peak spawning season) and months (in mature fish). In these analyses, the peak spawning season was considered to be May and June (Costa *et al.* 2008) and fish were assumed to be mature fish at 80 cm (Tixerant 1974).

Table 1. Main characteristics used in the macroscopic analysis of meagre testes

Characteristic	Classification levels
Coloration	White; Yellow; Orange; Red; Brown
Overall shape of gonad lobe (in ventral view)	Rectangular; Triangular; Lozenge; Too thin to characterize; Other
Shape of gonad cross-section	Rectangular; Triangular I (acute triangle; generally equilateral); Triangular IIa (rectangular; generally isosceles); Triangular IIb (rectangular; generally isosceles; with proximal sulcus on proximal side of testicle); Too thin to characterize
Fullness	Turgid (full); Not turgid ("half" full); Flaccid (empty-looking)
Thickness of gonad wall	Thin; Thick
Presence of ventral striae	Present; Absent; Gonad too thin to characterize
Reaction to abdominal pressure	Positive (leaks semen upon thumb-rubbing); Negative (no leakage of semen upon thumb-rubbing)
Reaction to pressure	Ruptures immediately; Offers resistance; Does not rupture ("rubberish")
Presence of blood "dots "	Present and conspicuous; Absent or inconspicuous

2.3. Histological analysis

Male gonads were fixed and preserved in 4% buffered formaldehyde (buffer: Na_2HPO_4 and $NaH_2PO_4*H_2O$) and stored in plastic boxes. Good preservation of large testes was assured by carefully injecting a small amount of fixative through the gonad wall with a syringe. When testis volume was too large, only the left lobe was preserved. Histological procedures were carried out on small pieces of meagre gonad (about 0.125 cm^3) taken from the ventral periphery of the medial region of the lobe. In general, the pieces included a full cross-section of the testes (from the periphery to the lumen); in larger testes, however, only the periphery and middle regions were represented. Histological preparation of gonads involved successive alcoholic dehydratations, infiltration, sectioning, mounting and staining. Technovit 7100 resin (Heraeus Kulzer) was used as the embedding medium and a Leica RM2155 micrometer was used to obtain thin sections (3–5 µm thick). The sections were stained with toluidine blue, a basophilic metacromatic stain. Three replicate sections were mounted in each slide. Permanent preparations were obtained using Neomount and Neoclear (Merck). Microscopic analyses were carried out at 40-400x magnification on a Zeiss stereomicroscope equipped with the Axioplan 2 imaging system. Digital pictures of histological slides were taken at 200–400x magnification with an AxioCam and processed and measured using AxioVision 4 (Zeiss). Image processing after capture was restricted to resizing, contrast and brightness adjustments, and minor background clean-ups.

The description of male gametogenesis follows Parenti and Grier (2004) and Dadzie and Abou-Seedo (2004), along with the recently proposed unifying terminology of Brown-Peterson *et al.* (2011). Slides were examined after randomizing slide boxes in order to minimize bias in structure identification. The final histological grading system developed for male meagre was based on a) overall testis structure, b) the relative abundance of the several germinal epithelium (GE) developmental stages, c) the continuity or discontinuity of the GE, d) abundance of spermatozoa in the lobule *lumen*, and f) the thickness of the testis wall (Dadzie and Abou-Seedo 2004, Brown-Peterson *et al.* 2011). Overall testis appearance was graded as "compact", "compact(at periphery)->tubular(closer to core)" and "tubular". The development stages of the GE included primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa. The relative abundance of each GE stage and spermatozoa was graded as "++" (very abundant), "+" (abundant), "-" (scarce) and "0" (absent), based on a consensus between the replicates on each slide. The continuity of the GE was graded as "compact", "continuous", "discontinuous" and "regenerating" (spermatogonia regeneration). Wall thickness was classified as "very thin", "thin", "thick" and "very thick". Cell diameters reported on descriptions of spermatogenesis stages were obtained from measurements of five cells per individual in each of five individuals randomly sampled from within the ones known to carry cysts of each developmental stage. Final testis development phases follow the recent standardization proposed by Brown-Peterson *et al.* (2011), whose conceptual model considers "immatures" (fish that never spawned), "developing" (fish whose testes are beginning to grow and developing), "spawning-capable" (fish that are developmentally and physiologically able to spawn in the current year), "regressing" (sexually mature fish that have finished spawning) and "regenerating" (sexually mature fish that are reproductively inactive). A full account of the histological details of each development phase can be found in Brown-Peterson *et al.* (2011).

2.4. Histological corroboration of the anatomical scale

To evaluate the extent to which the gross anatomical scale reflects the histological structure of testes in male meagre specimens and corroborate the use of the anatomical scale in routine sampling of meagre in the field, the results of the anatomical scale were compared with the ones obtained from histological analysis using the subset of testes in which both scales had been applied. These comparisons considered the histological classification to represent the "truth" because histology is widely regarded as the most accurate staging method for fish gonads (Hunter and Maciewicz 2003, Costa 2009, Ferreri *et al.* 2009).

3. Results

3.1. Anatomical classification scale

A total of 242 testes were subjected to macroscopic analyses. These included 234 testes taken from the monthly-length stratified sampling of sexed individuals and gonads from eight immature fish whose sex could not be macroscopically determined. Testes subjected to macroscopic analyses came from fish captured in estuarine (n=123) and coastal (n=114) fishing grounds from both the western (n=130) and the southern (n=112) Portuguese coasts. For five fish the type of fishing ground was unknown. The sample comprised more than three testes from each month and more than four testes from each 10-cm fish size class found in the size span of male meagre on the Portuguese coast (up to 178 cm). Exceptions to this were testes from the December to February period (when a single fish was sampled), testes from fish larger than 170 cm (two samples only) and testes from fish smaller than 30 cm (no sample).

The reproductive system of meagre males consists of a pair of elongated testes which lie within in the body cavity against the fish swimbladder. Upon dorsal observation, testes are rectangular in shape but variations occur between lozenge (middle part wider) and triangular (anterior part wider). The cross-section is generally triangular, with the base of the triangle fitting dorsally against the swimbladder and its tip oriented towards the belly. The surface of the testis is smooth and has two vascularized hili running along its dorsal surface. These hili fuse posteriorly to form a single sperm duct that opens to the genital pore.

The testes of mature meagre are creamy white to rosy between March and July, turning brownish thereafter. They appear full or half-full, filling the majority of the body cavity and easily rupturing upon handling or pressing from March to July, and become empty-looking and “rubberish” from August onwards (Figure 1A). In spring and early summer the hili are generally occluded underneath the swollen dorsal surface, becoming visible thereafter. From May onwards many testes exhibit a marked proximal groove and ventral striae. Positive reaction to abdominal pressure increases from March (50% of testes) to May (95% of testes) and decreases thereafter (Figure 1B). In late summer and autumn, residual milt is still observed in testis sections from fish showing no previous reaction to abdominal pressure, thus evidencing prolonged sperm storage in time.

Within the putative spawning season (May and June) the macroscopic appearance of meagre testes changes markedly with size (Figure 2). Fish <40 cm all have very thin thread-like testes with barely any width. From 40 to 80 cm the testes widen and thicken rapidly but remain empty-looking and relatively thin in many fish (Figure 2A, B). Over 80 cm no thin testes are observed and full to half-full gonads dominate (Figure 2A). The testes of fish <70 cm did not display ventral striae but these became apparent in fish >70 cm and were present in all individuals above 110 cm (Figure 2B). A positive reaction to abdominal pressure was not observed in fish below 40 cm, but took place in 40% of individuals between 40 and 70 cm, and in 85% of the fish larger than 70 cm (Figure 2C).

Nematodes from the genus *Philometra* were found in the testes of 22 out of 101 males checked for these parasites. The parasites were most frequently observed along the sperm duct and were easier to observe when gonads were less turgid. All testes with parasites were sampled on the southern coast, where the monthly prevalence reached 91% in September.

Based on the previous macroscopic characteristics, the gross anatomical grading system derived for meagre testes is displayed in Table 2 and Figure 3.

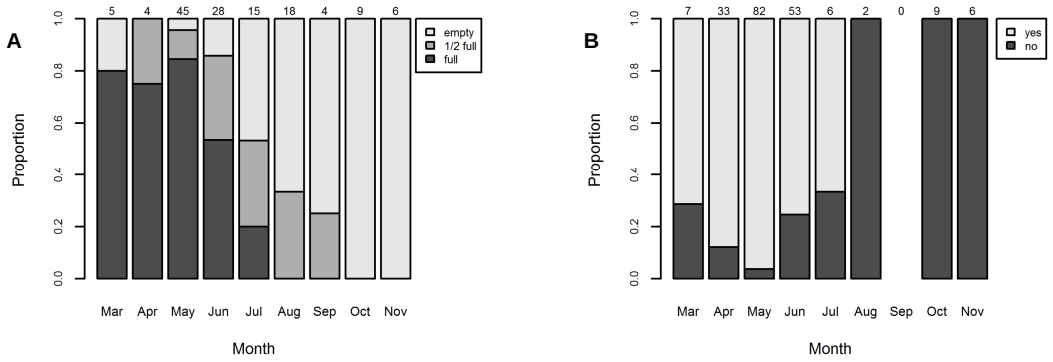

Figure 1. Monthly variations in the macroscopic appearance of testes of mature meagre (>80 cm; Tixerant 1974). *y*-axis represents the proportion of monthly samples that registered each feature. A) Fullness; B) Reaction to abdominal pressure. Numbers above bars are sample size.

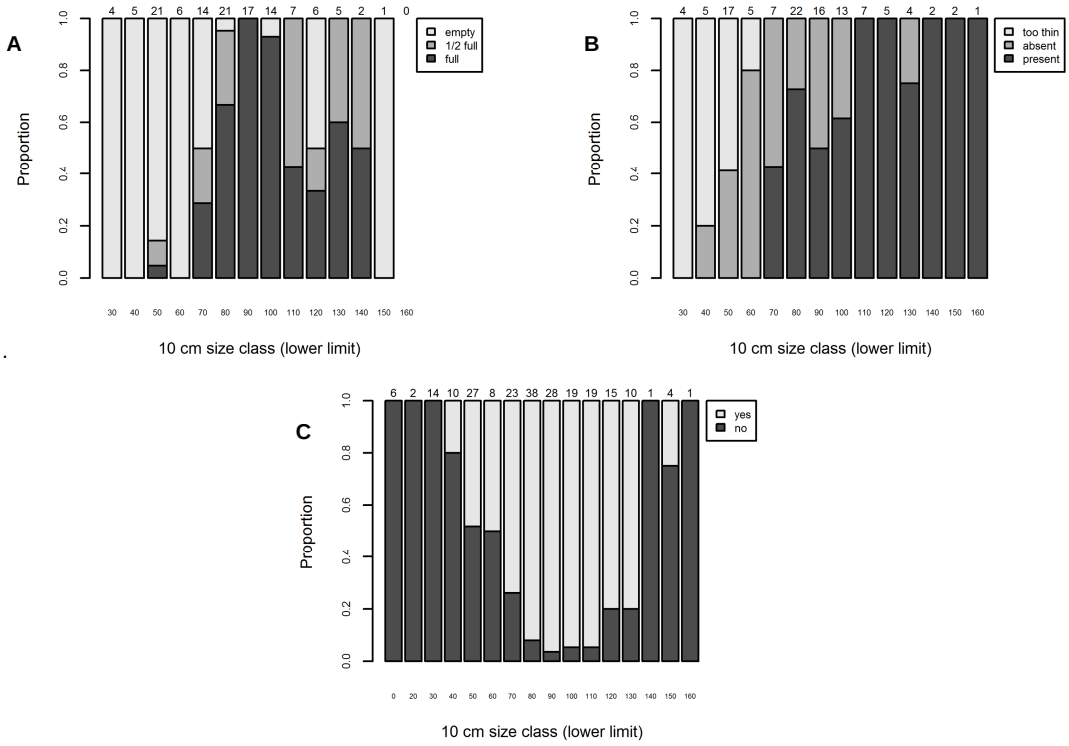

Figure 2. Size-related variations in the macroscopic appearance of testes of meagre during the putative spawning season (May and June). *y*-axis represents the proportion of samples that registered each feature. A) Fullness; B) Presence-absence of ventral striae ("too thin" indicates gonad so thin that identification of ventral striae could not be carried out); C) Reaction to abdominal pressure. Numbers above bars are sample size.

Table 2. Gross anatomical grading system derived for the meagre testis. W_g, weight of gonad; TL, total length (mean±s.e.); A, age (mean±s.e.); M, month(s) of occurrence. GSI (%) is calculated as (gonad weight/fish total weight)*100 (mean±s.e.).

Anatomical class	Macroscopic features	Other notes
0	Very thin thread-like lobes (transparent and with barely any width or thickness); sex of fish cannot be identified by naked eye.	W_g≈0 g (n=6); GSI(%):0.04±0.00 (n=6); TL(cm):37.3±1.5 (n=6) A(years):2.3±0.7 (n=3) M, June
I	White to rose or brown thread-like testes (translucent to opaque with small but measurable width and thickness); reduced thickness makes the observation of ventral striae and median cross-section very uncertain; no reaction to abdominal pressure.	W_g<4 g (n=39); GSI(%): 0.10±0.01 (*n*=39) TL(cm): 55.9±2.0 (n=39) A(years): 2.6±0.1 (n=37) M: April–July, October, December
II	White to rose opaque thread-like testes; median cross-section triangular or domed; no conspicuous ventral striae; robust to handling; some sperm may be present in the sperm duct; positive reaction to abdominal pressure but in reduced amount.	1 g<W_g<34 g (n=25); GSI(%): 0.29±0.03 (*n*=25) TL(cm): 64.0±2.2 (n=25) A(years): 3.4±0.2 (n=19) M: April–July, October
III	White to rose testes, sometimes reddish with increased vascularization; testes widen and increase in volume, becoming tubular in appearance; ventral striae usually present; triangular median cross-section (I or II), very turgid, the sperm duct occluded underneath the swollen surface; ruptures easily when handled, tearing itself up under its own weight; positive reaction to abdominal pressure, abundant semen throughout the gonad.	15 g<W_g<1420 g (n=84); GSI(%): 3.27±0.17 (*n*=84) TL(cm): 96.8±2.0 (n=83) A(years): 6.1±0.2 (n=58) M: March–July
IV	Brownish white to dark brown testes with much-reduced volume; median cross-section generally triangular with a pronounced proximal sulcus; conspicuous ventral striae in larger individuals; flaccid appearance with visible sperm duct; highly resistant to handling; some semen is visible in sectioned testes, particularly when they are squeezed; little or no reaction to abdominal pressure.	3 g<W_g<707 g (n=89); GSI(%): 0.60±0.06 (*n*=89) TL(cm): 117.3±3.6 (n=88) A(years): 8.8±0.8 (n=66) M: March, May–November

3.2. Histological classification scale

A total of 136 testes were subjected to histological analysis. These testes were selected at random from the pool of testes collected each month, but the selection was constrained to the overarching objective of obtaining good coverage of a) the size classes sampled, b) the main macroscopic types observed in the field and c) the main fishing grounds for the species. The final sample included at least four testes from each 10-cm fish size class between 40 cm and 170 cm and at least three testes from each month, with the exception of September (n=1) and November through February (n=0), when the meagre is scarce in Portuguese fisheries (Prista *et al.* 2008). Furthermore, testes from both estuarine (n=87) and coastal (n=31) fishing grounds and from both the western (n=99) and the southern (n=37) Portuguese coasts were represented. For 18 fish the fishing ground was unknown.

The internal structure of male testes is of lobular unrestricted type (*sensu* Parenti and Grier 2004). Active spermatogenesis occurs throughout the testis within spermatocysts that contain synchronously developing cell clones. Six developmental stages are present in male meagre germ epithelium: a) primary spermatogonia (Sg1), b) secondary spermatogonia (Sg2); c) primary spermatocytes (Sc1); d) secondary spermatocytes (Sc2); e) spermatids (St); and f) spermatozoa (Sz).

Primary spermatogonia (Sg1) are oval-shaped cells, 7-22 μm in diameter, that appear isolated or in small groups. Both the nucleus and cytoplasm of Sg1 are slightly basophilic (light blue). The nucleus frequently occupies over 2/3 of the cell surface and may have a conspicuous strongly basophilic nucleolus at the centre (dark blue). Sg1 are frequently accompanied by smaller Sertoli cells ~4-7 μm in diameter (Figure 4A).

Secondary spermatogonia (Sg2) are polygonal cells, 4-7 μm in diameter, that appear densely packed in nests with 8 to 35 cells. The nucleus has intermediate basophily (deep purple) and occupies nearly the entire cell. Cytoplasm is acidophilic and very reduced. No nucleoli are visible (Figure 4B).

Primary spermatocytes (Sc1) are polygonal to round cells, 4-8 μm in diameter, that appear more loosely clustered (frequently over 50 cells) and in larger numbers than Sg2 (Figure 4C). The nucleus of Sc1 is circular and strongly basophilic (dark blue), with no nucleolus visible. The cytoplasm has low basophily (light purple) and unlike Sg2 is clearly distinguishable under light microscopy.

Secondary spermatocytes (Sc2) are similar in appearance and numbers to Sc1 but smaller (3-6 μm), more basophilic (both nucleus and cytoplasm) and more densely packed (Figure 4C). Sc2 are a relatively brief developmental stage that are abundant only in 30% of spawning-capable individuals.

Spermatids (St) are markedly smaller than the previous spermatogenic stages, their nucleus remaining strongly basophilic and the cytoplasm has intermediate basophily. Their overall size (2-4 μm) is low compared with Sc2. They appear in very dense nests that may contain several hundred cells (Figure 4C, D).

Spermatozoa (Sz) first appear with their heads and tails aligned within recently burst St cysts in “parachute” form (Figure 4D). Their heads are tiny (1-3 μm) and strongly basophilic (dark blue), their tails being long and mildly basophilic (light purple). During spawning, they are released to the lumen of the lobules where they form very dense aggregations (Figure 4C).

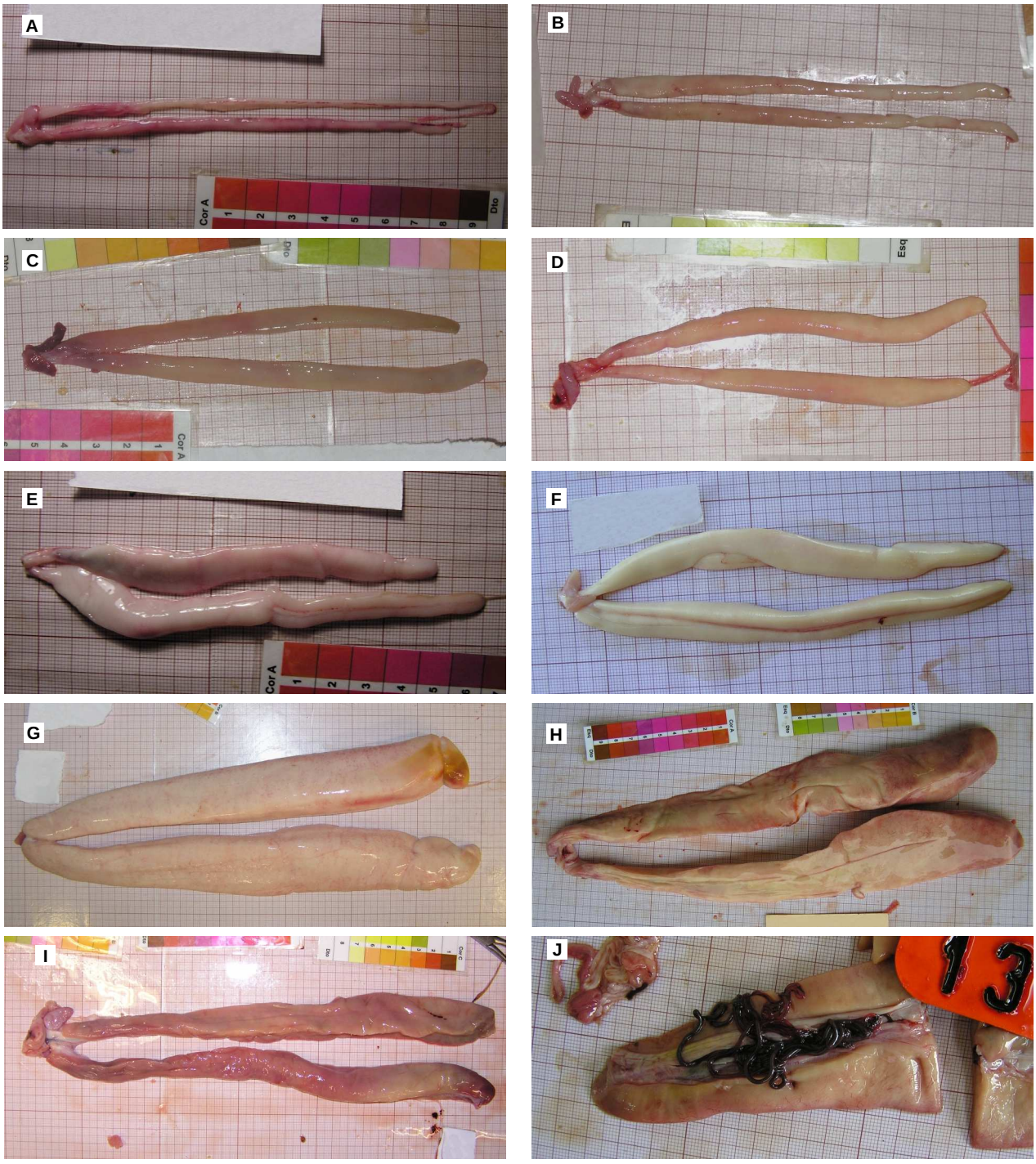

Figure 3. Macroscopic appearance of meagre male gonads [TL, total length in cm; M, month of capture; P, Place of capture, Southern Coast (SC) or Tagus Estuary (TE); H, Histological stage]. A) Anatomical class I [TL, 66; M, May; P, SC; H, Developing]; B) Anatomical class I [TL, 59; M, June; P, TE; H, spawning capable (virgin)]; C) Anatomical class II [TL, 76; M, July; P, TE; H, Developing]; D) Anatomical class II [TL, 69; M, June; P, TE; H, spawning capable (virgin)]; E) Anatomical class II [TL, 59; M, May; P, SC; H, spawning capable (mature)]; F) Anatomical class III [TL, 59; M, June; P, TE; H, spawning capable (virgin)]; G) Anatomical class III [TL, 100; M, May; P, TE; H, spawning capable (mature)]; H) Anatomical class IV [TL, 148; M, June; P, SC; H, regressing]; I) Anatomical class IV [TL, 144; M, October; P, SC; H, regenerating]; J) Close-up of live parasites from genus *Philometra*.

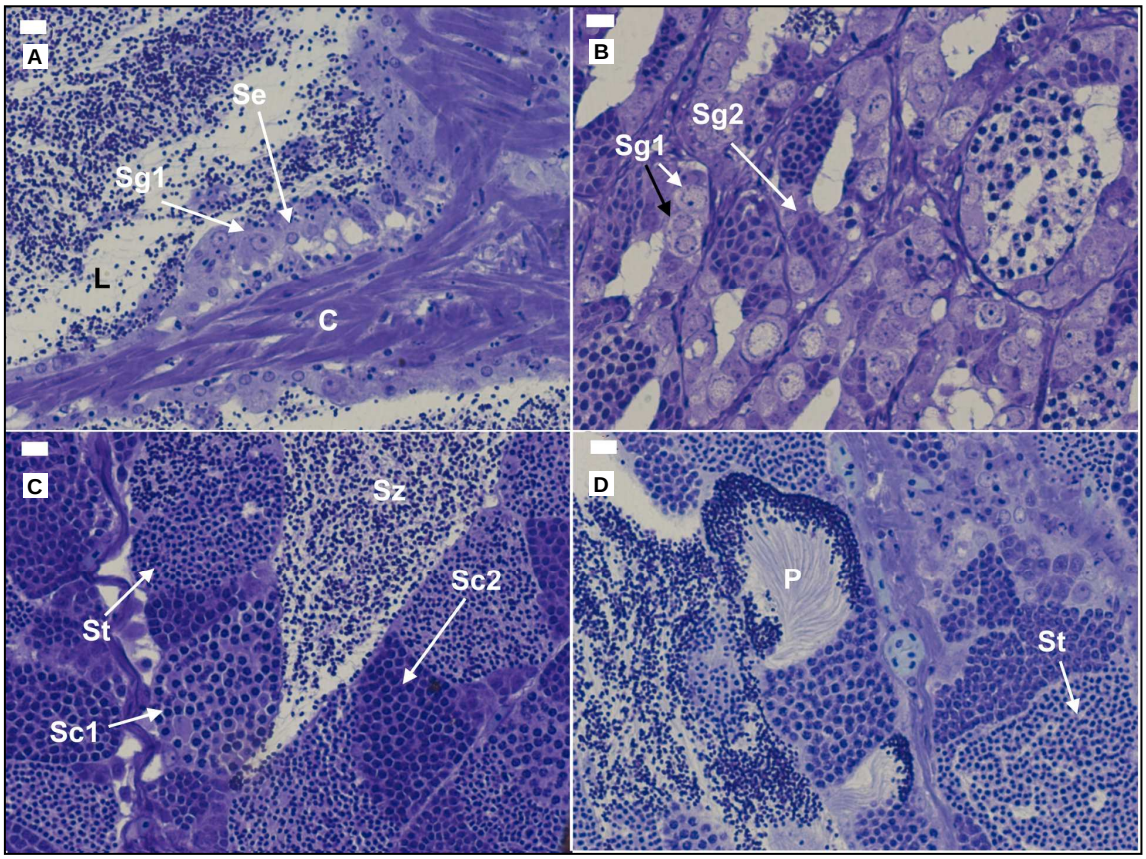

Figure 4. Histological stages of meagre spermatogenesis A) Primary spermatogonia (Sg1); B) Sg1 and secondary spermatogonia (Sg2). Black arrow, Sg1 in mitotic division; C) Primary (Sc1) and secondary (Sc2) spermatocytes, spermatids (St) and spermatozoa (Sz); D) Bursting St in "Parachute" form (P). Connective tissue (C), Lobule lumen (L), Sertoli cell (Se). Scale bar, 10 µm, 400x.

The testes of mature meagres display a tubular appearance throughout the year, active spermatogenesis taking place from March to June. During this period Sg1 are rare and the GE changes from continuous to discontinuous and then again to continuous but with Sg1 only (=regenerated) (Figure 5A) suffering significant developmental changes. Among these are a rapid reduction in Sg1 and Sg2 (Figure 5B) that gives rise to sustained high levels of Sc1 and St and abundant Sz both in the outer and inner lobules (Figure 5C-E). As the season progresses, the testis wall (*tunica albuginea*) and lobule connective tissue thicken (Figures 5F and 6A-D) and Sg1 start to increase abundance, lining up the naked lobule walls (Figure 5B). From July onwards, testes displaying Sg2, Sc1, Sc2 and St become increasingly rare, and Sg1 proliferate forming cords that line up the internal walls of the lobules, giving them a characteristic regenerated appearance (Figure 5A). Sz are still abundant but now concentrated mostly in the inner lobules and sperm duct, becoming more rare in peripheral lobules (Figure 5E).

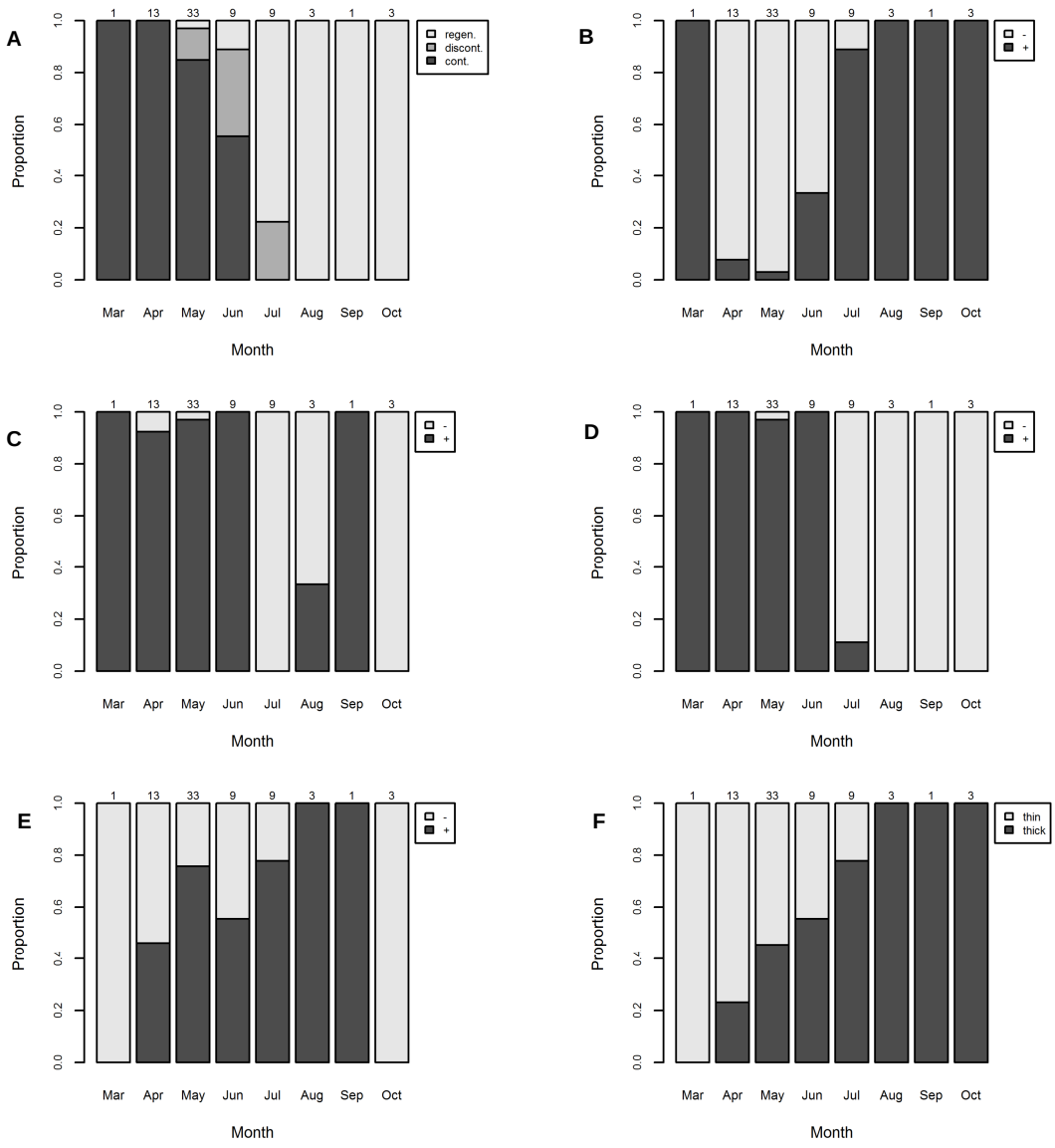

Figure 5. Month-related variations in the histologic characteristics of the testes of mature meagre (>80 cm; Tixerant 1974). *y*-axis represents the proportion of monthly samples that showed each feature. A) Germinal epithelium structure (cont.=continuous; discont.=discontinous; regen.=regenerated); B) primary spermatogonia; C) primary spermatocytes; D) spermatids; E) spermatozoa; F) tunica thickness. "+", abundant or very abundant, "-", not abundant or absent. Numbers above bars are sample size.

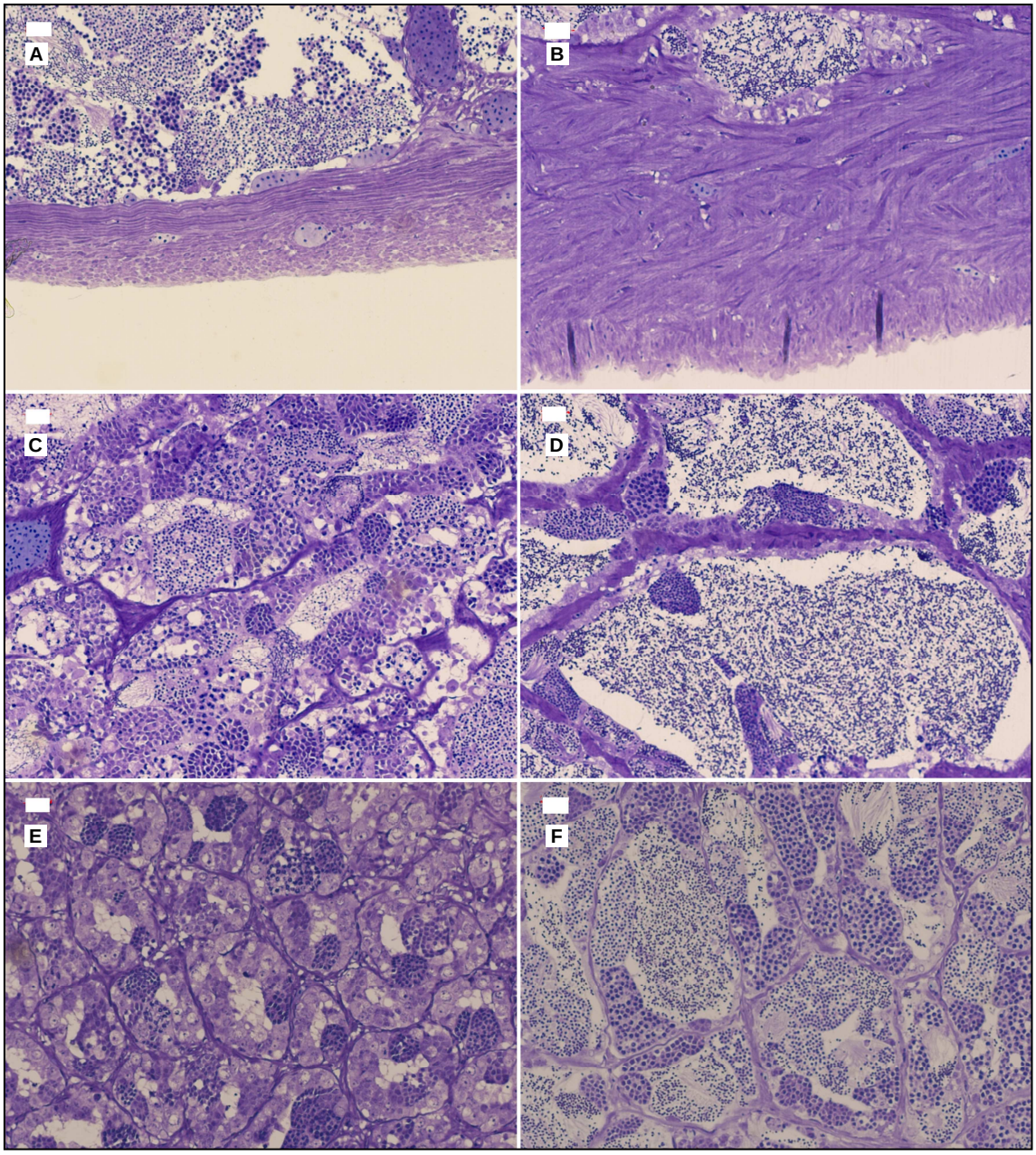

Figure 6. Details of month-related and size-related variability in the testes of meagre [TL, total length in cm; M, month of capture; P, place of capture, southern coast (SC) or Tagus Estuary (TE); H, Histological stage]. A) Thin wall during spawning months [TL, 140; M, April; P, SC; H, spawning-capable]; B) Thick wall characteristic of post-spawning [TL, 148; M, June; P, SC; H, regressing]; C) Thin connective tissue forming the lobular walls [TL, 144; M, March; P, SC; H, developing]; D) Thickened connective tissue [TL, 177; M, June; P, SC; H, Regressing]; E) Compact appearance with no sperm present [TL, 56; M, June; P, TE; H, developing]; F) Compact appearance with sperm present [TL, 44; M, June; P, TE; H, spawning-capable]. Scale bar, 20 µm, 200x.

The peripheral lobules of most fish <70 cm are generally compact, with relatively narrow lumina and a GE where Sg1 and Sg2 are abundant alongside more developed spermatogenic stages (Figure 7A, B). On the other hand, the testes of fish >80 cm all have a tubular appearance, with GE continuous to discontinuous, dominated by Sc1, St and Sz

(Figure 7C-E). Thick testis walls appear only in fish >70 cm (Figure 7F). Interestingly, some fish smaller than 50 cm already show signs of spermatogenic activity, displaying small inner lobules filled with Sz even if their testes are relatively small (Figures 6F and 7E). Despite the ongoing spawning season, it was noticed that some large fish sampled from coastal marine grounds already evidenced some signs of regeneration, namely Sg1 proliferation (Figure 7B).

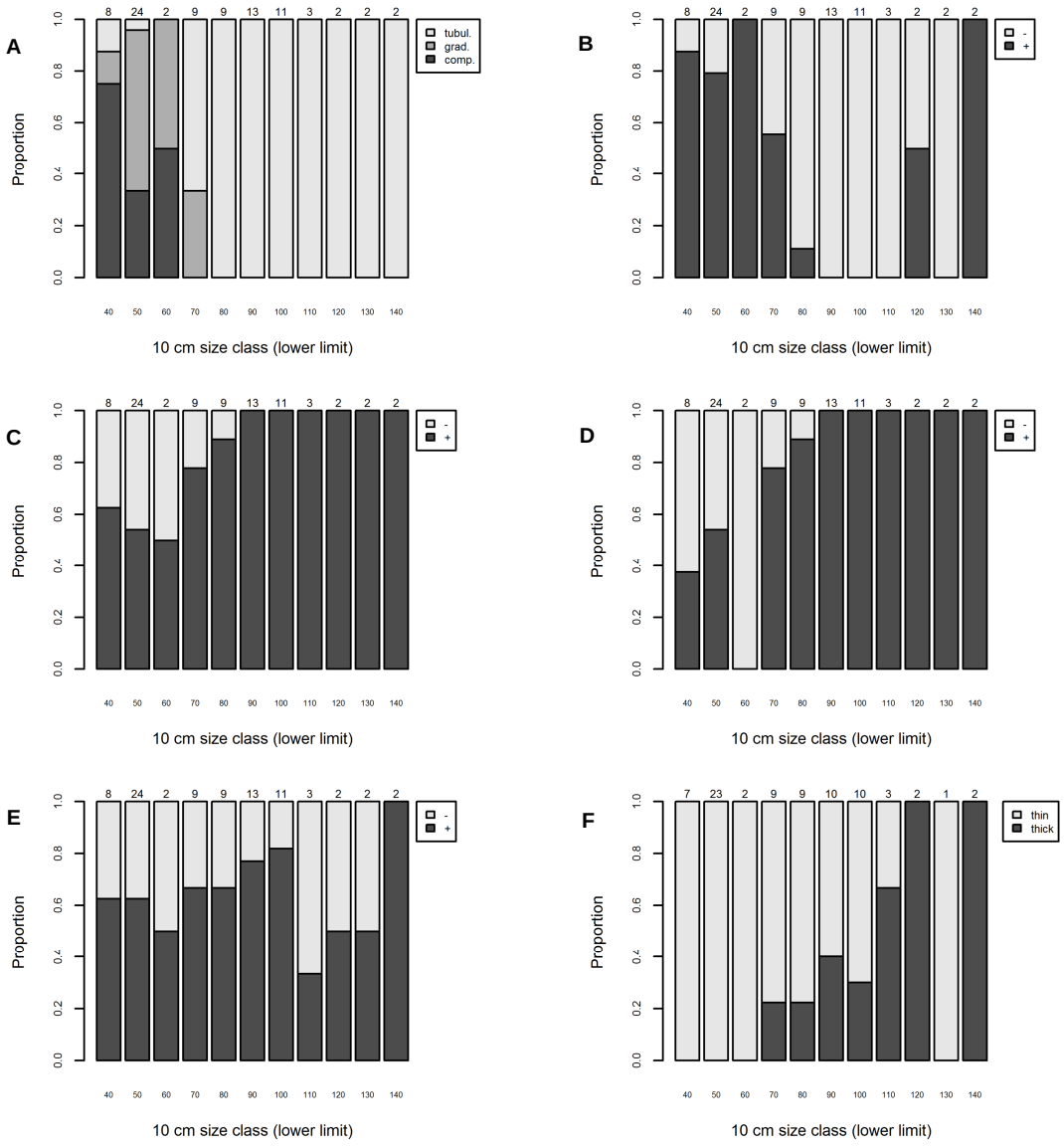

Figure 7. Size-related variations in the histologic characteristics of the testes of meagre during its putative spawning season (May and June). *y*-axis represents the proportion of samples that registered each feature. A) Overall testis structure (comp., compact; grad, gradient (compact at periphery, tubular at core); tubul, tubular); B) primary spermatogonia; C) primary spermatocytes; D) spermatids; E) spermatozoa; F) tunica thickness. "+", abundant or very abundant; "-", not abundant or absent. Numbers above bars are sample size.

Based on the previous characteristics the histological development of the meagre testis was divided into five main phases (Table 3, Figure 8). A subdivision of testes in spawning-capable phase into "spawning capable (virgin)" and "spawning capable (mature)" warrants a

separation between younger and smaller spawning-capable fish that still show signs of immaturity (namely abundant Sg1 and Sg2) and older and larger fish that do not display such signs (Table 3).

Table 3. Histological grading system of the meagre testis. Sg, spermatogonia; Sc1, primary spermatocytes; Sc2, secondary spermatocytes; St, spermatids; Sz, spermatozoa; GE, germinal epithelium; W_g, weight of gonad; GSI, Gonadosomatic index (calculated as in Table 2; mean±s.e.); TL, total length (mean±s.e.); A, age (mean±s.e.); M, month(s) of occurrence

Phase	Main Histological features	Other notes
Immature	Compact appearance; only Sg1 and Sg2 present.	W_g<1 g (*n*=4) GSI(%): 0.07±0.01 (*n*=4) TL(cm): 48.5±2.0 (n=4) A(years): 3.0±0.0 (n=4) M: March, June
Developing	Thin *tunica*; Lobular appearance (small compact lobules in developing virgins); continuous GE with spermatocysts in all stages; little or no Sz.	W_g<157 g (*n*=26) GSI(%): 0.36±0.11 (*n*=26) TL(cm): 67.6±4.4 (n=27) A(years): 3.9±0.4 (n=27) M: March–July
Spawning capable	Thin to intermediate width *tunica*; lobular appearance; wider lobules, very elongated in internal regions; GE of lobules ranging from continuous to totally discontinuous; Sc1 and St predominant; Sz very abundant in the internal regions and frequently also at periphery.	Spawning capable (all) 1<W_g<1422 g (n=77) GSI(%): 2.19±0.21 (*n*=77) TL(cm): 85.7±2.7 (n=79) A(years): 5.0±0.2 (n=78) M: April–July
	Spawning capable (virgin): Sg1 and Sg2 present and abundant;	*Spawning capable (virgin)* 1<W_g<173 g (*n*=34) GSI(%), 0.60±0.10 (*n*=34) TL(cm), 59.5±3.1 (n=34) A (years), 3.6±0.2 (n=33) M: April–July
	Spawning capable (mature): Sg1 absent; GE progresses from continuous in all lobules (early subphase) to discontinuous in all lobules (late subphase); Sc1 are present in large amounts (early subphase) and decrease over time, becoming rare (late subphase); St present in large amounts (early subphase) and decreasing to moderate amounts (late phase); a few late subphase individuals may show signs of Sg1 proliferation during the late subphase.	*Spawning capable (mature)* 58<W_g<1422 g (*n*=43) GSI(%): 3.45±0.22 (*n*=43) TL(cm): 100.9±2.1 (n=45) A(years): 6.0±0.2 (n=45) M: April–June
Regressing	Lobular appearance; intermediate to thick *tunica*; wide lobules; GE increasingly continuous composed of proliferating Sg1 (in cords) and a few scattered Sc1 and St; Sz may still be abundant in internal lobules and sperm duct, but decrease in abundance towards periphery.	1<W_g<412 g (*n*=23) GSI(%): 0.51±0.09 (*n*=23) TL(cm): 109.7±8.4 (n=24) A(years): 9.5±1.5 (n=22) M: May–October
Regenerating	Lobular appearance. Sg1 and Sg2 proliferate throughout the testis, thickening the GE and thinning the lobular lumen; later stages of spermatogenesis may be present but in reduced amounts (Sc1, Sc2, St); no Sz. *Tunica* still thick, becoming thinner.	86<W_g<90 g (*n*=2) GSI(%): 0.35±0.01 (*n*=2) TL(cm): 143.0±1.5 (n=2) A(years): 12 (n=1) M: October

3.3. Histological corroboration of the anatomical scale

A comparison of the results obtained in 104 testes subjected to both macroscopic and histologic analysis is presented in Table 4. A good correspondence was found between anatomical class 0 and histological phase immature, between the anatomical class II and the histological spawning-capable (virgin) phase, between anatomical class III and the histological

spawning-capable (mature) phase, and between anatomical class IV and the histological regressing phase, with 11%-16% error in one-to-one histological assignment of these

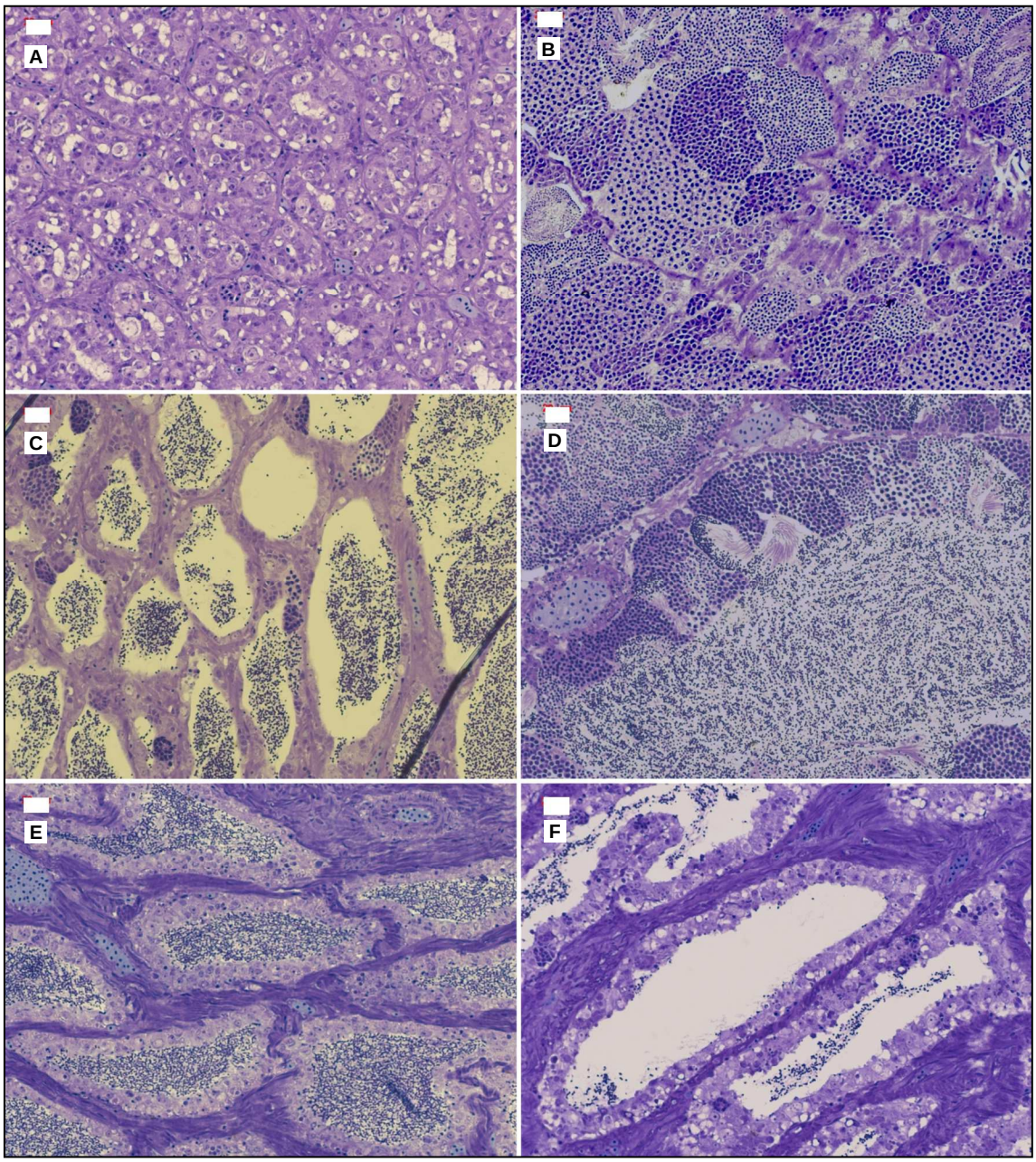

Figure 8. Histological phases of the meagre reproductive cycle. [TL, total length in cm; M, month of capture; P, place of capture, southern coast (SC) or Tagus Estuary (TE); Ma, Macroscopical phase]. A) Immature [TL, 51; M, March; P, TE; Ma, not determined]; B) developing [TL, 70; M, April; P, TE; Ma, not determined]; C) spawning-capable (virgin) [TL, 59; M, June; P, TE; Ma, Anatomical class I]; D) spawning-capable (mature) [TL, 100; M, May; P, TE; Ma, Anatomical class III]; E) regressing [TL, 127; M, September; P, SC; Ma, Anatomical class IV]; F) regenerating [TL, 144; M, October; P, SC; Ma, Anatomical class IV]. Scale bar, 20 µm, 200x.

anatomical classes. Testes classified as anatomical class I were found to be in histological developing phase or in spawning capable (virgin) phase, with a lower proportion being regressing or immature (Table 4). Alongside the evidence obtained for anatomical class II and

the covariate data displayed in Tables 2 and 3, the latter results indicate that smaller and younger meagres with inconspicuous testes and histological signs of immaturity already display a seasonal maturation cycle in testes development with minor spermatozoa production.

Gross anatomical scales and milt production are frequently used to assess size-at-maturity during the spawning season. Restricting the results displayed in Table 4 to the peak spawning season (May and June) indicates that all testes assigned to anatomical classes II, III and IV corresponded to individuals that were either in the spawning-capable phase or in the regressing phase and can therefore be safely assumed to be mature. In anatomical class I, one individual (7%) was immature and five individuals (33%) were still in the developing phase during the peak spawning season, being unlikely to spawn in the current season. This finding indicates that some error may be introduced into maturity ogives if class I individuals are assumed mature without a complementary histological analysis. The probability of being mature while expressing milt was 100% but 50% of spawning-capable virgins and nearly all regressing fish did not express milt and could therefore be classified as immature in analyses that consider milt expression to be the only maturity indicator (Table 5).

Table 4. Histological corroboration of the gross anatomical scale. Numbers displayed are percentages of column totals

	Anatomical class				
Histological phase	0 (*n*=1)	I (*n*=17)	II (*n*=18)	III (*n*=43)	IV (*n*=25)
Immature	100	6	–	–	–
Developing	–	47	11	–	8
Spawning-capable (virgin)	–	35	89	16	4
Spawning-capable (mature)	–	–	–	84	–
Regressing	–	12	–	–	84
Regenerating	–	–	–	–	4

Table 5. Meagre reaction to abdominal pressure during peak spawning season. Numbers are specimens

	Milt upon abdominal pressure?	
Histological phase	**No**	**Yes**
Immature	2	0
Developing	5	0
Spawning-capable (virgin)	14	14
Spawning-capable (mature)	1	29
Regressing	5	1
Regenerating	0	0

4. Discussion

This paper presents the first detailed study of the male reproductive characteristics of wild *Argyrosomus* spp. across the species size span. It is also one of the few available studies detailing testis histology of a large-sized sciaenid (but see Grier *et al.* 1987) and, to our knowledge the first detailed attempt at corroborating a gross anatomical scale of male gonads with histological evidence.

The testes of mature meagres belong to the unrestricted spermatogonial type, with spermatogonia appearing in both distal (peripheral) and proximal (core) regions. However, in the testes of developing younger fish the density of spermatogonia and Sertoli cells appears to be larger in peripheral regions than in proximal regions. Almeida *et al.* (2008) described the existence of a *germinative zone* at the periphery of *Gadus morhua* testes where higher spermatogonial concentrations took place. Grier *et al.* (1987) also described higher concentrations of spermatogonia in the recovering testes of *Sciaenops ocellatus*. Both authors suggest that these spermatogonia concentrations are linked to testis enlargement through lobule elongation. Other authors have considered the possibility that these asymmetries could reflect an intermediate form of (partially) restricted testis organization (Schulz *et al.* 2011). Our sample size of developing mature fish was low but it confirms that in meagre some highly dense nests of spermatogonia and Sertoli cells do take place in more peripheral lobules, thus building evidence towards the participation of these cells in testis enlargement.

Spermatogenesis is the process whereby diploid spermatogonia proliferate and evolve to form haploid spermatozoa (Schulz *et al.* 2011). Similar to other fish, the basic functional units of the meagre testes are the spermatocysts, i.e. envelopes of dynamic Sertoli cells that harbour clusters of synchronously developing cell clones (Schulz *et al.* 2011). Spermatocyst development is asynchronous throughout the testis with different spermatocyst development stages being present in each lobule during the developing and spawning-capable phases. Similar to descriptions of other species (e.g. Grier *et al.* 1987) and the currently accepted conceptual models for teleost testis development (Brown-Peterson *et al.* 2011, Schulz *et al.* 2011), in meagre the germinal epithelium is subjected to marked seasonal variation. Mitotic proliferation of primary spermatogonia takes place right after (sometimes overlapping) the end of the spawning period (Figure 4A). Then secondary spermatogonia (Sg2) evolve from mitotic divisions of scattered primary spermatogonia (Sg1) (Figure 4B) and undergo the first meiotic division to form primary spermatocytes (Sc1). Sc1 are the most frequent stage of spermatogenesis (Figures 4C), probably due to the long duration of the first meiotic prophase (Schulz *et al.* 2011). Final “parachute” formation of spermatozoa has been reported in *Otolithes ruber*, *Liza carinata* and *Perca flavescens* but does not appear to be a widespread phenomenon among teleosts (Dadzie and Abou-Seedo 2004). Overall, the maximum potential annual sperm production of large mature meagre appears to be fully determined at the end of the developing phase, since from then onwards spermatogonia become rare in mature individuals as a result of their evolution through spermatogenesis to form spermatozoa (Grier 2002). We predict that annual sperm production in meagre likely results from interactions between size and age, since

large spawning capable fish can carry testes with more than 10 times the weight of those from smaller spawning capable fish. Further studies on the histology of this species should help to clarify such issues.

Historically, macroscopic classifications of gonad development have constituted the methodological basis for most reproductive studies of fish, particularly in male reproduction. This is the case of meagre and other large-sized sciaenids, in which anatomical scales with four levels (Hermas 1995), five levels (Tixerant 1974), six levels (Abou Shabana 2012), seven levels (Griffiths 1996) and eight levels (Farmer 2008, Gil *et al.* 2013) have been previously used in describing the male reproductive cycle. In such species, the few studies that used histology sections to assess reproductive parameters did not detail the histological results (e.g. Murphy and Taylor 1989, 1990), were based on aquaculture observations of small young fish (Gil *et al.* 2013), used a very limited sample size (<30 individuals; Abou Shabana 2012) or did not report on the macroscopic appearance of male gonads (Grier *et al.* 1987). To our knowledge, the only comprehensive studies that reported on the histological features of meagre males were carried out with essentially juvenile fish (fish up to 84 cm in length and 62 months old) in aquaculture facilities and also did not provide information on macroscopic versus histological correlations of meagre testis (Schiavone *et al.* 2012, Gil *et al.* 2013).

The meagre results underscore the importance of carrying out validation studies of the gross anatomical scales before using them to routinely assess male reproductive status or establish management measures (Hunter and Maciewicz 2003). They also demonstrate how an objective gross anatomical scale can be developed from raw observational data using semi-quantitative graphical displays (Figures 5 and 7). Such displays helped to highlight objective characteristics to be used in the anatomical scale and the success of the final validation success might be explained by that in part. We have also shown that the final gross anatomical scale adopted for meagre provided a relatively reliable proxy for the main reproductive development phases of male gonads. Specifically, anatomical classes II and III both correspond to spawning-capable fish and can therefore be used to assess spawning season and determine size-at-maturity. Whether or not classes I and II should be fully considered mature in building maturity ogives used for advising managers is left open to discussion, since the reproductive output of spawning-capable virgins is likely much lower than that of adult fish. Additionally, we have shown that gross anatomical class IV adequately reflects the end of spawning season (regressing phase). We did not find a gross macroscopically level that accurately reflected the histological developing phase, probably because we did not find a fishery targeting such large individuals in the late autumn and winter months. Most importantly, we have shown that, though relatively accurate maturity data on males can be obtained from the use of gross anatomical scales, these are not objective enough and precise enough to resolve all the vital information for management that is provided by concurrent histological results. The latter is particularly noticeable in the significant proportion of small-sized fish that can be classified as reproductively active by some macroscopic criteria (e.g. milt extrusion) but not by all criteria (e.g. cross-section

shape) and in the fact that some testis classified as spawning-capable showed a quite distinct external appearance, gonad weight and gonadosomatic index (GSI) level.

Gross anatomical grading systems can be improved by reducing the number of classes and focusing on the most reliable characters (Hunter and Macewicz 2003). However, even in simpler systems, a frequent flaw is the confounding of fish that have just spawned and have regressing gonads with fish that are developing and yet to attain first maturity. In meagre males, we found that anatomical class IV rightly classifies most gonads as regressing with little overlap with developing fish. However, we note that *ca*. 3/4 of our samples were collected at the onset or during the fish spawning season, a time of the year that maximizes the classification success of anatomical grading systems (Hunter and Macewicz 2003, Lowerre-Barbieri *et al.* 2011). Another cause of concern with anatomical scales is the presence of skip spawners, i.e. fish that have already matured once but fail to spawn in a specific year. Existing knowledge of skip-spawning in male fish is very limited but these are generally identified as fish large enough to be mature that, during the spawning season, have a low GSI, a thick testicular wall and no sign of spermatogenic activity (Rideout and Tomkiewicz 2011). In our study, we found two mature fish (>80 cm) caught in the spawning season that had a thick wall and low GSI (<1%). Both these fish showed signs of active spermatogenesis, namely a few (but not many) spermatocysts in multiple developmental stages. Consequently, they likely belonged to fish at the end of the spawning season (regressing) and not to skip spawners. Even so, we caution that our samples are not fishery-independent and that we cannot exclude the possibility that some skip-spawning fish may spend the spawning season in areas away from the main fishing grounds (González-Quirós *et al.* 2011).

To date, the large sciaenid fisheries literature has frequently reported smaller size-at-maturity (L_{50}) in males than in females, with discrepancies ranging between a few cm and nearly 30 cm (e.g. Murphy and Taylor 1989, 1990, Nieland and Wilson 1993, Wilson and Nieland 1994, Griffiths 1996, 1997). Some of these studies have used milt extrusion as indicative of maturity (e.g. Wilson and Nieland 1994), others have applied gross anatomical scales (Hermas 1995, Griffiths 1996, 1997), and yet others have carried out histological analysis of gonads, considering sperm presence as indicative of maturity and not including a subclass of first maturing spawning-capable fish (e.g. Murphy and Taylor 1990). We have shown that milt expression is an unreliable indicator of maturity in meagre, because it wrongly classifies as immature 50% of spawning-capable virgins and 83% of testes in regressing condition (Table 5). This situation probably extends to males of other sciaenids and warns against the use of ripeness as a maturity indicator in building maturity ogives in sciaenid species. Implicitly acknowledging some of the problems associated with categorizing very small fish as mature, Griffiths (1997) categorized some small milt-extruding males of *Argyrosomus inodorus* as immature based on supplemental anatomical criteria, namely the presence of disproportionally small testes and the absence of drumming muscles. This author came to assume that these fish made a low contribution to the total reproductive output of the population. As for *Argyrosomus regius*, neither Tixerant (1974) nor Hermas (1995) reported mature males <70 cm, but in the

Gulf of Cadiz González-Quirós *et al.* (2011) noted that 26% of male meagres <70 cm were mature and suggested such precocious maturation could represent a reproductive strategy (e.g. sneaker small-sized males) or be revealing of different fish populations with lower size-at-maturity (e.g. Farmer 2008). While researching first maturation of aquacultured meagre, Schiavone *et al.* (2012) found puberty to be reached at two years and 26.8 cm (920 g) with still increasing GSI and hormone levels and Gil *et al.* (2013) determined the male length and age at 50% maturity to be 49.3 cm and 32.3 months, respectively. The growth rates of these fish were markedly different from those of wild populations (2 years old, 46 cm, 940 g) (Prista unpublished data) but Schiavone *et al.* (2012) also report a few one-year old males producing expressible milt.

Our histological analysis of meagre testes objectively confirmed the occurrence in wild populations of some small fish (<60 cm) with inconspicuous testes (low weight, low GSI) in spawning-capable condition that may (or may not) extrude milt upon squeezing. In histological terms these fish are considered to have reached maturity since they have spermatozoa and are therefore capable of participating in reproduction (Brown-Peterson *et al.* 2011). Whether or not these fish contribute significantly to the overall male reproductive output of the population and the exact extent of the implications for management of considering them as mature will be dealt with elsewhere. Here, we have shown that these fish have a mixture of adult and juvenile characters (non-tubular appearance and spermatogonia abundance throughout the year), being not only small-sized but also young. These fish are unlikely to have come from a different population and most likely represent virgin fish experiencing first maturation. We have also shown that these first maturing wild fish likely develop, spawn and regress despite being far from attaining their full reproductive potential. These results concur with those reported by Schiavone *et al.* (2012), which showed increasing levels of estradiol, testosterone and 11-KT plasma levels between the first and second spawning seasons, evidencing that hormonal control is not yet fully stabilized after the first reproductive event. In the wild, both meagre juveniles and meagre adults visit inshore and estuarine waters in spring and summer, so we hypothesize that hormonal control of testes development becomes increasingly coupled to the photoperiod variations and other environmental clues (e.g. temperature, turbidity) experienced by the fish during their seasonal presence in estuarine nursery areas and future spawning grounds (Prista *et al.* 2008) and that in the wild meagres may also display increasing levels of hormonal control as they migrate back-and-forth between coast and estuaries during their first years of life.

Overall, the evolutionary advantage of precocious maturity in wild fish remains unresolved. In aquaculture production, precocious maturity is considered detrimental because it diverts energy from growth to reproduction (Taranger *et al.* 2010) but it is possible that in wild populations the selection pressure on such precocious maturity is not very large since young meagres have fast growth rates (González-Quirós *et al.* 2011) and are able to quickly reach a size that protects them from most marine predators. If so, some adverse effects that precocious

maturity may have on growth might be counter-balanced by the advantages collected from a more precise environmental control of spermatogenesis.

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Chapter 5B

Reproductive phase determination in female meagre (*Argyrosomus regius*): histological development and corroboration of a gross anatomical scale

Nuno Prista[a,b,c], Cristina Nunes [c], José Lino Costa[a,b] and Maria José Costa[a,b]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[c]Divisão de Modelação e Gestão de Recursos de Pesca, Instituto Português do Mar e da Atmosfera, Avenida de Brasília, 1449-006 Lisboa, Portugal

To be submitted.

Reproductive phase determination in female meagre *Argyrosomus regius*: histological development and corroboration of a gross anatomical scale

Abstract: The meagre (*Argyrosomus regius*) is one of the world's largest sciaenids and supports regional fisheries and aquacultural interests in European and North African waters. However, its reproductive patterns are yet to be fully investigated. In the present study, we used semi-quantitative analyses to clarify the seasonal and size-related variability of the macroscopic appearance of meagre ovaries. We describe the histological stages and the reproductive phases of meagre females and determine the extent to which they corroborate a macroscopic scale. Our results indicate that the macroscopic scale is sufficiently accurate to be used in assessments of the meagre maturity. Additionally, we show that the development of meagre ovaries displays a well marked follicle stage characterized by lipid droplets and cortical alveoli and that a group of females does not proceed to spawning in the year their oocytes first acquire cortical alveoli and lipids. Finally, we show that consideration of ovarian wall structure and thickness alongside fish size aids to the accurate distinction of immatures and regenerating/developing mature females. We discuss these findings in terms of their contribution to the understanding of ovarian development within the sciaenid family and the importance of histologically corroborating macroscopical scales in reproduction analyses.

Keywords: histology, macroscopy, female, reproduction, *Argyrosomus regius*, validation

1. Introduction

The meagre, *Argyrosomus regius* (Asso, 1801), is one of the world's largest sciaenids, attaining over 180 cm in total length and 50 kg in weight (Quéméner 2002). Its distribution ranges from the English Channel to Senegal (including the Mediterranean Sea and the Black Sea). Most of the year the meagre occurs in coastal waters (<80 m deep) but in spring and summer the fish migrates to shallow coastal waters and/or large European estuaries that it uses as spawning and nursery grounds (Quéro and Vayne 1987). The largest meagre fisheries take place in Mauritania, Morocco, and Egypt, which together comprise over 80% of the ca. 10000 t world annual catch (Quéméner 2002, FAO 2009). In Europe, national landings are generally below 500 t/year (FAO 2009) but due to its large size, high ex-vessel prices and seasonal availability in inshore and nearshore waters, it is an important target species for local small-scale commercial and recreational fleets (Quéro and Vayne 1987, Quéméner 2002, Prista *et al.* 2008). This importance is underscored by the recent development of meagre aquaculture production and by the ecological significance of the meagre as a top marine predator in European coastal waters (Quéro and Vayne 1987, Quéméner *et al.* 2002). However, to date the biological characteristics of the meagre have remained poorly studied worldwide and its fisheries have yet to be routinely monitored or assessed (Prista *et al.* 2011).

Studies of gonad morphology at anatomical and histological levels are required to identify reproductive cycles, spawning seasons, spawning areas, and to determine size-at-maturity (Hunter and Macewicz 2003). In fisheries science, determinations of the reproductive state of a

fish population in a specific area, time, and/or size class, are the basis of several regulatory measures (e.g. minimum landing size, area closures) (Hunter and Macewicz 2003). Until recently, the reproduction of meagre had only been studied in North African waters and based on macroscopic observations of fish gonads and the monthly evolution of gonadosomatic indexes (Tixerant 1974, Hermas 1995). Recent interests in aquaculture production and concerns with data-poor regional fisheries and depleted local resources have sparked research and led to an array of studies some of which have addressed the reproduction of the species in captivity (Duncan *et al.* 2012, Schiavone *et al.* 2012, Gil *et al.* 2013, Mylonas *et al.* 2013a,b), and other in the wild (González-Quirós *et al.* 2011, Abou Shabana *et al.* 2012, Gil *et al.* 2013).

Macroscopic maturity scales (also known as the gross anatomical grading systems) of fish ovaries and testes are among the most frequently used indexes of fish reproductive condition (West 1990, Hunter and Macewicz 2003). The use of these methods is rooted in historical fish biology literature and their low cost and ease of applicability has resulted in integration into routine research protocols of many species (Kjesbu et al. 2003). However, many authors have pointed out the imprecisions and inaccuracies of macroscopical maturity scales in, e.g., assessments of size-at-maturity (Murua et al. 2003, Lowerre-Barbieri et al. 2011). Such flaws can be minimized through validation and corroborative studies in which histological observations are used to fine tune the macroscopic observations and identify its biases (West 1990, Hunter and Macewicz 2003, Brown-Peterson *et al.* 2011).

In this study we provide a detailed description of macroscopic (anatomical) and microscopic (histologic) ovarian development in wild meagre. First, we describe the variability of macroscopic features with fish size and season and use that information to build a macroscopic scale. Then we describe follicle development and derive histological reproductive phases for meagre. Finally, we compare the two and evaluate to what extent the macroscopical scale can be used to assess the reproductive phase of meagre. We discuss these results in terms of the progress achieved in the understanding of meagre reproduction in European waters.

2. Materials and methods

2.1. Sampling methodology

A total of 2418 meagres were sampled from 2003 to 2007 during a large-scale study that targeted the meagre fishery and biology on the Portuguese coast. Detailed coverage of the sampling methodologies, the fisheries and areas covered can be found in Prista *et al.* (2007, 2008) and Costa *et al.* (2008). Biological sampling was carried out monthly in two geographical areas that encompass the main meagre fisheries in Portugal: the Tagus estuary (on the central-western coast) and the coast of Olhão (on the southern coast). Monthly sampling goals were set at 10 females per 10-cm size class following the requirements of a concurrent age and growth study but the achieved goals largely depended on the seasonal availability and size span of the fish commercially available at each sampling occasion. In general, all meagre specimens were measured (total length) and weighed (total weight), and had their abdomen slightly squeezed for

expressible milt or roe. Whenever possible, the individuals were gutted and sexed, and their gonads were weighed (to the nearest 1 g), checked for the presence of the large-bodied female nematodes of the genus *Philometra* (Moravec *et al.* 2007), analyzed for macroscopic characteristics, and photographed (in dorsal and ventral view). Whenever possible otoliths were removed and age determinations were carried out according to Prista *et al.* (2009).

2.2. Macroscopic analysis

Fresh meagre ovaries were subjected to macroscopic classification with respect to a set of pre-defined morphological features (**Table 1**). The set included anatomical features that had

Table 1. Main characteristics used in the macroscopic analysis of meagre ovaries

Characteristic	Classification levels
Coloration	White; Yellow; Orange; Red; Brown
External vascularization (in dorsal view)	Present and active (conspicuous vessels, colored red with frequent ramifications); In resorption (less conspicuous vessels, colored gray, that give the gonad a scarred appearance); Absent or inconspicuous
Overall shape of gonad lobe (in ventral view)	Rectangular; Triangular; Lozenge; Too thin to characterize; Other
Shape of gonad cross-section	Circular; Ellipsoidal; Triangular (generally equilateral); Rectangular; Too thin to characterize
Fullness	Full (with eggs); 1/2-full; Empty; Turgid (but not full with eggs); Too thin to characterize
Thickness of gonad wall	Thin; Thick
Transparency of gonad wall	Transparent; Translucent; Opaque
Visibility of oocytes	Externally visible; Only internally visible; Not visible (externally and internally)
Opacity of oocytes	No translucent oocytes; <25% Translucent; >25% Translucent
Reaction to abdominal pressure	Positive (leaks eggs upon thumb-rubbing); Negative (does not leakage eggs)

been reported in previous studies of meagre and other large sciaenids (Tixerant 1974, Griffiths 1996, Wells 2002, Farmer 2003). To make the macroscopic analysis objective and reduce observation biases, different classificatory levels were set for each morphological feature after a preliminary analysis of the data obtained during the first year of the study. Additionally, the observations where carried out, one feature at a time, across the several fish sampled at each occasion, and only in the end were the gonads coarsely graded with regards to their overall macroscopic appearance.

2.3. Histological analysis

Meagre ovaries were fixed and preserved in 4% buffered formaldehyde (buffer: Na_2HPO_4 and $NaH_2PO_4*H_2O$) and stored in plastic boxes. Good preservation of large ovaries was assured by injecting a small amount of fixative through the ovary wall with a syringe. When ovary volume was too large, only the left lobe was preserved. Histological procedures were

carried out on small pieces of gonad (about 0.125 cm^3). As the meagre ovaries can be particularly large (up to 2 kg a piece), there was a need to select which part of the ovary would be prepared. A preliminary study involving comparisons of oocyte development stages across ovary locations (anterior, median, posterior; ventral, dorsal, lateral; ovarian wall, lumen) indicated no differences, so pieces of gonad were collected from ventral periphery of the median region of the left lobe. Histological preparation involved successive alcoholic dehydrations, infiltration, sectioning, mounting and staining. Technovit 7100 resin (Heraeus Kulzer) was used as the embedding medium and a Leica RM2155 micrometer was used to obtain thin sections (3–5 µm thick). The sections were stained with toluidine blue, a basophilic metacromatic stain (Sridharan and Shankar 2012). Three replicate sections were mounted in each slide. Permanent preparations were obtained using Neomount and Neoclear (Merck). Microscopic analyses were carried out at 40-400x magnification on a Zeiss stereomicroscope. Digital pictures of histological slides were taken at 40–630x magnification with an AxioCam. Image processing and measurements were carried out in AxioVision 4 (Zeiss). Image processing was restricted to resizing, contrast and brightness adjustments, with only minor background clean-ups.

Ovarian development involves a series of morphological changes that take place at the level of follicles complexes, connective tissues, blood vessels and the ovarian wall (or tunica albuginea) (Brown-Peterson *et al.* 2011). We studied these changes using a two-step approach. First we carried out an overall descriptive study on the morphology of the follicle complexes and defined a staging scheme. Then we carried out an analysis of the entire set of histological preparations and for each one recorded a set of ovarian characteristics we found useful in the characterization of the reproductive phase in meagre.

In our identifications and interpretations of the development of follicle complexes in meagre we used a simplified version of the staging scheme used by Grier (2012) in the study of follicle development in the cofamiliar red drum (*Sciaenops ocellatus*). According to Grier (2012) the follicle complex is composed of the oocyte itself, the surrounding follicle cells, a basement membrane and a theca. Oogenesis is the oocyte formation and folliculogenesis is the formation of the follicle. After follicle formation, the main stages of development are primary growth (PG), secondary growth (SG), oocyte maturation (OM) and ovulation (OV). Folliculogenesis and oogenesis are difficult to observe under light microscopy (Grier 2012) so we assumed that oogonia and oocytes in chromatin nucleolar stage were our initial follicle development stage and then assigned stages to follicle complexes based on a) oocyte shape, b) oocyte size, c) ooplasm basophilly, d) relative volume of the germinal vesicle, e) overall appearance of the zona pellucida, follicle cells and theca, and f) the abundance, size and basophilly of different ooplasm inclusions (lipid droplets, cortical alveoli, yolk granules) (Wallace and Selman 1981, West, 1990, Brown Peterson *et al.* 2011, Grier 2012). In addition to follicle complexes, we also analyzed the appearance of post-ovulatory follicle complexes (POCs), atretic stages of yolked oocytes and the ovarian wall. To our knowledge there is no established chronology on the degeneration of POCs and yolked oocytes in meagre. Consequently, we carried out our analysis of POCs degeneration based on the characteristics pointed out in the degeneration

chronologies presented by Hunter and Macewicz (1986), Fitzhugh and Hettler (1995) and Roumillat and Brouwer (2004) and defined atresia chronology after considering Barbieri *et al.* (1994) and Miranda *et al.* (1999). Ovarian wall was characterized based on Morrison (1990) and Ravaglia and Maggese (2002).

Routine analysis of the bulk of histological sections of meagre included the determination of the most advanced follicle complex (West 1990, Grier 2012) alongside observations on features that are relevant to the interpretation of the reproductive phases in teleosts, namely a) the relative density of healthy vitellogenic oocytes, b) the presence/absence of POCs, c) the presence/absence of atretic yolked follicles and their prevalence, d) the appearance and thickness of the ovarian wall, e) the relative compactness of PG stages, and f) an overall relative appreciation on the degree of regeneration of lamellar lining, the development of muscles bundles and capillaries in the interfollicular space, and level of oogonia proliferation (West 1990, Hunter and Macewicz 2003, Brown-Peterson *et al.* 2011, Grier 2012, Gil *et al.* 2013). In order to make our analysis more objective, histological scale slides were examined with no reference to fish size or month and several observations were registered in categorical scale. The latter was the case of the density of vitellogenic oocytes and PG stages (levels: very dense and compact, few dispersed), POCs and atresia presence (levels: yes or no), atresia intensity (levels: high intensity ≥5 cells affected, low intensity <5 cells affected), atresia prevalence (levels: all yolked stages affected, some healthy yolked stages remain, no yolked stages affected), and ovarian wall structure (<3 layers of muscle or capillaries, ≥3 layers of muscle or capillaries).

All measurements were carried out in AxioVision image processing software (Zeiss) in digital capture of photographs of histological slides. Oocyte diameters reported in descriptions were obtained from measurements of five cells per individual in each of five randomly sampled individuals found to carry the stage. In histological and microscopical analysis, oocyte diameter was considered to be length of the major axis of cell cut through the nucleus (West, 1990). The size of the germinal vesicle was measured parallel to the axis of cell size measurements. The ovarian wall width was taken as the minimum width found in the slide. The relationship of wall thickness to fish size and gonad reproductive phase was modeled using analysis of covariance (Quinn and Keough, 2002) and AICc model selection (Hurvich and Tsai 1989, Burham and Anderson 2002) using gonad thickness as response variable and fish length and reproductive phase as predictors. The models were fit in R 2.15.1 (R Development Core Team, 2012) and the general linear model assumptions checked according to Faraway (2002). The response variable was transformed (logarithm) to meet the assumption of homogeneity of variances.

2.4. Anatomical classes and histological reproductive phases

Anatomical and histological grading systems should reflect the major structural changes observed in the ovaries across the immature/mature gradient and the monthly maturation cycle (mature fish only). In our derivation of anatomical and histological reproductive scales for the

meagre ovary, we analyzed the variations in the frequency of occurrence of macroscopic and histological features across size (within the peak spawning season) and months (in mature fish). In these analyses, the peak spawning season was considered to be May and June (Costa *et al.* 2008) and fish were assumed to be mature fish at 90 cm. Final histological development phases of the meagre ovary follow the recent standardization proposed by Brown-Peterson *et al.* (2011) that classifies ovaries into “immature” (fish that never spawned), “developing” (fish whose ovaries are beginning to grow and developing), “spawning-capable” (fish that are developmentally and physiologically able to spawn in the current year), “regressing” (sexually mature fish that have finished spawning) and “regenerating” (sexually mature fish that are reproductively inactive). Following Brown-Peterson *et al.* (2011), specific subphases were considered for meagre specific aspects (e.g., "developing immature","developing mature").

2.5. Histological corroboration of the anatomical scale

To evaluate the extent to which the anatomical grading system reflects the histological development stage of the meagre ovary we compared the results from macroscopy and histology using the subset of gonads where both scales had been applied. In this comparisons we considered the histological classification to represent the “truth” because histology is widely regarded as the most accurate staging method for fish gonads (Hunter and Macewicz 2003, Costa 2009, Ferreri *et al.* 2009).

3. Results

3.1. Anatomical classification scale

A total of 351 ovaries were subjected to macroscopic analyses. These included 340 ovaries categorized as females in the field and gonads from 11 immature fish whose sex could not be macroscopically determined. The gonad sample comprised 6—92 ovaries from each month and 10—38 ovaries from each 10-cm fish length class with exceptions of January (no samples), fish smaller than 30 cm (two samples) and fish larger than 180 cm (three samples). The fish sampled were captured in both estuarine (n=154) and coastal (n=162) fishing grounds in both the western (n=166) and southern (n=185) regions. However, due to asymmetries in commercial fishing effort, local fishing traditions and the fish own migratory nature (Prista *et al.*, 2008), the majority of fish sampled came from the western estuarine fishery (43%) and the southern coastal fishery (46%) with only two fish sampled on the western coast and only three in southern estuaries. Furthermore, 80% of western samples were collected in May and June from fish between 29 cm. and 148 cm while the southern samples were available year-round and respected to fish between 49 cm. and 182 cm. In 35 fish the geographical area was known but not the exact type of fishing ground (estuarine or coastal).

Meagre females have a pair of elongated ovaries which lay within in the body cavity against the fish swimbladder. The two ovaries connect posteriorly and open to the exterior at the genital

pore. Left and right ovaries are similar in width and shape with rare malformations (e.g., one ovary much larger than the other). The surface of the ovaries is smooth and vascularized particularly on the dorsal surface where a long blood vessel runs longitudinally. In general, the ovaries present similar width on the anterior and medial regions and narrow a bit on the posterior region. The cross section at mid-ovary is circular to elliptical depending on gonad fullness.

The ovaries of mature meagre were found to be reddish-orange to pale yellow from February to June and adopt reddish-brown tonality thereafter. In the first half of the year, the cross section is frequently elliptical and the ovaries are wide and thick, occupying nearly all available space in the abdominal cavity. During this period, ovaries are commonly found full (Figure 1A) and highly vascularized displaying conspicuously branching blood vessels on the dorsal surface that extend sideways across the flanks to the ventral side. The ovary wall is frequently thin and transparent (Figure 1B-C) and oocytes are visible externally in nearly all ovaries (Figure 1D), translucent oocytes being observed in May and June in a small proportion of the gonads (less than 40%) (Figure 1D). Only n=16 fish were found to react to abdominal pressure by extruding roe and this also took place only in May and June. From June onward, the gonads become increasingly flat and flaccid, and the ovary wall thickens becoming translucent or opaque (Figure 1A-C). The vascularization is much less pronounced and assumes a grayish tonality, blending into the background as it is resorbed. During this period oocytes are rarely visible from the outside, being present in low numbers, and appearing opaque scattered through the gonad (Figure 1D).

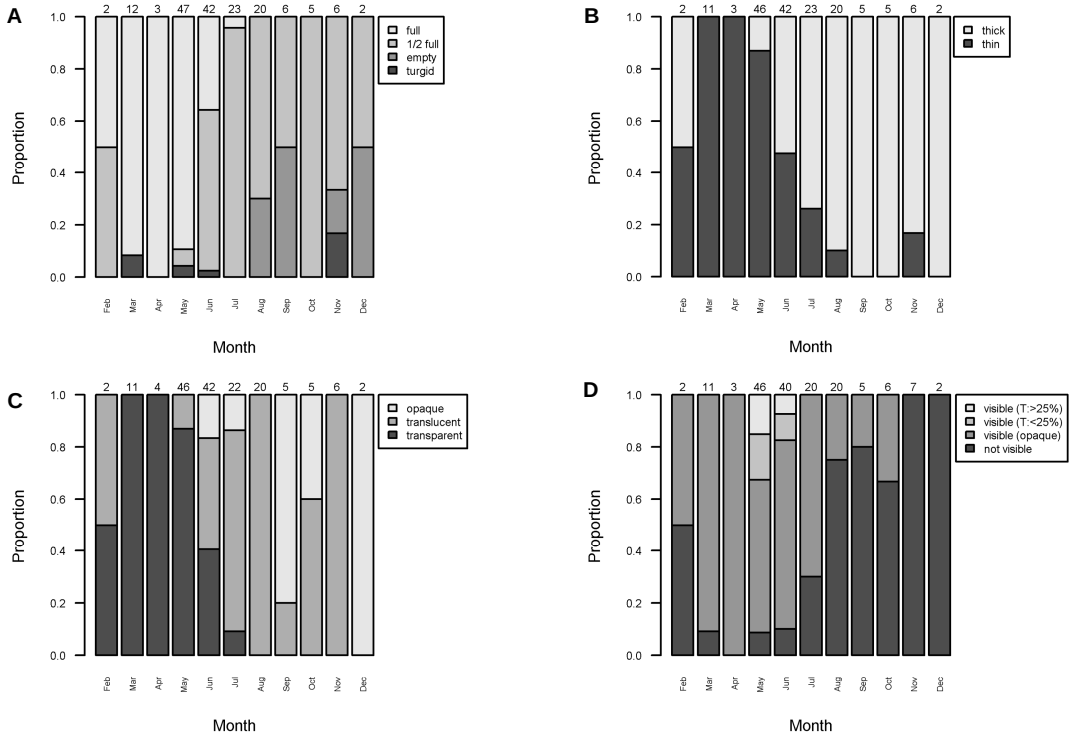

Figure 1. Monthly variations in the macroscopical appearance of ovaries of mature meagre (>90 cm). A) Fullness; B) Wall thickness; C) Wall transparency; D) External visibility of oocytes (T:<25% = less than 25% of translucent oocytes; T:>25% = more than 25% translucent oocytes). Numbers above bars are sample size.

During the spawning season the macroscopical appearance of meagre ovaries changes markedly with fish size. Up to 40 cm, thin thread-like transparent ovaries are relatively common and the sex of fish cannot generally be determined (Figure 2A). From 40 to 70 cm, ovaries are orange in color, appearing turgid (Figure 2A) and circular in cross-section and displaying a transparent wall with fine vascularization. From 60 cm upwards, the proportion of ovaries that appear full or half-full increases steadily, the cross-section becomes increasingly elliptical and the wall more vascularized. In fish larger than 80 cm gonads are nearly always full or half-full, displaying thin transparent or thicker opaque walls, through which oocytes were generally visible (Figure 2B-D). Roe extrusion was observed in a minor proportion of fish 80—150 cm collected from the western estuarine fishery (22%).

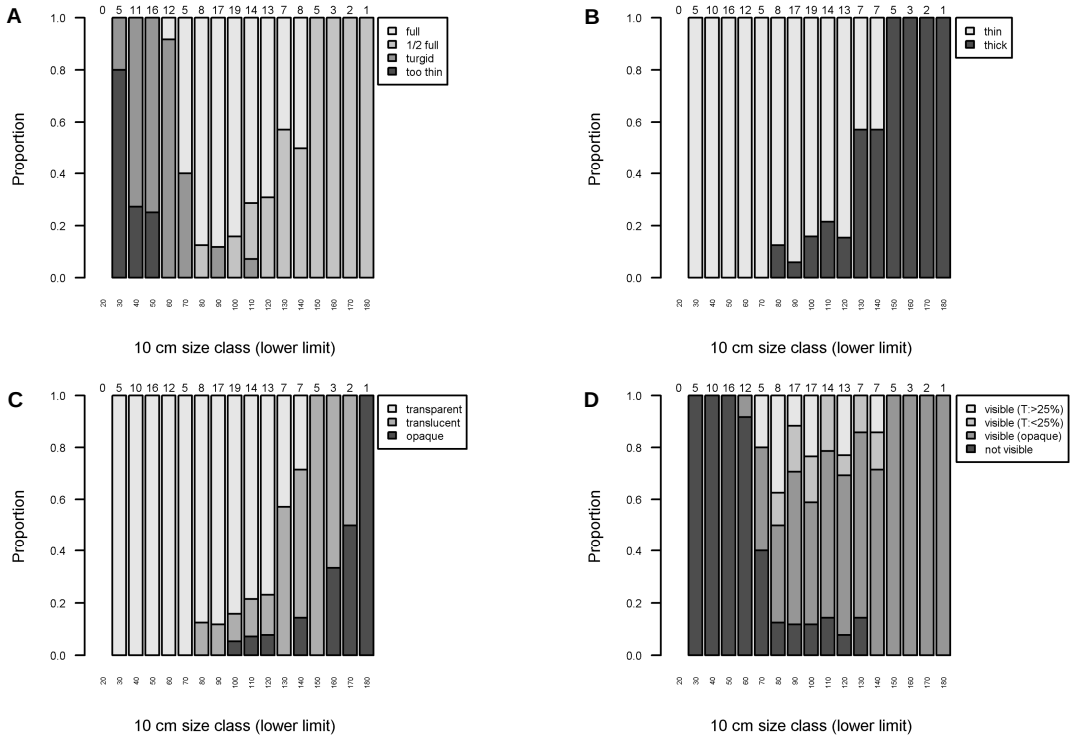

Figure 2. Size-related variations in the macroscopical appearance of ovaries of meagre during the peak spawning season (May and June). A) Fullness; B) Wall thickness; C) Wall transparency; D) External visibility of oocytes (T:<25% = less than 25% of translucent oocytes; T:>25% = more than 25% translucent oocytes). Numbers above bars are sample size.

Some geographical differences were noticed in mature fish (across seasons) and across the size gradient (within the spawning season). Ovaries from mature fish collected in the southern coastal were generally full in March and April and half-full from May onward while in the Tagus estuarine grounds displayed full gonads in May and June (only 3% half-full). Additionally, during peak spawning season, full gonads generally displayed thinner transparent walls and opaque oocytes being mostly sampled from the Tagus estuary fishery. A minor proportion of these gonads displayed translucent oocytes (Figure 2D). On the contrary, half-full

gonads generally had thicker translucent (or opaque) walls (Figure 2B-C) and lower densities of oocytes and were sampled essentially from large fish from the coastal waters of the southern coast. In the latter gonads oocytes were opaque (Figure 2D) and frequently seen only after a second look at the gonad.

Philometra was found in the ovaries of 29 out of 130 fish checked for the parasite. Parasite infections took place all year-round and both large and small size fish were infected. Interestingly, 93% of the fish infected were collected from southern waters where 34% of gonads carried the parasite.

Based on the macroscopic characteristics an anatomical grading system with seven classes (0 to VI) was derived for meagre ovaries. The main macroscopic features of this system are displayed in Table 2 and Figure 3.

3.2. Histological classification scale

A total of 286 ovaries were subjected to histologic analyses. The gonad sample comprised 4—70 ovaries from each month and 5—35 ovaries from each 10-cm fish length class with exceptions of January (no samples), fish smaller than 30 cm (no samples) and fish larger than 180 cm (two samples). Fish samples were captured both in estuarine (n=122) and coastal (n=137) fishing grounds and in the western (n=130) and southern (n=156) region. However, due to asymmetries in commercial fishing effort, local fishing traditions and the fish own migratory nature (Prista *et al.*, 2008), the majority of the ovaries came from fish sampled in the western estuarine fishery (42%) and the southern coastal fishery (48%) with only one fish sampled on the western coast and one fish sampled in southern estuaries. Furthermore, 80% of western samples were collected in May and June from fish between 36 cm and 148 cm while the southern samples were available year-round and respected to fish between 52 cm and 182 cm. In 26 ovaries the geographical area was known but not the exact type of fishing ground (estuarine or coastal). A single fish was caught offshore.

The meagre ovary is of cystovarian type. In mature fish, ovarian lamellae display several stages of follicle complex development during the spawning season. Four main development stages were observed in the histological sections of meagre: 1) Oogonia (OG) and Chromatin Nucleolar (CN), 2) Primary Growth (PG), 3) Secondary Growth (SG) and 4) Oocyte Maturation (OM) (Figure 4). These stages (and their substages) form a continuum of follicle development with changing follicle characteristics and increasing oocyte diameter that, alongside post-ovulatory follicle complex (POCs) formation, atresia of yolked oocytes and the thickness of the gonad wall, constitutes the corner-stone for understanding ovarian maturation in meagre.

Table 2. Gross anatomical grading system derived for the meagre ovaries. W_g = weight of gonad (g); TL = Fish total length (mean±s.e.); AG = age group (mean±s.e.); M = month(s) of occurrence. GSI (%) is calculated as (gonad weight/fish total weight)*100 (mean±s.e.)

Anatomical class	Macroscopic features	Other notes
0	Very thin thread-like ovaries (transparent and with barely any width or thickness); sex of fish cannot be identified at naked eye.	W_g<1 (n=7) GSI(%):0.0±0.0 (n=7) TL(cm):33.8±1.9 (n=7) AG(years):2±0.6 (n=3) M: Mar, May, Jun
I	Thread-like translucid ovaries; orange to reddish color; a few inconspicuous blood vessels may be present.	W_g≤2 (n=27) GSI(%):0.1±0.0 (n=27); TL(cm):45.9±1.4 (n=27) AG(years):2.5±0.1 (n=27) M: Mar—Jul, Dec
II	The ovaries occupy a small space within the abdominal cavity; orange to reddish opaque lobes, turgid with circular cross-section; thin transparent ovary wall; no oocytes externally visible at naked eye; no vascularization scars.	1≤W_g≤22 (n=75) GSI(%):0.2±0.0 (n=75) TL(cm):62±1.2 (n=76) AG(years):2.8±0.1 (n=56) M: Feb—Oct, Dec
III	The ovaries occupy most of the abdominal cavity; orange to pale yellow opaque lobes (at times slightly reddish in the posterior part); full in appearance with elliptical to circular cross-section; thin transparent membrane delicate to touch; opaque oocytes distinctively visible through the ovarian wall; generalized conspicuous vascularization displaying high degree of branching in both dorsal and ventral surfaces.	41≤W_g≤4040 (n=70) GSI(%):4.8±0.3 (n=68) TL(cm):113.6±2.8 (n=71) AG(years):7.7±0.6 (n=62) M: Feb—Jul
IV	The ovaries occupy most of the abdominal cavity; pale-yellow to rose opaque lobes (at times slightly reddish in the posterior part); full to very full appearance with elliptical to circular cross-section; very thin transparent ovarian wall sensitive to the touch; translucent oocytes visible as lighter dots among a background of opaque yellow oocytes; vascularization displaying high degree of branching in both dorsal and ventral surfaces.	156≤W_gv2292 (n=28) GSI(%):7.5±0.5 (n=28) TL(cm):106.9±3.4 (n=28) AG(years):6.8±0.5 (n=26) M: May, Jun
V	The ovaries occupy a relatively reduced volume within the abdominal cavity; yellowish translucent lobes; less full with elliptical cross-section; thick translucent ovarian wall very robust to handling; many opaque oocytes externally visible through the ovarian wall; conspicuous vascularization but with less branching than level III and IV; gonads occupy a median volume in body cavity.	49≤W_g≤1370 g (n=55) GSI(%):1.7±0.1 (n=53) TL(cm):152.1±2.8 (n=54) AG(years): 17.0±1.5 (n=44) M: May—Oct
VI	The ovaries occupy a relatively reduced volume within the abdominal cavity; brownish, dark orange or red opaque lobes; empty looking with elliptical cross section; ovarian wall thick, translucent to opaque, and robust to touch; no externally or internally visible oocytes; the vascularization is reabsorbed leaving a scarred pattern with grayish tone. Orange color more frequent in smaller individuals (66—126 cm) and red more frequent in larger individuals (98—172 cm).	4≤Wg≤831 (n=86) GSI(%):0.7±0.0 (n=86) TL(cm):105.6±2.9 (n=87) AG(years):6.4±0.6 (n=58) M: Feb—Dec

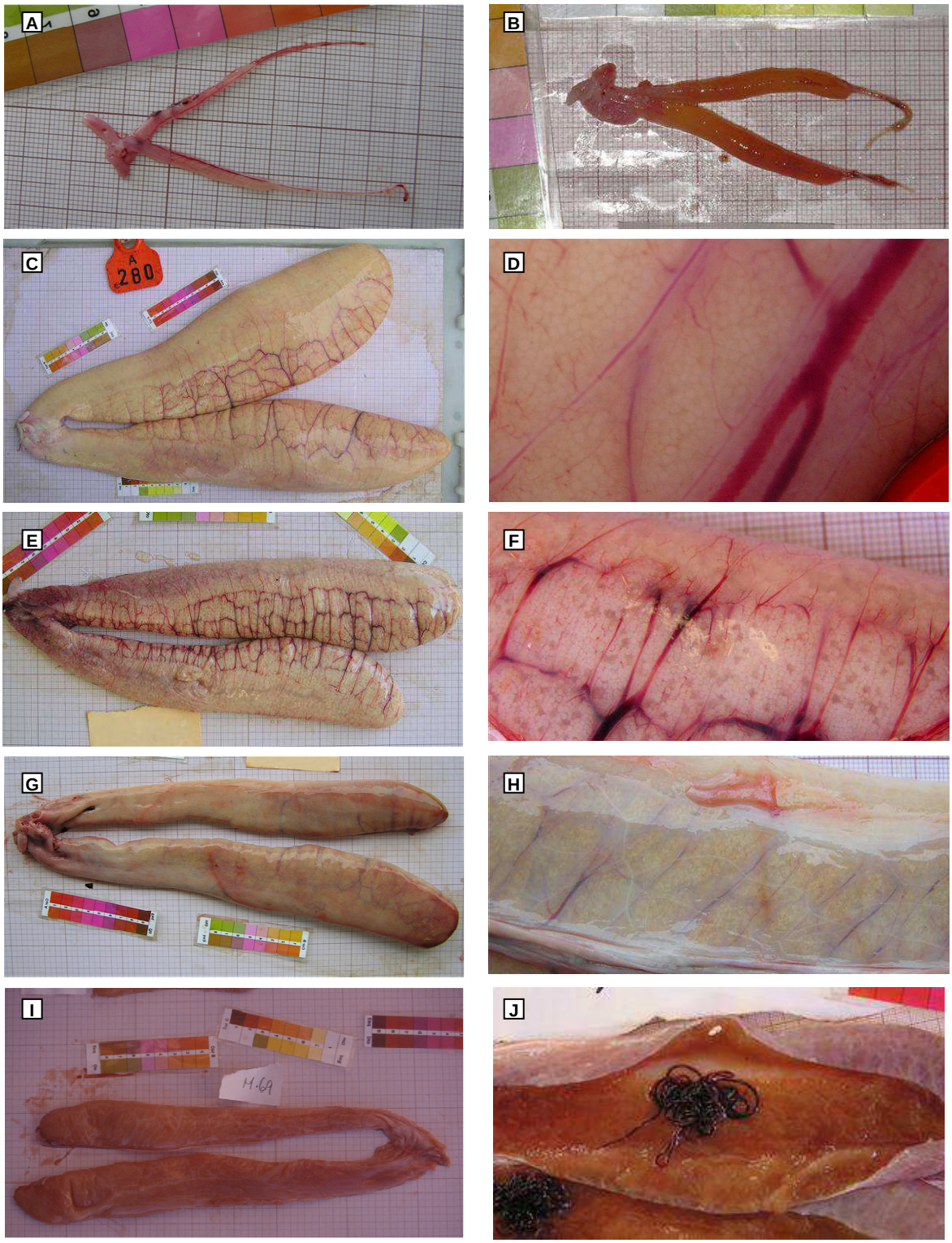

Figure 3. Macroscopical appearance of meagre ovaries [TL – total length in cm; M - month of capture; P - Place of capture: Southern Coast (SC), Tagus Estuary (TE); H – Histological stage]. A) Class I [TL: 41; M: June; P: TE; H: Immature] ; B) Class II (I) [TL: 49; M: June; P: TE; H: Developing immature], C) Class III [TL: 165; M: March; P: SC; H: spawning capable early, D) Class III, detail of oocytes [TL: 165; M: March; P: SC; H: spawning capable early, E) Class IV [TL: 100; M: June; P: TE; H: spawning capable actively spawning, F) Class IV, detail of oocytes [TL: 100; M: June; P: TE; H: spawning capable actively spawning, G) Class V [TL: 158; M: June; P: SC; H: spawning capable advanced], H) Class V, detail of oocytes [TL: 158; M: June; P: SC; H: spawning capable advanced], I) Class VI [TL: 130; M: November; P: SC; H: Developing mature], J) close-up of *Philometra* in class VI ovary.

Oogonia (OG) and *chromatin nucleolus* (CN)

Oogonia (OG) are oval shaped cells, 7—11 µm in diameter (*n*=11), that appear isolated or grouped in clusters with little or no basophilly. The nucleus occupies a large proportion of the cytoplasm (N/C: 39—72%) and displays one basophilic nucleolus. Chromatin nucleolus oocytes (NC) are oval shaped cells similar to OG but slightly larger (10—17 µm in diameter, N/C: 42—79%, *n*=10) and with chromatin threads in their nucleus.

Primary growth phase (PG)

Primary growth follicles are characterized by oocytes with a strong basophilic ooplasm and no yolk granules. Two sub-phases of primary growth can be distinguished and these can be distinguished by the presence/absence of oil droplets and cortical alveoli. Follicles in primary growth sub-phase 1 (PG-1) are smaller and more basophillic, do not have any lipid droplets or cortical alveoli and have rather indistinct follicle cells (=granulosa) and zona pellucida. Follicles in primary growth sub-phase 2 (PG-2) are larger and less basophillic, always carry lipid droplets and/or cortical alveoli, and their granulosa and zona pellucida are generally visible.

Primary growth sub-phase 1 (PG-1)

Early primary growth follicles sub-phase 1 (PG-1a): the oocytes are roundish to slightly polyhedric, 12—46 µm in diameter (*n*=23). The cytoplasm is strongly basophilic (dark blue) and homogeneous in coloration. The germinal vesicle is central and large (N/C: 40—69%) and has moderate basophilly (violet). Frequently, many nucleoli can be observed in the germinal vesicle, either dispersed or closer to the periphery; Concentric filaments with reduced basophilly (=nuage) appear in the ooplasm surrounding the germinal vesicle.

Late primary growth follicles sub-phase 1 (PG1-b): the oocytes are polyedric, 52-118 µm in diameter (*n*=26). The cytoplasm is strongly basophilic (dark blue) and the germinal vesicle has moderate basophily (violet). However, compared to PG-1a the germinal vesicle takes up smaller volume in the ooplasm (N/C: 25—50%) and the nucleoli are nearly always at its periphery. Nuage is frequent and patches of less basophillic material (=Balbiani bodies) appear in the ooplasm.

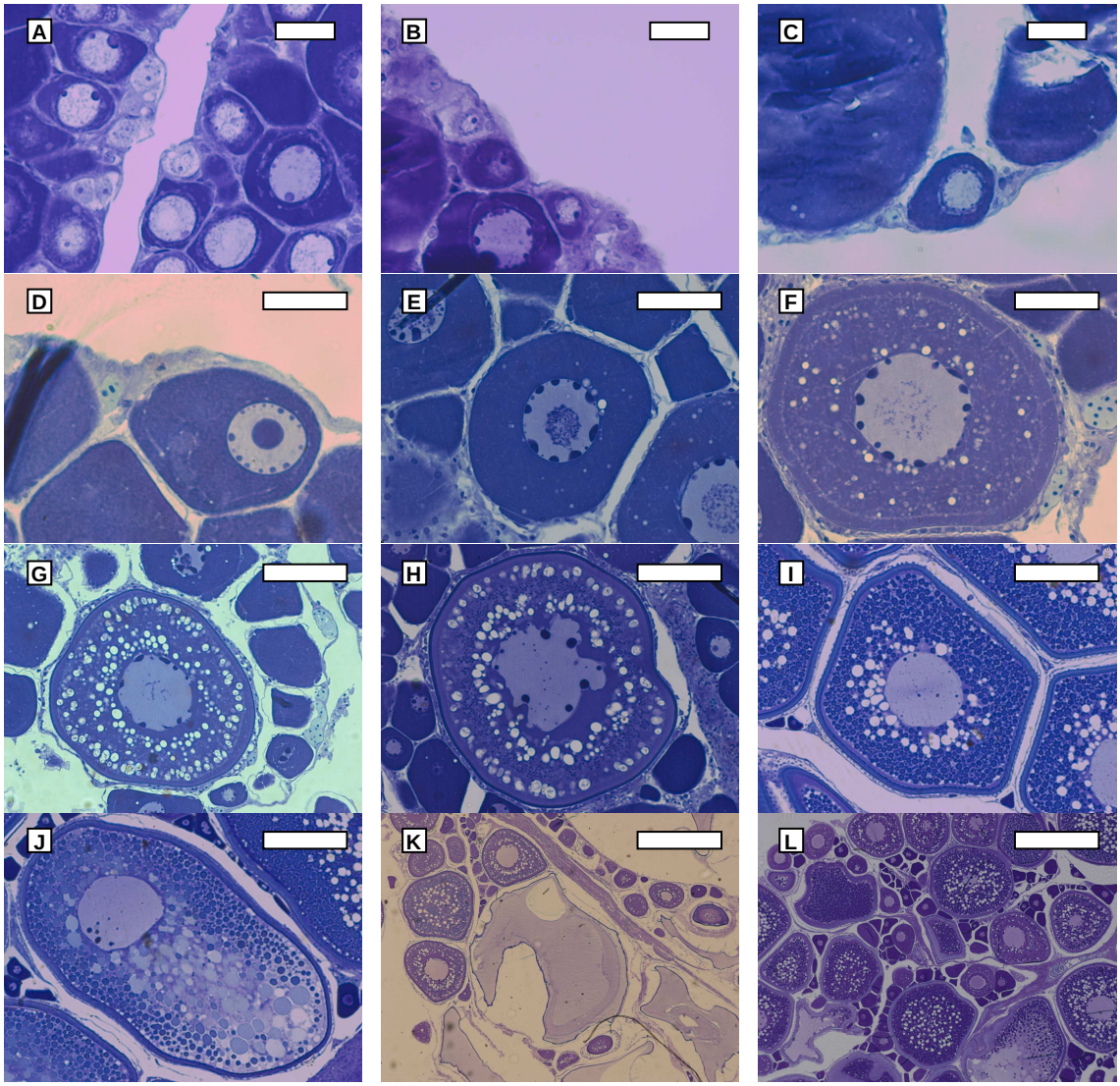

Figure 4. Stages of oogenesis an oocyte development in the meagre. A) Oogonia (Scale bar: ~20µm, 630x); B) Chromatin Nucleolar (Scale bar: ~20µm, 630x); C) Early primary growth follicles sub-phase 1 (PG-1a) (Scale bar: ~20µm, 630x); D) Late primary growth follicles sub-phase 1 (PG1-b) (Scale bar: ~50µm, 400x); E) Early primary growth follicles sub-phase 2 (PG-2a)(PG1-b) (Scale bar: ~50µm, 400x); F) Mid primary growth follicles sub-phase 2 (PG-2a) (Scale bar: ~100µm, 200x); G) Late primary growth follicles sub-phase 2 (PG-2a) (Scale bar: ~100µm, 200x); H) Early secondary growth follicles (SG-a)(Scale bar: ~100µm, 200x); I) Late secondary growth follicles (SG-b)(Scale bar: ~200µm, 100x); J) Early oocyte maturation follicles (OM-a)(Scale bar: ~200µm, 100x); K)Late oocyte maturation follicles (OM-b)(Scale bar: ~500µm, 40x); L) Overview of ovary section (Scale bar: ~500µm, 40x).

Primary growth sub-phase 2 (PG-2)

Early primary growth follicles sub-phase 2 (PG-2a): oocytes are polyhedric, 87—157 µm in diameter (*n*=19). The overall coloration is similar to PG-1b. The germinal vesicle has moderate size (N/C: 25—53%) and displays many peripheral nucleoli. The germinal vesicle envelope is smooth and conspicuous. Folicular cells are now distinct (namely the granulosa) but the zona pellucida is not yet visible. In PG-2a two

characteristic inclusions appear in the ooplasm: lipid droplets and cortical alveoli. Lipid droplets (=oil droplets *sensu* Grier 2012) appear in the ooplasm, either dispersed or aggregated closer to the germinal vesicle. The lipid droplets appear as white vacuoles in the ooplasm and grow quickly in volume (reaching rapidly more than 3 µm in diameter). Cortical alveoli are similar in shape and color to lipid droplets but are generally smaller (less than 3 µm in diameter) and display basophilic content. Initially, both structures appear scattered in the ooplasm but are present in very small numbers so it is frequent that only type is observed.

Mid primary growth follicles sub-phase 2 (PG-2b): the oocytes are polyhedric to roundish, 139—204 µm in diameter (*n*=16). The ooplasm suffers a large decrease in basophilly. The germinal vesicle is moderately sized (N/C 30—49%) and becomes irregular. The nucleoli are oval-shaped and located in peripheral position. Lipid droplets and cortical alveoli increase in number and size. The former become increasingly aggregated closer to the germinal vesicle while the latter move closer to the ooplasm periphery. The granulosa cells are now conspicuous.

Late primary growth follicles sub-phase 2 (PG2-c): the oocytes are polyhedric to roundish, 216—356 µm in diameter (*n*=11). Their coloration is similar to PG-2b. The germinal vesicle is moderately sized (N/C 25—55%) with and inconspicuous and irregular contour. The nucleoli are irregularly oriented at germinal vesicle periphery. Lipid droplets become extremely obvious in the cytoplasm forming one or two continuous concentric strings centered around on the germinal vesicle. The cortical alveoli become less conspicuous and are discernible only at the periphery of the ooplasm. The granulosa and the zona pellucida are conspicuous with the latter being clearly visible as a thin single layer band with strong marine-blue coloration.

Secondary growth phase (SG)

The secondary growth follicles are characterized by the appearance of strongly basophilic granules (yolk granules) in the ooplasm accompanied by a large increase in oocyte volume and the enlargement of the zona pellucida. Early and late SG follicles (SG-a and SG-b, respectively) can be distinguished based on the abundance and size of yolk globules, the size of the cell and the appearance of the zona pellucida.

Early secondary growth follicles (SG-a): the oocytes are round, 282—465 µm in diameter (*n*=10). The germinal vesicle is moderately sized (N/C 22—46%) and presents an inconspicuous and irregular envelope.The ooplasm coloration is not much different from PG-2c follicles but yolk globules with strong marine-blue coloration start appearing in the outer and middle cortex. Lipid droplets continue to increase in size and cortical alveoli are now at periphery of the ooplasm; the zona pellucida thickens gradually.

Late secondary growth follicles (SG-b): the oocytes are round, 477—725 µm in diameter (*n*=15). The germinal vesicle is smaller relative to cell diameter (N/C: 22—36%) with its contour becoming smooth but remaining relatively inconspicuous. Yolk granules

proliferate and increase in size. Lipid droplets continue to increase in volume at the periphery of the germinal vesicle; Cortical alveoli become increasingly inconspicuous at the oocyte periphery. The overall coloration of the oocyte approaches marine blue, greatly contrasting the violet and dark-blue that characterized the PG-1, PG-2 and SG-b. The zona pellucida becomes very thick and conspicuous, with clearly distinct internal and external areas.

Oocyte maturation phase (OM)

Oocyte maturation groups the final steps of follicle development prior to ovulation. In meagre this involves the coalescence of lipid and yolk droplets, the migration of the germinal vesicle to the animal pole and the progressive hydration of the oocyte. Early and late oocyte maturation (OM-a and OM-b, respectively) can be distinguished based on the overall appearance of the ooplasm and the size of the cell.

Early oocyte maturation follicles (OM-a): oocytes are round, 619—836 µm in diameter (*n*=10). The basophilly of ooplasm decreases drastically relative to previous stages. The yolk granules, the nucleoli and the zona radiata are now the most strongly stained structures. At the start of the OM-a stage the germinal vesicle is located central to the ooplasm and surrounded by lipid droplets. The lipid droplets then start to coalesce an form larger lipid droplets while polarity is established in the cell with the germinal vesicle appearing progressively closer to the animal pole. As maturation progresses, the cell hydrates and yolk globules coalesce with lipid droplets originating larger lighter blue globules. The germinal vesicle envelope becomes progressively more irregular and starts to disintegrate. The zona pellucida remains conspicuous.

Late oocyte maturation follicles (OM-b): occur just before ovulation and have fully hydrated oocytes. The oocyte is now very large with no germinal vesicle and homogeneous intracellular content of reduced basophilly (pink). Approximate oocyte diameter range 821—1108 µm (*n*=13) Yolk granules are no longer visible. The zona pellucida remains strongly basophilic but now appears as a single layer with marked invaginations into the ooplasm.

Ovulation (OV)

When follicle development is completed, ovulation takes place. Ovulation encompasses the parting of the germinal epithelium, basement membrane and follicular cell layer required for the release of the hydrated oocyte into the ovary lumen (Grier 2012). We have not observed signs of ovulation other than the presence of post-ovulatory complexes (POC) that we assumed to be indicative that had recently taken place.

Additional ovarian features that were found relevant to the interpretation of histological slides of the meagre ovaries during specific phases of the reproductive cycle are displayed in Figures 5,

6, and 7. From these, particular relevance is detained by post-ovulatory follicle complexes, atresia and the ovarian wall.

Post-ovulatory follicle complexes (POC)

Post-ovulatory follicle complexes (POC) are the remnants of the follicle that stay in the ovary after ovulation. These remnants include the basement membrane, the granulosa and the theca. In meagre, the POCs can be recognized by the convoluted appearance assumed by the granulosa cells after the ovum is released. Similar to other species a degenerative process of the POC structure gradually takes place. In our observations of meagre POCs we have distinguished between two types of POCs: early POCs and late POCs (Figure 5A). In early POCs the granulosa cells hypertrophy and convolute forming a continuous cord with their nuclei similarly oriented. Late POCs are smaller, the outer granulosa cells detaching from the theca and the inner granulosa cells loosing their linear arrangement and oriented nuclei and parting from each other to form an increasingly unstructured vacuolar mass (Figure 5B). Early and late POCs are morphologically similar to POCs reported by Hunter and Macewicz (1986) for skipjack tuna *Katsuwonus pelamis* kept at ~23ºC, 0 and 12 hours after spawning, respectively.

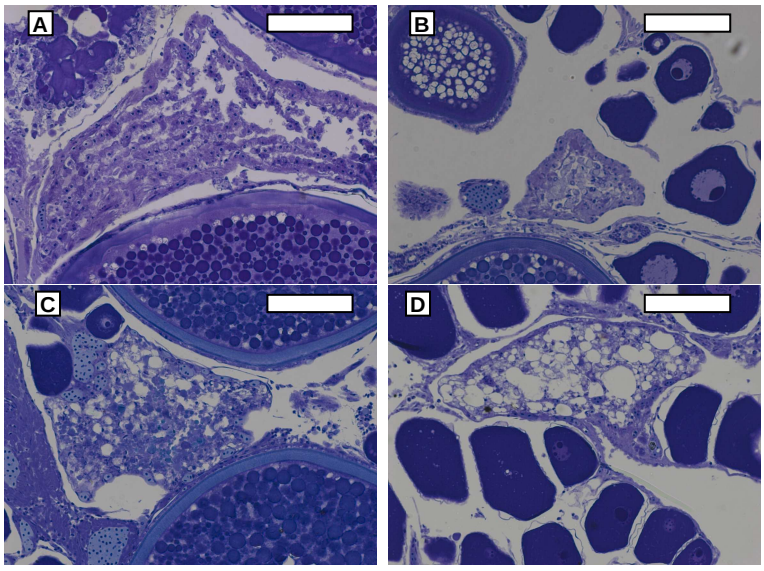

Figure 5. Post-ovulatory follicle complexes and vitellogenic atresia in the meagre ovary; A) Early post-ovulatory follicle complex (POC); B) Late post-ovulatory follicle complex. Note the smaller size and disorganized appearance of the granulosa relative to the early stage; C) Early vitellogenic atresia (α-atresia); D) late vitellogenic atresia. Note the conspicuous vacuoles and absence of yolk granules relative to α-atresia. Scale bar: ~100μm, 200x.

Atresia of yolked oocytes

Atresia is the process of degeneration and removal of the ovarian follicles from the ovary. In meagre atretic stages of unyolked oocytes were rarely observed but two stages of vitellogenic atresia were observed: α-atresia and β-atresia. A-atresia starts

with a proliferation of the blood vessels in the theca and the disintegration of the germinal vesicle. Then the contents of yolk granules and lipids start dissolving into the ooplasm (Figure 5C). Finally, the zona pellucida and olonema break, and yolk granules and lipids continue to coalesce and dissolve. Resorption of the cellular content continues until basophilly is definitively lost giving rise to a disorganized mass of vacuoles (Figure 5D). Atretic stages of unyolked oocytes were only rarely observed.

Ovary wall

The ovary wall of meagre is mildly basophillic and highly muscular varying widely in thickness and structure with both fish size and reproductive stage. The core structural components of the ovary wall are the squamous epithelium, smooth muscle fibers, nerves and blood vessels. In immature individuals the wall is thin and displays one to three layers of smooth muscle, capillaries and nerves (Figure 6A). In mature individuals, the wall displays several more such layers. As the spawning season approaches and the ovarian content increases in volume, capillaries become filled with erythrocytes and the muscle fibers become progressively more parallel as they stretch. During oocyte maturation, the different layers are completely compressed and barely distinguishable (Figure 6B). Then, as the ovary empties the wall layers become visible once more, with muscle fibers becoming highly disorganized. During this period, blood cells within capillaries loose basophilly and degenerate and the wall thickens (Figure 6C). A relationship appears to exist between the number of muscle and vessel layers and fish age, that may justify the increase in thickness with fish size.

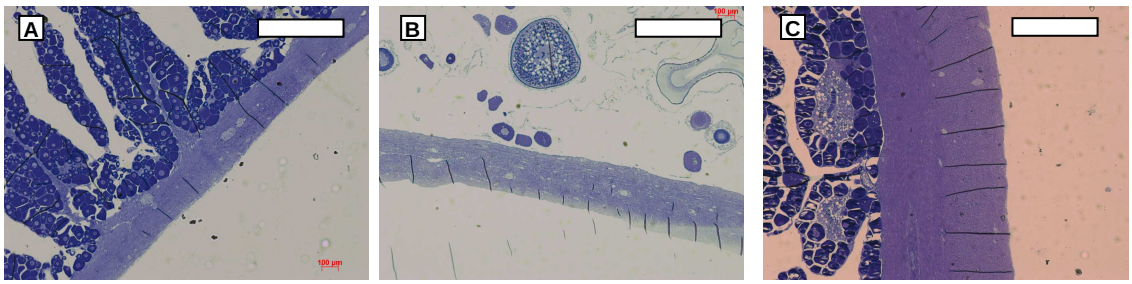

Figure 6. Ovarian wall in meagre. A) Thin wall in developing immature; B) Thin wall in spawning capable fish; C) Thick wall in regressing fish. Scale bar: ~500µm, 40x.

From February to July the most advanced follicle complex of the vast majority gonads of mature meagre area in SG phase (Figure 8A). OM follicles were observed between April and July but only in a minor proportion of females. Hydrated follicles (OM-b) were observed in May and June (Figure 8A). POCs were found from May to August (Figure 8B). Occasional vitellogenic atresia was present throughout the year but higher levels were only registered between June and October and always in a minor proportion of ovaries (Figure 8C). High density of healthy SG-b follicles took place essentially from February to June and low density from June to October. Nearly all mature individuals displayed a complex ovarian wall, with three

or more layers of capillaries, nerves and muscles. Wall thickness and vitellogenic oocyte density varied inversely, i.e., thinner walls being present when vitellogenic oocyte density was higher and thicker walls when the density of vitellogenic oocytes was lower or null (Table 3). From August until December the most advanced follicle displayed cortical alveoli and lipid droplets but no yolk granules (PG-2) (Figure 8A). No samples were obtained in January and February but PG-2 follicles were essentially loose (i.e., non compact) in ovaries collected from June to November becoming compact thereafter (Figure 8D). Muscle bundles (Figure 7C) co-occurred with the mass atresia in the end of summer but were found in nearly all mature individuals until the end of the year. Signs of regeneration in the lamella lining (Figure 7D) were noticed in individuals caught between August and November. PG-1 phase was observed in all slides but was never the most advanced stage in mature fish.

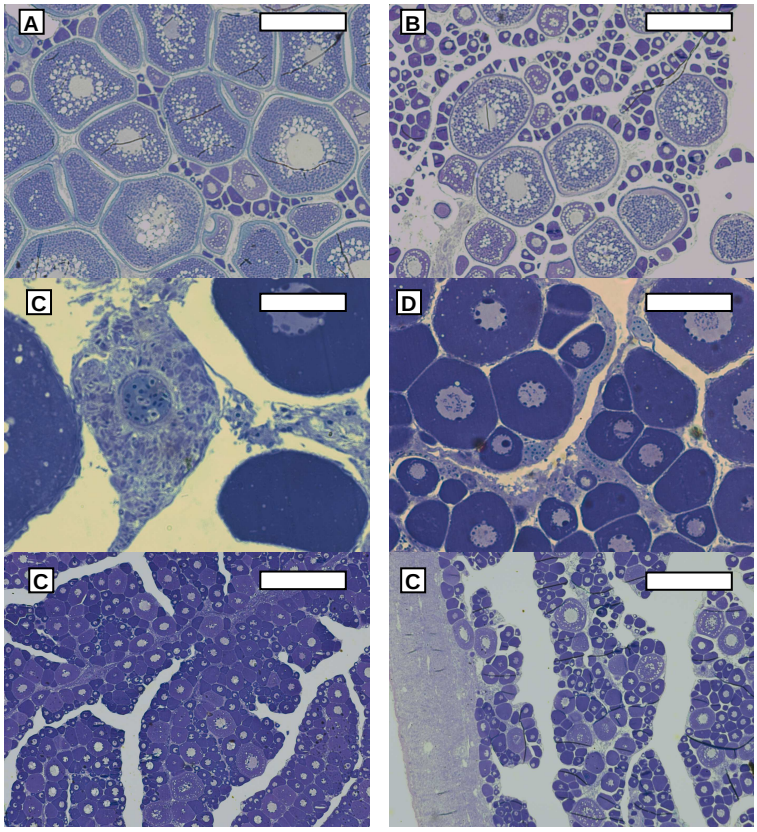

Figure 7. Additional features used to assign reproductive phase to meagre ovaries. A) High density of vitellogenic oocytes (Scale bar: ~500µm, 40x); B) Low density of vitellogenic oocytes (Scale bar: ~500µm, 40x); C) Muscle Bundle(Scale bar: ~50µm, 400x); D) Recovery of the germinal epithelium and lamella lining. Note the proliferation of pre-follicle and PG-1 follicles and capillaries (Scale bar: ~100µm, 200x); E) compact PG-1 and PG-2 follicles in developing mature fish (Scale bar: ~500µm, 40x); F) non-compact PG-1 and PG-2 in regenerating mature (Scale bar: ~500µm, 40x).

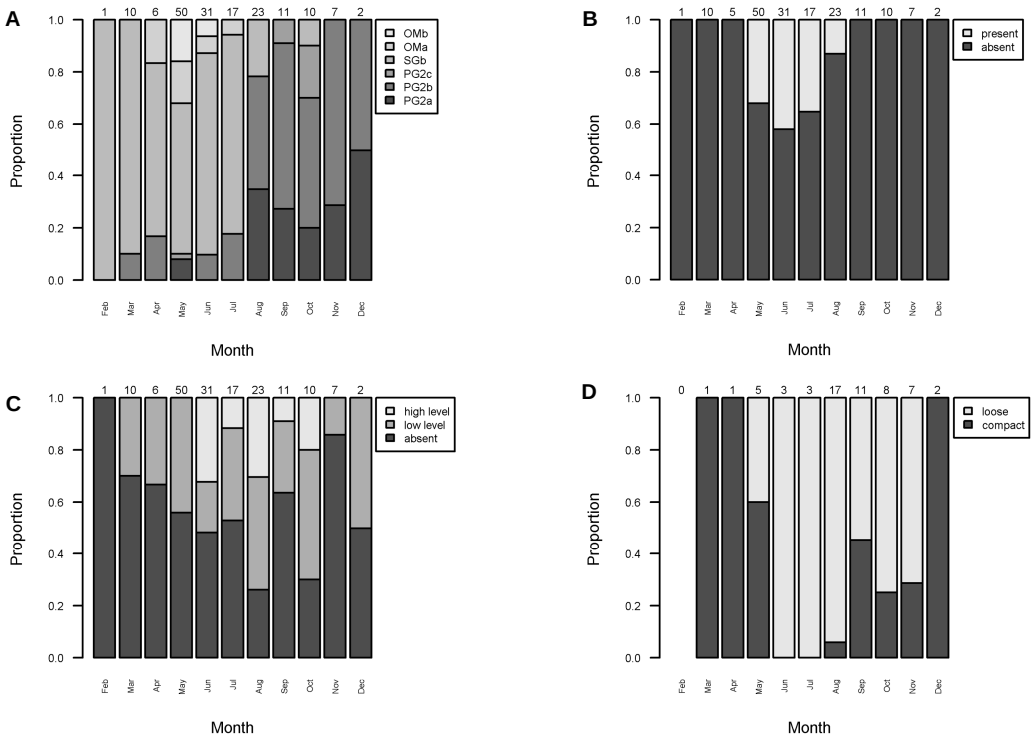

Figure 8. Monthly variations in the histological features of ovaries of mature meagre (>90 cm). A) Follicle development stage, B) Post-ovulatory follicle complexes, C) Vitellogenic atresia, D) Compactness of PG-2 stages in ovaries with PG-2 as the most advanced follicle stage. Numbers above bars are sample size.

Within the spawning season, the most advanced follicle of all ovaries from fish with length <40 cm was in PG-1 stage (Figure 9A). In fish lengths 40 cm to 70 cm PG-1 stage remained dominant but PG-2 follicles were detected in some ovaries. From 70 cm upwards, ovaries with most advanced follicle in SG and OM stage increase in frequency becoming dominant in fish >80 cm (Figure 9A). Along the size gradient, the first ovary in OM stage belonged to a 68.5 cm fish and displayed low vitellogenic density. Other than this specimen, in ovaries of fish <70 cm no other ovary containing yolked oocytes was observed. POCs were only observed in fish >70 cm (Figure 9B) and vitellogenic atresia was only detected in fish >60 cm with intense levels registered in mostly fish >100 cm (Figure 9C). The wall of fish <80 cm generally had at most two muscle and capillary layers and its thickness was below the thinnest walls registered in mature fish (Table 3).

Table 3. Wall thickness (µm) of the meagre ovary with follicles in different developmental stages. TL = Fish Total Length

Most advanced follicle stage	Specifics	n	Mean (± s.e.)	Range	Type of wall
OM-a	---	6	357±73	222—684	Thin, 3 or more layers
SG-b	high density SG-a	38	383±27	147—950	Thin, 3 or more layers
SG-b	medium or low density SG-b	32	1067±67	418—1702	Thick, 3 or more layers
PG-1 or PG-2	TL >90 cm	43	633±47	201—1468	Thick, 3 or more layers
PG-1 or PG-2	TL ≤ 90 cm	98	262±10	88—582	Thin, less than 3 layers

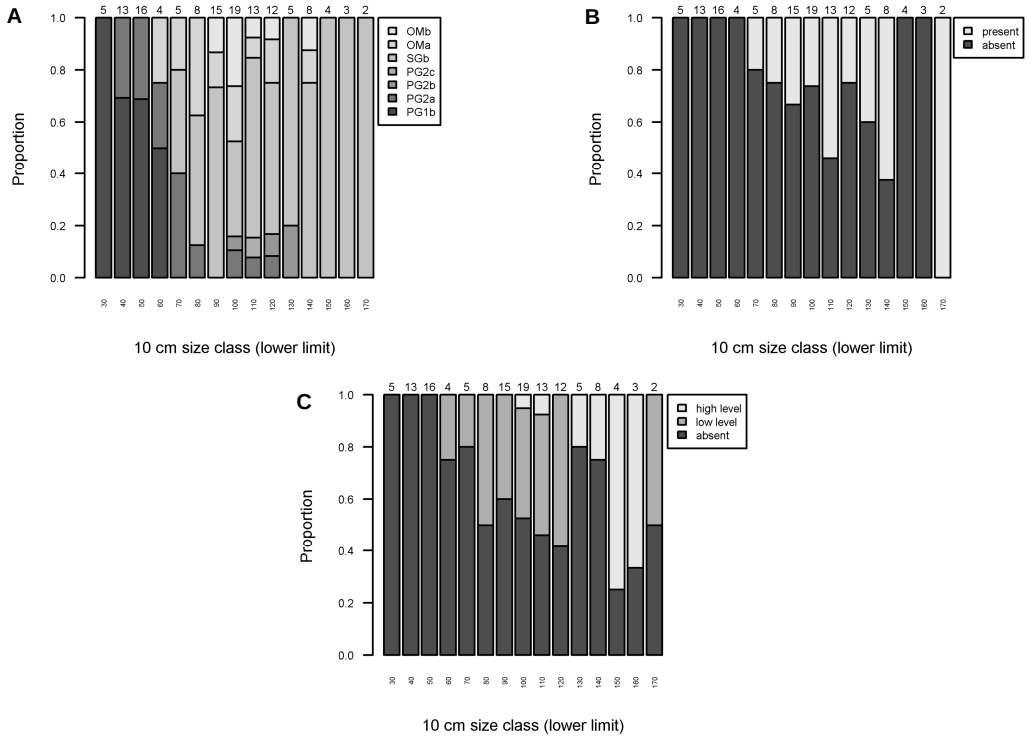

Figure 9. Size-related variations in the macroscopical appearance of ovaries of meagre during the peak spawning season (May and June). A) Follicle development stage; B) Post-ovulatory follicle complexes; C) Vitellogenic atresia. Numbers above bars are sample size.

Geographical differences were noticed in the histological sections of mature fish (across seasons) and across the size gradient (within the spawning season). In mature fish, OM follicles were observed in fish collected in both the western and southern regions but hydrated follicles (OM-b) were observed only in May and June and in fish sampled from the Tagus estuary fishery (western coast). POCs were found from May to August in both western and southern fisheries in ovaries collected from both estuarine or coastal waters. Late summer samples of large fish, with thick wall and low vitellogenic density were common in southern coast. During the spawning season, the ovaries of a few individuals between 100 and 140 cm had their most advanced follicle in stage PG-2. These fish showed signs of vitellogenic atresia and were all caught in the southern coast fishery with individuals of similar sizes of the western estuary displaying yolked stages. The only sample of >100 cm fish caught in estuarine waters of the southern coast was caught in late June. The ovary displayed follicles in SG-b stage with low density of vitellogenic oocytes, thin wall (418 µm thick) and no atresia. The only sample from fish >100 cm from the western coastal waters was caught in late November and displayed loose PG-2 follicles with no POCs or atresia. Finally, we note that only four individuals were observed with most advanced follicle in stage PG-2c and only two individuals with most advanced follicle in stage SG-a.

Based on the previous characteristics the histological development of the meagre ovaries can be divided into 5 main phases (Figure 10, Table 4). The width of the ovarian wall displayed significant linear relationship to fish length and reproductive phase (Figure 11). Final AICc

selected model indicated no coincident slopes and no interaction (parallel lines). In mature individuals ovarian wall thickness displayed a clear relationship to reproductive phase with thinner walls being registered in actively spawning and early spawning capable individuals. The wall thickened as spawning progresses achieving its maximum in advanced spawning and regressing individuals. Immatures and developing immatures displayed thinner walls than developing/regenerating matures (Table 4, Figure 11).

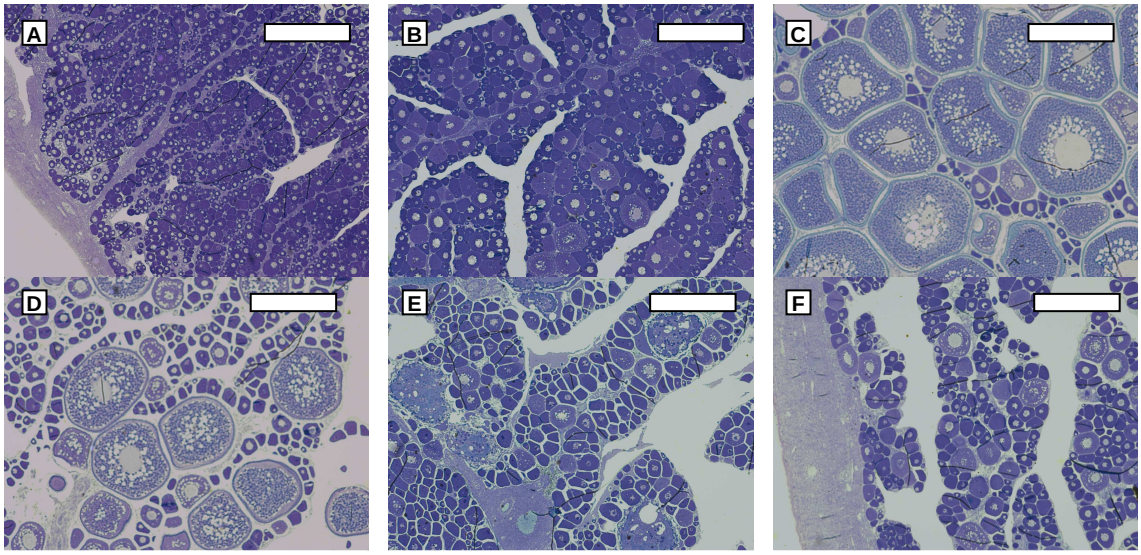

Figure 10. Histological development phases of meagre ovaries. A) Developing Immature; B) Developing Mature; C) Spawning Capable Early; D) Spawning capable advanced; E) Regressing; F) Regenerating. Scale bar: ~500µm, 40x.

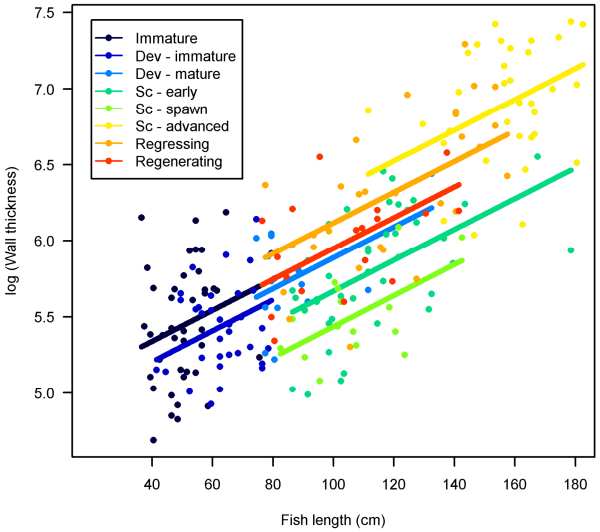

Figure 11. Relationship between ovarian wall width and fish length in the different reproductive phases of meagre. The straight lines are the values predicted from the linear model. Dev = Developing, Sc = Spawning capable. Note the y-axis in log-scale. Thickness in µm.

Table 4. Histological grading system of the meagre ovaries. PG – Primary Growth, SG – Secondary Growth, OM - Oocyte maturation, POCs – Post-ovulatory follicle complexes; W_g – weight of gonad. TL = Total length; AG = Age group; Wall = Thickness of the ovarian wall; M = Months of occurrence. Gonadosomatic Index (GSI, %) calculated as in Table 2

Phase	Main Histological features	Other notes
Immature	PG-1 is the most advanced follicle stage; low follicular density; abundant interfollicular tissue; no vitellogenic atresia or POCs; thin ovarian wall.	0≤Wg≤7 (n=42) GSI(%):0.1±0 (n=42) TL(cm):50.7±1.3 (n=42) AG(years):2.6±0.1 (n=41) Wall(μm):249±16 (n=40) M: Mar—Jun, Sep, Oct
Developing (immature)	PG-2 are the most advanced follicle stage; compact follicles with little interfollicular tissue (86%); no vitellogenic atresia or POCs; thin ovary wall with <3 layers.	2≤Wg≤21 (n=35) GSI(%):0.3±0 (n=35) TL(cm):63.5±1.7 (n=35) AG(years):2.8±0.1 (n=32) Wall(μm):236±13 (n=35) M: Feb—Jul, Oct, Dec
Developing (mature)	PG-2 or SG-a are the most advanced follicle stages; compact follicles (95%); no vitellogenic atresia or POCs; lamellae highly organized with smooth lining practically without blood vessels; muscle bundles absent; thin ovary wall with ≥3 layers and active blood vessels.	9≤Wg≤163 (n=22) GSI(%):0.6±0 (n=22) TL(cm):92.9±3.7 (n=22) AG(years):4.1±0.2 (n=14) Wall(μm):400±56 (n=18) M: Feb—Apr, Sep—Dec
	Early developing: PG-2 are the most advance follicle stage.	9≤Wg≤163 (n=20) GSI(%):0.6±0 (n=20) TL(cm):93.7±4.1 (n=20) AG(years):4.2±0.3 (n=12) Wall(μm):413±62 (n=16) M: Feb—Apr, Sep—Dec
	Late developing: SG-a is the most advance follicle stage.	41≤Wg≤53 (n=2) GSI(%):1±0.1 (n=2) TL(cm):85±3.5 (n=2) AG(years):4±0 (n=2) Wall(μm):294±36 (n=2) M: Mar—Apr
Spawning Capable	SG-b or OM are the most advanced follicle stages; follicles of all developmental stages may be present; vitellogenic atresia absent or in low levels; POCs may be present; ovarian wall thin to thick.	80≤Wg≤4040 (n=120) GSI(%):4.4±0.2 (n=119) TL(cm):124.9±2.7 (n=120) AG(years):10.4±0.8 (n=113) Wall(μm):607±45 (n=90) M: Feb—Ago, Oct
	Early spawning capable: SG-b is the most advanced stage and is present in high density; minor vitellogenic atresia frequent (39%); thin ovary wall; POCs frequent (30%).	95≤Wg≤4040 (n=57) GSI(%):4.9±0.2 (n=57) TL(cm):115.7±3.1 (n=57) AG(years):8±0.7 (n=54) Wall(μm):358±22 (n=40) M: Feb—Jul
	Late spawning capable: SG-b is the most advanced stage being present in low density; vitellogenic atresias frequent (58%), sometimes in high number(44%), but some healthy SG-b oocytes still remain; muscle bundles and blood vessels start appearing; very thick ovary wall, particularly in larger individuals; POCs frequent (31%).	80≤Wg≤1325 (n=36) GSI(%):1.8±0.1 (n=35) TL(cm):154.7±3.3 (n=36) AG(years):17.3±1.8 (n=33) Wall(μm):1064±65 (n=33) M: Apr, May—Ago, Oct
	Actively spawning subphase: OM is the most advanced developmental stage; very thin ovary wall; POCs frequent (30% of slides).	124≤Wg≤2292 (n=27) GSI(%):6.9±0.5 (n=27) TL(cm):104.8±3.6 (n=27) AG(years):6.6±0.5 (n=26) Wall(μm):308±40 (n=17) M: Apr—Jul
Regressing	PG-2 is the most advanced stage; intense folliculogenesis and oogenesis; vitellogenic atresias always present and affecting all remaining SG oocytes ; lamellae disorganized with irregular lining and extensive blood vessels and muscle bundles; non-compact follicles (76%); POCs rare (12%); ovary wall with intermediate thickness and ≥3 layers; many inactive vessels in ovarian wall, namely at its periphery.	16≤Wg≤404 (n=42) GSI(%):0.8±0 (n=42) TL(cm):119.3±4.2 (n=43) AG(years):8.4±0.8 (n=25) Wall(μm):604±58 (n=30) M, Apr—Dec
Regenerating	PG-2 are the most developed stage; intense folliculogenesis and oogenesis; No vitellogenic atresia or POCs; non-compact follicles (75%) ;Lamellae more organized with smoother lining and many blood vessels; Muscle bundles present; ovary wall with intermediate thickness and ≥3; Inactive vessels in ovarian wall.	12≤Wg≤387 (n=24) GSI(%):0.7±0.1 (n=24) TL(cm):106.9±4.7 (n=24) AG(years):5.4±0.8 (n=16) Wall(μm):498±62 (n=20) M: Jul—Nov

3.3. Correspondence between macroscopic and histological scales

The results obtained from the application of histological and macroscopical scales to 264 ovaries are displayed in Table 5. Ovaries classified macroscopically as class I and II largely corresponded to juvenile fish that were not spawning in the current annual cycle (immatures or developing immatures). Ovaries classified as class III, VI and V (and to a lesser extent also VI) all corresponded to mature fish. Table 6 displays conditional probabilities of interest for fisheries studies on meagre calculated based on data displayed in Table 5 (or seasonal subsets) and assuming the sample is representative of the population. It is noticeable that the macroscopical scale performs well in assessments of maturity but that it is difficult to identify actively spawning fish based on macroscopy.

Table 5. Histological corroboration of the macroscopic scale of meagre ovaries

	Gross anatomical class							
Histological phase	**0**	**I**	**II**	**III**	**IV**	**V**	**VI**	**Total**
Immature		21	20				1	42
Developing			30				4	34
Developing (mature)			2	2			14	18
Spawning capable (early)				52	5			57
Spawning capable (actively spawning)				7	19	1		27
Spawning capable (late)				2		32		34
Regressing			0	0		6	26	32
Regenerating			1			1	18	20
Total	0	21	53	63	24	40	63	264

Table 6. Accuracy of the gross anatomical scale when classifying meagre ovaries. Values calculated for "all year" were directly derived from table 5. These data assumes the meagre sample is representative of the population

Conditional Probability:	**Probability (%)**
Mature if III-VI	97
Mature if III-VI & Apr-Jul	98
Mature if III-VI & May+Jun	99
Immature if I-II	96
Immature if I-II & Apr-Jul	98
Immature if I-II & May+Jun	100
Actively Spawning if III	11
Actively Spawning if IV	79
Actively Spawning if V	2

4. Discussion

The present details the reproductive characteristics of wild meagre *Argyrosomus regius* females across the species size span and in different seasons of the year. The reproductive cycle of meagre has recently become the focus of significant research interest because of the potential the species exhibits for aquaculture diversification and difficulties in obtaining natural spawning in captivity (Duncan *et al.* 2012, Schiavone *et al.* 2012, Mylonas *et al.* 2013a,b). To date, few studies directly addressed the reproduction of wild meagre in European and Mediterranean waters. González-Quirós (2011) studied the life-history of meagre in the Gulf of Cadiz but covered larger females only from March to August and did not present histological results. Abou Shabana *et al.* (2012) analyzed 30 meagre ovaries from Egyptian waters but also with very limited seasonal and size span (few individuals >90cm). Gil *et al.* (2013) analyzed 37 wild females >100 cm from the Gulf of Cadiz but only between April and August. One additional work, provided histological and macroscopic details on the reproductive cycle of meagre but focused singly on male reproduction (Prista *et al.*, 2014). This scarcity of information extends to meagre populations from other geographical areas (Mauritania, Morocco) but also to *Argyrosomus* from the southern hemisphere (Tixerant 1974, Hermas 1995, Griffiths 1996, Farmer 2008, Silberschneider *et al.* 2009). As such, most of the information on ovarian histology that can be directly compared to the current results comes from the reproductive studies of american cofamiliars (e.g., Fitzhugh *et al.* 1993, Wells 2002, Wells and Jones 2002, Grier 2012). Probable causes for the shortage of studies on Eastern North Atlantic sciaenids (but see Grau *et al.* 2009) are difficulties reported in the acquisition of fresh samples from large highly valuable fish, particularly when they are migratory and have poorly characterized fisheries of essentially small scale artisanal or recreational nature (Prista *et al.* 2007).

Asynchronous ovarian development, batch spawning and indeterminate fecundity are common reproductive patterns in the Sciaenidae family, both in large-sized (Fitzhugh *et al.* 1993, Grier 2012) and smalle-sized species (Barbieri *et al.* 1994). In meagre, all development stages are present in ovaries of mature fish during the spawning season indicating this species has asynchronous ovarian development. Additionally, post ovulatory follicle complexes are found in meagre ovaries alongside healthy secondary growth follicles and/or oocytes undergoing maturation indicating the fish is also a batch spawner. This conclusion is further confirmed by information on multiple spawns associated to decreasing density of vitellogenic oocytes as the spawning season progresses registered in wild populations (Table 4, Figure 7A,B; Chapter 5C) and in captivity (Mylonas *et al.* 2013a). Finally, whole oocyte size frequencies of mature meagre were analyzed during the spawning season and no hiatus between pre-vitellogenic and vitellogenic oocytes was found (Chapter 5C). Alongside the high intensity atresia in late spawning capable ovaries and regressing ovaries (Table 4), these findings further corroborate the idea that meagre is a species with indeterminate fecundity (Hunter *et al.* 1992).

Follicle and ovarian development has been thoroughly studied in teleosts (Wallace and Selman 1981, Tyler and Sumpter 1996, Lubzens *et al.* 2010). The terminology and features used to describe the successive stages of oocyte formation vary with author and species but the

overall oocyte stages and reproductive phases initially proposed have been broadly maintained (Brown-Peterson *et al.* 2011). In mature meagre all main types of follicle stages can be found and we have grouped them into primary growth, secondary growth and oocyte maturation as recently suggested by Brown-Peterson *et al.* (2011). A comparison of our results with the recent morphological work carried out in the cofamiliar red drum *Sciaenops ocellatus* (Grier 2012) indicates that the structures observed and sequence of development match reasonably well with what has been observed in red drum descriptions with only minor differences being noticeable. However, some aspects of the meagre histology deserve highlighting because they contrast with recent standardization proposals (namely Brown-Peterson *et al.* (2011)) and because they have implications on studies of comparative histology and life-history across the members of the Sciaenid family and teleosts in general.

To date, oocyte stages defined by the presence of lipid droplets have been infrequently reported in sciaenid literature and it is not clear if they are absent or have not been noticed/reported in previous studies (but see Wells 2002, Berois *et al.* 2004, Grau *et al.* 2009 and Grier 2012). In histological sections of meagre we have found abundant lipids droplets that appear and proliferate in the oocyte at about the same time as cortical alveoli which has led us to consider a mixed lipid droplet and/or cortical alveoli substage (PG-2a-c) (Figure 4). Given the significance of lipids in the meagre gonads and gonads of other perciforms (Mayer *et al.* 1988, Grau *et al.* 1996, Holland *et al.* 2000, Abascal and Medina 2005) it would seem likely that lipid stages would have been more alluded to in reproductive studies of teleosts. Lipid droplets appear in routine histology slides as empty vacuoles because they loose their content during the alcoholic dehydrations and require histochemical or ultrastructural studies to be fully evidenced (Mayer *et al.* 1988, Grau *et al.* 1996, Abascal and Medina 2005, Grier 2012). Given their vacuolar appearance and their initial location in the cortex of the oocyte, it is possible that lipid droplets have been misidentified as cortical alveoli in some previous literature. However, it is also possible that authors have noticed lipids but preferred to continue to use the more familiar term "cortical alveoli" when referencing to the oocyte stage where lipid droplets frequently appear (Mayer *et al.* 1988, Selman and Wallace 1989, Brown-Peterson *et al.* 2011).

The exact positioning and significance of cortical alveoli and lipids within follicle development is currently under debate. Brown-Peterson *et al.* (2011) have advocated towards the inclusion of cortical alveoli stages at the beginning of secondary growth on grounds of evidence that relates cortical alveoli formation to gonadotropin dependent processes (Lubzens *et al.* 2010). Lowerre-Barbieri *et al.* (2011) also recommended using the cortical alveoli stage to identify maturity unless species have a long developmental rate and reproductive cycles longer than 1 year or skip spawning by arresting oocyte development while in cortical alveoli stage (e.g., Junquera *et al.* 2003). Conversely, Grier (2012) has emphasized that cortical alveoli synthesis and vitellogenesis are different processes that may (or may not) be coincident in time (Mayer *et al.* 1988, Abascal and Medina 2005). In his study of *Sciaenops ocellatus* he opted for a "staging compromise" considering that secondary growth starts with the appearance of yolk globules under light microscopy. Similarly, Abascal and Medina (2005) have opted for not

considering a cortical alveoli stage in *Thunnus thynnus* but rather a transitional lipid stage between primary and secondary growth on grounds of marked lipid presence but cortical alveoli being "scarce in, if not absent" in bluefin tuna oocytes. Lipid droplets are absent from some teleosts (Wallace and Selman 1981) but preponderant in others (Mayer *et al.* 1988, Grau *et al.* 1996, Abascal and Medina 2005, this study) and like cortical alveoli are also considered to be related to gonadotropins and vitellogenesis (Holland *et al.* 2000, Lubzens *et al.* 2010) thus being categorized as secondary growth.

In meagre we have opted to categorize follicles with lipids and cortical alveoli stages (and no yolk) within a final substage of primary growth. We base our decision on two main lines of evidence. On the one hand juvenile ovaries generally display stage PG-2b (or lower) follicles year-round (Table 4) which indicates cortical alveoli are present long before the fish are effectively mature and fully integrate the annual spawning cycle. On the other hand the ovaries of mature fish show no evidence of arrested oocyte development at cortical alveoli stage and display an annual cycle that involves progressing from a cortical alveoli stage (PG-2) towards yolked stages (SG) during the first semester and a regression back to cortical alveoli stages during late summer and autumn. In this annual cycle we have found a scarce number of ovaries with PG-2c and SG-a as the most advanced follicle (Figure 9) and it is likely this scarcity resulted from vitellogenesis in wild populations being a relatively fast process as has been described for meagre in captivity (2 months; Mylonas *et al.* 2013b). These two results indicate that if gonadotropin dependent vitellogenesis is actually taking place in PG-2a,b follicles then it is likely to be happening at a much slower rate than it happens annually in adult fish. Because secondary growth is frequently associated to fish maturity and used as threshold in the annual building of maturity ogives (Brown-Peterson *et al.* 2011, Lowerre-Barbieri *et al.* 2011) we found it better to follow Grier's (2012) compromise and for the time being consider cortical alveoli within a final substage of primary growth and accept the presence of yolk as the proof of significant vitelogenesis and upcoming maturity.

Our results consubstantiate the existence in meagre of a group of young females that display some signs of maturity (e.g., cortical alveoli, lipid droplets) but that do not yet develop yolk globules on an annual basis. Holland *et al.* (2000) have reported similar results in striped bass *Morone Saxatilis* where secondary growth appears to be initiated but not completed within a year. Some similar precocious maturity signals have been identified in wild male juveniles of meagre which at 3-4 years of age develop spermatozoa in very small amounts but do not appear to contribute significantly to spawning biomass (Prista *et al.* 2014). The adaptative value precocious maturity signs in meagre is difficult to envision as it represents an expenditure of energy that is diverted from fish growth (Taranger *et al.* 2010) and is thus likely to increase mortality in wild populations. One possibility is that it is a natural consequence of a progressive entrainment of the response of the brain-pituitary-gonad system to environmental clues that thrigger adult maturity (Lowerre-Barbieri *et al.* 2011). The fact that meagre juveniles co-occur with adults in estuarine grounds during the same seasons might be seen to corroborate this hypothesis (Prista *et al.* 2008). Recently, Campbell *et al.* (2006) studied cortical alveoli and lipid

formation in Coho salmon *Onchorhynchus kisutch* suggesting that growth achieved at a specific time of the year might dictate the evolution (or no-evolution) of ovaries towards ovulation. Considering the large energetic investment that is probably associated to gonad production on meagre it seems likely that a such a threshold in size exists and must be attained before the fish effectively proceed towards definitive vitellogenesis.

In meagre we have been able to distinguish between developing immatures (with PG-2 follicles) and regenerating/developing adults (also with PG-2 follicles) on grounds of the ovarian wall thickness of each phase. The importance of ovarian wall in distinguishing mature fish and immature fish has been frequently alluded to but present literature on the ovarian wall of teleosts is very fragmented and consists mostly of morphological studies (Takano 1968, Morrison 1990, Ravaglia and Maggese 2002). To the best of our knowledge, no detailed studies exist that directly analyze and quantify the seasonal- or size-related variations in wall thickness and wall structure in marine teleosts (but see Yamamoto 1963 in Takano 1968) and a single study has used quantitative measurements of wall thickness to separate reproductive phases within the Sciaenid family (Berois *et al.* 2004). One reason for this apparent scarcity may be difficulties in standardizing measurements of wall thickness (Lowerre-Barbieri *et al.* 2011) but in meagre we have found annual variability that is noticeable at the naked eye (Figure 3) and that appears to be well above imprecisions in measurement (Figure 11). Furthermore, a direct relationships appears to exist between microscopic features related to regression (resorption of capillary content, occlusion of older capillaries) (Figure 6C) and macroscopic features like the grayish vascular patterns characteristics of macroscopical class VI (Figure 3). The reason why thickness variations and macroscopical features were so noticeable in the meagre case may have to do with the large size/age span of the meagre and the large variations in size its ovaries experience throughout the year, but it may also be species specific. In the medaka *Oryzias latipes* the ovary wall is thought to have a secretory role and produce fluid that facilitates the extrusion of eggs (Takano 1968) and Bills *et al.* (2008) have recently suggested the contraction of ovarian wall musculature could play a significant role in ovulation helping the fish to extrude the eggs (Bills *et al.* 2008). During field work, local estuarine fishers reported us that the meagre rubs its belly on the estuarine bottom while spawning. This observation matches our own observations of numerous "reddish bellies" in meagre captured during spawning season in the Tagus estuarine grounds. This evidence is purely anecdotal but it does point out that the egg shedding may be difficult for meagre (this is mentioned by the fishers themselves) and that its highly muscled ovarian wall may be part of a set of physical adaptations and behaviors that facilitate egg extrusion.

Historically, macroscopic classifications of gonad development have constituted the methodological basis of most reproductive studies of fish. This is the case of meagre but also of other large sciaenids for which anatomical scales with five levels (González-Quirós *et al.* 2011), six levels (Hermas 1995, Potts *et al.* 2010), seven levels (Tixerant 1974, Griffiths 1996) and eight levels (Murphy and Taylor 1989, 1990, Farmer 2008, Silberschneider *et al.* 2009) have been previously designed and used with no apparent quantitative validation. Comparisons

between the histologically corroborated macroscopical scale presented in Table 5 and these prior macroscopic scales highlights some previously unnoticed features. As an example, Australian and South African studies (Griffiths 1996, Farmer 2008, Silberschneider *et al.* 2009) have assumed that flaccid gonads with a few opaque oocytes were "spent". Histological work as shown that in *Argyrosomus regius* those macroscopical characteristics reflect a "late spawning capable stage" characterized by low GSI, low density of vitellogenic oocytes and POCs, alongside atresia and a thick ovary wall (Table 4). Similar drastic reductions in vitellogenic pool have been noticed by Mylonas *et al.* (2013a) in ovary comparisons from captive fish before and after inducing spawning with an agonist of gonadotropin-releasing hormone (GnRHa). Considering some "spent" gonads as being in fact spawning capable is likely to expand the areal extent of spawning grounds and prolong reproductive seasons both of which have consequences in conservation efforts.

Gross anatomical scales used in fisheries research are not always validated histologically (Hunter and Macewicz 2003). In many species it is difficult (if not impossible) to distinguish macroscopically between developing immatures and regenerating/developing mature females, which is a key issue in fisheries reproductive studies (Lowerre-Barbieri *et al.* 2011). Several authors have cautioned on the use of non-validated macroscopic scales and/or defended a reduction in the number of macroscopic classes considered (West 1990, Hunter and Macewicz 2003, Kjesbu *et al.* 2003) as-well as the limitation of maturity ogives to samples collected within the spawning season to reduce the risk of such misidentification (Murua *et al.* 2003, Lowerre-Barbieri *et al.* 2011). If necessary, the macroscopic scale proposed in Table 2 can be simplified to a more conservative approach as has been suggested by Hunter and Macewicz (2003). However, we note that the semi-quantitative analysis of the size and month-related variability of meagre ovaries presented in this study conforms the major requirements set out for the development of macroscopical scales (Kjesbu *et al.* 2003) namely being able to reasonably discriminate hydrated oocytes into a separate class, having a set of class with clearly visible yolked oocytes (III-V), being able to separate the immatures and being independent of color. Altogether, even if we note that we have not conducted a formal validation of the accuracy of macroscopic scale because our sample was far from random and our macroscopical scale was not in fact independently validated but rather corroborated with histological results, it appears that, for the time being, these results strengthen reproductive phase interpretations in meagre to the point that they may be useful in the cutting down of sampling costs required to bring this European species to a more data-rich situation.

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Chapter 5C

Ovarian development and fecundity type in meagre (*Argyrosomus regius*)

Nuno Prista[a,b,c], Cristina Nunes [c], José Lino Costa[a,b] and Maria José Costa[a,b]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Divisão de Modelação e Gestão de Recursos de Pesca, Instituto Português do Mar e da Atmosfera, Avenida de Brasília, 1449-006 Lisboa, Portugal

To be submitted.

Ovarian development and fecundity type in meagre (*Argyrosomus regius*)

Abstract: The meagre (*Argyrosomus regius*) is one of the world's largest sciaenids and supports regional fisheries and aquaculture interests in European and North African waters. However, its reproductive development patterns have remained scarcely studied, particularly in what concerns wild populations. In the present study, we used histological and whole oocyte size frequency analyses to determine the ovarian development and fecundity type of meagre in Portuguese waters. We show that during the reproductive season, all stages of oocyte development are present in the ovaries and that the whole oocyte size frequency is continuous, displaying no discrete modes and no hiatus between pre-vitellogenic and vitellogenic oocytes. Additionally, we show that final oocyte maturation occurs in batches. We conclude that the meagre has asynchronous ovarian development and indeterminate fecundity and discuss these results in light of existing literature in the ovarian development of sciaenids and the importance of using stereological methods or the disector principle in analyses of oocyte size frequencies of asynchronous ovaries.

Keywords: oocyte frequency, histology, fecundity, reproduction, Sciaenidae

1. Introduction

Descriptive analyses of reproductive development are central to the understanding of fish biology and fish population dynamics (Murua and Saborido-Rey 2003, Brown-Peterson et al. 2011). Among these, the determination of the type of ovarian development organization, the spawning pattern and the type of fecundity assume major importance because they constrain how sampling and estimation should be carried out when estimates of annual fecundity need to be obtained (Murua et al. 2003). Such estimates are key-aspects in, e.g., determinations of stock biomass with the egg production method (Stratoudakis et al. 2006, Armstrong and Witthames 2011), interpretations of fish life-history (Lowerre-Barbieri et al. 2011) and investigations into the aquaculture potential of new species (Duncan et al. 2012).

To date, three major types of ovarian development organization have been recognized in marine teleosts: synchronous, group-synchronous and asynchronous (Wallace and Selman 1981, Murua and Saborido-Rey 2003). In synchronous ovaries, all oocytes develop and mature at the same time. Consequently, the ovaries are dominated by a single oocyte stage that evolves as the spawning season approaches. In group-synchronous ovaries, at least two populations of oocytes occur in the ovary as the spawning season approaches: one population of more advanced oocyte stages that evolves towards maturation (called a "clutch"); and a population of less developed oocyte stages (generally in primary growth) that is observed all year-round. In asynchronous ovaries, oocytes in several stages of development forming a continuum with no dominant populations or clear clutches except, eventually, a clutch of oocytes that undergoes final maturation.

Independently of the type of ovarian organization, oocytes grow in size as they proceed from oogenesis to final maturation and, in general progress towards maturation is assumed to take place when the oocytes enter vitellogenesis (West 1990, Grier 2012). Consequently, understanding the dynamics of ovarian development in terms of both oocyte staging and oocyte diameter is crucial for annual fecundity estimation (Murua et al. 2003). In fecundity studies fish species have been categorized as having "determinate fecundity" (when the total number of yolked oocytes is considered fixed prior to the spawning season) or "indeterminate fecundity" (when the total number of yolked oocytes cannot be considered fixed prior to the spawning season, i.e., when continuous recruitment of vitellogenic oocytes from the pre-vitellogenic pool takes place while the fish spawns). Correct identification of the type of fecundity is important because different sampling methods are applied in the estimation of individual annual fecundity of determinate and indeterminate spawners. If fecundity is determinate it is generally sufficient to quantify the number of vitellogenic oocytes present in the ovary before the start of the spawning season as long as one is able to appropriately select the time for sampling and estimate atretic losses (Kurita et al. 2003, Thorsen et al. 2006). However, if fecundity is indeterminate a more complex procedure is required that involves not only the estimation of the number of oocytes each female sheds per spawning event (batch fecundity) but also the estimation of the spawning interval (time period between two consecutive spawning events) and the estimation of the duration of the duration of the individual spawning season (Murua et al. 2003, Lowerre-Barbieri et al. 2011).

The meagre (*Argyrosomus regius*, Asso 1801) is one of the world's largest sciaenids and the only representative of the large sciaenid group in European waters. The meagre is a coastal fish (<80 m deep) that attains over 180 cm in total length and 50 kg in weight (Quéméner, 2002) and whose distribution extends from the English Channel to Senegal (including the Mediterranean Sea and Black Sea). Its major fisheries take place in Mauritania, Morocco, and Egypt, which together comprise over 80% of the ca. 10 000 t world annual catch (Quéméner 2002, FAO 2009). Similar to many of its co-familiars, the large size of meagre, its high ex-vessel prices, and its high seasonal availability in shallow waters, make it an important target species for local small-scale commercial fleets and the recreational sector (Quéro and Vayne 1987, Quéméner 2002, Prista et al. 2008). These characteristics alongside its fast growth rate and good quality of its meat have led to the recent development of aquaculture production and to increasing interest in its life-history patterns and life cycle (Quéméner et al. 2002, Prista et al. 2009, González-Quirós et al. 2011, Duncan et al. 2012, Schiavone et al. 2012, Gil et al. 2013, Prista et al. 2014).

Recent work on the ovarian cycle of meagre was inconclusive in what concerns the type of ovary development organization and the type of fecundity. Duncan et al. (2012) and Abou Shabana (2012) have categorized the meagre as a group-synchronous batch spawner but Gil et al. (2013) considered it to be an asynchronous batch spawner with determinate fecundity. To date other sciaenids have largely been considered as batch spawners, with both group-synchronous or asynchronous ovary development, and their fecundity has been estimated using

indeterminate fecundity procedures (Nieland and Wilson 1993, Barbieri et al. 1994, Wilson and Nieland 1994, Wells and Jones 2002, McDowell and Robillard 2013).

In this study, we present results from histological and whole oocyte analyses carried out in five wild meagre ovaries collected at the beginning, middle and end of the spawning season and determine the ovarian organization and fecundity type of meagre. We then discuss how this novel evidence changes the current interpretation on the type of ovarian organization and fecundity in this species. In doing this, we revisit published literature in other sciaenid species and discuss the possibility that non-standardized methodologies and terminologies may have given rise to some biased conclusions on ovarian development organization and fecundity type.

2. Materials and methods

We investigated ovarian development using a combination of histologic analysis and analysis of whole oocytes (West 1990).

Ovaries were obtained from a large scale study that addressed the life-history of meagre in Portuguese waters between 2004 and 2007. Details of field sampling and histological procedures are given in Chapter 5B. Briefly, fish were obtained from commercial landings, measured and weighed. Then, ovaries were removed and fixed in 4% buffered formaldehyde.

Histologic procedures involved the collection of small pieces of gonad (about 0.125 cm^3) from the ventral periphery of the medial region of the left lobe and their embedding in Technovit 7100 resin (Heraeus Kulzer). Thin sections (3–5 μm thick) were obtained using on a Leica RM2155 micrometer, stained with toluidine blue and definitively mounted on glass slides. Final microscopic analyses were carried out at 40-400x magnification on a Zeiss stereomicroscope. Among other, the following information was scored for each slide: the oocyte stages present (Table 1), the presence/absence of post-ovulatory follicle complexes (POCs), the incidence of vitellogenic atresia, and the diameters of the vitellogenic oocytes (Figure 1A, Chapter 5B).

Table 1. Oocyte stages considered in the analysis of histological slides from meagre ovaries (see Chapter 5B). Mean diameters and size range refer to maximum cell diameters through the nucleus. All measurements in μm

Stage	Substage	Main features	n	Mean diameter (s.e.)	Size range
Primary growth (PG)	PG-1a	No cortical alveoli or lipids, large germinal vesicle	23	26 (1)	12—46
	PG-1b	No cortical alveoli or lipids, large germinal vesicle	26	77 (4)	52—118
	PG-2a	Few cortical alveoli and lipids	19	116 (5)	87—157
	PG-2b	Many cortical alveoli and lipids; lower basophilly	16	173 (5)	139—204
	PG-2c	Concentric strings of lipid droplets and cortical alveoli	11	258 (14)	216—356
Secondary growth (SG)	SG-a	Small yolk granules appear	10	342 (17)	282—465
	SG-b	Large yolk granules, thick zona pellucida	15	621 (15)	477—725
Oocyte maturation (OM)	OM-a	Lipid-yolk coalescence; progressive hydration, nucleus migration to animal pole	10	756 (26)	619—836
	OM-b	Fully hydrated; homogeneous low basophilly ooplasm, no germinal vesicle	13	977 (24)	821—1108

From all the ovary slides analyzed in Chapter 5B, we selected subset of five mature females and determined its whole oocyte frequency. In the Portuguese coast the meagre is reproductively active (i.e., displays ovaries with healthy vitellogenic oocytes) from February to August and spawning (as indicated by the presence of hydrated oocytes and post-ovulatory follicles) takes place from May to August (Chapter 5B). We chose our five females so that our final sample represented ovarian development of meagre at the beginning, middle and end of the reproductive period (Table 2). For whole oocyte analysis we took ca. 1 cm^3 of ovarian content from the mid-ventral peripheral region of left ovary and carefully separated the oocytes from adjoining tissue into a gridded petri dish (Figure 1B). We then took digital pictures of all squares in the grid using a Leica MZ12 lens system equipped with a Leica DFC280 digital camera and measured the maximum diameter of whole oocytes in AxioVision image processing software (Zeiss) (Figure 1B). Initial trials revealed that some oocyte size classes represented less than 5% of the total number of oocytes in the petri dish grid. To assure detection and representativeness of whole oocyte measurements, we randomly selected digital photographs and measured all the oocytes in the successive selected squares until we reached a preset minimum of 500 oocytes. When that number was reached we completed the measurements in that square and stopped. During our whole oocyte measurements we did not distinguish between vitellogenic and non-vitellogenic oocytes so to investigate if the proportion of vitellogenic oocytes in the ovary decreased as the reproductive season progressed we estimated it as

$$prop(vit) = \frac{N_{vit}}{N_{tot}}$$

where N_{vit} is the number of whole oocytes with diameters in the range of diameters vitellogenic stages and N_{tot} is the number of oocytes larger the 50 µm detection threshold. Because the size of vitellogenic oocytes increases as the fish approach the spawning season and the final full-grown size is likely be dependent on fish size or fish age (West 1990), the range of diameters used to identify vitellogenic oocytes in each oocyte frequency was obtained from concurrent measurements of maximum diameters (through the nucleus) in the histological slides.

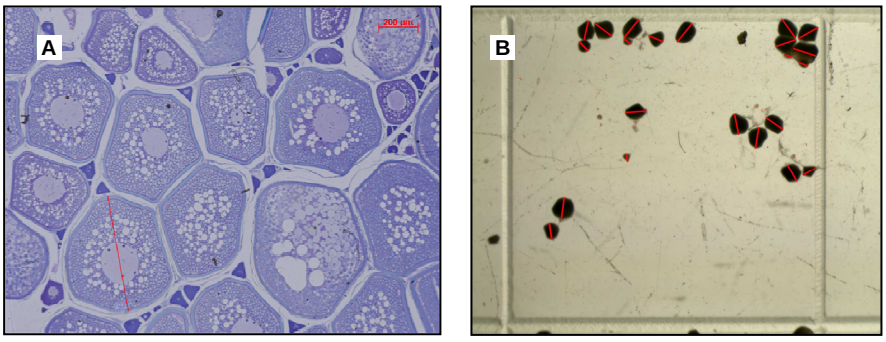

Figure 1. Example of oocyte counts and histological section (ovary R_553). A) Histological section with all oocyte stages visible (bar: 200 µm); B) Oocyte count (square area:1cm^2). Red lines represent measurements of maximum diameters.

Table 2. Characteristics of the meagre ovaries. Reproductive phase assigned according to Brown-Peterson at al. (2011); Oocyte stages as in Table II; VAt - Vitellogenic atresia, POCs - Post-ovulatory follicle complexes, GSI - Gonadosomatic index, TL - Total length, TW - Total weight, AG - Age group. Sc - Spawning capable; "0" = absent, "-" = few; "+" = many. GSI calculated as (gonad weight/fish total weight)*100

Fish ID	Date	Location	Reproductive phase	Oocytes	VAt	POCs	GSI (%)	TL (cm)	TW (kg)	AG (yr)
R609	2005-03-28	Olhão coast	Sc - early	PG-1a to SG-b	-	0	4.2	139	21.5	8
R553	2005-05-15	Tagus estuary	Sc - spawn	PG-1a to OM-a	0	0	8.5	120	15.7	8
R345	2004-05-31	Tagus estuary	Sc - early	PG-1a to SG-b	0	-	4.9	143	24.5	15
R348	2004-05-31	Tagus estuary	Sc - spawn	PG-1a to OM-b	-	-	10.5	118	12.8	9
R439	2005-08-30	Olhão coast	Regress	PG-1a to PG-2b	+	-	1.1	140	16.7	---

3. Results

Oocyte size frequencies of the meagre ovaries are displayed in Figure 2. During the spawning season all 20-µm size classes below 800 µm were found in the meagre ovary, i.e. no hiatus was observed in the oocyte diameters that might be separating non-vitellogenic from vitellogenic oocytes. No discrete modes were observed but two clear non-discrete modes were present: one at ~100 µm corresponding to primary growth oocytes (PG-1b and PG-2a) and one at ~600-700 µm that corresponded to secondary growth oocytes (SG-b) (Figure 2). Not so clear modes occurred at ~400 µm and 500 µm in some specimens and these may correspond to SG-a oocytes recruited from primary growth. In the three specimens whose ovaries contained oocytes larger than 800 µm, one/two detached modes appear at those larger sizes: these modes occur at ~900 µm and ~1000 µm (R_348 only) and corresponded to batches of oocytes undergoing final oocyte maturation.

The relative proportion of vitellogenic oocytes decreased substantially as the reproductive season progressed but mean diameters of the vitellogenic oocyte stages did not vary significantly between specimens or dates as observed by overlapping standard errors (Table 3).

4. Discussion

In recent years increasing concern has been expressed for the need to standardize terminologies and methodologies used in describing and assessing the reproductive strategies of fish (Hunter and Macewicz 2003, Murua and Saborido-Rey 2003, Murua et al. 2003, Brown-Peterson et al. 2011, Lowerre-Barbieri et al. 2011). Such standardization has been mainly advocated by those involved in fisheries and ecological research that frequently find the need to compare results of reproductive studies across geographical areas, stocks or species. However, some caution with such standardization should be considered when differences found in detailed analyses of individual species are important (Grier 2012).

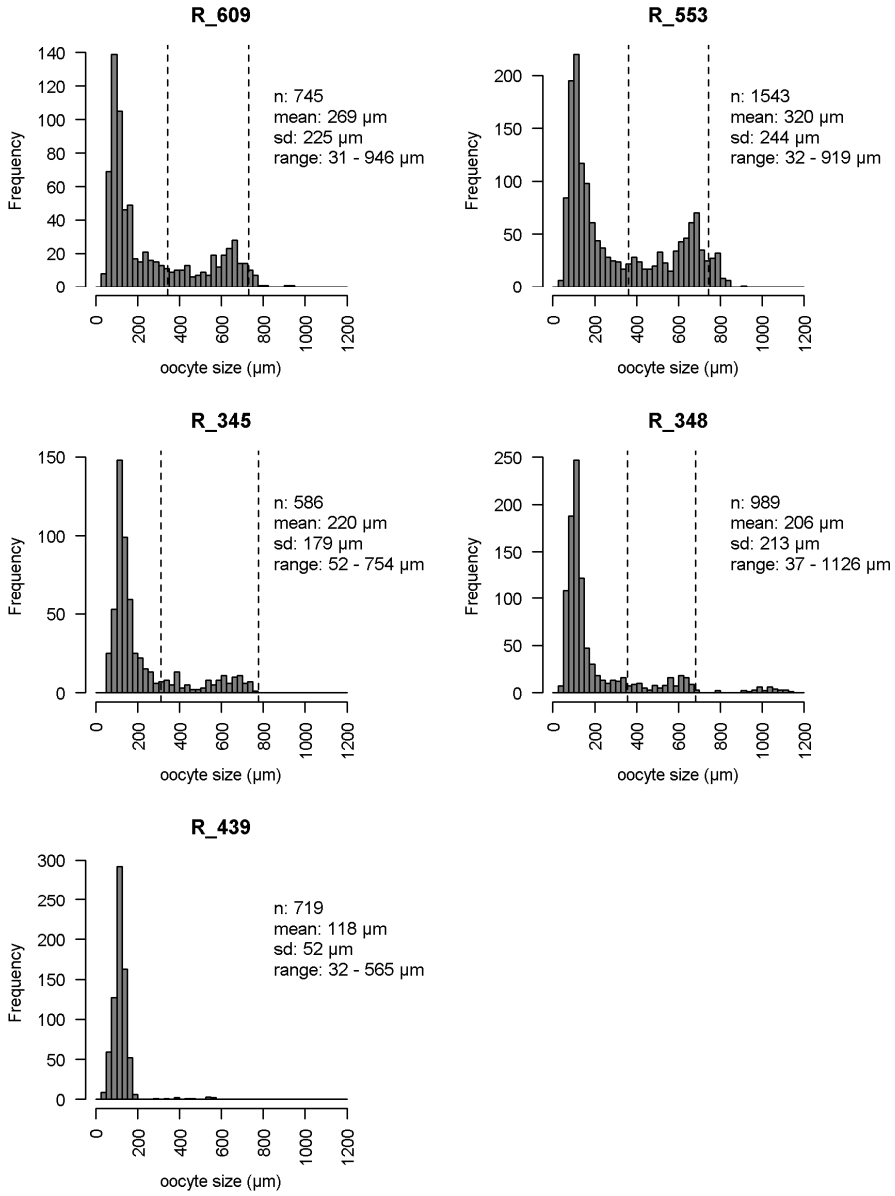

Figure 2. Oocyte size frequencies in the five meagre ovaries analyzed. Dashed lines indicate the range of secondary growth oocytes (SG-a and SG-b) measured in each ovary histological section. No secondary growth oocytes were observed in the histological slides of ovary R_439. Bins: 20 µm

Table 3. Mean diameters of vitellogenic oocytes SG-a and SG-b. Measurements taken from histological sections (µm)

Fish ID	Month/Day	Mean size SG-a ±2*s.e.	Mean size SG-b ±2*s.e.	Proportion of vitellogenic oocytes
R609	03/28	378±26 (n=4)	603±30 (n=14)	28%
R553	05/15	418±32 (n=8)	628±26 (n=14)	35%
R345	05/31	372±28 (n=11)	621±28 (n=27)	20%
R348	05/31	414±24 (n=12)	603±24 (n=16)	13%
R439	08/30	None observed	None observed	1%

According to currently accepted definitions of ovarian development organization and spawning types (Murua and Saborido-Rey 2003), the present results indicate that the meagre ovary has an asynchronous development pattern. This is revealed by the diversity of oocyte stages found in the histological slides during the spawning period which include all oocyte stages and substages from primary growth to oocyte maturation (Figure 1B, Table 2). Furthermore, the meagre should also be considered a batch spawner since vitellogenic oocytes do not undergo final maturation all at the same time but rather do it in batches, one batch per spawning event. The latter is evidenced by the signs of previous spawning events found in actively spawning females, namely the presence of post-ovulatory follicle complexes (POCs) in hydrated ovaries (Table I), and in the discrete mode (and concomitant hiatus) that is present when oocytes undergo final maturation (Figure 2).

Regarding the type of fecundity, the present results suggest that meagre has indeterminate fecundity. It would be thus impossible at the onset of the spawning season to identify and quantify the group of oocytes that will undergo final maturation, i.e., potential annual fecundity cannot be considered fixed before the onset of spawning because *de novo* vitellogenesis may still take place (Hunter et al. 1992, Murua et al. 2003). Our sample size is small but this categorization finds evident support in our data as they conform three out of four lines of evidence currently accepted for indeterminate fecundity (Hunter et al. 1992, Murua and Saborido-Rey 2003), namely a) the absence of a discrete hiatus between vitellogenic and non-vitellogenic oocytes at the start of the spawning season (Figure 2), b) the sustained size of vitellogenic oocytes throughout the season (Table 3) and c) the increase in prevalence of vitellogenic atresia at the end of season as the remaining yolk oocytes are resorbed (illustrated in Table 2 and further confirmed in Chapter 5B). In additional support of these lines of evidence we add that we have never observed mass atresia of PG-2 or SG-a oocytes in a wider array of fish examined (Chapter 5B) as would be expected from the resorption of intermediately sized oocytes. However, our meagre data does meet at least one line of evidence commonly accepted as indicative of determinate fecundity, namely the progressive reduction in the proportion of vitellogenic oocytes (Hunter and Macewicz 1992). This reduction is observed in Figure 2 and along side observations of reductions in the density of healthy vitellogenic oocytes suggests that *de novo* vitellogenesis may reduce significantly (or even stop) in mid-season. We have not examined females collected in June or July so we cannot confirm the eventual formation of a within-season hiatus. Such hiatus was reported by Grau et al. (2009) in their analysis of histological size frequencies in brown meagre *Sciaena umbra* and may be a reflection of the continuum that effectively exists between indeterminate fecundity and determinate fecundity (Ganias 2013). However, we stress that both PG2-c and SG-a are present in histological slides of meagre way into its spawning season so it is likely that *de novo* vitellogenesis continues and that fecundity should indeed be considered indeterminate for most practical purposes.

Our conclusions on the indeterminate fecundity of meagre are in line with those obtained by Duncan et al. (2012) and Abou Shabana (2012) that also observed a continuous size

distribution with two main modal classes in the ovaries of meagre. To our knowledge no histological work has been performed in South African *Argyrosomus* species but indeterminate fecundity was also reported by Farmer (2008) for *Argyrosomus japonicus* in Western Australia. These results are however substantially different from the ones reported by Gil et al. (2013) in *Argyrosomus regius* from the Gulf of Cadiz who concluded on determinate fecundity and estimated annual fecundity using gravimetric counts of total cortical alveoli and vitellogenic oocytes present in ripening/running females. The latter authors report oocyte size diameters with relatively low sample size (between 75 and 490 oocytes per ovary) obtained from histological counts of mature females and, most importantly, report not having used stereological correction factors in their counts because their aim was to "compare relative oocyte size-frequency distributions between individuals and not to quantify oocyte abundances". From a brief literature review we have found that whole-oocyte measurements or proper stereological methods have rarely been used in assessing oocyte size frequencies of sciaenid ovaries (but see Barbieri et al., 1994 and Wells and Jones 2002) and that authors frequently rely on transect counts of histological slides with their only concern being the measurement of diameters in cells sectioned through the nucleus. If one considers the large differences in nucleus size between pre-vitellogenic and vitellogenic oocytes (Chapter 5B), it is probable that under such methodological settings the larger oocytes have been disproportionally enumerated because larger cells with larger nucleus will be disproportionally represented in simple counts of histological slides, biasing down the estimates of smaller oocyte size classes (Peterson 1999; Murua et al., 2003). As an example, Kurita et al. (2003) compared regular profile counts with counts obtained using the more appropriate disector principle in an evaluation of the relative intensity of atresia in Atlantic herring. They found that because atresia reduces the cell size, underestimation occurs and a correction factor of 1.27 needs to be applied to relative intensity (i.e., proportion) of atretic stages estimated from profile counts.

In the meagre case, we were not able to compare whole oocyte size frequencies to histologically derived ones so we cannot be certain that stereological biases are the cause of the different perception on the existence of a hiatus in oocyte frequencies that we obtained. However, we note that Barbieri et al. (1994), Wells and Jones (2002) and Duncan et al. (2012) all have used whole oocyte measurements and their oocyte size frequency graphs display continuous patterns with all stages and size classes represented. In the medical world, the use of quantitative histology involving proper stereological methods or the disector principle has been subject of great endorsement given the consequences associated to biased estimates of, e.g., malignant cells in biopsies (Mayhew and Gundersen 1996, Peterson 1999). Similar calls for more rigorous methodologies have been made in fisheries science (Andersen 2003, Kurita et al. 2003) suggesting that oocyte size frequency results previously obtained from profile counts of histological slides should be interpreted with caution.

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Chapter 5D

Meagre *Argyrosomus regius* (Osteichthyes) as host of a gonad-infecting species of *Philometra* (Nematoda: Philometridae) off the Atlantic coast of Portugal

František Moravec[a], Nuno Prista[b,c], Maria José Costa[b,c]

[a]Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 37005 České Budějovice, Czech Republic
[b]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

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Meagre *Argyrosomus regius* (Osteichthyes) as host of a gonad-infecting species of *Philometra* (Nematoda: Philometridae) off the Atlantic coast of Portugal

Abstract: Subgravid females (up to 439 mm long) of the nematode *Philometra* sp. were found in meagre *Argyrosomus regius* (Asso, 1801) (Sciaenidae: Perciformes) off the southern Atlantic coast of Portugal in 2006. The general morphology of these nematodes somewhat resembles that of *Philometra lateolabracis* (Yamaguti, 1935), but the gravid females of the species from *A. regius* are apparently much longer. This is the first documented record of a gonad-infecting species of *Philometra* in marine fishes off the Atlantic coast of Europe. The possible importance of the gonad-parasitizing *Philometra* spp. as pathogens of marine fishes is stressed.

Keywords: life history, fishery-dependent sampling, market sampling, *Argyrosomus regius*

1. Introduction

Species of the nematode genus *Philometra* Costa, 1845 parasitic in the gonads of numerous marine fishes are widely distributed mainly in the tropical and subtropical regions of the Atlantic, Indian and Pacific Oceans (Moravec 2006). The ovoviviparous females are large-sized, with a body length from a few centimetres up to about 1 m in different species, whereas the conspecific males are generally of much smaller size, usually from 2 to 4 mm long. To date, 17 nominal species of the gonad-infecting *Philometra* spp. are known from marine fishes (Moravec 2006, Moravec *et al.* 2006a, Moravec & Salgado-Maldonado 2007) but the males remain unknown for many *Philometra* species, which makes the identification of these nematodes difficult. In Europe, the gonad-infecting *Philometra* spp. have been reported from marine, mainly perciform fishes in the Mediterranean region and the Black Sea (e.g. Rudolphi 1819, Willemoes-Suhm 1871, Stossich 1896, Janiszewska 1949, López- Neyra 1951, Kovaleva & Khromova 1967, Petter & Radujkovic 1986, 1989, Moravec *et al.* 2003, 2006b, Merella *et al.* 2004, Moravec & Genc 2004, Moravec 2006). However, to date *Philometra* have remained unreported in marine fish caught off the European Atlantic coast.

Meagre *Argyrosomus regius* (Asso, 1801) (Sciaenidae: Perciformes) is a large marine and brackish water fish (attaining over 180 cm and 50 kg total weight) whose distribution extends from Iceland to the Gulf of Guinea (including the Mediterranean and Black Seas) being regularly present between France and Senegal (and in the eastern Mediterranean) (Quéméner 2002). The fish supports minor (although lucrative) local fisheries, both recreational and commercial, throughout the European coasts and has become increasingly important to European aquaculture in recent years (Quéméner 2002, Costa *et al.* 2006). The species reproduction has so far only been thoroughly studied in Mauritania (Tixerant 1974) and Morocco (Hermas 1995) with only minor descriptions being made of the parasites found on *A. regius* gonads.

2. Materials and methods

Recent parasitological examinations of wild *Argyrosomus regius* captured off the Portuguese coast in 2006 revealed the presence of female *Philometra* specimens. The fish specimens, 1 male and 1 female, were mature individuals captured in March and June in Vila Real de Santo António and Olhão (male: total length = 144 cm, total weight = 19.0 kg; female: total length = 158 cm, total weight = 33.0 kg). The parasite specimens were collected from the fish gonads after being macroscopically detected from the exterior and were fixed and preserved in 96% ethanol. During this study, most of the specimens obtained were body fragments of subgravid *Philometra*; however, 2 complete specimens were also recovered. The specimens have been deposited in the Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic (Cat. No. N-70).

3. Results and discussion

The bodies of the 2 fixed, complete subgravid nematode females (Figures 1 and 2) are brown, 180 and 439 mm long and 585 to 843 µm maximally wide, respectively, somewhat tapering towards both ends; the posterior part of the body is distinctly narrower than the anterior part. The ratio of the maximum body width to the body length is 1:521−585. The cuticle is smooth. The cephalic end is rounded, 135 to 150 µm wide; cephalic papillae are very small and indistinct when viewed laterally under the light microscope. Scanning electron microscopy revealed the presence of 4 submedian pairs of minute papillae of the external circle and 1 pair of minute lateral papillae of the internal circle (Figure 1D), encircling the circular oral aperture; the mouth bottom is formed by the flat surfaces of the 3 oesophageal sectors. The oesophagus is narrow, somewhat swollen near the mouth to form a distinct inflation 63 µm long and 75 to 78 µm wide, which is not separated from the posterior cylindrical part of the oesophagus. The overall length of the oesophagus is 2.09 to 2.52 mm, representing 0.6 to 1.2% of the body length; the dorsal oesophageal gland extends anteriorly to the level of the nerve ring and posteriorly to the small ventriculus, which measures 30 × 81 µm in the larger specimen. The nerve ring is 340 to 394 µm from the anterior body end. The intestine is dark brown; its posterior end is atrophied, forming a ligament attached ventrally to the body wall near the posterior extremity (Figure 1G). The posterior end of the body is rounded, 109 to 136 µm wide, with 2 outlined lateral minute papilla-like caudal protrusions, found only in the larger specimen. The vagina and vulva are absent. The 2 ovaries are rather long and thin and are situated near the anterior and posterior ends of the body. The uterus occupies most of the body and is filled with eggs and developing embryos.

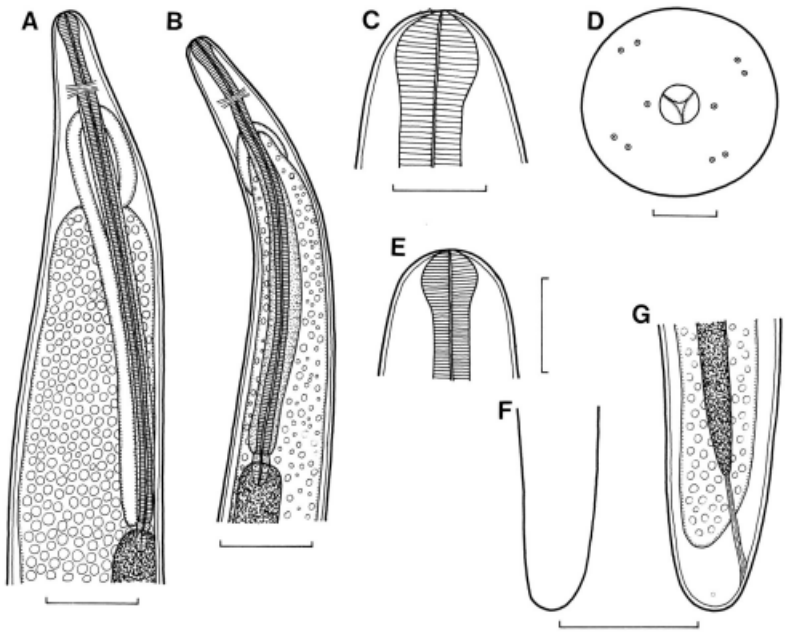

Figure 1. *Philometra* sp. from gonads of *Argyrosomus regius*, subgravid female. Anterior end of (A) larger (439 mm long) and (B) smaller (180 mm long) specimens. Cephalic end of (C) larger and (E) smaller specimens, lateral views. (D) Cephalic end, apical view (reconstructed from SEM micrograph). (F) Outline of caudal end of smaller specimen, lateral view. (G) Caudal end of larger specimen, lateral view. Scale bars: (A,B) = 500 µm, (C,E) = 100 µm, (D) = 30 µm, (F,G) = 300 µm.

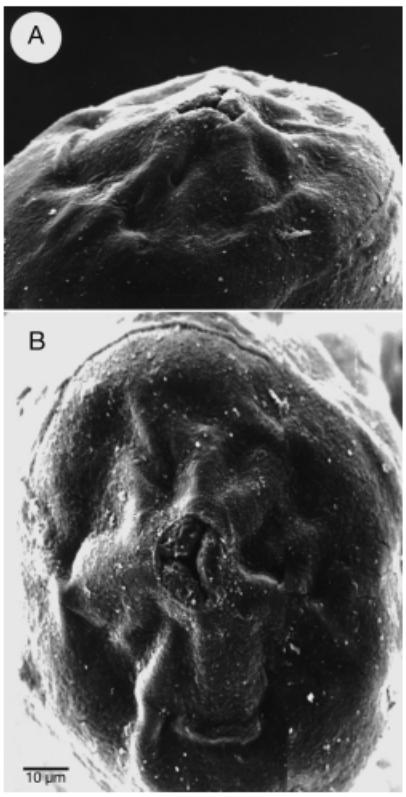

Figure 2. *Philometra* sp. from gonads of *Argyrosomus regius*. SEM micrographs of cephalic end of subgravid female. (A) Dorsal and (B) apical views.

The general female morphology of these nematodes is similar to that of *Philometra lateolabracis* (Yamaguti, 1935), a widespread, gonad-infecting parasite of many species of marine fishes, reported from different parts of the world. However, the maximum body length of the gravid (larvigerous) female of *P. lateolabracis* is only 230 mm (Moravec 2006), whereas one of the subgravid (ovigerous) females in the present study is nearly double that length (439 mm). Accordingly, conspecific gravid females with larvae can be expected to be even longer. Since the conspecific males are not yet known, the exact species identification of these Portuguese nematodes will only be possible when new material from this fish species, including better preserved specimens, both males and gravid females, is collected.

The *Philometra* specimens now collected from *Argyrosomus regius* represent the first detailed record of this nematode presence in perciforms from the North-Eastern Atlantic. Santos (1996) reports on 'a viviparous nematode 8 cm long' (probably a *Philometra* female) found in the body cavity of the European seabass, *Dicentrarchus labrax* (Linnaeus) (Moronidae) off the Portuguese coast. However, its location in the host suggests that it belonged to a different species than nematodes found in the present study (Moravec 2006). 'Very long nematodes' parasitic of *A. regius* have been reported in the gas bladder, stomach-walls and ovaries of fish caught off Mauritania (Tixerant 1974) but no such evidence was observed in the current specimens. On the other hand, Hermas (1995) presents a photograph a photograph of a similarly 'long nematode' found in male and female *A. regius* gonads off Agadir on the Moroccan Atlantic coast. Her work does not, however, provide detailed description of such specimens, so their taxonomic identification to family or genus is impossible. However, *Philometra* has been recorded in gonads of wild *A. japonicus* captured off the Western Australian coast where its prevalence was over 50% in mature specimens of both sexes (Farmer 2003). In the present work histological analysis of parasite gonads was not performed, so effective parasite damage to fish gonads could not be assessed. However, severe infections by these pathogenic parasites sometimes cause serious damage to the fish ovaries and thus may affect reproductive output at individual and population levels (Hine & Anderson 1982, Sakaguchi *et al.* 1987, Moravec *et al.* 2003, Clarke *et al.* 2006).

The exact identification of the *Philometra* species, its prevalence and eventual deleterious effects on *Argyrosomus regius* reproduction remain unknown. Given that (1) species of *Philometra* may prove to be significant pathogens in fish cultures, (2) *A. regius* is increasingly popular in Southern European aquaculture, and (3) mature *A. regius* female gonads are frequently used for human consumption throughout its distribution range, the authors suggest that a further, more detailed taxonomic and ecological study of this nematode parasite should be attempted in the near future.

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Chapter 6

Genetics

Chapter 6

Very high genetic fragmentation in a large marine fish, the meagre *Argyrosomus regius* (Sciaenidae, Perciformes): Impact of reproductive migration, oceanographic barriers and ecological factors.

Pierrick Haffray[a], Rachid Malha[b], Mahfoud Ould Taleb Sidi[c], Nuno Prista[d], Moshira Hassan[e], Gérard Castelnaud[f], Bilge Karahan-Nomm[g], Kutsal Gamsiz[g], Sherif Sadek[h], Jean-Sébastien Bruant[i], Philippe Balma[j], François Bonhomme[k]

[a]SYSAAF, Station SCRIBE/INRA, Campus de Beaulieu, 35042 Rennes, France.
[b]LABOGENA, Domaine de Vilvert, 78350, Jouy-en-Josas, France.
[c]IMROP, P.B. 22, Nouadhibou, Mauritania.
[d]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
[e]Reef Check, Wachmannstr. 25, 28209 Bremen, Germany
[f]CEMAGREF, 50 Av. de Verdun, 33612 Cestas, France.
[g]Ege University, Fisheries Faculty, Aquaculture Department, 35100 Izmir, Turkey
[h]Aquaculture Consultant Office (ACO), 12 B (House n° 9), Street n° 256, 11435 New Maadi, Cairo, Egypt
[i]FMD, BP 4, 17840 La Brée les Bains, France.
[j]Les Poissons du Soleil, BP 10, 34540 Balaruc-les-Bains, France.
[k]CNRS UMR 5554, Institut des Sciences de l'Evolution, Université de Montpellier 2, 2, rue des chantiers, 34200, Sète, France.

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Very high genetic fragmentation in a large marine fish, the meagre *Argyrosomus regius* (Sciaenidae, Perciformes): Impact of reproductive migration, oceanographic barriers and ecological factors

Abstract: The meagre *Argyrosomus regius* is a large sciaenid fish known to reproduce in the eastern Atlantic and Mediterranean Sea in just five distinct and restricted geographic areas: along the Mauritanian coast and at estuary openings (Gironde, Tagus, Guadalquivir and Nile). The biological traits of *A. regius* (high dispersal capabilities, high fecundity, long larval phase, overlapping generations, reproduction until 40 years of age) are, in principle, favourable to high gene flow, which should lead to genetic homogeneity over large geographic scales. Nevertheless, the high geographic distances between the few reproductive areas leads one ask whether there is genetic differentiation in this species. In the present study, the genetic differentiation of the wild *A. regius* was investigated across most of its natural range from the Atlantic Ocean (France, Portugal, Spain, Mauritania) to the Mediterranean Sea (Egypt, Turkey), using 11 microsatellite markers previously identified in another Sciaenid, the red drum *Sciaenops ocellatus*. At least two very distinct groups could be identified, separated by the Gibraltar Strait. Genetic divergences (F_{ST} values) were intermediate between the Atlantic samples (0.012–0.041), high between Egypt and the Atlantic (0.06–0.107) or Aegean Sea (0.081) and extremely high between the Aegean Sea and the Atlantic (0.098–0.168). *A. regius* exhibited a very high level of genetic differentiation rarely reported in marine fishes. These results also demonstrate the existence of a sixth independent reproduction area in the Aegean Sea. Factors potentially involved in this very high genetic fragmentation are discussed, including physical barriers, glaciation pulses and biological traits.

Keywords: *Argyrosomus*, Sciaenidae, genetic variation, genetic fragmentation, microsatellites, *Umbrina*, *Pseudotolithus*

1. Introduction

Marine fishes generally exhibit limited genetic differentiation across large geographic distances (> 1000 km). This pattern has been attributed to their high rates of dispersal and movement during both nektonic and planktonic phases (Gyllensten, 1985; Ward *et al.*, 1994). The level of within-species differentiation also depends on physical or biological barriers such as hydrology, oceanic fronts, geomorphology, historical sea-level variation and animal behaviour, which interact with complex species-specific life history traits. Therefore, within-species differentiation is difficult to predict even if such pattern is known for other closely related conspecific or confamilial species (Naciri *et al.*, 1999; Patarnello *et al.*, 2007).

The meagre *Argyrosomus regius* (Asso, 1801) is one of the world's largest Sciaenids, attaining over 1.80 m length and 50 kg body weight (FishBase, 2010). This coastal semi-pelagic species is distributed in the eastern Atlantic Ocean, from the Bay of Biscay to the coast of Senegal, and across the Mediterranean Sea, Black Sea and Gulf of Suez. Planktonic eggs (990 µm diameter) are spawned in open water and hatched within 48 h. Mouth opening is observed 2-3 days post hatch and yolk sac absorption within 7 days post hatch (Tixerant, 1974). Planktonic larvae develop in shallow lagoons and over mudflats when the temperature exceeds 20 °C (Quéro and Wayne, 1987). Juveniles migrate and spread toward deeper waters in their second year (60-200 m, 12 °C). *A. regius* reproductive biology combines several specific

reproductive traits (Tixerant, 1974; Quéro and Vayne, 1987; Hermas, 1995; Prista *et al.*, 2009). Adults migrate to coastal reproductive areas (10-15 m deep with high water flow associated with estuaries and/or tides) to spawn when temperatures reach 13 to 23 °C. The species exhibits also very high fecundity, late first reproduction (7 years old), long generation interval (> 40 years), overlapping generations, aggregation and schooling migration in nearshore waters. More over, only five restricted coastal spawning areas have been documented to date in the Lévrier Bay and the Banc d'Arguin (Mauritania) and at the opening of the Gironde (France), the Tagus (Portugal), the Guadalquivir (Spain) and the Nile (Egypt) estuaries (Tixerant, 1974; El-Hehyawi, 1974; Costa, 1986; Quéro and Vayne, 1987; Quéro, 1989a and b; González-Quirós *et al.*, 2011). Most of the abovementioned reproductive traits favour low or absent genetic differentiation, while the high geographic distances between the only five reproduction areas would act in the opposite direction. However, no genetic information has been reported to date for *A. regius*.

The objective of this study was thus to characterise the genetic variability of *A. regius* across its native range, using microsatellite nuclear markers previously isolated from another Sciaenid, the red drum *Sciaenops ocellatus* (Renshaw *et al.*, 2006). Two further Sciaenid species that live sympatrically with *A. regius* were also genotyped with the same markers to assess potential misidentification: the shi drum *Umbrina cirrosa* (Linnaeus, 1758) sympatric in the Mediterranean Sea and the law croaker *Pseudotolithus senegallus* (Cuvier, 1830) sympatric along the west coast of Africa. The results are expected to provide useful information for the preservation of wild stocks, improvements in fishery management and initiation of breeding programs for *A. regius* aquaculture.

2. Materials and methods

2.1. Sample collection

Fin clips from 378 wild *A. regius* were collected from twelve different locations in the Atlantic Ocean and eastern Mediterranean Sea (Table 1 and Figure 1) by experimental fishery (in F1 and F2) or obtained from catches landed by local fishermen (in P1, P2 and M1 to M4) or from fish farms rearing wild fish (S1, E1, E2, T1). Further information is given per site of collection:

1) Sites F1 and F2 (France): Fish from two year classes (2 years of age for F1 and 3 years of age for F2) were captured in 2008 in the Gironde estuary at Mortagne (F1) and at St Seurin d'Uzet (F2) using the CEMAGREF research vessel "L'Esturial" equipped with a bottom trawl (mesh size: 40 mm).
2) Site S1 (Spain): Wild fish from 4 years of age were collected in 2008 in the FMD hatchery (Oléron Island, France). These fish were captured at 5-10g in the Guadalquivir estuary in the summer 2005 by PIMSA fish farm (Seville, Spain).
3) P1 and P2 (Portugal): Fish came from two commercial landing areas located along the western (Tagus estuary and Peniche) and southern coasts (Quarteira to Vila Real de

Santo António) of Portugal. Samples from both sites included juveniles and adults collected from 2005 to 2007.

4) Sites M1 to M4 (Mauritania): Fins were collected in 2008 at commercial landing sites located at Nouakchott and Nouadhibou fishing harbours and in Nouamghar and Arkais Imragen villages (Parc National du Banc d'Arguin). The samples consisted of adult fish except in M4.
5) Sites E1 and E2 (Egypt): The samples were collected in 2009 from two fish farms located along the Manzalla Lake, close to Port Said. The fish consisted of wild juveniles captured as described by Sadek *et al.* (2009) between February and March 2008 along the Mediterranean coast between Alexandria and Port Said.
6) Site T1 (Turkey): Fins were collected in 2009 from wild fish reared in captivity in the Egemar Su Ürünleri A.Ş. fish farm (Akbük-Didim, Aydın, Turkey). Fish consisted of mature individuals grown in the fish farm since their capture at 5-10 g in the lagoons of the Menderes Delta, south of Izmir during July and August 2000.

The shi drum samples were collected in Turkey (n = 31) in the same geographical area as the *A. regius* samples. The law croaker samples were collected in Mauritania in Nouadhibou (n = 14) and in Arkais (n = 3), on the same days as the *A. regius* samples.

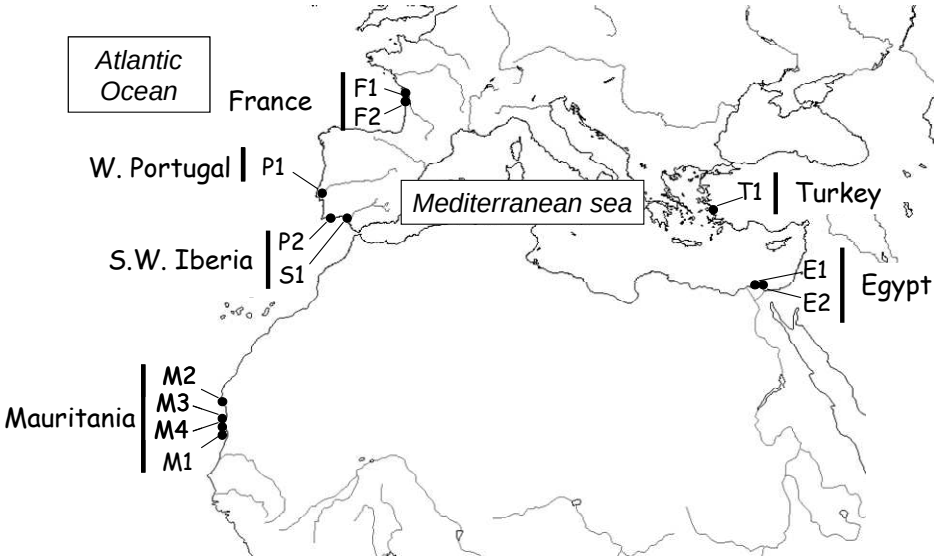

Figure 1. Geographic locations of the twelve collection sites (abbreviation: F1, F2, etc…) and populations (full name: France, etc…) of meagre *Argyrosomus regius* samples. Abbreviations, localities and coordinates are given in Table 1.

Table 1. Description of the twelve *Argyrosomus regius* collection sites by geographic origin, locality, coordinates, abbreviation, size, date of collection, mean standard body length (± SD), known or estimated (in bracket) age according to Hermas (1995) and Prista *et al.* (2009), and origin (E = experimental fishery; F = fisheries landings; A = aquaculture of wild fish).

Geographic origin	Locality	Coordinates	Abbreviation	n	Date of collection	Standard body length (cm)	Age (years)	Origin
Atlantic Ocean	Gironde estuary, Mortagne, France	45°27' N and 52°34' W	France-1 (F1)	35	25/06/2008	19.8 cm ± 2.2	2	E
	Gironde estuary, St Seurin sur Dizet, France	45°29' N and 55°23' W	France-2 (F2)	37	26/06/2008	35.6 cm ± 1.2	3	E
	Tagus estuary and Peniche, Portugal	39°21' N and 9°22' W	Portugal-1 (P1)	37	09/2005 to 05/2007	83.3 cm ± 30.9	2-12	F
	South coast (Algarve), Portugal	37°11'N and 7°24' W	Portugal-2 (P2)	30	08/2005 to 01/2007	84.9 cm ± 40.5	2-23	F
	Guadalquivir estuary, Spain	36°57' N and 6°14' W	Guadalquivir (S1)	30	09/02/2009	Data not available	4	A
	Nouakchott, Mauritania	18°06' N and 16°01' W	Mauritania-1 (M1)	12	19/05/2008	142 cm ± 7.9	(> 15)	F
	Nouadibou, Mauritania	20°54' N and 17°02' W	Mauritania-2 (M2)	9	21/05/2008	138.8 cm ± 13.1	(> 14)	F
	Nouamghar, Mauritania	19°21' N and 16°30' W	Mauritania-3 (M3)	12	22/05/2008	144.8 cm ± 12.8	(> 14)	F
	Arkais, Mauritania	20°07' N and 16°15' W	Mauritania-4 (M4)	29	23/05/2008	82.6 cm ± 14.8	(> 6)	F
Eastern Mediterranean	Port Said (Farm 1), Egypt	31.21' N and 32°02' E	Egypt-1 (E1)	30	12/10/2009	28.5 cm ± 2.9	0	A
Sea	Port Said (Farm 2), Egypt	31.21' N and 32°02' E	Egypt-2 (E2)	30	12/10/2009	33.7 cm ± 2.8	0	A
	Menderes Delta, Turkey	37.32' N and 27°10' E	Turkey (T1)	30	07/03/2009	115 cm ± 10	10	A

2.2. PCR amplification and microsatellite typing

All samples were stored in 95 % alcohol and genotyped using 14 red drum microsatellites (Renshaw *et al.*, 2006) combined to make two new panels: Soc11, Soc140, Soc400, Soc416, Soc423, Soc428, Soc592, Soc593 in panel 1 and Soc35, Soc44, Soc156, Soc410, Soc412, Soc432 in panel 2. PCR amplifications were performed in a final volume of 10 µl using a Qiagen® Multiplex PCR Kit and 50-100 ng of template DNA. Reactions were run for 30 cycles in an MJ thermal cycler (Model PTC-200). The PCR amplifications included an initial activation step at 95 °C for 15 minutes, denaturation at 94 °C for 30 seconds, primer annealing at 60 °C for 90 seconds, extension at 72 °C for 1 minute and final extension at 60 °C for 30 minutes. After PCR amplification, an Applied Biosystems 3730xl DNA Analyser with GeneMapper Analysis software (Applied Biosystems) was used to analyse the fluorescently tagged fragments for length polymorphisms.

2.3. Data analysis

2.3.1. Genetic variability and departure from Hardy-Weinberg equilibrium

The mean number of alleles per locus (N*A*) and the observed (H_{obs}) and unbiased expected (H_{exp}) heterozygosity (Nei, 1978) were computed for each collection site and locus using Genetix 4.05.2 (Belkhir *et al.*, 2004). The departure of genotypic frequencies from the expectations of Hardy-Weinberg equilibrium (HWE) was estimated within each site by the inbreeding coefficient or Wright's fixation index (F_{IS}) using Weir and Cockerham's (1984) *f*-estimator. The significance of the F_{IS} greater than zero (i.e. consistency with the null hypothesis on HWE) was estimated after 10000 random allelic permutations and using simple Bonferroni procedure (Rice, 1989) to correct for multiple testing and avoid type-1 errors (Rice, 1989). In *A. regius*, the presence of null alleles or other scoring errors were estimated for all loci and collection sites using the MICRO-CHECKER program version 2.2.3. (Van Oosterhout *et al.*, 2004). The program uses the Monte Carlo simulation method to generate expected allele size difference frequencies and to compare the estimated null allele frequency using four different methods.

2.3.2. Genetic differentiation between populations and phylogenetic relationships

The differentiation between collection sites was estimated using Weir and Cockerham's (1984) global fixation index (F_{ST}) estimator. F_{ST} were computed between sites of collection using Genetix 4.05.2 (Belkhir *et al.*, 2004). Study-wide significance levels across collection sites were adjusted using 10000 permutations on individual genotypes for the simple Bonferroni procedure.

The twelve collection sites were grouped into six new collection sites on the basis of the lack of significant pair-wise F_{ST} values between some of them and to increase the number of fish per site to 50 as recommended by Ruzzante (1998). The Mauritanian sites were pooled and treated as one population because of the limited number of samples per collection site, their

close geographic proximity and their lack of genetic differentiation (though M4 was significantly different from M3 it had no transitivity with M2 and M1). The six new collection sites that were thus recognised were “Egypt” (E1+ E2), “France” (F1+F2), “Mauritania” (M1+M2+M3+M4), southwest Iberia (named “S.W. Iberia”; S1+P2), west Portugal (named “W. Portugal”; P1) and “Turkey” (T). These groupings were used in all further analyses and comparisons.

The same genetic estimators (H_e, H_{obs}, N*A*, number of alleles, number of fish genotyped, F_{IS}, F_{ST}) and statistical tests were then computed. Allelic richness (*AR*), representing a measure of the number of alleles independent of the sample size, was estimated using Fstat 2.9.3.2. (Goudet, 1995). Difference in mean allelic richness and heterozygosity among the 6 collection sites were estimated using Friedman non-parametric test. The phylogenetic tree was drawn in MEGA 4.0 (Tamura *et al.*, 2007) based on the $D_{Reynolds}$ genetic distances and using the Neighbour-Joining algorithm (Saitou and Nei, 1987).

Different methods exist to estimate effective population size (or N_e) based on heterozygote excess, temporal variation, linkage disequilibrium or the Bayesian method and can produce different results. As the estimation of N_e was not the main objective of the present work, estimation was only made based on non-random gametic linkage disequilibrium using LDN_e software (Waples and Do, 2008). Minimum allelic frequency was fixed at 0.05, as the less biased frequency reported and putative 95% confidence intervals calculated with parametric or jackknife methods.

3. Results

3.1. Genotyping of markers in each species

Pseudotolithus senegallus: Three of the 14 genetic markers did not amplify (Soc35, Soc416, Soc428) and 4 markers were monomorphic (Soc140, Soc156, Soc400, Soc592). The last 7 markers could be amplified and had a maximum of 4 alleles per marker (Soc11, Soc44, Soc410, Soc412, Soc423, Soc432, Soc593). Soc410 exhibited 4 alleles not observed in *A. regius*. Four of the seventeen fish sampled did not amplify at any locus. The F_{IS} was not estimated for this species due to the low number of alleles observed per fish.

Umbrina cirrosa: Three markers did not amplify (Soc423, Soc428 and Soc593) and 3 were monomorphic (Soc 44, Soc140, Soc400). Four markers exhibited more than 3 alleles (Soc416, Soc35, Soc156 and Soc432). A new allele not reported in *A. regius* was observed for each marker. The four last markers showed a good capacity for amplification and also 1 to 3 alleles not observed in *A. regius*: Soc11 (3 new alleles), Soc410 (2 new alleles), Soc412 (1 new allele) and Soc592 (3 new alleles). The F_{IS} values calculated for each marker indicated an excess of homozygotes for most of the markers (data not shown).

Argyrosomus regius: The marker Soc416 amplified badly and the markers Soc 400 and Soc410 were monomorphic. These markers were not used in the genetic analysis of *A. regius*. One specimen collected in Mauritania that identified as *Pseudotolithus senegallus* based on its genotype characteristics was discarded from the data analyses.

Overall, eleven of the fourteen red drum microsatellites used in this study proved useful for further genetic investigations in *A. regius*: Soc11, Soc140, Soc423, Soc428, Soc592, and Soc593 from panel 1; and Soc35, Soc44, Soc156, Soc412 and Soc432 from panel 2.

3.2. Genetic variability of *A. regius*

The number of alleles per locus varied from 3 to 31 (Table 2). Soc593 and Soc156 exhibited the lowest number of alleles (3 and 4 respectively), and Soc11, Soc35, Soc44, Soc412 and Soc428 the highest (14, 31, 17, 24, and 25, respectively).

The genetic characteristics (H_e, H_{obs}, N*A*, F_{IS}) and the number of fish genotyped in the twelve collection sites are presented in Table 3 (upper section). The mean number of alleles per site varied from 4.09 in T1 to 8.63 in M4, with a mean for all sites of 6.28 ± 1.15 (mean ± SD). H_{obs} within site for all markers varied from 0.47 ± 0.28 in T1 to 0.67 ± 0.19 in M3 and H_{exp} varied from 0.46 ± 0.24 in T1 to 0.65 ± 0.20 in M1.

Null alleles were only detected for the loci Soc44 in F1 and E1, Soc423 in T1 and E2 and Soc 412 in E1. Only the E1 ($p < 0.001$) and M4 ($p < 0.05$) collection sites showed departure of genotypic frequencies from the expectations of HWE equilibrium.

F_{ST} pair-wise comparisons revealed no significant differences between: F1 and F2, Mauritanian sites (except between M3 and M4), S1 and P2 or between E1 and E2 (Table 4).

Table 2. Mean number of alleles per microsatellite marker (± SD) scored in meagre *Argyrosomus regius*, law croaker *Pseudotolithus senegallus* and shi drum *Umbrina cirrosa* genotyped in this study, taking the red drum *Sciaenosp ocellatus* genotyped by Saillant *et al.* (2009) as a reference. * = marker not used for genotyping by Saillant *et al.*, (2009).

	Argyrosomus regius	*Pseudotolithus senegallus*	*Umbrina cirrosa*	*Sciaenops ocellatus*
Soc11	14	3	7	10
Soc35	31	-	5	*
Soc44	17	2	1	24
Soc140	6	1	1	4
Soc156	4	1	4	5
Soc412	24	3	5	25
Soc423	6	3	-	18
Soc428	25	-	-	28
Soc432	8	4	3	9
Soc592	7	1	7	*
Soc593	3	2	-	*
Number of fish sampled	361	17	31	45
Mean number of alleles per locus for all markers	13.2 ± 9.7	2.2 ± 1.1	4.1 ± 2.4	15.4 ± 9.6
Mean number of alleles per locus with only the Saillant *et al.* (2009) common markers	13.2 ± 9.7	2.4 ± 1.1	3.5 ± 2.3	15.4 ± 9.6

Table 3. Genetic variability and F_{IS} estimates at 11 microsatellites loci in twelve *Argyrosomus regius* collection sites (upper panel) or the same samples pooled into six populations (lower panel). N = number of individuals; N*A* = mean number of alleles per locus; *AR* = Allelic richness estimated for n = 27; H_{exp} = Nei's (1987) gene diversity (across-locus standard deviation in brackets); H_{obs} = observed heterozygosity; F_{IS} = heterozygote deficiency within collection site (upper part of the Table) or within population (lower part of the Table) for all loci or per locus. Significant levels after Bonferroni correction (Rice, 1989) are shown in bold: * $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$ F_{IS} values with null allele are underlined.

	N	N*A*	ArR	He	Ho	Multilocus F_{IS}	P-value	Soc11	Soc35	Soc44	Soc140	Soc156	Soc412	Soc423	Soc428	Soc432	Soc592	Soc593
						All sites												
Egyp-t1 (E1)	30	5,81		0.54 (0.23)	0.49 (0.22)	0,114	**<0.001**	- 0.035	0.096	**0.444*** **	**0.275***	- 0.074	**0.191***	0.069	- 0.050	0.117	0.116	- 0.094
Egypt-2 (E2)	30	5.72		0.54 (0.25)	0.53 (0.27)	0,032	0.147	0.104	- 0.078	- 0.081	0.073	0.151	0.084	**0.335****	**0.106***	- 0.167	- 0.031	**- 0.035***
Spain (S1)	28	7.72		0.61 (0.20)	0.61 (0.23)	0,005	0.448	0.118	- 0.087	- 0.026	0.112	- 0.058	- 0.024	- 0.059	- 0.119	- 0.070	**0.176***	0.142
France-1 (F1)	35	6.27		0.64 (0.16)	0.61 (0.22)	0.041	0.095	- 0.026	- 0.111	**0.299****	0.004	**0.413***	- 0.031	- 0.185	- 0.011	0.072	**0.283****	- 0.084
France-2 (F2)	37	6.45		0.63 (0.18)	0.66 (0.21)	- 0.031	0.863	- 0.072	**0.114***	- 0.172	- 0.083	- 0.102	- 0.075	0.004	- 0.100	0.040	- 0.040	**0.254***
Mauritania-1 (M1)	12	6.72		0.65 (0.20)	0.66 (0.21)	- 0.052	0.865	- 0.038	- 0.038	- 0.052	**0.262***	- 0.100*	- 0.094	- 0.308	- 0.051	0.009	- 0.213	0.009
Mauritania-2 (M2)	9	6.36		0.63 (0.19)	0.61 (0.22)	0.058	0.141	0.138	0.096	0.130	0.158	- 0.067	- 0.142	0.094	**0.085***	- 0.191	- 0.032	**0.400***
Mauritania-3 (M3)	12	5.63		0.62 (0.19)	0.67 (0.19)	- 0.041	0.761	- 0.151	0.034	0.2090	- 0.054	- 0.158	- 0.234	0.172	- 0.073	0.074	- 0.222	- 0.200
Mauritania-4 (M4)	29	8.63		0.63 (0.21)	0.61 (0.20)	0.051	**0.037**	0.094	0.067	0.000	0.029	- 0.120	**0.119***	- 0.206	**0.118***	0.075	- 0.006	**0.263***
Portugal-1 (P1)	28	5.36		0.60 (0.19)	0.59 (0.21)	0.031	0.189	**0.197****	0.018	0.087	- 0.103	**0.286***	**0.160***	- 0.175	- 0.085	0.033	- 0.046	0.044
Portugal-2 (P2)	25	6.63		0.59 (0.21)	0.61 (0.21)	- 0.008	0.571	**0.226****	0.033	0.058	- 0.163	- 0.095	- 0.065	- 0.155	0.029	- 0.061	0.010	- 0.116
Turkey (T1)	27	4.09		0.46 (0.24)	0.47 (0.28)	0.003	0.464	**0.198***	0.070	0.118	- 0.040	- 0.106	- 0.027	**0.546*****	- 0.206	- 0.097	- 0.138	- 0.083
						Pooled sites												
Egypt (E1 + E2)	60	6.45	5.69	0.55 (0.24)	0.51 (0.24)	0.073	**0.002****	0.033	0.021	**0.185****	**0.174***	0.059	**0.135***	**0.195****	0.033	- 0.027	0.039	- 0.067
S.W. Iberia (P2 + S1)	53	7.72	7.17	0.61 (0.20)	0.61 (0.23)	0.003	0.455	**0.177***	- 0.033	0.020	- 0.021	- 0.084	- 0.041	- 0.102	- 0.052	- 0.055	0.102	0.091
France (F1 + F2)	72	7.27	6.13	0.64 (0.17)	0.64 (0.20)	0.006	0.399	- 0.047	0.003	0.045	- 0.036	**0.159***	- 0.043	- 0.087	- 0.056	0.051	**0.141***	0.082
Mauritania (M 1 to M4)	62	11.00	8.72	0.65 (0.20)	0.64 (0.19)	0.022	0.131	0.068	0.064	0.058	0.071	- 0.140	- 0.038	- 0.099	0.038	0.016	- 0.028	0.140
W. Portugal (P1)	28	5.36	5.33	0.60 (0.19)	0.59 (0.21)	0.032	0.196	**0.197****	0.018	0.087	- 0.103	**0.286***	**0.160***	- 0.175	- 0.085	0.033	- 0.046	0.044
Turkey (T1)	27	4.09	4.09	0.46 (0.24)	0.47 (0.28)	0.003	0.464	**0.198***	0.070	0.118	- 0.040	- 0.106	- 0.027	**0.546*****	- 0.206	- 0.097	- 0.138	- 0.083

The genetic characteristics (H_e, H_{obs}, N*A*, *AR* and F_{IS}) and the number of fish genotyped in the six collection sites are presented in Table 3 (lower section). The mean number of alleles per site varied from 4.09 in "Turkey" to 11.00 in "Mauritania", with a mean for all sites of 6.98 ± 2.37 (mean ± SD). The allelic richness differed significantly between sites (P < 0.001) and varied from 4.09 to 8.72. It was highest for the sites in "Mauritania" and "S.W. Iberia", intermediate in "France" and "W. Portugal" and the lowest in "Egypt" and "Turkey". H_e differed significantly between sites (P > 0.002), and within-site H_{obs} for all markers varied from 0.47 ± 0.28 in T1 to 0.64 ± 0.20 in G, and H_{exp} varied from 0.46 ± 0.24 in T1 to 0.65 ± 0.20 in M.

Heterozygote or homozygote excesses are given in Table 3 (lower section). When all markers were considered, only the Egyptian site showed a significant heterozygote deficiency. Null alleles were detected for Soc423 in Egypt and Turkey, for Soc11 in S.W Iberia and for Soc44 in Egypt.

Highly significant F_{ST} differentiations (P < 0.002) were observed between all 6 collection sites (Table 5, above the diagonal). The lowest F_{ST} values were found between the Atlantic samples (values from 0.012 < F_{ST} < 0.041). Differentiation was more moderate between the "Egypt" sample and all other populations (0.061 < F_{ST} < 0.107). The highest F_{ST} values were found between "Turkey" and all other sites (0.081 < F_{ST} < 0.168), the highest of these being observed between "Turkey" and "W. Portugal". Within the Mediterranean, despite a relative geographical proximity, high genetic differentiation was also observed between the two samples from "Egypt" and "Turkey" (0.081). The phylogenetic tree (Figure 2) illustrates the subdivision of *A. regius* populations into two distinct groups: Atlantic and Mediterranean.

Estimates of effective population size (N_e) are given in Table 6. Upper N_e values could not be differentiated from infinity (under the 95 % confidence interval) for the populations from France, W. Portugal and S.W. Iberia. The population from Mauritania showed a more limited N_e value of 111.0 (61.6 < N_e < 330.3). Only the population from Turkey presented a very low N_e of 17.4 (10.3 < N_e < and 33.6).

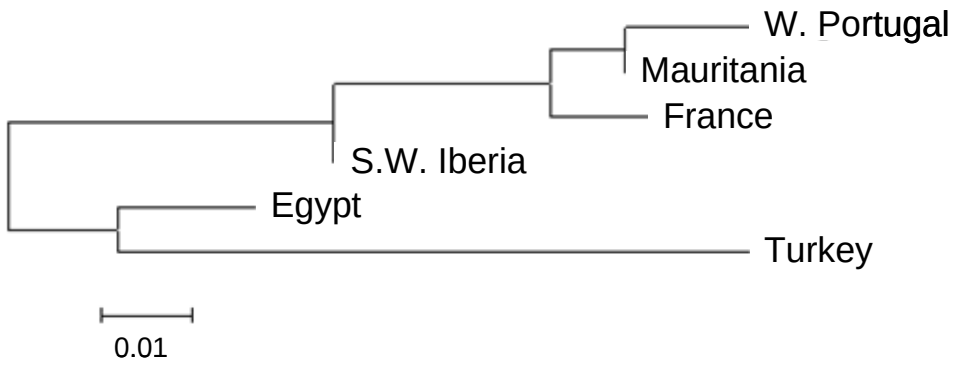

Figure 2. Evolutionary relationships of the six *Argyrosomus regius* populations. The optimal tree is inferred by Neighbour-Joining with a sum of branch length. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA 4.

Table 4. Pair-wise estimates of F_{ST} values among the twelve *Argyrosomus regius* collection sites: Weir and Cockerham (1984) θ above the diagonal and significance below the diagonal after simple Bonferroni correction (Rice, 1989).

	France-1 (F1)	France-2 (F2)	Portugal-1 (P1)	Portugal-2 (P2)	Spain (S1)	Mauritania-1 (M1)	Mauritania-2 (M2)	Mauritania-3 (M3)	Mauritania-4 (M4)	Egypt-1 (E1)	Egypt-2 (E2)	Turkey (T)
France-1 (F1)		0.003	0.021	0.023	0.035	0.021	0.026	0.033	0.038	0.112	0.108	0.159
France-2 (F2)	0.186		0.033	0.020	0.033	0.019	0.024	0.029	0.038	0.090	0.089	0.137
Portugal -1 (P1)	0.004	< 0.001		0.015*	0.014	0.044	0.019	0.044	0.034	0.109	0.011	0.168
Portugal.-2 (P2)	0.002	< 0.001	< 0.011		0.008	0.019	0.019	0.038	0.025	0.062	0.055	0.119
Spain (S1)	< 0.001	< 0.001	< 0.011	0.076		0.034	0.030	0.039	0.039	0.096	0.081	0.146
Mauritania-1 (M1)	0.019	0.009	< 0.001	0.017	0.002		0.007	0.001	0.006	0.068	0.065	0.113
Mauritania-2 (M2)	0.016.	0.009	0.005	0.039	0.006	0.693		0.023	0.009	0.057	0.061	0.118
Mauritani-3 (M3)	0.008	< 0.001	< 0.001	< 0.001	< 0.001	0.449	0.053		0.042	0.093	0.079	0.126
Mauritania-4 (M4)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.169	0.874	0.002		0.063	0.065	0.115
Egypt-1 (E1)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		0.001	0.093
Egypt-2 (E2)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.506		0.075**
Turkey (T)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Table 5. Pair-wise estimates of F_{ST} values among the six *Argyrosomus regius* populations: Weir and Cockerham (1984) θ above the diagonal and significance below the diagonal after simple Bonferroni correction (Rice, 1989)

	France	W. Portugal	Mauritania	S.W. Iberia	Egypt	Turkey
France		0.026	0.026	0.025	0.099	0.140
W. Portugal	<0.001		0.041	0.012	0.107	0.168
Mauritania	<0.001	<0.001		0.024	0.061	0.098
S.W. Iberia	0.003	<0.001	<0.001		0.073	0.126
Egypt	<0.001	<0.001	<0.001	<0.001		0.081
Turkey	<0.001	<0.001	<0.001	<0.001	<0.001	

Table 6. Effective population size (N_e) estimates for the 6 *Argyrosomus regius* populations based on linkage disequilibrium estimated with parametric and jackknife methods (Waples and Do, 2008). N_e = mean effective population size; Upper and lower 95 % confidence interval are shown; * Negative value estimated by LDN_e suggests N_e is not different from infinity.

Population	Ne	Parametric		Jackknife	
		Lower	Upper	Lower	Upper
France	-634.1*	394.1	Large	281.3	Large
W. Portugal	-117.5*	250.2	Large	136.0	Large
S.W. Iberia	-722.1*	182.5	Large	99.3	Large
Mauritania	111.0	68.7	237.2	61.6	330.3
Egypt	531.5	292.3	Large	151.6	Large
Turkey	17.4	10.7	31.8	10.3	33.6

4. Discussion

4.1. Genetic structuring

Genetic characterisation was made with microsatellite markers isolated from another species. The very limited number of null alleles in *A. regius* confirms the potential for cross-amplification between Sciaenids (Turner *et al.*, 1998) and ability to identify *U. cirrosa* and *P. senegallus* species. Incidentally, *A. regius* presents a lower mean number of alleles per locus per population than the red drum (Saillant *et al.*, 2009).

The non significant F_{ST} values between different collection sites justified their pooling into six distinct new sites. Among the Mauritanian sites, a sampling artefact can be suspected for sample M4, as it is differentiated from one Mauritanian sample but not the other two. A hierarchical Analysis of Molecular Variance could have been performed to account for regions and collection sites within regions, but we estimated that genetic differentiation was so high that any type of statistical method would give the same kind of results. Similarly, we did not estimate the effect of geographic distance on genetic differentiation since the geographic distances between sites are so great and different that this effect is obvious.

A. regius can be divided into at least two very distant genetic groups: Atlantic and Mediterranean. Our results reveal the existence of a previously unknown sixth distinct reproductive area for the species in the Aegean Sea, at the mouth of the Menderes river delta in Turkey. Interestingly, the S.W. Iberia population is somewhat intermediate between the Atlantic and Mediterranean, which is in accord with its geographical location. Reproduction has been also suggested in Morocco (Hermas, 1995) and in several other cryptic areas (JL Costa, pers. comm.; Champagnat and Domain, 1978; Dieuzeide, 1929; Quéro, 1989b; Chakroum *et al.*, 1983).

4.2. Why such high genetic differentiation?

A. regius F_{ST} values are very high and in the highest ever reported in marine fish from this the same geographic area (Bonhomme *et al.*, 2002; Nielsen *et al.*, 2003; Nielsen *et al.*, 2004;

Alarcón *et al.*, 2004; Kotoulas *et al.*, 2006; Maggio *et al.*, 2009; Gallarza *et al.*, 2009; González-Wangüemert *et al.*, 2010) or in smaller Sciaenids (Lankford *et al.*, 1999; Gold *et al.*, 2001; Ward *et al.*, 2007; Zhiqiang *et al.*, 2008; Xiao *et al.*, 2009). Such F_{ST} values have only been previously reported for coastal marine species strongly affected by post-glacial recolonization (Wilson and Veraguth, 2010) or for populations separated by the Atlantic ocean (Ball *et al.*, 2007) or by the Indian ocean for another *Argyrosomus* species (the mulloway *A. japonicus*) (Archangi, 2008).

Geographic and hydrological barriers were advocated to explain genetic differentiation in marine fish. The Cape Sagres separates the two Portuguese populations, which are less than 200 km apart. The Gibraltar Strait-Alboran Sea zone and the Siculo-Tunisian Strait limit genetic exchanges between the Atlantic Ocean and the southeast Mediterranean Sea (for further examples see Barhi-Sfar *et al.* (2000), Naciri *et al.* (1999) and Patarnello *et al.* (2007)). In the northern waters of the Aegean Sea, the lowest salinity and the coolest temperature induce a cyclonic circulation, causing its isolation from Egypt (Barhi-Sfar *et al.*, 2000). These barriers may have played an important role in the recent past of the species.

The subdivision into the two Atlantic and Mediterranean groups and the lower allelic richness and effective sizes of the Mediterranean populations could be due to the effects of vicariance, limited introgression after secondary contact, and/or population expansion following the successive coolings and warmings of the Mediterranean Sea during previous interglacial phases of the Quaternary (Borsa *et al.*, 1997; Bianchi and Morri, 2000; Patarnello *et al.*, 2007). Even though the present results do not demonstrate the impact of Pleistocene glaciation *per se*, these glaciations could have also restricted *A. regius* area in the Atlantic to a more southern part than its present distribution. Crowley (1981) estimates very low summer temperature (9.2 °C) in northern Portuguese waters at the time of the 18 000-year B.P. glacial maximum. Such a low temperature makes the reproduction of *A. regius* in the Tagus and the Gironde estuaries theoretically impossible at this time. These northern populations could, therefore, result from a recent expansion in the North Atlantic Ocean, which is in agreement with their lower allelic richness. In the Atlantic, this result confirms the early Tixerant (1974) hypothesis of differentiation between France and Mauritania based on differential otolith growth. In the Mediterranean Sea, the genetic differentiation between the two populations is in agreement with the two biogeographic areas defined by Bianchi and Morri (2000) as “North Aegean” and “Gulf of Gabes to Levant Sea”.

Biological factors have also been put forward to explain the genetic differentiation of marine organisms. However, early life-history traits (egg type, pelagic larval duration, and inshore-offshore spawning) have a limited involvement in the genetic differentiation of marine fishes (Gallarza *et al.*, 2009). For *A. regius*, Tixerant (1974) and Quéro (1989a) pointed out factors that varied between Mauritania, Egypt and France, such as the difference in water salinity. They also identified differences in the duration and the time of reproduction (3 weeks in early June in the Gironde, the 2 months of February and March in Egypt, and 9 months from October to June in Mauritania). But among all the biological factors, reproductive migration is probably the most important factor limiting adult movement and, therefore, limiting gene flow between reproductive

areas. This factor is reinforced by two others. The first is the limited number of potentially favourable sites for *A. regius* reproduction and for long term settlement. The need for an actual estuary was questioned by Tixerant (1974), as there is no estuary in Mauritania. A site favourable for reproduction would require intermediate temperature (14 °C to 23 °C), with an optimal window (19 °C to 21 °C) for reproduction and successful larval recruitment (Quéro and Vayne, 1987; Quéro, 1989a), high water flow and minimal water depth for spawning (> 10-15 m). The second requirement is the need for extensive mudflats (in the Atlantic) or lagoons (in the Mediterranean) habitats close to the reproduction sites to provide a suitable environment for larval recruitment and juvenile growth (Quéro and Vayne, 1987). The Gironde, Tagus, Guadalquivir and Menderes estuaries, the sea areas at the opening of the Nile river delta and the large mudflats in the Banc d'Arguin in Mauritania fulfil these basic requirements.

4.3. Potential biases

In this study, temporal variation in allele frequencies may be confounded with spatial variation because samples were composed of very different year classes among and within sites. However, the magnitude of the divergence between regions is so large that some noise due to temporal genetic variation is unlikely to have a great impact on the observed patterns of population structure reported here.

Bias could potentially arise from rearing wild fish in captivity by artificial selection for survival even if we estimate genetic variability of neutral markers. Only the population from Egypt had a significant heterozygote deficiency when all loci were considered. Inbreeding can be excluded, as heterozygote deficiency was not the case at all loci. As heterozygote deficiency was also estimated before the pooling of the two collection sites, Wahlund effect (presence of different genetic stocks in a single sample) seems improbable as such a hypothesis would imply that each of the two Egyptian stocks would have been composed of different origins in the same ratio. Assortative mating can also be excluded as *A. regius* is a mass spawner (Tixerant, 1974). An effect of artificial selection during captivity would require a strong association between the potentially neutral loci and survival, which is unrealistic with the limited number of markers used. Only a more detailed investigation on wild captured cohorts among different Egyptians coastal lakes could allow conclusions about these factors. In any case, the heterozygote deficiency observed in the Egyptian samples does not interfere with the general conclusion that there is very high geographic differentiation.

4.4. Effective population sizes

The estimations of population size differ greatly: from infinity (France, W. Portugal, S.W. Iberia) to very limited (Turkey). Several factors need to be considered for the interpretation of these results.

N_e estimates were obtained from populations composed of different year classes (W. Portugal, SW Iberia and Mauritania), a single year class (Egypt, Turkey) or two year classes (France). If estimates in the former three populations represent approximations of N_e (effective

size for the generation), the N_e estimates from the latter three populations most probably reflect N_b (effective number of breeders having produced the sample) than N_e (Waples, 2005). It can then be argued that the assumption of the statistical models based on discrete generations is severely violated as *A. regius* reproduces with overlapping generations until fourty years of age.

The long generation interval and overlapping generations are also factors that could cause genetic disequilibrium between year classes and underestimation of the true effective number of parents ("sweepstake recruitment hypothesis"; Hedgecock, 1994). Preliminary results from F1 and F2 yearly cohorts do not provide much if any support for this hypothesis as the two cohorts collected from the same population exhibited neither genetic differentiation nor heterozygote deficiency (caused in this case by temporal Wahlund effect).

High temporal heterogeneity in effective population size was also associated with a very low ratio of individuals producing new young-of-age year class to adult census population size in another large Sciaenid, the red drum *Scianops ocellatus* (Turner *et al.*, 2002). The high variation in *A. regius* captures (i.e. from 350 T in 1992 to 12200 T in 2001 in Mauritania or from 35 T in 1985 to 1356 T in 2006 in France ; FAO data) could also result and/or create unequal reproductive success and fluctuation in population size between cohorts (Hedrick, 2005).

Finally, all populations show very limited lower bounds of effective size, inferior to 500 (Table 6), which is very low for a marine fish and may indicate a population risk for long term viability (Franklin and Frankham, 1998). Among the six populations identified, Turkey and Mauritania had a finite upper estimate of N_e effective (33.6 or 330.3). For the population from Turkey, that is also the less variable and the more genetically differentiated, the actual small surface of the Menderes delta, and its shallow lagoons for the juvenile development, could explain the limited annual captures (< 50 T, K. Gamzis, pers. comm.). The Holocene sea-level variations (Ergin *et al.*, 2007; Kazanci *et al.*, 2009) are also factors that have greatly restricted the Menderes delta surface and potentially limited reproductive capacities and therefore Ne. The high genetic variation of the population from Mauritania is opposite to its limited N_e estimate. Since the Mauritanian "sample" combined small numbers of fish collected at four locations, the N_e estimate could be biased downward by mixture disequilibria from a two-locus Wahlund effect (see discussion in 4.3. about F_{ST} values and genetic differentiation between Mauritanian sites) and underestimate the real Ne, which should be considered higher.

More generally, the meagre shares several biological factors with the ten other large Sciaenids already threatened and for which the protection of seasonal aggregation areas and nursery grounds has been advocated (Cisneros-Mata *et al.*, 1995; Sadovy and Cheung, 2003; Liu and Sadovy de Mitchel, 2008). The limited values of N_e and the very long distances known between reproduction areas should be considered in the meagre management, as recent river water pollution, modification of water flow and overfishing at 1+ year of age were reported as factors associated with decreases in meagre abundance (Bebars *et al.*, 1996; Oczkowski and Nixon, 2008; Kazanci *et al.*, 2009; Sourget and Biais, 2009; Morales-Nin *et al.*, 2012).

5. Conclusion

This study provides the first genetic characterisation of *A. regius* across most of its natural range. The species is genetically highly structured, with a degree of differentiation rarely reported in a marine fish. The high genetic fragmentation highlights the genetic originality of each population and the need to consider their management regionally. This work also demonstrates the existence of an as yet unknown sixth reproductive area in the Aegean Sea. Future studies, including the genetic characterisation of fish from other areas (Morocco, Balearic Islands…) or other cryptic populations using microsatellite markers or mtDNA could offer further insight about *A. regius* ecology, biodiversity and recent evolution. These data will be useful for its preservation and for its exploitation by fisheries and aquaculture.

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Chapter 7

Fisheries management

Chapter 7

Use of SARIMA models to assess data-poor fisheries: a case study with a sciaenid fishery off Portugal

Nuno Prista[a,b], Norou Diawara[c], Maria José Costa[a,b] and Cynthia Jones[d]

[a]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c]Department of Mathematics and Statistics, Old Dominion University, Norfolk, Virginia 23529-0077, USA
[d]Center for Quantitative Fisheries Ecology, Old Dominium University, 800W 46th St., Norfolk, VA 23508, USA

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Use of SARIMA models to assess data-poor fisheries: a case study with a sciaenid fishery off Portugal

Abstract: Research on assessment and monitoring methods has primarily focused on fisheries with long multivariate data sets. Less research exists on methods applicable to data-poor fisheries with univariate data sets with a small sample size. In this study, we examine the capabilities of seasonal autoregressive integrated moving average (SARIMA) models to fit, forecast, and monitor the landings of such data-poor fisheries. We use a European fishery on meagre (Sciaenidae: *Argyrosomus regius*), where only a short time series of landings was available to model (n=60 months), as our case-study. We show that despite the limited sample size, a SARIMA model could be found that adequately fitted and forecasted the time series of meagre landings (12-month forecasts; mean error: 3.5 tons (t); annual absolute percentage error: 15.4%). We derive model-based prediction intervals and show how they can be used to detect problematic situations in the fishery. Our results indicate that over the course of one year the meagre landings remained within the prediction limits of the model and therefore indicated no need for urgent management intervention. We discuss the information that SARIMA model structure conveys on the meagre life-cycle and fishery, the methodological requirements of SARIMA forecasting of data-poor fisheries landings, and the capabilities SARIMA models present within current efforts to monitor the world's data-poorest resources.

Keywords: Seasonal ARIMA models; data-poor fisheries; fisheries monitoring; time series analysis; statistical process control; meagre

1. Introduction

Research, assessment, and management have traditionally focused on fisheries with the greatest landings and revenues (Scandol, 2005; Vasconcellos and Cochrane, 2005). Such fisheries are generally data-rich and have available the funds and expertise required to complete stock assessments and provide state-of-the-art advice to management. However, that is not the case for the vast majority of fisheries worldwide, which remain subjected to limited (if any) assessment and management (Vasconcellos and Cochrane, 2005). The latter have been collectively termed "data-poor fisheries" and are characterized by a low diversity and quantity of data, limitations in funding and expertise, and an overall shortage of assessment methods (Mahon, 1997; Scandol, 2005). Among the world's data-poorest fisheries are nearly all fisheries in developing countries, but also most fisheries in developed countries, namely the smaller-scale or less valuable commercial and recreational ones (NRC, 1998; Berkes *et al.*, 2001; EEA, 2005; Vasconcellos and Cochrane, 2005; ICES, 2008; Worm *et al.*, 2009; OSPAR, 2010).

Assessment of data-poor fisheries requires a significantly different approach from their data-rich counterparts. For data-poor fisheries, many deterministic multivariate stock assessment models cannot be used (e.g., NRC, 1998) and more pragmatic assessment methods must be put in place, particularly when fishery-independent data are not available and fishing effort cannot be quantified (Berkes *et al.*, 2001; Scandol, 2003; ICES, 2008). In many countries, the most readily available fisheries data are commercial

landings because of their connection to the economy and business (Vasconcellos and Cochrane, 2005). Commercial landings result from complex interactions between the environment, the fishing fleet, and the stocks, and therefore do not directly reflect the status of exploited populations. However, landing records contain valuable information that can be useful to managers if routine monitoring, rather than stock assessment, is established as a management objective (Scandol, 2003). In fact, even if they provide suboptimal indications on the status of the stocks, statistical analyses of landings can lead to the timely detection of phenomena such as sudden increases in fishing effort or marked population declines that could otherwise remain undetected (Caddy, 1999). Such detection is important — particularly within multispecies, budget-limited, management contexts — because it allows the prioritization of research and management actions toward the subset of fisheries and stocks most likely to be depleted (Scandol, 2003).

Autoregressive integrated moving-average (ARIMA) models are simple time series models that can be used to fit and forecast univariate data such as fisheries landings. With ARIMA models data are assumed to be the output of a stochastic process, generated by unknown causes, from which future values can be predicted as a linear combination of past observations and estimates of current and past random shocks to the system (Box *et al.*, 2008). In fisheries, ARIMA models (and their seasonal multiplicative version, SARIMA) have a long record of successful application that extends from modeling (e.g., Hare and Francis, 1994; Fogarty and Miller, 2004) to short-term forecasting of a variety of variables and resources for both data-rich and data-poor fisheries (Table 1). Specifically, SARIMA models, which are applicable to many already-available landings data sets, have been found to provide both annual and monthly forecasts that are comparable to, or even better than forecasts from many multivariate models, including some with fishing effort among the predictors (Stergiou *et al.*, 1997).

The good record, flexibility, and simplicity of SARIMA models have made them natural candidates for the modeling of data-poor fisheries (Rothschild *et al.*, 1996). However, to date, SARIMA models in fisheries have only been applied in detail on relatively long time series (≥120 months) (Table 1), and a single study has provided a few (but not detailed) results from shorter series (Lloret *et al.*, 2000). Such emphasis of previous SARIMA modeling on long time series finds little support in statistical literature where 50 months is generally regarded as the minimum sample size for model application (e.g., Pankratz, 1983; Chatfield, 1996a). Additionally, most literature to date has focused on SARI-MA models as tools to generate accurate forecasts of future landings. However, in addition to good forecasting, these models also possess significant capabilities for monitoring landings that have remained unexplored. These capabilities become apparent when SARIMA models are approached from a statistical process-control perspective and it is made known that SARIMA model forecasts include the assumption of persistence (through time) of the process that generated the data (Box *et al.*, 2008; Mesnil and Petitgas, 2009). Briefly, good landing forecasts are only attainable as long as significant changes do not take place in the fishery; therefore large forecast errors can be regarded as indications that can be changes in the fishery process took place that may require management intervention (Pajuelo and Lorenzo, 1995; Georgakarakos *et al.*, 2006; Box *et al.*, 2008).

Table 1 – Primary fisheries literature that presents seasonal autoregressive integrated moving-average models. Only studies with quantitative forecast results are displayed. "No." is the number of series, "Freq" is the sampling frequency (W=weekly, M=monthly, A=annual), "*n*" is the sample size of the fitting period (what is the unit of measurement? Here it is absolute. Units are established in the "Freq" column), "F" is number of forecasts, "models" indicates the type of models compared, and "PI" indicates if prediction intervals were presented (yes, no). "/" separates annual and monthly data sets when both were analyzed. "sp" = species, "nsp groups" = nonspecific groups, "Rel." = relative, "CPUE" = catch per unit of effort, "LPUE" = landings per unit of effort

Reference	Species	Variable	No.	Freq	*n*	F	Models[a]	PI
Saila *et al.* (1980)	*Jasus edwardsii*	CPUE	1	M	144	12	1,5	n
Mendelssohn (1981)	*Katsuwonus pelamis*	catch/effort	1	M	180	12	12	n
Fogarty (1988)	*Homarus americanus*	catch/CPUE	3/1	A/M	41–58/216	1/12	12	n
Jeffries *et al.* (1989)	*Pseudopleuronectes americanus*	Rel. abundance	2/3	A/M	27/156;324	2/12	—	y
Stergiou (1989)	*Sardina pilchardus*	catch	1	M	204	12	—	n
Noakes *et al.* (1990)	*Oncorhynchus nerka*	total returns	2	A	24	8	1,10,12,19,20	n
Stergiou (1990a)	*Engraulis encrasicolus*	catch	1	M	252	24	—	n
Stergiou (1990b)	Mullidae	catch	1	M	252	24	—	n
Campbell *et al.* (1991)	*Homarus americanus*	catch	4	A	61–97	10	12	n
Molinet *et al.* (1991)	*Penaeus* spp., *Lutjanus synagris*	landings/LPUE	2	M	132;180	24	—	n
Stergiou (1991)	*Trachurus* sp.	catch	1	M	252	12	1,8	n
Tsai and Chai (1992)	*Morone saxatilis*	harvest	1	A	27	4	3,4,12	n
Pajuelo and Lorenzo (1995)	1 nsp group	catch	1	M	131	24	—	y
Stergiou and Christou (1996)	4 sp; 12 nsp groups	catch	16	A	24	2	1–9	n
Stergiou *et al.* (1997)	4 sp; 12 nsp groups	catch	16	M	288	24	1–5,7–9	n
Park (1998)	*Theragra chalcogramma*	landings	1	M	264	24	—	n
Lloret *et al.* (2000)	30 sp; 36 nsp groups	catch	66[b]	M	51–200	12	—	y
Georgakarakos *et al.* (2002, 2006)	*Loligo vulgaris*, *Todarodes sagittatus*	landings	2	M	174	12	11,15,16	y
Pierce and Boyle (2003)	*Loligo forbesi*	LPUE	1	A/M	27/324	3/36	3, 12	y
Stergiou *et al.* (2003)	*Xiphias gladius*	catch	1	M	180	12	8,13	n
Zhou (2003)	*Oncorhynchus tshawytscha*	spawner density	2	A	11	4	1, 15	n
Hanson *et al.* (2006)	*Brevoortia tyrannus, B. patronus*	landings	2	A	57;63	10	3,14,15	n
Koutroumanidis *et al.* (2006)	*E. encrasicolus*, *Merluccius merluccius*, *Sarda sarda*	landings	3	M	216;252	12	17,18	n
Czerwinski *et al.* (2007)	*Hippoglossus stenolepis*	CPUE	1	W	107	31	15	n
Tsitsika *et al.* (2007)	Total pelagic, *E. encrasicolus, S. pilchardus, T. trachurus*	CPUE	4	M	180	12	11	y

[a] models compared: 1- Naïve, 2- Linear regression (LR), 3- Multiple LR, 4- Multiple LR with correlated errors, 5- Harmonic LR, 6- Fox surplus-yield, 7- Model combination, 8- Exponential, 9- Vector autoregressive, 10- Periodic autoregressive, 11- Multivariate ARIMA, 12- Transfer function noise, 13- X-11, 14- State space models, 15- Artificial neural networks, 16- Bayesian dynamic modeling, 17- Genetic modeling for optimal forecasting, 18- Fuzzy expected intervals, 19- Stock-recruitment, 20- Sibling;

[b] includes 12 series with 51–64 months.

In this study, we report the first detailed application of SARIMA models for monitoring of data-poor fisheries landings. We use data from a previously unassessed Portuguese fishery on meagre (Sciaenidae: *Argyrosomus regius*) as our example. The meagre is a valuable top predator from European coastal waters but its stocks have not been analytically assessed because of limitations in data, personnel, and funding existing at the national level. At the time of our analysis only a short time series of monthly landings (60 months) was available for this fishery, a situation that replicates conditions found in many other data-poor fisheries worldwide. We show that the short time series was not a problem for SARIMA modeling and forecasting and that prediction intervals from SARIMA models can be used to provide this fishery with basic monitoring. We suggest that SARIMA models should be more widely considered to extend the cover-age of monitoring to all exploited marine resources.

2. Materials and methods

2.1. Meagre (*Argyrosomus regius*) and its fisheries

Meagre is one of the world's largest and most valuable sciaenids (up to 180 cm, 50 kg, and with a US$ 15 per kg exvessel price). It ranges from France to Senegal, and the largest fisheries take place off Mauritania, Morocco, and Egypt. In Europe, the meagre constitutes a prized trophy-fish for anglers and an important income for small-scale commercial fishermen along the Atlantic shores of France, Spain, and Portugal. Its biology and life cycle remain scarcely documented, but recent concerns about the overexploitation of juveniles and interests in aquaculture production have sparked some research. Currently, the fish is known to be fairly long-lived (up to 44 yr) (Prista *et al.*, 2009), to present fast juvenile growth (Morales-Nin *et al.*, 2010) and to spawn at 3–4 yr old (N. Prista, unpub. data). Data on adult growth and reproduction have not been published, but preliminary reports indicate a life-cycle characterized by fast growth, high fecundity, and a long reproductive span, and that the estuaries of the Gironde (France), Tagus (Portugal), and Guadalquivir (SW Spain) rivers constitute the main spawning habitats (Quéméner, 2002; Prista *et al.*, 2008; N. Prista, unpub. data). Marked seasonal variations in landings linked to juvenile and adult migrations have been identified in local fisheries (Quéro and Vayne, 1987; Prista *et al.*, 2008). Overall, adults are thought to come inshore from spring to early summer to spawn but their overwintering grounds are still unknown; juveniles are thought to use estuaries as nursery areas during the warmer months and overwinter in adjoining coastal grounds (Quéro and Vayne, 1987; Quéméner, 2002; Prista *et al.*, 2008; N. Prista, unpub. data).

Recently, substantial conservation risks have been identified in European meagre fisheries that are related to the overexploitation of juvenile and adults schools in estuaries and nearby coastal areas (Quéméner, 2002; Prista *et al.*, 2008). To protect juveniles, precautionary management measures have been put in place (namely minimum landing size regulations) but the actual status of the meagre stocks was never assessed. This lack of assessment mainly

results from a lack of sufficient multivariate time-series data and because national assessment priorities, funding, and expertise are generally allocated to the largest national and transnational fisheries instead of the less-significant, albeit numerous and regionally important, ones. The fish being largely absent from routine fishery-independent surveys (Quéro and Vayne, 1987; F. Cardador, personal commun. 2008. INRB, I.P./IPIMAR, Av. Brasília, 1449-006 Lisboa, Portugal) and difficulties related to its sampling at port and the estimation of fishing effort (Prista *et al.*, 2007, 2008) further contribute to its unassessed status. In this type of setting, if simple methods are not put in place that can, at least, detect the most alarming signals in the landings data it is likely that stock collapses can occur without being detected.

2.2. Data set and data transformations

The Lisboa region in Central West Portugal (henceforth termed “Lisboa region”) (38°25′N to 38°59′N lat., ~9°15′W long.) is the main fishing area for meagre off the Iberian Peninsula (between 29% and 45% of annual landings of meagre, all gears combined, in 2001−05). In this region, most of the catch is associated with the Tagus estuary and its adjoining coastal area. The catch derives essentially from a small-scale artisanal fleet in which gillnets, trammel nets, and longlines are used to catch meagre during its spawning and nursery season (Prista *et al.*, 2008). To minimize overfishing of juvenile fish, a minimum landing size of 42 cm was established in 2002 that complements an array of other gear-related and effort-related management regulations that are not specific to meagre.

To test SARIMA models in the monitoring of the Lisboa meagre landings, we obtained a time series of meagre monthly landings from the Portuguese General Directorate for Fisheries and Aquaculture (DGPA). The landings data resulted from mandatory reports of fish sales obtained at all ports of the Lisboa region (N = 14) from May 2002 to April 2008 (i.e., 72 monthly values) as part of a routine data collection program (Figure 1). We used the first 60 months to fit the SARIMA models and the last 12 months as a hold-out period to evaluate forecasting performance and to monitor the fishery. Some previous data were available on this fishery, but those data were found to be unreliable because of contamination with landings from Portuguese vessels operating off North African waters. No significant management interventions occurred on the fishery during the course of our study.

Before fitting a SARIMA model, the time series must be checked for violations of the weak stationarity assumption of the models (Brockwell and Davis, 2002; Box *et al.*, 2008). In SARIMA models, trend and seasonal nonstationarities are handled directly by the model structure so that only the nonstationarity of variance needs to be addressed before model fitting. The meagre time series (x_t, t = 1,...,60) was seasonal and exhibited no trend (Figure 1A), but annual variance-mean plots indicated an increase in variance with the series mean. To correct this, we evaluated Box-Cox transformations (Box and Cox, 1964) and found that a $\log_{10}$ transformation successfully stabilized the variance of the series. Accordingly, we log-transformed the data,

subtracted its mean, and then used the mean-centered log-trans-formed data set (y_t, t = 1,...,60) as input to the SARIMA analyses (Figure 1B).

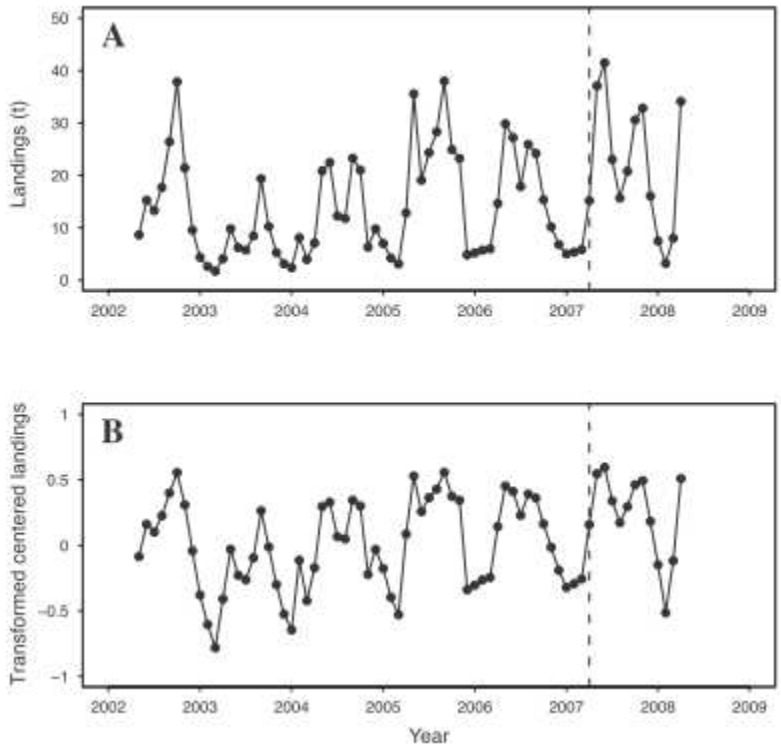

Figure 1. Time series of monthly meagre (*Argyrosomus regius*) landings, in tons, in the Lisboa region of the Portuguese coast (May 2002 to April 2008). The dashed vertical line is the forecast origin (April 2007) and separates the fitting period (May 2002 to April 2007, left) from the hold-out period (May 2007 to April 2008, right). (**A**) Raw data. (**B**) Log_{10}-transformed mean-centered data.

2.3. Data modeling

We fitted SARIMA models to the meagre data using a semi-automated approach based on a combination of the Box-Jenkins method with small-sample, bias-corrected Akaike information criteria (AIC_c) model selection (Rothschild *et al.*, 1996; Brockwell and Davis, 2002). This approach involved three major steps: 1) selection of the candidate model set; 2) estimation of the model and determination of AIC_c; and 3) a diagnostic check. Details on the notation and model selection procedures used to fit SARIMA models to short time series are given in Appendices 1 and 2.

Selection of the candidate model set was carried out by first analyzing sample estimates of the autocorrelation function (ACF) and partial autocorrelation function (PACF) in order to select the three major orders of the SARIMA models: d, D, and S. In the meagre case, we concluded that a configuration with d=0, D=1, and S=12 should be adopted (see Results section). Consequently, a SARIMA$(p,0,q)\times(P,1,Q)_{12}$ was selected as the basic model structure of the candidate set, with p, q, P, and Q left to vary. There is no a priori method to determine the maximum value that p, q, P, and Q can take, but the maximum orders of the models are obviously restricted by sample size. In our analysis, we conditioned p, q, P, and Q to the upper

boundary *max(p+q+SP+SQ)*=24 and *p*+*q*≤12 (Table 2), which caused the maximum possible term of any SARIMA model to be x_{t-36} and the maximum possible number of parameters to be 13. We found this procedure to provide a good compromise between model complexity and the convergence of estimation algorithms.

Table 2 – Candidate set of seasonal autoregressive integrated moving-average models. The "rule" column displays the mathematical expression used to determine the autoregressive components (*p*) and moving-average components (*q*) of the candidate models. "Max AR term" and "Max MA term" columns display the maximum autoregressive (AR) and moving-average (MA) lags included in the model equations, with respect to the original (x_t) and 12-month differenced $\log_{10}$-transformed mean-centered data ($w_t = \nabla_{12}^1 y_t = \nabla_{12}^1 (\log_{10} x_t - 4.022)$), respectively

Model structure	No. of models	Rule	Max AR term	Max MA term
$(p,0,q)\times(0,1,0)_{12}$	325	$q<25-p$; $p\leq 24$	w_t-24; x_t-36	z_t-12
$(p,0,q)\times(1,1,0)_{12}$	91	$q<13-p$; $p\leq 12$	w_t-24; x_t-36	z_t-12
$(p,0,q)\times(0,1,1)_{12}$	91	$q<13-p$; $p\leq 12$	w_t-12; x_t-24	z_t-24
$(p,0,q)\times(1,1,1)_{12}$	1	$q=0$; $p=0$	w_t-12; x_t-24	z_t-12

Model estimation was carried out by using maximum likelihood methods, after conditional sum of squares estimation of the starting values (Brockwell and Davis, 2002). Given the large number of models requiring estimation (Table 2), we developed a semi-automated software routine in R, vers. 2.5.1 (R Development Core Team, 2007) that estimated the models and output their AIC_c values. This routine used several functions incorporated in the R packages "stats" (R Development Core Team, 2007), "tseries" (Trapletti and Hornik, 2007), and "FinTS" (Graves, 2008). After estimation, the model with the minimum AIC_c was selected for further analysis.

Diagnostic checks on the AIC_c-selected model involved the following steps: 1) verification of the resemblance of residuals to white noise (ACF plots, Ljung-Box test, cumulative periodogram test); 2) tests on the normality of residuals (Jarque-Bera and Shapiro-Wilks tests); and 3) confirmation of model stationarity, invertibility, and parameter redundancy (Shapiro *et al.*, 1968; Ljung and Box, 1978; Jarque and Bera, 1987; Box *et al.*, 2008). All tests were carried out at a significance level of α=0.05. The variance explained by the model was determined as $1-\hat{\sigma}^2/\sigma_{y_t}^2$ (Stergiou, 1990a).

2.4. Forecasts and model performance

We evaluated 12 months of model forecasts, using the last month of the fitting data set as the forecast origin (i.e., April 2007). Forecasts were obtained in the mean-centered transformed scale ($\hat{y}_h$, *h*=1,...,12) and in the original scale of the data ($\hat{x}_h$, *h*=1,...,12), after correcting for back-transformation bias (Pankratz, 1983). SARIMA model performance was assessed by

comparing *h*-step forecasts ($\hat{x}_h$ and $\hat{y}_h$) with monthly landings observed between May 2007 and April 2008 (x_h and y_h). This was done by evaluating monthly forecast errors (e.g., $e_h = \hat{x}_h - x_h$) and then considering a set of accuracy measures: 1) annual root mean-square error (RMSE); 2) mean error (ME); 3) absolute percent error (APE_h); 4) mean absolute percent error (MAPE); and 5) annual percent error (PE) (Mendelssohn, 1981; Hyndman and Koehler, 2006). From these, RMSE was evaluated in the transformed scale to allow its comparison to $\hat{\sigma}$, and all others were computed in the more user-friendly original scale of the data. Additionally, we compared the forecasting performance of the SARIMA model against two simple naïve forecasting models (naïve model 1 or NM1, and naïve model 2 or NM2) (Noakes *et al.*, 1990; Stergiou *et al.*, 1997). The latter represented *ad hoc* forecasting models likely to be used in data-poor fisheries with short time series of landings: with NM1, future landings were assumed to be equal to the landings registered in the previous year; and with NM2, future landings were assumed to be equal to the average monthly landings registered in the fitting period. We also evaluated the Kitanidis and Bras (1980) coefficient of persistence (P) that summarizes forecasting results by comparing them with those of a naïve model where landings at time *t*+1 are assumed equal to landings at time *t*. This coefficient takes values smaller than or equal to 1, with P=1 representing perfect model forecasts.

2.5. Monitoring of fisheries

SARIMA models predict the future on the assumption that the statistical properties of the process generating the data remain the same over time (Box *et al.*, 2008). When framed within the perspective of statistical process control (e.g., Scandol, 2005; Box *et al.*, 2008; Mesnil and Petitgas, 2009), this characteristic allows the predictions of well-developed SARIMA models to be used as "guidelines" to monitor future observations. When a SARIMA model is found that appropriately fits the landings data, a significant departure of its forecasts from future observations can be seen as an indication that changes in the underlying fishery process have occurred (=out-of-control situation). In contrast, if such a significant departure does not take place, then there is no indication for such changes (= in-control situation). From a data-poor fisheries perspective, such a distinction means that if funding is limited and multiple fisheries require assessment, research and management efforts should be allocated to fisheries displaying out-of-control decreasing trends in production rather than to fisheries that remain stable or display in-control increasing trends (Scandol, 2003, 2005).

The distinction between in-control and out-of-control landings requires a set of detection limits. To date, process-control detection limits for fisheries indicators have been derived mostly from historical reference data (Scandol, 2003; Mesnil and Petitgas 2009; Petitgas, 2009). However, most fisheries have only a few years of collected data and consequently historical limits are difficult to estimate. In such situations, model-based detection limits like the prediction intervals (PIs) of SARIMA models (Chatfield, 1993; Box *et al.*, 2008) provide easy-to-compute

detection limits that explicitly take into account the correlation structure of the data. SARIMA PIs resemble confidence intervals for model forecasts and consist of upper and lower boundaries that encompass a 1−*α* probability region for future forecasts (Chatfield, 1993). Their main use is to convey the uncertainty around forecasts (De Gooijer and Hyndman, 2006). However, because prediction intervals encompass only future observations, as long as no structural changes take place in the underlying process (Chatfield, 1993), their boundaries can be used to monitor univariate data such as fisheries landings.

To date, the prediction intervals (PIs) from SARIMA models have seldom been reported in fisheries literature and, when they have, with little detail and discussion (**Table 1**). To monitor the landings of the meagre fishery we used two types of PIs: single step PIs ($PI_{ss,h}$) and multistep PIs ($PI_{ms,h}$). Single step PIs refer to a single monthly forecast (e.g., h=3) and are useful for determining whether a specific monthly observation is an outlier at a given significance level α. Multistep PIs encompass a 1−*α* prediction region that is a simultaneous PI for all observations registered up to a certain h-step and are useful in detecting systematic departures from historical patterns. We calculated $PI_{ss,h}$ as $\hat{y}_h \pm t_{df,\alpha/2}\sqrt{PMSE_h}$ where $PMSE_h$ is the expected mean squared prediction error at step h and $df=N-DS-d-r$ (Chatfield, 1993; Harvey, 1989). In the calculation of multistep PIs, we used a conservative approach based on a first-order Bonferroni inequality, whereby $PI_{ms,h}$ is given as $\hat{y}_h \pm t_{df,\alpha/2h}\sqrt{PMSE_h}$ and joint prediction intervals of, at least, 1−*α* around the point forecasts are obtained (Chan *et al.*, 2004).

3. Results

3.1. Data modeling

Large autocorrelations were recorded for lags 1, 2, 11, 12, 23, and 24 with values 0.68, 0.32, 0.44, 0.46, 0.28 and 0.31, respectively (Figure 2). The sharp decrease in autocorrelation values after lag 2 (0.07 at lag 3) indicated no evidence of a long-term trend; consequently, there was no need to include a first-lag difference term in the SARIMA model structure (d=0). In contrast, large autocorrelation values were registered at annual lags (and its multiples) which indicated the need to include a 12-month difference term in the models (S=12, D=1) (Figure 2). The ACF and PACF plots of the differenced series provided further support for these conclusions (Figure 2). Accordingly, a SARIMA$(p,0,q)\times(P,1,Q)_{12}$ was selected as the basic structure of the SARIMA candidate set.

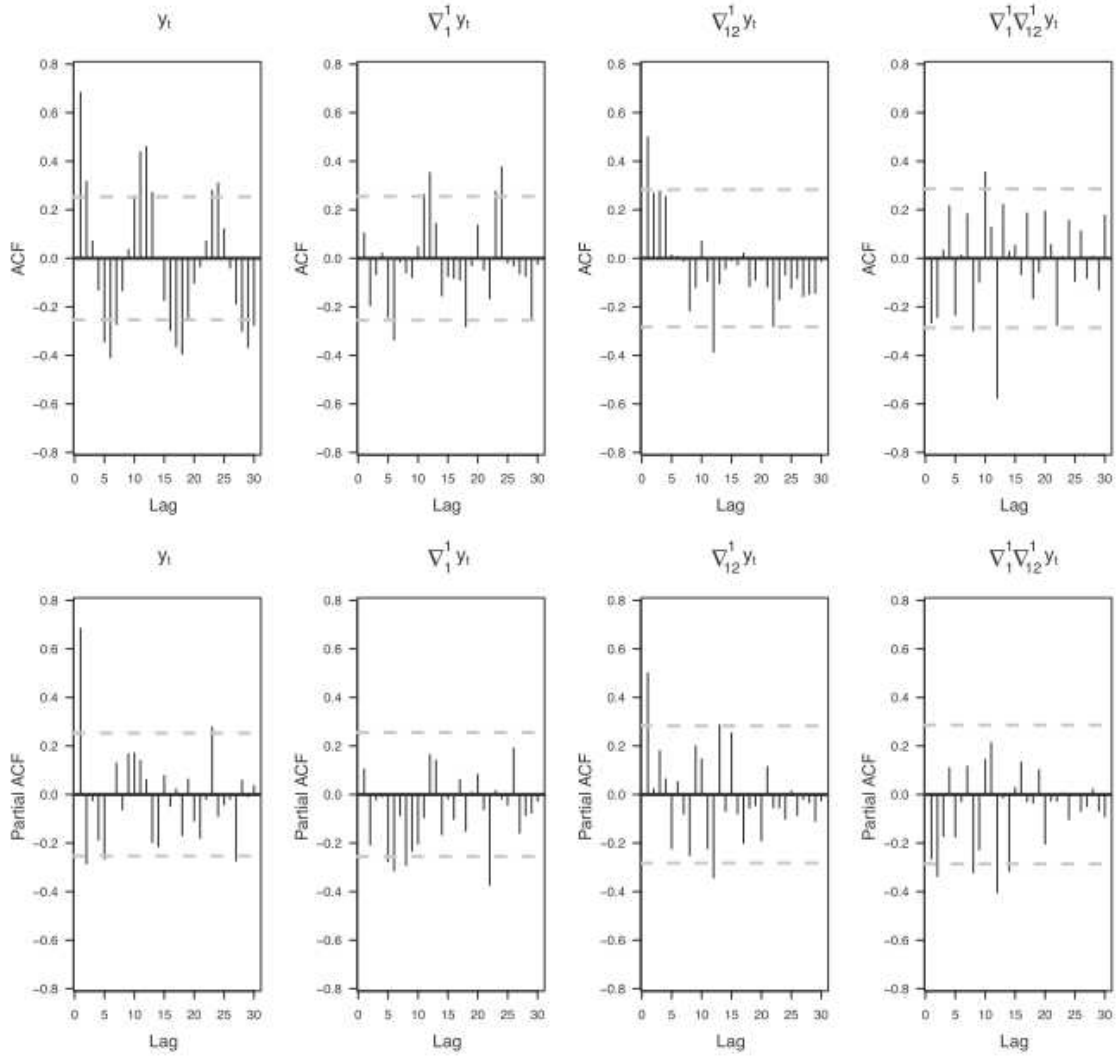

Figure 2. Sample autocorrelation function (ACF) and partial autocorrelation function (PACF) of the transformed meagre (*Argyrosomus regius*) landings. ACF/PACF plots for $\log_{10}$-transformed mean-centered data (y_t, far left), lag-1 differenced series ($\nabla_1^1 y_t$), lag-12 differenced series ($\nabla_{12}^1 y_t$), and lag-1 and lag-12 differenced series ($\nabla_1^1 \nabla_{12}^1 y_t$, far right) are displayed. Horizontal dashed lines represent the 95% confidence limits valid under the null hypothesis of white noise error structure.

Out of all models in the candidate set, a SARIMA$(0,0,5)\times(1,1,0)_{12}$ was selected as the best model for the meagre data (-2 ln $(L)=-26.32$, $n=48$, $r=7$, $AIC_c=-9.52$). This model had the following equation:

$$(1+0.65_{\{.10\}}B^{12})\ \nabla_{12}^1\ y_t = (1+0.63_{\{.19\}}B+0.56_{\{.15\}}B^2+0.51_{\{.17\}}B^3+0.93_{\{.18\}}B^4+0.60_{\{.21\}}B^5)z_t,$$

with a noise variance estimate of $\hat{\sigma}^2 = 0.025$ and where y_t = the mean-centered log-transformed meagre series (i.e., $y_t=\log_{10}x_t-4.022$) and the values in { } are the standard errors of the estimates.

Diagnostic checks indicated that the SARIMA model was stationary and invertible and did not have redundant parameters. The residuals were white noise (Ljung-Box $Q=3.35$, P-value>0.05) and passed asymptotic normality tests (Shapiro-Wilk $W=0.97$, P-value >0.05;

Jarque-Bera LM=4.91, P-value >0.05) indicating the model fitted the data and errors were normally distributed. The model explained 78.2% of the variance of the series.

The final process equation selected for the meagre data was

$$\log_{10} X_t = 0.35\log_{10}X_{t-12}+0.65\log_{10}X_{t-24}+Z_t+0.63Z_{t-1}+0.56Z_{t-2}+ 0.51Z_{t-3}+0.93Z_{t-4}+0.60Z_{t-5} ,$$

where $Z_t \sim N$ (0, 0.025).

3.2. Model forecasts and performance

The model forecasts presented two local maxima (May 2007 and September 2007) followed by a four-month period of low landings (December 2007 through March 2008) and an increase in the last month (April 2008) (Figure 3, Table 3). This pattern in forecasts matched the one in observed landings and the only deviations were that the actual maxima took place one to two months later and the winter trough was sharper than that predicted by the model (Figure 3). RMSE during the hold-out period (0.234) was ≈1.5 times the RMSE of the fitting period. Eight of the 12 forecasts registered negative errors, but the low ME and PE indicated that underestimation was minor in global terms. APE was large in August, September, December, and April, reflecting the delay in cessation of the 2007 fishing season and the hastening of the 2008 fishing season. Maximum APE coincided with the lowest landings (February), and the minimum APE with the first month forecasted (May) (Table 3). MAPE was 40.3%, reflecting the lagged seasonality and the low landings observed during the winter period.

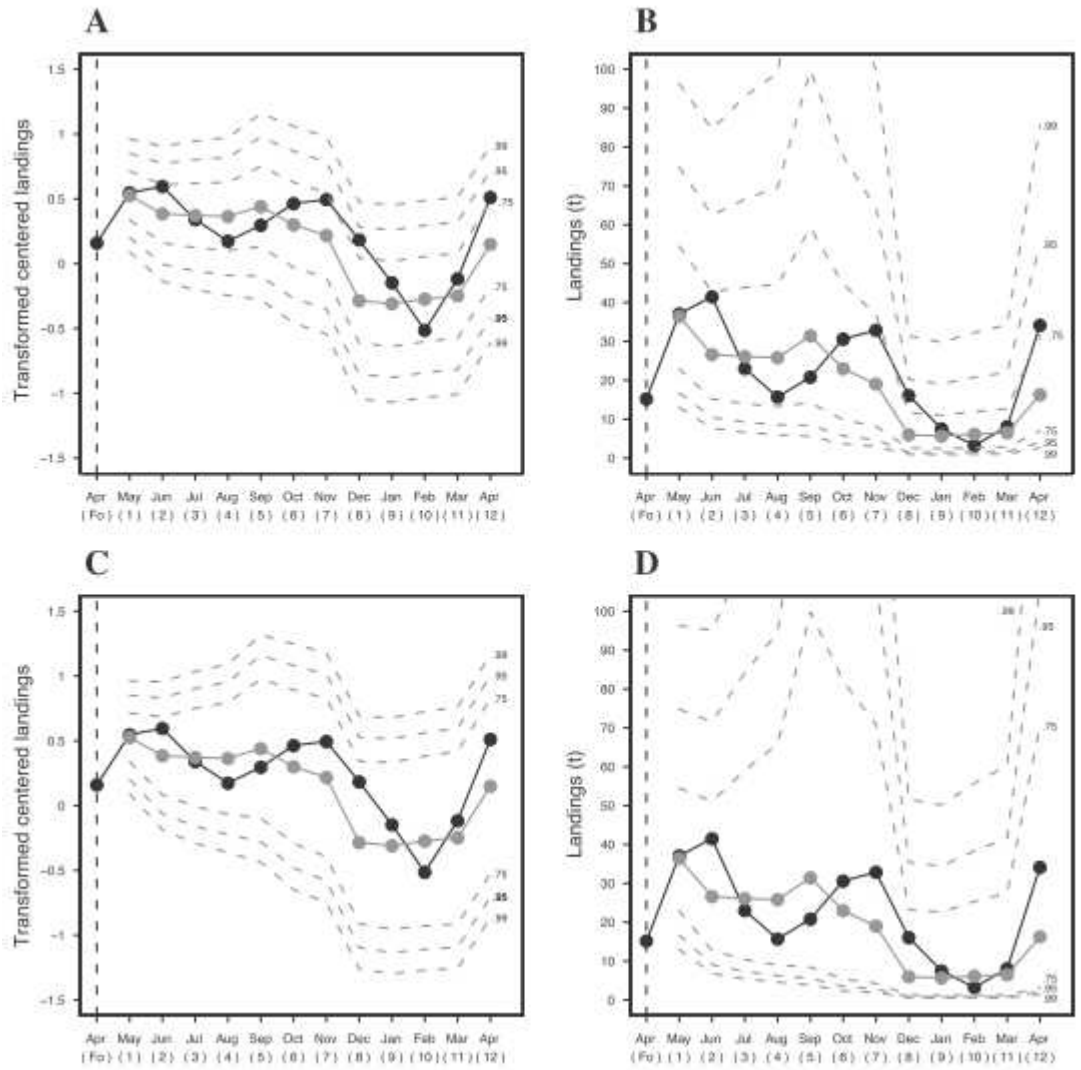

Figure 3. Forecasts and forecast prediction intervals (PIs) of meagre (*Argyrosomus regius*) landings. The dashed vertical line is the forecast origin ("Fo", April 2007). The gray circles and line represent the monthly forecasts. The black circles and line represent observed monthly landings. The dashed gray lines represent the upper and lower 75%, 95%, and 99% prediction intervals. (**A** and **B**) Single step prediction intervals ($PI_{ss,h}$) of transformed centered landings and back-transformed landings, respectively. (**C** and **D**) Multistep prediction intervals ($PI_{ms,h}$) of transformed centered landings and back-transformed landings, respectively.

As with SARIMA forecasts, naïve model predictions also lagged observed values by one or two months. However, the SARIMA forecasts registered the best performance in all accuracy measures, resulting in a 10% to 18% reduction in RMSE, 49% to 60% reduction in ME, 6% to 10% reduction in MAPE, and ≈15% reduction in PE (Table 3). The coefficient of persistence of the SARIMA model was also better (P=0.46) than the one registered by NM1 (P=0.23) and NM2 (P=0.03).

Table 3 – Forecasts of meagre (*Argyrosomus regius*) landings (May 2007 to April 2008). Observed landings (x_h), forecasted landings ($\hat{x}_h$), monthly forecast errors (e_h), monthly absolute percent error (APE_h), mean error (ME), and mean absolute percent error (MAPE) are displayed for the two naïve models (NM1 and NM2) and the seasonal autoregressive integrated moving-average model (SAR). Annual root mean-square error of the mean-centered

transformed data (RMSE) and annual percent error (PE) for NM1, NM2 and SAR were 0.261 and 30.2%, 0.285 and 38.9%, and 0.234 and 15.4%, respectively

Month	Step (h)	Obs (x_h)	Forecasts ($\hat{x}_h$)			Forecast errors (e_h)			APE_h		
			NM1	NM2	SAR	NM1	NM2	SAR	NM1	NM2	SAR
May-07	1	37.1	29.9	21.0	36.4	–7.2	–16.1	–0.7	19.4	43.5	1.8
Jun-07	2	41.5	27.2	18.1	26.6	–14.3	–23.4	–14.9	34.4	56.5	35.8
Jul-07	3	23.0	17.9	14.7	26.1	–5.2	–8.3	+3.1	22.4	36.2	13.3
Aug-07	4	15.7	25.9	18.4	25.8	+10.2	+2.8	+10.1	65.3	17.6	64.7
Sep-07	5	20.8	24.2	26.3	31.4	+3.4	+5.5	+10.6	16.3	26.2	51.1
Oct-07	6	30.6	15.3	21.9	23.0	–15.2	–8.7	–7.6	49.8	28.5	24.9
Nov-07	7	32.9	10.2	13.3	19.0	–22.7	–19.6	–13.9	69.0	59.5	42.2
Dec-07	8	16.1	6.8	6.8	6.0	–9.3	–9.2	–10.1	57.7	57.5	62.8
Jan-08	9	7.5	5.0	4.8	5.7	–2.5	–2.7	–1.8	32.8	35.7	24.5
Feb-08	10	3.2	5.4	5.2	6.1	+2.1	+2.0	+2.9	66.6	61.9	90.7
Mar-08	11	8.0	5.8	4.1	6.5	–2.2	–3.9	–1.5	27.3	48.6	19.0
Apr-08	12	34.1	15.2	10.8	16.3	–18.9	–23.4	–17.9	55.5	68.4	52.4
Mean	1:12	22.5	15.7	13.8	19.1	–6.8	–8.8	–3.5	43.1	45.0	40.3
Sum	1:12	270.5	188.8	165.4	228.9	–81.7	–	–41.6	–	–	–

3.3. Monitoring of fisheries

During the hold-out period, observed landings remained entirely within the 95% prediction intervals of the SARIMA forecasts (Figure 3), indicating that the observed forecast errors were within the range of values expected from random variability. Consequently the time series for meagre landings may be described as having remained in-control during the forecasting period. The PIs were symmetrical in the log-transformed scale (Figure 3, A and C), but asymmetrical in the original scale of the data (Figure 3, B and D). This pattern was expected from predictions of log-transformed data and indicates that sudden increases in monthly landings (positive forecast errors) are considered “more acceptable” than sudden decreases (negative forecast errors). Individual forecast errors that could have signaled an alarm ranged from 4.3 to 23.0 t (negative errors) to 13.5–68.3 t (positive errors). In relative terms, alarms would have been triggered by a higher than 54–75% drop, or by a higher than 105–238% increase, in monthly landings (Table 4). Compared to monthly PIs, multistep PIs were wider as a result of the increasing number of comparisons performed (Table 4). Even so, it is noticeable that such widening took place mainly on their upper boundary, and only a 12% increase was observed on their lower boundary.

Table 4: Prediction intervals of meagre (*Argyrosomus regius*) landings (May 2007 to April 2008). Point forecasts ($\hat{x}_h$) and 95% boundaries of the single step ($PI_{ss,h}$) and multistep ($PI_{ms,h}$) prediction intervals are displayed. The prediction boundaries are given as absolute errors ($|e_h|$) and absolute percent errors (APE_h) in each monthly forecast step (h). In each cell, the left and right values represent the lower and upper boundaries, respectively

Month	Step (h)	$\hat{x}_h$	$PI_{ss,h}$		$PI_{ms,h}$	
			$\|e_h\|$	APE_h	$\|e_h\|$	APE_h
May-07	1	36.4	19.7–38.4	54–105	19.7–38.4	54–105
Jun-07	2	26.6	16.2–35.8	61–135	17.5–45.0	66–169
Jul-07	3	26.1	16.9–40.5	65–155	18.8–58.0	72–222
Aug-07	4	25.8	17.3–43.7	67–169	19.6–68.8	76–266
Sep-07	5	31.4	23.0–68.3	73–217	25.9–120.0	82–382
Oct-07	6	23.0	17.3–54.7	75–238	19.5–103.6	85–451
Nov-07	7	19.0	14.3–45.2	75–238	16.2–89.7	85–472
Dec-07	8	6.0	4.5–14.2	75–238	5.1–29.4	86–491
Jan-08	9	5.7	4.3–13.5	75–238	4.9–28.8	86–509
Feb-08	10	6.1	4.6–14.6	75–238	5.3–32.2	87–525
Mar-08	11	6.5	4.9–15.5	75–238	5.7–35.1	87–539
Apr-08	12	16.3	12.3–38.7	75–238	14.2–89.9	87–553

4. Discussion

4.1. Interpretation of the models

Univariate SARIMA models based on landings do not have explanatory variables, but several studies have found the mathematical formulation in the models to correlate well with fish life history and fleet dynamics (Stergiou, 1990b; Stergiou *et al.*, 1997; Lloret *et al.*, 2000). In Europe, adult and juvenile meagre are thought to perform spring–summer migrations to major estuaries, remaining there until mid-summer (adults) and autumn (juveniles). These migrations are well known to local fishermen that actively target the meagre schools while they reside in estuarine grounds (Quéro and Vayne, 1987; Prista *et al.*, 2008). Such interactions between fish migrations and directed fishing effort are likely the cause of the strong seasonal component of the SARIMA model because target effort tends to intensify the natural seasonal signal generated by fish migrating through a fishery (Lloret *et al.*, 2000; Prista *et al.* 2008). In the case of central Portugal, such intensification is likely modulated at an interannual level by the expectations created for local fishermen by catches obtained in pre ceding years (represented in the seasonal autoregressive term) and, at an intra-annual level, by random environmental and anthropogenic perturbations occurring on the fishery system (represented in the set of nonseasonal moving-average terms).

4.2. Model fit and forecast performance

The univariate SARIMA model presented a good fit to the short time series of meagre landings, explaining most of its variance and adequately modeling the seasonality and

correlation structure of the data. Similar results were obtained in other studies of short and long time series: up to 68% (Lloret *et al.*, 2000, series ≤64 months), 75% (Saila *et al.*, 1980), 77% (Stergiou *et al.*, 2003), 84–96% (Stergiou, 1989, 1991; Stergiou *et al.*, 1997), and 93% (Pajuelo and Lorenzo, 1995). Taken together, these results indicate that SARIMA models should be adequate for data sets of monthly landings in general, and not just those with larger sample sizes. Bearing in mind that the minimum series length usually stated for SARIMA model fitting is 50 (Pankratz, 1983; Chatfield, 1996b), such generalized applicability may make SARIMA models particularly useful for fisheries with less reliable historical records or where only recently landings have been sampled.

In addition to a good fit, the SARIMA model also provided good short-term forecasts of meagre landings. The fact that all observed values were located within the predicted intervals of the model, and that naïve forecasts presented similarly lagged seasonality, indicates that the main forecast errors more likely resulted from natural variations in the timing of fish migrations and fishing seasons (Quéro and Vayne, 1987; Prista *et al.*, 2008) or from specifics of SARIMA forecasts and accuracy measures (namely, correlation and APE sensitivity to near-zero observations) (Hyndman and Koehler, 2006; Box *et al.*, 2008) than from model misspecification. At the annual level, the 15% error achieved is comparable to results previously obtained in larger data sets and well within the 10–20% range considered acceptable for market-planning and fisheries management (e.g., Mendelssohn, 1981; Pajuelo and Lorenzo, 1995; Hanson *et al.*, 2006). Additionally, SARIMA forecasts clearly outperformed naïve forecasting in all accuracy metrics, underscoring the large benefits of using these models instead of simpler alternatives (Saila *et al.*, 1980; Stergiou, 1991; Stergiou *et al.*, 1997). Considered together with the overall good forecasting performance reported by Lloret *et al.* (2000) in their shorter series, these results build confidence that SARIMA models are useful for forecasting short time series of landings and thus can substantially contribute to the planning and management of many data-poor fisheries.

4.3. Use of SARIMA models to forecast landings of data-poor fisheries

SARIMA models forecast future landings by directly handling the seasonality and autocorrelation structure of the data and assuming the continuity over time of past time series behavior (Box *et al.*, 2008). These models are known to be well adapted to forecast highly seasonal and autocorrelated data (Stergiou *et al.*, 1997; Georgakarakos *et al.*, 2006). Additionally, some authors have reported better SARIMA forecasting performances in fisheries with lower interannual variability, namely those that target benthic and demersal long-lived species (Lloret *et al.*, 2000). The data for meagre are autocorrelated and present a relatively stable seasonal pattern. Also, the meagre is long-lived and a targeted fish in central Portugal (Prista *et al.*, 2009; Prista *et al.* 2). Therefore, it is possible such features contributed to the good forecasts obtained from the SARIMA model. However, we note that the landings of many short-lived pelagic species and species with variable seasonal patterns have also been well

forecasted with SARIMA models (Stergiou, 1990a; Stergiou *et al.*, 1997; Georgakarakos *et al.*, 2006; Tsitsika *et al.*, 2007) and that the meagre landings also display substantial annual and monthly stochasticity Therefore, such general patterns should not be considered as strict limitations to SARIMA forecasting. More importantly, we note that SARIMA models can forecast well only if they have been adequately identified and estimated, and always under the assumption that the future is behaving like the past (Chatfield, 1993). Consequently, factors like data quality, presence of outliers, and model selection criteria are also very important for model performance. We discuss these next.

The quality of the input data for SARIMA models is determined mainly by the temporal stability of the statistical properties of the fisheries process and the consistency of its sampling over time. Consequently, although accuracy is required for some model applications (e.g., Zhou, 2003), data inaccuracies do not necessarily undermine SARIMA forecasts as long as factors such as fishing practices, regulatory measures, or data collection practices can be assumed to remain constant. When dealing with shorter series, a careful check whether these assumptions hold becomes particularly important because model identification and estimation are very dependent on the few observations available (Hyndman and Kostenko, 2007) and statistical techniques used to incorporate the effects of process changes in the models (e.g., Fogarty and Miller, 2004) are difficult to implement. In the case of meagre, the use of a short and recent time series better supported the assumption that data collection procedures, fishing techniques, fishery regulations, unreported landings, discards, and law enforcement practices did not change over time. In contrast, it is probable that these assumptions were not met in some less successful applications of the model to longer time series (e.g., Park, 1998).

Outliers are known to cause trouble in time series model identification, estimation, and forecasts—an effect that is amplified in shorter time series (Chatfield, 1993; Trívez and Nievas, 1998). The effects of outliers on forecasting performance are most disastrous when they occur near the forecasting origin because there they not only condition model structure and parameter estimates but are directly incorporated into the forecasts (Chatfield, 1993). The meagre data set presented no apparent outliers and this likely contributed to the good fit and forecasting performance achieved. If outliers were present, specific modeling techniques could have been used to estimate their influence, smooth them, or incorporate them into the model (e.g., Chen and Liu, 1993; Lloret *et al.*, 2000). We note, however, that any outlier during the hold-out period could still have changed our perception of model performance, even if it did not compromise the overall adequacy of the SARIMA model to forecast the landings.

In time series analysis, adequate model specification is considered the most important driver of forecasting accuracy (Chatfield, 1996b). The difficulties of specifying an appropriate model increase for data sets with lower information content, such as those of highly variable short time series from more complex processes (Hyndman and Kostenko, 2007; Appendix 2). To date, fisheries applications of SARIMA models have essentially relied on Box-Jenkins (BJ) model selection procedures to specify a model, and models with $p \leq 2$ and $q \leq 2$ have generally been selected (e.g., Mendelssohn, 1981; Pajuelo and Lorenzo, 1995; Lloret *et al.*, 2000).

Compared to these, the model for meagre seems overparameterized, but we note that all of its parameters are statistically significant and that the low $RMSE_{forec.}$ to $RMSE_{fit}$ ratio indicates an excellent correspondence between fit and forecasting performances (Chatfield, 1996b). In fact, although reduced model parameterization is considered beneficial to accuracy in forecasting, the most important aspect of time series analysis is not the number of parameters, but the degree to which the model approximates the statistical process underlying the data and whether or not it achieves the forecasting objectives (Chatfield, 1996b; Burnham and Anderson, 2002). In the case of meagre, had Box-Jenkins procedures been used, the selected models would be simpler and would still adequately fit the data: $(1,0,0)\times(1,1,0)_{12}$ or $(0,0,1)\times(0,1,1)_{12}$. However, they would have performed worse than our AIC_c-selected model in most performance metrics (RMSE: 0.245 and 0.302, APE: 1.7–92.7% and 20.6–72.4%, MAPE: 44.1% and 44.0%, PE: 13.7% and 31.7%, respectively). These results show the impact that different model selection techniques may have on forecasting performance with SARIMA models and stress the importance of considering objective data-driven criteria like AIC_c for circumventing the subjectivities of model selection in smaller data sets (Hurvich and Tsai, 1989; Burnham and Anderson, 2002).

5. Conclusions

5.1. Use of SARIMA models in monitoring fisheries

From a strictly forecasting perspective, SARIMA models have often been criticized for the excessive reliance on past time series behavior and their difficulty in predicting future structural changes (Georgakarakos *et al.*, 2002; Koutroumanidis *et al.*, 2006). Our results show that these drawbacks can become major advantages when SARIMA models are used for monitoring fisheries. At present, none of the European meagre fisheries is subjected to routine analytical assessment. By fitting SARIMA models to already available landings data we were able to carry out a first baseline evaluation of one such fishery, using limited funds and minimal time.

Our study provides a first example of how SARIMA models can be used to monitor data-poor fisheries. In the case of meagre, the data displayed no trend and the 95% SARIMA prediction intervals fully encompassed all monthly landings, thus indicating a stable “in-control” fishery. Note that by stating this, at no point do we suggest that the meagre fishery is sustainable long-term because landings do not necessarily reflect stock abundance and our study was limited in time. We suggest only that, since no motive for alarm exists in landings data, and because funds, personnel, and expertise are limited at the national level, attention should be allocated to fisheries that, contrary to the meagre, display decreasing trends or out-of-control situations. Similar types of pragmatic reasoning are generally of great help to fisheries managers handling multiple data-poor fishery scenarios because they help them prioritize management actions for the subset of “problematic” resources in a statistically sound way (Scandol, 2003, 2005).

Underlying the usefulness of SARIMA models in monitoring the meagre fishery and other data-poor fisheries is the use of prediction intervals as reference points to signal alarming trends or sudden level shifts in the fisheries process (Caddy, 1999; Scandol, 2003; Mesnil and Petitgas, 2009). SARIMA PIs have been previously reported in the literature (Table 1), but their use in monitoring was not explored or formalized. These intervals are currently the focus of much statistical research on how to deal with their tendency toward "over-optimism," i.e., the fact that nominal 95% prediction intervals generally contain less than 95% of future observations (Chatfield, 1993). Fortunately, from a fisheries conservation perspective such over-optimism does not constitute a major problem because narrower PIs will be more sensitive to changes in the fisheries process.

Statistical process control (SPC) monitoring of univariate fisheries indicators has become the focus of increased research attention (Scandol, 2003, 2005; ICES, 2008; Mesnil and Petitgas, 2009; Petitgas, 2009). The use of SARIMA PIs is similar to that of SPC control-charts, which makes them interesting candidates for the simultaneous monitoring of multiple fisheries and fisheries indicators (Caddy, 1999; Scandol, 2005; Petitgas, 2009). For such cases, SARIMA PIs offer the advantage of being model-based and do not require extensive historical reference data. They are also free from the assumption of statistical independence that frequently troubles the estimation of SPC detection limits (Mesnil and Petitgas, 2009). The simulation framework proposed by Scandol (2003, 2005) for SPC charts provides a means whereby SARIMA PIs can be calibrated toward specific detection rates and management goals. Such calibration was beyond the objectives our study but constitutes an interesting research route for those in charge of more holistic fisheries management.

5.2. SARIMA models in assessments of data-poor fisheries

Formal stock assessment has traditionally been considered as the starting point of any fisheries assessment (Mahon, 1997; Berkes *et al.*, 2001). Such an approach is highly desirable but will not be implemented easily, nor quickly, in the many existing data-poor fisheries (Vasconcellos and Cochrane, 2005). In fact, NRC (1998) estimated that 16% of U.S. stocks are not subjected to assessment; and the European Environmental Agency (EEA, 2005) estimated that, depending on the region considered, 20–90% of commercial stocks exploited in the Northeast Atlantic and Mediterranean are not routinely assessed. These figures are much worse in developing countries and when discard and bycatch species are included in the estimates (Vasconcellos and Cochrane, 2005). Addressing such situations requires increased focus on alternative stock indicators and assessment methods that can be used to monitor more fisheries by using available (or easily obtainable) data, funds, and human resources (e.g., Caddy, 1999; Scandol, 2005; Mesnil and Petitgas, 2009; OSPAR, 2010; ICES 1). Univariate time series models fitted to landings data may be, for some time longer, the best possible approach to extend assessment and management coverage to many of these unassessed resources.

SARIMA modeling and process-control schemes do not constitute alternatives to analytical stock assessment models. Rather, whenever possible, they should be seen as statistical tools to support expert judgment, funding allocation, and management decisions in the most data-limited and assessment-limited settings (Scandol, 2003; 2005). SARIMA modeling and model-based monitoring have a range of characteristics that make them worthy of future exploration in data-poor contexts. Among these are their appropriateness to numerous resources and variables, their strong statistical background and ecological plausibility, their good forecasting performance and easy-to-estimate detection limits, and their applicability to both long and short time series. Furthermore, SARIMA models can also be used to model the nonspecific groupings that dominate many landings data sets, or can be upgraded if multivariate data become available (Stergiou *et al.*, 1997; Vasconcellos and Cochrane, 2005). Finally, the availability of SARIMA models in open-source software packages and their routine use in sectors other than fisheries (e.g., sales, economics, engineering) (Brockwell and Davis, 2002; Box *et al.*, 2008) may be decisive advantages in budget-limited and expertise-limited countries.

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Appendix 1

ARIMA and SARIMA models

An extensive review of ARIMA and SARIMA models can be found in, e.g., Box *et al.* (2008) and Brockwell and Davis (2002). A mean-centered time series x_t can be modeled as an ARIMA(p,d,q), where p, d, q are nonnegative integers, if it can be adequately fitted with the process equation

$$\varphi(B)(1-B)^d X_t = \theta(B) Z_t \,,$$

where for a time interval T, $(X_t)_{\,t \in T}$ is a sequence of random variables, B is a backshift differencing operator $B^h X_t = X_{t-h}$ (h nonnegative integer), $(1-B)^d X_t = \nabla_1^d X_t$ is stationary, $\varphi(B)$ and $\theta(B)$ are linear filters defined as $\varphi(B)=1-\varphi_1 B-\varphi_2 B^2-\ldots-\varphi_p B^p$ and $\theta(B)=1+\theta_1 B+\theta_2 B^2+\ldots+\theta_q B^q$ and $(Z_t)_{t \in T}$ is a sequence of uncorrelated random variables with zero mean and variance σ^2 (termed white noise). In ARIMA models the orders p, q, and d define the structure of the model, by specifying the autoregressive (AR) and moving average (MA) components of an autoregressive–moving average process (ARMA[p,q]). d is the degree of differentiation ($d \geq 1$) required for X_t to become stationary. This differentiation involves the loss of d observations in the series.

The SARIMA $(p,d,q)\times(P,D,Q)_S$ models, where P, D, Q, and S are nonnegative integers, extend the modeling capabilities of ARIMA(p,d,q) models to seasonal processes. The SARIMA process equation is given by

$$\varphi(B)\Phi(B^S)(1-B)^d(1-B^S)^D X_t = \theta(B)\Theta(B^S) Z_t \,,$$

where X_t, Z_t, $\varphi(B)$ and $\theta(B)$ are defined as above, $(1-B)^d(1-B^S)^D X_t = \nabla_1^d \nabla_S^D X_t$ is stationary, and $\Phi(B^S)$ and $\Theta(B^S)$ are seasonal linear filters defined as $\Phi(B^S)=1-\Phi_1 B^S-\Phi_2 B^{2S}-\ldots-\Phi_P B^{PS}$ and $\Theta(B^S)=1+\Theta_1 B^S+\Theta_2 B^{2S}+\ldots+\Theta_Q B^{QS}$. In SARIMA, P defines the seasonal autoregressive component of the model (SAR) and Q the seasonal moving average component of the model (SMA). S represents the seasonal period (e.g., 12 months) and D is the degree of seasonal differentiation. Together, S and D account for seasonal nonstationarity in X_t through a data transformation that involves the loss of DS observations in the series.

Appendix 2

Selection of ARIMA and SARIMA models

ARIMA and SARIMA models are usually fitted by using a sequence of three general steps collectively known as the Box-Jenkins (BJ) method: 1) identification of the model; 2) estimation of the model; and 3) a diagnostic check of the model (Box *et al.*, 2008). In the identification stage, a model structure $(p,d,q)\times(P,D,Q)_S$ is selected by comparisons of sample ACF and PACF with theoretical ACF/PACF profiles of AR, MA and ARMA processes. In the estimation stage,

the model structure is fitted to the data and its parameters are estimated, generally by using conditional sum of squares or maximum likelihood methods. In the diagnostic check stage, the goodness-of-fit and assumptions for the model are evaluated and, if necessary, the BJ procedure is repeated until a suitable model is found. This model is then used to forecast future values (Box *et al.*, 2008). In-depth theoretical coverage of the BJ method is given in Box *et al.* (2008) and extensive practical applications are provided in Pankratz (1983) and Brockwell and Davis (2002).

The model identification stage of the BJ method is widely considered its most subjective step because it relies primarily on graphical interpretations of ACF/PACF estimates obtained from a single sample. This interpretation requires substantial analytical expertise and knowledge of the time series (both of which are problematic in data-poor scenarios) and is troublesome when complex ARMA processes have generated the data (Harvey, 1989; Shumway and Stoffer, 2006). Furthermore, it can also be confounded by existing correlations among ACF/PACF estimates (Box *et al.*, 2008). The minimum sample size generally advised for SARIMA model fitting is 50 observations (Pankratz, 1983; Chatfield, 1996b), but see Hyndman and Kostenko (2007) for an absolute lower limit. When sample size is large (e.g., $n \geq 100$), ACF/PACF estimates have lower variability and are more likely to approximate the theoretical ACF/PACF estimates of the underlying process. In such cases, less subjectivity exists in identification of the model. However, when sample size is small, the interpretation of ACF/PACF patterns becomes increasingly confounded by the large variance of the sample estimates, particularly at larger lags ($\geq n/4$) (Box *et al.*, 2008). This variability substantially increases the subjectivity of the model identification stage of the BJ method and is the main issue to be dealt with when analyzing shorter time series.

AIC approach

To circumvent the subjectivity of the identification of the model with the BJ method and to aid in the determination of the final orders of the ARMA processes a wide variety of model selection criteria have been developed (De Gooijer *et al.*, 1985). The most frequently used are Akaike's information criteria (AIC) (Akaike, 1974) and its small-sample, bias-corrected equivalent, AIC_c (Hurvich and Tsai, 1989). Contrary to the Box-Jenkins method, AIC/AIC_c selection of a model involves the a priori estimation by maximum likelihood methods of a set of model structures (here termed the candidate set). This estimation is followed by the determination of the AIC/AIC_c values for each individual model. The model with minimum AIC/AIC_c is then selected as the model that is closest to the statistical process "generating" the data. In SARIMA models, AIC is calculated as

$$AIC = -2\ln(L) + 2r \,,$$

where $\ln(L)$ is the log-likelihood of the model, $r = p+q+P+Q+1$, and the AIC_c is given by

$$AIC_c = -2\ln(L) + 2r + 2r(r+1)/(n-r-1) \,,$$

where $n=N-DS-d$ is the number observations used to fit the model. AIC/AIC_c constitute objective methods to achieve model parsimony through a trade-off between the variance explained by the model and penalty terms caused by excessive model parameters. Both of them are well founded in the principles of information and likelihood theory and have been applied extensively in time series, fisheries, and ecological literature (e.g., Brockwell and Davis, 2002; Burnham and Anderson, 2002; Hanson *et al.*, 2006). Burnham and Anderson (2002) suggest AIC_c is used when $n/r \leq 40$, which prompts the consideration of this small-sample, bias-corrected version of AIC in studies of short time series

Discussion

General Discussion

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4. General discussion

The scientific work presented in this thesis aimed at (1) increasing scientific and societal awareness about the historical and present significance of the meagre in Portuguese waters; (2) contributing to the sustained elevation of this European data-poor species to a more data-rich situation; and (3) carry out a first appraisal on the life-history of the Portuguese meagre and, if necessary, recommend fisheries management actions.

The meagre has been an important fishery in the Iberian Peninsula since prehistoric times. This fact is confirmed by abundant osteological remains found at archaeological sites (Lentacker, 1986; Izquierdo & Muñiz, 1990). The work presented in Chapter 1 (Gabriel *et al.*, 2012) showed that otoliths are the main meagre bone structure present at those sites. The abundant presence of this calcified structure is not surprising if one considers the size and robustness of typical Sciaenid otoliths (Schwarzhans, 1993). However, the results in Chapter 1 demonstrated that meagre fished by Mesolithic communities included both juveniles and adults, and indicating that meagre fishing has been carried out in estuarine environments since prehistoric times. Considering the significant anthropogenic pressure that occurs in today's estuarine systems, including in the Tagus and Sado estuaries (Costa and Cabral, 1999), the presence of meagre in both current and prehistoric estuarine environments appears to indicate that the overall environmental conditions required by the fish have not changed so dramatically that its natural migratory rhythms are drastically affected.

Accurate knowledge of the spatio-temporal distribution of fisheries is essential to fisheries management and the conservation of marine species. Current meagre fisheries in Portugal were described in detail for the first time in Chapter 2 (Prista, Jones *et al.*, 2008; Prista, Santos *et al.*, 2008). In Portugal, adult meagres are actively targeted by local a local small-scale fleet that operates within the Tagus estuary (Prista, Jones *et al.*, 2008) and a significant by-catch of a tuna trap that operates in the Southern coast near the mouth of the Guadiana River (Santos *et al.*, 2002; Prista, Santos *et al.*, 2008). This exploitation pattern is similar to the one verified in the Gulf of Cádiz where meagre is caught by an artisanal fleet operating in the vicinities of the Guadalquivir entrance (González-Quirós *et al.,* 2011) and in France where large meagres are caught in the Gironde Estuary during the fish spawning season (Quéro and Vayne, 1987; Lagardère and Mariani, 2006). Adult meagres are also fished along shallow coastal areas of the Banc d'Arguin (Tixerant, 1974) and in coastal lagoons of the Nile Delta (Rizk and Hashem, 1981; Bebars *et al.*, 1997).

Studies on the growth and reproduction of meagre and other large valuable fish like tuna or swordfish have always been constrained by difficulties in obtaining biological samples with sufficient sample size at reasonable costs (e.g., Pilling *et al.*, 2006–2009). In the case of *Argyrosomus*, the high ex-vessel prices of adult specimens and the absence of the fish from fishery-independent surveys have proven the most significant challenge to biological studies. Examples of the latter include: (1) Quéro and Vayne (1987) who report on only 920 meagre collected during research cruises in Bay of Biscay from 1965 to 1987; (2) Costa *et al.* (2005) who did not capture adult specimens in otter trawl surveys carried out in Tagus spawning grounds; and (3) the generalized absence of meagre from IPIMAR coastal surveys in Portuguese waters (F. Cardador, INRB/IPIMAR, pers. comm., 2008). Similar difficulties were reported from other geographical areas including South Africa (Griffiths, 1996) and Australia (Farmer, 2008). Research described in Chapter 3 (Prista *et al.*, 2007) addressed these difficulties by seeking a collaborative strategy with market dealers and developing a new sampling methodology, termed Commercial Mark-Recapture (CMR), that successfully decreased sampling costs while ensuring a large unbiased sample. During meagre sampling in Portugal the application of commercial mark-recapture (CMR) in the Southern coast was coupled to a collaboration with Western coast retailers (that supplied adult fish from the Tagus spawning ground) and specimens from juvenile fishery independent surveys carried out by other colleagues, thus assuring the collection of a significant amount of biological material from all size classes (Costa *et al.*, 2008). Other large valuable fish species from Portuguese waters may come to benefit from the application of the CMR. Among these are other data-poor species (e.g., *Polyprion americanus*, wreckfish) and some internationally-exploited marine resources (e.g., *Xiphias gladius*, swordfish; *Thunnus thynnus*, bluefin tuna).

Age and growth studies constitutes a major source of information for fisheries scientists and are of fundamental importance in modern-day fish population modeling and management advice (Campana, 2001). The age and growth of European meagre was described for the first time using otolith thin sections in Chapter 4 (Prista *et al.*, 2009). Previous studies involving meagre age determination used methodologies that, although sophisticated at the time, are presently considered inaccurate (Tixerant, 1974; Hermas, 1995). Using otolith thin sections the previous maximum lifespan of meagre (31 years, Tixerant, 1974) was updated to 43 years old (Prista *et al.*, 2009) and two companion papers are presently being prepared that demonstrate the validity of the age determination criteria and present data on the growth of Portuguese meagre. For the time being, Prista *et al.* (2009) appears to have been accepted as standard reference in age determinations carried out in southern Spain and the coast of France (González-Quirós *et al.* 2011; Morales-Nin, 2012; N. Prista, unpub. data). Such standardization of procedures is expected to increase the comparability of results, contributing significantly to a better understanding of the meagre life-cycle in this geographical area.

To date the reproduction of wild meagre from European waters was only studied in the Gulf of Cádiz (González-Quirós *et al.,* 2011). Most other studies refer to aquaculture production (e.g., Schiavone *et al.*, 2012; Duncan *et al.*, 2012) or to different geographical areas and were based

on gross anatomical scales of gonad maturity and GSI analyses (Tixerant, 1974, Hermas, 1995). Notable exceptions are the work of Lagardère and Mariani (2006) who report on passive acoustic recordings made in the Gironde estuary. Most previous research indicated that the meagre spawned only in the Gironde and Nile estuaries and in the Banc d'Argin (Quéméner, 2002). The research included in Chapter 5 (Prista *et al.*, 2014, Chapters 5B and 5C) and other unpublished evidence including data from passive acoustic recordings carried out in the Tagus estuary (N. Prista and M.C.P. Amorim, unpub. data) show that this estuary is a spawning ground for the species. The presence of late spawning capable fish with post-ovulatory follicle complexes in the Southern Tuna trap during early summer is indicative that spawning may also take place in that area. Anecdotal reports of incidental captures of large schools of adult meagre by Spanish purse-seiners operating at the Guadiana river mouth (N. Prista, pers. obs.), the presence of juveniles inside the Guadiana estuary (Bexiga, 2002; Chícharo 2006; N. Prista, pers. obs.), and the considerable chorusing activity registered inside this estuary during spawning months (N. Prista and M.C.P. Amorim, unpub. data) also indicate that the Guadiana estuary (and most probably also its adjoining coastal area) is a spawning ground for this species. Other possible spawning grounds are the Mira estuary and the Sado estuary. On the Mira estuary commercial and recreational fisher's report adults catches during the reproductive season (J.L. Costa, CO-FCUL, pers. comm., 2008). However, in the Sado there seems to be no account of its present fishery. North of the Tagus, no adult spawning ground or estuarine nursery has been identified, including in the regularly monitored estuarine environments of the Mondego and Douro rivers (Costa *et al.*, 2004; Costa *et al.*, 2005).

One important component of reproductive studies is the determination of size-at-maturity of fish populations. Size-at-maturity estimates for male meagre were provided by González-Quirós *et al.* (2011) (males only) and by Costa *et al.* (2008) for male and females. The estimates presented by the latter study are currently being revised based on the new histological work presented in Chapter 5 and will be published in a companion paper that is currently being prepared. However, it is worth mentioning that Costa *et al.* (2008) reported 50% male maturity at 53.5 cm and 50% female maturity at 82.0 cm. These estimates were derived from combined macroscopical and histological observations and considered sperm production as an indication of male maturity. Similarly, González-Quirós *et al.* (2011) estimated size at maturity of males to be 61.6 cm. This size is also much smaller than females (all females <76 cm total length were found to be inactive or immature). The latter authors reference Micale *et al.* (2002) and Wallace and Selman (1981) as protocols for their histological work but left unclear the specific criteria they used to assess male maturity and did not provide data on the gonadossomatic index or gonad appearance. The results of Chapter 5A are in concordance with the existence of small males (<60 cm) that produce sperm. However, the histological analyses and the covariate age data indicated that these males are young, display several histological signs of immaturity, and produce little sperm, being unlikely to contribute significantly to the annual reproductive output of the population. Evidence collected from females (Chapter 5B) appears to corroborate such view with young females displaying some maturity signs (cortical alveoli) but not proceeding

towards spawning in the current season. Altogether, these set of results suggests that in fish with a large size range, long lifespan and fast growth, direct interpretation of maturity based on standardized rules of thumb like sperm production (in males) or presence of cortical alveoli (in femailes) can be troublesome and that detailed studies of reproductive development should be made before management measures can be appropriately supported.

Adequate knowledge of stock structure is an important subject for fisheries management and an interesting subject from both an ecological and evolutionary perspective. Presently, meagre captures are considerably higher off Morocco, Mauritania and Egypt than off most European fishing grounds, namely Portugal (FAO, 2012a). Under a single stock situation overexploitation of the meagre in Northern African waters would likely have direct impact on the European meagre populations, compromising both capture production and conservation efforts in Portugal, Spain and France. The genetic evidence presented in Chapter 6 (Haffray *et al.*, 2012) indicates that Mediterranean and Atlantic meagre stocks are quite distinct and that substantial population structure may also exist within the Atlantic range of the species. The possibility that fish from these regions might constitute different stocks had already been noticed by Tixerant (1974) who reported on substantial differences in the internal and external morphology of otoliths colllected in Mauritania, France and the Eastern Mediterranean. During the course of the present study similar evidence was collected from otolith thin sections with some fish caught off Morocco displaying an annuli pattern markedly distinct from the one displayed by Portuguese meagre (N. Prista, unpub. data). On the contrary, fish from the western and southern Portugal are not distinguishable based on such gross analysis to their internal structure. The study of Haffray *et al.* (2012) indicates that the meagre populations in Europe are likely to be much more fragmented than previously thought. It also indicates that populations from Western and Southern coast of Portugal may in fact be spatially segregated. Such separation of the Iberian populations into a Western and Southern component was also suggested by González-Quirós *et al.* (2011) that considered the Southern Portugal and Gulf of Cádiz meagre to form a continuum. Such a separation finds further support in the incidence of *Philometra* reported in Chapters 5A, 5B and 5D as this parasite was overwhelmingly associated to fish gonads collected from the Southern coast. The currently planned reanalysis of Costa *et al.* (2008) growth and reproduction data and future tagging studies on meagre (B. Quintella, CO-FCUL, pers. comm., 2013) will likely contribute to clarify this issue. For the time being, considering the relatively small size of the fisheries involved and the gaps that still remain on our knowledge of the distribution of meagre in Iberian waters, the iberian meagre fisheries are probably better-off managed as a single Iberian stock.

Meagre management was approached from a data-poor fisheries perspective in Chapter 7 (Prista *et al.*, 2011). The statistical process control technique used monitor meagre in that chapter requires only monthly landings data. No prior biological knowledge on meagre stock structure or its biological properties is necessary. This study indicated that the meagre fishery in the Tagus was stable from a statistical process control perspective. However, as mentioned by Prista *et al.* (2011) its intent was to provide a completely un-assessed data-poor stock with

some degree of monitoring and *not* to make an assessment on the status or the sensitivity to fishing of the meagre population that spawns in the estuary. In respect to the latter, the yield-per-recruit models developed by Jones and Wells (2001) and González-Quirós et al. (2011) likely provide for a much better approach.

5. Final Remarks

Prior to this research, the meagre was large valuable fish hardly known to European science and aquaculture. Presently, the fish and its fisheries have left their originally data-poor situation and been broadly characterized not only in Portugal but also in several other European countries. Additionally, its aquaculture production has started in Portugal and other Mediterranean countries and is currently expanding (FAO, 2012b). The fish is still fished mostly by small-scale local fisheries and recreational anglers and continues to be expensive and hard to sample. Consequently it is expected that the meagre will continue to be a data-poor resource for some time longer and that it will remain difficult to monitor it or assess it at the levels practiced in other commercial species exploited at industrial level. To those interested in pursuing research on meagre and/or wishing to draw from its fishery and life-cycle interpretations that may generalize to other species, a few research lines can be suggested. On the one hand, there seems to be great potential in the further exploration of comparisons between present day otoliths and those found in archaeological remains. Under some assumptions, such studies could provide interesting evidence into the growth parameters of the meagre populations when they were experiencing much lower fishing effort but also insights into the impact of climate change and other anthropogenic pressures on fish communities. An additional line of research that is suggested involves the study of the migration of adult and juvenile meagre. Such developments would not only be important for the fisheries management but also provide a background for validating its high level of genetic differentiation. Furthermore, to date, the overwintering grounds of meagre remain a mystery and restricted to sparse records of huge schools of adult fish being caught in purse-seines (by mere chance) extremely far from currently known spawning grounds (Quéro and Vayne, 1993). Discovering the overwintering grounds of the meagre will prove to be an interesting methodological challenge whereby different tactics such as interviews with fishers across a large geographic range, passive hydroacoustics, and sophisticated tags may have to be combined.

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