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Towards biological and ecological knowledge of *Lophius* spp. in the NW Mediterranean Sea for a sustainable fishery

Ana Isabel Colmenero Ginés

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Ana Isabel Colmenero Ginés

PhD Thesis

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TOWARDS BIOLOGICAL AND ECOLOGICAL KNOWLEDGE OF *Lophius* spp. IN THE NW MEDITERRANEAN SEA FOR A SUSTAINABLE FISHERY

Memoria presentada por **Ana Isabel Colmenero Ginés** para optar
al grado de Doctora por la Universitat de Barcelona
Programa de Doctorado en Ciencias del Mar

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A mis padres

Cerca del mar, porque yo

nací en el Mediterráneo…

(J. M. Serrat 1971)

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En el transcurso de esta Tesis he aprendido muchas cosas, del mar, de la ciencia, de las personas que me rodean, pero sobre todo de mí misma. Después de tantos años surcando el Mar de Bering nunca me hubiera imaginado que dejaría Alaska por embarcarme en esta nueva aventura, que finalmente ha llegado a buen puerto. Pero lo hice, y de una manera fortuita llegué al Instituto de Ciencias del Mar hace ya 11 años de la mano de Toni Lombarte y Laura Recasens a los cuales tengo que agradecerles eternamente que contaran conmigo porque sin ese primer empujón nada de esto hubiera sido posible.

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ABSTRACT

In the northwestern Mediterranean Sea, anglerfishes *Lophius budegassa* Spinola, 1807 and *Lophius piscatorius* Linnaeus, 1758 are among the most valuable demersal species and support an important fishery. Both anglerfishes are mainly caught in mixed bottom trawl fisheries operating in the continental shelf and upper slope. Their landings, which are primarily composed of *L. budegassa* (86%) and a small percentage of *L. piscatorius* (14%), have increased lately with the development of directed fisheries reaching 9,486 t with a value of 65 million of Euros over the period 2000-2016. Up to date, European Commission has been conducted stock assessment for *L. budegassa* inhabiting Mediterranean waters but no effort has been made to assess *L. piscatorius*.

Due to the commercial importance of both *Lophius* species, the aim of this Thesis is to improve the knowledge of the population parameters of *L. budegassa* and *L. piscatorius* of the northwestern Mediterranean Sea in order to determine the state of exploitation of both species and establish an effectively management. To achieve this aim, a set of specific objectives are addressed: to determine the behavioural activity and sensory mechanisms for reducing the levels of their ecological competition; to describe the reproductive parameters for a better understanding of the life cycle of both *Lophius* spp.; to examine morphological characteristics of ova in the two species of *Lophius* for aid in identification of eggs from ichthyoplankton samples; to provide new data on population dynamics as well as on population structure and biometric relationships for modelling the stock of both *Lophius* spp.; to explore the sustainability indicators for monitoring the state of *Lophius* population relative to exploitation; and to analyse the prevalence of the microsporidian parasite *Spraguea lophii* for hypothesize about the health status of the population of *Lophius* spp. and the possible side effects of severe infections on their morphology.

The results demonstrate that although *L. budegassa* and *L. piscatorius* occupy the same habitat in the northwestern Mediterranean Sea, differences noted in their life history traits support the hypothesis that both species of *Lophius* have different ecological strategies in order to lessen competition between them. According to the differences found in the life history traits for both species, it is highly recommended to carry out a differentiated management for each *Lophius* species in the northwestern Mediterranean Sea.

In conclusion, this Thesis improves the current understanding of the biology and ecology of *L. budegassa* and *L. piscatorius*; and it is the first study to provide information on the reproductive strategy of *L. piscatorius* in the northwestern Mediterranean Sea; contributing to increase the scientific knowledge of both *Lophius* spp. which will be useful to improve future stock assessments and ensure proper management actions.

En el noroeste del mar Mediterráneo, las especies de rape *Lophius budegassa* Spinola, 1807 y *Lophius piscatorius* Linnaeus, 1758 se encuentran entre las especies demersales más valiosas sobre las cuales se efectúa una actividad pesquera importante. Ambas especies son capturadas generalmente en pesquerías mixtas de arrastre de fondo que operan en la plataforma continental y en el talud superior. Las descargas se componen principalmente de *L. budegassa* (86%) y un pequeño porcentaje de *L. piscatorius* (14%), y han aumentado en los últimos años debido al desarrollo de una pesca más dirigida, alcanzando 9,486 t y un valor de 65 millones de euros durante el período 2000-2016. Hasta la fecha, la Comisión Europea ha realizado evaluaciones de las poblaciones de *L. budegassa* que habitan las aguas del Mediterráneo, pero no se ha hecho ningún esfuerzo respecto a *L. piscatorius*.

Atendiendo a la importancia comercial de estas especies, el objetivo de esta Tesis ha sido mejorar el conocimiento de los parámetros de la población de *L. budegassa* y *L. piscatorius* del noroeste del Mar Mediterráneo para determinar su estado de explotación y establecer una gestión efectiva. Para alcanzar dicho objetivo, se plantearon un conjunto de objetivos específicos: determinar su comportamiento y los mecanismos sensoriales para reducir los niveles de competencia ecológica; describir los parámetros reproductores para mejorar la comprensión del ciclo de vida; examinar las características morfológicas de los óvulos para identificar los huevos a partir de muestras de ictioplancton; proporcionar nuevos datos sobre la dinámica de la población, así como sobre la su estructura y las relaciones biométricas para modelar los stocks; explorar los indicadores de sostenibilidad para monitorear el estado de la población en relación con la explotación; y analizar la prevalencia del parásito microspordio *Spraguea lophii* para formular hipótesis sobre el estado de salud de la población de *Lophius* y los posibles efectos secundarios de infecciones graves en su morfología.

Los resultados han demostrado que *L. budegassa* y *L. piscatorius* ocupan el mismo hábitat en el noroeste del Mediterráneo, si bien las diferencias observadas en sus parámetros del ciclo de vida apoyan la hipótesis de que ambas especies tienen diferentes estrategias ecológicas para disminuir la competencia entre ellas. De acuerdo con las diferencias encontradas, se recomienda realizar una gestión diferenciada para cada especie de *Lophius* en el noroeste del Mar Mediterráneo.

En conclusión, esta Tesis mejora la comprensión actual de la biología y la ecología de *L. budegassa* y *L. piscatorius*; y es el primer estudio que proporciona información sobre la

estrategia reproductiva de *L. piscatorius* en el noroeste del Mar Mediterráneo; contribuyendo a aumentar el conocimiento científico de ambas especies que ayudará a mejorar las evaluaciones futuras de las poblaciones y asegurar acciones de gestión adecuadas.

Al nord-oest de la mar Mediterrània, les espècies de rap *Lophius budegassa* Spinola, 1807 i *Lophius piscatorius* Linnaeus, 1758 es troben entre les espècies demersals més valuoses sobre les quals s'efectua una activitat pesquera important. Les dues espècies són capturades generalment en pesqueres mixtes d'arrossegament de fons que operen a la plataforma continental i en el talús superior. Les descàrregues es componen principalment de *L. budegassa* (86%) i un petit percentatge de *L. piscatorius* (14%), i han augmentat en els últims anys degut al desenvolupament d'una pesca més dirigida, aconseguint 9,486 t i un valor de 65 milions d'euros durant el període 2000-2016. Fins ara, la Comissió Europea ha realitzat avaluacions de les poblacions de *L. budegassa* que habiten les aigües de la Mediterrània, però no s'ha fet cap esforç pel que fa a *L. piscatorius*.

Atenent a la importància comercial d'aquestes espècies, l'objectiu d'aquesta Tesi ha estat millorar el coneixement dels paràmetres de la població de *L. budegassa* i *L. piscatorius* del nord-oest del Mar Mediterrani per determinar el seu estat d'explotació i establir una gestió efectiva. Per assolir aquest objectiu, es van plantejar un conjunt d'objectius específics: determinar el seu comportament i els mecanismes sensorials per reduir els nivells de competència ecològica; descriure els paràmetres reproductors per millorar la comprensió del cicle de vida; examinar les característiques morfològiques dels òvuls per identificar els ous a partir de mostres d' ictioplancton; proporcionar noves dades sobre la dinàmica de la població, així com sobre la seva estructura i les relacions biomètriques per modelar els estocs; explorar els indicadors de sostenibilitat per monitoritzar l'estat de la població en relació amb l'explotació; i analitzar la prevalença del paràsit microsporidi *Spraguea lophii* per formular hipòtesis sobre l'estat de salut de la població de *Lophius* i els possibles efectes secundaris d'infeccions greus en la seva morfologia.

Els resultats han demostrat que *L. budegassa* i *L. piscatorius* ocupen el mateix hàbitat al nord-oest de la Mediterrània, tot i que les diferències observades en els seus paràmetres del cicle de vida donen suport a la hipòtesi que les dues espècies tenen diferents estratègies ecològiques per disminuir la competència entre elles. D'acord amb les diferències trobades, es recomana realitzar una gestió diferenciada per a cada espècie de *Lophius* al nord-oest del Mar Mediterrani.

En conclusió, aquesta Tesi millora la comprensió actual de la biologia i l'ecologia de *L. budegassa* i *L. piscatorius*; i és el primer estudi que proporciona informació sobre l'estratègia reproductiva de *L. piscatorius* al nord-oest del Mar Mediterrani; contribuint a augmentar el coneixement científic de les dues espècies que ajudarà a millorar les avaluacions futures de les poblacions i assegurar accions de gestió adequades.

CHAPTER 1

INTRODUCTION

Phylogeny of Lophiiformes

The order Lophiiformes (Teleostei, Actinopterygii) contains a diverse group of marine fish distributed throughout the world's oceans. Commonly referred to as anglerfishes, they are characterized by the structure of the first dorsal fin spine, typically placed out on the tip of the snout and modified to serve as a luring apparatus (Pietsch 2005). This order includes approximately 377 valid living species (Eschmeyer and Fong 2017), distributed among 66 genera and 18 families. The families themselves are organised into five suborders (Pietsch 1984, Pietsch and Grobecker 1987, Pietsch 2005) (Figure 1).

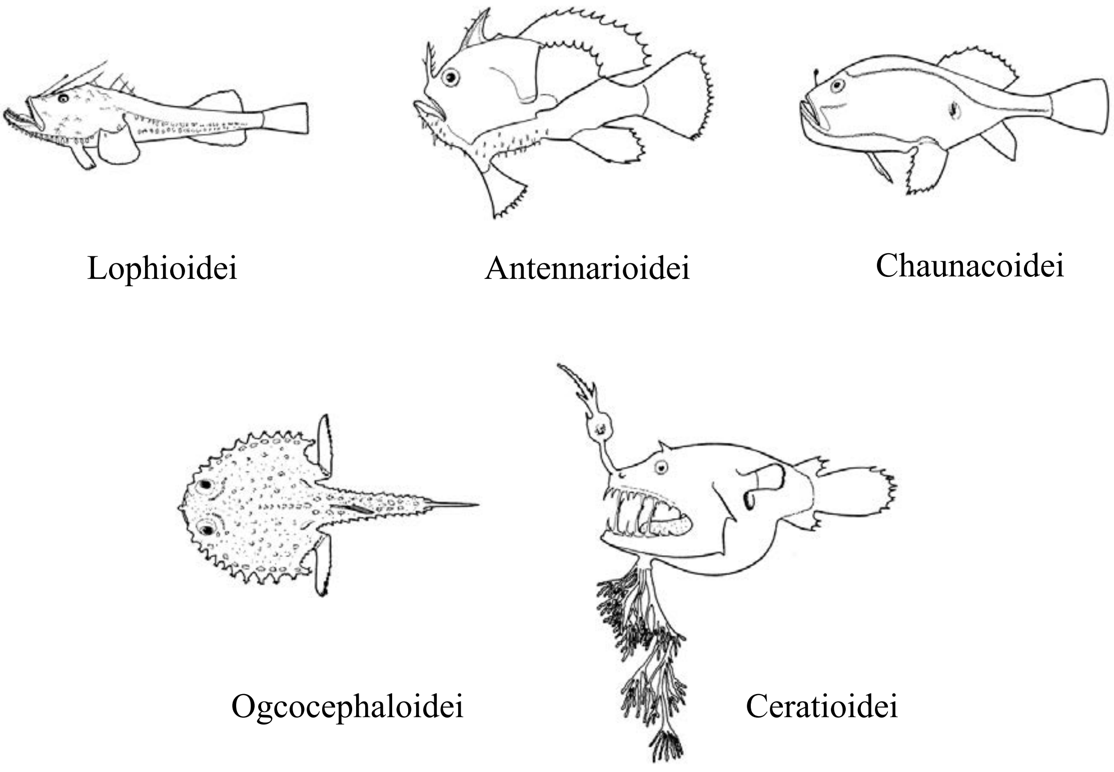


Figure 1. Suborder organisation of 18 families of the order Lophiiformes (Source: Nelson 2006).

The phylogenetic analysis of the order Lophiiformes is based on 23 morphological characters proposed by Pietsch and Grobecker (1987) (Figure2).

The suborder Lophioidei (Caruso 1981, 1983), contains a single family, the Lophiidae, with four genera (*Sladenia* Regan, 1908; *Lophiodes* Goode and Bean, 1896; *Lophiomus* Gill, 1882; and *Lophius* Linnaeus, 1758) (Figure 3) and 25 species (Caruso 1985).

The genus *Lophius*, commonly known as anglerfish, monkfish, or goosefish, belong to a family of bathydemersal fishes, which includes 7 species distributed worldwide. The hypotheses

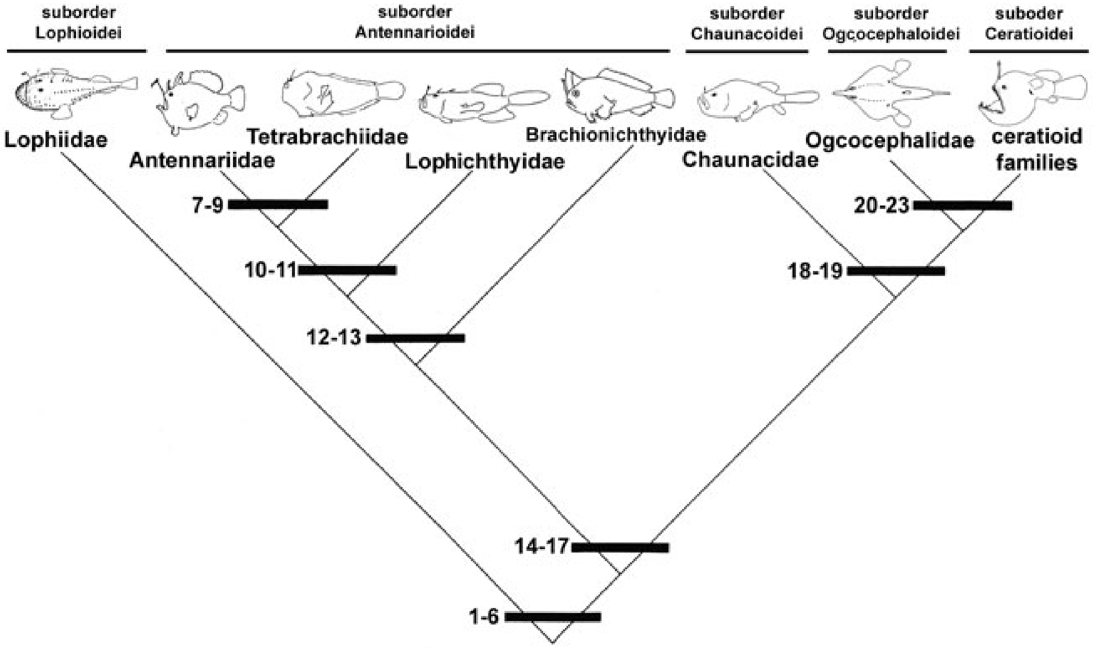


Figure 2. Phylogenetic relationships of Lophiiformes proposed by Pietsch and Grobecker (1987).

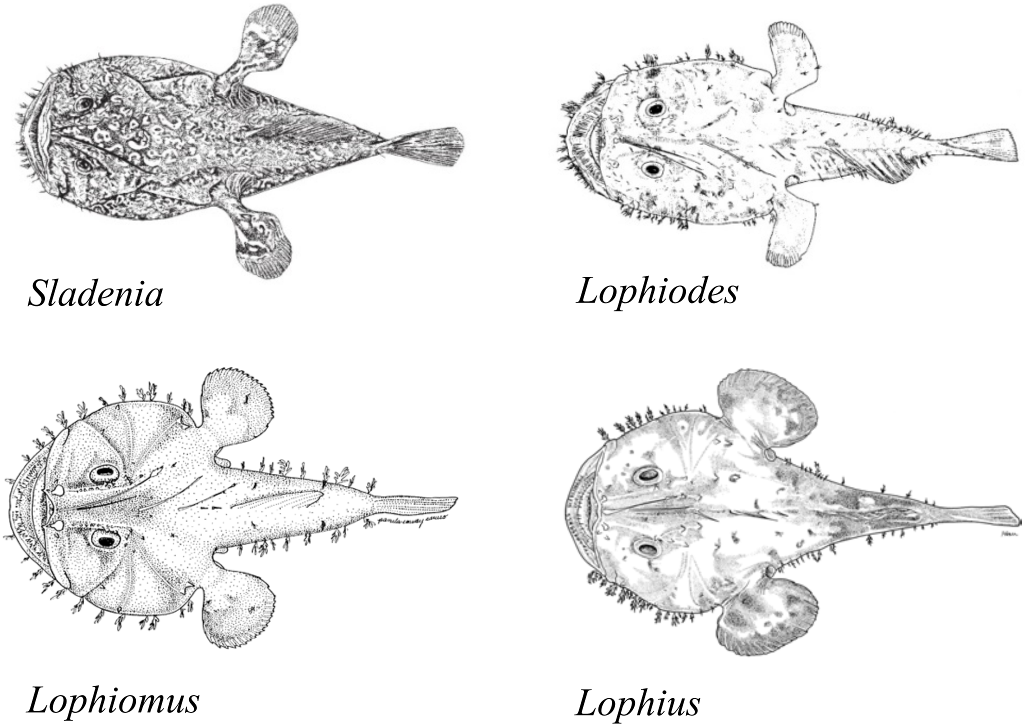


Figure 3. Genera classification of the family Lophiidae (Source: Caruso 2005).

proposed by Caruso (1977) for the phylogeny of the genus was that small numbers of pectoral and dorsal fin rays and vertebrae represented the primitive state and large numbers the derived state. Among species of *Lophius*, *Lophius vaillanti* Regan, 1903 has the fewest pectoral fin rays (19–24), so according to Caruso's hypothesis, it may be phylogenetically the most primitive species of the genus. *Lophius americanus* Valenciennes, 1837 and *Lophius piscatorius* Linnaeus, 1758 have relatively large numbers of vertebrae (30–31) and dorsal fin rays, so may represent the most derived types, although the similarity of the morphological characters could be the result of convergence processes between distantly related species (Caruso 1983, Leslie and Grant 1994).

The genus *Lophius* has a rhomboid shape body, and it is characterized by a depressed head and body with a flattened ventral side. The mouth is wide and cavernous, with strong jaws with slender depressible and recurved teeth. The lower jaw projects well beyond the upper one. The skin is loose, thin and scaleless protected by a mucous layer. The dorsal skin has a brownish colour that changes accordingly with the surroundings, while the ventral skin is white, showing almost no pigmentation. Lateral margins of head, lower jaw and body hold numerous fleshy tendrils; and the dorsal surface of head has prominent bony spines. First ray of the dorsal fin is modified into a mobile fishing apparatus, the illicium (line) with a terminal esca (bait), which serves as a lure, attracting prey to the mouth (Caruso 1986, Afonso-Dias 1997).

The two Mediterranean species *Lophius budegassa* Spinola, 1807 and *L. piscatorius* are very similar, being distinguished mainly by the colour of the peritoneum and by the number of rays in the second dorsal and anal fins. *Lophius budegassa* has black peritoneum with 9-10 rays in the second dorsal fin and 8-9 in the anal fin. *Lophius piscatorius* has white peritoneum with 11-12 rays in the second dorsal fin and 9-10 in the anal fin (Caruso 1986) (Figure 4).

Biogeography and current distribution

The origins of the related species of *Lophius* scattered throughout the world's oceans are linked to geological and biogeographical events, such as tectonic activity, genetic divergence, colonization, and geographic isolation, regression and expansion (Fariña et al. 2008). Grant and Leslie (1993) suggested that the genus *Lophius* arose from a common ancestor of *Lophiomus*, a monotypic genus distributed throughout the western Pacific and Indian Ocean, by the closure of the Tethys Sea. The tropical eastern Atlantic *L. vaillanti* represents the most morphologically

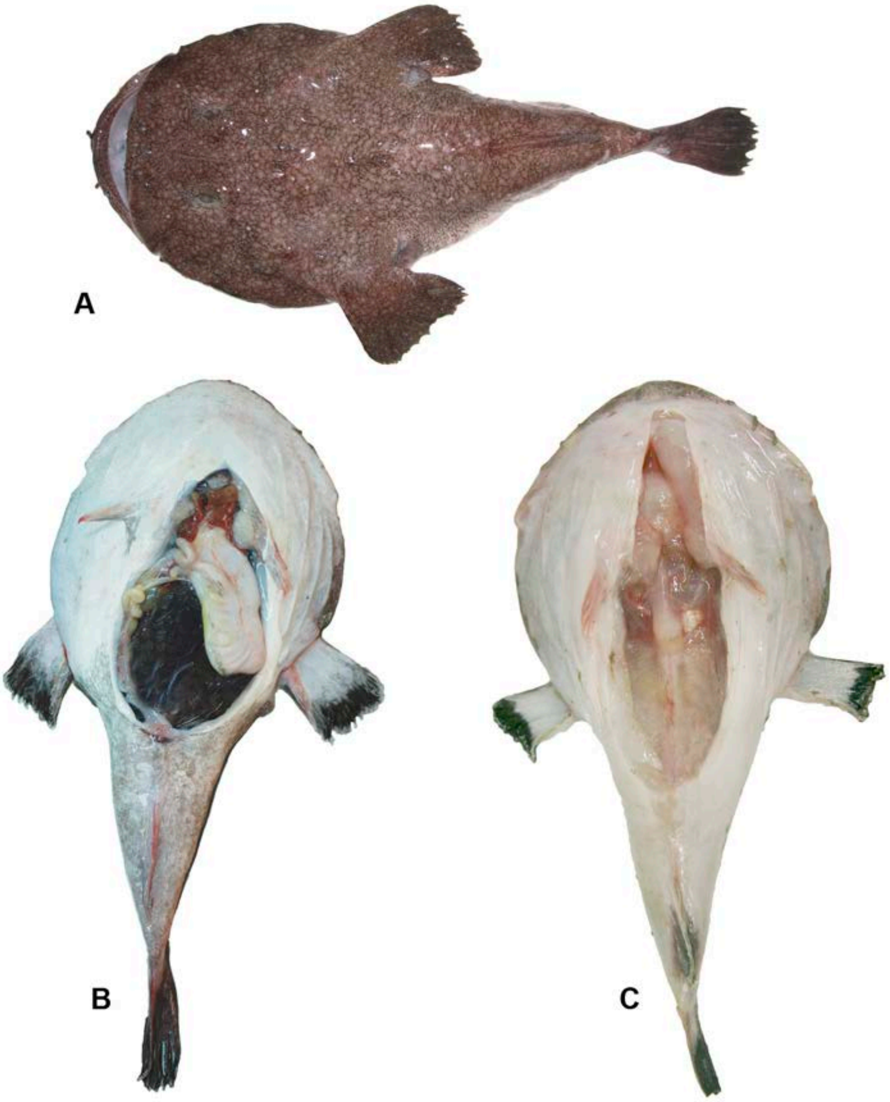


Figure 4. Dorsal view of (A) *Lophius* sp. and ventral view of (B) *L. budegassa* and (C) *L. piscatorius* showing the colour of the peritoneum.

and genetically primitive of the *Lophius* species. Tectonic separation of the South American and African Plates split the ancestral tropical population and led to the appearance of *Lophius gastrophysus* Miranda Ribeiro, 1915 in the West and the South Atlantic. A northward expansion of the range and dispersal from ancestral South Atlantic populations gave rise to *L. americanus*. An ancestral European species of *Lophius* arose from an ancestral North American species by a northeasterly expansion or dispersal of larvae or adults. Palaeo-oceanographic events in the Mediterranean Sea allowed the appearance of the two European species, *L. piscatorius* and *L. budegassa*. Dispersal of a European ancestor along the West African coast led to the appearance of *Lophius vomerinus* Valenciennes, 1837 in the eastern South Atlantic. This species

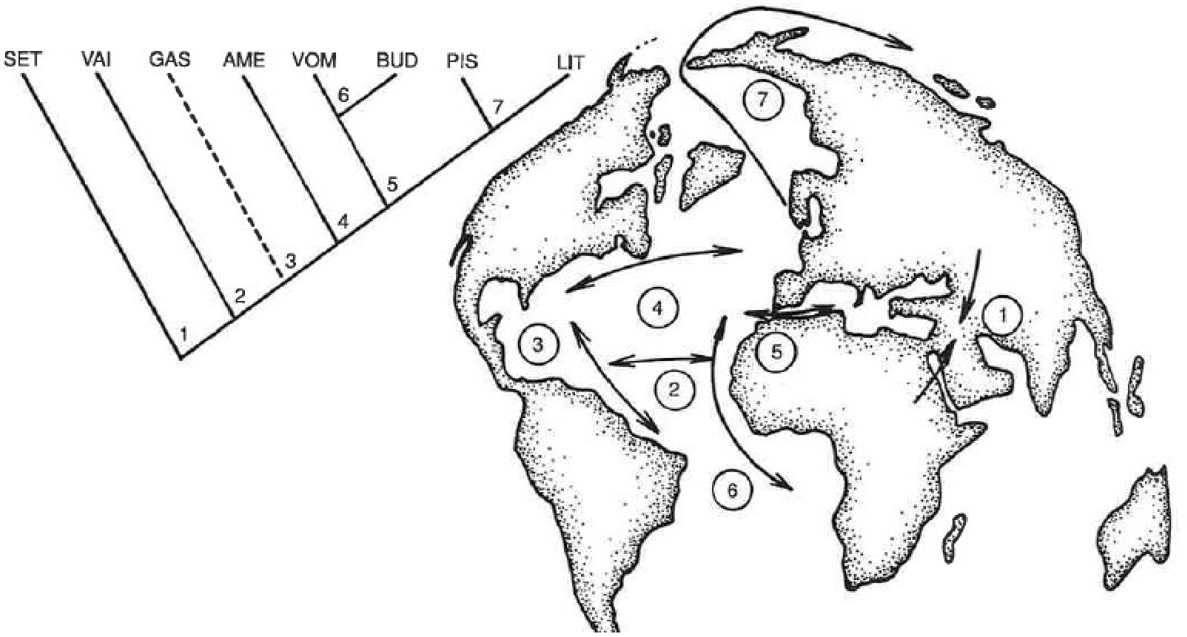


Figure 5. Geographical theory for the initial separation of *Lophius* ancestor based on Grant and Leslie 1993 (SET: *Lophiomus setigerus* Vahl, 1797; VAI: *L. vaillanti*; GAS: *L. gastrophysus*; AME: *L. americanus*; VOM: *L. vomerinus*; BUD: *L. budegassa*; PIS: *L. piscatorius*; LIT: *L. litulon*).

is considered a probable sister taxon of *L. budegassa*. *Lophius litulon* Jordan, 1902 arose by long-distance dispersal from the North Atlantic to the western North Pacific through a warmer Arctic Ocean after the Pliocene opening of the Bering Strait (Figure 5). *Lophius piscatorius* and *L. litulon* are sister taxa. The lack of colonization in the eastern North Pacific or posterior extinctions may explain the non-existence of *Lophius* in other areas of the Pacific.

Currently, the shortspine african angler (*L. vaillanti*) is found in the eastern Atlantic (Maartens and Booth 2005). The devil anglerfish (*L. vomerinus*) occupies the southeast Atlantic and the northern and western Indian Ocean (Walmsley et al. 2005). The blackfin goosefish (*L. gastrophysus*) inhabits the western Atlantic, and the goosefish (*L. americanus*) occurs in the northwest Atlantic (Caruso 1983). The yellow goosefish (*L. litulon*) is distributed in the northwest Pacific, in the Gulf of Po-Hai, in the Yellow Sea, and in the East China Sea (Yoneda et al. 1997). The white anglerfish (*L. piscatorius*) is distributed in the northeast Atlantic Ocean from the southwestern Barents Sea and Iceland to the Strait of Gibraltar, including the Mediterranean and the Black Sea (Caruso 1986, Solmundsson et al. 2010). The black anglerfish (*L. budegassa*) coexists with the white anglerfish over most of its range, although it has a more southerly distribution in the Atlantic Ocean, from the British Isles to Senegal (Caruso 1986) (Figure 6).

Fisheries of Lophius species

The genus *Lophius* have been exploited worldwide; at the beginning as bycatch in mixed fisheries, but in the last decades, they have acquired great importance from a commercial point of view and their world catch has increased with the improvements in fishing technology (1,701,847 t from 2000 to 2015, FAO) (Figure 7) leading to a target anglerfish fisheries

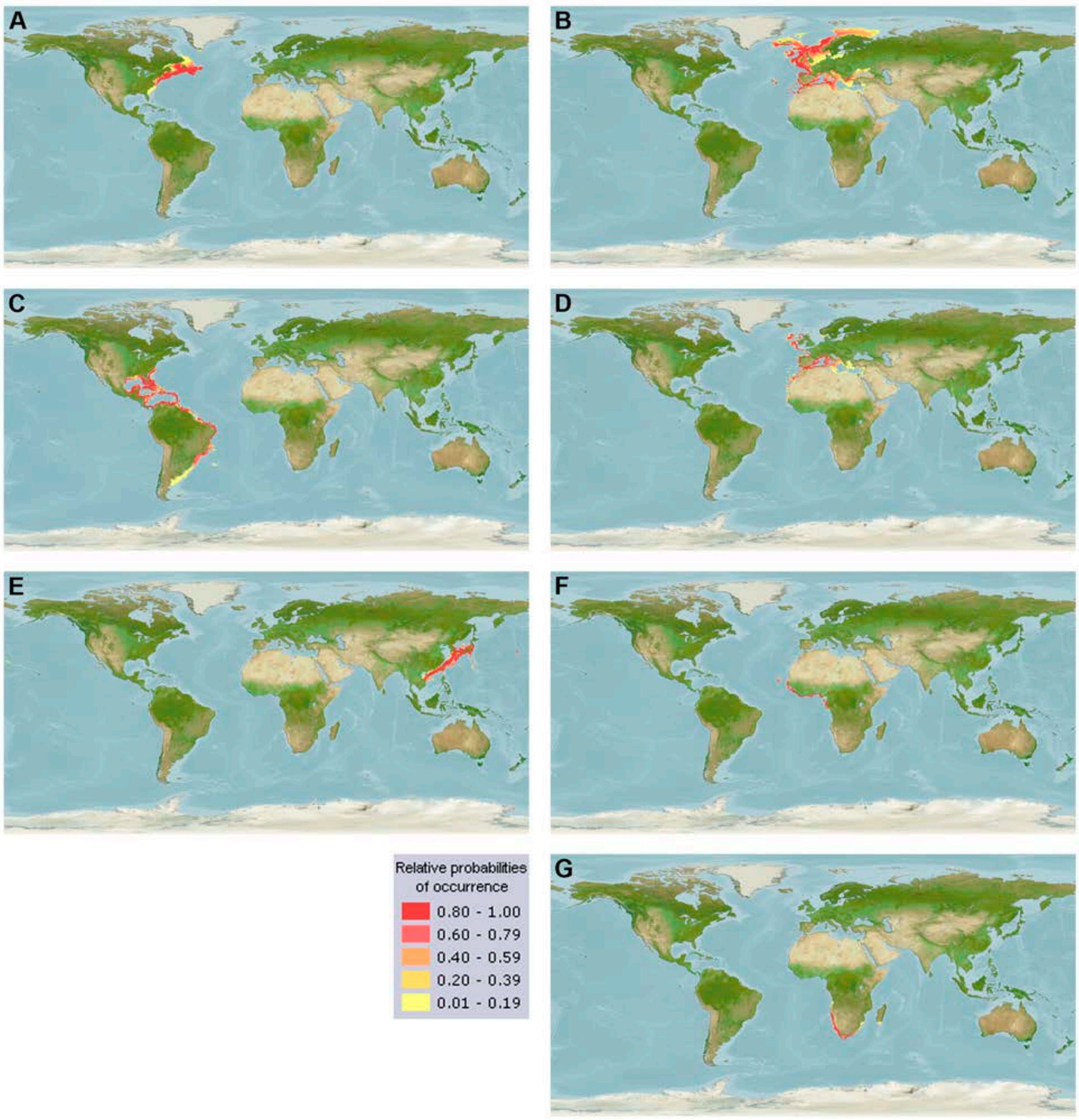


Figure 6. World map showing the current distribution of *Lophius* species. **A**, *L. americanus*; **B**, *L. piscatorius*; **C**, *L. gastrophysus*; **D**, *L. budegassa*; **E**, *L. litulon*; **F**, *L. vaillanti*; **G**, *L. vomerinus* (Source: Aquamaps).

(Fariña et al. 2008). *Lophius* are caught with trawl and fixed nets, mostly gillnets; and they are managed through one or a combination of a total allowable catch (TAC), effort control, mesh size restrictions, and seasonal closures.

The two European anglerfish species (*L. budegassa* and *L. piscatorius*) are among the most valuable bottom fisheries in western and southern European waters. Both anglerfishes are mainly caught in mixed bottom trawl fisheries operating in the continental shelf and upper slope, together with other commercially important species, such as european hake (*Merluccius merluccius* Linnaeus, 1758), striped mullet (*Mullus surmuletus* Linnaeus, 1758), red mullet (*Mullus barbatus* Linnaeus, 1758), rose shrimp (*Aristeus antennatus* Risso, 1816) or norway lobster (*Nephrops norvegicus* Linnaeus, 1758) (Demestre and Lleonart 1993, Demestre et al. 1997, Sardà 1998, Recasens et al. 2008). Discards are negligible since these species have high commercial value in the entire size range (Díaz et al. 2008), and are usually caught, landed and recorded together in the ports statistics. According to official data, total landings of *Lophius* spp. in the northwestern Mediterranean Sea have increased over the period 2000-2016 reaching 9,486 t; with a value of 65 million of Euros (Unpublished data from the Directorate of Fishing and Maritime Affaires; Government of Catalonia) (Figure 8).

They were composed primarily of *L. budegassa* (86%) and generally only a small percentage of *L. piscatorius* (14%) but, for landings in Atlantic waters, the opposite is true with *L. piscatorius* (94%) dominating the catch (Dobby et al. 2008). The 96% of the landings were taken in trawlers and the 4% in other fishing gear including seiners, longliners and artisanal fleet.

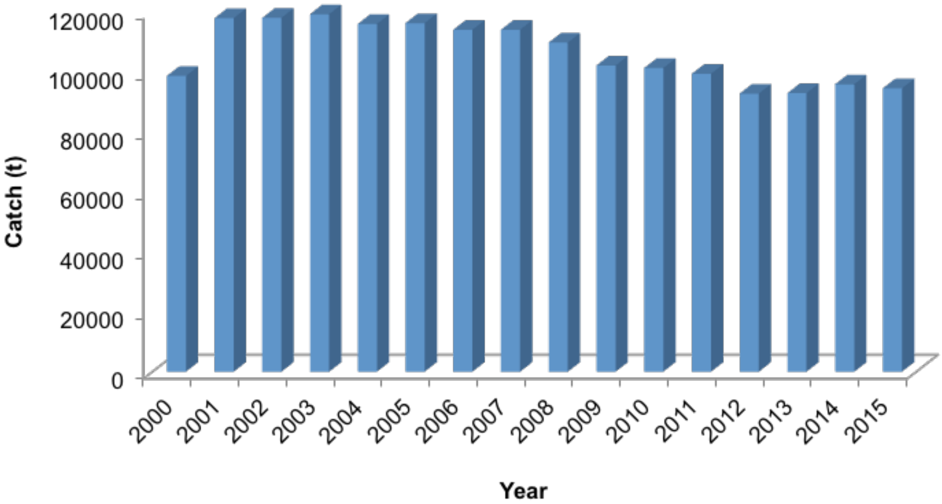


Figure 7. Evolution of *Lophius* world catches from 2000 to 2015 (FAO 2017).

The management of the Mediterranean fisheries is based on effort control. Total allowable catches (TACs) is considered unfeasible (except for swordfish and bluefin tuna) because of the multi-specific character of the fisheries, although other technical measures, such as minimum landing sizes and minimum mesh sizes, are implemented but not always enforced (Lleonart and Maynou 2003). According to Spanish regulations trawling is carried out in fishing grounds beyond the 3 first nautical miles from the coast or deeper than 50 m and shallower than 1000 m, and the activity is limited to a maximum of 5 days per week and 12 hours per day (Real Decreto N°1440/1999 of 10 September 1999 and Orden APA/79/2006 of 19 January 2006). The actual management regulations applied for black anglerfish generally are those applied to bottom trawling (European Union Council Regulation N°1967/2006), with recommendations aimed at reducing the fishing effort of the fleet in order to avoid loss in stock productivity and decreases in landings (Cardinale et al. 2015); together with a local regulation stablishing the minimum landing size at 30 cm TL (Real Decreto N°1615/2005). Although the European Commission previously has conducted stock assessments of *L. budegassa* in the western Mediterranean Sea, there is no corresponding assessment for *L. piscatorius*. The lack of information about the structure of the population of white anglerfish in this region and the lack of knowledge of the basic biology of this species are the main reasons for the absence of any assessment.

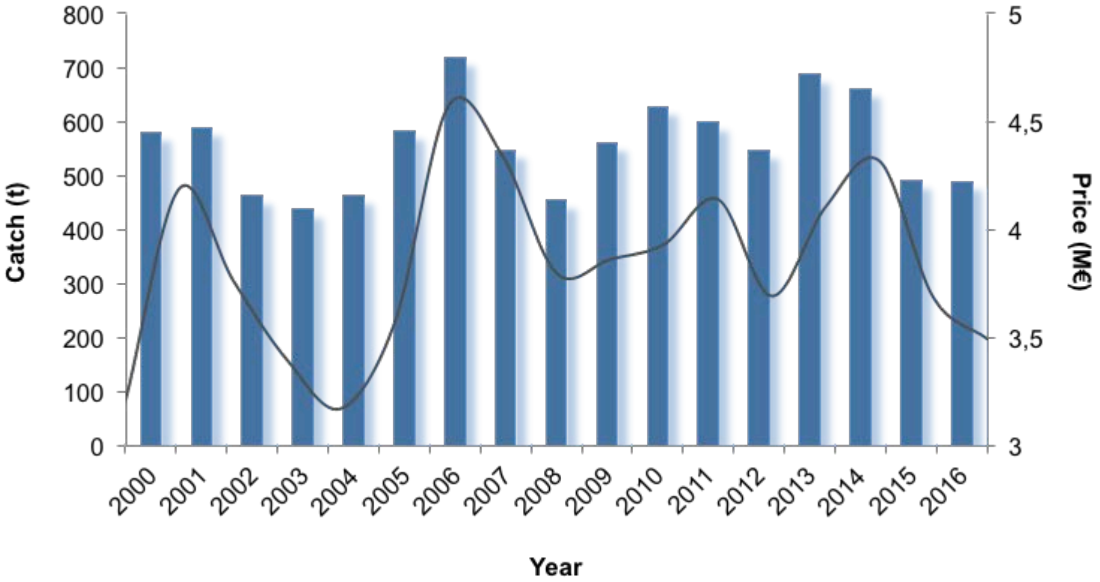


Figure 8. Annual catch (t) and price (M€) of *Lophius* spp. in the Catalan coast.

The importance of biological data on fisheries research

In order to determine the state of exploitation of the resources we need to have a biological data on the species we are evaluating. These data are basically sexual maturity, age, natural mortality and the parameters of length-weight relationships. Some of these parameters are also very important to establish management measures that allow us to carry out a sustainable exploitation of fishing resources. It is well recognized that fishing can induce evolutionary changes in individual life history traits (Enberg et al. 2010) and although phenotypic plasticity allows individuals to respond to harvest-induced alterations in environmental conditions, any changes in these traits might have strong repercussions for population dynamics and sustainability harvesting (Ernande et al. 2004).

In general, species sharing important traits related to their ecological niches can coexist in the same area due to a temporal segregation in the phase of maximum behavioural activity (Carothers and Jaksic 1984); and a rhythmic catch variations in trawl hauling at a certain location can be considered to be the product of rhythmic displacements of marine populations into and out of the sampling window (Aguzzi and Company 2010). Awareness of diel changes in behaviour and habitat use are significant in establishing management policies for fishes because their activity rhythms influences their susceptibility to overfishing.

A study of reproductive biology is important for an understanding of population dynamics, and it is critical for assessing the effects of harvesting on fish populations when attempting to develop appropriate management strategies. Recruitment is recognized as a key process for maintaining sustainable populations, and the relationship between the reproductive output of the population and the resulting recruitment is central to understanding how a fish population will respond to constant stressors such as fishing (Chambers and Trippel 1997). Studies on gonad morphology, reproductive pattern, reproductive behaviour, reproductive cycle, spawning season duration, size at maturity, sex ratio, size at sexual transition, and fecundity could help to evaluate reproductive potential (Koslow et al. 1995) and to serve as a basis for limits on fishing that aim in order to keep recruitment at sustainable levels (García-Díaz et al. 2006). Maturity status and sex identification can be useful indices since the juvenile-to-adult ratio can indicate important aspects of the dynamics of a fish population (Reynolds and Babb 1978). A large ratio of juveniles to adults can be an indication of exploitation, optimal spawning conditions in a particular year, or consistently successful recruitment. A population with recruitment difficulties will be characterized by fewer juveniles relative to adults.

On the other hand, natural mortality and growth parameters (asymptotic length and growth rate) are required to carry out an effective management of the fishery. Biometric relationships such as length-weight are also useful biological parameters, having a wide application in fish biology and fisheries management for predicting weight from length data or for the calculation of production and biomass of a fish stock. The conversion factor (total weight - gutted weight) is also useful in fisheries management due to the commercial landings of this species are available in gutted weight (Landa and Antolínez 2017). The ratio of males to females in a population can also be important because may provide an indication of anthropogenic influences since the sex ratio 1:1 indicates healthy fish communities (Larsson et al. 2000).

Fish populations are sometimes characterized according to their health status. Inspection of individual fish in a random sample can provide data on the proportion of a population with lesions, parasites, or other anomalies (Wilson et al. 1996). A sample with a high proportion of individuals with lesions or parasites is indicative of a population that is likely under stress from either disease or environmental conditions. Stressed individuals may consume less food, grow more slowly, reproduce less successfully, and suffer a higher mortality rate (Pope et al. 2010).

Biological and ecological traits of Lophius budegassa and L. piscatorius

The *Lophius* spp. of the Mediterranean Sea are demersal fishes living in sandy, muddy and rocky bottoms along the continental shelf and slope. *Lophius budegassa* inhabits depths from the shoreline to 800 m and co-occurs with *L. piscatorius* over all its bathymetric range, although white anglerfish has a deeper distribution reaching depths >1000 m (Afonso-Dias and Hislop 1996, Carlucci et al. 2009).

Some authors described migratory behaviour in both juveniles and adults. Horizontal displacements were observed for *L. budegassa* and *L. piscatorius* in response to seasonal changes in water temperature, food availability or gonadal maturation (Landa et al. 2001, Yoneda et al. 2002, Laurenson et al. 2005). Although anglerfishes are mainly bottom dwellers, vertical movements were also reported by Hislop et al. (2000), but the cause of this behaviour is unknown.

Mediterranean anglerfishes share common behavioural traits within the lophiids, especially in their feeding strategy. They are opportunistic predators showing a sit-and-wait ambush behaviour, usually lying half-buried on muddy to gravely bottoms (Figure 9), using their angling apparatus, the *illicium*, as a lure (Crozier 1985, Laurenson and Priede 2005, Preciado et al. 2006). Although an active predatory behaviour has also been described by Laurenson et al. (2004) in the Shetland Isles. They have a wide, cavernous mouth with numerous sharp, backward pointing teeth to prevent the prey from escaping.

The diet spectrum is size-dependent with a similar pattern for both species: invertebrates (crustaceans and cephalopods) make up a significant part of the diet of small juveniles, the consumption of invertebrates decreases with age, and larger juveniles and adults are mainly ichthyophagous (Fariña et al. 2008, Negzaoui-Garali et al. 2008, Stagioni et al. 2013). In the northwestern Mediterranean Sea, the small sizes of *L. budegassa*, more abundant on the continental shelf, mainly feed on small benthic fishes such as *Gobius niger* Linnaeus, 1758; *Lesueurigobius friesii* Malm, 1874; while the large sized individuals, distributed in deeper waters associated with the continental slope, usually feed on *M. merluccius* and *Gadiculus argenteus* Guichenot, 1850. Regarding *L. piscatorius*, the small sized individuals, mainly found in inshore waters, feed on fishes such as *Ophidion barbatum* Linnaeus, 1758 and *Cepola*

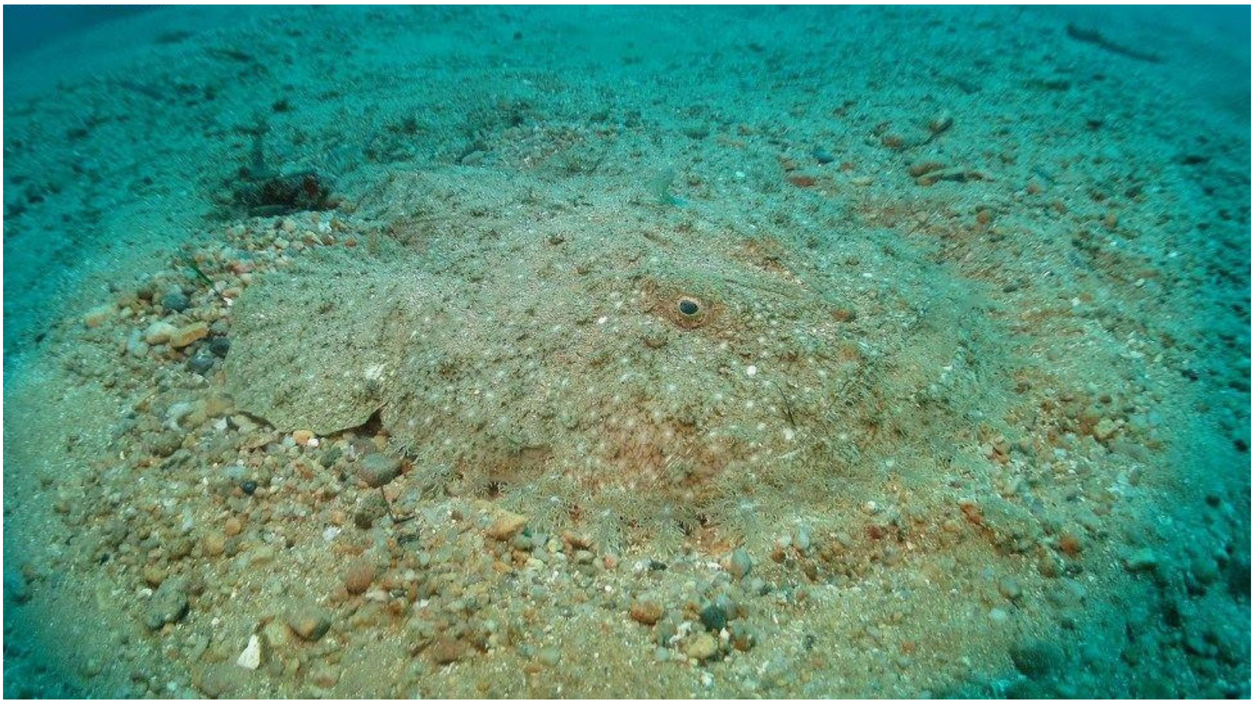


Figure 9. *Lophius* sp. half-buried on gravel bottom in Tossa de Mar (Image by S. Solans; L'Àmfora Centre d'Immersió).

macrophthalma Linnaeus, 1758; while large individuals, distributed at the edge of the continental shelf and upper slope, prey also on *C. macrophthalma* as well as *Trachinus draco* Linnaeus, 1758 (López et al. 2016) (Figure 10).

Differences in the diet composition of both *Lophius* spp. could be explained by differences in the temporal activity rhythms and their spatial distribution in order to reduce competition and allow coexistence (Schoener 1986).

Relating to reproduction, both anglerfishes have group-synchronous oocyte development and determinate fecundity (Afonso-Dias and Hislop 1996). The process of oocytes maturation is similar to that in other teleosts, although the morphology of ovaries differs markedly from them. The gonad of female anglerfish has 2 ribbon-like ovarian lobes connected to each other at their posterior end. One side of the "ribbon" consists of an ovigerous membrane from which a single layer of oocyte clusters, which contain oocytes at different developmental stages, and as the ovaries develop, one group of oocytes becomes clearly demarcated from the others (Afonso-Dias and Hislop 1996). The other side is nonovigerous and secretes a gelatinous material during maturation that fills the ovarian lumen, and

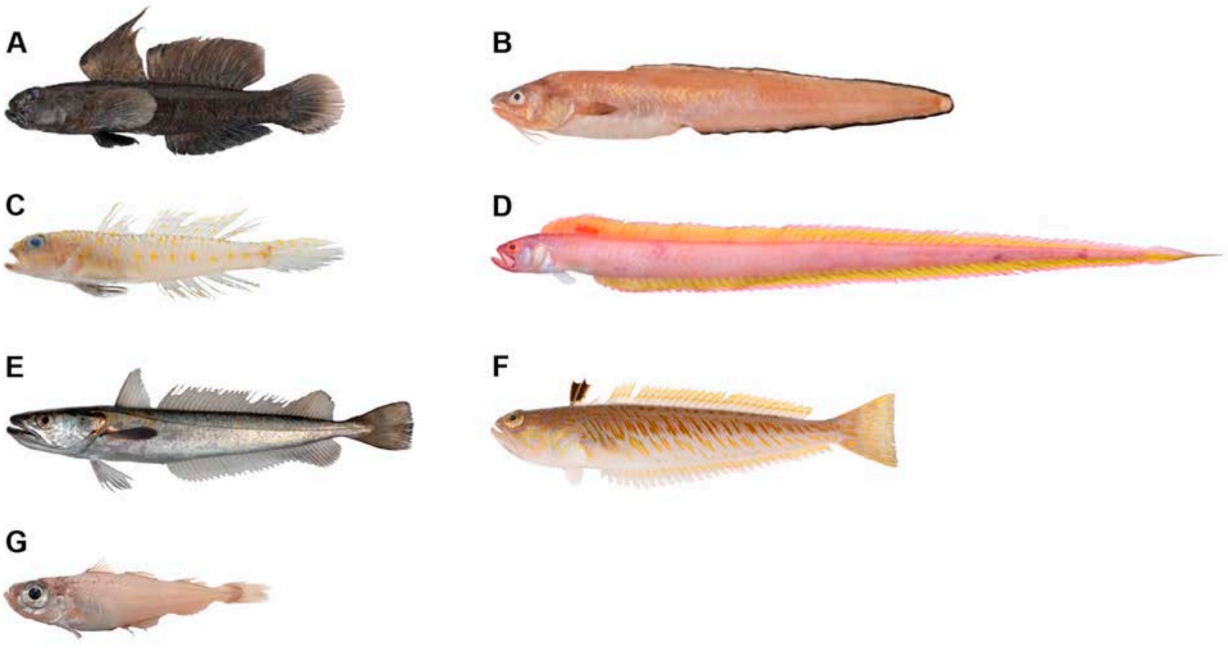


Figure 10. Main prey species reported for *L. budegassa* and *L. piscatorius* in the NW Mediterranean Sea. **A** *G. niger*; **B** *O. barbatum*; **C** *L. friesii*; **D** *C. macrophthalma*; **E** *M. merluccius*; **F** *T. draco*; **G** *G. argenteus* (Images source: Iglésias 2013).

consists of individual chambers where mature oocytes develop. In ripe females, the egg ribbons can be 10 m long and up to 1 m wide which may contain more than a million eggs (Bowman 1920). The gonad mass forms up to 35–50% of total body mass (Armstrong et al. 1992, Yoneda et al. 2001, Walmsley et al. 2005) representing a considerable energetic contribution to reproduction. Females spawn buoyant gelatinous egg masses, which could facilitate their dispersal, and fertilization is external. Anglerfish occupy the water column as eggs and larvae, and then they shift to a benthic existence as juveniles and adults (Lo Bianco 1931, Sabatés 1988, Fariña et al. 2008).

Another aspect of anglerfish biology is the determination of their age and growth. Information about growth parameters (asymptotic length and growth rate) had been obtained using several methods such as otoliths (Tsimenidis and Ondrias 1980, Crozier 1989, Woodroffe et al. 2003, Hernández et al. 2015), illicium (Dupouy et al. 1984, Duarte et al. 1997, Landa et al. 2001, Landa et al. 2013) and vertebrae (Armstrong et al. 1992, Yoneda et al. 1997, Johnson et al. 2008). Regardless of preference for the structure used, all authors reported great difficulties in age interpretation, mainly because of the location of the first annulus and the existence of false annuli (Fariña et al. 2008). The length-frequency distributions of catches (García-Rodríguez et al. 2005, Carlucci et al. 2009, Ofstad et al. 2013) and the analysis of tagging-recapture individuals (Laurenson et al. 2005, Landa et al. 2008) have been also used in the estimation of these parameters.

Study area

The Mediterranean Sea with its approximately 2.5 million km^2 is considered the largest semi-enclosed sea on Earth (Tyler 2003), bounded on the north by Europe, on the south by Africa, and on the east by Asia. It has an average depth of 1500 m, with a deepest point of 5267 m in the Calypso Deep (Ionian Sea), a water volume of 3.7 million km^3 and a residence time of 80-100 years (Ambroggi 1977, Vanney and Gennesseaux 1985). It is divided into two main basins, the western and the eastern depressions of 1.65 million km^2 and 0.85 million km^2 respectively, connected through the Strait of Sicily. The western basin is linked to the Atlantic Ocean by the Gibraltar Strait, while the eastern basin merge to the Sea of Marmara and the Black Sea by the Dardanelles and the Bosporus Straits respectively, and with the Red Sea by the artificial Suez Chanel (Figure 11).

The Mediterranean Sea comprises three main water masses (Izzo and Moretti 1999): the Modified Atlantic Water (MAW), found in the surface layer, with a thickness of 50–200 m and characterised by a salinity of 36.2 psu (practical salinity units) near Gibraltar to 38.6 psu in the Levantine basin; the Levantine Intermediate Water (LIW), formed in the Levantine basin, lying in depth between 200 and 800 m, and characterised by temperatures of 13–15.5°C and salinity of 38.4–39.1 psu; the Mediterranean Deep Water (MDW), formed in both the Western and Eastern basins. The Western Mediterranean Deep Water (WMDW) is characterised by a temperature of 12.7°C and a salinity of 38.4 psu, while the Eastern Mediterranean Deep Water (EMDW) is characterised by a temperature of 13.6°C and a salinity of 38.7 psu (Figure 12). All along its course, MAW is seasonally warmed or cooled, but overall its salt content increases and it becomes denser. In autumn, in the northern parts of both basins, MAW remains at the surface. In winter, cold and dry air masses induce marked evaporation and direct cooling of MAW, resulting in a dramatic increase in its density, which makes it sink. This sinking occurs in a series of specific zones, generally located in the northern parts of the basins, and is responsible for the formation of the deeper waters in the Mediterranean.

The surface waters come from the Atlantic Ocean through the Strait of Gibraltar and spreads throughout the whole basin flowing from the west to the east and turning into intermediate waters in the eastern Mediterranean Sea. Denser deep-Mediterranean waters flow

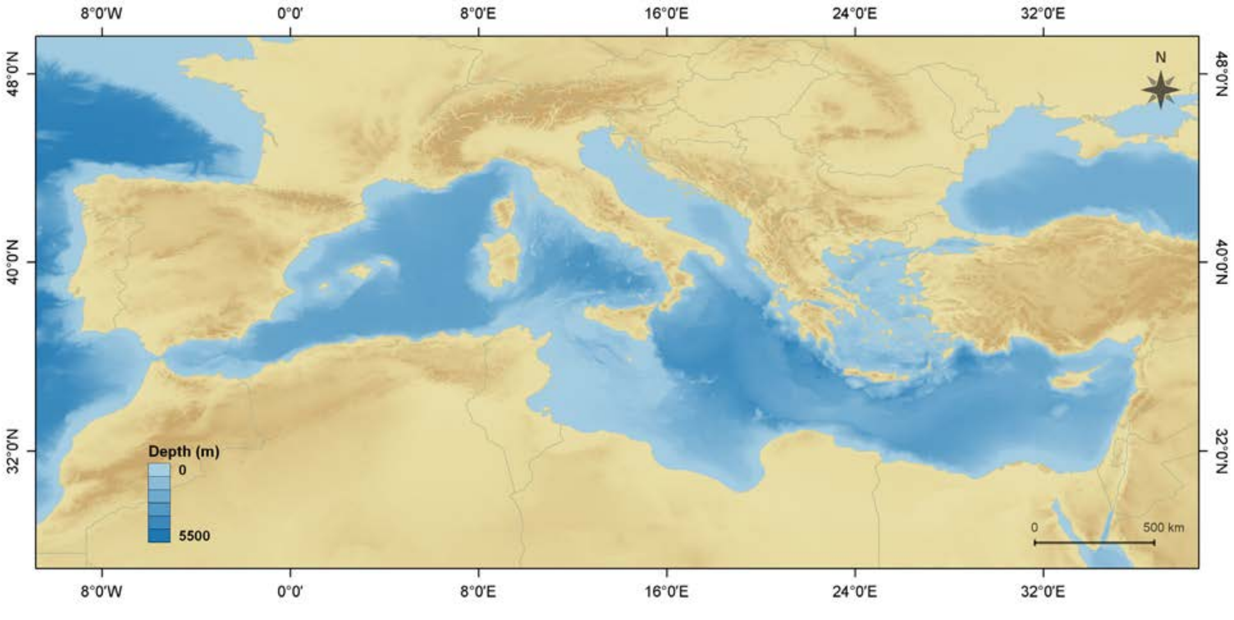
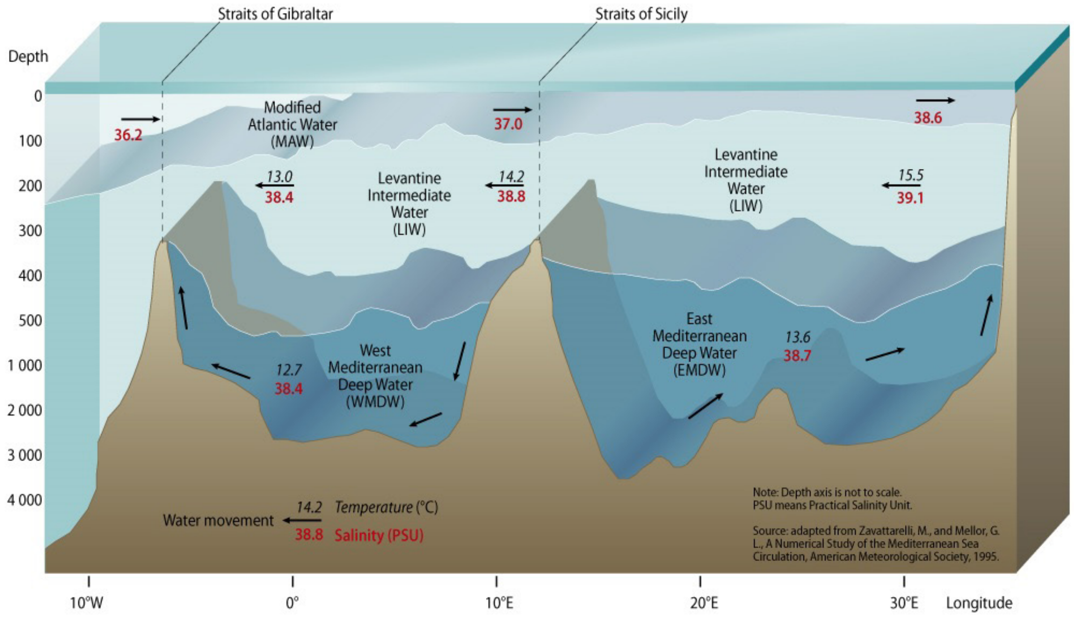


Figure 11. The Mediterranean basin (Data source: NOAA 2017).

beneath the Atlantic waters in the opposite direction (Astraldi et al. 2002, Bas 2002).

The large-scale circulation of the Mediterranean Sea has recently been described as being composed of sub-basin scale and meso-scale gyres interconnected and bounded by currents and jets with strong seasonal and inter-annual variability (Figure 13). This general circulation flow impinges on the coastal regions and strongly influences the local dynamics of currents. In fact, shelf areas in the Mediterranean are comparatively small and are separated from the deepest regions by steep continental shelf breaks. This configuration makes possible the intrusion of the large-scale flow field on the coastal/shelf areas and the direct influence of the large-scale currents on the coastal flow. Transport of material from the coastal areas to the open ocean is enhanced by this mechanism with important consequences for the maintenance of the ecological cycles in the basin. Eddies and local current systems are also essentially part of the general circulation, due to the complexity of the topography and the presence of islands (Izzo and Moretti 1999).

The Mediterranean is an oligotrophic sea, with low productivity which decreases from north to south and from west to east whilst an opposite trend is observed for temperature and salinity (Barale and Gade 2008) (Figure 14). Its basin has a surface temperature that ranges from 13°C in winter to 27°C in summer (Millot 1999), although it is homoeothermic below 200 m depth (12.5-14.5°C), with high salinity (38.0-39.5‰) and high oxygen levels (4.5-5 ml/l)(Hopkins 1985).



Note: Depth axis is not to scale.
PSU means Practical Salinity Unit.

Source: adapted from Zavattarelli, M., and Mellor, G. L., A Numerical Study of the Mediterranean Sea Circulation, American Meteorological Society, 1995.

Figure 12. Vertical distribution of Mediterranean Sea water masses (Source: Zavatarielli and Mellor 1995).

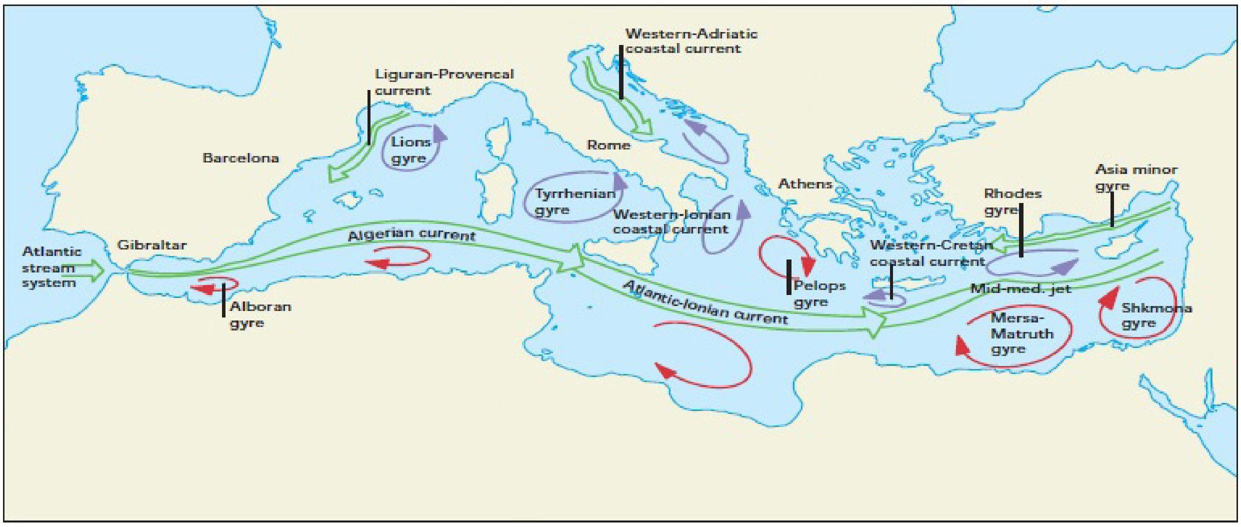


Figure 13. Main circulation in the Mediterranean Sea (Source: Pinardi et al. 1997).

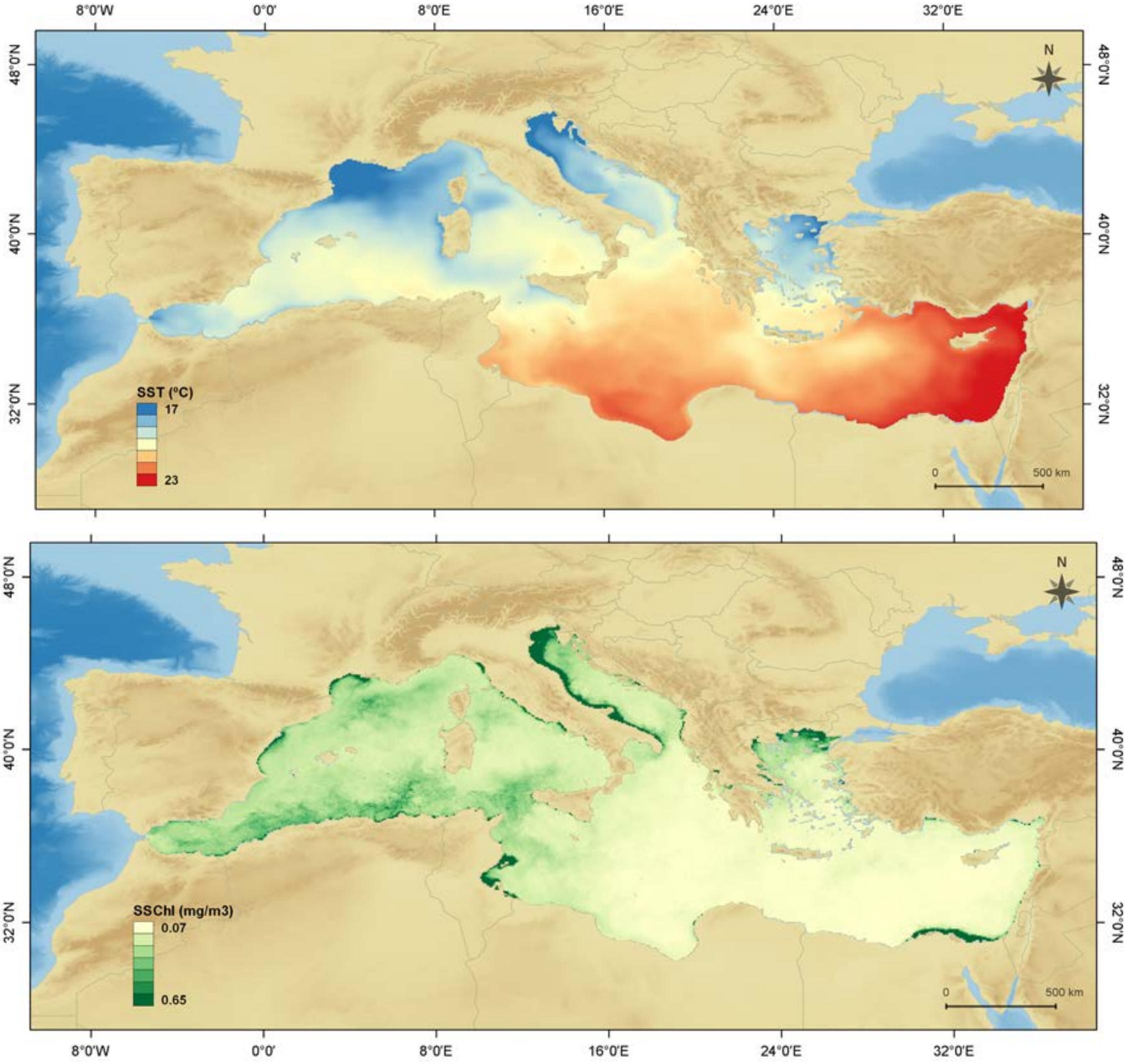


Figure 14. Mean sea surface temperature and chlorophyll concentration of the Mediterranean Sea in 2015 (Data source: EC 2017).

Samples used in this Thesis were collected in the northwestern Mediterranean Sea, in the fishing grounds off the Catalan coast, from 40º 5.980'N to 43º 39.310'N and from 0º 32.922'E to 3º 35.718'E (Figure 15). This area is characterized by a narrow continental shelf, less than 20 km wide, although it becomes wider between the largest submarine canyons south of the Gulf of Lions (La Fonera and Blanes canyons) and in the Ebro shelf (Salat et al. 2002).

The dynamics of the northwestern Mediterranean is characterized by a main surface current, called the Northern Current that follows the northern continental slope front, from the eastern Ligurian Sea to the Gulf of Valencia, and a southern current, following the north Balearic front (Font et al. 1988, Millot 1999) (Figure 16).

The Northern current extends down to a depth of 300-400 m (Castellón et al. 1990, Salat 1996) and displays a significant mesoscale activity such as meanders, filaments and eddies which contribute to increase the primary production of the study area (Estrada 1996).

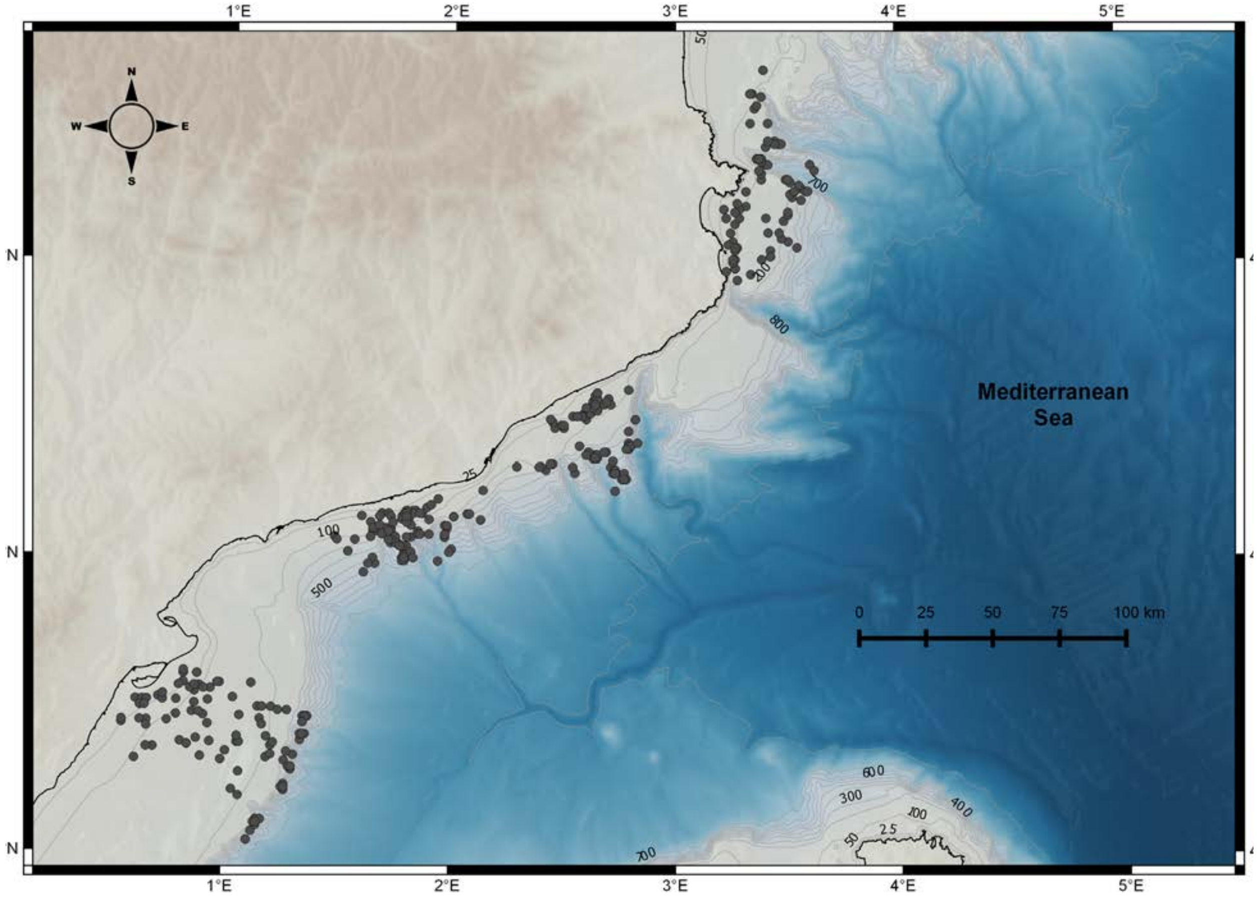


Figure 15. Map of the study area; northwestern Mediterranean Sea (Grey dots indicate sample stations).

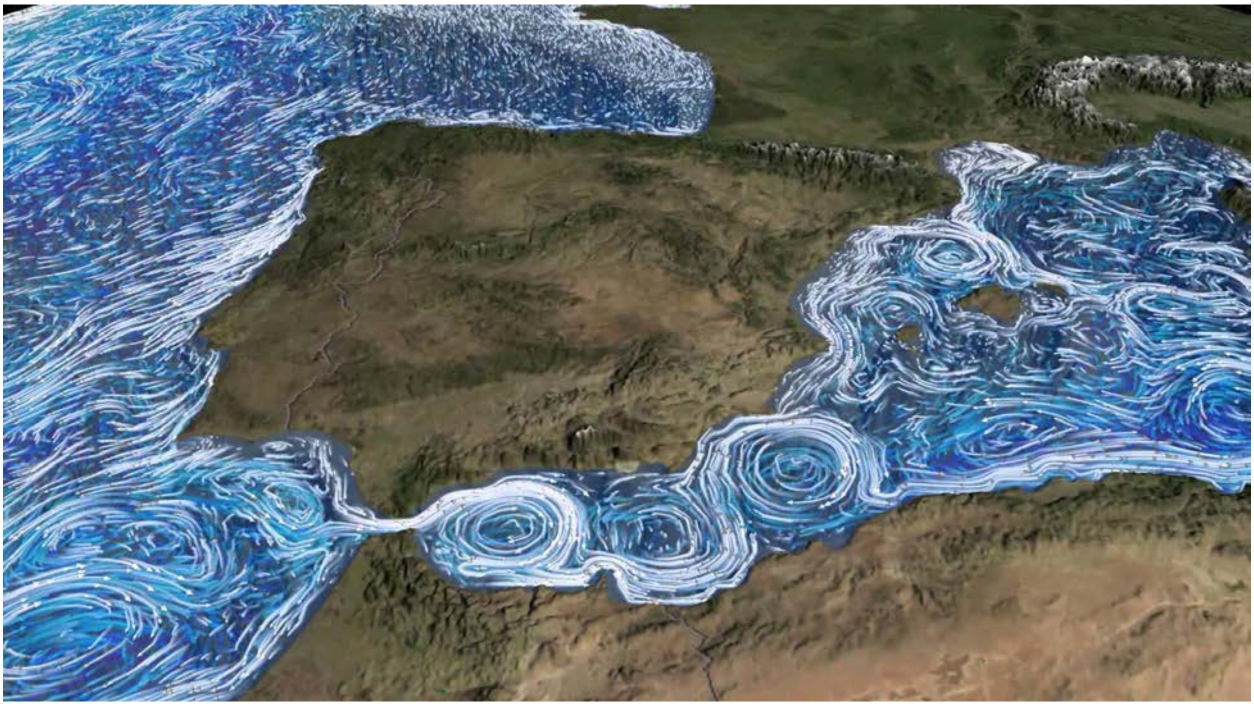


Figure 16. Surface circulation in the northwestern Mediterranean Sea (Source: Shirah et al. 2011).

Thesis Objectives

The aim of this Thesis is to improve the knowledge of the population parameters of *Lophius budegassa* and *Lophius piscatorius* of the northwestern Mediterranean Sea in order to determine the state of exploitation of both species and establish an effectively management.

In order to achieve this aim, a set of specific objectives are addressed in the different chapters of the Thesis:

1. To determine the behavioural activity and sensory mechanisms for reducing the levels of ecological competition between *L. budegassa* and *L. piscatorius* (Chapter 2.1)

2. To describe the reproductive parameters (gonad morphology, spawning season, size at sexual maturity, oocyte development and fecundity) for a better understanding of the life cycle of both *Lophius* spp. (Chapter 2.2; Subchapters 1 and 2)

3. To examine morphological characteristics of ova in the two species of *Lophius* for aid in identification of eggs from ichthyoplankton samples (Chapter 2.2; Subchapter 3)

4. To provide new data on population dynamics (growth parameters and natural mortality) as well as on population structure (sex ratio) and biometric relationships for modelling the stock of both *Lophius* spp. (Chapter 2.3)

5. To explore sustainability indicators for monitoring the state of *Lophius* population relative to exploitation (Chapter 2.3)

6. To analyse the prevalence of the microsporidian parasite *Spraguea lophii* for hypothesize about the health status of the population of *Lophius* spp. and the possible side effects of severe infections on their morphology (Chapter 2.4)

Report of the Supervisors

Dr. Mª Pilar Sánchez Zalacaín and Dr. Víctor Manuel Tuset Andújar, supervisors of the present PhD Thesis entitled *Towards biological and ecological knowledge of Lophius spp. in the NW Mediterranean Sea for a sustainable fishery* certify that the research studies included in this Thesis has been carried out by Ana Isabel Colmenero Ginés in its totality and they have been submitted to peer-review specialized international journals.

As supervisors, we have participated in designing, guiding and correcting earlier drafts of the chapters and manuscripts written by the PhD candidate. The contribution of the PhD candidate to each manuscript together with details of each publication and the Impact Factors of each journal (InCites Journal Citation Reports from Web of Sciences) are detailed below:

Colmenero AI, Aguzzi J, Lombarte A, Bozzano A (2010) Sensory constraints in temporal segregation in two species of anglerfish, *Lophius budegassa* and *L. piscatorius*. *Marine Ecology Progress Series* 416:255-265
Impact factor (2010): 2.483
5 - Year Impact factor (2010): 2.993

Colmenero AI, Tuset VM, Recasens L, Sánchez P (2013) Reproductive biology of Black Anglerfish (*Lophius budegassa*) in the northwestern Mediterranean Sea. *Fishery Bulletin* 111:390-401
Impact factor (2013): 1.783
5 - Year Impact factor (2013): 1.619

Colmenero AI, Tuset VM, Sánchez P (2017) Reproductive strategy of white anglerfish (*Lophius piscatorius*) in Mediterranean waters: implications for management. *Fishery Bulletin* 115:60-73
Impact factor (2016): 0.879
5 - Year Impact factor (2016): 1.677

Colmenero AI, Tuset VM, Fortuño J-M, Sánchez P (2015) The chorion ultrastructure of ova of *Lophius* spp. *Journal of Fish Biology* 86:1881-1886
Impact factor (2015): 1.246
5 - Year Impact factor (2015): 1.837

Colmenero AI, Tuset VM, Sánchez P (2017) Population parameters and sustainability indicators to assess *Lophius* fisheries in the northwestern Mediterranean Sea (Submitted to *Deep Sea Research Part I*)
Impact factor (2016): 2.480
5 - Year Impact factor (2016): 2.735

Colmenero AI, Barría C, Feist SW, Tuset VM (2015) Observations on the occurrence of *Spraguea lophii* in Mediterranean lophiids. *Parasitology Research* 114:1977-1983
Impact factor (2015): 2.027
5 - Year Impact factor (2015): 2.096

We certify that Ana Isabel Colmenero Ginés has participated actively on the development of the research and the elaboration associated to each of the papers listed. In particular, her contribution included the following tasks:

Participation in setting objectives and in the experimental design of each one of the chapters developed.

- Sampling design and field work.
- Sample processing and analyses in laboratory.
- Statistical data analyses and interpretation of results.
- Writing, reviewing and editing processes of the manuscripts.

We also assure that all the co-authors of the scientific articles included in the present Thesis have not been used for the elaboration of other PhD Thesis.

Dra. Mª Pilar Sánchez Zalacaín
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CHAPTER 2

RESULTS

CHAPTER 2.1

SENSORY CONSTRAINTS IN TEMPORAL SEGREGATION IN TWO SPECIES OF ANGLERFISH (*Lophius budegassa* AND *L. piscatorius*)

SENSORY CONSTRAINTS IN TEMPORAL SEGREGATION IN TWO SPECIES OF ANGLERFISH (*Lophius budegassa* AND *L. piscatorius*)

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Abstract. The temporal segregation of 2 ecologically equivalent and sympatric species, the black anglerfish *Lophius budegassa* and white anglerfish *L. piscatorius* was studied in relation to day–night behaviour and the underlying morphological variation in the sensory organs. Temporally scheduled trawl sampling was conducted on the western Mediterranean shelf, and diel fluctuations in catches were used as a proxy of behavioural rhythms. *L. budegassa* was more abundant at night than *L. piscatorius*, which mostly occurred in daytime catches. The observed differences in the timing of maximum activity (i.e. catches) were consistent with differences in otolith sagittae weight, eye/lens sizes and optical sensitivity. *L. budegassa* presented significantly heavier otolith sagittae than *L. piscatorius*, indicating that the behaviour of the black anglerfish is mostly acoustically driven (a typical characteristic of species living in dim light conditions). Preference for a dim light environment was also confirmed by the high optical sensitivity observed in this species achieved by enlarging the photoreceptors' diameter. In contrast, *L. piscatorius* showed larger eyes and lenses than *L. budegassa*, but only in individuals larger than 50 cm. Large eyes usually improve photon capture and the fineness with which the image is sampled; however, relatively low values of sensitivity and resolution were observed in large *L. piscatorius*. This indicates that a trade-off might exist between the increase in eye size as an adaptive response to the impending deep meso- and bathypelagic life and a visual constraint imposed by the quality of the water at the bottom level where contrast can be degraded by suspended particles. These results indicate that interspecific competition with sympatric and ecologically equivalent species may result in different adaptations to diurnal/nocturnal behaviour, which are sustained by morphological specialisations in sensory organs.

INTRODUCTION

Temporal segregation in activity rhythms of ecologically equivalent and sympatric species may promote their coexistence in overlapping distribution areas (Kronfeld-Schor and Dayan 2003). Thus, species reduce interspecific competition by segregating their timing of maximum behavioural activity into different windows of the day-night cycle (Pittman and McAlpine 2003).

Although activity patterns in fishes are often said to be plastic (Ali 1992), i.e. not always are consistent either within species or between individuals, trawling can be considered a reliable method for assessing the behavioural rhythms in those fish living in deep water areas where direct observation is unfeasible (Aguzzi and Company 2010). This method provides interesting insight into species' biological clock regulation when catches are conducted concomitantly with measurements of physical factors that are key habitat parameters controlling rhythms (e.g. the light or tidal cycles as Zeitgebers) (Aguzzi et al. 2008). However, the field study of biological rhythms has to be supported by observations of the anatomy and functional mechanisms of the sensory capabilities that each species displays (Naylor 2005). In fact, to understand why a species occupies a temporal niche, it is crucial to identify the environmental characteristics under which individuals determine their appropriate period of activity.

Taking into account the fact that sensory systems represent the filters between the environmental inputs and behavioural outputs, habitat choice will affect the environmental conditions under which sensory systems are employed, and the timing of behavioural activity may then be sustained by differences in sensory capabilities (Endler and McLellan 1988, Dusenbery 1992, Myrberg Jr and Fuiman 2002, Weissburg et al. 2005). Even though sensory modalities underlying spatial segregation phenomena have previously been highly documented in fish (Aguirre and Lombarte 2000, Bozzano and Catalán 2002, Lombarte et al. 2003, Sabatès et al. 2003, Schulz-Mirbach et al. 2008, Seehausen et al. 2008), there is still scant information on the role played by sensory modalities in relation to the temporal segregation. Additionally, although the survival of a species demands some co-evolution of its sensory systems, adaption to a temporal niche causes implementation of compensatory mechanisms that counterbalances the quality of each sensory modality, which means that the poor performance of one system is compensate for by the good performance of another (Schellart and Popper 1992).

With this in mind, 2 ecologically equivalent lophiid species, the black anglerfish *Lophius budegassa* and the white anglerfish *L. piscatorius*, represent a good example in which segregation can be analysed in relation to sensory modalities. In fact, these species show partial overlap in their bathymetric distribution range in the Mediterranean. Both species are present on the continental shelf but reach different depths on the slope. *L. budegassa* occurs down to a depth of 800 m (Carlucci et al. 2009), while *L. piscatorius* has been detected deeper, down to almost 2600 m (Afonso-Dias and Hislop 1996, Hislop et al. 2000). The black and white anglerfishes share common behavioural traits within the lophiids, especially in their feeding strategy, as they exhibit sit-and-wait ambush behaviour using their angling apparatus, the illicium, as a lure. In addition, both species show similar food preferences and therefore compete for similar prey items (Crozier 1985, Laurenson and Priede 2005, Preciado et al. 2006, Negzaoui-Garali et al. 2008).

Interspecific competition of ecologically equivalent species is reduced in sympatric zones by varying population densities and distributions, but also by varying the timings of their activity (Linnell and Strand 2000, Kronfeld-Schor and Dayan 2003). Accordingly, in the western Mediterranean, the reported overlap in the bathymetric range of lophiids indicates that there could be a concomitant process of morphological divergence occurring that may sustain species activity within different time windows (Seehausen et al. 2008). The activity rhythms of *Lophius piscatorius* and *L. budegassa* were analysed in the field using temporally scheduled trawl sampling and considering potential quantitative differences in the morpho-functionalities of their inner ears (acoustic and vestibular functions) and eyes (visual functions), which are the sensory systems involved in remote detection of objects. With regard to the 3 otolithic organs of the fish's inner ear, the sacculus together with its otolith, the sagitta, shows the highest morphological variability, and this structure is located in the lower part of the inner ear, which is generally associated with the hearing sense (Platt and Popper 1981, Popper and Fay 1993, Popper and Lu 2000). The size and shape of this structure show adaptations to different environmental factors, such as depth, water temperature and substrate type (Lombarte and Fortuño 1992, Aguirre and Lombarte 1999, Paxton 2000, Torres et al. 2000, Gauldie and Crampton 2002, Volpedo and Echeverria 2003). With respect to vision, the capability to hunt at night or at the maximum depth at which a fish can operate seems very likely to be determined by its eye size, because large eyes both increase the retinal sampling frequency and therefore improve resolution, and capture more photons, improving sensitivity, although many exceptions to this exist (Land and Nilsson 2002).

The aim of the present work was to determine whether anglerfish reduce competition in their overlapping distribution range by displaying temporal segregation in their behavioural activity and whether this is consistent with differences in their sensory modalities.

MATERIALS AND METHODS

Field sampling

Four days of temporally scheduled trawl sampling were carried out on the western Mediterranean lower continental shelf (100 to 110 m depth) during June 2000 at latitude 41º N to characterize the activity rhythms of the black and white anglerfishes. Thirty-two hauls were made along parallel and closely situated transects. The field survey was carried out onboard the research vessel "García del Cid" (38 m length; 1200 HP), which was equipped with otter trawl nets of 27.5 mm headrope size (OTMS; Sardà et al. 1998).

The duration of the hauls was approximately 90 min, and their number, separated into day- and night-time trawls, is designated by 2 h time intervals in Table 1. A global positioning system (GPS) recorded the ship velocity and the initial and final position (latitude and longitude) for all hauls, while an echosounder provided depth measures. SCANMAR telemetric sensors connected to the mouth of the net recorded wing openings (m) and depths (m). Data were stored on an onboard computer. The setting and retrieving time of each haul were considered as the exact moment of the net landing and rising from the seabed, according to SCANMAR information.

Light intensity was directly sampled between consecutive hauls. The photometer employed (LI-193SA Spherical Quantum Sensor; LI-COR) was mounted on a conductivity, temperature and depth sensor (CTD), and it measured the light intensity as the photon fluency rate (PFR, $\mu Em^{-2}s^{-1}$) within the photosynthetic active radiation (PAR) range of 400 to 700 nm.

Activity rhythm determination

Fluctuations in the quantity of collected animals were used as proxy of their behavioural rhythms when considering these as the product of their movement into and out of the sampling window. For each haul, all individuals of both *Lophius* spp. were counted. Because both species present overall similar morphological characteristics and colorations, animals were distinguished by the colour of the peritoneum and by the number of rays in the second dorsal

Table 1. Numbers of trawl samples of *Lophius piscatorius* and *L. budegassa* and photon fluency rate (PFR) readings used for mean estimations at each 2 h time interval in the 24 h waveform analysis. –: no samples collected at the corresponding time interval during the 4 d of fishing activity; np: night period. Missing values at certain time intervals (i.e. –) were replaced by computing an average (± SD) estimate from all temporally adjacent values.

Time interval (h)	No. of hauls	No. of PFR readings
12:00–14:00	3	–
14:00–16:00	1	1
16:00–18:00	3	2
18:00–20:00	5	4
20:00–22:00	4	np
22:00–00:00	3	np
00:00–02:00	–	np
02:00–04:00	1	np
04:00–06:00	4	4
06:00–08:00	4	4
08:00–10:00	4	2
10:00–12:00	–	4

fin (Caruso 1986): black peritoneum and 9 to 10 rays in *L. budegassa*; white peritoneum and 11 to 12 rays in *L. piscatorius*.

Time series of catches for each species were then elaborated by estimating the animals' bottom surface density variations over the 24 h cycle. A density value per haul was obtained by dividing the number of sampled animals by the swept area (km^2), as estimated by SCANMAR and GPS measures (Sparre et al. 1998).

Waveform analysis was conducted on time series of surface density and PFR estimates to assess the phase of the activity rhythms (i.e. the timing of peaks) in relation to the day-night cycle. A standard period of 24 h was subdivided in 2 h time intervals (Aguzzi et al. 2003a, b). All densities and PFR values computed from samplings that took place within a given 2 h time interval were averaged to obtain the 24 h consensus waveform (see Table 1 for the number of hauls and PFR readings pooled in each 2 h time interval). The phase of the time series in surface density estimates was identified in each waveform plot by computing a daily mean as a threshold. This threshold was obtained by re-averaging all mean waveform values together. The resulting threshold value was represented as a horizontal line in the plot. Surface density estimates above that threshold indicated the presence of a significant increase in catches;

adapted from Hammond and Naylor (1977). Mean density and light intensity estimates were plotted together in relation to the time of sunset and sunrise at the latitude of the study area (Greenwich Meridian Time: 19:27 and 04:19 on the 22 June).

Periodicity in the time series of density data was screened between 600 min (10 hrs) and 1800 min (30 hrs) with a chi-square periodogram analysis ("El temps"; A. Díez-Noguera pers. comm.). The periodogram analysis requires a series of data obtained at a constant time interval (for details on the procedure, see Hammond & Naylor 1977). This condition is difficult to satisfy in trawl-based sampling studies. Gaps in the time series were therefore replaced by the values obtained from waveforms at the corresponding 2 h time intervals (Aguzzi et al. 2003a, b). In the periodograms, the highest significant ($p < 0.001$) peak represented the maximum percentage of total data variance fitted by the corresponding periodicity. The peak value was chosen for period attribution of the analysed time series.

Morphometric measurements

Morphological measurements of the sensory organs were conducted on 274 *Lophius budegassa* and 137 *L. piscatorius,* ranging from 4.5 to 80 cm and 15 to 97.5 cm in total length (TL), respectively. Otolith sagittae were removed, mechanically cleaned (deprived of tissue debris with tweezers), dried at room temperature and finally weighed to the nearest 0.01 mg with a balance with a sensibility of 0.001 mg. Otolith weight was used as a measure of size because in lophiids, otolith shape and area obtained by orthogonal projection are highly variable (Tuset et al. 2008). The eyes were enucleated, and their diameters were measured with callipers to the nearest 0.01 mm. The corneas were excised to extract the lenses, which were then measured using callipers to the nearest 0.01 mm. The vitreous was removed and the eye cups were fixed in a mixture of paraformaldehyde (2%) and glutaraldehyde (2%) in phosphate buffer (0.1 M). The caudal portion of the fixed retina from one small (*L. budegassa* of 14 cm TL and *L. piscatorius* of 15 cm TL) and one large individual of both species (*L. budegassa* of 66 cm TL and *L. piscatorius* of 77 cm TL) were embedded in resin (Technovit 7.0) and sectioned in the transverse and tangential planes.

Since the studied anglerfish stretch their distribution from shallow water to the deep mesopelagic zone, their visual systems encounter different light conditions; therefore, the optical sensitivity, i.e. the capability of seeing well in dim light conditions, was calculated both employing the Land (1981) sensitivity equation for monochromatic light at the preferred wavelength of the photoreceptor

$$S = (\pi/4)^2 A^2(d/f)^2(1 - e^{-kl}) \quad (1)$$

found in deep environments and the Warrant and Nilsson (1998) equation for white light

$$S = (\pi/4)^2 A^2(d/f)^2[kl/(2.3 + kl)] \quad (2)$$

visible in shallow waters. In these equations, sensitivity (S) is related to the diameter of the eye's aperture (A), the photoreceptor diameter (d), the focal length (f), the absorption coefficient of the photoreceptor (k) and photoreceptor length (l). In the present study, the eye's aperture, i.e. the diameter of the pupil, was considered equivalent to the lens diameter (Fernald 1990). Although these species show a pupil response, and hence the maximum pupil aperture should be employed in the sensitivity formulae, the analysed individuals were caught with diurnal commercial trawls and therefore the pupil was partially closed. The focal length (f), i.e. the distance from the centre of the lens to the retina, was calculated from Matthiessen's ratio, where $f = 2.55r$ and r is the radius of the lens. The absorption coefficient of monochromatic and white light is unknown in these species; therefore, the average k (0.035 μm^{-1}) for bony fish was employed (Warrant and Nilsson 1998). From f, the inter-receptor angle $\Delta\phi$, i.e. the feature of the eye's resolving power, can be calculated in accordance with Land & Nilsson (2002) where $\Delta\phi = s/f = L/(nf)$ and s is the photoreceptor spacing. The photoreceptor spacing is the inverse of the visual cell number (n) counted in the retinal linear transect L (3 linear 100 μm transects in the present study). Finally, the spatial frequency $1/2\Delta\phi$, a way of representing the optical resolution at the photoreceptor level, is obtained.

A comparative analysis of the relative increase of otolith weight and eye and lens diameters with respect to body length was performed for both species. Although the total size interval was 4.5 to 97.5 cm for both species, the range over which data of eye and lens diameter were available was 22.5 to 80 cm. To determine whether eye diameter and otolith weight presented isometric or allometric growth patterns, a comparison of the rate of their increase was performed according to different size classes. A *t*-test for the slopes using normalised data (i.e. logarithmically transformed) was employed to test the significance of the differences between pairs of curves (Zar 1984). The presence of a significant relationship was assessed at the significant level of $p < 0.05$.

RESULTS

Activity rhythms

In total, 3810 of *Lophius budegassa* and 2170 individuals of *Lophius piscatorius* were collected from our temporally scheduled trawling survey. Both species showed different temporal patterns in their catchability in relation to the measured light intensity cycle (Fig. 1).

The outputs of a 24 h waveform analysis on the time series of surface density estimates and PFR (i.e. light intensity) data at depths of 100 to 110 m are shown in Fig. 2.

Lophius budegassa chiefly presented a nocturnal catch pattern. A significant increase in catches occurred over a temporal amplitude that started between 20:00 and 22:00 h and ended between 04:00 and 06:00 h. A decrease in catches occurred for PFR values above 0.93 μE m^{-2} s^{-1}.

Lophius piscatorius showed a significant increase in catches (i.e. values above the daily mean) at daytime. The resulting temporal amplitude of the catch peak spanned from 12:00–14:00 to 22:00–00:00 h. Increased catches were observed for PFR values above 1.25 μE m^{-2} s^{-1}. Catches decreased within a few hours after the onset of darkness, and the onset of this decrement occurred for PFR values below 0.87 μE m^{-2} s^{-1}.

Both species showed a significant ($p < 0.001$) diel periodicity very close to a 24 h cycle (Fig. 3). *Lophius budegassa* presented a significant increase in catches that repeated itself every 1430 min, which is equal to 23 h and 50 min. A similar temporal pattern was detected for *L. piscatorius* with a period of 1455 min, which is equal to 24 h and 15 min, in catch fluctuations.

Morphometric relationships in sensory organs

Otolith weight increased progressively during fish growth, ranging from 2.7 to 102.6 mg in *Lophius piscatorius* and from 0.6 to107.3 mg in *Lophius budegassa* (Fig. 4). For equivalent fish lengths, *L. budegassa* had 50% heavier otoliths than *L. piscatorius*, and with increasing length, the difference between the species was observed to be fairly constant. A significant difference between the curves depicting this relationship for these 2 species was observed ($p < 0.05$).

During fish growth, progressive eye and lens enlargement was observed in both anglerfish species (Fig. 5). However, a shift in their trends was observed during growth. In fact, the eye diameters of small *Lophius budegassa* (TL < 50 cm) were larger than those of *L. piscatorius* of comparable size, with a mean value (± SD) of 20.7 ± 3.7 mm (n = 82) in the former group

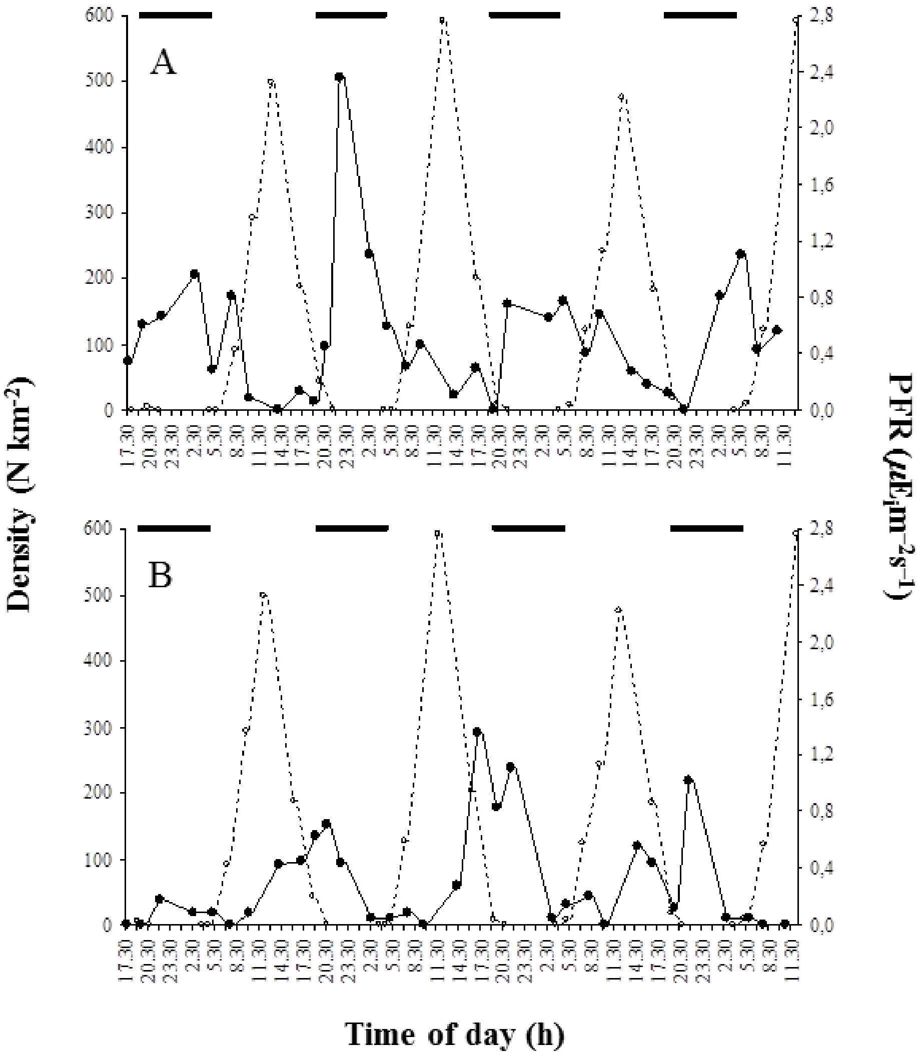

Figure 1. *Lophius budegassa* and *L. piscatorius*. Density (no. km^{-2}; continuous lines) estimates of (A) *L. budegassa* and (B) *L. piscatorius* in relation to the light intensity cycles (i.e. photon fluency rate, PFR, in µE m^{-2}s^{-1}; dashed line) as recorded during 4 d of fishing activity at 100 m around the summer solstice (i.e. June). Black top bars indicate the night duration.

and 17.5 ± 3.6 mm (n = 37) in the latter. In larger individuals (TL ≥ 50 cm), the mean eye diameters (± SD) of *L. budegassa* measured 26.8 ± 2.8 mm (n = 24), and those of *L. piscatorius* measured 29.8 ± 2.2 mm (n = 22). When the eye diameters of small individuals of both species were plotted separately from the diameters of large individuals, significant differences were observed in both cases ($p < 0.05$).

A similar trend was observed for lens diameter, although the shift was recorded at 58 cm. The mean lens diameter (± SD) of small individuals of *Lophius budegassa* and *L. piscatorius* measured 7.9 ± 1.5 mm (n = 59) and 6.4 ± 1.4 mm (n = 37), respectively, while large individ-

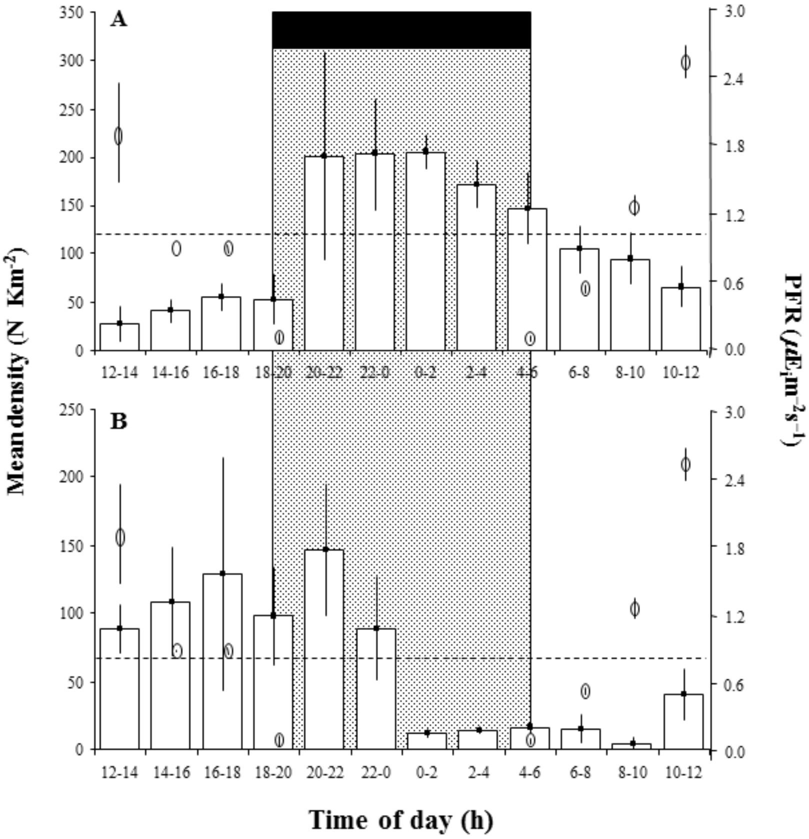

Figure 2. *Lophius budegassa* and *L. piscatorius*. Waveform analysis outputs from time series of catches depicting mean estimates in abundance (no. km^{-2}, bars) of (A) *L. budegassa* and (B) *L. piscatorius* and light intensity measures (photon fluency rate, PFR, circles) as recorded during 4 d at 100 to 110 m depth around the summer solstice (22 June to 3 July 2000). The recorded light intensity ranged between 0.0038 and 2.77 μE m^{-2}s^{-1}. The vertical shaded area with the dark grey bar on the top indicates the night duration. Daily means (horizontal dashed lines as computed by averaging all waveform values; no. km^{-2}) are 114 for *L. budegassa* and 64 for *L. piscatorius*.

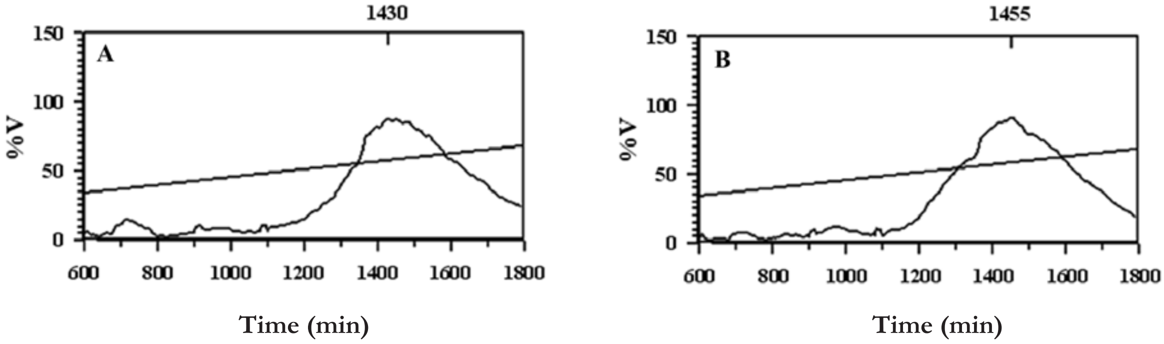

Figure 3. *Lophius budegassa* and *L. piscatorius*. Outputs of periodogram analysis conducted on the time series of catches of (A) *L. budegassa* and (B) *L. piscatorius* as recorded during 4 d at 100 to 110 m depth around the summer solstice (22 June to 3 July 2000). Periodogram units (%V) refer to the percentage of variance explained by fitting to the data set of modelled harmonics of increasing periodicity. The straight sloping line in each panel indicates the significance threshold for periodicity determination ($p < 0.001$).

uals had lenses of 9.9 ± 1.0 mm (n = 21) and 10.7 ± 0.8 mm (n = 19), respectively. Although significant differences ($p < 0.05$) between the slopes of lens diameter were observed over the total size range of the individuals examined, when the 2 size groups were plotted separately, no significant differences were found ($p > 0.05$).

The anglerfishes showed duplex retinas where rods and single and double cones were visible. Single cones were the predominant cone type, and their density and the size of their outer segments were employed to calculate the spatial frequency and the optical sensitivity, respectively. Although a more accurate method for calculating sensitivity would be to use rod size, the use of the single cones is partially due to the fact that the rod diameter was difficult to measure accurately in the retina of the analysed individuals. In addition, since a large number of single cones was observed in these species, the role of these photoreceptors in improving sensitivity is highly probable. The measurements taken from the anglerfish eyes are summarised in Table 2. Using obtained data and the sensitivity equations, the eye of the anglerfish showed an in- crease in sensitivity with growth both to white and monochromatic light. While a small increase in sensitivity was observed for *Lophius piscatorius*, in *L. budegassa* the

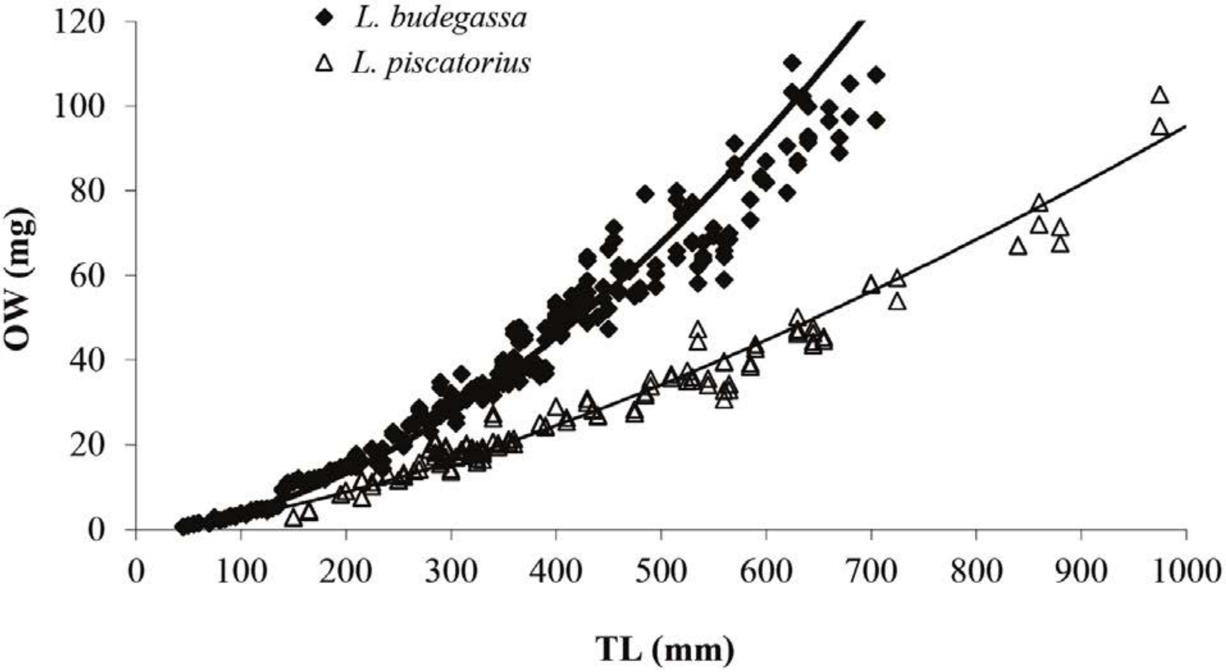

Figure 4. *Lophius budegassa* and *L. piscatorius*. Relationship between otolith weight (OW) and total length (TL); *L. budegassa* ($OW = 0.0011\ TL^{1.7725}$; $r^2 = 0.9813$; n = 274) and *L. piscatorius* ($OW = 0.0035\ TL^{1.4802}$; $r^2 = 0.9417$; n = 137).

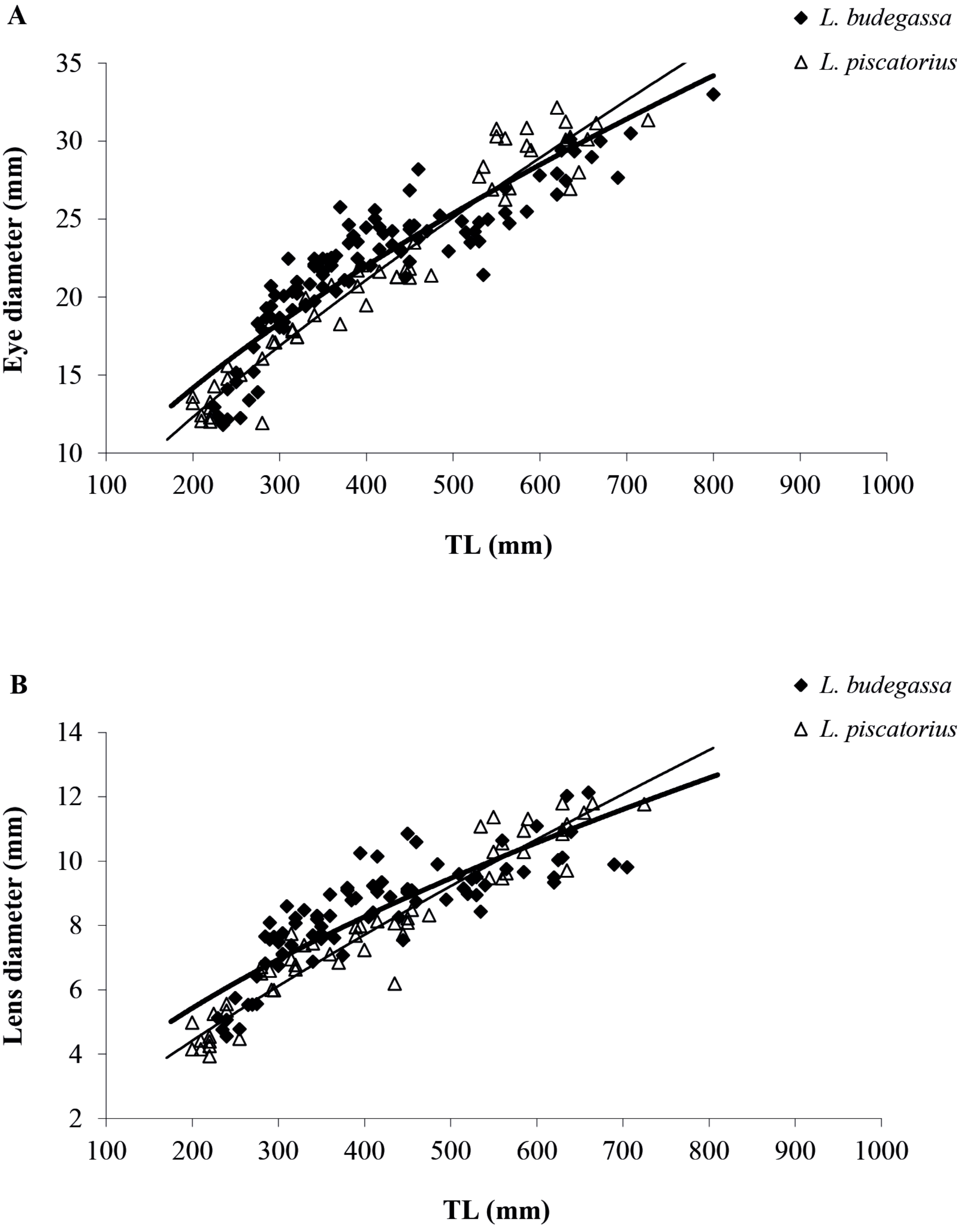

Figure 5. *Lophius budegassa* and *L. piscatorius*. (A) Relationship between eye diameter and total length (TL); *L. budegassa* (eye diameter = 0.4882 $TL^{0.6357}$; $r^2 = 0.7745$; n = 106) and *L. piscatorius* (eye diameter = 0.1998 $TL^{0.7778}$; $r^2 = 0.9472$; n = 59). (B) Relationship between lens diameter and TL; *L. budegassa* (lens diameter = 0.2186 $TL^{0.6063}$; $r^2 = 0.7138$; n = 80) and *L. piscatorius* (lens diameter = 0.0628 $TL^{0.8027}$; $r^2 = 0.9231$; n = 56).

sensitivity values of the large individual were triple those of the small individual for both types of light (Table 2). These increases seem to be attributable to the considerable enlargement of the photoreceptor diameter during growth. The retinal receptor spacing and the relative spatial frequency were similar in the 2 species (Table 2).

Table 2. *Lophius piscatorius* and *L. budegassa*. Morphological parameters taken from 1 ind. for each size, employed to calculate sensitivity for the preferred wavelength (λ_{max}) and white light and for the spatial frequency in anglerfish.

Species	Size (cm)	Lens diameter (mm)	Focal length (mm)	Cone length (µm)	Cone diameter (µm)	Sensitivity white (µm2 sr)	Sensitivity (λmax) (µm2 sr)	Interreceptor angle (degrees)	Spatial frequency (cycle per degree)
L. budegassa	14	3.88	4.95	9.7	4.12	0.83	1.85	0.14	3.56
L. budegassa	77	12.05	15.36	12.85	6.44	2.57	5.7	0.08	6.15
L. piscatorius	15	3.78	4.82	8.4	3.53	0.54	1.2	0.14	3.66
L. piscatorius	63	10.91	13.91	12.96	4.01	1.01	2.2	0.09	5.57

Because interspecific differences of a sensory organ cannot be interpreted in isolation from other sensory systems, otolith weight and eye diameter were compared, and the allometric coefficient values indicated that in *Lophius budegassa*, otolith weight increased more than eye size, while an opposite trend was observed for *L. piscatorius* (Fig. 6). A comparison of the slopes showed significant ($p < 0.05$) differences between the species.

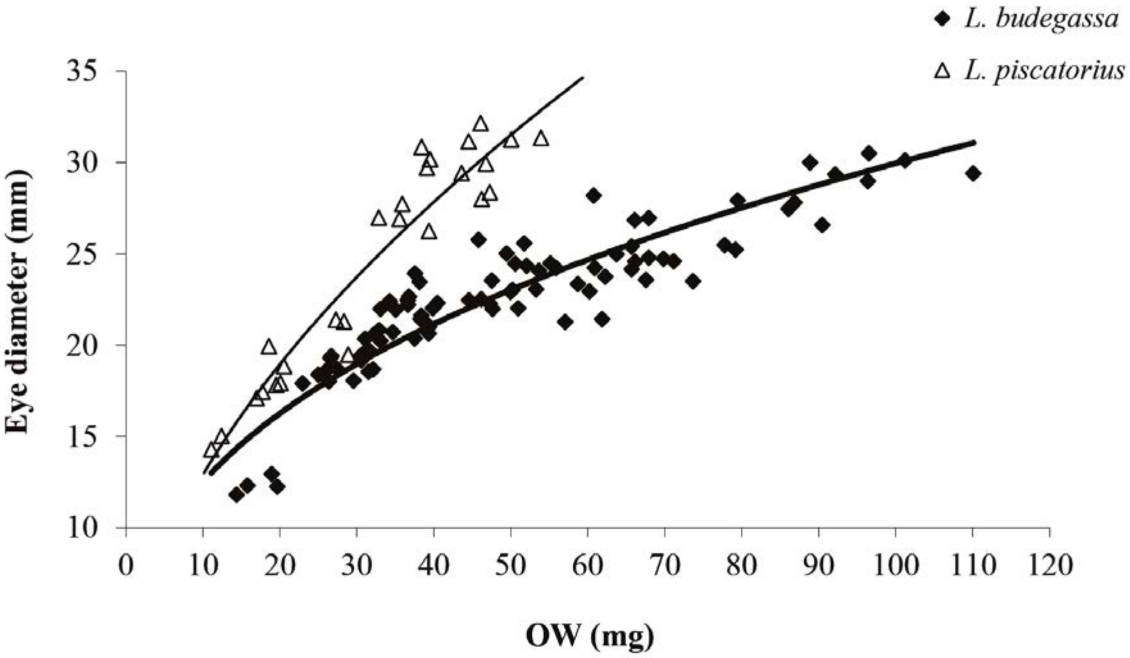

Figure 6. *Lophius budegassa* and *L. piscatorius*. Relationship between eye diameter and otolith weight (OW): *L. budegassa* (eye diameter = 5.2128 OW $^{0.3798}$; $r^2 = 0.8215$; n = 87) and *L. piscatorius* (eye diameter = 3.5919 OW $^{0.5551}$; $r^2 = 0.9289$; n = 26). Total lengths of individuals ranged from 22.5 to 80 cm.

DISCUSSION

Temporal segregation

The black and the white angelfishes coexist on the Mediterranean shelf, but their relative abundance was observed to vary on a diel scale, with *Lophius budegassa* more abundant at night and *L. piscatorius* more abundant during the day. In general, species sharing important traits related to their ecological niches can coexist in the same area due to a temporal segregation in the phase of maximum behavioural activity (Carothers and Jaksic 1984). Although trawling is an active method of sampling that collects all animals down to the seabed independently of their level of behavioural activation, rhythmic catch variations in trawl hauling at a certain location can be considered to be the product of rhythmic displacements of marine populations into and out of the sampling window (Aguzzi and Company 2010). When that sampling window consistently occurs at a certain depth and location, catchability rhythms can be precisely studied in relation to the light intensity fluctuation at the site. Rhythmic population movements occur over the day–night cycle in several different ways: within the water column (i.e. vertical–bentholeagic), in and out from the substrate (i.e. endobenthic), or at the benthic boundary layer, along bathymetric gradients (i.e. horizontal–nektobenthic). Anglerfishes do not burrow and rarely display vertical migration (Hislop et al. 2000), but they do possess some swimming capability (Velasco et al. 2008). The flattened morphology typical of demersal fish (Caruso 1986) allows us to assume that the activity rhythms of these species occur as nektobentic displacements. In fact, tagged and recaptured *L. piscatorius* demonstrated in- and offshore movements in the northeastern Atlantic (Laurenson and Priede 2005, Landa et al. 2008a), Therefore, these species may rhythmically swim between the shelf and the slope, with the main part of the population moving into shallow water areas during 2 opposite times in the day–night cycle.

Although preyed species are known to shift their activity patterns to avoid predation (Fenn and Macdonald 1995, Fraser et al. 2004), it is also common to observe a similar phenomenon among predators that compete for the same prey (Schoener 1986, Soria-Barreto and Rodiles-Hernández 2008). While this competition is reduced for species of mixed predatory and scavenging activity, pressure is likely to be stronger for more specialised piscivores such as anglerfish. Both *Lophius piscatorius* and *L. budegassa* mainly prey on whiting *Micromesistius poutassou*, cod *Gadus morhua*, and other gadids (Crozier 1985, Laurenson and Priede 2005, Preciado et al. 2006, Negzaoui-Garali et al. 2008). Because they show similar food preferences and compete for the same available resources, their temporal segregation seems to occur in order to avoid interference competition.

Sensory specialisation

The observed differences in the timing of maximum activity of *Lophius piscatorius* and *L. budegassa* were consistent with differences in their otolith weight and eye size. Otoliths represent an important part of the fish's hearing apparatus, and they are an indicator of how the teleostean inner ear works (Platt and Popper 1981, Paxton 2000). The inner ear in species that live in poor light environments, such as sea slope-dwelling fishes (Paxton 2000, Lombarte and Cruz 2007), nocturnal species, such as holocentrids (Smale et al. 1995), and cave-adapted species (Schulz-Mirbach et al. 2008), are characterised by relatively large otolith sagittae. Furthermore, sound-producing species, which are specialised in acoustic communication, such as sciaenids, are also characterised by bigger otoliths than species that are not sound producers (Cruz and Lombarte 2004). The heavier otoliths found in *L. budegassa* confirm this as a preferentially nocturnal species compared to *L. piscatorius*. In fact, species that are predominantly active at night or that dwell in deep water have been found to have large (and therefore heavy) otoliths, indicating that their behaviour is mostly acoustically driven, which is a characteristic usually found where light is insufficient to support visual communication (Lychakov and Rebane 2000, Paxton 2000, Parmentier et al. 2001, Lombarte and Cruz 2007, Tuset et al. 2010).

However, the visual systems of many organisms have evolved to see well in dim light conditions employing different optical strategies to enhance the of an image, both in terms of spatial resolution (by increasing the focal length as a consequence of enlarging the axial diameter of the eye) and sensitivity (improving photon capture, which is mostly achieved by having a large pupil) (Warrant 1999). Although the major adaptation for improving sensitivity is having a low F-number (focal length divided by the pupil diameter), most fish have an F-number close to 1 as their optics adhere to Matthiessen's ratio. Therefore, large eyes may reflect a need for increasing spatial resolution (Land and Nilsson 2002). Because better resolution and sensitivity are achieved with bigger eyes, evolution has benefited animals that must perform considerable visual tasks with large eyes. Although there are many exceptions, epipelagic fishes with nocturnal activity or species inhabiting mesopelagic waters tend to have bigger eyes than diurnal shallow water species (Marshall 1971, Warrant and Locket 2004, Pulcini et al. 2008, Antonucci et al. 2009). In this context, the data we present here fit well with observations of the eye design in other diurnal and nocturnal teleosts. In fact, *Lophius piscatorius*, the species exhibiting a preference for diurnal activity, had a significant smaller eye and lens than *L. budegassa*, the species captured mainly at night. The preference for diurnal activity of *L. piscatorius* was clearly reflected in the retinal anatomy of this species, especially in the cone diameter that was smaller than that of *L. budegassa*. This characteristic was responsible for the low optical sensitivity observed in this species. Instead, *L. budegassa* showed a sensitivity higher than 1, typical of crepuscular or mid-water species (Land and Nilsson 2002). Optical sensitivity increased greatly with size in this species, confirming the possibility of seeing well in dim light. In fact, a similar sensitivity of 5.7 μm^2 sr was found in the blue marlin *Makaira nigricans*, a species whose eyes are adapted to the severe change in light intensity that this species encounters during its diving that may stretch several hundred metres (Fritsches et al. 2003).

The interspecific comparison of the eye size of *Lophius piscatorius* and *L. budegassa* was reversed when *L. piscatorius* reached 50 to 55 cm TL, and above this threshold, *L. piscatorius* showed significantly larger eyes than *L. budegassa*. This trend reversal probably reflects important changes in the life history of *L. piscatorius*. Even though there are uncertainties about several key events during the life history of *L. piscatorius*, many authors agree that this species spawns in deep or very deep water (>1000 m) (Hislop et al. 2001). At these depths, mature males of 50 to 60 cm are commonly observed (Duarte et al. 2001), whereas females of *L. piscatorius* reach their first maturity at a larger size. In addition, changes in the feeding behaviour of *L. piscator*ius have also been observed at this size (Laurenson and Priede 2005). The change in the visual scenery with depth, from being extended in the epipelagic zone to semi extended in the mesopelagic zone and even to point source light (bioluminescence) in the bathypelagic zone, could be one of the driving forces in the evolution of larger eyes in large *L. piscatorius*. Instead, the relatively low resolution obtained for this species is quite surprising, as larger fish with larger eyes usually have higher resolving power (Collin and Pettigrew 1989, Shand 1997, Bozzano and Catalán 2002). Probably, for a species living on the bottom where the water is often full of suspended particles, the environment acts as a constraint for increasing acuity. Similarly, an unexpected low resolution was also observed in the blue marlin, a species with a huge eye size (Fritsches et al. 2003).

Although interspecific temporal segregation is one of the mechanisms employed by different fish species to allow their coexistence, there is still little information on how it occurs and its adaptive value. In other groups, such as birds, adaptations to night vision include large eye size, and it has been demonstrated that birds with large eyes become active earlier (at low light intensity) than birds with small eyes (Thomas et al. 2002). In fishes, spatial segregation is a more common mechanism of coexistence, and concomitant spatial segregation in activity rhythms and different eye sizes has been observed in several ecologically equivalent and sympatric species. In cichlids of African lakes, vision greatly contributes to their habitat segregation and even to their speciation (Seehausen et al. 2008, Hofmann et al. 2009). In this family, 2 highly morphologically similar species, *Haplochromis hiatus* and *H. iris*, prey on similar food items and inhabit different depths. Accordingly, the deeper water species (*H. iris*) has larger eyes compared to the shallower species (Van Der Meer and Anker 1984). However, eye size and visual capability in fish are likely to be determined by a number of different selection pressures and constraints, and therefore, the existence of large eyes does not necessarily mean that they have evolved only to see better in dim light conditions. For example, fast swimming also requires good vision, and hence, large eyes are also found in swordfish and tuna. The association of evolutionary changes in eye size with different behavioural and ecological traits in *Lophius* spp. needs to be further investigated by morphological and physiological analyses to trace the history of changes in the relationship between morphological characters and activity.

However, sensory constrains appear to be an important feature enhancing partitioning in time by enabling closely related species such as *Lophius piscatorius* and *L. budegassa* to inhabit the same area at different times of the day. Although these species rely on a combination of sensory stimuli to perform their daily tasks, under the paradigm of compensatory specialization, their sensory systems experience high interspecific pressure that may lead to different diurnal or a nocturnal behaviour.

CHAPTER

2.2

REPRODUCTIVE STRATEGY

SUBCHAPTER 1

REPRODUCTIVE BIOLOGY OF BLACK ANGLERFISH (*Lophius budegassa*) IN THE NORTHWESTERN MEDITERRANEAN SEA

REPRODUCTIVE BIOLOGY OF BLACK ANGLERFISH (*Lophius budegassa*) IN THE NORTHWESTERN MEDITERRANEAN SEA

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Abstract. The gonadal morphology and reproductive biology of the Black Anglerfish (*Lophius budegassa*) were studied through examination of 4410 specimens collected between June 2007 and December 2010 in the northwestern Mediterranean Sea. Ovaries and testes presented traits common among fishes of the order Lophiiformes. Spawning occurred between November and March. Size at first maturity (L_{50}) was 33.4 cm in total length (TL) for males and 48.2 cm TL for females. The Black Anglerfish is a total spawnerv with group-synchronous oocyte development and determinate fecundity. Fecundity values ranged from 87,569 to 398,986 oocytes, and mean potential fecundity was estimated at 78,929 (standard error of the mean [SE] 13,648) oocytes per kilogram of mature female. This study provides the first description of the presence of 2–3 eggs that shared the same chamber and a semicystic type of spermatogenesis for the Black Anglerfish. The new information from this study allows for a better understanding of Black Anglerfish reproduction, knowledge that will be useful for the assessment and management of this species.

INTRODUCTION

Lophius, a genus commonly known as anglerfishes or monkfishes, includes 7 species broadly distributed and exploited worldwide. Most of these species inhabit the northwestern Atlantic, as do the Goosefish (*Lophius americanus*) and Blackfin Goosefish (*L. gastrophysus*), or the northeastern Atlantic, as do the Cape Monk (*L. vomerinus*), Shortspine African Angler (*L. vaillanti*), Black Anglerfish (*L. budegassa*), and White Anglerfish (*L. piscatorius*), although Black Anglerfish and White Anglerfish also live in the Mediterranean Sea and Yellow Goosefish (*L. litulon*) can be found only in the northwestern Pacific (Fariña et al. 2008). In the past, species of *Lophius* have been captured as bycatch in mixed fisheries, but an increase in their economic value, together with the overexploitation of other groundfish species, has led to the development of targeted anglerfish fisheries (Hislop et al. 2001). In the northwestern Mediterranean Sea, landings of Black Anglerfish and White Anglerfish accumulated to just over 6000 metric tons during the last 10 years (data obtained from the General Directorate of Fishing and Maritime Affairs of the Catalan Government, Spain). The scarce reproductive information available for these species does not allow for a proper assessment or informed management of anglerfish fisheries.

This study focuses on the Black Anglerfish, a demersal fish distributed along the Mediterranean Sea as well as the northeastern Atlantic from the British Isles to Senegal (Caruso 1986). This species is found over the continental shelf and upper slope at depths of up to 800 m and inhabits sandy, muddy, and rocky bottoms (Carlucci et al. 2009). They occupy the water column as eggs and larvae, and then they shift to a benthic existence as juveniles and adults (Fariña et al. 2008). This species co-occurs with the White Anglerfish over all its bathymetric range, although the White Anglerfish has a deeper distribution that reaches to depths >1000 m (Afonso-Dias and Hislop 1996). Despite of the overlapping distributions of these species, Colmenero et al. (2010) concluded that no ecological competition exists between these species because of a temporal segregation in their biorhythms; the Black Anglerfish is more active at nighttime, and the White Anglerfish is more active during daytime.

Most studies of these species have been done in northeastern Atlantic waters, and they often have dealt with age and growth (Dupouy et al. 1984, Woodroffe et al. 2003, Landa et al. 2008a), feeding habits (Crozier 1985, Laurenson and Priede 2005, Preciado et al. 2006), geographical and depth distribution (Caruso 1985, Landa et al. 2008b, Velasco et al. 2008), and reproduction (Afonso-Dias and Hislop 1996, Duarte et al. 2001, Laurenson 2006). In the Mediterranean Sea, studies have been less numerous and for the most part have focused on biological aspects similar to the ones examined in the studies just described (Tsimenidis and Ondrias 1980, Tsimenidis 1984, Ungaro et al. 2002, Maravelias and Papaconstantinou 2003, García-Rodríguez et al. 2005, La Mesa and De Rossi 2008, Negzaoui-Garali and Ben Salem 2008, Negzaoui-Garali et al. 2008, Carlucci et al. 2009, Colmenero et al. 2010). However, only Tsimenidis (1980) and Carbonara et al. (2005) focused on reproductive traits of the Black Anglerfish.

Information about the duration of the spawning season, the size at first maturity, reproductive strategy, maturation of oocytes, and fecundity are very relevant for studies of the biology and population dynamics used in stock assessments for management of fishery resources. Of all these reproductive features, fecundity is the most difficult biological parameter to obtain, although it is critical for accurate stock assessments (Trippel et al. 1997). In the peculiar case of species of *Lophius*, studies on fecundity are scarce because of the difficulty of 1) acquisition of suitably mature individuals in the maturity phases of spawning capable or actively spawning and 2) the use of a proper method for fecundity estimation, which is especially complicated because of the gonad morphology of these species. During reproduction, a gelatinous material is secreted into the lumen, and enumeration and measurement of eggs embedded in this mucus matrix is extremely difficult.

Although species of *Lophius* have similar traits throughout the world, some biological aspects and patterns of fisheries and exploitation present interspecific and spatial variations (Fariña et al. 2008). For that reason, reproductive parameters of the Atlantic stock of the Black Anglerfish cannot be applied to the stock in the Mediterranean. In addition, knowledge of the reproductive biology of this species in the northwestern Mediterranean Sea is very limited. Therefore, this study is the first one to take a detailed approach to the examination of reproductive traits of the Black Anglerfish in the Mediterranean Sea, and the results of this study can contribute to improvement of stock assessment and effective management for this species in this region.

MATERIALS AND METHODS

Sample collection

Monthly samples of Black Anglerfish were obtained from 467 sampling stations situated in the fishing grounds off the Catalan coast in the northwestern Mediterranean Sea from 40°5.980′ N to 43°39.310′ N and from 0°32.922′ E to 3°35.718′ E between June 2007 and December 2010. Specimens were collected onboard commercial trawl fishery vessels at depths of 20–600 m and identified following Caruso (1986). The trawl fleet belonged to the ports of Roses, Blanes, Arenys de Mar, Vilanova i la Geltrú, and Sant Carles de la Ràpita (Fig. 1).

For this study, 4410 specimens were measured to the nearest centimeter in total length (TL) weighed to the nearest gram in total weight (TW) and gutted weight (GW), and measured with an accuracy of 0.01 g in gonad weight (GNW) and liver weight (LW).

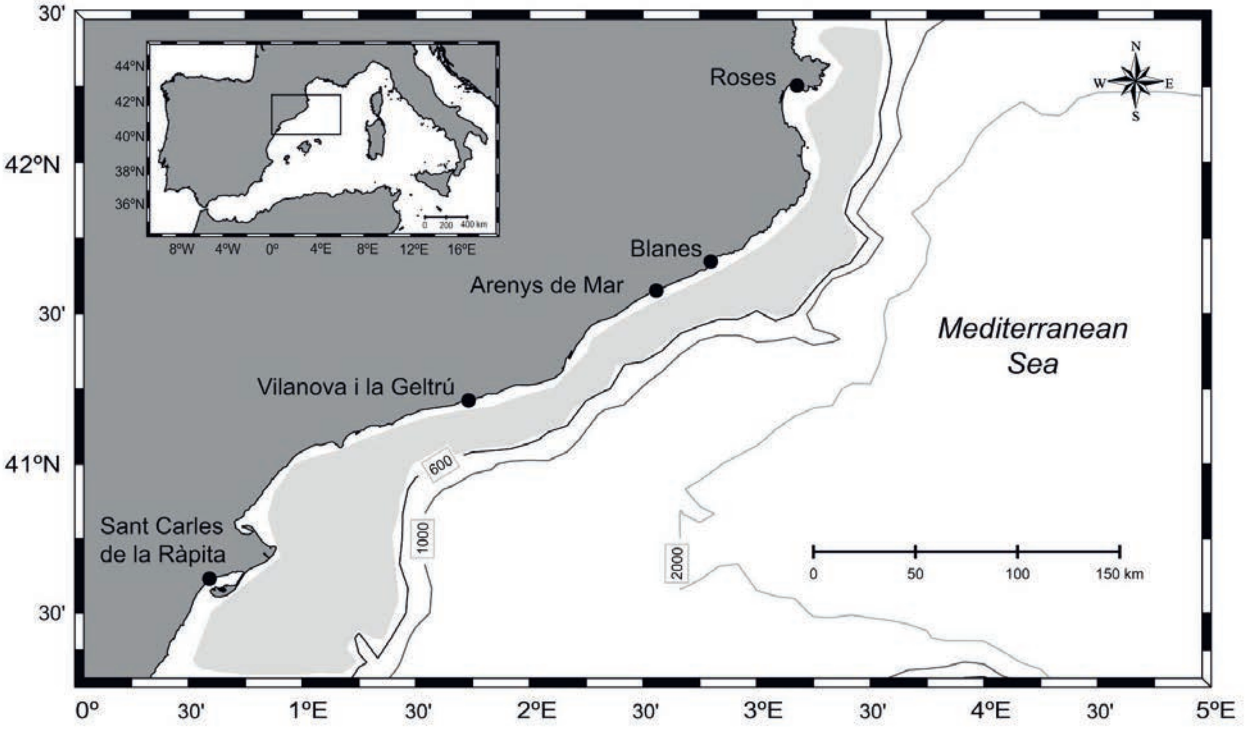

Figure 1. Map of study area where specimens of Black Anglerfish (*Lophius budegassa*) were collected in the northwestern Mediterranean Sea between June 2007 and December 2010 to examine the reproductive biology of this species. The zone shaded in light gray closest to the Catalan coast indicates the sampling area. The cities shown on the coast were the ports of commercial trawl fishery vessels from which specimens were collected.

Macroscopic and histological description of gonads

Of the total number of specimens, 3562 fish had gonads removed and their sex determined, and they were assigned macroscopically to a gonadal stage on the basis of a scale with 5 maturity phases that were described in previous studies: immature (phase I), developing/regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (Afonso-Dias and Hislop 1996, Brown-Peterson et al. 2011) (Table 1).

Sex easily was assessed macroscopically in mature individuals. However, gonads from small individuals (approximately <20 cm TL) were indistinguishable macroscopically because ovaries and testes were small, translucent, and string-like. Fish that were too small to determine their sex or assign to a gonadal phase were classified as indeterminate.

Table 1. Macroscopic and microscopic description of the 5 maturity phases in the reproductive cycle of male and female Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010; adapted from Afonso-Dias and Hislop (1996) and Brown-Peterson et al. (2011).

Phase	Males	Females
Immature (I)	Testes are long, narrow, and tubular shaped. They are translucent with no visible vascularisation. The medial seminiferous duct is distinct. Only spermatogonia and primary spermatocytes are present.	Ovaries are very narrow, thin, and flattened-tube shaped. They are translucent; no oocyte clusters visible and minimal vascularization. Only oogonia and primary growth oocytes are present.
Developing / Regenerating (II)	Testes are small with visible blood vessels around the seminal duct. Spermatogonias, primary and secondary spermatocytes are predominant. Spermatids are scarce.	Ovaries are small. Still no noticeable individual oocyte clusters. They acquire a cream colour and vascularization is visible. Only oogonia and primary growth oocytes are present.
Spawning capable (III)	Testes increase in length and width. They have a firm texture and cream color. Seminal duct is highly vascularized. Germ cells at all stages of spermatogenesis are present. Spermatids are predominant with a lot of spermatozoa in the lumen of the sperm duct.	Ovaries increase in width and length. They have light orange color, and blood vessels are prominent. The edges of the ovaries start to curl and they occupy a larger proportion of the body cavity. A mucus matrix starts to develop. Primary growth, cortical alveolar, and primary and secondary vitellogenic oocytes are present.
Actively spawning (IV)	Testes are large and firm and have a creamy coloration. Large amounts of sperm produced when testes are dissected. Abundant quantities of spermatozoa are present in the seminiferous tubules.	Ovaries are extremely long and width and occupy most of the body cavity. The colour of the oocytes is orange, and they are visible macroscopically. Ovaries are characterized by the presence of large hyaline oocyte clusters enclosed in a transparent gelatinous matrix that is completely developed. High vascularization is present. Oocytes are in tertiary vitellogenesis, migratory nucleus and hydration.
Regressing (V)	Testes are small, flaccid, and have brown or red areas in their beige surface. They are still highly vascularized. Sperm and residual spermatozoa can be found in the lumina of the sperm duct. Spermatogonia are present in the testes cortex.	Ovaries are flaccid and highly vascularized and often have longitudinal striations. Their colour is dark pink or red. Atresia and postovulatory follicles, together with primary growth stages, are present. Cortical alveolar, primary and secondary vitellogenesis can be found.

To corroborate the macroscopic classification of some unclear and undetermined gonads, 372 specimens were histologically examined. They were fixed in 10% buffered formalin solution before they were dehydrated and embedded in a methacrylate polymer resin. Cross sections, each 3–4 µm thick, were made with a manual microtome Leica Reichert-Jung 2040 (Leica Microsystems, Wetzlar, Germany) stained with Lee's stain (methylene blue and basic fuchsin), and mounted in a synthetic resin of dibutyl phthalate xylene (DPX) on microscope slides. Gonads were classified according to their morphology and the presence of specific inclusions (oil droplets, yolk granules, yolk vesicles, or postovulatory follicles) (Wallace and Selman 1981). The ovarian and testicular phases were defined by the developmental stage of the most advanced cell within the gonad (Yoneda et al. 1998b).

Spawning season and size at first maturity

The spawning season was established from the analysis of the monthly variation of the maturity phases and the changes in gonadosomatic (GSI) and hepatosomatic (HSI) indices for each sex (Afonso-Dias and Hislop 1996). Because immature specimens were not considered, 1437 males and 1167 females were used to determine both indices, which were calculated according to Yoneda et al. (2001) with these equations:

$$\mathrm{GSI} = (\mathrm{GNW} \,/\, \mathrm{GW}) \times 100$$

$$\mathrm{HSI} = (\mathrm{LW} \,/\, \mathrm{GW}) \times 100$$

Size at first maturity (L_{50}) was determined through the examination of males and females in mature phases (phase III, phase IV, or phase V) and immature individuals collected during the spawning period (Duarte et al. 2001). Total length of all individuals was used to estimate L_{50}, defined as the size at which 50% of all fish sampled were at sexually mature phases. Maturity curves were determined with a logistic curve (Pope et al. 1975):

$$\mathrm{P} = 100 \,/\, (1 + \exp\,[a + b\mathrm{TL}])$$

where P is the percentage of mature individuals as a function of size class (TL), and *a* and *b* are specific parameters that can change during the life cycle. A logarithmic transformation was applied to this equation to calculate the parameters *a* and *b* by means of linear regression.

Reproductive strategy and fecundity

Patterns of ovarian organization and fecundity were tested by oocyte size-frequency distributions (West 1990). For our analysis, 36 fish, with lengths between 20.0 and 72.5 cm TL, were randomly selected from all maturity phases. From these fish, 4428 oocytes—with more than 300 oocytes from each maturity phase (I=961; II=1106; III=1046; IV=381; V=934)—were measured for their diameter with an image analysis program (Image-Pro Plus, vers. 5.0, Media Cybernetics, Inc., Rockville, MD) in combination with an Axioskop 2 Plus microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY) and a ProgRes C14 digital microscope camera (Jenoptik AG, Jena, Germany). Diameters were measured to the nearest 0.01 μm. The mean oocyte diameter by developmental stage was determined by calculating the diameter of all oocytes encountered in each subsample. Measurements were taken only of oocytes that were sectioned through the nucleus (Afonso-Dias and Hislop 1996).

Before fecundity was estimated, the gonads of 7 individuals were divided into 3 sections (anterior, middle, and posterior) to test differences in mean oocyte density within the ovary through the use of a one-way analysis of variance (ANOVA). This use of 3 sections ensured that the analyzed subsample represented the entire ovary (Murua et al. 2003). Batch fecundity (BF), the total number of mature eggs produced in a single spawning batch by an individual female, was estimated through the use of the gravimetric method on the basis of the relation between ovary weight and the density of oocytes in the ovary (Hunter and Goldberg 1980). Three ovarian tissue samples of known weight, representing 10% of the total ovarian weight, were extracted from different areas of the same ovary (anterior, middle, and posterior ovarian lobe). These subsamples were collected from 15 specimens with ovaries in phases III and IV with neither postovulatory follicles nor atretic oocytes present. Because the oocytes could not be extracted from their mucogelatinous matrix without destroying them, whole tissue subsamples were mounted on several slides for analysis and covered with a cover slip.

Images of each ovarian tissue sample were taken with a Canon Powershot SD870 IS digital camera (Canon USA, Melville, NY) and oocytes were counted manually with Image-Pro Plus software. Fecundity values were obtained through examination of Black Anglerfish with lengths of 46–65 cm TL, TW of 1096–5592 g, GW of 986–3600 g, and GNW of 88.70–2300 g. Batch fecundity for each female was calculated as a product of the number of secondary vitellogenic oocytes per unit of weight multiplied by the total ovarian weight (Yoneda et al. 2001). Relative batch fecundity (RBF), the total number of mature eggs released by a female during the spawning batch per gram of body weight of gutted fish, was calculated as BF divided by GW (Pavlov et al. 2009):

$$\mathrm{BF} = (\text{oocyte number} \,/\, \text{sampled GNW}) \times \text{total GNW}$$

$$\mathrm{RBF} = \mathrm{BF} \,/\, \mathrm{GW}$$

Linear regression analysis was used to examine the relationships between BF and fish TL, TW, and GW (Armstrong et al. 1992). Linear regression analysis also was applied to analyze the relationship between RBF and TL. Mean potential fecundity was also calculated as the number of vitellogenic oocytes divided by kilogram of mature female (Murua et al. 2003).

RESULTS

Gonadal morphology

Ovarian structure consists of a flattened band with 2 distinctive lobes that are folded up and connected to each other at their posterior end. The lobes form a single organ attached to the abdominal cavity by a black mesenteric tissue called the mesovarium. One side of the ovarian wall is made of an ovigerous membrane and connective tissue. The nonovigerous side is made of epithelial cells. A single layer of oocyte clusters projects from the ovigerous membrane to the lumen (Fig. 2A). Inside of each gonad, the clusters can be in different development stages. Only the oocytes situated closest to the tip of the clusters have progressed through all maturity stages, and the other oocytes are only oogonia or in the primary growth stage (Fig. 2B).

A gelatinous material is secreted into the lumen during the late phases of gonad maturation, producing the mucus matrix characteristic of the reproduction of *Lophius* species (Fig. 2C). Hydration of the oocytes occurs just before spawning, and postovulatory follicles (Fig. 2D) are found during the regression phase of the reproductive cycle. Ripe eggs, which are usually situated on the tip of the oocyte cluster, rupture the follicles and are pressed into the layer of mucus. In this study, every chamber examined contained at least 1 egg in the gelatinous matrix (Fig. 2E), although the presence of 2 (Fig. 2F) or 3 eggs (Fig. 2G) floating in separate chambers also was noted (Fig. 2H).

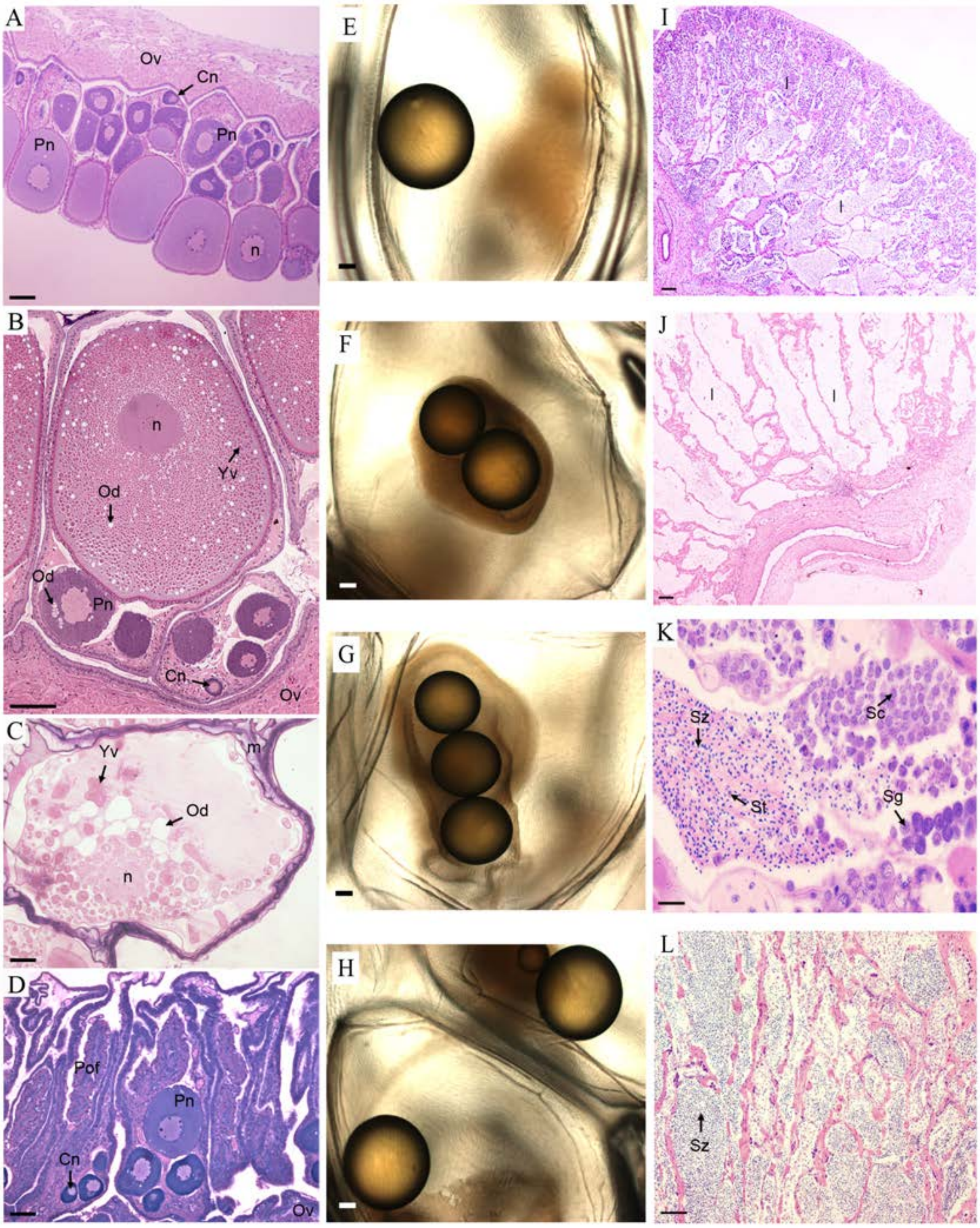

Figure 2. Micrographs of transverse sections of ovaries, oocytes inside the gelatinous matrix, and testes of Black Anglerfish (*Lophius budegassa*) collected in the northwestern Mediterranean Sea between June 2007 and December 2010. The final 4 stages of ovary development are shown in the left column: (**A**) Phase II: developing or regenerating; (**B**) Phase III: spawning capable; (**C**) Phase IV: actively spawning; (**D**) Phase V: regressing. Oocytes are featured in the middle column: (**E**) 1 oocyte in a chamber (486 μm in diameter); (**F**) 2 oocytes floating in a chamber (427 μm and 359 μm in diameter) and (**G**) 3 oocytes in a chamber (341 μm, 330 μm, and 322 μm in diameter); and (**H**) closed-up division between chambers. In the right column, transverse sections of testes show (**I**) its lobular organization, (**J**) empty lobules, (**K**) seminal lobule during spermatogenesis, and (**L**) spermatozoa in the lumen of the seminal lobule. Ov=ovigerous membrane; n=nucleus; Od=oil droplet; Yv=yolk vesicle; m=mucus matrix; Cn=chromatin nucleolar; Pn=perinucleolar; Pof=postovulatory follicle; l=seminal lobule; Sg=spermatogonia; Sc=spermatocyte; St=spermatid; Sz=spermatozoa. Scale bars=100 μm.

Oocyte diameter appeared to differ depending on the quantity of oocytes present in each chamber, with the oocytes that were isolated in their chambers found to be larger in size than other oocytes. A diameter of 486 μm was obtained for the oocyte that was the single oocyte in its chamber; diameters of 427 μm and 359 μm were found for the 2 oocytes floating together in a chamber; and diameters of 341 μm, 330 μm, and 322 μm were observed for the 3 oocytes that shared the same chamber. Measurements of more oocyte diameters are needed to confirm these preliminary observations.

The testes are a pair of elongated and tubular structures located in the dorsal portion of the abdominal cavity, and they are bean-shaped in transverse section. The organization of the testes is lobular: the connective tissue extends from the testicular capsule to form lobules that have their blind ends on the surface of the gonads, converging ventrally towards the sperm duct (Fig. 2I). These lobules are fused to the posterior end of each testicular lobe to form a common sperm duct that leads to a genital pore (Fig. 2J). Spermatogenesis takes place in a capsule-like sac called a cyst, but it is not completed within the cyst. Each cyst contains spermatogonia or developing spermatocytes (Fig. 2K). Before the end of the spermatogenesis, the cyst breaks up and spermatids are released into the lumina of the lobules, where spermatogenesis is then completed and spermatids transform into spermatozoa (Fig. 2L). The cysts appear to be arranged in order of maturation, with a gradient of germ cells of increasing maturation from the cortex to the sperm duct. The morphology of the spermatozoa head seems to be elongated.

Spawning season and size at first maturity

Monthly distribution of macroscopic classification of the maturity phases (Fig. 3A and B) revealed that the period of maximum occurrence of females in the spawning capable phase (III) was from November to January. The presence of females in the actively spawning phase (IV) was observed from November to March, with a maximum peak in January. Females in the immature, regressing, and developing/regenerating phases (I, V, and II, respectively) were found throughout the year, with the highest percentage of immature individuals seen in May. A slight increase in phase-III females was observed in August, and that increase would likely result in spawning activity in September, indicating the possibility of a secondary breeding season. Males in all maturity phases were observed throughout the year, with 2 maxima of mature males occurring in December and July.

For mature males and females, GSI and HSI indices were calculated. In males, GSI was fairly constant throughout the year, with a maximum index value of 1.06 reached in January (Fig. 3C). The mean GSI for females was highest from December to March, with a peak of maximum activity in January (4.94) and February (2.43) (Fig. 3D). The mean HSI for females and males followed the same pattern. The highest value for males was found in September (2.50), and the lowest value in February (1.65) (Fig. 3C). In females, HSI values ranged from 3.19 in January to

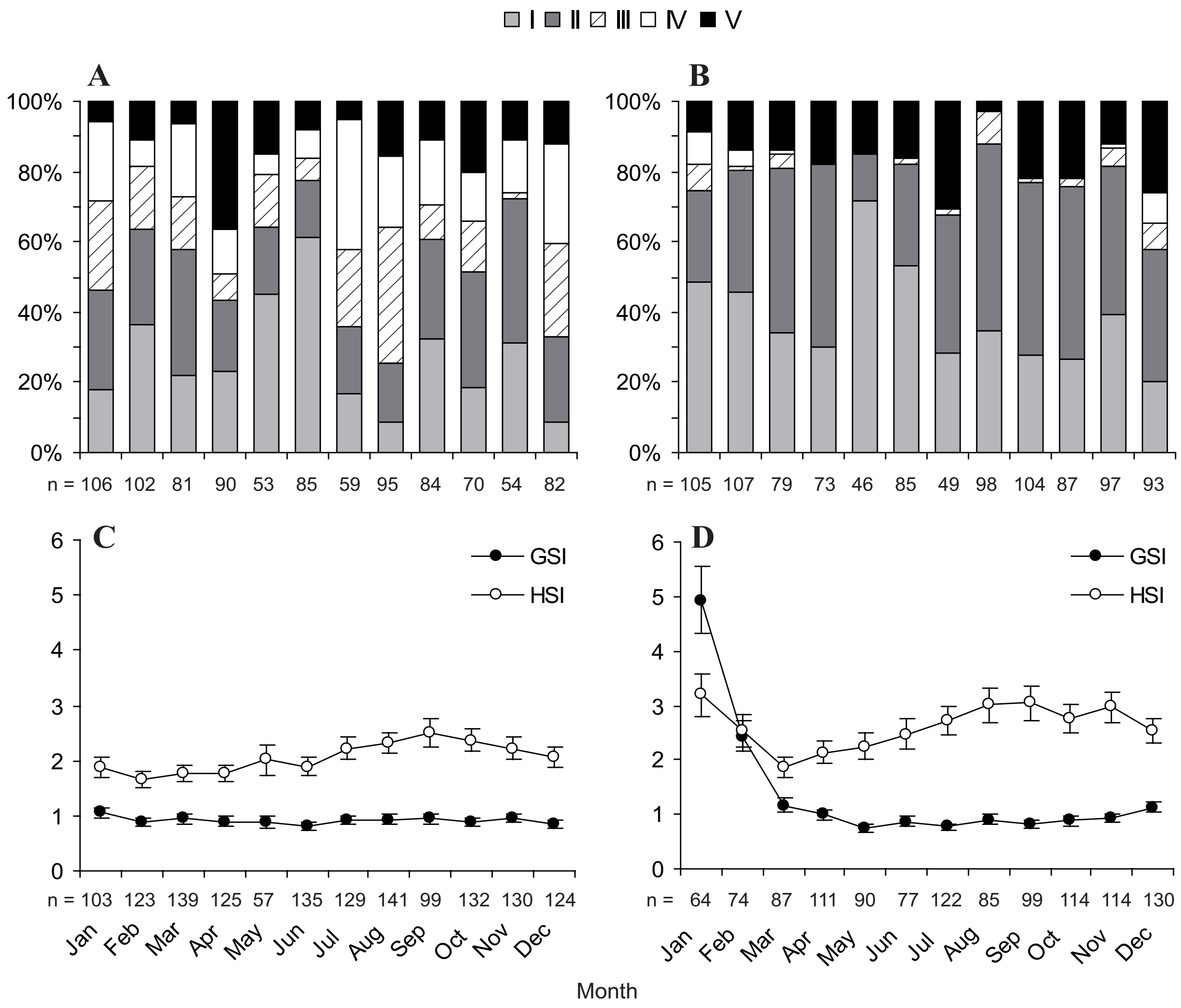

Figure 3. Monthly distributions of (**A**) males and (**B**) females in the 5 phases of gonad maturity of Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010 and monthly changes in the mean gonadosomatic index (GSI) and hepatosomatic index (HSI) for (**C**) males and (**D**) females. On the basis of macroscopic examination, specimens were assigned to the following phases: immature (phase I), developing or regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (phase V). Error bars indicate ± 1 standard error of the mean.

1.86 in March (Fig. 3D). The highest HSI values were found just at the beginning of the main spawning season. GSI and HSI results, together with observations of maturity phases throughout the year, indicate that there is one main spawning season from November to March.

Comparison of L_{50} curves showed a clear difference between males and females. The size at 50% sexual maturity was 33.4 cm TL for males (Fig. 4A) and 48.2 cm TL for females (Fig. 4B).

Reproductive strategy and fecundity

The size-frequency distributions of oocyte diameters in each of the 5 maturity phases indicate that oocytes in different stages of development were found in each maturity phase (Table 2; Fig. 5).

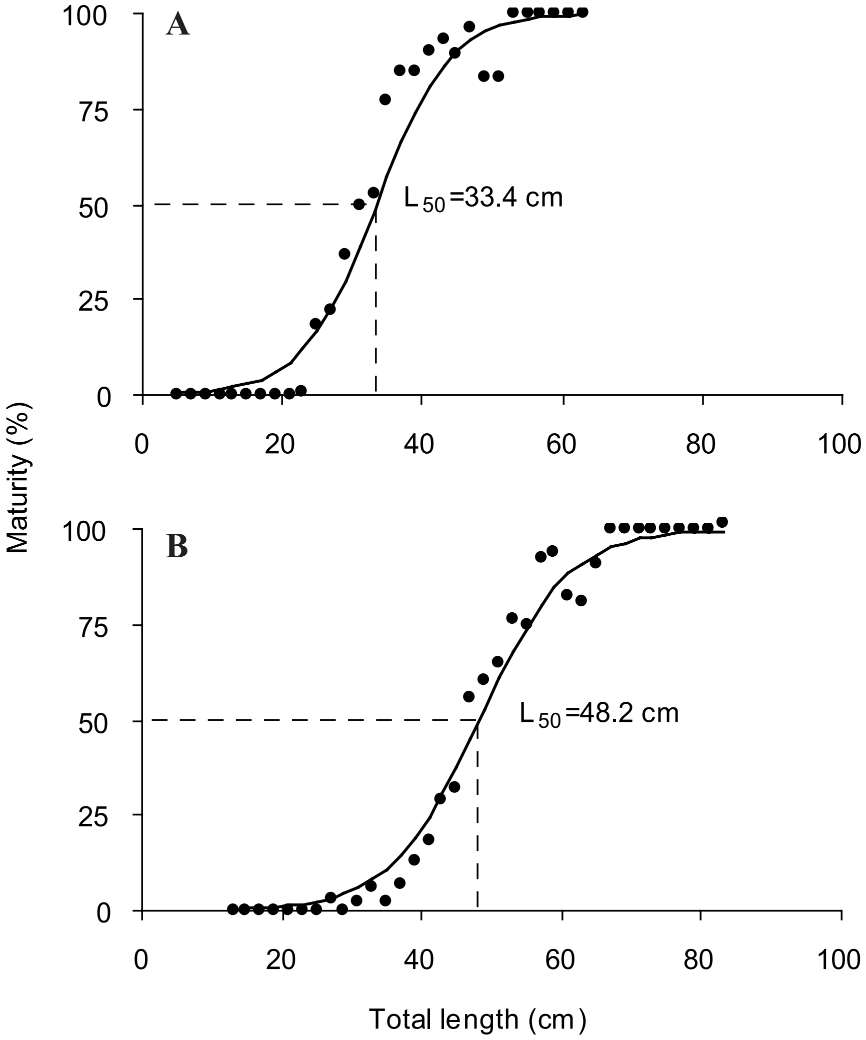

Figure 4. Maturity ogives used to estimate length at maturity (L_{50}) for (**A**) male and (**B**) female Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010.

Table 2. Oocyte histological characteristics of Black Anglerfish (*Lophius budegassa*) collected between June 2007 and December 2010 in the northwestern Mediterranean Sea; descriptions follow those of Wallace and Selman (1981). Mean oocyte diameters, which were measured to the nearest 0.01 μm, are provided by developmental stage with standard errors of the mean (SE).

Oocyte developmental stage	Oocyte diameter (μm)	Histological characteristics
Chromatin nucleolar	36.55 (SE 21.27)	Nucleus contains a large nucleolus and some small peripheral nucleoli. Yolk granules are not present in the cytoplasm.
Perinucleolar	110.78 (SE 62.54)	Nucleus grows and several big peripheral nucleoli and vacuoles are present. No yolk granules are present in the cytoplasm.
Cortical alveolar	256.35 (SE 80.81)	Nucleus is central. Cortical alveolar vesicles and oil droplets appear in the cytoplasm. Yolk granules are still not present in the cytoplasm.
Primary vitellogenic	364.54 (SE 37.31)	Yolk granules appear between cortical alveolar vesicles. Nucleus remains central.
Secondary vitellogenic	406.20 (SE 39.60)	Yolk granules fill the cytoplasm. Nucleus is still central.
Tertiary vitellogenic	544.55 (SE 185.49)	Yolk granules are in contact with the nucleus, which is still central, and the oocyte size increased from size at previous stages.
Migratory nucleus	883.42 (SE 153.33)	Yolk granules and oil droplets start to fuse. Nucleus migrates to one pole of the oocyte.
Hydration	1125.09 (SE 176.93)	Yolk granules form a single mass. Nucleus is not present in the cytoplasm.

During phase I, only oocytes in the primary growth stage (chromatin nucleolar and perinucleolar) with a narrow range of diameters were present (Fig. 5A). In phase II, cortical alveolar vesicles were found in the cytoplasm together with oocytes in the stage of primary growth with diameters that had increased notably (Fig. 5B). In phase III, yolk granule stages (Murua et al. 2003) were present along with the previous 2 types of oocytes. The oocytes increased in size as the yolk accumulated, and a wider oocyte diameter range distribution was observed during this phase (Fig. 5C). In phase IV, oocytes were observed in different stages (primary growth, vitellogenesis, migratory nucleus, and hydration). Two populations of oocytes were recognized in phase IV: a population of larger oocytes (defined as a clutch) and a population of smaller oocytes from which the clutch was recruited (Fig. 5D). In phase V, oocytes in the primary growth stage were found along with postovulatory follicles and atretic oocytes (Fig. 5E).

The presence of oocytes in different developmental stages within the same cluster and the frequency distribution of oocyte diameter along all maturity phases indicate that oocyte development in the Black Anglerfish is group-synchronous. The existence of a gap that separates the yolked oocyte stock, the ones to be spawned during the current breeding season, from the unyolked oocytes, the ones to be spawned in the coming breeding season, together with the increase of the mean diameter of the advanced vitellogenic oocytes, indicates that annual fecundity is determinate.

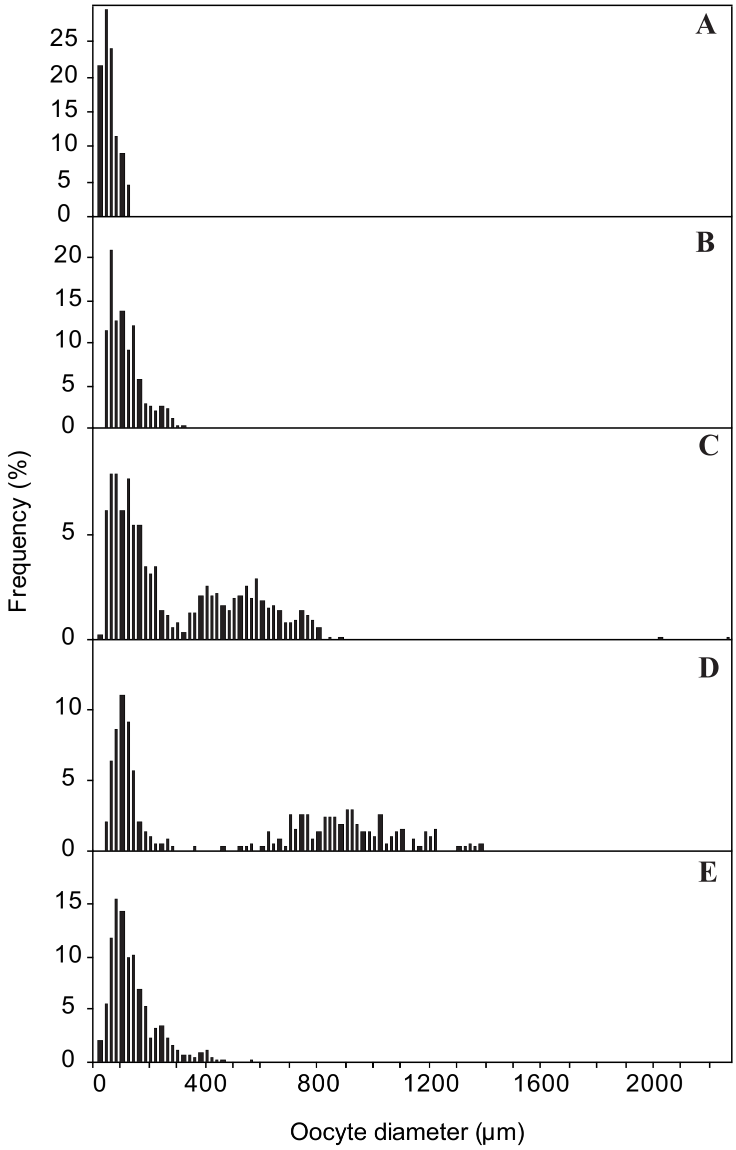

Figure 5. Size-frequency distributions of oocyte diameters at each phase of gonad maturity of Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. The 5 maturity phases are (**A**) phase I, immature, *n*=961; (**B**) phase II, developing or regenerating, *n*=1106; (**C**) phase III, spawning capable, *n*=1046; (**D**) phase IV, actively spawning, *n*=381; (**E**) phase V, regressing, *n*=934.

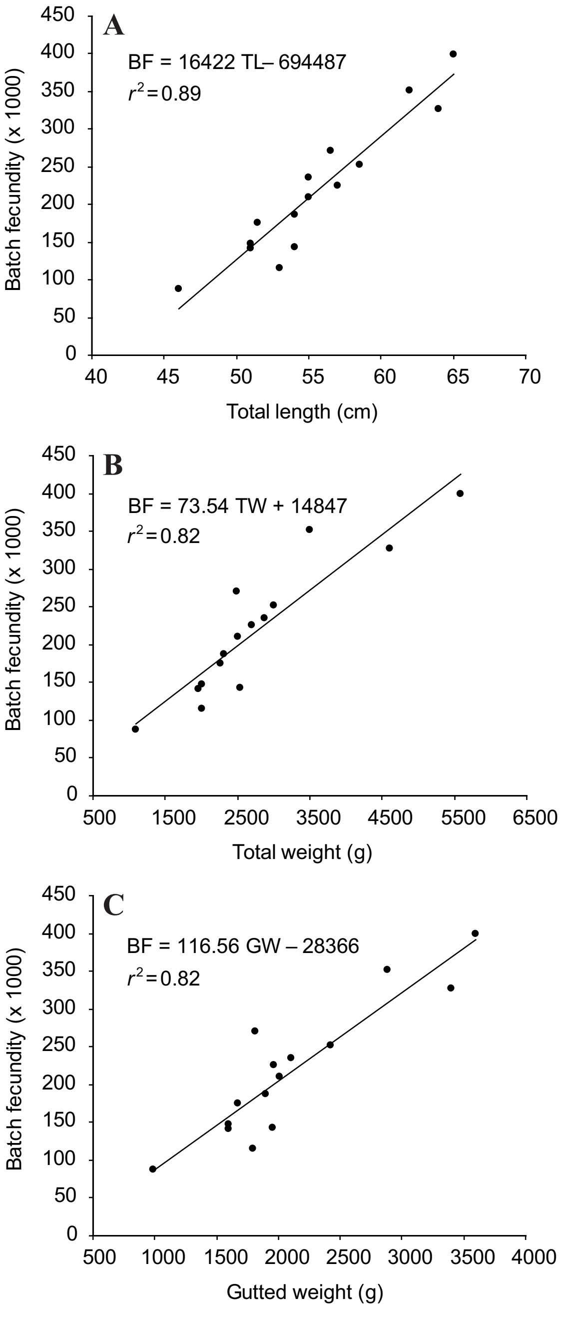

Figure 6. Relationships between batch fecundity (BF) and (**A**) total length (TL), (**B**) total weight (TW), and (**C**) gutted weight (GW) for Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. r^2=coefficient of determination.

Significant differences in oocyte densities among ovary sections were not observed (ANOVA, $F_{(2,42)}$=0.002, P=0.998). Batch fecundity ranged between 87,569 and 398,986 oocytes, and mean BF was 218,020 oocytes (standard error of the mean [SE] 90,018). Relative batch fecundity was estimated at 102 (SE 20) oocytes per gram of female (GW), and mean potential fecundity (oocytes kg^{-1}) was 78,929 (SE13,648) oocytes per kilogram of mature female.

Batch fecundity tended to increase linearly with TL (linear regression, coefficient of determination [r^2]=0.89, $F_{1,13}$=106.57, P<0.001), TW (linear regression, r^2=0.82, $F_{1,13}$=60.79, P<0.001), and GW (linear regression, r^2=0.82, $F_{1,13}$=59.31, P<0.001), indicating that fecundity is dependent on size and body weight (Fig. 6). No significant correlation was found between RBF and TL, indicating that RBF is not size dependent (linear regression, r^2=0.16, $F_{1,13}$=2.50, P=0.138).

DISCUSSION

This study indicates that oocyte development and the fecundity pattern of the Black Anglerfish are similar to findings for other species of *Lophius*: White Anglerfish (Fulton 1898), Cape Monk (Leslie and Grant 1990), Goosefish (Armstrong et al. 1992) and Yellow Goosefish (Yoneda et al. 2001). The eggs of Black Anglerfish appear to be shed as a part of a single event and are likely released only once during the spawning season, the pattern of a total spawner. This type of spawning has been observed also in Goosefish (Feinberg 1984) and White Anglerfish (Afonso-Dias and Hislop 1996), although the possibility of spawning several batches over the spawning season is not unfeasible because this behavior has been described for Yellow Goosefish (Yoneda et al. 2001).

Batch fecundity estimates for Black Anglerfish reveal a positive relationship between number of oocytes and fish length and weight; therefore, large spawners have a higher contribution to egg production than do smaller ones. Previous authors found lower fecundity values than the ones observed in this study. In the Tyrrhenian Sea, Carbonara et al. (2005) determined mean potential fecundity as 54,057 oocytes per kilogram of mature female and total fecundity as 211,687 oocytes from data obtained from a single individual (59 cm TL). In the case of the Aegean Sea, where fecundity values varied from 105,800 to 284,200 oocytes, fecundity was determined from an undefined number of individuals and size range (Tsimenidis 1980).

Another relevant feature of the reproduction of the Black Anglerfish is the presence of a gelatinous matrix, which has been noted for other *Lophius* species (Fulton 1898, Leslie and Grant 1990, Armstrong et al. 1992, Yoneda et al. 2001). The matrix consists of individual chambers where hydrated oocytes are released. In our study, we detected the presence of 2 or 3 eggs in some chambers (Fig. 2). This phenomenon also has been described in Goosefish, and it has been assumed that such eggs in that species might be fertilized (Armstrong et al. 1992, Everly 2002). Trippel et al. (1997) concluded that for the same species larger eggs have a higher probability of hatching and of subsequent larval survival than do smaller ones. It is unknown if the smaller eggs of Black Anglerfish that share a chamber hatch at a different rate or produce less viable larvae than do the larger eggs that are alone in a chamber. Finally, the semicystic kind of spermatogenesis has been described only once before in the family Lophiidae, for the Blackmouth Angler (*Lophiomus setigerus*) (Yoneda et al. 1998a). Muñoz et al. (2002) reported that semicystic spermatogenesis may be related to the secretion of abundant, thick seminal fluid, the function of which is to keep the spermatozoa together to enable fertilization of the entire egg mass.

The variation in spawning seasonality of Black Anglerfish between spring (La Mesa and

De Rossi 2008) and winter (Tsimenidis 1980, Duarte et al. 2001, Carbonara et al. 2005) may be associated to local oceanographic features. Eddies and fronts enhance productivity, often function as physical barriers that retain larvae and juveniles, and favor the feeding behavior of recruits and their subsequent transport toward the main nursery areas (Sánchez and Gil 2000). During spring and summer, temporary eddies are generated in the Adriatic Sea (Mediterranean Sea) and in the Bay of Biscay (Atlantic) (Artegiani et al. 1997a, b). In contrast, in wintertime eddies are generated in the Aegean and the Tyrrhenian Seas, and the northern component of the outflow water from the Mediterranean Sea influences the Atlantic Iberian coast (Iorga and Lozier 1999).

Finally, maturity sizes between individuals off the Atlantic Iberian coast, 53.6 cm TL in females and 38.6 cm TL in males (Duarte et al. 2001), and individuals in the northwestern Mediterranean Sea, 48.2 cm TL in females and 33.4 cm TL in males in our study, were very similar in comparison to the sizes observed for individuals in the Aegean Sea, 34 cm TL in females and 24 cm TL in males (Tsimenidis 1980). These variations in L_{50} could be related to environmental and anthropogenic factors (e.g., temperature, food availability, or fishing pressure) (Trippel et al. 1997).

CONCLUSIONS

The Black Anglerfish is a bycatch species in commercial fisheries off the Catalan coast of Spain. Despite not being a target species for these fisheries, the rise of its economic value has led to an increase of captures in the northwestern Mediterranean Sea. The lack of information about reproduction and fecundity of the Black Anglerfish off the Catalan coast has been a problem for management of the fishery for Black Anglerfish. The results of our study improve the understanding of the reproductive dynamics of this species. From the morphological point of view, the structure and the development of ovaries and testes do not differ from the development of other Lophiiformes, although the Black Anglerfish presents variation in its spawning season that is linked to its geographic area.

Our most important results are for L_{50} Males and females both reach L_{50} at large sizes: 33.4 cm TL for males and 48.2 cm TL for females. As a consequence, the large catch and retention of individuals below the L_{50}, 57% of males and 83% of females landed, indicate that overfishing could be a concern for this species in the northwestern Mediterranean Sea. Therefore, our study provides new data for the Black Anglerfish that are needed for a better understanding of the biology and ecology of this species, and this knowledge will useful in assessment and management of the stock exploited by the fisheries of the northwestern Mediterranean Sea.

SUBCHAPTER 2

REPRODUCTIVE STRATEGY OF WHITE ANGLERFISH (*Lophius piscatorius*) IN MEDITERRANEAN WATERS: IMPLICATIONS FOR MANAGEMENT

REPRODUCTIVE STRATEGY OF WHITE ANGLERFISH (*Lophius piscatorius*) IN MEDITERRANEAN WATERS: IMPLICATIONS FOR MANAGEMENT

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Abstract. Reproductive parameters of the white anglerfish (*Lophius piscatorius*) in the northwestern Mediterranean Sea were studied in 556 specimens collected monthly aboard commercial fishing vessels that were trawling at depths of 12–836 m. The main spawning season occurred from February through June. The size at maturity was estimated to be 48.8 cm in total length (TL) for males, 59.9 cm TL for females, and 51.3 cm TL for both sexes combined. The white anglerfish has group-synchronous oocyte development and determinate fecundity. It is a total spawner (spawns all its eggs once during a spawning season) and has a batch fecundity ranging from 661,647 to 885,214 oocytes, a relative batch fecundity of 66–128 oocytes per gram of female gutted weight, and a potential fecundity with values from 54,717 to 104,506 oocytes per kilogram of female total weight. This study is the first to provide the reproductive biology of white anglerfish in the northwestern Mediterranean Sea and provide valuable information that can be used to improve the stock assessment and ensure proper management of this species.

INTRODUCTION

The genus *Lophius*, commonly known as anglerfish, monkfish, or goosefish, belong to a family of bathydemersal fishes, which live and feed on the bottom of the seafloor generally below 200 m (Caruso 1986). It includes 7 species distributed around the world. The white anglerfish (*Lophius piscatorius*) is found in the northeast Atlantic Ocean and the Mediterranean Sea, and the black anglerfish (*Lophius budegassa*) coexists with white anglerfish over most of its range, although the black anglerfish has a more southerly distribution in the Atlantic (Caruso 1986). The shortspine African angler (*Lophius vaillanti*) is found in the eastern Atlantic (Maartens and Booth 2005). The devil anglerfish (*Lophius vomerinus*) occupies the southeast Atlantic and the northern and western Indian Ocean (Walmsley et al. 2005). The blackfin goosefish (*Lophius gastrophysus*) inhabits the western Atlantic, and the goosefish (*Lophius americanus*) occurs in the northwest Atlantic (Caruso 1983). Finally, the yellow goosefish (*Lophius litulon*) is distributed in the northwest Pacific, in the Gulf of Po-Hai, in the Yellow Sea, and in the East China Sea (Yoneda et al. 1997).

We focused on white anglerfish, which can be found on the continental shelf and slope, inhabiting depths from the shoreline to >1000 m (Afonso-Dias and Hislop 1996). In the Mediterranean Sea, this species cohabits with black anglerfish, and even though the distributions of both species overlap, no ecological competition exists between them because of a temporal segregation in their daily biorhythms (Colmenero et al. 2010). Both of these species of *Lophius* play an important role in the trophic structure of benthodemersal ecosystems because they represent major predators, along with the European hake (*Merluccius merluccius*) (Díaz et al. 2008). In the community structure of the northwestern Mediterranean Sea, species of anglerfish are considered top predators (Coll et al. 2006, Valls et al. 2014). They are also reported to be important in the deep-sea community (depths from 200 m to the bottom of the ocean) because they are the most abundant species (Labropoulou and Papaconstantinou 2000, Maiorano et al. 2010).

Despite the fact that the deep sea is the largest ecosystem on the planet, is highly diverse, and has a wealth of resources, it is still mostly unknown and poorly understood in comparison with shallow-water areas: therefore environmental management in deep waters is difficult (Ramirez-Llodra et al. 2010). In the last few decades, the decline of traditional fisheries on the continental shelf, the increasing demand for food sources, and rapid technological developments have resulted in an increasing exploitation of deep-sea resources (Koslow et al. 2000, Ramirez-Llodra et al. 2011) and in an incremental increase in the global mean depth of fishing (Watson and Morato 2013).

This rise in deep-sea fishing has affected catches of *Lophius* species, given the growing demand for human consumption of this group of fish that is leading to an increase in worldwide commercial exploitation and targeting of anglerfishes (Fariña et al. 2008). Total catch reported globally for white anglerfish reached more than 26,500 metric tons (t) in 2014 (FAO 2016) and total catch of anglerfishes in the northwestern Mediterranean Sea for the same year added up to 660 t (data obtained from the General Directorate of Fishing and

Maritime Affairs of the Catalan Government, Spain). Landings in our study area were composed primarily of black anglerfish (86%) and generally only a small percentage of white anglerfish (14%) (data obtained from the General Directorate of Fishing and Maritime Affairs of the Catalan Government, Spain), but, for landings in Atlantic waters, the opposite is true; white anglerfish (94%) dominate the catch (Dobby et al. 2008). Although the European Commission previously has conducted stock assessments of black anglerfish in the western Mediterranean Sea, there is no corresponding assessment for white anglerfish. The lack of information about the structure of the population of white anglerfish in this region and the lack of knowledge of the basic biology of this species are the main reasons for the absence of any assessment. The actual management regulations applied for black anglerfish generally are those applied to bottom trawling (European Union Council Regulation 1967/2006), with recommendations aimed at reducing the fishing effort of the fleet in order to avoid loss in stock productivity and decreases in landings (Cardinale et al. 2015).

The small quantity of white anglerfish available from landings in Mediterranean waters makes studies of this species challenging. Studies conducted in the Mediterranean Sea have been scarce, and they have been focused on temporal and spatial distribution of this species (Ungaro et al. 2002, Colmenero et al. 2010), age and growth (Tsimenidis and Ondrias 1980, Tsimenidis 1984), feeding ecology (López et al. 2016), morphometrics (Negzaoui-Garali and Ben Salem 2008), parasites (Colmenero et al. 2015a), and ova characteristics (Colmenero et al. 2015b). Among these studies, only Ungaro et al. (2002) analyzed some of the biological features of this species by using data available from trawl surveys, including data on distribution, abundance, stock demography, and size at maturity. The latter work is valuable but is limited because sampling occurred only in the spring and summer; a whole year of sampling is recommended to obtain more accurate biological information.

A study of reproductive ecology is important for an understanding of population dynamics, and it is critical for assessing the effects of harvesting on fish populations when attempting to develop appropriate management strategies. Recruitment is recognized as a key process for maintaining sustainable populations, and the relationship between the reproductive output of the population and the resulting recruitment is central to understanding how a fish population will respond to constant stressors such as fishing (Chambers and Trippel 1997). Although knowing more about the relationships between life history strategies and productivity with depth could help managers understand the potential response of a deep-sea species to fishing (Drazen and Haedrich 2012), it is first necessary to conduct biological studies of fish to gain knowledge of the reproductive system of a species (Koslow et al. 1995). Such studies include gonad morphology (external and cellular description of the ovary and testis), reproductive pattern (hermaphroditism or gonochorism), reproductive behavior, reproductive cycle, spawning season duration, size at maturity, sex ratio, size at sexual transition, and fecundity.

All of this information can be applied at the population level to evaluate reproductive potential and to serve as a basis for limits on fishing that aim in order to keep recruitment at sustainable levels (García-Díaz et al. 2006). Because reproductive strategy varies within species, depending on the area of distribution of each species and the depth distribution of each species in each area (Rotllant et al. 2002), there is a need for knowledge about reproduction of deep-sea fish species. Such information is needed particularly in the Mediterranean Sea because the data available for this region are limited (Morales-Nin et al. 1996, D'Onghia et al. 2008, Muñoz et al. 2010, Bustos-Salvador et al. 2015), and, furthermore, target species of fisheries have been the focus of only a few studies (Rotllant et al. 2002, Recasens et al. 2008).

The goal of this study was to describe the reproductive parameters—gonadal morphology, spawning season, size at sexual maturity, oocyte development, and fecundity—of white anglerfish in the northwestern Mediterranean Sea in order to provide valuable information and scientific background to improve stock assessments and effective management for *Lophius* species in Mediterranean waters.

MATERIALS AND METHODS

Sampling and data collection

Between June 2007 and December 2010, 556 white anglerfish, with total lengths (TLs) of 9–120 cm, were collected monthly aboard commercial fishing vessels that were trawling at depths of 12–836 m. Fish were sampled from 467 stations located in the fishing grounds off the Catalan coast in the northwestern Mediterranean Sea from 40°5.980′N to 43°39.310′N and from 0°32.922′E to 3°35.718′E (Fig. 1).

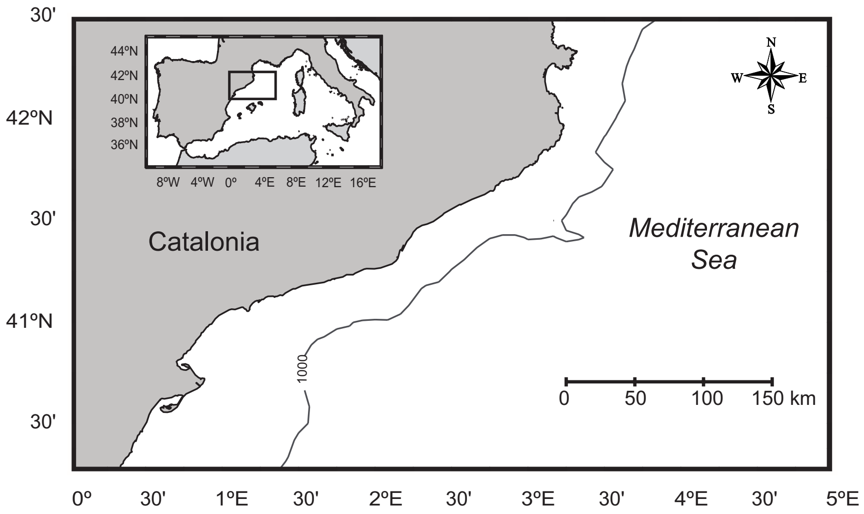

Figure 1. Map of the northwestern Mediterranean Sea showing the study area where white anglerfish (*Lophius piscatorius*) were collected from fishing grounds off the Catalan coast between June 2007 and December 2010.

For each individual, TL was measured to the nearest centimeter, total weight (TW) and gutted weight (GW) were measured to the nearest gram, and gonad weight (GNW) and liver weight (LW) were measured to the nearest 0.01 g. The sex of all fish was determined and assigned macroscopically to a gonadal stage on the basis of a scale of 5 maturity phases proposed by Colmenero et al. (2013): immature (phase I), developing or regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (phase V).

Fish that were too small (<20 cm TL) for their sex to be determined or for assignment to a gonadal phase were classified as indeterminate. Macroscopic gonadal stage was validated histologically, according to the most advanced cell within the gonad (West 1990). Gonads were fixed in 10% buffered formalin solution, dehydrated in ascending solutions of alcohols and embedded in a methacrylate polymer resin, sectioned at a thickness of 4 µm with a manual microtome Leica Reichert-Jung 2040 (Leica Microsystems, Wetzlar, Germany), stained with Lee's stain (methylene blue and basic fuchsin), and mounted in a synthetic resin of dibutyl phthalate xylene on microscope slides. Gonads were classified according to their size and color and the presence or absence of specific inclusions (oil droplets, yolk, postovulatory follicles, or sperm), as well as the type of oocytes (Wallace and Selman 1981).

Reproductive biology

The spawning season was estimated by analyzing the monthly variation in the percentage of maturity phases and the changes in gonadosomatic index (GSI) and hepatosomatic index (HSI) for mature fish of each sex (Afonso-Dias and Hislop 1996, Colmenero et al. 2013). Because indeterminate individuals (n=27) were not considered, 251 males and 278 females were used to determine both indices, which were calculated according to Yoneda et al. (2001) as:

$$GSI = (GNW \;/\; GW) \times 100$$

$$HSI = (LW \;/\; GW) \times 100$$

The lengths at which 25%, 50%, and 75% of sampled fish reached sexual maturity were estimated by fitting the proportion of sexually mature males and females (phase III, phase IV, or phase V) and for both sexes combined to the logistic equation (Colmenero et al. 2013):

$$P = 100 \;/\; (1 + \exp\,[a + b\mathrm{TL}])$$

where P = the percentage of mature individuals as a function of size class (measured in TL); and a and b are specific parameters that can change during the life cycle.

A logarithmic transformation was applied to this equation to calculate the parameters a and b by means of linear regression.

Oocyte development and fecundity

Oocyte diameters obtained from 21 randomly selected specimens at all phases of maturity were measured to the nearest 0.01 µm with an image analysis program (Image-Pro Plus, vers. 5.0, Media Cybernetics, Inc., Rockville, MD) in combination with an Axioskop 2 Plus microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY), and a ProgRes C14 digital microscope camera (Jenoptik AG, Jena, Germany). Only oocytes sectioned through the nucleus were taken into account. The developmental stages of the oocytes were categorized according to the descriptions in Colmenero et al. (2013) that were adapted from Wallace and Selman (1981). The mean oocyte diameter by developmental stage was determined by calculating the diameter of all oocytes encountered in each subsample, and the range was set with the smallest and largest oocytes found at each developmental oocyte stage.

Fecundity was determined by using the gravimetric method described by Hunter and Goldberg (1980). Because homogeneity in oocyte distribution within ovaries of white anglerfish has already been established (Afonso-Dias and Hislop 1996), ovarian tissue subsamples of approximately 500 mg were taken randomly from 2 specimens with ovaries in phase III that had neither postovulatory follicles nor atretic oocytes present. Whole tissue subsamples were placed on several slides and covered with cover slips, then photographed with a Canon Powershot SD870 IS digital camera (Canon USA, Melville, NY). Oocytes were counted manually with Image-Pro Plus.

Batch fecundity (BF), the total number of hydrated oocytes produced in a single spawning event by an individual female, of each female was determined by means of this equation:

$$BF = (\textit{oocyte number} \ / \ \textit{sampled GNW}) \times \textit{total GNW}$$

where BF is the product of the number of secondary vitellogenic oocytes per unit of weight multiplied by the total ovarian weight (Yoneda et al. 2001). Relative batch fecundity (RBF), the total number of mature eggs released by a female during the spawning batch per gram of female GW, was calculated with the following equation (Pavlov et al. 2009):

$$RBF = BF \ / \ GW$$

Potential fecundity was calculated as the number of vitellogenic oocytes divided by TW in kilograms for each mature female and then averaged (Murua et al. 2003).

RESULTS

Gonad morphology

The gonad of female white anglerfish has 2 ribbon-like ovarian lobes connected to each other at their posterior end. One side of the “ribbon” consists of an ovigerous membrane from which a single layer of oocyte clusters, which contain oocytes at different developmental stages, projects into the lumen. The other side is nonovigerous and secretes a gelatinous material during maturation that fills the ovarian lumen, where mature oocytes develop (Fig. 2).

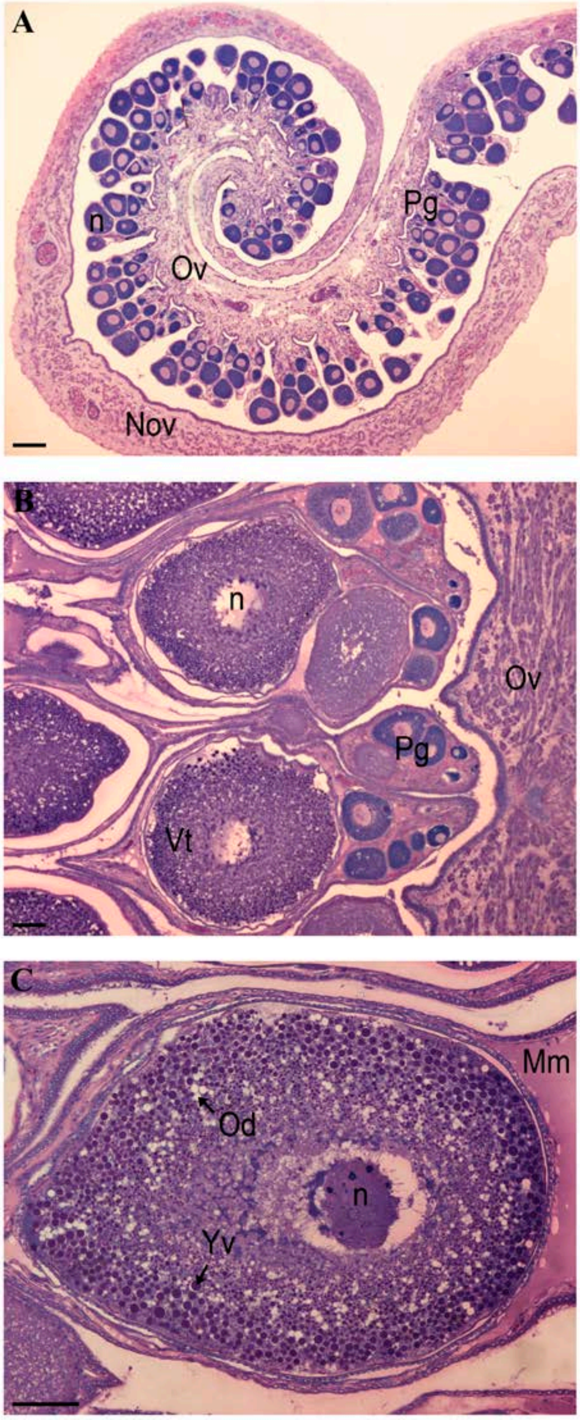

Figure 2. Histological sections from ovaries of female white anglerfish (*Lophius piscatorius*) in 3 phases of maturity: (**A**) immature, (**B**) spawning capable, and (**C**) actively spawning. Ov=ovigerous membrane, Nov=nonovigerous membrane, n=nucleus, Pg=primary growth stage, Vt=vitellogenesis stage, Od=oil droplet, Yv=yolk vesicle, Mm=mucus matrix. Scale bars=100 µm.

During maturation, the gonad increases in size until it fills the abdominal cavity (Fig. 3).

Testes are a pair of elongated organs with a bean shape in transverse section. Spermatogenesis takes place in a capsule-like sac called a *cyst*, but it is completed in the lumina of the lobules. The cysts appear to be arranged with a gradient of germ cells of increasing maturation from the cortex to the sperm duct (Fig. 4).

Spawning season

The monthly distribution of maturity phases (Fig. 5) revealed a peak in reproduction during spring, when a major portion of the spawning females and the highest value of GSI (0.77) were found. Spawning capable females (phase III) were caught primarily between April and June, and females in the actively spawning phase (IV) were observed in November, December, and March—the latter month having the maximum occurrence (11%). Females in immature, regressing, and developing or regenerating phases (I, V, and II, respectively) were found year-round, although the highest percentage of immature individuals (49%) was observed in January.

The GSI values followed the same pattern shown in these maturity phases: highest during spring, decreasing during summer and autumn, and increasing again during winter.

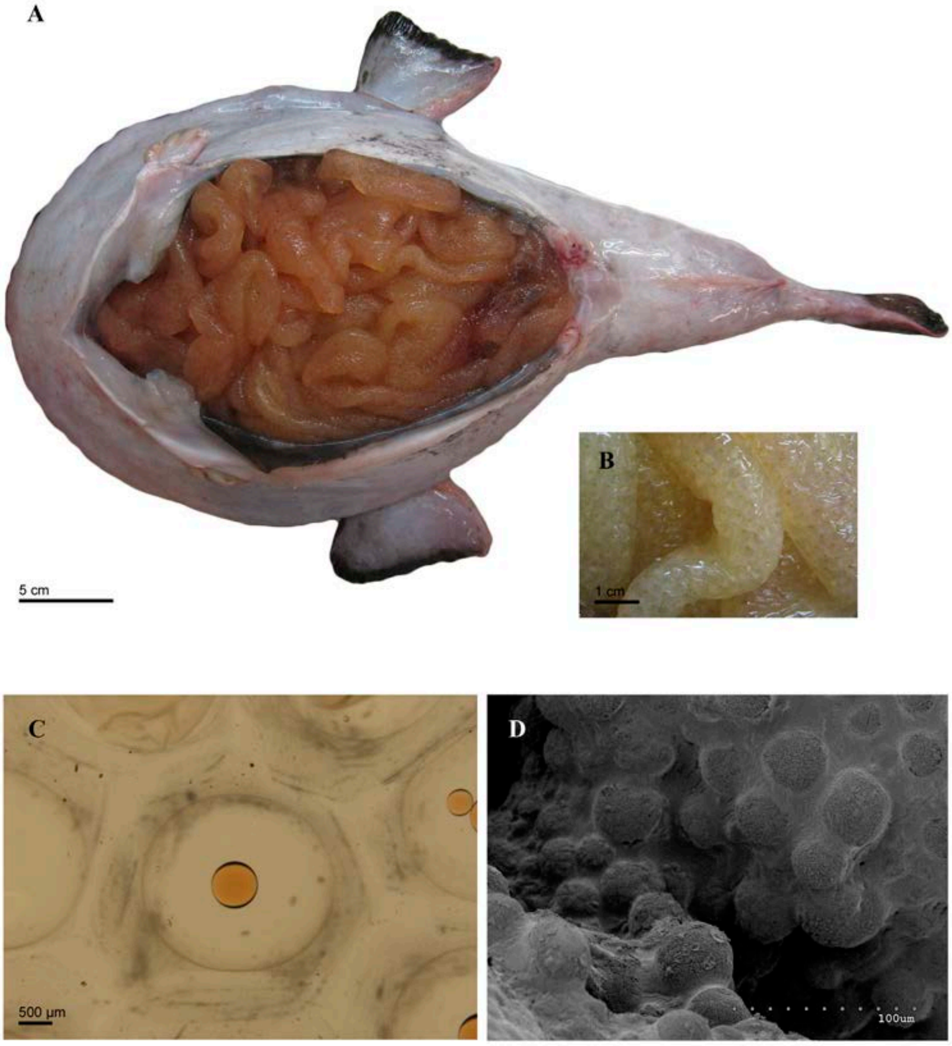

Figure 3. Images of a female of the species *Lophius* showing (**A**) an ovary that occupies the entire abdominal cavity; (**B**) a close-up of the female gonad; and (**C**) a hexagonal chamber of the mucoid veil, which contains an unfertilized egg of white anglerfish (*Lophius piscatorius*) and (**D**) a scanning electron micrograph of yolk vesicles from the inside of the ovum of a white anglerfish.

Males in all maturity phases were observed throughout the year, but with a maximum percentage of mature males (66%) in February and March. Immature males were found primarily in July (69%). The mean GSI for females increased as their ovaries developed and peaked in phase IV. For males, the mean GSI increased with testicular development and reached a maximum in phase IV (Table 1). The mean HSI for females and males increased during the summer and autumn months and decreased during winter and spring. On the basis of these observations, a main spawning season was found from February through June and a secondary one occurred in November and December.

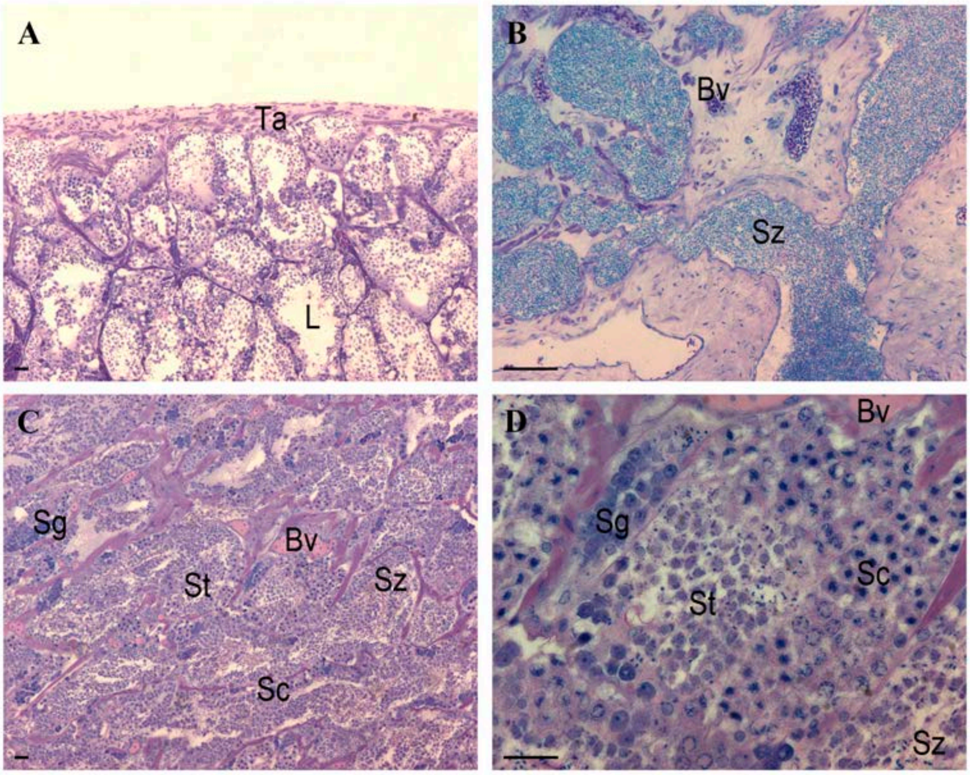

Figure 4. Histological sections of testes from actively spawning male white anglerfish (*Lophius piscatorius*) showing (**A**) lobular organization, (**B**) spermatozoa in the lumen of the seminal lobules and in the sperm duct, (**C**) seminal lobules during spermatogenesis, and (**D**) a close-up of the seminal lobules. Ta=tunica albuginea, L=seminal lobule, Bv=blood vessel, Sz=spermatozoa, Sg=spermatogonia, Sc=spermatocyte, St=spermatid, Scale bars=25 μm.

Table 1. Gonadosomatic (GSI) and hepatosomatic (HSI) indices at each maturity phase for male and female white anglerfish (*Lophiums piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. SE=standard error.

Sex	Maturity phase	GSI range	Mean GSI (SE)	HSI range	Mean HSI (SE)	*n*
Males	I	0.01–0.41	0.10 (0.01)	1.04–4.65	2.37 (0.07)	106
	II	0.06–1.07	0.25 (0.03)	0.27–5.11	2.67 (0.14)	54
	III	0.21–1.30	0.61 (0.05)	1.92–6.72	3.20 (0.18)	35
	IV	0.30–1.70	0.70 (0.09)	0.40–5.39	3.34 (0.28)	17
	V	0.19–1.11	0.50 (0.06)	2.04–5.10	3.35 (0.22)	17
Females	I	0.01–0.86	0.23 (0.02)	0.92–5.33	2.37 (0.11)	66
	II	0.04–1.22	0.40 (0.02)	0.42–7.79	2.87 (0.10)	133
	III	0.61–1.65	1.13 (0.52)	2.15–5.83	3.99 (1.85)	2
	IV	1.59–3.86	2.81 (0.52)	2.68–8.50	5.80 (1.20)	4
	V	0.18–2.44	0.66 (0.06)	0.36–8.59	3.03 (0.17)	60

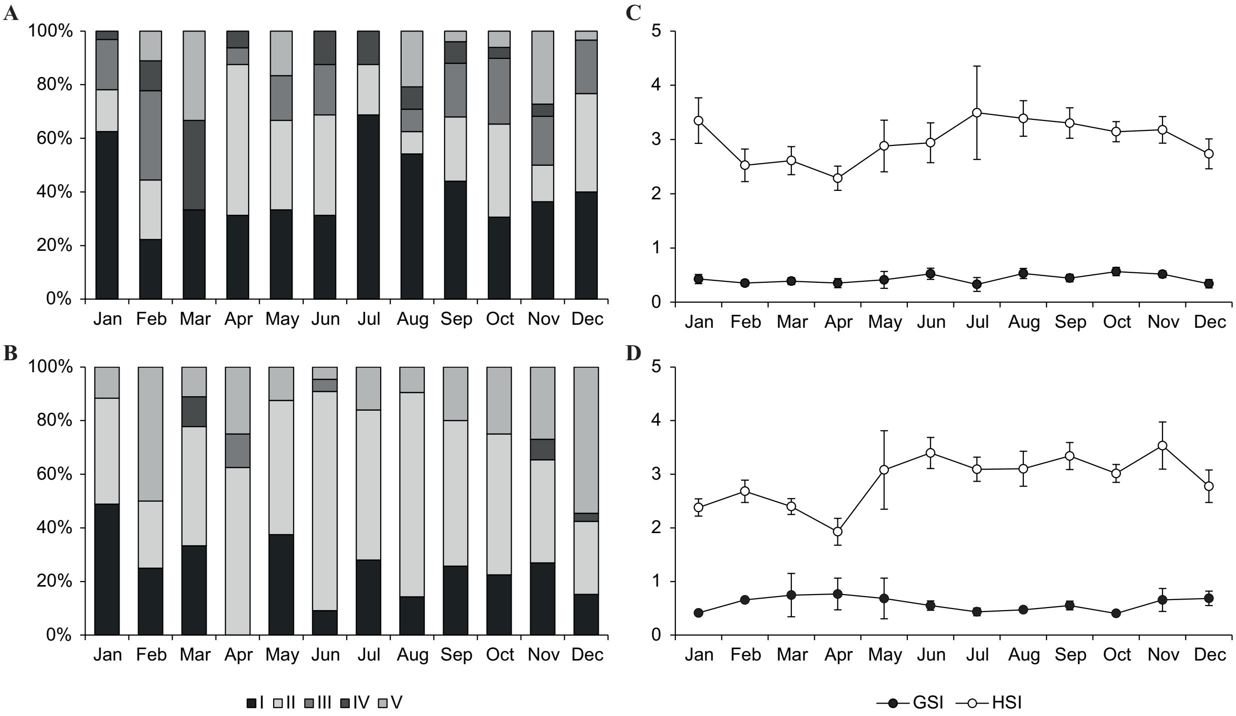

Figure 5. Monthly distribution of maturity phases of gonads for (**A**) males (n=251) and (**B**) females (n=278) and monthly changes in the mean gonadosomatic (GSI) and hepatosomatic (HSI) indices for (**C**) males (n=135) and (**D**) females (n=202) of white anglerfish (*Lophius piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. On the basis of macroscopic examination, specimens were assigned to the following 5 phases: immature (phase I), developing or regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (phase V). Error bars indicate standard error of the mean.

Size at sexual maturity

The maturity ogive for males indicates that the length at which 50% of them reached sexual maturity (L_{50}) was 48.4 cm TL (Fig. 6A). Maturity in males occurred at about 37% of their maximum observed TL. The smallest mature male found was 32.5 cm TL, and the largest immature male was 50 cm TL. The maturity ogive for females indicates that L_{50} was 59.9 cm TL (Fig. 6B). Female maturity occurs at about 30% of their maximum observed TL. Like the smallest male, the smallest mature female was 32.5 cm TL. The largest immature female measured 56 cm TL. The maturity ogive for the sexes combined indicates an L_{50} of 51.3 cm TL. The lengths at which 25% and 75% of fish attained maturity were 43.5 and 53.4 cm TL for males, 48.6 and 71.1 cm TL for females, and 44.7 and 58 cm TL for the sexes combined.

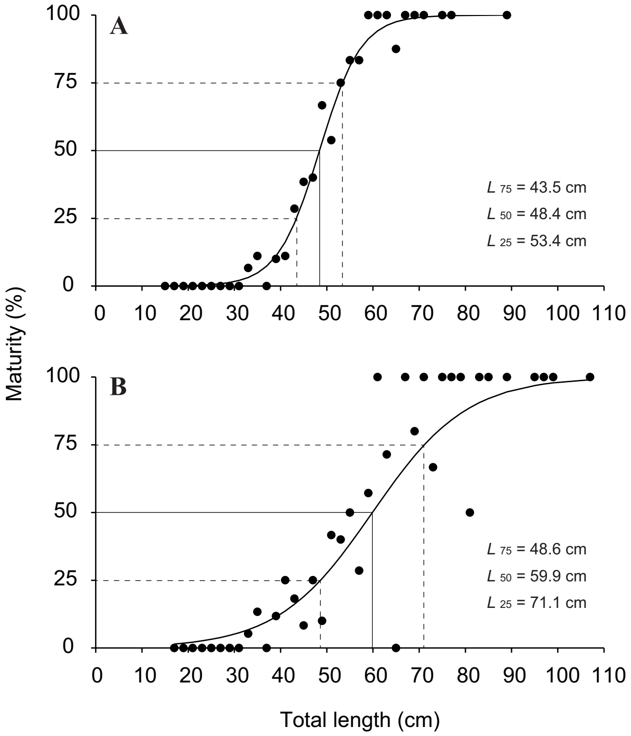

Figure 6. Maturity ogives for (**A**) male and (**B**) female white anglerfish (*Lophius piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010.

Oocyte development and fecundity

Oocytes in different developmental stages were found in each maturity phase. They were organized in clusters where a gradient in the size of the oocyte was observed. A group of oocytes differentiated from others as the ovaries developed, indicating that white anglerfish has group-synchronous oocyte development and can be considered to have determinate fecundity (Fig. 7).

Ovaries at each maturity phase contained primary oogonia- and perinucleolar-stage oocytes. Chromatin nucleolar were difficult to find and were present only in immature phase. Females at the cortical alveolar stage were not found in our samples. Vitellogenic and hydrated oocytes were located in females capable of spawning. Oocyte diameters at each stage of oocyte development are shown in Table 2.

Batch fecundity ranged from 661,647 to 885,214 oocytes from 2 females that measured 76 and 105 cm TL, 6331 and 16,178 g TW, and 5182 and 13,330 g GW, respectively. Relative batch fecundity ranged from 66 to 128 oocytes/g GW (average of 97 oocytes/g GW [standard deviation, SD 43]). Potential fecundity values moved from 54,717 to 104,506 oocytes/kg TW with a mean of 79,612 oocytes/kg TW (SD 35,206).

Table 2. Oocyte diameters, ranges and means with standard errors (SEs), and histological characteristics of ovarian follicles in white anglerfish (*Lophius piscatorius*), collected from the northwestern Mediterranean Sea between June 2007 and December 2010. SE=standard error, at each stage of oocyte development.

Stages of oocyte development	Mean oocyte diameter (µm) (SE)	Oocyte diameter (µm) range	Histological characteristics
Primary growth stage	82.79 (2.34)	12–203	Nucleus contains a large nucleolus and some peripheral nucleoli. Yolk granules are not present in the cytoplasm.
Cortical alveolar stage	256.10 (3.08)	207–316	Cortical alveolar vesicles and oil droplets appear in the cytoplasm. Yolk granules are not yet present in the cytoplasm. Nucleus is central within the yolk.
Vitellogenesis	729.31 (17.58)	324–876	Yolk granules appear between cortical alveolar vesicles. As vitellogenesis advances, yolk granules fill the cytoplasm until they are in contact with the nucleus, which remains in central position.
Migratory nucleus	939.31 (7.77)	902–1008	Yolk granules and oil droplets start to fuse. Nucleus migrates to one pole of the oocyte.
Hydration	1672.50 (4.77)	1523–1750	Yolk granules form a single mass. Nucleus is not present in the cytoplasm.

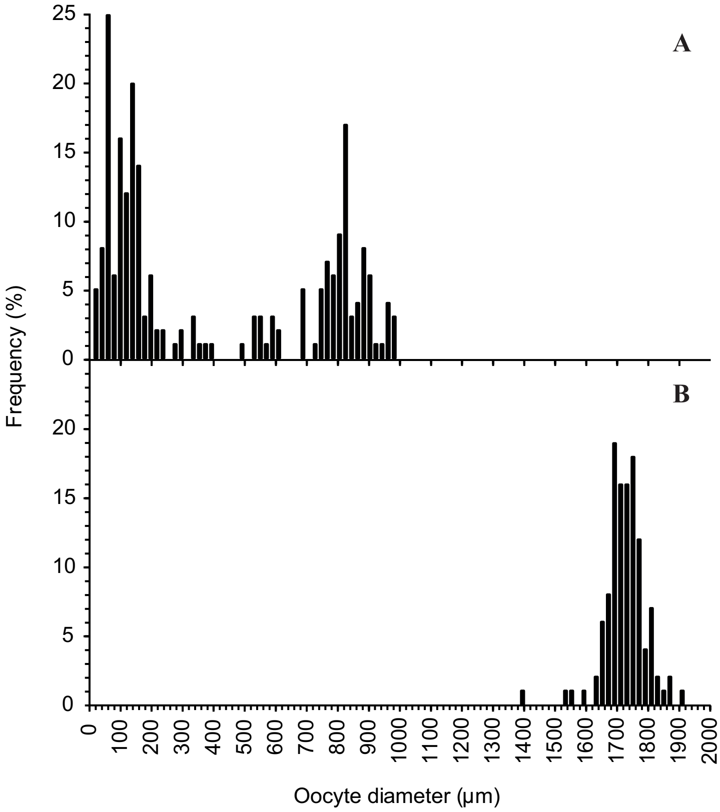

Figure 7. Distribution of oocyte diameters in (**A**) spawning capable and (**B**) actively spawning female white anglerfish (*Lophius piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010.

DISCUSSION

Reproductive relevance for sustainable management

Fishing activity during spawning seasons may affect population parameters, specifically composition of the size distribution, mortality rate, sexual structure of the population, size at maturity, and changes in the spawning season. These parameters, in turn, can increase the risk of over-exploitation of a stock.

Fishing during spawning periods may result in targeting a specific size class of the population and thus increasing the chance of catching the older (and larger) age classes and making the stock vulnerable to reproductive collapse (van Overzee and Rijnsdorp 2015). Because spawning is generally limited to specific areas and times (Cushing 1990), the conservation of resources can be enhanced by limiting fishing activity in a spatiotemporal frame. Furthermore, fishing pressure has been documented to have reduced initial size at maturity—an issue that is a concern particularly for late-maturing species (Stewart et al. 2010). If size of capture is below the size at first maturity, there is a genuine risk of recruitment overfishing. Therefore, knowledge of the spawning season and the size at maturity can help managers establish closed seasons and prevent fishing at this vulnerable time in the life cycle of fish species by preserving breeding individuals and establishing a legal minimum size.

The results of our study of white anglerfish in the northwestern Mediterranean Sea indicate that a long spawning period occurs during mid-winter and late spring, from February through June, although a secondary breeding period has been observed in November and December. These results agree with those obtained in studies that were focused on the northeastern Atlantic Ocean, where this species spawns from November through June (Fulton 1898, Afonso-Dias and Hislop 1996, Hislop et al. 2001). However, a previous study in the northwestern Mediterranean Sea identified a spawning season during spring–summer (Ungaro et al. 2002). Discrepancies between the latter study and our work may be explained by the differences in sampling periods.

Nevertheless, spawning seasonality, which is associated with environmental conditions and local oceanographic features, varies between species as well as by geographical area. An example of this variability in spawning seasonality can be observed in 2 locations along the Atlantic–Iberian coast: on the Portuguese and western Spanish coasts, spawning of the white anglerfish takes place during winter–spring (Duarte et al. 2001), whereas on the northern Spanish coast (Bay of Biscay), spawning occurs during summer (Quincoces et al. 1998b). In fact, spawning activity for one of its congenerics, the black anglerfish, in the northwestern Mediterranean seems to occur from November through March and a secondary spawning occurs in August and September (Colmenero et al. 2013). Although a little overlap exists between spawning seasons of both of these *Lophius* species in Mediterranean waters, the main period is markedly different, and that difference lessens competition among these species.

Usually, species of *Lophius* have long spawning periods ranging between 4 and 6 months. Black anglerfish off the Spanish-Atlantic coasts spawn from November through February (Duarte et al. 2001), and in the Bay of Biscay the peak spawning period is from May through July (Quincoces et al. 1998a). The goosefish off the East Coast of the United States has its reproductive period from May through June (Armstrong et al. 1992), spawning for the blackfin goosefish off the Brazilian coasts takes place during spring and summer (Valentim et al. 2007), and the yellow goosefish spawns between February and May in the East China Sea and the Yellow Sea (Yoneda et al. 2001). The devil anglerfish off the coast of South Africa has a well-defined summer breeding season (Griffiths and Hecht 1986), and individuals of this species off the coast of Namibia spawn throughout the year with a slight increase between autumn and spring (Maartens and Booth 2005).

Most deep-sea fish species reach sexual maturity at sizes larger than those of species that inhabit the continental shelf reach maturity, and, in some cases, males mature at smaller sizes than females (Rotllant et al. 2002, Pajuelo et al. 2008). A similar pattern was observed for white anglerfish—one in which females mature sexually at larger sizes (59.9 cm TL) than those recorded for males (48.4 cm TL). This pattern has also been found for white anglerfish in other areas (Ofstad and Laurenson 2007) and for other species of anglerfish. Female black anglerfish, for example, mature at 48.2 cm TL, whereas males attain first maturity at 33.4 cm TL (Colmenero et al. 2013), and female devil anglerfish reach sexual maturity at 58.2 cm TL, whereas males of this species mature at 39.9 cm TL (Maartens and Booth 2005). For the goosefish, L_{50} for females and males was estimated at 48.5 and 36.9 cm TL, respectively (Armstrong et al. 1992), and female yellow goosefish mature at 56.7 cm TL and males of this species mature at 36.2 cm (Yoneda et al. 2001). This dissimilarity in size at maturity is usually associated with a trade-off between life history traits, where early maturity involves a larger size but a slower growth (Stearns and Koella 1986, Charnov 2008).

Reproductive strategy

The reproductive strategy of white anglerfish is one of discontinuous oogenesis with synchronous development of vitellogenic oocytes and is, therefore, this species is considered a total spawner (Afonso-Dias and Hislop 1996). The oocytes ovulate at once, and the eggs are released in either a unique event or over a short period of time, as part of a single episode during the spawning season (Murua and Saborido-Rey 2003, Pavlov et al. 2009). This pattern of oocyte development and spawning patterns is also found in other species of *Lophius* (Leslie and Grant 1990, Armstrong et al. 1992, Colmenero et al. 2013). Yoneda et al. (2001) suggested that yellow goosefish may have the potential to spawn more than once a year, on the basis of the observation of a captive specimen that released several infertile egg masses. However, this spawning behavior cannot be considered normal.

Female anglerfish spawn their eggs in a mucoid veil that floats near the surface. The veil consists of individual chambers that contain 1–3 eggs and has an opening that provides water circulation. In our study we recognized in some chambers the presence of 2 eggs sharing the same chamber. Although this way of releasing eggs is not common among fish species, some Scorpaeniformes, such as the shortfin turkeyfish (*Dendrochirus brachypterus*) (Fishelson 1978) or the shortspine thornyhead (*Sebastolobus alascanus*) (Erickson and Pikitch 1993), also spawn buoyant gelatinous egg masses. It has been proposed that the advantages of releasing eggs in these veils facilitate their dispersal; the egg veil floats near the surface and is subject to the actions of wind, currents, and waves. The veil also serves as protection for eggs against predation because of the presence of obnoxious or toxic substances in the veils (Armstrong et al. 1992). Moreover, the veil may help with the fertilization of eggs. When males are present and the egg ribbon is laid, the ribbon keeps the eggs together and prevents their dispersion through the water. The males then eject milt near the veil to guarantee fertilization of all the eggs (Dahlgren 1928). Armstrong et al. (1992) suggested that sperm reach oocyte chambers through the pores that connect the chambers when the ribbon is extruded from the female and starts to absorb water.

Another feature of the reproduction of the white anglerfish to highlight is its type of spermatogenesis, which is known to be semicystic. Spermatogenesis starts inside the cysts that contain germinal cells in different stages of development from spermatogonia to spermatids, but it is not completed within the cyst. During spermatogenesis, the cyst breaks and spermatids are released from the cyst into the lumen of the lobule, where they become spermatozoa. This kind of spermatogenesis has been described previously in the family Lophiidae only in the blackmouth angler (*Lophiomus setigerus*) (Yoneda et al. 1998b) and in black anglerfish (Colmenero et al. 2013). This specialized spermatogenesis also has been found in other deep-sea species of Neoceratiidae (Jespersen 1984) and of Macruridae (Fernandez-Arcaya et al. 2013), in the shore clingfish (*Lepadogaster lepadogaster*) (Mattei and Mattei 1978), in species of Blennidae (Lahnsteiner and Patzner 1990), in a species of *Ophidion* (Mattei et al. 1993), in the dusky jawfish (*Opistognathus whitehursti*) (Manni and Rasotto 1997), and in species of *Scorpaena* (Muñoz et al. 2002, Sàbat et al. 2009), which also release their eggs in gelatinous substances.

Fecundity

Because of their particular reproduction behavior, which includes a high parental investment in the offspring, white anglerfish are likely to spawn once a year, and the population dynamics of this species are expected to be highly sensitive to external biological and ecosystem factors (ICES 2012). Spawning occurs in deep waters because mature white anglerfish have been described by Hislop et al. (2001) as migrating to deeper water before spawning. The same behavior is seen in yellow goosefish: adult fish migrate to deeper waters in response to seasonal changes in water temperature and gonadal maturation (Yoneda et al. 2002). These vertical migrations into deeper waters where commercial fishing and scientific surveys cannot reach could be the reason that very few mature females were captured during our study—a trend that is common in other studies of *Lophius* species (Ofstad and Laurenson 2007). Clearly, this low number of mature females will affect the estimation of such reproductive parameters as fecundity.

Generally, deep-sea species have low fecundity and large egg sizes (Gage and Tyler 1991, Herring 2002). The white anglerfish has determinate fecundity with values between 661,647 to 885,214 oocytes—levels that are high in comparison with other deep-sea species that inhabit the same depth strata but that are similar to the mean potential fecundity of its Mediterranean congeneric, the black anglerfish (Colmenero et al. 2013). Fecundity values vary among populations as a result of adaptations to local environmental conditions, and they are related to abiotic factors, such as temperature and salinity (Nissling and Dahlman 2010, Thorsen et al.

2010), and to biotic factors, such as food supply, population density, allocation of energy to reproduction, and fish size (Treasurer 1981, Merrett 1994, Nash et al. 2000).

In this study, we were not able to determine correlations between fecundity and these factors because only 2 actively spawning females were collected. Eggs of white anglerfish have been reported to have a mean diameter of 2.72 mm (SD 0.08) (Colmenero et al. 2015b), a size that is considered large for pelagic eggs, which typically range from 0.5 to 5.5 mm in diameter (Ahlstrom and Moser 1980). Larger eggs have more yolk, which increases the potential for larval survival (Duarte and Alcaraz 1989). The only information available about egg diameters for other species of *Lophius* is for yellow goosefish, which occupy a bathymetric range that is similar to that occupied by white anglerfish and have a similar egg size (Yoneda et al. 2001). In contrast, the black anglerfish has an egg diameter of 1.88 mm (SD 0.12), a size that is nearly 1.5 times smaller than the diameters reported for the white anglerfish and yellow goosefish, and inhabits shallower waters than those inhabited by the other 2 species (Colmenero et al. 2015b). A comparative study of egg sizes in deep-sea species found that egg size increased significantly with depth (Fernandez-Arcaya, personal commun.). Egg size is important to offspring survival in many organisms, and large eggs survive better than small ones in environments where dissolved oxygen is low (Hendry and Day 2003).

GENERAL REMARKS

In this study, we estimated the spawning season, size at sexual maturity, and fecundity of white anglerfish. Considering the parameter values that we obtained, we can conclude that this species is one that employs a *K* reproductive strategy. In general, this strategy is defined by a large body size, longevity, late maturation, and low fecundity (Pianka 1970, 1974). A wide range of deep-sea demersal fish species generally display life history characteristics consistent with *K*-selection (Adams 1980, Gage and Tyler 1991). These traits make deep-sea fish stocks highly vulnerable to fishing and capable of little resilience to over-exploitation, increasing the urgency for the conservation and management of this group of animals (Koslow et al. 2000, Morato et al. 2006, Norse et al. 2012).

Theoretically, the *K*-strategy for deep-sea fish species should imply a low fecundity; however, some species, such as the North Pacific armorhead (*Pseudopentaceros wheeleri*), wreckfish (*Polyprion americanus*), and splendid alfonsino (*Beryx splendens*), have high fecundities (Sedberry et al. 1996, Lehodey et al. 1997, Humphreys 2000). White anglerfish and species of *Lophius* in general also should be included in this group because of their high fecundity (Afonso-Dias and Hislop 1996, Colmenero et al. 2013). This variability in reproductive strategy is the result of adaptation to environmental changes, such as temperature, bathymetric pressure, light, and food availability (Herring 2002, Brown-Peterson et al. 2011). Likely, the high fecundity and the low economic value of the white anglerfish, at least until the last decades of the 20th century, has allowed the stock to be sustainable within acceptable limits. With the recent expansion of anglerfish fisheries, sustainability is in question, and our study is the first step toward an informed assessment of this deep-sea resource and its management with an ecosystem perspective.

SUBCHAPTER 3

THE CHORION ULTRASTRUCTURE OF OVA OF *Lophius* SPP.

THE CHORION ULTRASTRUCTURE OF OVA OF *Lophius* SPP.

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Abstract. The chorion surface ultrastructure of unfertilized eggs of black anglerfish *Lophius budegassa* and white anglerfish *Lophius piscatorius* was examined by scanning electron microscopy. Species-specific differences were observed.

INTRODUCTION

Identification of fish eggs is one of the most difficult problems faced when working with ichthyoplankton (Olivar 1987). Some characteristics observed under light microscopy, which include egg shape, egg size, chorion structure, yolk structure, presence or absence of oil globule and perivitelline space, have been proposed by several authors in order to solve this problem (Ahlstrom and Moser 1980, Markle and Frost 1985). Many of these characteristics are the same over large groups of fishes and the use of a scanning electron microscope (SEM) helps to discern differences that then aid in identification of eggs. In marine teleosts, the structure of the egg chorion has been considered as an important taxonomic characteristic for identifying eggs of different species because it is an indicator of the systematic relationships of the species (Ivankov and Kurdyayeva 1973, Chen et al. 2007) and it has a morphology that is species specific (Hagström and Lönning 1968). Although there are major differences in the structure of the chorion in different species of teleosts with respect to thickness, number of lamellae and the presence or absence of pores due to the diversity in habitat and breeding habits of teleosts (Kuchnow and Scott 1977), geographic differences in chorion structure were found between eggs from the same species (Lönning 1972). These variations may be correlated with differences in salinity, temperature and viscosity of the seawater, necessitating adjustment in the structure of pelagic eggs to secure adequate buoyancy (Lönning 1972). For this reason, it is important to characterize eggs of different species from different geographic locations. The chorion is perforated by the micropyle, through which a spermatozoan can penetrate the chorion for fertilization.

The microstructure of the micropyle in unfertilized eggs is an important characteristic in fish identification (Chen et al. 1999, Li et al. 2000). The aim of the present work is to examine morphological characteristics of ova in the two species of *Lophius* inhabiting the Mediterranean Sea by means of light microscopy and SEM, provide some information about their chorion ultrastructure and to use these findings for aid in taxonomic classification from ichthyoplankton samples.

MATERIALS AND METHODS

In this study, specimens of black anglerfish *Lophius budegassa* Spinola 1807 and white anglerfish *Lophius piscatorius* L. 1758 were collected on board commercial trawler fishing vessels in the north-western Mediterranean Sea at depths of 20–600 m and identified following Caruso (1986). Unfertilized eggs were removed from gonads, measured under a light microscope and prepared for SEM using two different techniques: cryo-SEM (Klein et al. 1992, Sempere and Santamarina 2011) and critical-point drying (Boehlert 1984). For cryo-SEM, unfixed fresh ova were placed on a polycarbonate filter and mounted on aluminium stubs by means of conducting gel. Ova were then immediately plunged into liquid nitrogen slush. Once samples were frozen, they were introduced into the cryo preparation chamber Quorum PP3000T (www.quorumtech.com) where water sublimed at −90° C within 5 min, leaving ova free of impurities. After ice sublimation, ova were sputter coated with platinum and transferred inside the chamber of the Hitachi S-3500N SEM (www.hitchi-hitec.com) maintained at −130° C, where samples were examined and photographed with an acceleration voltage of 5 kV or less. Ova shrinkage of *c.* 4 % was observed using this method. For critical-point drying, samples used were fixed in 10% formalin. Before observation under SEM, ova were rinsed with filtered seawater and prepared following Olivar (1987) protocol: (1) post-fixed in 2% osmium tetroxide, (2) dehydrated in a graded series of ethanol, (3) critical-point dried (Bal-Tec CPD 030; www.leica-microsystems.com) and (4) coated with a layer of gold-palladium prior to viewing. This method showed shrinkage of *c.* 40%. Ova and oil globule diameter of fresh unfertilized eggs were measured under a light microscope (Table I).

Table I. Characteristics of fresh ova examined under light microscope in Mediterranean *Lophius* spp.

	Ova diameter (mm)		Oil globule diameter (mm)	
Species	**Mean ± S.D.**	**Range**	**Mean ± S.D.**	**Range**
Lophius budegassa	1.88 ± 0.12	1.52 - 2.05	0.48 ± 0.03	0.41 - 0.56
Lophius piscatorius	2.72 ± 0.08	2.45 - 2.90	0.63 ± 0.02	0.59 - 0.68

Measurements of chorion characteristics including thickness, ova size, pore diameter, minimum distance between pores, pore density and micropyle diameter of fixed ova were obtained using photographs from both SEM techniques (Table II).

Table II. Characteristics of fixed ova examined under scanning electron microscope in Mediterranean *Lophius* spp.

	Ova diameter (mm)		Pore diameter (µm)		Minimun distance (µm)		Density (number of pores per 100 µm2)	
Species	**Mean ± S.D.**	**Range**	**Mean ± S.D.**	**Range**	**Mean ± S.D.**	**Range**	**Mean ± S.D.**	**Range**
Lophius budegassa	1.11 ± 0.10	1.01 - 1.29	0.12 ± 0.03	0.06 - 0.22	1.39 ± 0.22	0.93 - 1.98	56 ± 14	40 - 70
Lophius piscatorius	1.67 ± 0.08	1.52 - 1.75	0.10 ± 0.02	0.06 - 0.16	1.09 ± 0.17	0.62 - 1.48	91 ± 7	81 - 100

All measurements were performed using Image-Pro Plus software (www.mediacy.com). Differences in these characteristic values were analysed statistically by using a Mann – Whitney *U*-test. Statistical analyses were performed using SPSS Statistics software (www.01.ibm.com/software/analytics/spss/products/statistics/downloads.html) with statistical significance level set at $P < 0{\cdot}05$. All data presented are mean ± S.D.

RESULTS

The unfertilized eggs of both species of *Lophius* are transparent and spherical in shape [Fig. 1(a)]. Ova of both species have a single oil globule, yellow-ochre in colour and located slightly off-centre position [Fig. 1(b)].

Some eggs of the species of *Lophius* hold multiple oil globules because before fertilization there is a coalescence of many oil globules into a single one and some of the studied ova might be in the middle of this process. Another reason could be the easy rupture of this structure during gonad manipulation. Both ova and oil globules of *L. piscatorius* are

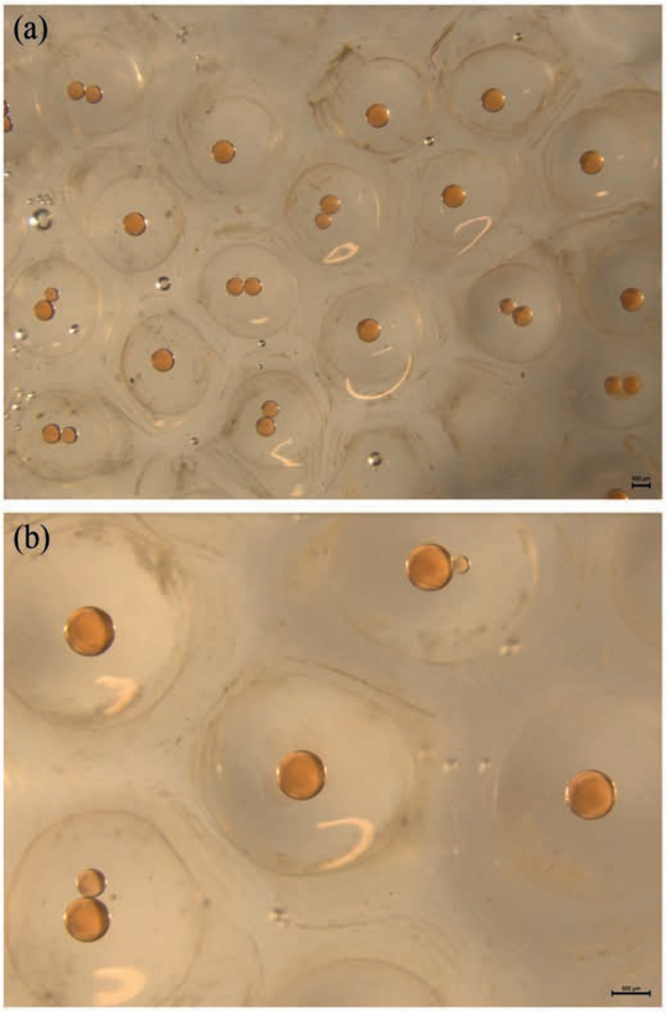

Figure 1. Unfertilized eggs of *Lophius piscatorius*. (a) General view and (b) closed-up of ova and oil globules.

significantly larger than *L. budegassa* (Mann – Whitney *U*-test, $P < 0{\cdot}05$). The chorion of *L. budegassa* and *L. piscatorius* are smooth and transparent [Fig. 2(a), (b)], with oval pores distributed evenly over the surface. Both species have similar pore patterns although *L. piscatorius* had more pores per unit area [Fig. 2(c), (d)].

The pore diameter and minimum distance between pores are significantly less in *L. budegassa* (Mann – Whitney *U*-test, $P < 0{\cdot}05$). The granular matter observed on the surface of the chorion might be remains of its mucous layer dissolved by the fixation methods used in SEM. In *L. piscatorius*, the chorion consists of a thin outer layer and a thicker lamellated inner layer (1·15 ± 0·08 μm) with five lamellae [Fig. 3(a)]. The micropyle observed on an ovum prepared for cryo-SEM was funnel-shaped with an aperture diameter of 74·87 ± 0·68 μm that leads to the micropyle canal which traverses the entire chorion layer [Fig. 3(b)].

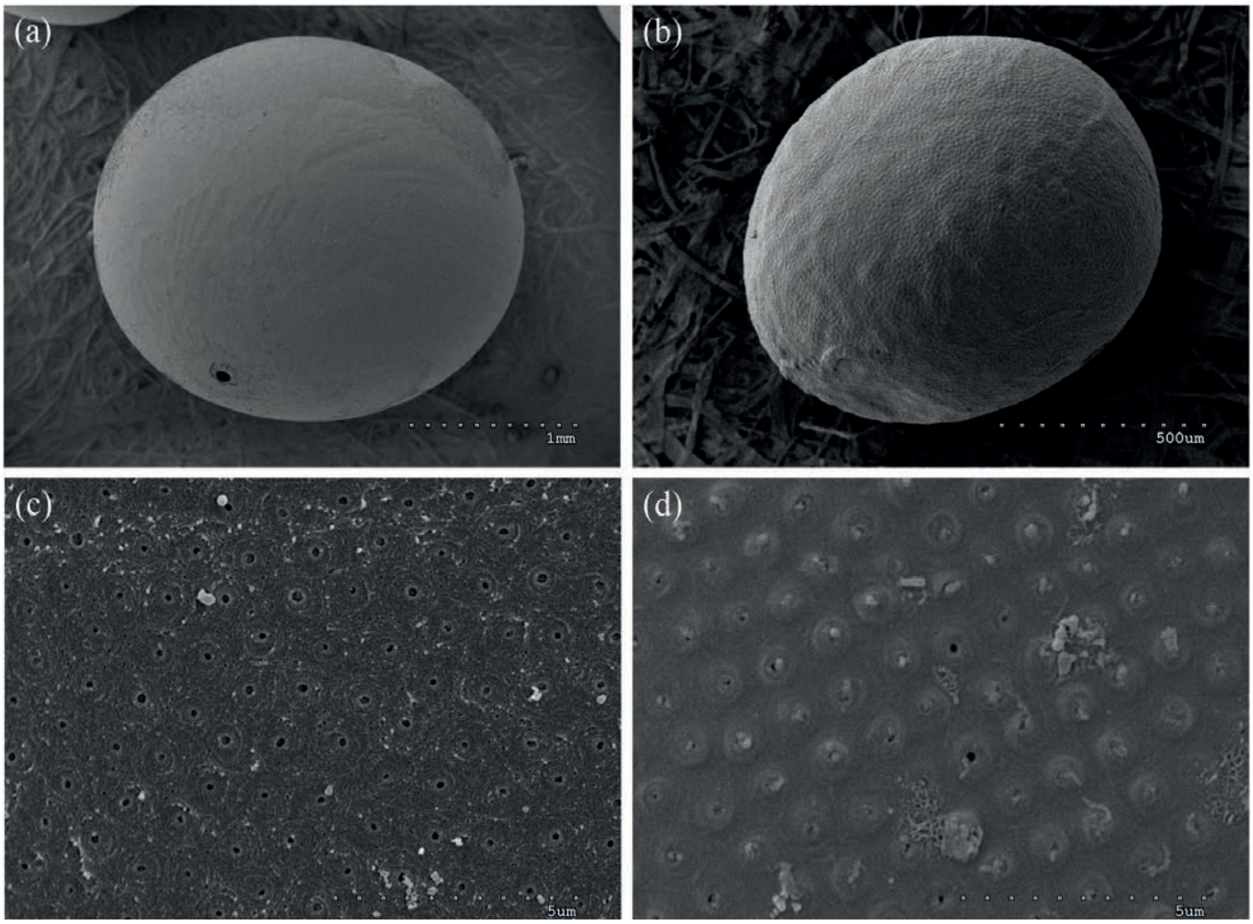

Figure 2. Scanning electron micrographs of (a) ovum of *Lophius piscatorius* and (b) ovum of *Lophius budegassa*; chorion surface with pores of (c) *L. piscatorius* and (d) *L. budegassa*.

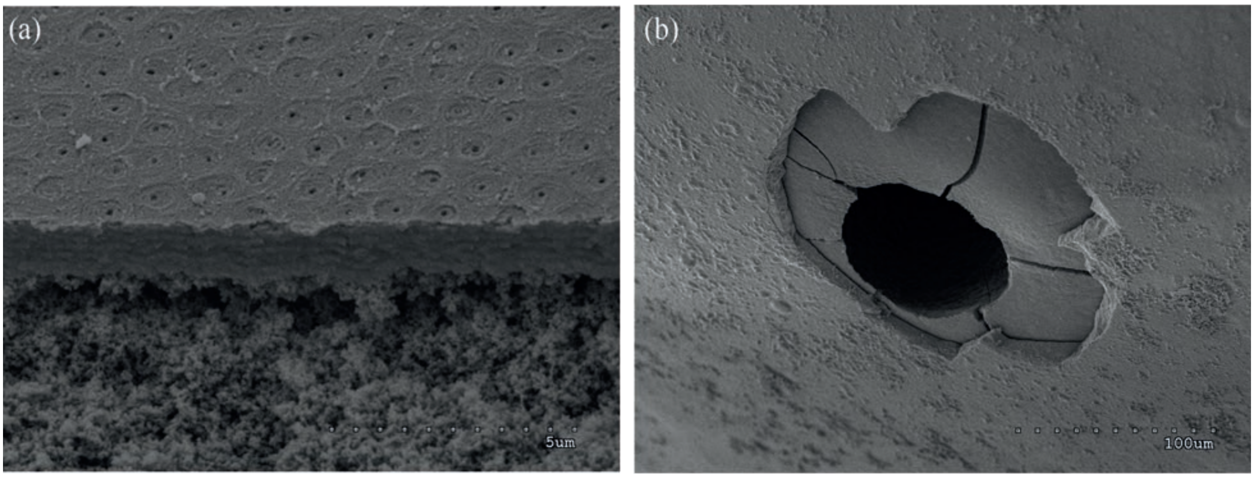

Figure 3. Scanning electron micrographs of (a) chorion lamellar laer and (b) micropyle aperture of ovum of *Lophius piscatorius*.

DISCUSSION

These results show that ova size, oil globule diameter, pore diameter, minimum distance between pores and pore density are useful characteristics for distinguishing the ova of *L. budegassa* and *L. piscatorius*. *Lophius piscatorius* has the largest ova, which measures 2·72 ± 0·08 mm in diameter, being within the range given by Bowman (1920) for Atlantic specimens, whilst ova diameter of *L. budegassa* was measured in this study as 1·88 ± 0·12 mm, which is the first available data from non-fixed ova. Egg size is important to offspring survival in many organisms where a positive correlation between adult female size and egg size is common (Hendry and Day 2003). This ecological premise appears to be appropriate in the two species studied here, because the maximum size of *L. piscatorius* (200 cm) is double that of *L. budegassa* (100 cm) (Caruso 1986) and their ova are 70% larger. The ova of these species can be considered large for pelagic eggs, which range from 0·5 to 5·5 mm in diameter (Ahlstrom and Moser 1980). Not surprisingly, larger ova also have larger oil globule diameters (*L. piscatorius*: 0·63 ± 0·02 mm; *L. budegassa*: 0·48 ± 0·03 mm), both for larval buoyancy and as a concentrated energy source (Eldridge et al. 1977, Markle and Frost 1985). In addition, the chorion of *L. piscatorius*, which protects the embryo from the external environment (Stehr and Hawkes 1979, Olivar 1987), has more pores per unit area. In the ovary, the pore canals distributed throughout the chorion contribute to the transportation of nutrients from the follicle cell to the developing oocyte (Nagahama 1983, Groot and Alderdice 1985) and hence the larger eggs of species of *Lophius* could need more pores in order to satisfy this nutritional intake. It is still unknown if the pores remain open after fertilization but, if this occurs, they could expose the embryo to the natural environment or contaminants (Stehr and Hawkes 1979). In conclusion, although both species occupy the same habitat, differences noted in the characteristics of their ova support the hypothesis that both species of *Lophius* have different ecological strategies, as was previously observed in Colmenero et al. (2010) study of the species' biorhythms.

CHAPTER 2.3

POPULATION DYNAMICS AND SUSTAINABILITY INDICATVORS TO ASSESS *Lophius* FISHERIES IN THE NORTHWESTERN MEDITERRANEAN SEA

POPULATION PARAMETERS AND SUSTAINABILITY INDICATORS TO ASSESS *Lophius* FISHERIES IN THE NORTHWESTERN MEDITERRANEAN SEA

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ABSTRACT. Population dynamics of *Lophius budegassa* and *L. piscatorius* were studied, providing the first estimates of growth parameters for *L. piscatorius* in Mediterranean waters. Monthly time series samples of 5923 *L. budegassa* and 628 *L. piscatorius* ranging in total length from 4 to 105 cm and from 9 to 120 cm respectively; were collected aboard commercial trawlers from 2007 to 2010 in the northwestern Mediterranean Sea. The length-frequency analysis showed that both anglerfishes have a high growth rate during their first year of life: 18.8 cm for *L. budegassa* and 19.4 cm for *L. piscatorius*. The von Bertalanffy growth parameters for *L. budegassa* were: $L_\infty = 128.97$, $k = 0.106$ years^{-1} and $t_0 = -0.10$ years. For *L. piscatorius* were obtained: $L_\infty = 146.92$, $k = 0.114$ years^{-1} and $t_0 = -0.30$ years; then somatic growth of *Lophius* species differed in the asymptotic length, being higher in *L.* piscatorius. The natural mortality rates estimated for both *Lophius* species using the growth parameters derived from the length-frequency analysis revealed lower natural mortalities than the estimator based on t_{max}. Sex ratio was found to be significantly different from 1:1 for *L. budegassa* (1977 males, 1923 females), but not for *L. piscatorius* (249 males, 280 females). In both species sex ratio varied with length and females reached greater size than males. Our results suggest that Mediterranean *Lophius* species have a growth rate and a life spam characteristics of a *K*-strategy species that makes them vulnerable and not able to sustain high levels of exploitation. This, together with the sustainability indicators of *Lophius* spp. reveals a high level of exploitation of their populations and emphasises the need for management actions to conserve stocks.

INTRODUCTION

Lophius are bathydemersal fishes (Caruso 1986) that include seven species exploited worldwide. In the last decades, they have acquired great importance from a commercial point of view and their world catch has increased with the improvements in fishing technology (1,519,022 t since 2010, FishStatJ) leading to a target anglerfish fisheries (Fariña et al. 2008). In the Mediterranean Sea inhabit two species on sandy, muddy and rocky bottoms along the continental shelf and slope, the black anglerfish *Lophius budegassa* Spinola, 1807 and the white anglerfish *Lophius piscatorius* Linnaeus, 1758; from shallow waters to 800 m the former and deeper than 1000 m the later (Afonso-Dias and Hislop 1996, Carlucci et al. 2009). Despite their overlapping distribution no ecological competition exists between them due to a temporal segregation in their daily biorhythms (Colmenero et al. 2010). Both anglerfishes play an important role in the trophic structure of the benthodemersal ecosystems of the northwester Mediterranean Sea (Valls et al. 2014) and occupy the higher trophic levels (Coll et al. 2006, López et al. 2016) as top predators (Colmenero et al. 2017).

Anglerfishes are bycatch species of commercial relevance, which are mainly caught in mixed bottom trawl fisheries operating in the continental shelf and upper slope, together with other commercially important species, such as european hake (*Merluccius merluccius*), striped mullet (*Mullus surmuletus*), red mullet (*Mullus barbatus*), rose shrimp (*Aristeus antennatus*) or norway lobster (*Nephrops norvegicus*) (Demestre and Lleonart 1993, Demestre et al. 1997, Sardà 1998, Recasens et al. 2008). Discards are negligible since these species have high commercial value in the entire size range (Díaz et al. 2008), and are usually caught, landed and recorded together in the ports statistics. According to official data, over the period 2000-2016 total landings of *Lophius* in the northwestern Mediterranean Sea have added up to 9,486 t with a value of 65 million of Euros (Unpublished data from the Directorate of Fishing and Maritime Affaires; Government of Catalonia). Due to the commercial importance of *Lophius* species, it is important to assess the status of their stock in order to set catch limits and reduce the risk of stock collapse, although there is poor data available for these species.

Since many methods for assessment fisheries require information on life history parameters (Cope and Punt 2009), recent studies have been performed on sexuality, reproduction (spawning season, size at sexual maturity (L_{50})) and fecundity (Colmenero et al. 2013, Colmenero et al. 2015b) however information regarding natural mortality and growth parameters (asymptotic length and growth rate) is not available yet although they are crucial for sustainability and for an effective management of their fishery. Age and growth studies have been focused in other geographical areas such as Atlantic Ocean, eastern Mediterranean waters as well as Eat China and Yellow Seas, using several methods such as otoliths (Tsimenidis and Ondrias 1980, Crozier 1989, Woodroffe et al. 2003, Hernández et al. 2015), *illicium* (Dupouy et al. 1984, Duarte et al. 1997, Landa et al. 2001, Landa et al. 2013) and vertebrae (Armstrong et al. 1992, Yoneda et al. 1997, Johnson et al. 2008). The length-frequency distributions of catches (García-Rodríguez et al. 2005, Carlucci et al. 2009, Ofstad et al. 2013) and the analysis of tagging-recapture individuals (Laurenson et al. 2005, Landa et al. 2008a) have been also used in the estimation of these parameters. Current assessment techniques require technical abilities, biological knowledge, and time-series data on catch, effort and abundance (Walters and Martell 2004). Although it represents an impediment to assessing small-scale and data-poor fisheries; other evaluation methods can be applied for this kind of fisheries (Froese 2004, Prince et al. 2015).

Understanding the biology and life history characteristics of a species is crucial for the successful management of the fishery. Therefore, and due to the lack of information in the Mediterranean Sea on the sex ratio, biometric relationships, natural mortality and growth of the anglerfishes, the goals of this study are to provide scientific background on life history of *L. piscatorius* and *L. budegassa* for achieving an appropriate stock assessment and an efficient management for both species in this area; and explore the sustainability indicator proposed by Froese (2004) to check the actual status of their stocks.

MATERIAL AND METHODS

Data collection

Lophius specimens were collected monthly between June 2007 and December 2010 aboard commercial trawlers at depths of 12–836 m, at 466 sampling stations located in the fishing grounds of the northwestern Mediterranean Sea (Fig. 1). A total of 5923 specimens of *L. budegassa* and 628 *L. piscatorius* with sizes ranging from 4 to 105 cm TL and from 9 to 120 cm TL, respectively, were caught during the sampling period. A subsample of 4217 individuals of *L. budegassa* and 556 of *L. piscatorius* was used to obtain biological information. Total length (TL, cm), total weight (TW, g) and gutted weight (GW, g) of each specimen were recorded. Lengths were rounded down to the nearest half centimetre and weights were measured to the nearest 0.1 g. Fish were sexed and assigned macroscopically to a gonadal stage following a scale with 5 maturity phases proposed by Colmenero et al. (2013) applicable to both sexes: I, immature; II, developing and regenerating; III, spawning capable; IV, actively spawning; V,

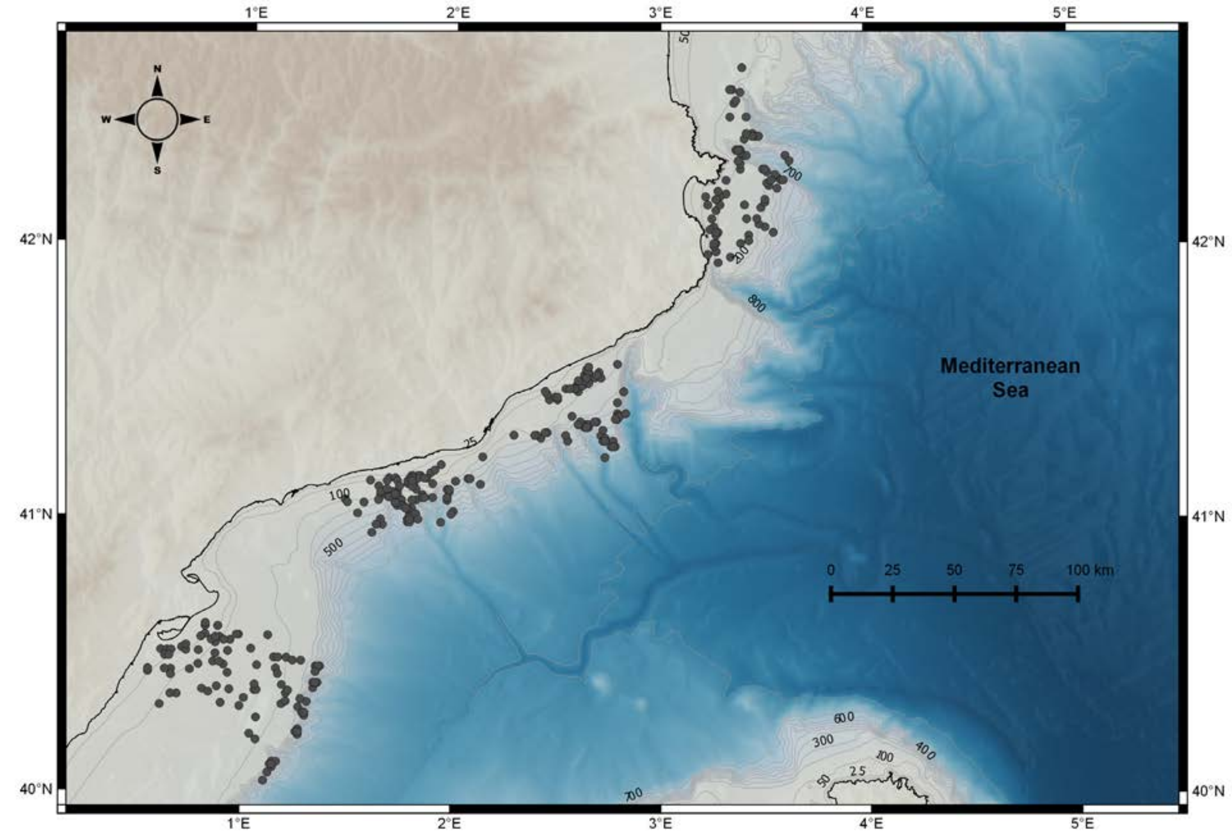


Figure 1. Stations sampled on the northwestern Mediterranean Sea from June 2007 to December 2010.

regressing. Fish that were too small to determine their sex or assign to a gonadal phase (approximately < 20 cm TL) were classified as indeterminate. All data presented are mean ± SD.

Biometric relationships

The relationship between length-weight was described for both sexes of both *Lophius* species using total length, total weight and gutted weight by fitting all data to the potential equations:

$$\mathrm{TW} = a\mathrm{TL}b$$

$$\mathrm{GW} = a\mathrm{TL}b$$

where a and b are the parameters to be estimated, with b being the coefficient of allometry. Student's t-test was used to determine if total length, total weight and gutted weight were significantly different from males and females within the same *Lophius* species and among species. A covariance analysis (ANCOVA) was used to test the influence of sex on the different relationships found for length-weight within the same species and between black and white anglerfish.

Estimation of growth and mortality parameters

The von Bertalanffy growth function (von Bertalanffy 1938) was used to estimate the growth parameters of *L. budegassa* and *L. piscatorius*:

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

where L_t is the length at time t, L_∞ is the asymptotic length at which growth is zero, k is the growth coefficient which determines how fast the fish approaches its L_∞ and t_0 defines the point in time when the fish has zero length. For the estimation of L_∞, k and t_0 parameters, two different routines of FiSAT II Program (Gayanilo Jr. et al. 2005) were carried out: Direct fit of length-frequency data by Shepherd's method and modal progression analysis by Bhattacharya's method (Bellido et al. 2000, García-Rodríguez et al. 2005). The results were compared to the goodness of fit using the Akaike information criterion (AIC) with a routine of R v. 3.1.2 (R Core Team 2014). The selection of the best parameters was considered depending on the minimum value of AIC (Panhwar et al. 2010). Based on von Bertalanffy growth parameters (Pauly and Munro 1984), the performance index (Ø') was calculated as:

$$\text{ø}' = \log_{10} k + 2 \log_{10} L_\infty$$

The natural mortality rate (M) for both species of *Lophius* was estimated using several empirical estimators in order to consider the highly uncertainty of M, particularly for data-poor stocks as is the case of *Lophius spp.* in the Mediterranean Sea.

From empirical estimator of M based on life-history parameters, Pauly (1980) is the most widely applied. In order to be compared our M estimates with other *Lophius* studies, M was calculated using the routine of FiSAT II Program based on Pauly's estimator:

$$\log M = -\,0.0066 - 0.279 \log L_\infty + 0.6543 \log k + 0.4634 \log T$$

where L_∞ and k are variables described above and T is the mean water temperature inhabited by the fish in the study area (13.4 ºC for both species). We also used the updated Pauly's estimator recommended by Then et al. (2015):

$$M = 4.118\, k^{0.73}\, L_\infty^{-0.333}$$

excluding the mean annual temperature from the equation because this variable was not a strong predictor of M in their analysis.

The second estimator more widely used in obtaining M is the Hoenig (1983) estimator, which is based on maximum age. Here we used an updated version also recommended by Then et al. (2015):

$$M = 4.899\, t_{max}^{-0.916}$$

where t_{max} was calculated solving the von Bertalanffy growth equation.

Sex ratio

Sex ratio was analysed considering total numbers of males and female (M:F) and the numbers of each sex at 5 cm length classes for both *Lophius* species. In order to assess significant differences in the proportion of both sexes, a chi-squared test was performed for each size class interval and for the whole sample.

All statistical analyses were carried out using Stata/SE 12.0 software with a significant level of $p < 0.05$. Variables were tested for normality using visual methods (histogram and standardized normal probability plot) and through normality tests (Shapiro-Wilk for sample sizes with $n \leq 2000$ and Shapiro-Francia with $2000 < n \leq 5000$ observations) (Shapiro and Francia 1972). Samples that came from a non-normal distribution were log transformed for normality.

Sustainability indicators

The three size-based indicators presented by Froese (2004) were used to monitor the *Lophius* population status relative to exploitation: a) Indicator 1 “Let them spawn”, which indicates the percentage of mature fish ($\geq L_{50}$) in the catch, with 100% as target; b) Indicator 2 “Let them grow”, which is estimated as percentage of fish caught at optimum length (L_{opt}) for harvest, with 100% between ± 10% L_{opt} as target. If length at first maturity (L_{50}) is available, L_{opt} can be estimated from the following equation (Froese and Binohlan 2000):

$$\log L_{opt} = 1.053 \log (L_{50}) - 0.0565$$

and c) Indicator 3 “Let the mega-spawners live”, which shows the percentage of mega-spawners (fish of a size larger than optimum length plus 10%) in the catch, with 0% as target. Values of 30-40% representing a healthy age structure, whereas less than 20% will be a matter of concern.

RESULTS

Length-weigh analysis

From the subsamples collected during the sampling period, *L. budegassa* females (n = 1923) varied in size from 11 to 72.5 cm TL, and between 13 to 5592 g and from 11 to 4376 g in total and gutted weight respectively. Males (n = 1977) varied from 10.5 to 62 cm TL; and between 13 to 3320 g TW and from 11 to 2922 g GW. Indeterminate specimens (n = 317) ranged in size from 4.5 to 38.5 cm TL, and from 1 to 700 g TW and 1 to 548 g GW. Significant differences were found in the mean total length, the mean total and gutted weight between males (30 ± 9 cm TL; 490 ± 363 g TW; 406 ± 304 g GW) and females (34 ± 13 cm TL; 887 ± 970 g TW; 719 ± 781 g GW) (*t*-test, total length: $t = 7.55$, $p < 0.05$; total weight: $t = 9.41$, $p < 0.05$; gutted weight: $t = 8.90$, $p < 0.05$).

Females (n = 280) of *L. piscatorius* varied from 16 to 107 cm TL, 52 to 14600 g TW and 42 to 12688 GW. Males (n = 249) size ranged from 14.5 to 89 cm TL, and weight from 32 to 7812 g and from 26 to 6728 g, total and gutted respectively. Indeterminate individuals (n = 27) varied in size from 14 to 41 cm TL, in total weight from 30 to 836 g and in gutted weight from 24 to 700 g. Significant differences were not detected in the mean total length, the mean total weight and the mean gutted weight between males (41 ± 13 cm TL; 1330 ± 1204 g TW; 1052 ± 979 g GW) and females (43 ± 17 cm TL; 1797 ± 2209 g TW; 1461 ± 1832 g GW) (*t*-test, total length: $t = 0.82$, $p = 0.41$; total weight: $t = 1.37$, $p = 0.17$; gutted weight: $t = 1.26$, $p = 0.21$).

Comparing both *Lophius* species, not significant differences were noted between males and females for total length (*t*-test; $t = -13.66$, $t = - 9.32$, $p < 0.05$, respectively), total weight (*t*-test; $t = -14.08$, $t = - 9.33$, $p < 0.05$, respectively) and gutted weight (*t*-test; $t = -13.87$, $t = - 9.30$, $p < 0.05$, respectively).

The results provided by ANCOVA indicated that there were significant differences between males and females for *L. budegassa* in both length-total weight ($F_{1,\,3898} = 26.80$, $p < 0.001$) and length-gutted weight ($F_{1,\,3898} = 18.92$, $p < 0.001$) relationship, as well as for *L. piscatorius* in length-total weight ($F_{1,\,527} = 4.32$, $p = 0.038$) and length-gutted weight ($F_{1,\,527} = 4.14$, $p = 0.042$) (Table 1, Fig. 2). Comparing length-total weight and length-gutted weight relationships for males of both *Lophius* species not significant differences were found between them ($F_{1,\,2224} = 3.02$, $p = 0.083$; $F_{1,\,2224} = 0.14$, $p = 0.709$, respectively) but significant differences were attained comparing the same relationships between females of both species ($F_{1, 2201} = 13.21$, $p < 0.001$; $F_{1, 2201} = 24.44$, $p < 0.001$, respectively) (Table 1, Fig. 2).

Table 1. Parameters of the relationship between length and weight (total and gutted) by sex of *Lophius budegassa* and *Lophius piscatorius*.

Species	Function	r^2	n
L. budegassa			
Indeterminate	$TW = 0.0164 * TL^{2.9333}$	0.9596	317
	$GW = 0.011 * TL^{3.0232}$	0.9841	317
Males	$TW = 0.0183 * TL^{2.9255}$	0.9634	1977
	$GW = 0.0123 * TL^{2.9837}$	0.9784	1977
Females	$TW = 0.0146 * TL^{3.0125}$	0.9759	1923
	$GW = 0.0107 * TL^{3.0404}$	0.9858	1923
Both sexes	$TW = 0.0142 * TL^{3.0092}$	0.9778	4217
	$GW = 0.011 * TL^{3.0232}$	0.9841	4217
L. piscatorius			
Indeterminate	$TW = 0.0095 * TL^{3.1334}$	0.9514	27
	$GW = 0.0067 * TL^{3.1823}$	0.9590	27
Males	$TW = 0.0153 * TL^{2.9873}$	0.9731	249
	$GW = 0.0122 * TL^{2.9941}$	0.9823	249
Females	$TW = 0.022 * TL^{2.9025}$	0.9782	280
	$GW = 0.0165 * TL^{2.9227}$	0.9825	280
Both sexes	$TW = 0.0174 * TL^{2.9588}$	0.9765	556
	$GW = 0.0136 * TL^{2.97}$	0.9825	556

Growth and natural mortality

The growth parameters and the growth performance values obtained for both Mediterranean lophiids in our study together with the values estimated by other authors in the Mediterranean Sea and Atlantic waters are presented in Table 2.

We identified eight modal classes for *L. budegassa* corresponding to ages from 0 to 6 years and the length frequency distribution show three recruitment modes during the first year of life. The mean size at the first age was 18.8 cm and the older (age 6) grew on average 9.20 cm. For *L. piscatorius* we recognized nine modes that belong to ages 0 to 8 years and two of these recruitment modes were observed during the first year of life. The maximum growth until age 1 was 19.4 cm and at the older age 8 grew on average 9.22 cm (Table 3).

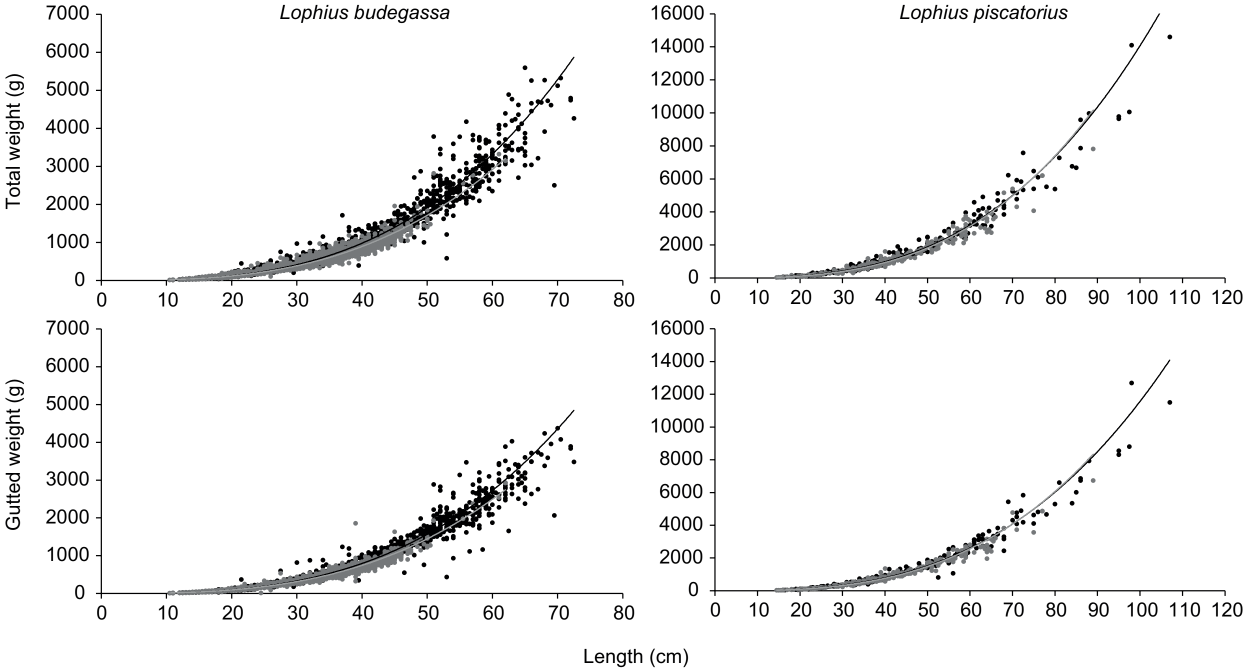


Figure 2. Length-weight relationships for (•) male and (•) female of *Lophius budegassa* and *Lophius piscatorius*.

The natural mortality rates estimated for *L. budegassa* and *L. piscatorius* using the growth parameters derived from the length-frequency analysis (both Pauly's estimators) revealed lower natural mortalities than the Hoening's estimator based on t_{max}. For *L. budegassa* the following natural mortality values were obtained: Pauly's M = 0.19 year^{-1}, updated Pauly's M = 0.16 year^{-1} and updated Hoenig's M = 0.39 year^{-1}. For *L. piscatorius* the mortality values were: Pauly's M = 0.20 year^{-1}, updated Pauly's M = 0.16 year^{-1} and updated Hoenig's M = 0.42 year^{-1}.

Population structure

Sex ratio (M:F) was found to be 1:0.97 for *L. budegassa* (1977 males, 1923 females) and 1.12:1 for *L. piscatorius* (249 males, 280 females). The M:F proportion in *L. budegassa* differ significantly from the 1:1 ($\chi^2 = 381.502$, $p < 0.05$) but the sex ratio of *L. piscatorius* does not differ significantly from 1:1 ($\chi^2 = 19.490$, $p = 0.301$). In both species, the sex ratio varies with length and females reached greater size than males (Fig. 3).

In individuals of *L. budegassa* with small sizes (10 to 35 cm) no differences between the number of males and females were observed (χ^2, $p \geq 0.05$). The proportion of males increased up to 60 % between 35 and 45 cm, they decreased down to 30 % in the length interval 45-50 cm and disappeared at 60-65 cm where females dominated (100%) at sizes > 70 cm.

Table 2. Growth parameters estimated for sex combined species of *Lophius budegassa* and *Lophius piscatorius* in the Mediterranean Sea and Atlantic waters.

Species	Area	Subarea	Source	Method	L_∞ (cm)	k (y^{-1})	t_0 (y)	Ø'	n	Size range (cm)
L. budegassa	Mediterranean Sea	Northwestern	Present study	Modal lengths	128.97	0.106	-0.10	3.25	5923	4-105
		Northwestern Ionian	Carlucci et al. 2009	Modal lengths	68.53	0.290	-0.10	3.13	-	3.5-114
		Northwestern Ionian	Carlucci et al. 2009	*Illicium*	68.39	0.100	-1.43	2.67	565	4-71
		Western	García-Rodríguez et al. 2005	Modal lengths	102.00	0.150	-0.05	3.2	1301	4-86.5
		Western	García-Rodríguez et al. 2005	*Illicium*	90.00	0.080	-0.10	2.81	170	-
	Atlantic Ocean	Iberian waters	Landa et al. 2001	*Illicium*	93.50	0.101	0.38	2.95	1038	5-93
		Bay of Biscay	Quincoces et al. 1998	*Illicium*	100.00	0.110	1.46	3.04	2006	12.5-136.5
		Iberian waters	Duarte et al. 1997	*Illicium*	101.69	0.080	-0.20	2.91	336	19.4-65.5
		Northeastern	Dupouy et al. 1986	*Illicium*	94.00	0.090	0.66	2.9	281	-
L. piscatorius	Mediterranean Sea	Northwestern	Present study	Modal lengths	146.92	0.114	-0.30	3.39	628	9-120
	Atlantic Ocean	Faroese waters	Ofstad et al. 2013	*Illicium*	231.90	0.048	-1.16	3.41	9057	0.7-142
		Porcupine Bank	Landa et al. 2013	*Illicium*	162.31	0.088	-0.89	3.37	979	5-125
		Northeastern	Landa et al. 2008	Tag-recapture	105.31	0.174	0	3.29	32	15-135
		Faroese waters	Ofstad and Laurenson 2007	*Illicium*	210.73	0.050	-0.88	3.35	5794	11-142
		Northeastern	Laurenson et al. 2005	Tag-recapture	140.00	0.104	0	3.31	20	25-83
		Iberian waters	Landa et al. 2001	*Illicium*	163.50	0.065	-0.38	3.24	844	14-140
		Bay of Biscay	Quincoces et al. 1998	*Illicium*	150.00	0.088	-0.02	3.30	1385	12.5-136.5
		Iberian waters	Duarte et al. 1997	*Illicium*	121.54	0.102	0.03	3.18	165	5-80
		Iberian waters	Landa and Pereda 1997	*Illicium*	132.05	0.110	0.66	3.28	514	-
		Irish Sea	Crozier 1989	Otolith	105.56	0.176	0.38	3.29	3739	9.1-133.1
		Northeastern	Dupouy et al. 1986	Illicium	140.00	0.102	0.52	3.30	1028	-

Significant differences were noted in the proportion of males and females in larger sizes of 35 cm (χ^2, $p < 0.05$). The presence of *L. piscatorius* males was a 100% in 10-15 cm class size with significant differences on the sex ratio (χ^2, $p < 0.05$). The proportion between males and females remained between 40% and 50% from 15 to 55 cm; increased up to 60% in the size class interval 55-60 cm and dropped down to 30% at 75-80 cm; but not significant differences were found in the sex ratio (χ^2, $p \geq 0.05$). At lengths greater than 90 cm, 100% were females; and significant differences were observed again in the M:F proportion.

Sustainability indicators

The percentage of mature specimens in the catch was 5.0% for *L. budegassa* and 20.4% for *L. piscatorius*. The optimum length obtained for *L. budegassa* was 42.0 cm and the size range of ± 10% L_{opt} went from 37.8 to 46.3 cm TL, representing the 4.8% of the catch. For *L. piscatorius* L_{opt} was found to be 55.5 cm and the ± 10% L_{opt} size range varied from 49.9 to 61.0 cm TL, which the 10.3% of the fish captured, was within that range (Fig. 4 a, b). The portion of mega-spawners present in the catch corresponded to 2.2% for *L. budegassa* and 12.9% for *L. piscatorius*. Applying the results obtained from the three indicators to time series of length-frequency data for both Mediterranean lophiids we noted for *L. budegassa* and *L. piscatorius* very low percentages of mature and optimum size specimens compared with the target of 100% together with a declining tendency of mega-spawners since 2008, which are always below 20% (Fig. 4 c, d).

Table 3. Modal classes for sex combined length-frequency distributions calculated for *Lophius budegassa* and *Lophius piscatorius* in the northwestern Mediterranean Sea with standard deviation (S.D.) and separation index (S.I.).

	L. budegassa			*L. piscatorius*		
Modal class	**Mean TL (cm)**	**S.D.**	**S.I.**	**Mean TL (cm)**	**S.D.**	**S.I.**
1	5.24	1.49	-	13.05	1.66	-
2	12.40	2.19	3.04	19.38	3.84	2.11
3	18.78	2.62	2.22	34.25	3.13	2.68
4	25.71	2.44	2.17	47.35	3.33	2.36
5	35.48	2.82	2.32	54.74	1.96	2.08
6	43.59	2.16	2.16	67.43	3.58	2.25
7	53.65	2.08	2.25	78.08	2.74	2.12
8	62.85	2.57	2.16	87.06	2.69	2.09
9	-	-	-	96.28	1.89	2.10

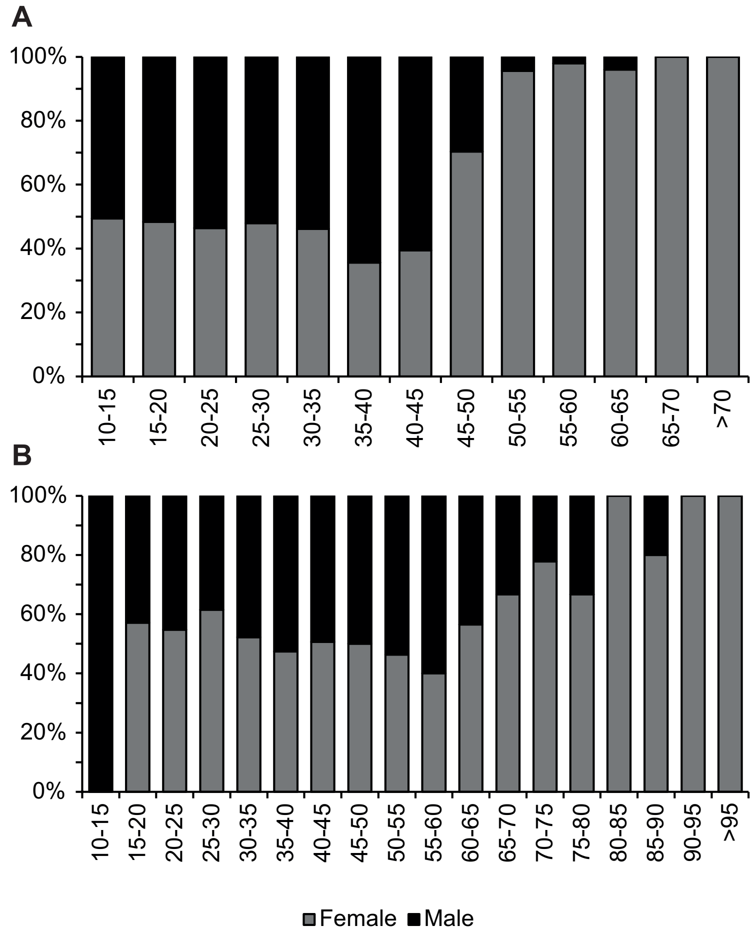


Figure 3. Sex ratio for (A) *Lophius budegassa* and (B) *Lophius piscatorius* by 5 cm length class interval.

DISCUSSION

Our findings mainly revealed that growth pattern of *Lophius* species inhabiting the Mediterranean Sea differ in the asymptotic length, being higher in *L. piscatorius*. However, the population structure was similar in both species, where females were larger and weightier than males. This sexual variability in the somatic growth pattern has been already reported for these species in other geographical areas (Table 1) (Ofstad and Laurenson 2007, Carlucci et al. 2009), and it has been also described in other species inhabiting the slope and the deep sea such as *Merluccius merluccius, Phycis blennoides, Mora moro* or *Lepidion lepidion* (Rotllant et al. 2002, Piñeiro and Sainza 2003). Differences in growth coincide with the onset of sexual maturity (Kozlowski and Teriokhin 1999), with males growing faster than females before reaching sexual maturity and the reverse afterwards (Landa and Piñeiro 2000, Lucio et al. 2000). It may be also associated to ontogenetic migrations with depth as occurs in *Lophius* species where older females move towards deeper waters (Laurenson et al. 2001, Yoneda et al. 2002) experiencing changes in temperature

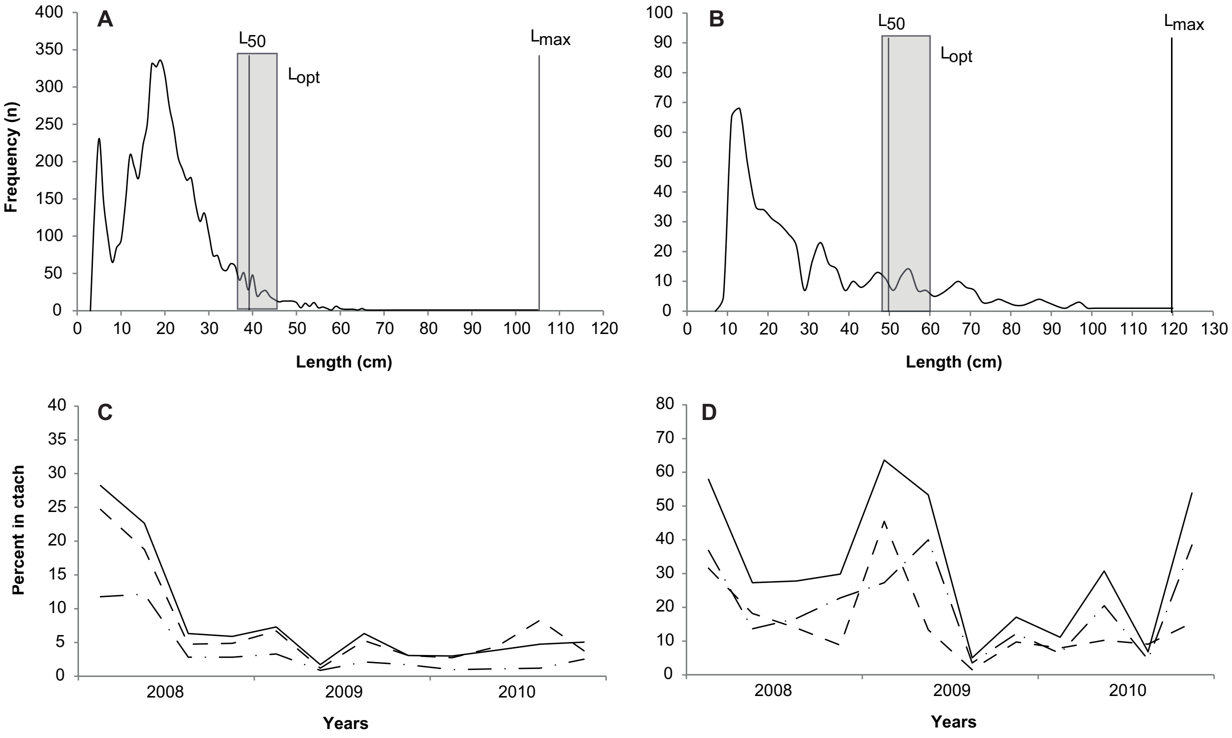


Figure 4. Length-frequency data and percentage of mature, optimum size and mega-spawners of (A,C) *Lophius budegassa* and (B,D) *Lophius piscatorius* from 2008 to 2010 in the northwestern Mediterranean Sea.

and salinity that might have an impact on growth and other physiological processes (Higgins et al. 2015), such as the velocity of digest process (slower in colder temperatures) that affect longevity (Cailliet et al. 2001, Valenzano et al. 2006), differences in oxygen consumption (Pauly 1994) and/or to differences in the level of surplus energy between reproduction and somatic growth (Rljnsdorp and Ibelings 1989). Furthermore, the rate of compensatory growth might also provide a greater reproductive success (Clarke 1983) to maximize egg producing biomass since larger females tend to produce more and bigger eggs than smaller ones (Colmenero et al. 2015b) as occurs with females of *L. piscatorius* attaining a higher asymptotic length and expelling out more eggs than *L. budegassa* (Colmenero et al. 2013).

Different hard parts (otoliths, *illicium* and vertebrae) have been used for the estimation of age and growth in *Lophius* species (Dupouy et al. 1984, Armstrong et al. 1992, Landa et al. 2013); and although they are the best option, the analysis of length-frequency distributions has been also common for this purpose (García-Rodríguez et al. 2005, Carlucci et al. 2009).

In fact, most of the studies performed on *L. budegassa* and *L. piscatorius* in Mediterranean and Atlantic waters have obtained growth coefficient values (k) around 0.1 year^{-1}, independently of the methodology used (Table 2). However, higher values (k = 0.29 year^{-1}) have been obtained using modal progression analysis (Carlucci et al. 2009), and lower ones (k = 0.05 year^{-1}) counting rings in the *illicium* (Ofstad and Laurenson 2007). When comparing our results with those published in the Mediterranean Sea and Atlantic waters, differences are reflected in the different values for growth parameters and Ø' (Table 2). Our findings suggest a slower growth in the populations of both *Lophius* species inhabiting Atlantic waters, whereas *L. budegassa* attain higher sizes in the Mediterranean Sea. According to Sequeira et al. (2009), several factors could explain it: (a) the different method used (whole and sectioned otoliths, length frequency analysis, back calculation), (b) the heterogeneity of the sample (different size distribution probably caused by different gear being deployed), (c) different environmental conditions, (d) different latitudes, and (e) different fishing pressures. Nevertheless, studies in other demersal fishes seems to suggest that the Mediterranean populations tend to grow faster than Atlantic ones (Tuset 2000, Pajuelo et al. 2002, Piñeiro and Sainza 2003, Sequeira et al. 2009), likely due to the growth plasticity of the species and the influence of factors that vary geographically, as temperature or food availability (Landa et al. 2013).

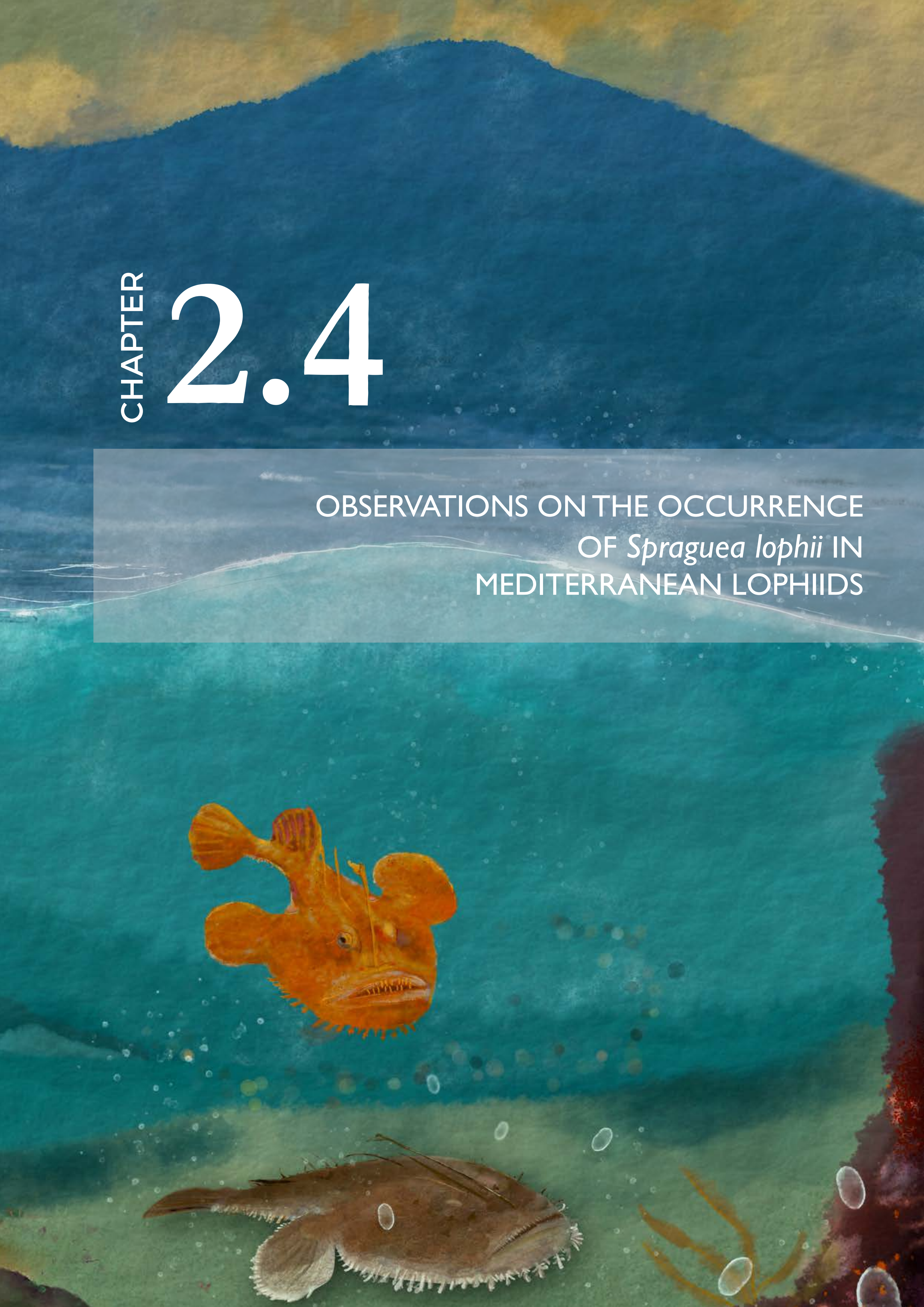
The modal progression analysis indicated that the mean growth rate during the first year of life for Mediterranean lophiids was double than the rest of age classes being the values for *L. piscatorius* slightly higher than its congener *L. budegassa*. It is consistent with the observations in Atlantic waters where age class 0-1 attains lengths around 20 cm; more than twice that from age 1 to 2 (Landa et al. 2008a, Ofstad et al. 2013). This confirms that both *Lophius* species have a high growth rate during their early life; thus smaller fish grow faster than older ones favouring a clear ontogenetic change of both anglerfishes. Moreover, several recruitment modes were noted despite that these species are total spawner (Murua and Saborido-Rey 2003), it suggests a local variability in the spawning seasonality or different conditions for feeding during larvae growth. In the first case, studies have showed that mature females occur in specific periods (Colmenero et al. 2013, Colmenero et al. 2017). Therefore, the second hypothesis may gain strength knowing that physical and oceanographic features of the area such as hydrographic heterogeneity, geomorphology of the continental shelf, and the presence of a hydrographic front along the shelf slope of the Catalan are variable (Sabatès et al. 1989).

The use of mortality estimators based on maximum age such as Hoenig's estimator was recommended by Then et al. (2015) when possible, instead of estimators based on growth parameters (Pauly's estimator) due to the first one performed the best among other models tested based on cosss-validation predictor error, model residual patterns, model parsimony and biological considerations. In this context, our data emphasize that the mortality rate for *L. piscatorius* adopted for stock assessment following Pauly's estimator (0.2 year^{-1}) is nowadays adequate (ICES 2012), but incorrect for *L. budegassa* (0.15 year^{-1} versus 0.19 year^{-1}, (ICES 2012); present study, respectively). Although these results are reversed when updated Pauly's estimator is used (0.16 year^{-1} for both *Lophius* species) being natural mortality for *L. budegassa* correctly selected, but not the one applied to *L. piscatorius*. The higher values of M obtained through Hoenig's estimator could be the consequence of a sampling bias since the size frequency come from commercial catches and possibly the largest sizes are not captured by the trawling fishing fleet because these boats only reach 800 meters and larger individuals inhabit in deeper waters (Yoneda et al. 2002).

Although an analysis by sex was not possible to perform in this work due to our data characteristics, the sex-ratio analysis showed a clear difference in the dynamic population of them in both species according to Duarte et al. (2001). The sexual proportion was distributed close to equilibrium observing significant differences in the large sizes, which were entirely composed by females. Skewed sex ratios have been reported for other population of *Lophius* (Duarte et al. 2001, Ofstad and Laurenson 2007, Richards et al. 2008) and several explanations can be addressed to explain this pattern, studies indicate that males have a slower growth rate after a certain length or stops, and females appear to have longer lifespan (Yoneda et al. 1997, Richards et al. 2008). Moreover, it could be related to behavioural differences such sex-specific movement patterns where females move into deep waters with increasing size and maturity (Laurenson et al. 2001, Richards et al. 2008).

Considering the growth parameters, mortality and size at maturity estimated for *Lophius* species, the stock evaluation allows concluding that these species are overfished in the north-western Mediterranean Sea. Although the absolute percentage of the evaluation indicators may be affected by biological parameters obtained length composition, the trend is correct (Froese 2004). Data on the size structure of anglerfish retained by bottom trawlers indicate that 5.3% of landed *L. budegassa* were > 39.4 cm (the size of 50% maturity for sex combined) and the 20.4% of *L. piscatorius* were > 51.3 cm (L_{50} for sex combined) (Colmenero et al. 2017). In addition, individuals of lengths of about 10-13 cm (settlement size) (Hislop et al. 2001) are also captured by the trawl fleet. In both cases more than the 80% of the *Lophius* population were prevented from reproducing since they are caught by the trawling fleet, which will not allow rebuilding and maintenance of a healthy spawning stock. The slow growth rates and late maturity of both species confirm that a large part of the population captured by the fishery has not matured, which if unmanaged could lead to recruitment-overfishing (Walmsley et al. 2005). The percentage of fish caught at ± 10% L_{opt} was 4.8% for *L. budegassa* and 10.3% for *L. piscatorius*, being both values far away from the target of the second sustainability indicator "Let then grow" which would be to catch all fish within the optimum length range. On the other hand, the 2.2% of *L. budegassa* and the 12.9% of *L. piscatorius* caught in the catch were mega-spawners. According to this, and considering that values of 30-40% of mega-spawners reflect a healthy population, both *Lophius* species have little resilience against recruitment failures.

According to the Marine Strategy Framework Directive (2008/56/EC) (MSFD), age and size structure is considered one of the important characteristics determining the "health" of a population. These data together with the sustainability indicators obtained for *Lophius* spp. reveal a high exploitation level of their stocks in the study area, why management actions are highly recommended to conduct as soon as possible. A regulation based on a limitation on the maximum depth at which fishing activity can be carried out could preserve could preserve the oldest specimens and favour a biomass of eggs and recruitment (Hixon et al. 2014), although this type of regulation would be difficult to carry out since larger specimens of *Lophius* are fished together with other commercially important species, such as *A. antennatus* or *N. norvegicus*. The present study provides valuable information of the life history parameters for *L. budegassa* and *L. piscatorius* in Mediterranean waters, as well as an important baseline for future studies.



CHAPTER 2.4

OBSERVATIONS ON THE OCCURRENCE OF *Spraguea lophii* IN MEDITERRANEAN LOPHIIDS

OBSERVATIONS ON THE OCCURRENCE OF *Spraguea lophii* IN MEDITERRANEAN LOPHIIDS

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Abstract. In the Mediterranean Sea, anglerfish *Lophius budegassa* and *L. piscatorius* support an important fishery, and landings have increased in recent years. These species are infected by the microsporean *Spraguea lophii*, a parasite that infects their nervous system giving rise to multiple cysts. Due to the high value of these fish and conspicuous nature of the infection, we determined the apparent prevalence of this parasite in both *Lophius* spp. Specimens were obtained from commercial catches along the NW Mediterranean Sea during 2013. The parasite was observed in the nerves from the peripheral nervous system of the individuals and was detected at the prevalence of 69.2% (202/209) and 100% (58/58) in *L. budegassa* and *L. piscatorius*, respectively. Non-significant differences were noted between sexes of *L. budegassa* (χ^2=0.683; *p*=0.409), although a positive correlation was noted between host size and prevalence (χ^2=6.134; *p*=0.013). During the sampling, two specimens of *L. budegassa* with atypical morphological characteristics, pigment anomalies and blindness, and infected with *S. lophii* xenomas were described.

INTRODUCTION

Microsporidia are intracellular parasites related to fungi whose spores infect a broad range of hosts, such as arthropods, molluscs and vertebrates including immunocompromised humans (Hinkle et al. 1997, Lom and Dyková 2005, Williams 2009, Stentiford et al. 2013). Some microsporidians parasitize a variety of fish species and are capable of causing serious disease affecting various tissues and organs and resulting mortality in both wild stocks and aquaculture (Antonio and Hedrick 1995, Maíllo et al. 1998). Spores are released into the environment in faeces (from infected hosts or their predators), via the urinary tract or post mortem (Kramer 1976, Maíllo et al. 1998). They are the only microsporidian stage that can survive for extended periods in the environment and are responsible for its dissemination (Vávra and Lukes 2013). Although horizontal transmission by direct ingestion of the spores is the main transmission route among the aquatic microsporidia (Stentiford et al. 2013); many species infect germinal cells (oocytes, sperm) and are vertically transmitted to offspring (Galbreath et al. 2004) and autoinfection may occur. In humans, the sources of microsporidian infections and the modes of transmission remain unknown, but may be the result of the ingestion of poorly cooked infected fish (Curry 1999), as well as by the use of well water and groundwater containing microsporidian spores (Dowd et al. 1998). Inactivation of spores in fish can be achieved by freezing the fish at −20 °C for 48 h, treatment at 60 °C for 15 min and microwaving at 750W for more than 60 s Leiro et al. (2012).

Spraguea lophii (Doflein, 1898) Vávra and Sprague, 1976 is a microsporidian that infect the brain and ganglion cells in the peripheral nervous system of black anglerfish *Lophius budegassa* Spinola, 1807 and white anglerfish *Lophius piscatorius* Linnaeus, 1758, inhabiting the North Atlantic and Mediterranean regions (Thelohan 1895, Döflein 1898, Campbell et al. 2013). Once the spores of *S. lophii* reach the host digestive tract they enter the intestine and insert their sporoplasms into neurofibrils in the epithelial layer, intestinal connective tissue or muscular layers. If the neurofibrils become assembled into a nerve fibre, sporoplasms may migrate up to the central nervous system and finally reach its ganglion cells where sporogony occurs (Weissenberg 1976, Lom and Dyková 1992). The infection of *S. lophii* is manifested as cystic structures called xenoparasitic complexes (or xenomas) located in nerve ganglia running along the spine into the head. These cysts sometimes measure several centimetres cross and each contains large numbers of xenomas harbouring thousands of spores (Amigó et al. 1995, Freeman et al. 2004, Casal et al. 2012, Mansour et al. 2013). The host cell and the parasite are morphologically and physiologically integrated in the xenomas (Vávra and Lukes 2013) but the cell biology of this reaction is not known, nor is it understood whether the xenoparasitic complex occurs as fish tissue encapsulates the infection for its own defence or if the microsporidian transforms the host cell for its own shelter (Williams 2009). After the initial infection, other nerves may become heavily infected causing serious pathogenicity as pigment anomalies and blindness (Bucke et al. 1994, Landa et al. 1998, Bañón and Armesto 2004, Ragonese and Giusto 2006, Arculeo et al. 2010). However, no data exist to suggest that *S. lophii* infections cause mortalities in anglerfishes (Freeman et al. 2011).

The *Lophius* spp. of the Mediterranean Sea are demersal fishes living in sandy, muddy and rocky bottoms, cohabiting on the continental shelf. Despite the overlapping distribution of both species, no ecological competition exist between them due to a temporal segregation of their biorhythms (Colmenero et al. 2010). The demand for anglerfish for human consumption has increased in the last two decades, with more than 27,500 t of *L. budegassa* and *L. piscatorius* captured in 2012 (FAO, 2014). In the NW Mediterranean Sea, 5,450 t were caught in the last 10 years, with a value of 37 million of Euros (Unpublished data from the Directorate of Fishing and Maritime Affaires; Government of Catalonia). Due to the relevance of these species for the humans, the goals of the present study were (i) to evaluate the current presence of the parasite *S. lophii* in individuals of *L. budegassa* and *L. piscatorius* from the NW Mediterranean Sea, (ii) to compare the data with previous studies in *L. budegassa* inferring temporal changes and (iii) to hypothesize about possible side effects of severe infections on morphology of *Lophius* spp.

MATERIALS AND METHODS

Individuals of *L. budegassa*, with a size range between 7.0 to 52.5 cm in total length (TL) (n=292), and *L. piscatorius*, from 17.0 to 53.0 cm (TL) (n=58), were randomly selected from commercial catches along the NW Mediterranean Sea during 2013. Each fish was measured, dissected, sex determined (male, female and indeterminate), and examined macroscopically for the presence of microsporidia *S. lophii*. The prevalence was the only quantitative descriptor of parasite populations calculated due to the difficulty in quantifying the clustered individuals in the xenomas. For that, the spinal cord, the medulla oblongata and the nerves from the peripheral nervous system of each specimen were inspected. Prevalence was estimated according to Bush et al. (1997):

$$\text{Prevalence} = (\text{number of hosts infected} \,/\, \text{number of hosts examined}) \times 100$$

To assess the independence of prevalence of the parasite in relation to the fish sex and size, a chi-squared test ($p<0.05$) was performed. Finally, results were compared with a previous study conducted by Maíllo et al. (1998) to analyse the temporal variation. Statistical analysis was performed in XLStat 2012, a statistical plug-in for MS Excel 2011 spreadsheet program.

Two specimens of *L. budegassa* were obtained from commercial catches and scientific surveys presenting atypical characteristics (blindness and orange colouration respectively) and both with several *S. lophii* xenomas present.

RESULTS AND DISCUSSION

Of the 292 *L. budegassa* analyzed, 202 (69.2%) were infected by the parasite *S. lophii*; whilst all specimens of *L. piscatorius* (n=58) presented parasitism. The occurrence of this parasite in *L. budegassa* had been reported in the Mediterranean Sea (Maíllo et al. 1998), but this is the first report of its occurrence in *L. piscatorius* in this location. However, spores had been noted in both species in the Atlantic waters (Canning and Lom 1986, Cañás et al. 2010). Currently, five (*L. piscatorius*, *L. budegassa*, *L. americanus*, *L. litulon* and *L. gastrophysus*) of seven lophiid species have their nervous tissues parasitized by microsporidia of this genus (Weissenberg 1976, Takvorian and Cali 1986, Freeman et al. 2004, Casal et al. 2012, Campbell et al. 2013).

In the current investigation, infection was always observed in the vagus nerve situated near the kidneys. In some cases microsporidian xenomas were also present in the spinal cord, the medulla oblongata, and in the glossopharyngeal and trigeminal nerves (Fig. 1). Occurrence in these locations are in agreement with previous descriptions for these and other lophiids (Mansour et al. 2013). Members of the genus *Spraguea* normally infect nervous tissues and rarely other tissues of lophiid hosts (Casal et al. 2012).

Non-significant differences were noted between males and females of *L. budegassa* (χ^2=0.683; p=0.409) (Fig. 2a). However a positive relationship was observed between host size and prevalence (χ^2=6.134; p=0.013) increasing the presence of the parasite in individuals larger than 30 cm (Fig. 2b). Although both species feeds mainly on fishes and crustaceans (Crozier 1985, Negzaoui-Garali et al. 2008), cannibalism is also common in larger specimens (Gordoa and Macpherson 1990, Armstrong et al. 1996, Laurenson and Priede 2005). Since one of the transmission pathways of the parasite is by direct ingestion of the spores, feeding habits could explain the increased severity of infection in larger individuals (Gibson and Jones 1993). The possibility of autoinfection may also be a factor influencing severity in larger fish. However, gender does not appear to be an influential factor since male and female anglerfish select the same prey items (Cañás et al. 2010). The difference in

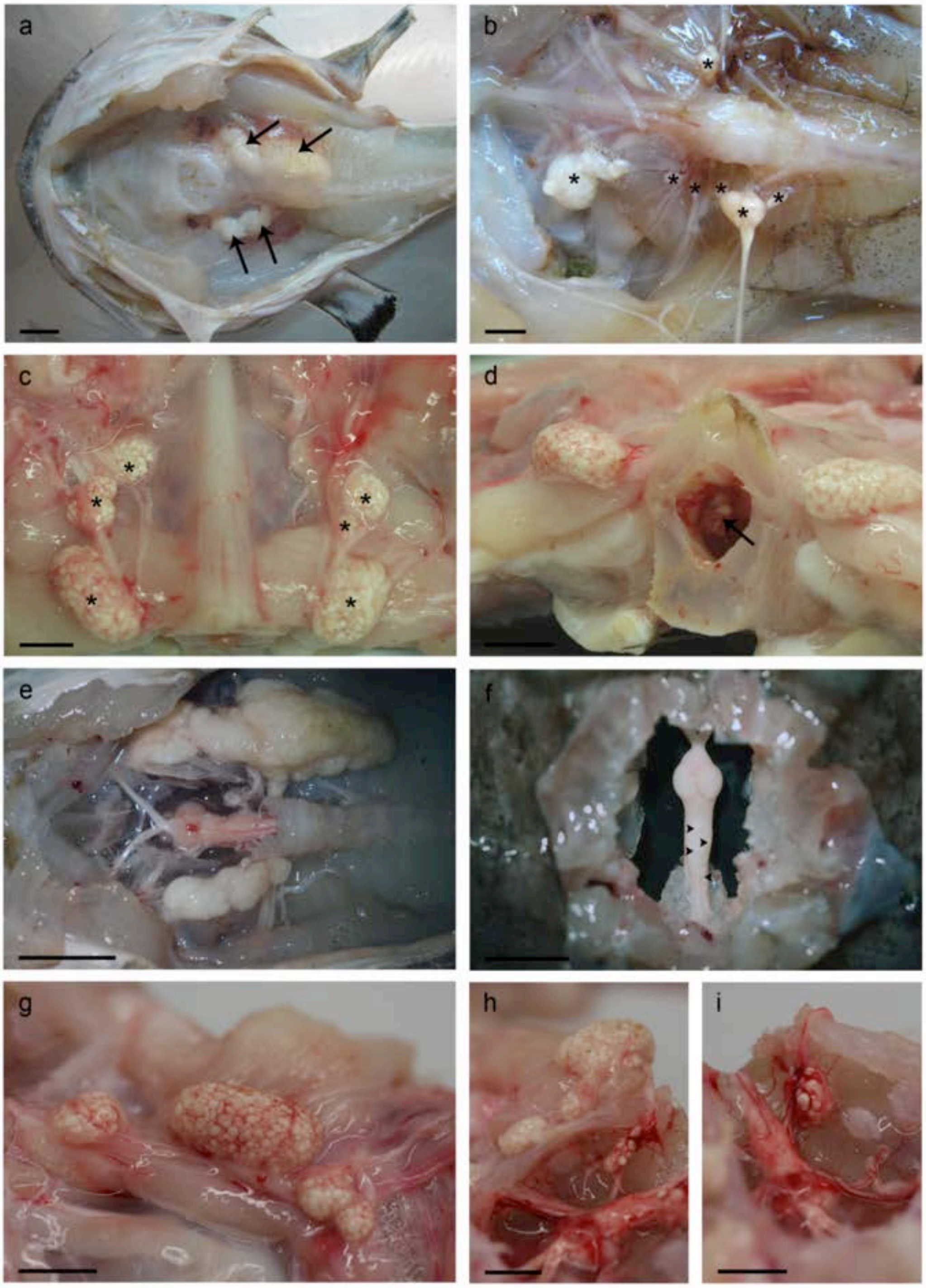

Figure 1. *Spraguea lophii* infection in *Lophius* spp. showing the organization of xenomas and spores on nervous tissues. **a** Ventral view of a dissected *Lophius piscatorius* showing the microsporidian cysts situated near the kidneys (*arrows*). **b, c** Nerves exiting the central nervous system enlarged with microsporidian infection (*asterisks*). **d** Xenomas developing in the spinal nerves inside the vertebral column (*arrow*). **e** Ventral view of the brain showing heavily infected vagus and glossopharyngeal nerves. (f) Dorsal view of the brain showing spores of *S. lophii* in the medulla oblongata (*arrowheads*). **g, h, i** Groups of xenomas that run into the brain cavity. *Scale bars* = 1 cm.

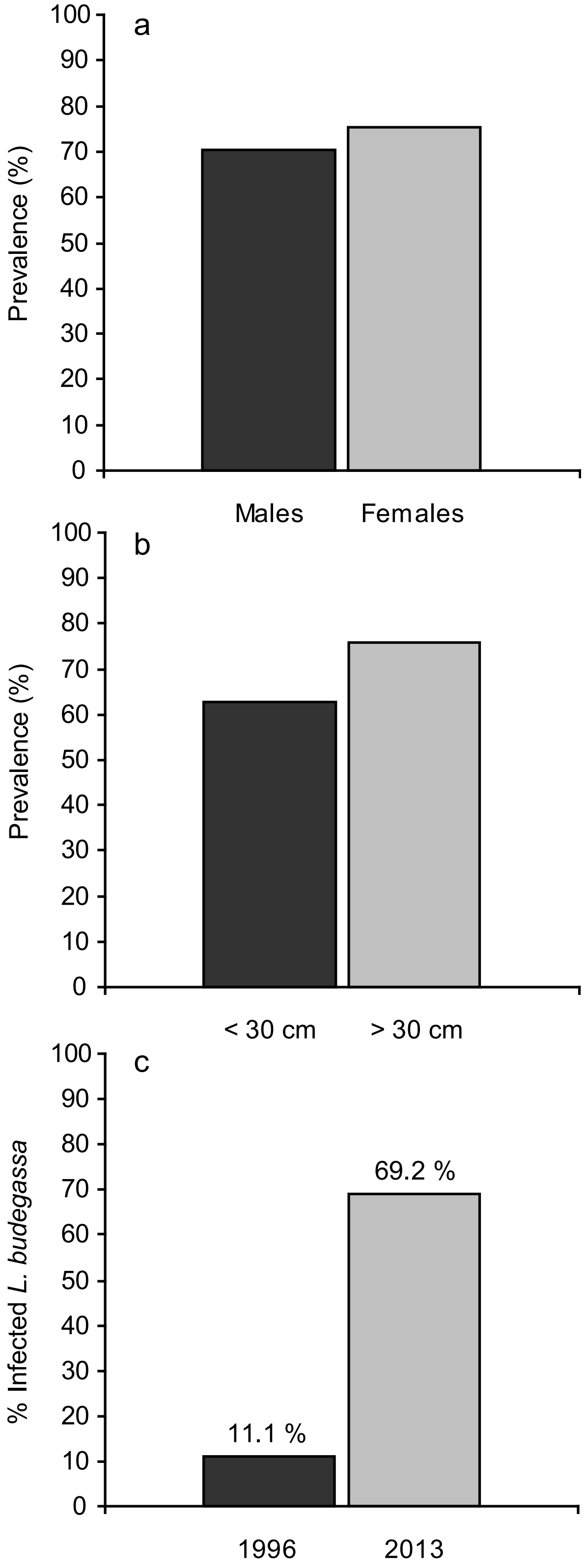

Figure 2. Prevalence of *Spraguea lophii* in *Lophius budegassa* from the NW Mediterranean Sea by sex (**a**) and by size (**b**) and increase of parasitism in the last 18 years (**c**).

parasite prevalence between the two species is difficult to explain since both species have similar depth distribution and also feed on the same prey (Laurenson and Priede 2005, Preciado et al. 2006).

The 69.2% of individuals of *L. budegassa* analysed in the present study were infected with *S. lophii* (Fig. 2c) compared with 11.1% prevalence reported by Maíllo et al. (1998).

Some studies had demonstrated an increase of the temperature average on the Mediterranean Sea, associated with the accelerating trend in global warming from the early 1990s (Carillo et al. 2012, Skliris et al. 2012). It is hypothesised that increase in water temperatures could significantly impact prevalence and intensity of parasitism through thermal stress leading to reduced immunocompetence and increasing the susceptibility of the host to the parasite (Antonio and Hedrick 1995, Marcogliese 2008).

Although, parasite impact on host population level dynamics is unknown, severe infections may be associated with anatomical anomalies such as eye deformities (Bucke et al. 1994).

However, in the study of Bucke et al. (1994) histological assessment of the eye and optic nerve did not reveal the presence of *Spraguea* (unpublished data). In the current study, we found two specimens of

L. budegassa with morphological anomalies. A blind specimen of 23.3 cm in length was apparently lacking an eye on gross examination (Fig. 3a, b). However, a rudimentary and deformed sclera with degenerate retinal tissue under the dermis and reduced optic nerve was found (Fig. 3c).

Body colouration was dark-brown similar to other *Lophius* spp. This specimen was infected with microsporidian xenomas of *S. lophii* in the vagus nerve near the kidneys as well as the spinal cord and medulla oblongata region of the hind brain. Xenomas were also observed in the trigeminal nerve. Bucke et al. (1994) considered that this anomaly was caused by the parasite *S. lophii* affecting the nervous system and did not consider genetic alterations or pollution effects as likely aetiologies. The other atypical specimen of *L. budegassa* (Fig. 4) was 33.6 cm in length was bright orange in colour and without the ocular anomalies in the previous specimen.

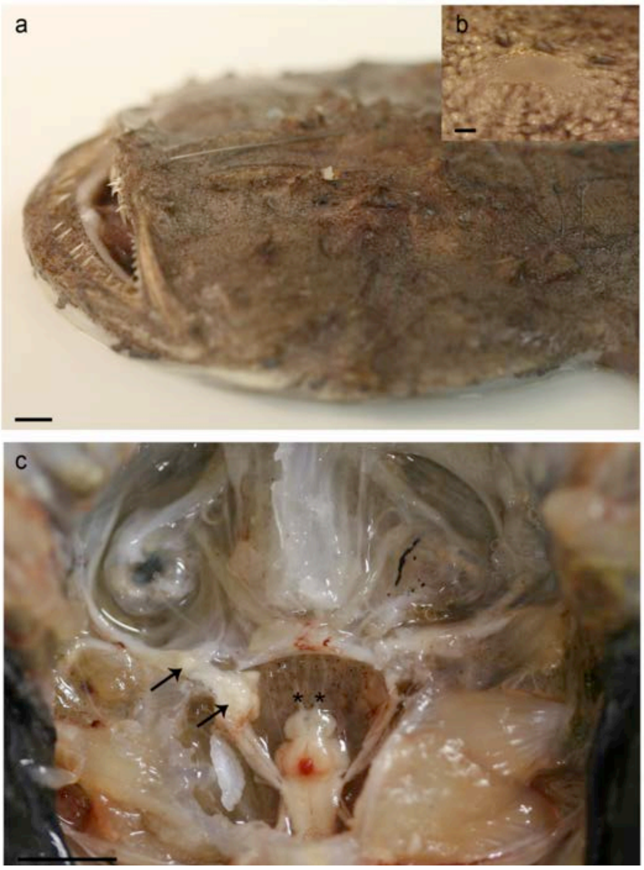

Figure 3. Blind specimen of *Lophius budegassa*. **a** General view (*scale bar* = 1 cm). **b** Close-up of the ocular region showing a depigmented area where the eye should be present (*scale bar* = 1 mm). **c** Ventral view revealing microsporidian xenomas in the trigeminal nerve (*arrows*). A deformed sclera and degenerate retinal tissue are also visible. Optic nerves are reduced (*asterisks*) (*scale bar* = 1 cm).

Similar cases have been reported previously (Cendrero and de Cárdenas 1979, Fariña and Fernández 1981, Alonso-Allende 1983, Allué and Sánchez 1986, Bucke et al. 1994, Pereda and Gancedo 1994, Landa et al. 1998, Bañón and Armesto 2004, Ragonese and Giusto 2006, Landa et al. 2007, Arculeo et al. 2010). In teleosts, colour changes are produced by both endocrine and neural system and depend to chromatophoral responses to ecological, physiological or ethological conditions (Fujii 2000). Consequently, the cause of this anomaly may be multifactorial and therefore difficult to determine. Some studies attribute alterations in skin pigmentation to parasites (Roberts and Bullock 1980, Oetinger and Nickol 1981). In lophiids the cutaneous mucous glands, where the peripheral nervous system are known

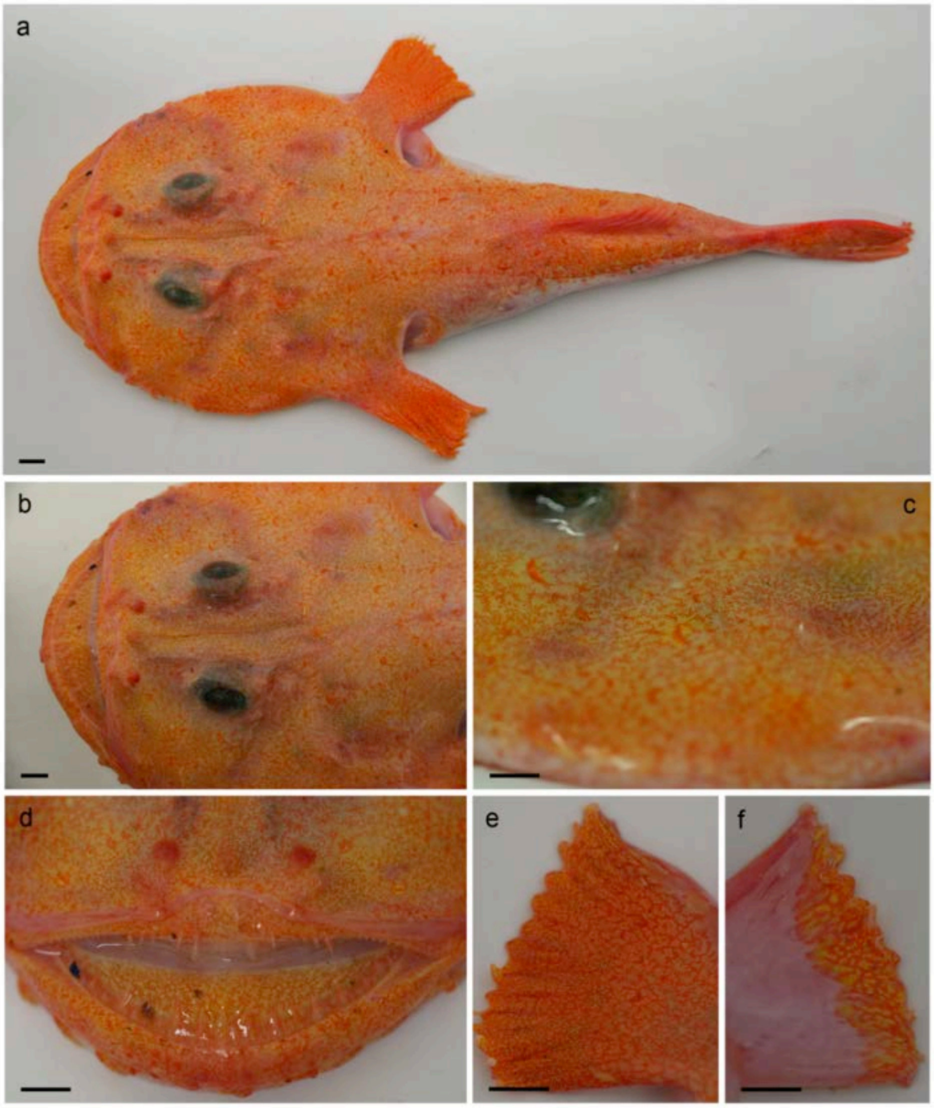

Figure 4. Specimen of *Lophius budegassa* with atypical colouration. **a** Dorsal view. **b** Head region. **c** Detail of the skin showing the orange colouration. **d** Close-up of the mouth. Dorsal (**e**) and ventral (**f**) part of the pectoral fin. *Scale bars* = 1 cm.

to extend, are one of the points of infection of *S. lophii* (Freeman et al. 2011) which may produce changes in skin colouration.

Although human infections with microsporidians from several genera are known to occur in immunocompromised human patients, there are no records of infection with *Spraguea* spp. (Leiro et al. 2012). However, some authors suggest a possible fish-human transmission route for infections by other species of microsporidia (Cali et al. 2005). Since there is evidence that suggest an increase of parasites, disease transmission and possibly virulence, associated with global warming (Marcogliese 2008); there is a need for better understanding of microsporidian ecology in the marine environment, including *Spraguea* spp. and others infecting commercial fish species that enter the human food chain.

CHAPTER 2.5

SUMMARY OF RESULTS

CHAPTER 2.1. SENSORY CONSTRAINTS IN TEMPORAL SEGREGATION IN TWO SPECIES OF ANGLERFISH (*Lophius budegassa* AND *L. piscatorius*)

Activity rhythms

A total of 3,810 individuals of *Lophius budegassa* and 2,170 individuals of *L. piscatorius* were collected from a temporally scheduled trawling survey. Both species showed different temporal patterns in their catchability in relation to the measured light intensity cycle (Fig. 1 of Chapter 2.1). The outputs of a 24 h waveform analysis on the time series of surface density estimates and PFR (i.e. light intensity) data at depths of 100 to 110 m are shown in Fig. 2 of Chapter 2.1.

Lophius budegassa chiefly presented a nocturnal catch pattern. A significant increase in catches occurred over a temporal amplitude that started between 20:00 and 22:00 h and ended between 04:00 and 06:00 h. A decrease in catches occurred for PFR values above 0.93 $\mu E\ m^{-2}\ s^{-1}$. *Lophius piscatorius* showed a significant increase in catches at daytime. The resulting temporal amplitude of the catch peak spanned from 12:00–14:00 to 22:00–00:00 h. Increased catches were observed for PFR values above 1.25 $\mu E\ m^{-2}\ s^{-1}$. Catches decreased within a few hours after the onset of darkness, and the onset of this decrement occurred for PFR values below 0.87 $\mu E\ m^{-2}\ s^{-1}$.

Both species showed a significant ($p < 0.001$) diel periodicity very close to a 24 h cycle (Fig. 3 of Chapter 2.1). *Lophius budegassa* presented a significant increase in catches that repeated itself every 1,430 min, which is equal to 23 h and 50 min. A similar temporal pattern was detected for *L. piscatorius* with a period of 1,455 min, which is equal to 24 h and 15 min, in catch fluctuations.

Morphometric relationships in sensory organs

Otolith weight increased progressively during fish growth, ranging from 2.7 to 102.6 mg in *L. piscatorius* and from 0.6 to107.3 mg in *L. budegassa* (Fig. 4 of Chapter 2.1). For equivalent fish lengths, *L. budegassa* had 50% heavier otoliths than *L. piscatorius*, and with increasing length, the difference between the species was observed to be fairly constant. A significant difference between the curves depicting this relationship for these 2 species was observed ($p < 0.05$).

During fish growth, progressive eye and lens enlargement was observed in both anglerfish species (Fig. 5 of Chapter 2.1). However, a shift in their trends was observed during growth. In fact, the eye diameters of small *L. budegassa* (TL < 50 cm) were larger than those of *L. piscatorius* of comparable size, with a mean value (± SD) of 20.7 ± 3.7 mm (n = 82) in the former group and 17.5 ± 3.6 mm (n = 37) in the latter. In larger individuals (TL ≥ 50 cm), the mean eye diameters (± SD) of *L. budegassa* measured 26.8 ± 2.8 mm (n = 24), and those of *L. piscatorius* measured 29.8 ± 2.2 mm (n = 22). When the eye diameters of small individuals of both species were plotted separately from the diameters of large individuals, significant differences were observed in both cases ($p < 0.05$).

A similar trend was observed for lens diameter, although the shift was recorded at 58 cm. The mean lens diameter (± SD) of small individuals of *L. budegassa* and *L. piscatorius* measured 7.9 ± 1.5 mm (n = 59) and 6.4 ± 1.4 mm (n = 37), respectively, while large individuals had lenses of 9.9 ± 1.0 mm (n = 21) and 10.7 ± 0.8 mm (n = 19), respectively. Although significant differences ($p < 0.05$) between the slopes of lens diameter were observed over the total size range of the individuals examined, when the 2 size groups were plotted separately, no significant differences were found ($p > 0.05$).

The anglerfishes showed duplex retinas where rods and single and double cones were visible. Single cones were the predominant cone type, and their density and the size of their outer segments were employed to calculate the spatial frequency and the optical sensitivity, respectively. Although a more accurate method for calculating sensitivity would be to use rod size, the use of the single cones is partially due to the fact that the rod diameter was difficult to measure accurately in the retina of the analysed individuals. In addition, since a large number of single cones was observed in these species, the role of these photoreceptors in improving sensitivity is highly probable. The measurements taken from the anglerfish eyes are summarised in Table 2 of Chapter 2.1. Using obtained data and the sensitivity equations, the eye of the anglerfish showed an in- crease in sensitivity with growth both to white and monochromatic light. While a small increase in sensitivity was observed for *L. piscatorius*, in *L. budegassa* the sensitivity values of the large individual were triple those of the small individual for both types of light (Table 2 of Chapter 2.1). These increases seem to be attributable to the considerable enlargement of the photoreceptor diameter during growth. The retinal receptor spacing and the relative spatial frequency were similar in the 2 species (Table 2 of Chapter 2.1).

Because interspecific differences of a sensory organ cannot be interpreted in isolation from other sensory systems, otolith weight and eye diameter were compared, and the allometric coefficient values indicated that in *L. budegassa*, otolith weight increased more than eye size, while an opposite trend was observed for *L. piscatorius* (Fig. 6 of Chapter 2.1). A comparison of the slopes showed significant ($p < 0.05$) differences between the species.

CHAPTER 2.2. REPRODUCTIVE STRATEGY

SUBCHAPTER 1. REPRODUCTIVE BIOLOGY OF BLACK ANGLERFISH (*Lophius budegassa*) IN THE NORTHWESTERN MEDITERRANEAN SEA

Gonadal morphology

Ovarian structure consists of a flattened band with 2 distinctive lobes that are folded up and connected to each other at their posterior end. The lobes form a single organ attached to the abdominal cavity by a black mesenteric tissue called the mesovarium. One side of the ovarian wall is made of an ovigerous membrane and connective tissue. The nonovigerous side is made of epithelial cells. A single layer of oocyte clusters projects from the ovigerous membrane to the lumen (Fig. 2A of Chapter 2.2, Subchapter 1). Inside of each gonad, the clusters can be in different development stages. Only the oocytes situated closest to the tip of the clusters have progressed through all maturity stages, and the other oocytes are only oogonia or in the primary growth stage (Fig. 2B of Chapter 2.2, Subchapter 1).

A gelatinous material is secreted into the lumen during the late phases of gonad maturation, producing the mucus matrix characteristic of the reproduction of *Lophius* species (Fig. 2C of Chapter 2.2, Subchapter 1). Hydration of the oocytes occurs just before spawning, and post-ovulatory follicles (Fig. 2D of Chapter 2.2, Subchapter 1) are found during the regression phase of the reproductive cycle. Ripe eggs, which are usually situated on the tip of the oocyte cluster, rupture the follicles and are pressed into the layer of mucus. Every chamber examined contained at least 1 egg in the gelatinous matrix (Fig. 2E of Chapter 2.2, Subchapter 1), although the presence of 2 (Fig. 2F of Chapter 2.2, Subchapter 1) or 3 eggs (Fig. 2G of Chapter 2.2, Subchapter 1) floating in separate chambers also was noted (Fig. 2H of Chapter 2.2, Subchapter 1).

The testes are a pair of elongated and tubular structures located in the dorsal portion of the abdominal cavity, and they are bean-shaped in transverse section. The organization of the testes is lobular: the connective tissue extends from the testicular capsule to form lobules that have their blind ends on the surface of the gonads, converging ventrally towards the sperm duct (Fig. 2I of Chapter 2.2, Subchapter 1). These lobules are fused to the posterior end of each testicular lobe to form a common sperm duct that leads to a genital pore (Fig. 2J of Chapter 2.2, Subchapter 1). Spermatogenesis takes place in a capsule-like sac called a cyst, but it is not completed within the cyst. Each cyst contains spermatogonia or developing spermatocytes (Fig. 2K of Chapter

2.2, Subchapter 1). Before the end of the spermatogenesis, the cyst breaks up and spermatids are released into the lumina of the lobules, where spermatogenesis is then completed and spermatids transform into spermatozoa (Fig. 2L of Chapter 2.2, Subchapter 1). The cysts appear to be arranged in order of maturation, with a gradient of germ cells of increasing maturation from the cortex to the sperm duct. The morphology of the spermatozoa head seems to be elongated.

Spawning season and size at first maturity

Monthly distribution of macroscopic classification of the maturity phases (Fig. 3A and B of Chapter 2.2, Subchapter 1) revealed that the period of maximum occurrence of females in the spawning capable phase (III) was from November to January. The presence of females in the actively spawning phase (IV) was observed from November to March, with a maximum peak in January. Females in the immature, regressing, and developing/regenerating phases (I, V, and II, respectively) were found throughout the year, with the highest percentage of immature individuals seen in May. A slight increase in phase III females was observed in August, and that increase would likely result in spawning activity in September, indicating the possibility of a secondary breeding season. Males in all maturity phases were observed throughout the year, with 2 maxima of mature males occurring in December and July.

For mature males and females, GSI and HSI indices were calculated. In males, GSI was fairly constant throughout the year, with a maximum index value of 1.06 reached in January (Fig. 3C of Chapter 2.2, Subchapter 1). The mean GSI for females was highest from December to March, with a peak of maximum activity in January (4.94) and February (2.43) (Fig. 3D of Chapter 2.2, Subchapter 1). The mean HSI for females and males followed the same pattern. The highest value for males was found in September (2.50), and the lowest value in February (1.65) (Fig. 3C of Chapter 2.2, Subchapter 1). In females, HSI values ranged from 3.19 in January to 1.86 in March (Fig. 3D of Chapter 2.2, Subchapter 1). The highest HSI values were found just at the beginning of the main spawning season. GSI and HSI results, together with observations of maturity phases throughout the year, indicate that there is one main spawning season from November to March.

Comparison of L_{50} curves showed a clear difference between males and females. The size at 50% sexual maturity was 33.4 cm TL for males (Fig. 4A of Chapter 2.2, Subchapter 1) and 48.2 cm TL for females (Fig. 4B of Chapter 2.2, Subchapter 1).

Reproductive strategy and fecundity

The size-frequency distributions of oocyte diameters in each of the 5 maturity phases indicate that oocytes in different stages of development were found in each maturity phase (Table 2; Fig. 5 of Chapter 2.2, Subchapter 1). During phase I, only oocytes in the primary growth stage (chromatin nucleolar and perinucleolar) with a narrow range of diameters were present (Fig. 5A of Chapter 2.2, Subchapter 1). In phase II, cortical alveolar vesicles were found in the cytoplasm together with oocytes in the stage of primary growth with diameters that had increased notably (Fig. 5B of Chapter 2.2, Subchapter 1). In phase III, yolk granule stages were present along with the previous 2 types of oocytes. The oocytes increased in size as the yolk accumulated, and a wider oocyte diameter range distribution was observed during this phase (Fig. 5C of Chapter 2.2, Subchapter 1). In phase IV, oocytes were observed in different stages (primary growth, vitellogenesis, migratory nucleus, and hydration). Two populations of oocytes were recognized in phase IV: a population of larger oocytes (defined as a clutch) and a population of smaller oocytes from which the clutch was recruited (Fig. 5D of Chapter 2.2, Subchapter 1). In phase V, oocytes in the primary growth stage were found along with postovulatory follicles and atretic oocytes (Fig. 5E of Chapter 2.2, Subchapter 1).

The presence of oocytes in different developmental stages within the same cluster and the frequency distribution of oocyte diameter along all maturity phases indicate that oocyte development is group-synchronous. The existence of a gap that separates the yolked oocyte stock, the ones to be spawned during the current breeding season, from the unyolked oocytes, the ones to be spawned in the coming breeding season, together with the increase of the mean diameter of the advanced vitellogenic oocytes, indicates that annual fecundity is determinate.

Significant differences in oocyte densities among ovary sections were not observed (ANOVA, $F_{(2,42)} = 0.002$, $p = 0.998$). Batch fecundity ranged between 87,569 and 398,986 oocytes, and mean BF was 218,020 ± 90,018. Relative batch fecundity was estimated at 102 ± 20 oocytes/g of female (GW), and mean potential fecundity (oocytes/kg) was 78,929 ± 13,648 oocytes/kg of mature female. Batch fecundity tended to increase linearly with TL (linear regression, coefficient of determination [r^2] = 0.89, $F_{1,13} = 106.57$, $p < 0.001$), TW (linear regression, $r^2 = 0.82$, $F_{1,13} = 60.79$, $p < 0.001$), and GW (linear regression, $r^2 = 0.82$, $F_{1,13} = 59.31$,

$p < 0.001$), indicating that fecundity is dependent on size and body weight (Fig. 6 of Chapter 2.2, Subchapter 1). No significant correlation was found between RBF and TL, indicating that RBF is not size dependent (linear regression, $r^2 = 0.16$, $F_{1,13} = 2.50$, $p = 0.138$).

SUBCHAPTER 2. REPRODUCTIVE STRATEGY OF WHITE ANGLERFISH (*Lophius piscatorius*) IN MEDITERRANEAN WATERS: IMPLICATIONS FOR MANAGEMENT

Gonad morphology

The gonad of female white anglerfish has 2 ribbon-like ovarian lobes connected to each other at their posterior end. One side of the "ribbon" consists of an ovigerous membrane from which a single layer of oocyte clusters, which contain oocytes at different developmental stages, projects into the lumen. The other side is nonovigerous and secretes a gelatinous material during maturation that fills the ovarian lumen, where mature oocytes develop (Fig. 2 of Chapter 2.2, Subchapter 2). During maturation, the gonad increases in size until it fills the abdominal cavity (Fig. 3 of Chapter 2.2, Subchapter 2). Testes are a pair of elongated organs with a bean shape in transverse section. Spermatogenesis takes place in a capsule-like sac called a cyst, but it is completed in the lumina of the lobules. The cysts appear to be arranged with a gradient of germ cells of increasing maturation from the cortex to the sperm duct (Fig. 4 of Chapter 2.2, Subchapter 2).

Spawning season

The monthly distribution of maturity phases (Fig. 5 of Chapter 2.2, Subchapter 2) revealed a peak in reproduction during spring, when a major portion of the spawning females and the highest value of GSI (0.77) were found. Spawning capable females (phase III) were caught primarily between April and June, and females in the actively spawning phase (IV) were observed in November, December, and March—the latter month having the maximum occurrence (11%). Females in immature, regressing, and developing or regenerating phases (I, V, and II, respectively) were found year-round, although the highest percentage of immature individuals (49%) was observed in January. The GSI values followed the same pattern shown in these maturity phases: highest during spring, decreasing during summer and autumn, and increasing again during winter. Males in all maturity phases were observed throughout the year, but with a maximum percentage of mature males (66%) in February and March. Immature males were found primarily in July (69%). The mean GSI for females increased as their ovaries developed and peaked in phase IV. For males, the mean GSI increased with testicular development and reached a maximum in phase IV (Table 1 of Chapter 2.2, Subchapter 2). The mean HSI for females and males increased during the summer and autumn months and decreased during winter and spring. On the basis of these observations, a main spawning season was found from February through June and a secondary one occurred in November and December.

Size at sexual maturity

The maturity ogive for males indicates that the length at which 50% of them reached sexual maturity (L_{50}) was 48.4 cm TL (Fig. 6A of Chapter 2.2, Subchapter 2). Maturity in males occurred at about 37% of their maximum observed TL. The smallest mature male found was 32.5 cm TL, and the largest immature male was 50 cm TL. The maturity ogive for females indicates that L_{50} was 59.9 cm TL (Fig. 6B of Chapter 2.2, Subchapter 2). Female maturity occurs at about 30% of their maximum observed TL. Like the smallest male, the smallest mature female was 32.5 cm TL. The largest immature female measured 56 cm TL. The maturity ogive for the sexes combined indicates an L_{50} of 51.3 cm TL. The lengths at which 25% and 75% of fish attained maturity were 43.5 and 53.4 cm TL for males, 48.6 and 71.1 cm TL for females, and 44.7 and 58 cm TL for the sexes combined.

Oocyte development and fecundity

Oocytes in different developmental stages were found in each maturity phase. They were organized in clusters where a gradient in the size of the oocyte was observed. A group of oocytes differentiated from others as the ovaries developed, indicating a group-synchronous oocyte development with determinate fecundity (Fig. 7 of Chapter 2.2, Subchapter 2). Ovaries at each maturity phase contained primary oogonia- and perinucleolar-stage oocytes. Chromatin nucleolar were difficult to find and were present only in immature phase. Females at the cortical alveolar stage were not found in our samples. Vitellogenic and hydrated oocytes were located in females capable of spawning. Oocyte diameters at each stage of oocyte development are shown in Table 2 of Chapter 2.2, Subchapter 2.

Batch fecundity ranged from 661,647 to 885,214 oocytes from 2 females that measured 76 and 105 cm TL; 6,331 and 16,178 g TW, and 5,182 and 13,330 g GW, respectively. Relative batch fecundity ranged from 66 to 128 oocytes/g GW (average of 97 ± 43 oocytes/g GW). Potential fecundity values moved from 54,717 to 104,506 oocytes/kg TW with a mean of 79,612 ± 35,206 oocytes/kg TW.

SUBCHAPTER 3. THE CHORION ULTRASTRUCTURE OF OVA OF *Lophius* SPP.

The unfertilized eggs of both species of *Lophius* are transparent and spherical in shape (Fig. 1A of Chapter 2.2, Subchapter 3). Ova of both species have a single oil globule, yellow-ochre in colour and located slightly off-centre position (Fig. 1B of Chapter 2.2, Subchapter 3). Both ova and oil globules of *L. piscatorius* are significantly larger than *L. budegassa* (Mann – Whitney *U*-test, $p < 0.05$). The chorion of *L. budegassa* and *L. piscatorius* are smooth and transparent (Fig. 2A, B of Chapter 2.2, Subchapter 3), with oval pores distributed evenly over the surface. Both species have similar pore patterns although *L. piscatorius* had more pores per unit area (Fig. 2C, D of Chapter 2.2, Subchapter 3). The pore diameter and minimum distance between pores are significantly less in *L. budegassa* (Mann – Whitney *U*-test, $p < 0.05$). The granular matter observed on the surface of the chorion might be remains of its mucous layer dissolved by the fixation methods used in SEM. In *L. piscatorius*, the chorion consists of a thin outer layer and a thicker lamellated inner layer (1.15 ± 0.08 μm) with five lamellae (Fig. 3A of Chapter 2.2, Subchapter 3). The micropyle observed on an ovum prepared for cryo-SEM was funnel-shaped with an aperture diameter of 74.87 ± 0.68 μm that leads to the micropyle canal which traverses the entire chorion layer (Fig. 3B of Chapter 2.2, Subchapter 3).

CHAPTER 2.3. POPULATION DYNAMICS AND SUSTAINABILITY INDICATORS TO ASSESS *Lophius* FISHERIES IN THE NORTHWESTERN MEDITERRANEAN SEA

Length-weigh analysis

Lophius budegassa females (n = 1,923) varied in size from 11 to 72.5 cm TL, and between 13 to 5,592 g and from 11 to 4,376 g in total and gutted weight respectively. Males (n = 1,977) varied from 10.5 to 62 cm TL; and between 13 to 3,320 g TW and from 11 to 2,922 g GW. Indeterminate specimens (n = 317) ranged in size from 4.5 to 38.5 cm TL, and from 1 to 700 g TW and 1 to 548 g GW. Significant differences were found in the mean total length, the mean total and gutted weight between males (30 ± 9 cm TL; 490 ± 363 g TW; 406 ± 304 g GW) and females (34 ± 13 cm TL; 887 ± 970 g TW; 719 ± 781 g GW) (*t*-test, total length: $t = 7.55$, $p < 0.05$; total weight: $t = 9.41$, $p < 0.05$; gutted weight: $t = 8.90$, $p < 0.05$).

Females (n = 280) of *L. piscatorius* varied from 16 to 107 cm TL, 52 to 14,600 g TW and 42 to 12,688 GW. Males (n = 249) size ranged from 14.5 to 89 cm TL, and weight from 32 to 7,812 g and from 26 to 6,728 g; total and gutted respectively. Indeterminate individuals (n = 27) varied in size from 14 to 41 cm TL, in total weight from 30 to 836 g and in gutted weight from 24 to 700 g. Significant differences were not detected in the mean total length, the mean total weight and the mean gutted weight between males (41 ± 13 cm TL; 1,330 ± 1,204 g TW; 1,052 ± 979 g GW) and females (43 ± 17 cm TL; 1,797 ± 2,209 g TW; 1,461 ± 1,832 g GW) (*t*-test, total length: $t = 0.82$, $p = 0.41$; total weight: $t = 1.37$, $p = 0.17$; gutted weight: $t = 1.26$, $p = 0.21$).

Comparing both *Lophius* species, not significant differences were noted between males and females for total length (*t*-test; $t = -13.66$, $t = - 9.32$, $p < 0.05$, respectively), total weight (*t*-test; $t = -14.08$, $t = - 9.33$, $p < 0.05$, respectively) and gutted weight (*t*-test; $t = -13.87$, $t = - 9.30$, $p < 0.05$, respectively).

The results provided by ANCOVA indicated that there were significant differences between males and females for *L. budegassa* in both length-total weight ($F_{1,\,3898} = 26.80$, $p < 0.001$) and length-gutted weight ($F_{1,\,3898} = 18.92$, $p < 0.001$) relationship, as well as for *L. piscatorius* in length-total weight ($F_{1,\,527} = 4.32$, $p = 0.038$) and length-gutted weight ($F_{1,\,527} = 4.14$, $p = 0.042$) (Table 1, Fig. 2 of Chapter 2.3). Comparing length-total weight and length-gutted weight relationships for males of both *Lophius* species not significant differences were found between them ($F_{1,\,2224} = 3.02$, $p = 0.083$; $F_{1,\,2224} = 0.14$, $p = 0.709$, respectively) but significant differences were attained comparing the same relationships between females of both species ($F_{1,\,2201} = 13.21$, $p < 0.001$; $F_{1,\,2201} = 24.44$, $p < 0.001$, respectively) (Table 1, Fig. 2 of Chapter 2.3).

Growth and natural mortality

The growth parameters and the growth performance values obtained for both Mediterranean lophiids in our study together with the values estimated by other authors in the Mediterranean Sea and Atlantic waters are presented in Table 2 of Chapter 2.3. We identified eight modal classes for *L. budegassa* corresponding to ages from 0 to 6 years and the length frequency distribution show three recruitment modes during the first year of life. The mean size at the first age was 18.8

cm and the older (age 6) grew on average 9.20 cm. For *L. piscatorius* we recognized nine modes that belong to ages 0 to 8 years and two of these recruitment modes were observed during the first year of life. The maximum growth until age 1 was 19.4 cm and at the older age 8 grew on average 9.22 cm (Table 3 of Chapter 2.3).

The natural mortality rates estimated for *L. budegassa* and *L. piscatorius* using the growth parameters derived from the length-frequency analysis (both Pauly's estimators) revealed lower natural mortalities than the Hoening's estimator based on t_{max}. For *L. budegassa* the following natural mortality values were obtained: Pauly's M = 0.19 year^{-1}, updated Pauly's M = 0.16 year^{-1} and updated Hoenig's M = 0.39 year^{-1}. For *L. piscatorius* the mortality values were: Pauly's M = 0.20 year^{-1}, updated Pauly's M = 0.16 year^{-1} and updated Hoenig's M = 0.42 year^{-1}.

Population structure

Sex ratio (M:F) was found to be 1:0.97 for *L. budegassa* (1,977 males, 1,923 females) and 1.12:1 for *L. piscatorius* (249 males, 280 females). The M:F proportion in *L. budegassa* differ significantly from the 1:1 ($\chi^2 = 381.502, p < 0.05$) but the sex ratio of *L. piscatorius* does not differ significantly from 1:1 ($\chi^2 = 19.490, p = 0.301$). In both species, the sex ratio varies with length and females reached greater size than males (Fig. 3 of Chapter 2.3). In individuals of *L. budegassa* with small sizes (10 to 35 cm) no differences between the number of males and females were observed (χ^2, $p \geq 0.05$). The proportion of males increased up to 60 % between 35 and 45 cm, they decreased down to 30 % in the length interval 45-50 cm and disappeared at 60-65 cm where females dominated (100%) at sizes > 70 cm. Significant differences were noted in the proportion of males and females in larger sizes of 35 cm (χ^2, $p < 0.05$). The presence of *L. piscatorius* males was a 100% in 10-15 cm class size with significant differences on the sex ratio (χ^2, $p < 0.05$). The proportion between males and females remained between 40% and 50% from 15 to 55 cm; increased up to 60% in the size class interval 55-60 cm and dropped down to 30% at 75-80 cm; but not significant differences were found in the sex ratio (χ^2, $p \geq 0.05$). At lengths greater than 90 cm, 100% were females; and significant differences were observed again in the M:F proportion.

Sustainability indicators

The percentage of mature specimens in the catch was 5.0% for *L. budegassa* and 20.4% for *L. piscatorius*. The optimum length obtained for *L. budegassa* was 42.0 cm and the size range of ± 10% L_{opt} went from 37.8 to 46.3 cm TL, representing the 4.8% of the catch. For *L. piscatorius* L_{opt}

was found to be 55.5 cm and the ± 10% L_{opt} size range varied from 49.9 to 61.0 cm TL, which the 10.3% of the fish captured, was within that range (Fig. 4 A, B of Chapter 2.3). The portion of mega-spawners present in the catch corresponded to 2.2% for *L. budegassa* and 12.9% for *L. piscatorius*. Applying the results obtained from the three indicators to time series of length-frequency data for both Mediterranean lophiids we noted for *L. budegassa* and *L. piscatorius* very low percentages of mature and optimum size specimens compared with the target of 100% together with a declining tendency of mega-spawners since 2008, which are always below 20% (Fig. 4 C, D of Chapter 2.3).

CHAPTER 2.4. OBSERVATIONS ON THE OCCURRENCE OF *Spraguea lophii* IN MEDITERRANEAN LOPHIIDS

Of the 292 *L. budegassa* analysed, 202 (69.2%) were infected by the parasite *Spraguea lophii*; whilst all specimens of *L. piscatorius* (n = 58) presented parasitism. Infection was always observed in the vagus nerve situated near the kidneys. In some cases microsporidian xenomas were also present in the spinal cord, the medulla oblongata, and in the glossopharyngeal and trigeminal nerves (Fig. 1 of Chapter 2.4). Non-significant differences were noted between males and females of *L. budegassa* ($\chi^2 = 0.683$; $p = 0.409$) (Fig. 2A of Chapter 2.4). However a positive relationship was observed between host size and prevalence ($\chi^2 = 6.134$; $p = 0.013$) increasing the presence of the parasite in individuals larger than 30 cm (Fig. 2B of Chapter 2.4).

In this study, we also found two specimens of *L. budegassa* with morphological anomalies and heavily infected with the microsporidian parasite *S. lophii*. A blind specimen of 23.3 cm in length was apparently lacking an eye on gross examination (Fig. 3A, B of Chapter 2.4). However, a rudimentary and deformed sclera with degenerate retinal tissue under the dermis and reduced optic nerve was found (Fig. 3C of Chapter 2.4). Body colouration was dark-brown similar to other *Lophius* spp. This specimen was infected with xenomas of *S. lophii* in the vagus nerve near the kidneys as well as the spinal cord and medulla oblongata region of the hind brain. Xenomas were also observed in the trigeminal nerve. The other atypical specimen of *L. budegassa* (Fig. 4 of Chapter 2.4) was 33.6 cm in length, bright orange in colour and without the ocular anomalies in the previous specimen.

CHAPTER 3

DISCUSSION

Biological and ecological knowledge of species are fundamental for stock assessments which basic objectives are to determine the status of fish populations and provide the information necessary to make regulations that prevent overfishing and attain optimum yield from the fisheries. Stock assessments are based on models of population dynamics that require three primary categories of data: catch, abundance, and population parameters (NMFS 2001, Kilduff et al. 2009). Nevertheless, it is also necessary knowledge on other biological and ecological aspects to enable a sustainable fisheries and management of the resource (Morgan 2008, Cope and Punt 2009).

Behavioural rhythms have been introduced in the stock assessment models of some commercially exploited species (Naylor 2005) because animals may be present or not in a certain area of sampling depending on their activity cycle (Aguzzi and Sardà 2008). In the Mediterranean Sea, *Lophius* species coexist on the continental shelf being *Lophius budegassa* more abundant at night and *Lophius piscatorius* more abundant during the day. Although preyed species are known to shift their activity patterns to avoid predation (Fenn and Macdonald 1995, Fraser et al. 2004), it is also common to observe a similar behaviour among predators that compete for the same prey (Schoener 1986, Soria-Barreto and Rodiles-Hernández 2008). While this competition is reduced for species of mixed predatory and scavenging activity, pressure is likely to be stronger for more specialised piscivores such as anglerfish (Crozier 1985, Preciado et al. 2006). Since both *L. piscatorius* and *L. budegassa* show similar food preferences and compete for the same available resources (Laurenson and Priede 2005, Negzaoui-Garali et al. 2008), their temporal segregation seems to occur in order to avoid interference competition.

The observed differences in the timing of maximum activity of both *Lophius* species were also consistent with differences in their otolith weight and eye size. The inner ear in species that live in poor light environments, such as sea slope-dwelling fishes (Paxton 2000, Lombarte and Cruz 2007), nocturnal species (Smale et al. 1995), and cave-adapted species (Schulz-Mirbach et al. 2008), are characterised by relatively large otolith sagittae. The heavier otoliths found in *L. budegassa* confirm this as a preferentially nocturnal species compared to *L. piscatorius*. On the other hand, the visual systems of many organisms have evolved to see well in dim light conditions employing different optical strategies to enhance the of an image, both in terms of spatial resolution and sensitivity which are achieved with bigger eyes (Warrant 1999). Although there are many exceptions, epipelagic fishes with nocturnal activity or species inhabiting mesopelagic waters tend to have bigger eyes than diurnal shallow water species (Marshall 1971, Warrant and Locket 2004, Pulcini et al. 2008, Antonucci et al. 2009). In this context, *L. piscatorius*, the species exhibiting a preference for diurnal activity, had a significant smaller eye and lens than *L. budegassa*, the species captured mainly at night. The preference for diurnal activity of *L. piscatorius* was clearly reflected in the retinal anatomy of this species, especially in the cone diameter that was smaller than that of *L. budegassa*.

The interspecific comparison of the eye size of *L. piscatorius* and *L. budegassa* was reversed when *L. piscatorius* reached 50 to 55 cm TL, and above this threshold, *L. piscatorius* showed significantly larger eyes than *L. budegassa*. This trend reversal probably reflects important changes in the life history of *L. piscatorius* since many authors agree that this species spawns in deep water (>1000 m) (Hislop et al. 2001). In addition, changes in the feeding behaviour of *L. piscatorius* have also been observed at this size (Laurenson and Priede 2005, López et al. 2016). The change in the visual scenery with depth, from being extended in the epipelagic zone to semi extended in the mesopelagic zone and even to point source light (bioluminescence) in the bathypelagic zone, could be one of the driving forces in the evolution of larger eyes in large *L. piscatorius*. Although interspecific temporal segregation is one of the mechanisms employed by different fish species to allow their coexistence, there is still little information on how it occurs and its adaptive value. However, sensory constrains appear to be an important feature enhancing partitioning in time by enabling closely related species such as *L. piscatorius* and *L. budegassa* to inhabit the same area at different times of the day leading to different diurnal or a nocturnal behaviour. Knowledge of diel changes in behaviour and habitat use are significant in obtaining the correct information for stock assessments for both *Lophius* species because their activity rhythms influences their susceptibility to overfishing.

An understanding of the reproductive biology of the studied species is also an important aspect and provide sound information for stock assessment since reproduction largely determines productivity and therefore their population's resilience to exploitation (Morgan 2008). According to that, fishing activity during spawning seasons may affect population parameters, specifically composition of the size distribution, mortality rate, sexual structure of the population, size at maturity, and changes in the spawning season. These parameters, in turn, can increase the risk of over-exploitation of a stock. Fishing during spawning periods may also result in targeting a specific size class of the population and thus increasing the chance of catching the older (and larger) age classes and making the stock vulnerable to reproductive collapse (van Overzee and Rijnsdorp 2015). Because spawning is generally limited to specific areas and times (Cushing 1990), the conservation of resources can be enhanced by limiting fishing activity in a spatiotemporal frame. Furthermore, fishing pressure has been documented to have reduced initial size at maturity which negatively affects the fecundity; an issue that is a concern particularly for late-maturing species (Stewart et al. 2010). If size of capture is below the size at first maturity, there is a genuine risk of recruitment overfishing. In this context, the reproductive strategy of *L. budegassa* and *L. piscatorius* is one of discontinuous oogenesis with synchronous development of vitellogenic oocytes and, therefore, both species are considered total spawners. The oocytes ovulate at once, and the eggs are released in either a unique event or over a short period of time, as part of a single episode during the spawning season (Murua and Saborido-Rey 2003, Pavlov et al. 2009). Because of their particular reproductive behaviour, which includes a high parental investment in the offspring, both Mediterranean anglerfishes are likely to spawn once a year, and the population dynamics of these species are expected to be highly sensitive to external biological and ecosystem factors (ICES 2012).

Female anglerfish spawn their eggs in a mucoid veil that floats near the surface (Fulton 1898, Leslie and Grant 1990, Armstrong et al. 1992, Yoneda et al. 2001). The veil consists of individual chambers that contain 1–3 eggs and has an opening that provides water circulation. It has been proposed that the advantages of releasing eggs in these veils facilitate their dispersal; the egg veil floats near the surface and is subject to the actions of wind, currents, and waves. The veil also serves as protection for eggs against predation because of the presence of obnoxious or toxic substances in the veils (Armstrong et al. 1992). Moreover, the veil may help with the fertilization of eggs. When males are present and the egg ribbon is laid, the ribbon keeps the eggs together and prevents their dispersion through the water. The males then eject milt near the veil to guarantee fertilization of all the eggs (Dahlgren 1928). Armstrong et al. (1992) suggested that sperm reach oocyte chambers through the pores that connect the chambers when the ribbon is extruded from the female and starts to absorb water. Spermatogenesis of *L. budegassa* and *L. piscatorius* is known to be semicystic which starts inside the cysts that contain germinal cells in different stages of development from spermatogonia to spermatids, but it is not completed within the cyst. During spermatogenesis, the cyst breaks and spermatids are released from the cyst into the lumen of the lobule, where they become spermatozoa.

Muñoz et al. (2002) reported that semicystic spermatogenesis may be related to the secretion of abundant, thick seminal fluid, the function of which is to keep the spermatozoa together to enable fertilization of the entire egg mass.

In the northwestern Mediterranean Sea, spawning season for *L. budegassa* occurs between late autumn and early spring; November and March with a secondary spawning happening in August and September. Spawning activity for its congener *L. piscatorius* seems to take place during mid-winter and late spring, from February through June, although a secondary breeding period has been observed in November and December. Although a little overlap exists between spawning seasons of both *Lophius* species in Mediterranean waters, the main period is markedly different which lessens competition among these species.

Differences in relation to size at sexual maturity have been also noted for *L. budegassa* and *L. piscatorius* during this Thesis. Females of *L. budegassa* mature sexually at larger sizes (48.2 cm TL) than those recorded for males (33.4 cm TL); like *L. piscatorius* where females mature at 59.9 cm TL and males attain first maturity at 48.4 cm TL. Most deep-sea fish species, such as *L. piscatorius*, reach sexual maturity at sizes larger than those of species that inhabit the continental shelf (*L. budegassa*) where males mature at smaller sizes than females (Rotllant et al. 2002, Pajuelo et al. 2008). These variations in size at sexual maturity could be related to a trade-off between life history traits together with environmental and anthropogenic factors (e.g., temperature, food availability, or fishing pressure) (Stearns and Koella 1986, Charnov 2008).

Fecundity estimation is usually a difficult biological parameter to obtain, but it is critical for accurate stock assessments (Trippel et al. 1997). In the particular case of species of *Lophius*, studies on fecundity are scarce because of the difficulty of acquisition of suitably mature individuals in the maturity phases of spawning capable or actively spawning; and the use of a proper method for fecundity estimation, which is especially complicated because of the gonad morphology of these species. During reproduction, a gelatinous material is secreted into the lumen, and enumeration and measurement of eggs embedded in this mucus matrix is extremely difficult. Findings showed that in the northwestern Mediterranean Sea *L. budegassa* has determinate fecundity with values ranging from 87,569 to 398,986 oocytes, and mean potential fecundity was estimated at 78,929 oocytes per kilogram of mature female. *L. piscatorius* inhabiting the Mediterranean Sea has also determinate fecundity with values between 661,647 to 885,214 oocytes; levels that are high in comparison with other deep-sea species that inhabit the same depth strata but that are similar to the mean potential fecundity of its Mediterranean congeneric. Fecundity values vary among populations as a result of adaptations to local environmental conditions, and they are related to abiotic factors, such as temperature and salinity (Nissling and Dahlman 2010, Thorsen et al. 2010), and to biotic factors, such as food supply, population density, allocation of energy to reproduction, and fish size (Treasurer 1981, Merrett 1994, Nash et al. 2000).

The spawning stock biomass of an exploited species is an important variable in fisheries management (Somarakis et al. 2004). Ichthyoplankton based methods are increasingly being used around the world to estimate this biomass and monitor trends in abundance (Gonçalves et al. 2009). These include egg production methods (Gunderson 1993), that are commonly used for obtaining fishery-independent abundance indices for fish species with pelagic eggs. To develop this methodology requires the knowledge of the structure of the egg chorion, which has an important taxonomic character (Ivankov and Kurdyayeva 1973, Olivar 1987, Chen et al. 2007) since their morphology is species-specific (Hagström and Lönning 1968). Although there are major differences in the structure of the chorion from different species of teleosts with respect to thickness, number of lamella, and the presence or absence of pores due to the diversity in habitat and breeding habits of teleosts (Kuchnow and Scott 1977), geographic differences in chorion structure were found between eggs from the same species (Lönning and Solemdal 1972). These variations may be correlated with differences in salinity, temperature, and viscosity of the seawater, necessitating adjustment in the structure of pelagic eggs to secure adequate buoyancy (Lönning 1972), for this reason it is important to characterise eggs of different species from different geographic locations for aid in taxonomic classification from ichthyoplankton samples. In this Thesis, findings showed that ova size, oil globule diameter, pore diameter, minimum distance between pores and pore density are useful characteristics for distinguishing the ova of both *Lophius* species. *L. piscatorius* has the largest ova, which measures 2.72 ± 0.08 mm in diameter, whilst ova diameter of *L. budegassa* was measured in this study as 1.88 ± 0.12 mm, which is the first available data from non-fixed ova. Egg size is important to offspring survival in many organisms where a positive correlation between adult female size and egg size is common (Hendry and Day 2003). This ecological premise appears to be appropriate in the two species studied here, because the maximum size of *L. piscatorius* (200 cm) is double that of *L. budegassa* (100 cm) (Caruso 1986) and their ova are 70%

larger. The ova of these species can be considered large for pelagic eggs, which range from 0.5 to 5.5 mm in diameter (Ahlstrom and Moser 1980). Not surprisingly, larger ova also have larger oil globule diameters (*L. piscatorius*: 0.63 ± 0.02 mm; *L. budegassa*: 0.48 ± 0.03 mm), both for larval buoyancy and as a concentrated energy source (Eldridge et al. 1977, Markle and Frost 1985). In addition, the chorion of *L. piscatorius*, which protects the embryo from the external environment (Stehr and Hawkes 1979, Olivar 1987), has more pores per unit area. In the ovary, the pore canals distributed throughout the chorion contribute to the transportation of nutrients from the follicle cell to the developing oocyte (Nagahama 1983, Groot and Alderdice 1985) and hence the larger eggs of species of *Lophius* could need more pores in order to satisfy this nutritional intake. It is still unknown if the pores remain open after fertilization but, if this occurs, they could expose the embryo to the natural environment or contaminants (Stehr and Hawkes 1979). Results demonstrated that although *L. budegassa* and *L. piscatorius* occupy the same habitat, differences noted in their reproductive strategy and the characteristics of their ova support the hypothesis that both species of *Lophius* have different ecological strategies to reduce competition.

Otherwise, the life history parameters such as growth parameters (asymptotic length and growth rate) and natural mortality are also required to carry out an effective management of their fishery. The growth pattern of the studied *Lophius* species differs in the asymptotic length, being higher in *L. piscatorius*. However, the population structure was similar in both species, where females were larger and weightier than males. This sexual variability in the somatic growth pattern coincide with the onset of sexual maturity (Kozlowski and Teriokhin 1999), with males growing faster than females before reaching sexual maturity and the reverse afterwards (Landa and Piñeiro 2000, Lucio et al. 2000). It may be also associated to ontogenetic migrations with depth as occurs in *Lophius* species where older females move towards deeper waters (Laurenson et al. 2001, Yoneda et al. 2002) exposing to changes in temperature and salinity that might have an impact on growth and other physiological processes (Higgins et al. 2015), such as the velocity of digest process (slower in colder temperatures) that affect longevity (Cailliet et al. 2001, Valenzano et al. 2006), differences in oxygen consumption (Pauly 1994) and/or to differences in the level of surplus energy between reproduction and somatic growth (Rljnsdorp and Ibelings 1989). Furthermore, the rate of compensatory growth might also provide a greater reproductive success (Clarke 1983) to maximize egg producing biomass since larger females tend to produce more and bigger eggs than smaller ones as occurs with females of *L. piscatorius* attaining a higher asymptotic length and expelling out more eggs than *L. budegassa*.

Findings also revealed a faster growth in the populations of both *Lophius* species inhabiting the Mediterranean Sea, where *L. budegassa* attain higher sizes. Nevertheless, studies in other demersal fishes seems to suggest that the Mediterranean populations tend to grow faster than Atlantic ones (Tuset 2000, Pajuelo et al. 2002, Piñeiro and Sainza 2003, Sequeira et al. 2009), likely due to the growth plasticity of the species and the influence of factors that vary geographically, as temperature or food availability (Landa et al. 2013). The modal progression analysis indicated that the mean growth rate during the first year of life for Mediterranean lophiids was double than the rest of age classes being the values for *L. piscatorius* slightly higher than its congener *L. budegassa*. This confirms that both *Lophius* species have a high growth rate during their early life; thus smaller fish grow faster than older ones favouring a clear ontogenetic change of both anglerfishes. Studies indicate that males have a slower growth rate after a certain length or stops, and females appear to have longer lifespan (Yoneda et al. 1997, Richards et al. 2008). This could be related to behavioural differences such sex-specific movement patterns where females move into deep waters with increasing size and maturity (Laurenson et al. 2001, Richards et al. 2008); which affects sexual proportion favouring females at larger sizes.

The mortality rate for *L. piscatorius* adopted for stock assessment following Pauly's estimator (0.2 year^{-1}) is nowadays adequate (ICES 2012), but incorrect for *L. budegassa* (0.15 year^{-1} versus 0.19 year^{-1}). Although these results are reversed when updated Pauly's estimator is used (0.16 year^{-1} for both *Lophius* species) being natural mortality for *L. budegassa* correctly selected, but not the one applied to *L. piscatorius*.

Considering the growth parameters, mortality and size at maturity estimated for *Lophius* species, the stock evaluation allows concluding that these species are overfished in the northwestern Mediterranean Sea. Data on the size structure of anglerfish retained by bottom trawlers indicate that 5.3% of landed *L. budegassa* were > 39.4 cm (the size of 50% maturity for sex combined) and the 20.4% of *L. piscatorius* were > 51.3 cm (L_{50} for sex combined). In addition, individuals of lengths of about 10-13 cm (settlement size) (Hislop et al. 2001) were also captured by the trawl fleet. In both cases more than the 80% of the *Lophius* population were excluded from reproduction since were being caught by the trawling fleet, which will not allow rebuilding and maintaining a healthy spawning stock. The slow growth rates and late maturity of both species confirm that a large part of the population captured by the fishery has not matured, which if unmanaged could lead to recruitment-overfishing (Walmsley et al. 2005). The percentage of fish caught at ± 10% L_{opt} was 4.8% for *L. budegassa* and 10.3% for *L. piscatorius*, being both values far away from the target of the second sustainability indicator "Let then grow" which would be to catch all fish within the optimum length range. On the other hand, the 2.2% of *L. budegassa* and the 12.9% of *L. piscatorius* caught in the catch were mega-spawners. According to this, and considering that values of 30-40% of mega-spawners reflect a healthy population, both *Lophius* species have little resilience against recruitment failures.

Finally, the study of marine fish parasites was also initially developed to give us an approach about the health status of *Lophius* populations, as well as having an added interest in public health (for the possible accidental ingestion of live parasites by eating raw or undercooked fish). The 69.2% of *L. budegassa* analysed were infected by the parasite *Spraguea lophii*; whilst the 100% of the specimens of *L. piscatorius* presented parasitism. Infection was observed in the vagus nerve situated near the kidneys, in the spinal cord, the medulla oblongata, and in the glossopharyngeal and trigeminal nerves. A positive relationship was observed between host size and prevalence increasing the presence of the parasite in individuals larger than 30 cm. However, gender did not appear to be an influential factor. Since one of the transmission pathways of the parasite is by direct ingestion of the spores, feeding habits could explain the increased severity of infection in larger individuals (Gibson and Jones 1993, Cañás et al. 2010). Some studies had demonstrated an increase of the temperature average on the Mediterranean Sea, associated with the accelerating trend in global warming from the early 1990s (Carillo et al. 2012, Skliris et al. 2012). It is hypothesised that increase in water temperatures could significantly impact prevalence and intensity of parasitism through thermal stress leading to reduced immunocompetence and increasing the susceptibility of the host to the parasite (Antonio and Hedrick 1995, Marcogliese 2008).

Although parasite impact on host population level dynamics is unknown, severe infections may be associated with anatomical anomalies such as eye deformities (Bucke et al. 1994). Two specimens of *L. budegassa* with morphological anomalies and infected with microsporidian xenomas of *S. lophii* were presented in this Thesis. A blind specimen, lacking of eye but with a rudimentary and deformed sclera with degenerate retinal tissue under the dermis and reduced optic nerve. The other atypical specimen of *L. budegassa* was bright orange in colour.

The increase of *S. lophii* parasites in the last 18 years together with the anomalies found is indicative of a population that is under stress which individuals may consume lees food, reproduce less successfully, grow slowly and suffer higher mortalities (Pope et al. 2010). Although human infections with microsporidians from several genera are known to occur in immunocompromised human patients, there are no records of infection with *Spraguea* spp. (Leiro et al. 2012). However, some authors suggest a possible fish-human transmission route for infections by other species of microsporidia (Cali et al. 2005). Since there is evidence that suggest an increase of parasites, disease transmission and possibly virulence, associated with global warming (Marcogliese 2008); there is a need for better understanding of microsporidian ecology in the marine environment, including *Spraguea* spp. and others infecting commercial fish species that enter the human food chain.

In conclusion, the findings obtained in this Thesis are relevant and of great importance since demonstrate that although *L. budegassa* and *L. piscatorius* occupy the same habitat in the northwestern Mediterranean Sea they have acquired different ecological and biological strategies in order to lessens competition between them. According to the differences observed, differentiated stock assessments for each *Lophius* species are necessary in order to provide fisheries managers with the correct information to take proper management actions. Considering the health status of both *Lophius* populations and the high exploitation level of their stocks in the study area, management actions are highly recommended to be conducted in the near future.

CHAPTER 4

CONCLUSIONS

Main conclusions derived from this Thesis are presented below:

1. Interspecific competition of sympatric and ecologically equivalent species such as *L. budegassa* and *L. piscatorius* may result in different adaptations to diurnal/nocturnal behaviour sustained by morphological specializations in sensory organs.

2. The main spawning season for females of *Lophius* species in Mediterranean waters is markedly different, occurring during winter for *L. budegassa* and spring for *L. piscatorius*. In addition, a secondary breeding period has been observed for females of both *Lophius* species; during summer for *L. budegassa* and winter for *L. piscatorius*.

3. Males of both *Lophius* species are ready to spawn throughout the year but the percentage of mature males increases when occurs the main spawning season for females.

4. *Lophius piscatorius* reach sexual maturity (L_{50}) at larger sizes that *L. budegassa*; and males of both *Lophius* species reach sexual maturity at small sizes than females.

5. Histological examination of ovaries indicates that *L. budegassa* and *L. piscatorius* are total spawners with group-synchronous oocyte development and determinate fecundity.

6. *Lophius piscatorius* and *L. budegassa* have similar relative fecundity, although the former has larger egg and oil droplet than the later which may indicate a higher investment of energy to offspring for the deeper species.

7. Both *Lophius* species release their eggs in a buoyant gelatinous matrix made of individual chambers containing from 1 to 3 eggs. This matrix serves as protection for eggs, facilitates their dispersal and could help with fertilization.

8. Males of *L. budegassa* and *L. piscatorius* have a semicystic type of spermatogenesis which may enable fertilization of the entire egg mass.

9. The mean growth rate during the first year of life for Mediterranean *Lophius* species was double than the rest of age classes being the values for *L. piscatorius* slightly higher than its congener *L. budegassa*.

10. Somatic growth pattern of *Lophius* species inhabiting the Mediterranean Sea differ in the asymptotic length, being higher in *L. piscatorius*.

11. The population structure is similar in both *Lophius* species, where females are larger and weightier than males. Differences in growth coincide with the onset of sexual maturity with males growing faster than females before reaching sexual maturity and the reverse afterwards.

12. The sexual proportion for both *Lophius* species was distributed close to equilibrium observing significant differences in the large sizes, which were entirely composed by females.

13. According to updated Pauly's estimator for natural mortality, the mortality rate adopted for stock assessment of *L. piscatorius* is adequate, but incorrect for *L. budegassa*.

14. The sustainability indicators to monitor both *Lophius* populations status relative to exploitation indicate a high exploitation level of their stocks in the study area.

15. The prevalence of the microsporidian parasite *Spraguea lophii* is higher in *L. piscatorius* than *L. budegassa*. This prevalence, in both cases, has a positive correlation with the host size although it seems not to be influenced by the fish sex.

16. An increase of the 58% in the prevalence of *S. lophii* in *L. budegassa* has been observed in the last 18 years indicating that the population is likely under stress.

17. Severe infections of *S. lophii* may be related with anatomical anomalies such as eye deformities and alterations in skin pigmentation.

FINAL REMARKS

- Although *L. budegassa* and *L. piscatorius* occupy the same habitat in the northwestern Mediterranean Sea, differences noted in their life history traits support the hypothesis that both species of *Lophius* have different ecological strategies in order to lessen competition between them.

- Up to date, European Commission has been conducted stock assessment for *L. budegassa* inhabiting Mediterranean waters but no effort has been made to assess *L. piscatorius*. According to the differences found in the life history traits for both species, it is highly recommended to carry out a differentiated management for each *Lophius* species in the northwestern Mediterranean Sea.

- This Thesis improves the current understanding of the biology and ecology of *Lophius budegassa* and *Lophius piscatorius*; and it is the first study to provide information on the reproductive strategy of *L. piscatorius* in the northwestern Mediterranean Sea; contributing to increase the scientific knowledge of both *Lophius* spp. which will be useful to improve future stock assessments and ensure proper management actions.

CHAPTER 5

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ANNEXES

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Sensory constraints in temporal segregation in two species of anglerfish, *Lophius budegassa* and *L. piscatorius*

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ABSTRACT: The temporal segregation of 2 ecologically equivalent and sympatric species, the black anglerfish *Lophius budegassa* and white anglerfish *L. piscatorius*, was studied in relation to day–night behaviour and the underlying morphological variation in the sensory organs. Temporally scheduled trawl sampling was conducted on the western Mediterranean shelf, and diel fluctuations in catches were used as a proxy of behavioural rhythms. *L. budegassa* was more abundant at night than *L. piscatorius*, which mostly occurred in daytime catches. The observed differences in the timing of maximum activity (i.e. catches) were consistent with differences in otolith sagittae weight, eye/lens sizes and optical sensitivity. *L. budegassa* presented significantly heavier otolith sagittae than *L. piscatorius*, indicating that the behaviour of the black anglerfish is mostly acoustically driven (a typical characteristic of species living in dim light conditions). Preference for a dim light environment was also confirmed by the high optical sensitivity observed in this species achieved by enlarging the photoreceptors' diameter. In contrast, *L. piscatorius* showed larger eyes and lenses than *L. budegassa*, but only in individuals larger than 50 cm. Large eyes usually improve photon capture and the fineness with which the image is sampled; however, relatively low values of sensitivity and resolution were observed in large *L. piscatorius*. This indicates that a trade-off might exist between the increase in eye size as an adaptive response to the impending deep meso- and bathypelagic life and a visual constraint imposed by the quality of the water at the bottom level where contrast can be degraded by suspended particles. These results indicate that interspecific competition with sympatric and ecologically equivalent species may result in different adaptations to diurnal/nocturnal behaviour, which are sustained by morphological specialisations in sensory organs.

KEY WORDS: Sensory systems · Activity rhythm · Temporal segregation · Otolith · Eye · Anglerfish

INTRODUCTION

Temporal segregation in activity rhythms of ecologically equivalent and sympatric species may promote their coexistence in overlapping distribution areas (Kronfeld-Schor & Dayan 2003). Thus, species reduce interspecific competition by segregating their timing of maximum behavioural activity into different windows of the day–night cycle (Pittman & McAlpine 2001).

Although activity patterns in fishes are often said to be plastic (Ali 1992), i.e. not always consistent either within species or between individuals, trawling can be considered a reliable method for assessing the behavioural rhythm in those fish living in deep water areas where direct observation is unfeasible (reviewed by Aguzzi & Company 2010). This method provides interesting insight into species' biological clock regulation when catches are conducted concomitantly with measurements of physical factors that are key habitat parameters controlling rhythms (e.g. the light or tidal cycles as Zeitgeber; reviewed by Aguzzi et al. 2008). However, the field study of biological rhythms has to be supported by observations of the anatomy and functional mechanisms of the sensory capabilities that each species displays (Naylor 2005). In fact, to understand why a species occupies a temporal niche, it is crucial to identify the environmental characteristics under which individuals determine their appropriate period of activity.

Taking into account the fact that sensory systems represent the filters between environmental inputs and behavioural outputs, habitat choice will affect the

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environmental conditions under which sensory systems are employed, and the timing of behavioural activity may then be sustained by differences in sensory capabilities (Endler & McLellan 1988, Dusenbery 1992, Myrberg & Fuiman 2002, Weissburg 2005). Even though sensory modalities underlying spatial segregation phenomena have previously been highly documented in fish (Aguirre & Lombarte 2000, Bozzano & Catalán 2002, Lombarte et al. 2003, Sabatés et al. 2003, Schulz-Mirbach et al. 2008, Seehausen et al. 2008), there is still scant information on the role played by sensory modalities in relation to temporal segregation. Additionally, although the survival of a species demands some co-evolution of its sensory systems, adaptation to a temporal niche causes implementation of compensatory mechanisms that counterbalance the quality of each sensory modality, which means that the poor performance of one system is compensated for by the good performance of another (Schellart & Popper 1992).

With this in mind, 2 ecologically equivalent lophiid species, the black anglerfish *Lophius budegassa* and the white anglerfish *L. piscatorius*, represent a good example in which segregation can be analysed in relation to sensory modalities. In fact, these species show partial overlap in their bathymetric distribution range in the Mediterranean. Both species are present on the continental shelf but reach different depths on the slope. *L. budegassa* occurs down to a depth of 800 m (Carlucci et al. 2009), while *L. piscatorius* has been detected deeper, down to almost 2600 m (Afonso-Dias & Hislop 1996, Hislop et al. 2000). The black and white anglerfishes share common behavioural traits within the lophiids, especially in their feeding strategy, as they exhibit sit-and-wait ambush behaviour using their angling apparatus, the illicium, as a lure. In addition, both species show similar food preferences and therefore compete for similar prey items (Crozier 1985, Laurenson & Priede 2005, Preciado et al. 2006, Negzaoui-Garali et al. 2008).

Interspecific competition of ecologically equivalent species is reduced in sympatric zones by varying population densities and distributions, but also by varying the timing of their activity (Linnell & Strand 2000, Kronfeld-Schor & Dayan 2003). Accordingly, in the western Mediterranean, the reported overlap in the bathymetric range of lophiids indicates that there could be a concomitant process of morphological divergence occurring that may sustain species activity within different time windows (Seehausen et al. 2008). The activity rhythms of *Lophius piscatorius* and *L. budegassa* were analysed in the field using temporally scheduled trawl sampling and considering potential quantitative differences in the morpho-functionalities of their inner ears (acoustic and vestibular functions) and eyes (visual functions), which are the sensory systems involved in remote detection of objects. With regard to the 3 otolithic organs of the fish's inner ear, the sacculus together with its otolith, the sagitta, shows the highest morphological variability, and this structure is located in the lower part of the inner ear, which is generally associated with the hearing sense (Platt & Popper 1981, Popper & Fay 1993, Popper & Lu 2000). The size and shape of this structure show adaptations to different environmental factors, such as depth, water temperature and substrate type (Lombarte & Fortuño 1992, Aguirre & Lombarte 1999, Paxton 2000, Torres et al. 2000, Gauldie & Crampton 2002, Volpedo & Echeverria 2003). With respect to vision, the capability to hunt at night or at the maximum depth at which a fish can operate seems very likely to be determined by its eye size, because large eyes both increase the retinal sampling frequency and therefore improve resolution, and capture more photons, improving sensitivity, although many exceptions to this exist (Land & Nilsson 2002).

The aim of the present work was to determine whether anglerfish reduce competition in their overlapping distribution range by displaying temporal segregation in their behavioural activity and whether this is consistent with differences in their sensory modalities.

MATERIALS AND METHODS

Field sampling. Four days of temporally scheduled trawl sampling were carried out on the western Mediterranean lower continental shelf (100 to 110 m depth) during June 2000 at latitude 41° N to characterise the activity rhythms of the black and white anglerfishes. Thirty-two hauls were made along parallel and closely situated transects. The field survey was carried out onboard RV 'García del Cid' (38 m in length, 1200 HP), which was equipped with otter trawl nets of 27.5 mm headrope size (OTMS; Sardà et al. 1998).

The duration of the hauls was approximately 90 min, and their number, separated into day- and night-time trawls, is designated by 2 h time intervals in Table 1. A global positioning system (GPS) recorded the ship velocity and the initial and final position (latitude and longitude) for all hauls, while an echosounder provided depth measures. SCANMAR telemetric sensors connected to the mouth of the net recorded wing openings (m) and depths (m). Data were stored on an onboard computer. The setting and retrieving time of each haul were considered as the exact moment of the net landing and rising from the seabed, according to SCANMAR information.

Table 1. Numbers of trawl samples of *Lophius piscatorius* and *L. budegassa* and photon fluency rate (PFR) readings used for mean estimations at each 2 h time interval in the 24 h waveform analysis. –: no samples collected at the corresponding time interval during the 4 d of fishing activity; np: night period. Missing values at certain time intervals (i.e. –) were replaced by computing an average (±SD) estimate from all temporally adjacent values

Time interval (h)	No. of hauls	No. of PFR readings
12:00–14:00	3	–
14:00–16:00	1	1
16:00–18:00	3	2
18:00–20:00	5	4
20:00–22:00	4	np
22:00–00:00	–	np
00:00–02:00	3	np
02:00–04:00	1	np
04:00–06:00	4	4
06:00–08:00	4	4
08:00–10:00	4	2
10:00–12:00	–	4

Light intensity was directly sampled between consecutive hauls. The photometer (LI-193SA Spherical Quantum Sensor; LI-COR) was mounted on a conductivity, temperature and depth sensor (CTD), and it measured the light intensity as the photon fluency rate (PFR, $\mu E\ m^{-2}\ s^{-1}$) within the photosynthetic active radiation (PAR) range of 400 to 700 nm.

Activity rhythm determination. Fluctuations in the quantity of collected animals were used as a proxy of their behavioural rhythms when considering these as the product of their movement into and out of the sampling window. For each haul, all individuals of both *Lophius* spp. were counted. Because both species present overall similar morphological characteristics and colorations, animals were distinguished by the colour of the peritoneum and by the number of rays in the second dorsal fin (Caruso 1986): black peritoneum and 9 to 10 rays in *L. budegassa*; white peritoneum and 11 to 12 rays in *L. piscatorius*.

Time series of catches for each species were then elaborated by estimating the animals' bottom surface density variations over the 24 h cycle. A density value per haul was obtained by dividing the number of sampled animals by the swept area (km^2), as estimated by SCANMAR and GPS measures (Sparre et al. 1989).

Waveform analysis was conducted on time series of surface density and PFR estimates to assess the phase of the activity rhythms (i.e. the timing of peaks) in relation to the day–night cycle. A standard period of 24 h was subdivided into 2 h time intervals (Aguzzi et al. 2003a,b). All densities and PFR values computed from samplings that took place within a given 2 h time interval were averaged to obtain the 24 h consensus waveform (see Table 1 for the number of hauls and PFR readings pooled in each 2 h time interval). The phase of the time series in surface density estimates was identified in each waveform plot by computing a daily mean as a threshold. This threshold was obtained by re-averaging all mean waveform values together. The resulting threshold value was represented as a horizontal line in the plot. Surface density estimates above that threshold indicated the presence of a significant increase in catches (adapted from Hammond & Naylor 1977). Mean density and light intensity estimates were plotted together in relation to the time of sunset and sunrise at the latitude of the study area (Greenwich Meridian Time: 19:27 and 04:19 h on 22 June).

Periodicity in the time series of density data was screened between 600 min (10 h) and 1800 min (30 h) using a chi-square periodogram analysis ('El temps'; A. Díez-Noguera pers. comm.). The periodogram analysis requires a series of data obtained at a constant time interval (for details on the procedure, see Hammond & Naylor 1977). This condition is difficult to satisfy in trawl-based sampling studies. Gaps in the time series were therefore replaced by the values obtained from waveforms at the corresponding 2 h time intervals (Aguzzi et al. 2003a,b). In the periodograms, the highest significant ($p < 0.001$) peak represented the maximum percentage of the total data variance fitted by the corresponding periodicity. The peak value was chosen for period attribution of the analysed time series.

Morphometric measurements. Morphological measurements of the sensory organs were conducted on 274 *Lophius budegassa* and 137 *L. piscatorius,* ranging from 4.5 to 80 and 15 to 97.5 cm in total length (TL), respectively. Otolith sagittae were removed, mechanically cleaned (deprived of tissue debris with tweezers), dried at room temperature and finally weighed to the nearest 0.01 mg with a balance with a sensitivity of 0.001 mg. Otolith weight was used as a measure of size because in lophiids, otolith shape and area obtained by orthogonal projection are highly variable (Tuset et al. 2008). The eyes were enucleated, and their diameters were measured with callipers to the nearest 0.01 mm. The corneas were excised to extract the lenses, which were then measured using callipers to the nearest 0.01 mm. The vitreous was removed and the eye cups were fixed in a mixture of paraformaldehyde (2%) and gluteraldehyde (2%) in phosphate buffer (0.1 M). The caudal portion of the fixed retina from one small (*L. budegassa* of 14 cm TL and *L. piscatorius* of 15 cm TL) and one large individual of both species (*L. budegassa* of 66 cm TL and *L. piscatorius* of 77 cm TL) were embedded in resin (Technovit 7.0) and sectioned in the transverse and tangential planes.

Since the studied anglerfish stretch their distribution from shallow water to the deep mesopelagic zone, their visual systems encounter different light conditions; therefore, the optical sensitivity, i.e. the capability of seeing well in dim light conditions, was calculated both employing the Land (1981) sensitivity equation for monochromatic light at the preferred wavelength of the photoreceptor

$$S = (\pi/4)^2\, A^2(d/f)^2(1 - \mathrm{e}^{-kl}) \qquad (1)$$

found in deep environments and the Warrant & Nilsson (1998) equation for white light

$$S = (\pi/4)^2\, A^2(d/f)^2[kl/(2.3 + kl)] \qquad (2)$$

visible in shallow waters. In these equations, sensitivity (S) is related to the diameter of the eye's aperture (A), the photoreceptor diameter (d), the focal length (f), the absorption coefficient of the photoreceptor (k) and photoreceptor length (l). In the present study, the eye's aperture, i.e. the diameter of the pupil, was considered equivalent to the lens diameter (Fernald 1990). Although these species show a pupil response, and hence the maximum pupil aperture should be employed in the sensitivity formulae, the analysed individuals were caught with diurnal commercial trawls and therefore the pupil was partially closed. The focal length (f), i.e. the distance from the centre of the lens to the retina, was calculated from Matthiessen's ratio, where $f = 2.55r$ and r is the radius of the lens. The absorption coefficient of monochromatic and white light is unknown in these species; therefore, the average k (0.035 $\mu\mathrm{m}^{-1}$) for bony fish was employed (Warrant & Nilsson 1998). From f, the inter-receptor angle $\Delta\phi$, i.e. the feature of the eye's resolving power, can be calculated in accordance with Land & Nilsson (2002) where $\Delta\phi = s/f = L/(nf)$ and s is the photoreceptor spacing. The photoreceptor spacing is the inverse of the visual cell number (n) counted in the retinal linear transect L (3 linear 100 µm transects in the present study). Finally, the spatial frequency $1/2\Delta\phi$, a way of representing the optical resolution at the photoreceptor level, is obtained.

A comparative analysis of the relative increase of otolith weight and eye and lens diameters with respect to body length was performed for both species. Although the total size interval was 4.5 to 97.5 cm for both species, the range over which data on eye and lens diameter were available was 20 to 80 cm. To determine whether eye diameter and otolith weight presented isometric or allometric growth patterns, a comparison of the rate of their increase was performed according to different size classes. A t-test for the slopes using normalised data (i.e. logarithmically transformed) was employed to test the significance of the differences between pairs of curves (Zar 1984). The presence of a significant relationship was assessed at a significance level of $p < 0.05$.

RESULTS

Activity rhythms

In total, 3810 individuals of *Lophius budegassa* and 2170 individuals of *L. piscatorius* were collected from our temporally scheduled trawling survey. Both species showed different temporal patterns in their catchability in relation to the measured light intensity cycle (Fig. 1). The outputs of a 24 h waveform analysis on the time series of surface density estimates and PFR (i.e. light intensity) data at depths of 100 to 110 m are shown in Fig. 2.

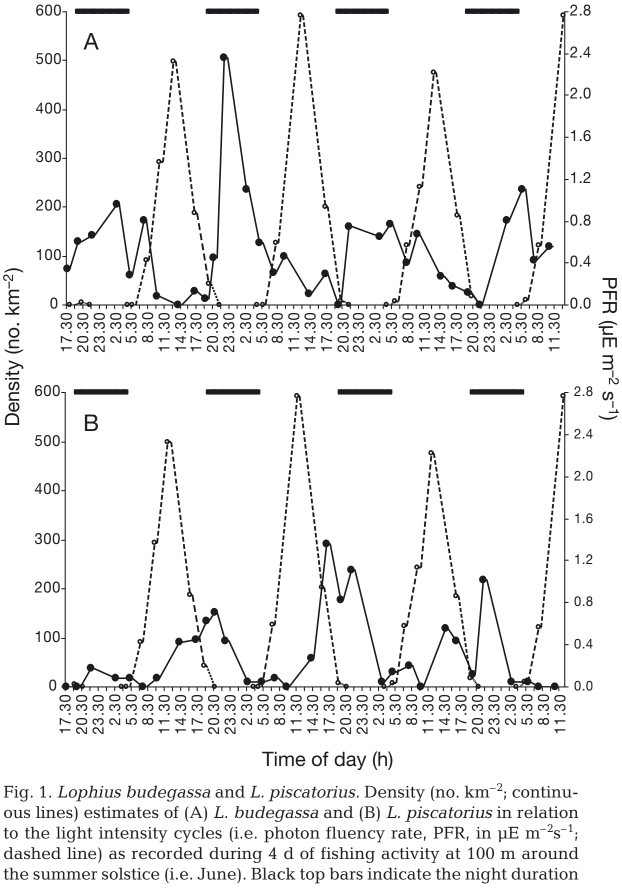

Fig. 1. *Lophius budegassa* and *L. piscatorius*. Density (no. km^{-2}; continuous lines) estimates of (A) *L. budegassa* and (B) *L. piscatorius* in relation to the light intensity cycles (i.e. photon fluency rate, PFR, in µE m^{-2}s^{-1}; dashed line) as recorded during 4 d of fishing activity at 100 m around the summer solstice (i.e. June). Black top bars indicate the night duration

Lophius budegassa chiefly presented a nocturnal catch pattern. A significant increase in catches occurred over a temporal amplitude that started between 20:00 and 22:00 h and ended between 04:00 and 06:00 h. A decrease in catches occurred for PFR values above 0.93 µE m^{-2} s^{-1}.

Lophius piscatorius showed a significant increase in catches (i.e. values above the daily mean) at daytime. The resulting temporal amplitude of the catch peak spanned from 12:00–14:00 to 22:00–00:00 h. Increased catches were observed for PFR values above 1.25 µE m^{-2} s^{-1}. Catches decreased within a few hours after the onset of darkness, and the onset of this decrement occurred for PFR values below 0.87 µE m^{-2} s^{-1}.

Both species showed a significant ($p < 0.001$) diel periodicity very close to a 24 h cycle (Fig. 3). *Lophius budegassa* presented a significant increase in catches that repeated itself every 1430 min, which is equal to 23 h and 50 min. A similar temporal pattern was detected for *L. piscatorius* with a period of 1455 min, which is equal to 24 h and 15 min, in catch fluctuations.

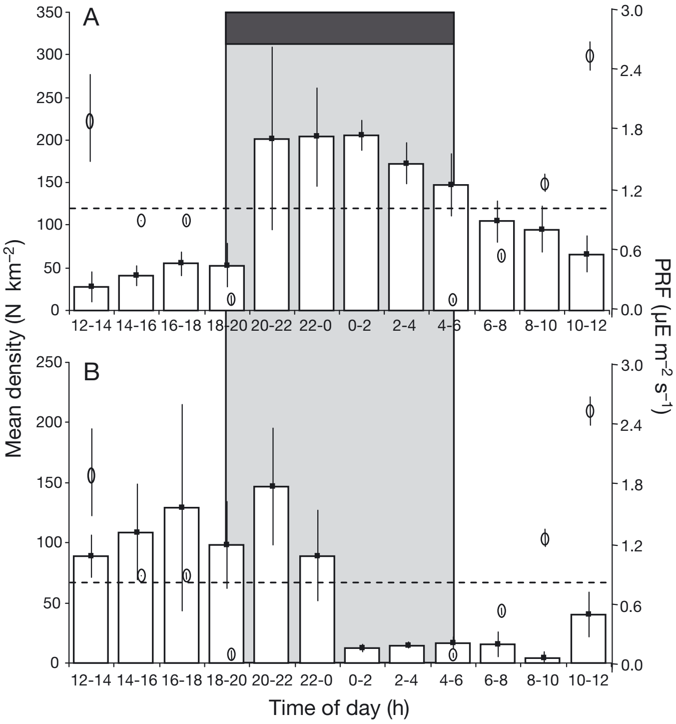

Fig. 2. *Lophius budegassa* and *L. piscatorius.* Waveform analysis outputs from time series of catches depicting mean estimates in abundance (no. km^{-2}, bars) of (A) *L. budegassa* and (B) *L. piscatorius* and light intensity measures (photon fluency rate, PFR, circles) as recorded during 4 d at 100 to 110 m depth around the summer solstice (22 June to 3 July 2000). The recorded light intensity ranged between 0.0038 and 2.77 µE m^{-2}s^{-1}. The vertical shaded area with the dark grey bar on the top indicates the night duration. Daily means (horizontal dashed lines as computed by averaging all waveform values; no. km^{-2}) are 114 for *L. budegassa* and 64 for *L. piscatorius*

Morphometric relationships in sensory organs

Otolith weight increased progressively during fish growth, ranging from 2.7 to 102.6 mg in *Lophius piscatorius* and from 0.6 to 107.3 mg in *L. budegassa* (Fig. 4). For equivalent fish lengths, *L. budegassa* had 50% heavier otoliths than *L. piscatorius*, and with increasing length, the difference between the species was observed to be fairly constant. A significant difference between the curves depicting this relationship for these 2 species was observed ($p < 0.05$).

During fish growth, progressive eye and lens enlargement was observed in both anglerfish species (Fig. 5). However, a shift in their trends was observed during growth. In fact, the eye diameters of small *Lophius budegassa* (TL < 50 cm) were larger than those of *L. piscatorius* of comparable size, with a mean value (±SD) of 20.7 ± 3.7 mm (n = 82) in the former group and 17.5 ± 3.6 mm (n = 37) in the latter. In larger individuals (TL ≥ 50 cm), the mean eye diameters (±SD) of *L. budegassa* measured 26.8 ± 2.8 mm (n = 24), and those of *L. piscatorius* measured 29.8 ± 2.2 mm (n = 22). When the eye diameters of small individuals of both species were plotted separately from the diameters of large individuals, significant differences were observed in both cases ($p < 0.05$).

A similar trend was observed for lens diameter, although the shift was recorded at 58 cm. The mean lens diameter (±SD) of small individuals of *Lophius budegassa* and *L. piscatorius* measured 7.9 ± 1.5 mm (n = 59) and 6.4 ± 1.4 mm (n = 37), respectively, while large individuals had lenses of 9.9 ± 1.0 mm (n = 21) and 10.7 ± 0.8 mm (n = 19), respectively. Although significant differences ($p < 0.05$) between the slopes of lens diameter were observed over the total size range of the individuals examined, when the 2 size groups were plotted separately, no significant differences were found ($p > 0.05$).

The anglerfishes showed duplex retinas where rods and single and double cones were visible. Single cones were the predominant cone type, and their density and the size of their outer segments were employed to calculate the spatial frequency and the optical sensitivity, respectively. Although a

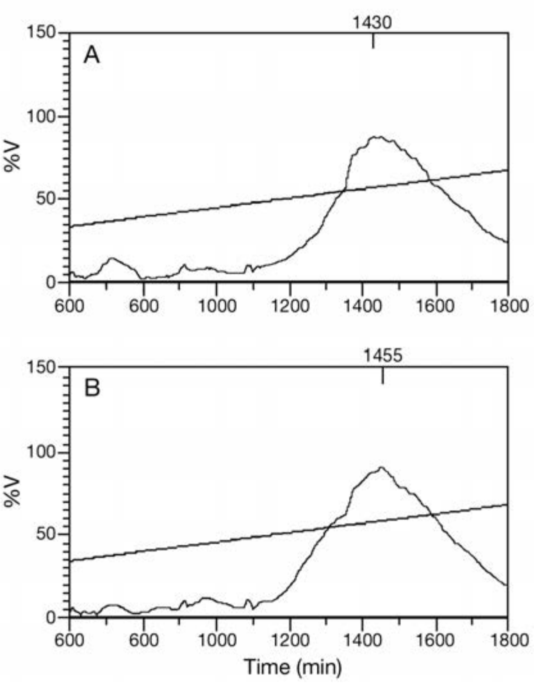

Fig. 3. *Lophius budegassa* and *L. piscatorius.* Outputs of periodogram analysis conducted on the time series of catches of (A) *L. budegassa* and (B) *L. piscatorius* as recorded during 4 d at 100 to 110 m depth around the summer solstice (22 June to 3 July 2000). Periodogram units (%V) refer to the percentage of variance explained by fitting to the data set of modelled harmonics of increasing periodicity. The straight sloping line in each panel indicates the significance threshold for periodicity determination ($p < 0.001$)

more accurate method for calculating sensitivity would be to use rod size, the use of the single cones is partially due to the fact that the rod diameter was difficult to measure accurately in the retina of the analysed individuals. In addition, since a large number of single cones was observed in these species, the role of these photoreceptors in improving sensitivity is highly probable. The measurements taken from the anglerfish eyes are summarised in Table 2. Using obtained data and the sensitivity equations, the eye of the anglerfish showed an increase in sensitivity with growth both to white and monochromatic light. While a small increase in sensitivity was observed for *Lophius piscatorius*, in *L. budegassa* the sensitivity values of the large individual were triple those of the small individual for both types of light (Table 2). These increases seem to be attributable to the considerable enlargement of the photoreceptor diameter during growth. The retinal receptor spacing and the relative spatial frequency were similar in the 2 species (Table 2).

Because interspecific differences of a sensory organ cannot be interpreted in isolation from other sensory systems, otolith weight and eye diameter were compared, and the allometric coefficient values indicated that in *Lophius budegassa*, otolith weight increased more than eye size, while an opposite trend was observed for *L. piscatorius* (Fig. 6). A comparison of the slopes showed significant ($p < 0.05$) differences between the species.

DISCUSSION

Temporal segregation

The black and white anglerfishes coexist on the Mediterranean shelf, but their relative abundance was observed to vary on a diel scale, with *Lophius budegassa* more abundant at night and *L. piscatorius* more abundant during the day. In general, species sharing important traits related to their ecological niches can coexist in the same area due to temporal segregation in the phase of their maximum behavioural activity (Carothers & Jaksić 1984). Although trawling is an active method of sampling that collects all animals down to the seabed independently of their level of behavioural activation, rhythmic catch variations in trawl hauling at a certain location can be considered to be the product of rhythmic displacements of marine populations into and out of the sampling window

Table 2. *Lophius piscatorius* and *L. budegassa.* Morphological parameters taken from 1 ind. for each size, employed to calculate sensitivity for the preferred wavelength (λ_{max}) and white light and for the spatial frequency in anglerfish

Species	Size (cm)	Lens diameter (mm)	Focal length (mm)	Cone length (µm)	Cone diameter (µm)	Sensitivity white (μm^2 sr)	Sensitivity (λ_{max}) (μm^2 sr)	Inter receptor angle (degrees)	Spatial frequency (cycle per degree)
L. budegassa	14	3.88	4.95	9.7	4.12	0.83	1.85	0.14	3.56
L. budegassa	77	12.05	15.36	12.85	6.44	2.57	5.7	0.08	6.15
L. piscatorius	15	3.78	4.82	8.4	3.53	0.54	1.2	0.14	3.66
L. piscatorius	63	10.91	13.91	12.96	4.01	1.01	2.2	0.09	5.57

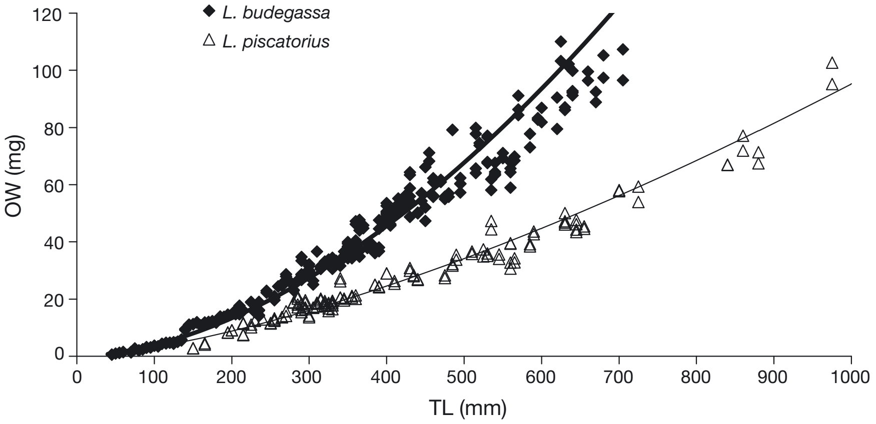

Fig. 4. *Lophius budegassa* and *L. piscatorius.* Relationship between otolith weight (OW) and total length (TL): *L. budegassa* (OW = 0.0011 $TL^{1.7725}$; $r^2 = 0.9813$; n = 274) and *L. piscatorius* (OW = 0.0035 $TL^{1.4802}$; $r^2 = 0.9417$; n = 137)

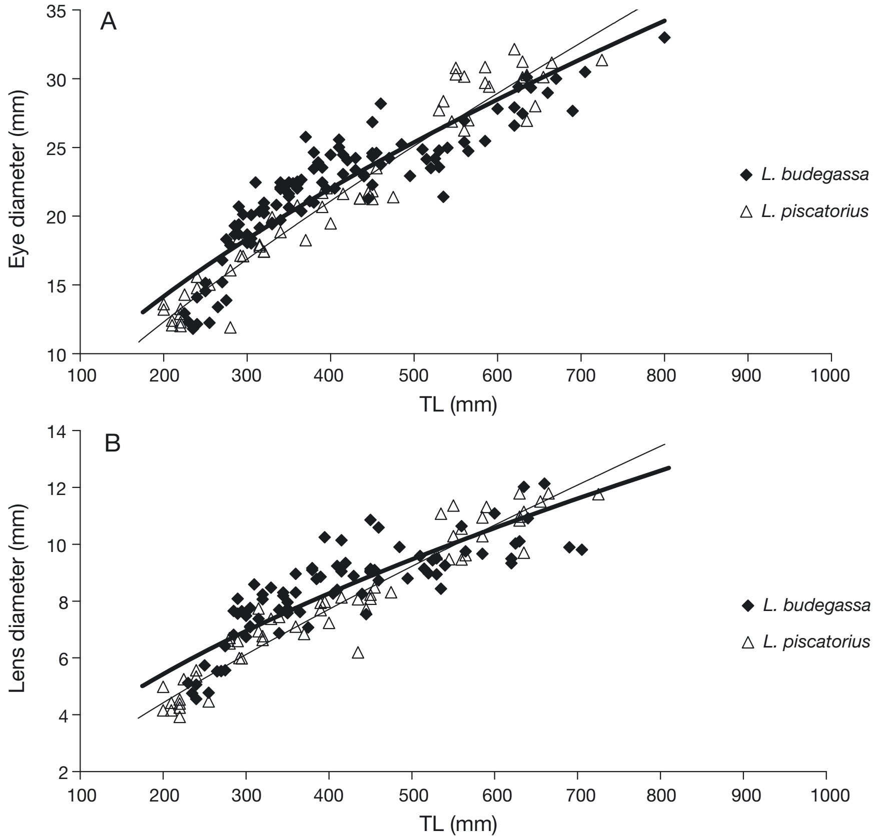

Fig. 5. *Lophius budegassa* and *L. piscatorius.* (A) Relationship between eye diameter and total length (TL): *L. budegassa* (eye diameter = 0.4882 $TL^{0.6357}$; $r^2 = 0.7745$; n = 106) and *L. piscatorius* (eye diameter = 0.1998 $TL^{0.7778}$; $r^2 = 0.9472$; n = 59). (B) Relationship between lens diameter and TL: *L. budegassa* (lens diameter = 0.2186 $TL^{0.6063}$; $r^2 = 0.7138$; n = 80) and *L. piscatorius* (lens diameter = 0.0628 $TL^{0.8027}$; $r^2 = 0.9231$; n = 56)

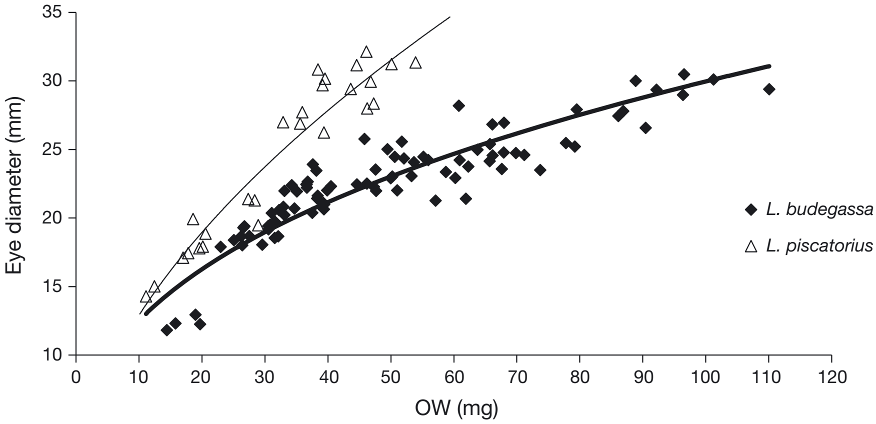

Fig. 6. *Lophius budegassa* and *L. piscatorius*. Relationship between eye diameter and otolith weight (OW): *L. budegassa* (eye diameter = 5.2128 OW $^{0.3798}$; r^2 = 0.8215; n = 87) and *L. piscatorius* (eye diameter = 3.5919 OW $^{0.5551}$; r^2 = 0.9289; n = 26). Total lengths of individuals ranged from 22.5 to 80 cm

(reviewed by Aguzzi & Company 2010). When that sampling window consistently occurs at a certain depth and location, catchability rhythms can be precisely studied in relation to the light intensity fluctuation at the site. Rhythmic population movements occur over the day−night cycle in several different ways: within the water column (i.e. vertical−bentholeagic), in and out from the substrate (i.e. endobenthic), or at the benthic boundary layer, along bathymetric gradients (i.e. horizontal−nektobenthic). Anglerfishes do not burrow and rarely display vertical migration (Hislop et al. 2000), but they do possess some swimming capability (Velasco et al. 2008). The flattened morphology typical of demersal fish (Caruso 1986) allows us to assume that the activity rhythms of these species occur as nektobentic displacements. In fact, tagged and recaptured *L. piscatorius* demonstrated in- and offshore movements in the northeastern Atlantic (Laurenson et al. 2005, Landa et al. 2008), Therefore, these species may rhythmically swim between the shelf and the slope, with the main part of the population moving into shallow water areas during 2 opposite times in the day−night cycle.

Although prey species are known to shift their activity patterns to avoid predation (Fenn & Macdonald 1995, Fraser et al. 2004), it is also common to observe a similar phenomenon among predators that compete for the same prey (Schoener 1986, Soria-Barreto & Rodiles-Hernández 2008). While this competition is reduced for species of mixed predatory and scavenging activity, pressure is likely to be stronger for more specialised piscivores such as anglerfish. Both *Lophius piscatorius* and *L. budegassa* generally mainly prey on whiting *Micromesistius poutassou*, cod *Gadus morhua* and other gadids (Crozier 1985, Laurenson & Priede 2005, Preciado et al. 2006, Negzaoui-Garali et al. 2008). Because they show similar food preferences and compete for the same available resources, their temporal segregation seems to occur to avoid interference competition.

Sensory specialisation

The observed differences in the timing of maximum activity of *Lophius piscatorius* and *L. budegassa* were consistent with differences in their otolith weight and eye size. Otoliths represent an important part of the fish's hearing apparatus, and they are an indicator of how the teleostean inner ear works (Platt & Popper 1981, Paxton 2000). The inner ear in species that live in poor light environments, such as sea slope-dwelling fishes (Paxton 2000, Lombarte & Cruz 2007), nocturnal species, such as holocentrids (Smale et al. 1995), and cave-adapted species (Schulz-Mirbach et al. 2008), are characterised by relatively large otolith sagittae. Furthermore, sound-producing species, which are specialised in acoustic communication, such as sciaenids, are also characterised by bigger otoliths than species that are not sound producers (Cruz & Lombarte 2004). The heavier otoliths found in *L. budegassa* confirm this as a preferentially nocturnal species compared to *L. piscatorius*. In fact, species that are predominantly active at night or that dwell in deep water have been found to have large (and therefore heavy) otoliths, indicating that their behaviour is mostly acoustically driven, which is a characteristic usually found where light is insufficient to support visual communication (Lychakov & Rebane 2000, Paxton 2000, Parmentier et al. 2001, Lombarte & Cruz 2007, Tuset et al. 2010).

However, the visual systems of many organisms have evolved to see well in dim light conditions by employing different optical strategies to enhance the perception of an image, both in terms of spatial resolution (by increasing the focal length as a consequence of enlarging the axial diameter of the eye) and sensitivity (improving photon capture, which is mostly achieved by having a large pupil; Warrant 1999). Although the major adaptation for improving sensitivity is having a low F-number (focal length divided by the pupil diameter), most fish have an F-number close to 1 as their optics adhere to Matthiessen's ratio. Therefore, large eyes may reflect a need for increasing spatial resolution (Land & Nilsson 2002). Because better resolution and sensitivity are achieved with bigger eyes, evolution has benefited animals that must perform considerable visual tasks with large eyes. Although there are many exceptions, epipelagic fishes with nocturnal activity or species inhabiting mesopelagic waters tend to have bigger eyes than diurnal shallow water species (Marshall 1971, Warrant & Locket 2004, Pulcini et al. 2008, Antonucci et al. 2009). In this context, the data we present here fit well with observations of the eye design in other diurnal and nocturnal teleosts. In fact, *Lophius piscatorius*, the species exhibiting a preference for diurnal activity, had a significant smaller eye and lens than *L. budegassa*, the species captured mainly at night. The preference for diurnal activity of *L. piscatorius* was clearly reflected in the retinal anatomy of this species, especially in the cone diameter that was smaller than that of *L. budegassa*. This characteristic was responsible for the low optical sensitivity observed in this species. Instead, *L. budegassa* showed a sensitivity higher than 1, typical of crepuscular or mid-water species (Land & Nilsson 2002). Optical sensitivity increased greatly with size in this species, confirming the possibility of seeing well in dim light. In fact, a similar sensitivity of 5.7 μm^2 sr was found in the blue marlin *Makaira nigricans*, a species whose eyes are adapted to the severe change in light intensity that this species encounters during its diving that may stretch several hundred metres (Fritsches et al. 2003).

The interspecific comparison of the eye size of *Lophius piscatorius* and *L. budegassa* was reversed when *L. piscatorius* reached 50 to 55 cm TL, and above this threshold, *L. piscatorius* showed significantly larger eyes than *L. budegassa*. This trend reversal probably reflects important changes in the life history of *L. piscatorius*. Even though there are uncertainties about several key events during the life history of *L. piscatorius*, many authors agree that this species spawns in deep or very deep water (>1000 m) (reviewed by Hislop et al. 2001). At these depths, mature males of 50 to 60 cm are commonly observed (Duarte et al. 2001), whereas females of *L. piscatorius* reach their first maturity at a larger size. In addition, changes in the feeding behaviour of *L. piscatorius* have also been observed at this size (Laurenson & Priede 2005). The change in the visual scenery with depth, from being extended in the epipelagic zone to semi-extended in the mesopelagic zone and even to point-source light (bioluminescence) in the bathypelagic zone, could be one of the driving forces in the evolution of larger eyes in large *L. piscatorius*. Instead, the relatively low resolution obtained for this species is quite surprising, as larger fish with larger eyes usually have higher resolving power (Collin & Pettigrew 1989, Shand 1997, Bozzano & Catalán 2002). Probably, for a species living on the bottom where the water is often full of suspended particles, the environment acts as a constraint for increasing acuity. Similarly, an unexpected low resolution was also observed in the blue marlin, a species with a huge eye size (Fritsches et al. 2003).

Although interspecific temporal segregation is one of the mechanisms employed by different fish species to allow their coexistence, there is still little information on how it occurs and its adaptive value. In other groups, such as birds, adaptations to night vision include large eye size, and it has been demonstrated that birds with large eyes become active earlier (at low light intensity) than birds with small eyes (Thomas et al. 2002). In fishes, spatial segregation is a more common mechanism of coexistence, and concomitant spatial segregation in activity rhythms and different eye sizes has been observed in several ecologically equivalent and sympatric species. In cichlids of African lakes, vision greatly contributes to their habitat segregation and even to their speciation (Seehausen et al. 2008, Hofmann et al. 2009). In this family, 2 highly morphologically similar species, *Haplochromis hiatus* and *H. iris*, prey on similar food items and inhabit different depths. Accordingly, the deeper water species (*H. iris*) has larger eyes compared to the shallower species (van der Meer & Anker 1983). However, eye size and visual capability in fish are likely to be determined by a number of different selection pressures and constraints, and therefore, the existence of large eyes does not necessarily mean that they have evolved only to see better in dim light conditions. For example, fast swimming also requires good vision, and hence, large eyes are also found in swordfish and tuna. The association of evolutionary changes in eye size with different behavioural and ecological traits in *Lophius* spp. needs to be further investigated by morphological and physiological analyses to trace the history of changes in the relationship between morphological characters and activity.

However, sensory constraints appear to be an important feature enhancing partitioning in time by enabling closely related species such as *Lophius piscatorius* and *L. budegassa* to inhabit the same area at different times of the day. Although these species rely on a combination of sensory stimuli to perform their daily tasks, under the paradigm of compensatory specialisation, their sensory systems experience high interspecific pressure that may lead to different diurnal or a nocturnal behaviour.

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Reproductive biology of Black Anglerfish (*Lophius budegassa*) in the northwestern Mediterranean Sea

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Abstract—Gonadal morphology and reproductive biology of the Black Anglerfish (*Lophius budegassa*) were studied by examining 4410 specimens collected between June 2007 and December 2010 in the northwestern Mediterranean Sea. Ovaries and testes presented traits common among fishes of the order Lophiiformes. Spawning occurred between November and March. Size at first maturity (L_{50}) was 33.4 cm in total length (TL) for males and 48.2 cm TL for females. Black Anglerfish is a total spawner with group-synchronous oocyte development and determinate fecundity. Fecundity values ranged from 87,569 to 398,986 oocytes, and mean potential fecundity was estimated at 78,929 (standard error of the mean [SE] 13,648) oocytes per kilogram of mature female. This study provides the first description of the presence of 2–3 eggs sharing the same chamber and a semicystic type of spermatogenesis for Black Anglerfish. This new information allows for a better understanding of Black Anglerfish reproduction—knowledge that will be useful for the assessment and management of this species.

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Lophius, a genus commonly known as anglerfishes or monkfishes, includes 7 species broadly distributed and exploited worldwide. Most of these species inhabit the northwestern Atlantic, as do Goosefish (*Lophius americanus*) and Blackfin Goosefish (*L. gastrophysus*), or the northeastern Atlantic, as do Cape Monk (*L. vomerinus*), Shortspine African Angler (*L. vaillanti*), Black Anglerfish (*L. budegassa*), and White Anglerfish (*L. piscatorius*), although Black Anglerfish and White Anglerfish also live in the Mediterranean Sea and Yellow Goosefish (*L. litulon*) can be found only in the northwestern Pacific (Fariña et al., 2008). In the past, species of *Lophius* have been captured as bycatch in mixed fisheries, but an increase in their economic value, together with the overexploitation of other groundfish species, has led to the development of targeted anglerfish fisheries (Hislop et al., 2001). In the northwestern Mediterranean Sea, landings of Black Anglerfish and White Anglerfish have accumulated to just over 6000 metric tons during the last 10 years.[1] The scarce reproductive information available for these species does not allow for a proper assessment or informed management of anglerfish fisheries.

This study focuses on Black Anglerfish, a demersal fish distributed along the Mediterranean Sea, as well as the northeastern Atlantic from the British Isles to Senegal (Caruso, 1986). This species is found over the continental shelf and upper slope at depths of up to 800 m and inhabits sandy, muddy, and rocky bottoms (Carlucci et al., 2009). They occupy the water column as eggs and larvae, and then they shift to a benthic existence as juveniles and adults (Fariña et al., 2008). This species co-occurs with White Anglerfish over all its bathymetric range, although White Anglerfish has a deeper distribution that reaches to depths >1000 m (Afonso-Dias and Hislop, 1996). Despite the overlapping distributions of these species, Colmenero et al. (2010) concluded that no ecological competition exists between these species because of a temporal segregation in their biorhythms; Black Anglerfish is more active at nighttime, and White Anglerfish is more active during daytime.

Most studies of these species have been undertaken in northeastern Atlantic waters, and they often have dealt with age and growth (Dupouy et al., 1984; Landa et al., 2008a; Woodroffe et al., 2003), feeding habits (Crozier, 1985; Laurenson

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[1] Tudó Vila, P. 2012. Unpubl. data. Directorate of Fishing and Maritime Affaires, Government of Catalonia, Avinguda Diagonal, 523-525, 08029 Barcelona, Spain.

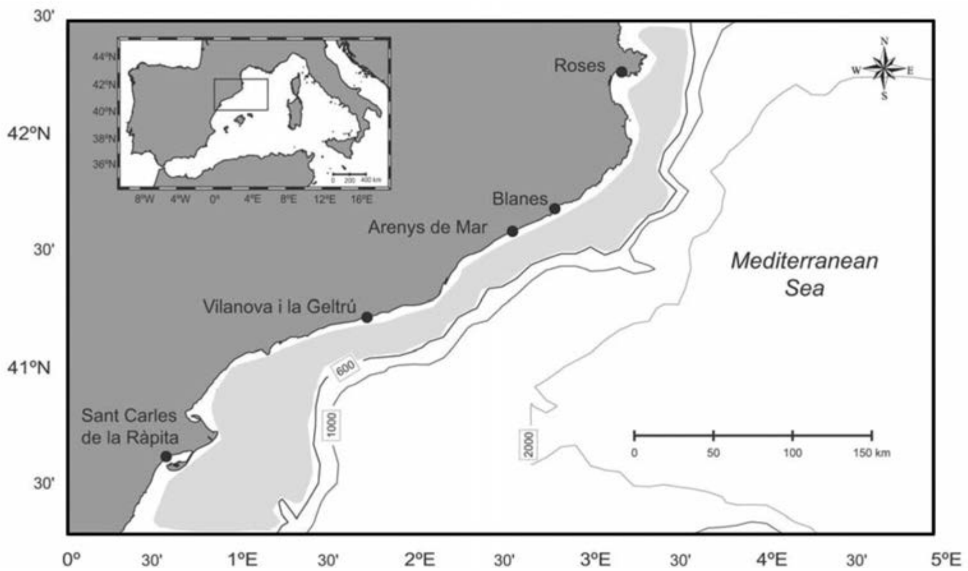

Figure 1

Map of study area where specimens of Black Anglerfish (*Lophius budegassa*) were collected in the northwestern Mediterranean Sea between June 2007 and December 2010 to examine the reproductive biology of this species. The zone shaded in light gray closest to the Catalan coast indicates the sampling area. The cities shown on the coast were the ports of commercial trawl fishery vessels from which specimens were collected.

and Priede, 2005; Preciado et al., 2006), geographical and depth distribution (Caruso, 1985; Landa et al., 2008b; Velasco et al., 2008), and reproduction (Afonso-Dias and Hislop, 1996; Duarte et al., 2001; Laurenson, 2006). In the Mediterranean Sea, studies have been less numerous and for the most part have focused on biological aspects similar to the ones examined in the studies just described (Carlucci et al., 2009; Colmenero et al., 2010; García-Rodríguez et al., 2005; La Mesa and De Rossi, 2008; Maravelias and Papaconstantinou, 2003; Negzaoui-Garali and Ben Salem, 2008; Negzaoui-Garali et al., 2008; Tsimenidis, 1984; Tsimenidis and Ondrias, 1980; Ungaro et al., 2002). However, only Tsimenidis (1980) and Carbonara et al. (2005) focused on reproductive traits of Black Anglerfish.

Information about the duration of the spawning season, fish size at first maturity, reproductive strategy, maturation of oocytes, and fecundity are very relevant for studies of the biology and population dynamics used in stock assessments for management of fishery resources. Of all these reproductive features, fecundity is the most difficult biological parameter to obtain, although it is critical for accurate stock assessments (Trippel et al., 1997). In the peculiar case of species of *Lophius*, studies on fecundity are scarce because of the difficulty of 1) acquisition of suitably mature individuals in the maturity phases of spawning capable or actively spawning and 2) the use of a proper method for fecundity estimation, which is especially complicated because of the morphological features of the gonads of these species. During reproduction, a gelatinous material is secreted into the lumen, and enumeration and measurement of eggs embedded in this mucus matrix is extremely difficult.

Although species of *Lophius* have similar traits throughout the world, some biological aspects and catch trends of fisheries present interspecific and spatial variations (Fariña et al., 2008). For that reason, reproductive parameters of the Atlantic stock of Black Anglerfish cannot be applied to the stock in the Mediterranean. In addition, knowledge of the reproductive biology of this species in the northwestern Mediterranean Sea is very limited. Therefore, our study is the first one to take a detailed approach to the examination of reproductive traits of Black Anglerfish in the Mediterranean Sea, and the results of this study can contribute to improvement of stock assessment and effective management of this species in this region.

Materials and methods

Sample collection

Monthly samples of Black Anglerfish were obtained from 467 sampling stations situated in the fishing grounds off the Catalan coast in the northwestern Mediterranean Sea from 40°5.980′N to 43°39.310′N

Table 1

Macroscopic and microscopic description of the 5 maturity phases in the reproductive cycle of male and female Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010; adapted from Afonso-Dias and Hislop (1996) and Brown-Peterson et al. (2011).

Phase	Males	Females
Immature (I)	Testes are long, narrow, and tubular shaped. They are translucent with no visible vascularization. The medial seminiferous duct is distinct. Only spermatogonia and primary spermatocytes are present.	Ovaries are very narrow, thin, and flattened-tube shaped. They are translucent; no oocyte clusters visible and minimal vascularization. Only oogonia and primary growth oocytes are present.
Developing/ Regenerating (II)	Testes are small with visible blood vessels around the seminal duct. Spermatogonias, primary and secondary spermatocytes are predominant. Spermatids are scarce.	Ovaries are small. Still no noticeable individual oocyte clusters. They acquire a cream color and vascularization is visible. Only oogonia and primary growth oocytes are present.
Spawning capable (III)	Testes increase in length and width. They have a firm texture and cream color. Seminal duct is highly vascularized. Germ cells at all stages of spermatogenesis are present. Spermatids are predominant with a lot of spermatozoa in the lumen of the sperm duct.	Ovaries increase in width and length. They have a light orange color, and blood vessels are prominent. The edges of the ovaries start to curl and they occupy a larger proportion of the body cavity. A mucus matrix starts to develop. Primary growth, cortical alveolar, and primary and secondary vitellogenic oocytes are present.
Actively spawning (IV)	Testes are large and firm and have a creamy coloration. Large amounts of sperm produced when testes are dissected. Abundant quantities of spermatozoa are present in the seminiferous tubules.	Ovaries are extremely long and wide and occupy most of the body cavity. The color of the oocytes is orange, and they are visible macroscopically. Ovaries are characterized by the presence of large hyaline oocyte clusters enclosed in a transparent gelatinous matrix that is completely developed. High vascularization is present. Oocytes are in tertiary vitellogenesis, migratory nucleus and hydration.
Regressing (V)	Testes are small, flaccid, and have brown or red areas in their beige surface. They are still highly vascularized. Sperm and residual spermatozoa can be found in the lumina of the sperm duct. Spermatogonia are present in the testes cortex.	Ovaries are flaccid and highly vascularized and often have longitudinal striations. Their color is dark pink or red. Atresia and postovulatory follicles, together with primary growth stages, are present. Cortical alveolar, primary and secondary vitellogenesis can be found.

and from 0°32.922′E to 3°35.718′E between June 2007 and December 2010. Specimens were collected onboard commercial trawl fishery vessels at depths of 20–600 m and identified according to Caruso (1986). The trawl fleet belonged to the ports of Roses, Blanes, Arenys de Mar, Vilanova i la Geltrú, and Sant Carles de la Ràpita (Fig. 1). For this study, 4410 specimens were measured to the nearest centimeter in total length (TL), weighed to the nearest gram in total weight (TW) and gutted weight (GW), and measured with an accuracy of 0.01 g in gonad weight (GNW) and liver weight (LW).

Macroscopic and histological description of gonads

Of the total number of specimens, 3562 fish had gonads removed and their sex was determined, and they were assigned macroscopically to a gonadal stage on the basis of a scale with 5 maturity phases that were described in previous studies: immature (phase I), developing or regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (phase V) (Afonso-Dias and Hislop, 1996; Brown-Peterson et al., 2011) (Table 1). Sex was easily assessed macroscopically in mature individuals. However, gonads from small individuals (approximately <20 cm TL) were indistinguishable macroscopically because ovaries and testes were small, translucent, and string-like. Fish that were too small to determine their sex or assign to a gonadal phase were classified as indeterminate.

To corroborate the macroscopic classification of some unclear and undetermined gonads, 372 specimens were histologically examined. They were fixed in 10% buffered formalin solution before they were dehydrated and embedded in a methacrylate polymer resin.

Cross sections, each 3–4 µm thick, were made with a manual microtome Leica Reichert-Jung 2040 (Leica Microsystems,[2] Wetzlar, Germany), stained with Lee's stain (methylene blue and basic fuchsin), and mounted in a synthetic resin of dibutyl phthalate xylene (DPX) on microscope slides. Gonads were classified according to the morphological features and the presence of specific inclusions (oil droplets, yolk granules, yolk vesicles, or postovulatory follicles) (Wallace and Selman, 1981). The ovarian and testicular phases were defined by the developmental stage of the most advanced cell within the gonad (Yoneda et al., 1998b).

Spawning season and size at first maturity

The spawning season was established from the analysis of the monthly variation of the maturity phases and the changes in gonadosomatic (GSI) and hepatosomatic (HSI) indices for each sex (Afonso-Dias and Hislop, 1996). Because immature specimens were not considered, 1437 males and 1167 females were used to determine both indices, which were calculated according to Yoneda et al. (2001) with the following equations:

$$GSI = (GNW \ / \ GW) \times 100. \tag{1}$$

$$HSI = (LW \ / \ GW) \times 100. \tag{2}$$

Size at first maturity (L_{50}) was determined through the examination of males and females in mature phases (phase III, phase IV, or phase V) and immature individuals collected during the spawning period (Duarte et al., 2001). Total length of all individuals was used to estimate L_{50}, defined as the size at which 50% of all fish sampled were at sexually mature phases. Maturity curves were determined with a logistic curve (Pope et al., 1975):

$$P = 100 \ / \ (1 + \exp \ [a + b\mathrm{TL}]), \tag{3}$$

where P = the percentage of mature individuals as a function of size class (TL); and
a and b = specific parameters that can change during the life cycle.

A logarithmic transformation was applied to this equation to calculate the parameters a and b by means of linear regression.

Reproductive strategy and fecundity

Patterns of ovarian organization and fecundity were tested by oocyte size-frequency distributions (West, 1990). For our analysis, 36 fish, with lengths between 20.0 and 72.5 cm TL, were randomly selected from all maturity phases. From these fish, 4428 oocytes—with more than 300 oocytes from each maturity phase (I=961; II=1106; III=1046; IV=381; V=934)—were measured for their diameter with an image analysis program (Image-Pro Plus, vers. 5.0, Media Cybernetics, Inc., Rockville, MD) in combination with an Axioskop 2 Plus microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY) and a ProgRes C14 digital microscope camera (Jenoptik AG, Jena, Germany). Diameters were measured to the nearest 0.01 µm. The mean oocyte diameter by developmental stage was determined by calculating the diameter of all oocytes encountered in each subsample. Measurements were taken only of oocytes that were sectioned through the nucleus (Afonso-Dias and Hislop, 1996).

Before fecundity was estimated, the gonads of 7 individuals were divided into 3 sections (anterior, middle, and posterior) to test differences in mean oocyte density within the ovary by using a one-way analysis of variance (ANOVA). This use of 3 sections ensured that the analyzed subsample represented the entire ovary (Murua et al., 2003). Batch fecundity (BF), the total number of mature eggs produced in a single spawning batch by an individual female, was estimated by using the gravimetric method on the basis of the relation between ovary weight and the density of oocytes in the ovary (Hunter and Goldberg, 1980). Three ovarian tissue samples of known weight, representing 10% of the total ovarian weight, were extracted from different areas of the same ovary (anterior, middle, and posterior ovarian lobe). These subsamples were collected from 15 specimens with ovaries in phases III and IV with neither postovulatory follicles nor atretic oocytes present. Because the oocytes could not be extracted from their mucogelatinous matrix without destroying them, whole tissue subsamples were mounted on several slides for analysis and covered with a cover slip.

Images of each ovarian tissue sample were taken with a Canon Powershot SD870 IS digital camera (Canon USA, Melville, NY), and oocytes were counted manually with Image-Pro Plus software. Fecundity values were obtained by examining Black Anglerfish with total lengths of 46–65 cm, TW of 1096–5592 g, GW of 986–3600 g, and GNW of 88.70–2300 g. Batch fecundity for each female was calculated as a product of the number of secondary vitellogenic oocytes per unit of weight multiplied by the total ovarian weight (Yoneda et al., 2001). Relative batch fecundity (RBF), the total number of mature eggs released by a female during the spawning batch per gram of body weight of gutted fish, was calculated as BF divided by GW (Pavlov et al., 2009):

$$BF = (oocyte\ number \ / \ sampled\ GNW) \times total\ GNW. \tag{4}$$

$$RBF = BF \ / \ GW. \tag{5}$$

Linear regression analysis was used to examine the relationships between BF and fish TL, TW, and GW (Armstrong et al., 1992). Linear regression analysis also was applied to analyze the relationship between RBF and TL. Mean potential fecundity was

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[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

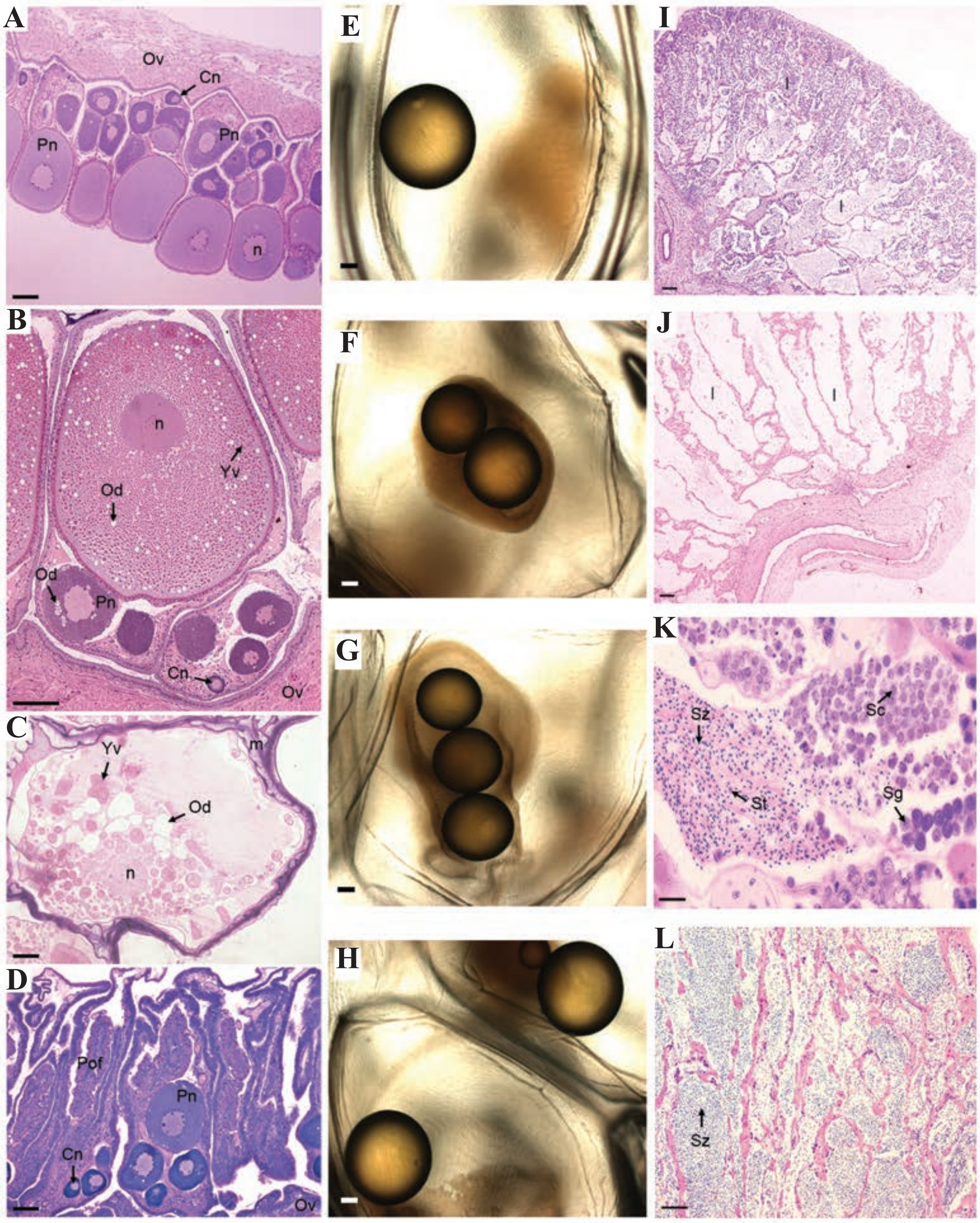

Figure 2

Micrographs of transverse sections of ovaries, oocytes inside the gelatinous matrix, and testes of Black Anglerfish (*Lophius budegassa*) collected in the northwestern Mediterranean Sea between June 2007 and December 2010. The final 4 stages of ovary development are shown in the left column: (**A**) Phase II: developing or regenerating; (**B**) Phase III: spawning capable; (**C**) Phase IV: actively spawning; (**D**) Phase V: regressing. Oocytes are featured in the middle column: (**E**) 1 oocyte in a chamber (486 µm in diameter), (**F**) 2 oocytes floating in a chamber (427 µm and 359 µm in diameter), (**G**) 3 oocytes in a chamber (341 µm, 330 µm, and 322 µm in diameter), and (**H**) closed-up division between chambers. In the right column, transverse sections of testes show (**I**) its lobular organization, (**J**) empty lobules, (**K**) seminal lobule during spermatogenesis, and (**L**) spermatozoa in the lumen of the seminal lobule. Ov=ovigerous membrane; n=nucleus; Od=oil droplet; Yv=yolk vesicle; m=mucus matrix; Cn=chromatin nucleolar; Pn=perinucleolar; Pof=postovulatory follicle; l=seminal lobule; Sg=spermatogonia; Sc=spermatocyte; St=spermatid; Sz=spermatozoa. Scale bars=100 µm.

also calculated as the number of vitellogenic oocytes divided by kilogram of mature female (Murua et al., 2003).

Results

Gonadal morphology

Ovarian structure consists of a flattened band with 2 distinctive lobes that are folded up and connected to each other at their posterior end. The lobes form a single organ attached to the abdominal cavity by a black mesenteric tissue called the mesovarium. One side of the ovarian wall is made of an ovigerous membrane and connective tissue. The nonovigerous side is made of epithelial cells. A single layer of oocyte clusters projects from the ovigerous membrane to the lumen (Fig. 2A). Inside each gonad, the clusters can be in different development stages. Only the oocytes situated closest to the tip of the clusters have progressed through all maturity stages, and the other oocytes are only oogonia or in the primary growth stage (Fig. 2B).

A gelatinous material is secreted into the lumen during the late phases of gonad maturation, producing the mucus matrix characteristic of the reproduction of *Lophius* species (Fig. 2C). Hydration of the oocytes occurs just before spawning, and postovulatory follicles (Fig. 2D) are found during the regression phase of the reproductive cycle. Ripe eggs, which are usually situated on the tip of the oocyte cluster, rupture the follicles and are pressed into the layer of mucus. In this study, every chamber examined contained at least 1 egg in the gelatinous matrix (Fig. 2E), although the presence of 2 (Fig. 2F) or 3 eggs (Fig. 2G) floating in separate chambers was also noted (Fig. 2H).

Oocyte diameter appeared to differ depending on the quantity of oocytes present in each chamber; the oocytes that were isolated in their chambers were found to be larger in size than other oocytes. A diameter of 486 µm was obtained for the oocyte that was the single oocyte in its chamber. Diameters of 427 µm and 359 µm were found for the 2 oocytes floating together in a chamber, and diameters of 341 µm, 330 µm, and 322 µm were observed for the 3 oocytes that shared the same chamber. Measurements of more oocyte diameters are needed to confirm these preliminary observations.

The testes are a pair of elongated and tubular structures located in the dorsal portion of the abdominal cavity, and they are bean-shaped in transverse section. The organization of the testes is lobular: the connective tissue extends from the testicular capsule to form lobules that have their blind ends on the surface of the gonads, converging ventrally towards the sperm duct (Fig. 2I). These lobules are fused to the posterior end of each testicular lobe to form a common sperm duct that leads to a genital pore (Fig. 2J). Spermatogenesis takes place in a capsule-like sac called a cyst, but it is not completed within the cyst. Each cyst contains spermatogonia or developing spermatocytes (Fig. 2K). Before the end of the spermatogenesis, the cyst breaks up and spermatids are released into the lumina of the lobules, where spermatogenesis is then completed and spermatids transform into spermatozoa (Fig. 2L). The cysts appear to be arranged in order of maturation, with a gradient of germ cells of increasing maturation from the cortex to the sperm duct. The shape of the spermatozoa head seems to be elongated.

Spawning season and size at first maturity

Monthly distribution of macroscopic classification of the maturity phases (Fig. 3, A and B) revealed that the period of maximum occurrence of females in the spawning capable phase (III) was from November to January. The presence of females in the actively spawning phase (IV) was observed from November to March, with a maximum peak in January. Females in the immature, regressing, and developing and regenerating phases (I, V, and II, respectively) were found throughout the year, with the highest percentage of immature individuals seen in May. A slight increase in phase-III females was observed in August, and that increase would likely result in spawning activity in September, indicating the possibility of a secondary breeding season. Males in all maturity phases were observed throughout the year, with 2 maxima of mature males occurring in December and July.

For mature males and females, GSI and HSI indices were calculated. In males, GSI was fairly constant throughout the year and a maximum index value of 1.06 was reached in January (Fig. 3C). The mean GSI for females was highest from December to March, with a peak of maximum activity in January (4.94) and February (2.43) (Fig. 3D). The mean HSI for females and males followed the same pattern. The highest value for males was found in September (2.50), and the lowest value in February (1.65) (Fig. 3C). In females, HSI values ranged from 3.19 in January to 1.86 in March (Fig. 3D). The highest HSI values were found just at the beginning of the main spawning season. GSI and HSI results, together with observations of maturity phases throughout the year, indicate that there is one main spawning season from November to March.

Comparison of L_{50} curves showed a clear difference between males and females. The size at 50% sexual maturity was 33.4 cm TL for males (Fig. 4A) and 48.2 cm TL for females (Fig. 4B).

Reproductive strategy and fecundity

The size-frequency distributions of oocyte diameters in each of the 5 maturity phases indicate that oocytes in different stages of development were found in each maturity phase (Table 2; Fig. 5). During phase I, only oocytes in the primary growth stage (chromatin nucleolar and perinucleolar) with a narrow range of diameters were present (Fig. 5A). In phase II, cortical alveolar

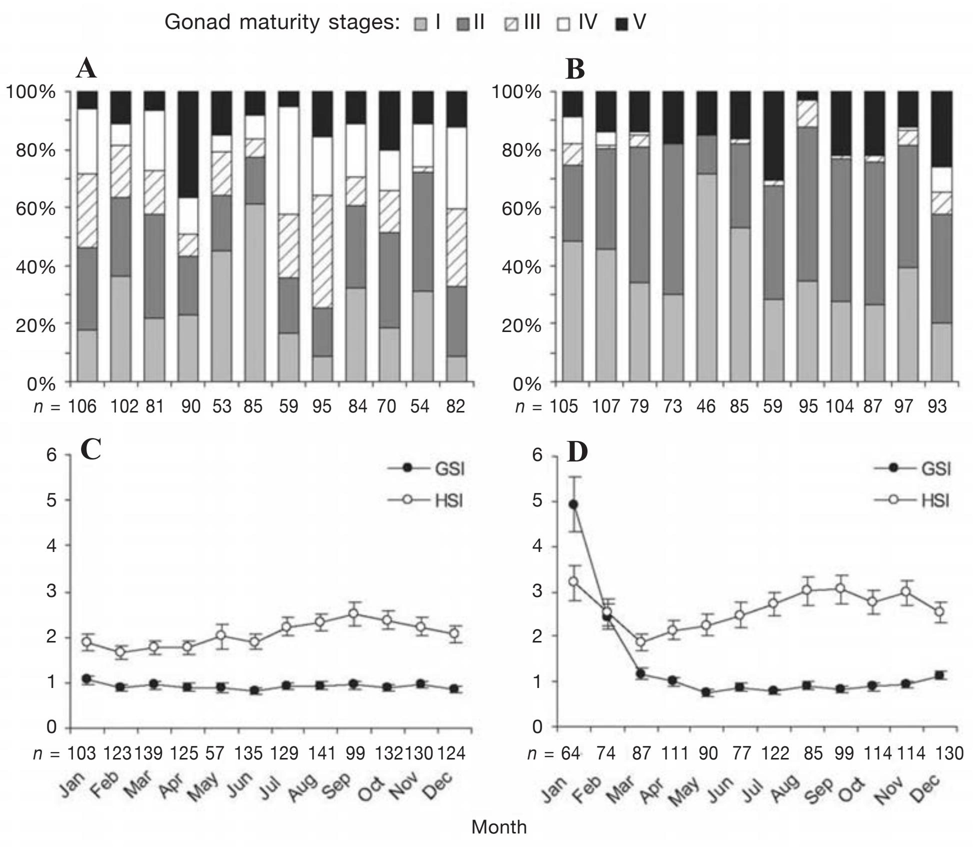

Figure 3

Monthly distributions of (**A**) males and (**B**) females in the 5 phases of gonad maturity of Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010 and monthly changes in the mean gonadosomatic index (GSI) and hepatosomatic index (HSI) for (**C**) males and (**D**) females. On the basis of macroscopic examination, specimens were assigned to the following phases: immature (phase I), developing or regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (phase V). Error bars indicate ±1 standard error of the mean.

vesicles were found in the cytoplasm together with oocytes in the stage of primary growth with diameters that had increased notably (Fig. 5B). In phase III, yolk granule stages (Murua et al., 2003) were present along with the previous 2 types of oocytes. The oocytes increased in size as the yolk accumulated, and a wider oocyte diameter range distribution was observed during this phase (Fig. 5C). In phase IV, oocytes were observed in different stages (primary growth, vitellogenesis, migratory nucleus, and hydration). Two populations of oocytes were recognized in phase IV: a population of larger oocytes (defined as a clutch) and a population of smaller oocytes from which the clutch was recruited (Fig. 5D). In phase V, oocytes in the primary growth stage were found along with postovulatory follicles and atretic oocytes (Fig. 5E).

The presence of oocytes in different developmental stages within the same cluster and the frequency distribution of oocyte diameter along all maturity phases indicate that oocyte development in Black Anglerfish is group-synchronous. The existence of a gap that separates the yolked oocyte stock, the ones to be spawned during the current breeding season, from the unyolked oocytes, the ones to be spawned in the coming breeding season, together with the increase of the mean diameter of the advanced vitellogenic oocytes, indicates that annual fecundity is determinate.

Significant differences in oocyte densities among

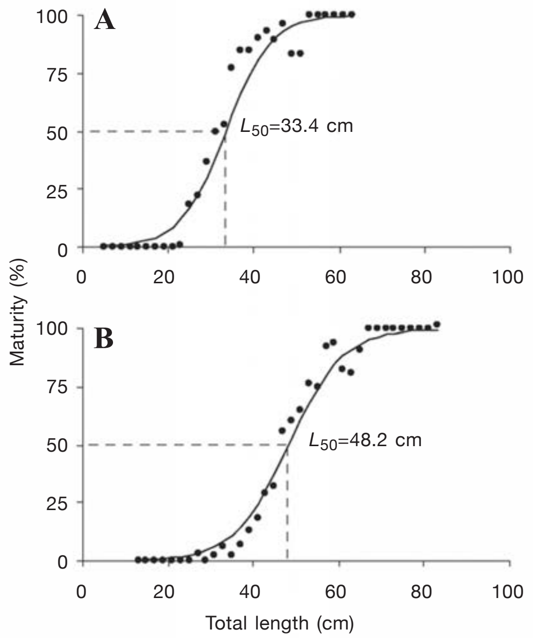

Figure 4

Maturity ogives used to estimate length at maturity (L_{50}) for (**A**) male and (**B**) female Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010.

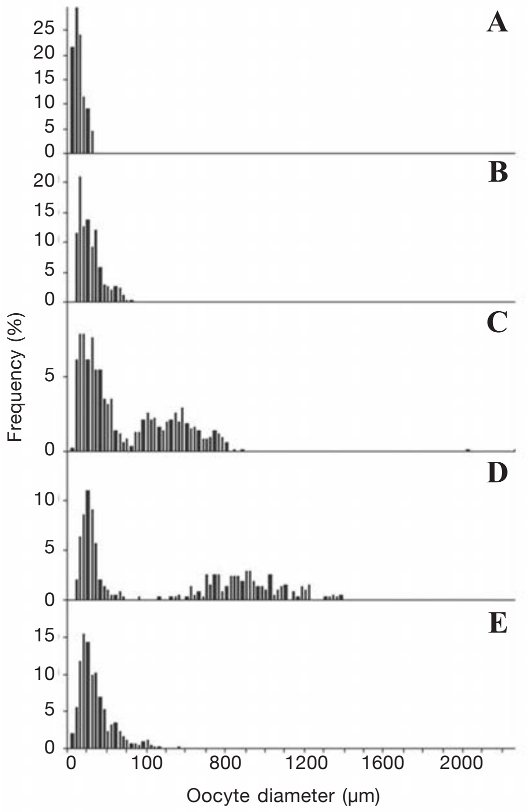

Figure 5

Size-frequency distributions of oocyte diameters at each phase of gonad maturity of Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. The 5 maturity phases are (**A**) phase I, immature, n=961; (**B**) phase II, developing or regenerating, n=1106; (**C**) phase III, spawning capable, n=1046; (**D**) phase IV, actively spawning, n=381; (**E**) phase V, regressing, n=934.

ovary sections were not observed (ANOVA, $F_{(2,42)}$=0.002, P=0.998). Batch fecundity ranged between 87,569 and 398,986 oocytes, and mean BF was 218,020 oocytes (standard error of the mean [SE] 90,018). Relative batch fecundity was estimated at 102 (SE 20) oocytes per gram of female (GW), and mean potential fecundity was 78,929 (SE 13,648) oocytes per kilogram of mature female.

Batch fecundity tended to increase linearly with TL (linear regression, coefficient of determination [r^2]=0.89, $F_{1,13}$=106.57, P<0.001), TW (linear regression, r^2=0.82, $F_{1,13}$=60.79, P<0.001), and GW (linear regression, r^2=0.82, $F_{1,13}$=59.31, P<0.001), indicating that fecundity is dependent on size and body weight (Fig. 6). No significant correlation was found between RBF and TL, indicating that RBF is not size dependent (linear regression, r^2=0.16, $F_{1,13}$=2.50, P=0.138).

Discussion

This study indicates that oocyte development and the fecundity pattern of Black Anglerfish are similar to findings for other species of *Lophius*: White Anglerfish (e.g., Fulton, 1898), Cape Monk (Leslie and Grant, 1990), Goosefish (Armstrong et al., 1992) and Yellow Goosefish (Yoneda et al., 2001). The eggs of Black Anglerfish appear to be shed as a part of a single event and are likely released only once during the spawning season—the pattern of a total spawner. This type of spawning has been observed also in Goosefish (e.g., Feinberg, 1984) and White Anglerfish (Afonso-Dias and Hislop, 1996), although the possibility of spawning several batches over the spawning season is not unfeasible because this behavior has been described for Yellow Goosefish (Yoneda et al., 2001).

Batch fecundity estimates for Black Anglerfish reveal a positive relationship between number of oocytes

Table 2

Oocyte histological characteristics of Black Anglerfish (*Lophius budegassa*) collected between June 2007 and December 2010 in the northwestern Mediterranean Sea; descriptions follow those of Wallace and Selman (1981). Mean oocyte diameters, which were measured to the nearest 0.01 µm, are provided by developmental stage with standard errors of the mean (SE).

Oocyte developmental stage	Oocyte diameter (µm)	Histological characteristics
Chromatin nucleolar	36.55 (SE 21.27)	Nucleus contains a large nucleolus and some small peripheral nucleoli. Yolk granules are not present in the cytoplasm.
Perinucleolar	110.78 (SE 62.54)	Nucleus grows and several big peripheral nucleoli and vacuoles are present. No yolk granules are present in the cytoplasm.
Cortical alveolar	256.35 (SE 80.81)	Nucleus is central. Cortical alveolar vesicles and oil droplets appear in the cytoplasm. Yolk granules are still not present in the cytoplasm.
Primary vitellogenic	364.54 (SE 37.31)	Yolk granules appear between cortical alveolar vesicles. Nucleus remains central.
Secondary vitellogenic	406.20 (SE 39.60)	Yolk granules fill the cytoplasm. Nucleus is still central.
Tertiary vitellogenic	544.55 (SE 185.49)	Yolk granules are in contact with the nucleus, which is still central, and the oocyte size has increased from size at previous stages.
Migratory nucleus	883.42 (SE 153.33)	Yolk granules and oil droplets start to fuse. Nucleus migrates to one pole of the oocyte.
Hydration	1125.09 (SE 176.93)	Yolk granules form a single mass. Nucleus is not present in the cytoplasm.

and fish length and weight; therefore, large spawners have a higher contribution to egg production than do smaller ones. Previous authors have found lower fecundity values than the ones observed in this study. In the Tyrrhenian Sea, Carbonara et al. (2005) determined mean potential fecundity as 54,057 oocytes per kilogram of mature female and total fecundity as 211,687 oocytes from data obtained from a single individual (59 cm TL). In the case of the Aegean Sea, where fecundity values varied from 105,800 to 284,200 oocytes, fecundity was determined from an undefined number of individuals and size range (Tsimenidis, 1980).

Another relevant feature of the reproduction of Black Anglerfish is the presence of a gelatinous matrix, which has been noted for other *Lophius* species (Armstrong et al., 1992; Fulton, 1898; Leslie and Grant, 1990; Yoneda et al., 2001). The matrix consists of individual chambers where hydrated oocytes are released. In our study, we detected the presence of 2 or 3 eggs in some chambers (Fig. 2). This phenomenon also has been described for Goosefish, and it has been assumed that such eggs in that species may have been fertilized (Armstrong et al., 1992; Everly, 2002). Trippel et al. (1997) concluded that for the same species, larger eggs have a higher probability of hatching and of subsequent larval survival than do smaller ones. It is unknown whether the smaller eggs of Black Anglerfish that share a chamber hatch at a different rate or produce less viable larvae than the larger eggs that are alone in a chamber. Finally, the semicystic kind of spermatogenesis has been described only once before in the family Lophiidae, for Blackmouth Angler (*Lophiomus setigerus*) (Yoneda et al., 1998a). Muñoz et al. (2002) reported that semicystic spermatogenesis may be related to the secretion of abundant, thick seminal fluid, the function of which is to keep the spermatozoa together to enable fertilization of the entire egg mass.

The variation in spawning seasonality of Black Anglerfish between spring (La Mesa and De Rossi, 2008) and winter (Carbonara et al., 2005; Duarte et al., 2001; Tsimenidis, 1980) may be associated with local oceanographic features. Eddies and fronts enhance productivity, often function as physical barriers that retain larvae and juveniles, and provide favorable conditions for the feeding behavior of recruits and their subsequent transport toward the main nursery areas (Sánchez and Gil, 2000). During spring and summer, temporary eddies are generated in the Adriatic Sea (Mediterranean Sea) and in the Bay of Biscay (Atlantic) (Artegiani et al., 1997a, 1997b). In contrast, in wintertime eddies are generated in the Aegean and the Tyrrhenian seas, and the northern component of the outflow water from the Mediterranean Sea influences the Atlantic Iberian coast (Iorga and Lozier, 1999).

Finally, maturity sizes between individuals off the Atlantic Iberian coast, 53.6 cm TL in females and 38.6 cm TL in males (Duarte et al., 2001), and individuals in the northwestern Mediterranean Sea, 48.2 cm TL in females and 33.4 cm TL in males in our study, were very similar, in contrast to the sizes observed for individuals in the Aegean Sea, 34 cm TL in females and 24 cm TL in males (Tsimenidis, 1980). These variations in L_{50} could be related to environmental and anthropogenic factors (e.g., temperature, food availability, or fishing pressure) (Trippel et al., 1997).

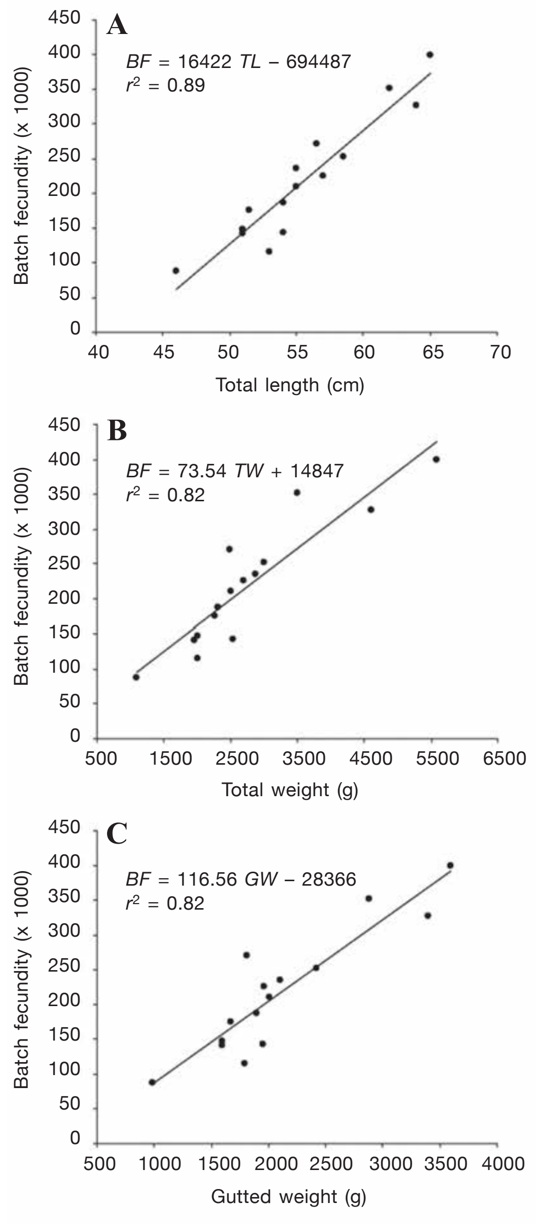

Figure 6

Relationships between batch fecundity (BF) and (**A**) total length (TL), (**B**) total weight (TW), and (**C**) gutted weight (GW) for Black Anglerfish (*Lophius budegassa*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. r^2=coefficient of determination.

Conclusions

Black Anglerfish is a bycatch species in commercial fisheries off the Catalan coast of Spain. Despite not being a target species for these fisheries, the rise of its economic value has led to an increase of captures in the northwestern Mediterranean Sea. The lack of information about reproduction and fecundity of the Black Anglerfish off the Catalan coast has been a problem for management of the fishery for Black Anglerfish. The results of our study improve the current understanding of the reproductive dynamics of this species. From the morphological point of view, the structure and the development of ovaries and testes do not differ from the development of gonads in other Lophiiformes, although Black Anglerfish presents variation in its spawning season—a variation that is linked to its geographic area.

Our most important results are for L_{50}. Males and females both reach L_{50} at large sizes: 33.4 cm TL for males and 48.2 cm TL for females. As a consequence, the large catch and retention of individuals below the L_{50}, 57% of males and 83% of females landed, indicate that overfishing of this species could be a concern in the northwestern Mediterranean Sea. Therefore, our study provides new data that are needed for a better understanding of the biology and ecology of Black Anglerfish; this knowledge will be useful in assessment and management of the stock that is exploited by the fisheries of the northwestern Mediterranean Sea.

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Abstract—Reproductive parameters of the white anglerfish (*Lophius piscatorius*) in the northwestern Mediterranean Sea were studied in 556 specimens collected monthly aboard commercial fishing vessels that were trawling at depths of 12–836 m. The main spawning season occurred from February through June. The size at maturity was estimated to be 48.8 cm in total length (TL) for males, 59.9 cm TL for females, and 51.3 cm TL for both sexes combined. The white anglerfish has group-synchronous oocyte development and determinate fecundity. It is a total spawner (spawns all its eggs once during a spawning season) and has a batch fecundity ranging from 661,647 to 885,214 oocytes, a relative batch fecundity of 66–128 oocytes per gram of female gutted weight, and a potential fecundity with values from 54,717 to 104,506 oocytes per kilogram of female total weight. This study is the first to provide the reproductive biology of white anglerfish in the northwestern Mediterranean Sea and provide valuable information that can be used to improve the stock assessment and ensure proper management of this species.

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Reproductive strategy of white anglerfish (*Lophius piscatorius*) in Mediterranean waters: implications for management

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The genus *Lophius*, commonly known as anglerfish, monkfish, or goosefish, belong to a family of bathydemersal fishes, which live and feed on the bottom of the seafloor generally below 200 m (Caruso, 1986). It includes 7 species distributed around the world. The white anglerfish (*Lophius piscatorius*) is found in the northeast Atlantic Ocean and the Mediterranean Sea, and the black anglerfish (*Lophius budegassa*) coexists with white anglerfish over most of its range, although the black anglerfish has a more southerly distribution in the Atlantic (Caruso, 1986). The shortspine African angler (*Lophius vaillanti*) is found in the eastern Atlantic (Maartens and Booth, 2005). The devil anglerfish (*Lophius vomerinus*) occupies the southeast Atlantic and the northern and western Indian Ocean (Walmsley et al., 2005). The blackfin goosefish (*Lophius gastrophysus*) inhabits the western Atlantic, and the goosefish (*Lophius americanus*) occurs in the northwest Atlantic (Caruso, 1983). Finally, the yellow goosefish (*Lophius litulon*) is distributed in the northwest Pacific, in the Gulf of Po-Hai, in the Yellow Sea, and in the East China Sea (Yoneda et al., 1997).

We focused on white anglerfish, which can be found on the continental shelf and slope, inhabiting depths from the shoreline to >1000 m (Afonso-Dias and Hislop, 1996). In the Mediterranean Sea, this species cohabits with black anglerfish, and even though the distributions of both species overlap, no ecological competition exists between them because of a temporal segregation in their daily biorhythms (Colmenero et al., 2010). Both of these species of *Lophius* play an important role in the trophic structure of benthodemersal ecosystems because they represent major predators, along with the European hake (*Merluccius merluccius*) (Díaz et al., 2008). In the community structure of the northwestern Mediterranean Sea, species of anglerfish are considered top predators (Coll et al., 2006; Valls et al., 2014). They are also reported to be important in the deep-sea community (depths from 200 m to the bottom of the ocean) because they are the most abundant species (Labropoulou and Papaconstantinou, 2000; Maiorano et al., 2010).

Despite the fact that the deep sea is the largest ecosystem on the planet, is highly diverse, and has a wealth of resources, it is still mostly unknown and poorly understood in comparison with shallow-water areas: therefore environmental management in deep waters is difficult (Ramirez-Llodra et al., 2010). In the last few decades, the decline of traditional fisheries on the continental shelf, the increasing demand for food sources, and rapid technological developments have resulted in an increasing exploitation of deep-sea resources (Koslow et al., 2000; Ramirez-Llodra et al., 2011) and in an incremental increase in the global mean depth of fishing (Watson and Morato, 2013).

This rise in deep-sea fishing has affected catches of *Lophius* species, given the growing demand for human consumption of this group of fish that is leading to an increase in worldwide commercial exploitation and targeting of anglerfishes (Fariña et al., 2008). Total catch reported globally for white anglerfish reached more than 26,500 metric tons (t) in 2014 (FAO Global Capture Production database, website) and total catch of anglerfishes in the northwestern Mediterranean Sea for the same year added up to 660 t (Tudó Vila[1]). Landings in our study area were composed primarily of black anglerfish (86%) and generally only a small percentage of white anglerfish (14%) (Tudó Vila[1]), but, for landings in Atlantic waters, the opposite is true; white anglerfish (94%) dominate the catch (Dobby et al., 2008). Although the European Commission previously has conducted stock assessments of black anglerfish in the western Mediterranean Sea, there is no corresponding assessment for white anglerfish. The lack of information about the structure of the population of white anglerfish in this region and the lack of knowledge of the basic biology of this species are the main reasons for the absence of any assessment. The actual management regulations applied for black anglerfish generally are those applied to bottom trawling (European Union Council Regulation 1967/2006), with recommendations aimed at reducing the fishing effort of the fleet in order to avoid loss in stock productivity and decreases in landings (Cardinale et al.[2]).

The small quantity of white anglerfish available from landings in Mediterranean waters makes studies of this species challenging. Studies conducted in the Mediterranean Sea have been scarce, and they have been focused on temporal and spatial distribution of this species (Ungaro et al., 2002; Colmenero et al., 2010), age and growth (Tsimenidis and Ondrias, 1980; Tsimenidis, 1984), feeding ecology (López et al., 2016), morphometrics (Negzaoui-Garali and Ben Salem, 2008), parasites (Colmenero et al., 2015a), and ova characteristics (Colmenero et al., 2015b). Among these studies, only Ungaro et al. (2002) analyzed some of the biological features of this species by using data available from trawl surveys, including data on distribution, abundance, stock demography, and size at maturity. The latter work is valuable but is limited because sampling occurred only in the spring and summer; a whole year of sampling is recommended to obtain more accurate biological information.

A study of reproductive ecology is important for an understanding of population dynamics, and it is critical for assessing the effects of harvesting on fish populations when attempting to develop appropriate management strategies. Recruitment is recognized as a key process for maintaining sustainable populations, and the relationship between the reproductive output of the population and the resulting recruitment is central to understanding how a fish population will respond to constant stressors such as fishing (Chambers and Trippel, 1997). Although knowing more about the relationships between life history strategies and productivity with depth could help managers understand the potential response of a deep-sea species to fishing (Drazen and Haedrich, 2012), it is first necessary to conduct biological studies of fish to gain knowledge of the reproductive system of a species (Koslow et al., 1995). Such studies include gonad morphology (external and cellular description of the ovary and testis), reproductive pattern (hermaphroditism or gonochorism), reproductive behavior, reproductive cycle, spawning season duration, size at maturity, sex ratio, size at sexual transition, and fecundity.

All of this information can be applied at the population level to evaluate reproductive potential and to serve as a basis for limits on fishing that aim in order to keep recruitment at sustainable levels (García-Díaz et al., 2006). Because reproductive strategy varies within species, depending on the area of distribution of each species and the depth distribution of each species in each area (Rotllant et al., 2002), there is a need for knowledge about reproduction of deep-sea fish species. Such information is needed particularly in the Mediterranean Sea because the data available for this region are limited (Morales-Nin et al., 1996; D'Onghia et al., 2008; Muñoz et al., 2010; Bustos-Salvador et al., 2015), and, furthermore, target species of fisheries have been the focus of only a few studies (Rotllant et al., 2002; Recasens et al., 2008).

The goal of this study was to describe the reproductive parameters—gonadal morphology, spawning season, size at sexual maturity, oocyte development, and fecundity—of white anglerfish in the northwestern Mediterranean Sea in order to provide valuable information and scientific background to improve stock assessments and effective management for *Lophius* species in Mediterranean waters.

Materials and methods

Sampling and data collection

Between June 2007 and December 2010, 556 white anglerfish, with total lengths (TLs) of 9–120 cm, were

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[1] Tudó Vila, P. 2015. Unpubl. data. Directorate of Fishing and Maritime Affairs, Government of Catalonia, Avinguda Diagonal 523-525, 08029 Barcelona, Spain.

[2] Cardinale, M., D. Damalas, and C. G. Osio (eds.). 2015. Scientific, Technical and Economic Committee for Fisheries (STECF)—Mediterranean Assessments, part 2 (STECF-15-06), 396 p. Publications Office of the European Union, Luxembourg. [Available from website.]

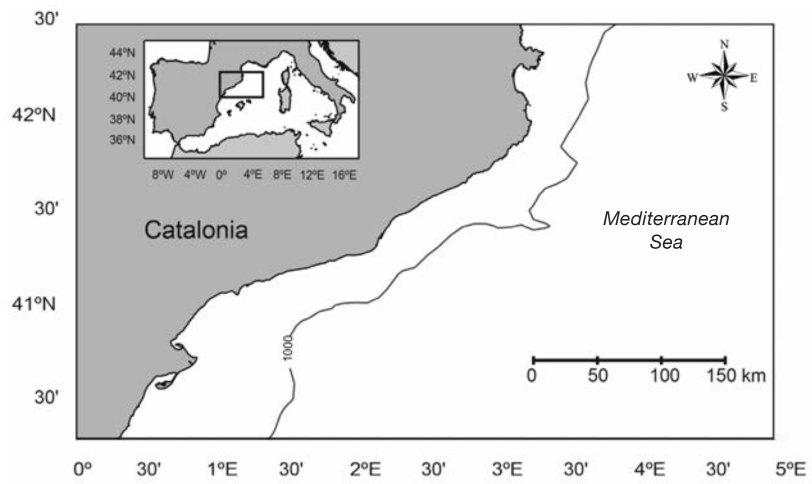

Figure 1

Map of the northwestern Mediterranean Sea showing the study area where white anglerfish (*Lophius piscatorius*) were collected from fishing grounds off the Catalan coast between June 2007 and December 2010.

collected monthly aboard commercial fishing vessels that were trawling at depths of 12–836 m. Fish were sampled from 467 stations located in the fishing grounds off the Catalan coast in the northwestern Mediterranean Sea from 40°5.980′N to 43°39.310′N and from 0°32.922′E to 3°35.718′E (Fig. 1). For each individual, TL was measured to the nearest centimeter, total weight (TW) and gutted weight (GW) were measured to the nearest gram, and gonad weight (GNW) and liver weight (LW) were measured to the nearest 0.01 g. The sex of all fish was determined and assigned macroscopically to a gonadal stage on the basis of a scale of 5 maturity phases proposed by Colmenero et al. (2013): immature (phase I), developing or regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (phase V).

Fish that were too small (<20 cm TL) for their sex to be determined or for assignment to a gonadal phase were classified as indeterminate. Macroscopic gonadal stage was validated histologically, according to the most advanced cell within the gonad (West, 1990). Gonads were fixed in 10% buffered formalin solution, dehydrated in ascending solutions of alcohols and embedded in a methacrylate polymer resin, sectioned at a thickness of 4 μm with a manual microtome Leica Reichert-Jung 2040[3] (Leica Microsystems, Wetzlar, Germany), stained with Lee's stain (methylene blue and basic fuchsin), and mounted in a synthetic resin of dibutyl phthalate xylene on microscope slides. Gonads were classified according to their size and color and the presence or absence of specific inclusions (oil droplets, yolk, postovulatory follicles, or sperm), as well as the type of oocytes (Wallace and Selman, 1981).

Reproductive biology

The spawning season was estimated by analyzing the monthly variation in the percentage of maturity phases and the changes in gonadosomatic index (GSI) and hepatosomatic index (HSI) for mature fish of each sex (Afonso-Dias and Hislop, 1996; Colmenero et al., 2013). Because indeterminate individuals (n=27) were not considered, 251 males and 278 females were used to determine both indices, which were calculated according to Yoneda et al. (2001) as

$$GSI = (GNW\ /\ GW) \times 100 \qquad (1)$$

and

$$HSI = (LW\ /\ GW) \times 100. \qquad (2)$$

The lengths at which 25%, 50%, and 75% of sampled fish reached sexual maturity were estimated by fitting the proportion of sexually mature males and females (phase III, phase IV, or phase V) and for both sexes combined to the logistic equation (Colmenero et al., 2013):

$$P = 100\ /\ (1 + \exp\ [a + bTL]), \qquad (3)$$

where P = the percentage of mature individuals as a function of size class (measured in TL); and
a and b are specific parameters that can change during the life cycle.

A logarithmic transformation was applied to this equation to calculate the parameters a and b by means of linear regression.

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[3] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Oocyte development and fecundity

Oocyte diameters obtained from 21 randomly selected specimens at all phases of maturity were measured to the nearest 0.01 µm with an image analysis program (Image-Pro Plus, vers. 5.0, Media Cybernetics, Inc., Rockville, MD) in combination with an Axioskop 2 Plus microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY), and a ProgRes C14 digital microscope camera (Jenoptik AG, Jena, Germany). Only oocytes sectioned through the nucleus were taken into account. The developmental stages of the oocytes were categorized according to the descriptions in Colmenero et al. (2013) that were adapted from Wallace and Selman (1981). The mean oocyte diameter by developmental stage was determined by calculating the diameter of all oocytes encountered in each subsample, and the range was set with the smallest and largest oocytes found at each developmental oocyte stage.

Fecundity was determined by using the gravimetric method described by Hunter and Goldberg (1980). Because homogeneity in oocyte distribution within ovaries of white anglerfish has already been established (Afonso-Dias and Hislop, 1996), ovarian tissue subsamples of approximately 500 mg were taken randomly from 2 specimens with ovaries in phase III that had neither postovulatory follicles nor atretic oocytes present. Whole tissue subsamples were placed on several slides and covered with cover slips, then photographed with a Canon Powershot SD870 IS digital camera (Canon USA, Melville, NY). Oocytes were counted manually with Image-Pro Plus.

Batch fecundity (BF), the total number of hydrated oocytes produced in a single spawning event by an individual female, of each female was determined by means of this equation:

$$BF = (oocyte\ number\ /\ sampled\ GNW) \times total\ GNW, \quad (4)$$

where BF is the product of the number of secondary vitellogenic oocytes per unit of weight multiplied by the total ovarian weight (Yoneda et al., 2001). Relative batch fecundity (RBF), the total number of mature eggs released by a female during the spawning batch per gram of female GW, was calculated with the following equation (Pavlov et al., 2009):

$$RBF = BF\ /\ GW. \quad (5)$$

Potential fecundity was calculated as the number of vitellogenic oocytes divided by TW in kilograms for each mature female and then averaged (Murua et al., 2003).

Results

Gonad morphology

The gonad of female white anglerfish has 2 ribbon-like ovarian lobes connected to each other at their posterior end. One side of the “ribbon” consists of an ovigerous membrane from which a single layer of oocyte clusters, which contain oocytes at different developmental stages, projects into the lumen. The other side is nonovigerous and secretes a gelatinous material during maturation that fills the ovarian lumen, where mature oocytes develop (Fig. 2). During maturation, the gonad increases in size until it fills the abdominal cavity (Fig. 3). Testes are a pair of elongated organs with a bean shape in transverse section. Spermatogenesis takes place in a capsule-like sac called a *cyst*, but it is completed in the lumina of the lobules. The cysts appear to be arranged with a gradient of germ cells of increasing maturation from the cortex to the sperm duct (Fig. 4).

Spawning season

The monthly distribution of maturity phases (Fig. 5) revealed a peak in reproduction during spring, when a major portion of the spawning females and the highest value of GSI (0.77) were found. Spawning capable females (phase III) were caught primarily between April and June, and females in the actively spawning phase (IV) were observed in November, December, and March—the latter month having the maximum occurrence (11%). Females in immature, regressing, and developing or regenerating phases (I, V, and II, respectively) were found year-round, although the highest percentage of immature individuals (49%) was observed in January. The GSI values followed the same pattern shown in these maturity phases: highest during spring, decreasing during summer and autumn, and increasing again during winter. Males in all maturity phases were observed throughout the year, but with a maximum percentage of mature males (66%) in February and March. Immature males were found primarily in July (69%). The mean GSI for females increased as their ovaries developed and peaked in phase IV. For males, the mean GSI increased with testicular development and reached a maximum in phase IV (Table 1). The mean HSI for females and males increased during the summer and autumn months and decreased during winter and spring. On the basis of these observations, a main spawning season was found from February through June and a secondary one occurred in November and December.

Size at sexual maturity

The maturity ogive for males indicates that the length at which 50% of them reached sexual maturity (L_{50}) was 48.4 cm TL (Fig. 6A). Maturity in males occurred at about 37% of their maximum observed TL. The smallest mature male found was 32.5 cm TL, and the largest immature male was 50 cm TL. The maturity ogive for females indicates that L_{50} was 59.9 cm TL (Fig. 6B). Female maturity occurs at about 30% of their maximum observed TL. Like the smallest male, the smallest mature female was 32.5 cm TL. The largest immature female measured 56 cm TL. The maturity ogive for the sexes combined indicates an L_{50} of 51.3

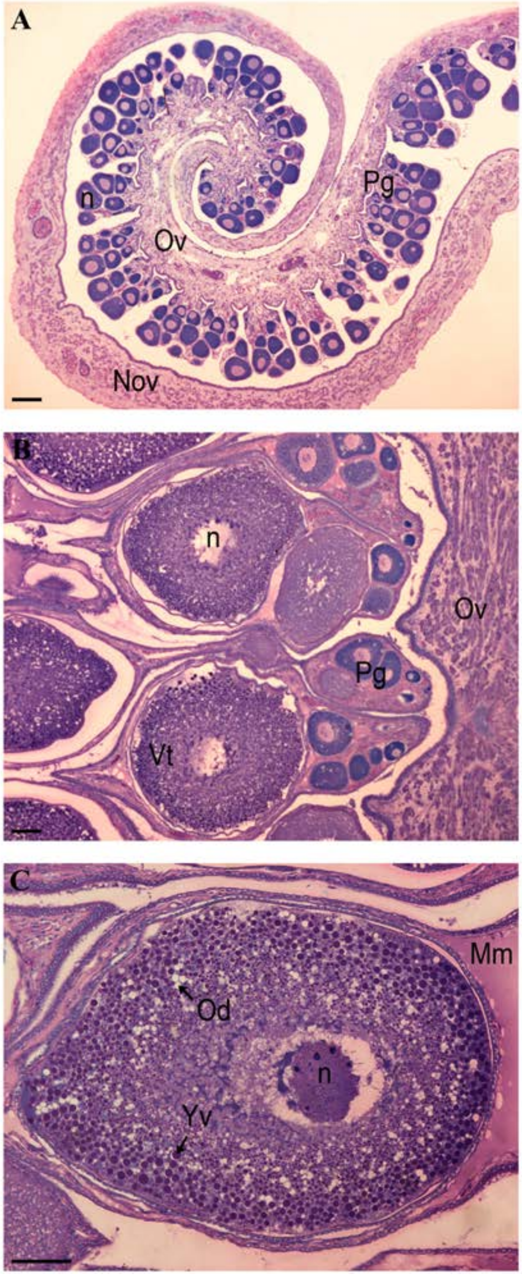

Figure 2

Histological sections from ovaries of female white anglerfish (*Lophius piscatorius*) in 3 phases of maturity: (**A**) immature, (**B**) spawning capable, and (**C**) actively spawning. Ov=ovigerous membrane, Nov=nonovigerous membrane, n=nucleus, Pg=primary growth stage, Vt=vitellogenesis stage, Od=oil droplet, Yv=yolk vesicle, Mm=mucus matrix. Scale bars=100 µm.

cm TL. The lengths at which 25% and 75% of fish attained maturity were 43.5 and 53.4 cm TL for males, 48.6 and 71.1 cm TL for females, and 44.7 and 58 cm TL for the sexes combined.

Oocyte development and fecundity

Oocytes in different developmental stages were found in each maturity phase. They were organized in clusters where a gradient in the size of the oocyte was observed. A group of oocytes differentiated from others as the ovaries developed, indicating that white anglerfish has group-synchronous oocyte development and can be considered to have determinate fecundity (Fig. 7). Ovaries at each maturity phase contained primary oogonia- and perinucleolar-stage oocytes. Chromatin nucleolar were difficult to find and were present only in immature phase. Females at the cortical alveolar stage were not found in our samples. Vitellogenic and hydrated oocytes were located in females capable of spawning. Oocyte diameters at each stage of oocyte development are shown in Table 2.

Batch fecundity ranged from 661,647 to 885,214 oocytes from 2 females that measured 76 and 105 cm TL, 6331 and 16,178 g TW, and 5182 and 13,330 g GW, respectively. Relative batch fecundity ranged from 66 to 128 oocytes/g GW (average of 97 oocytes/g GW [standard deviation, SD 43]). Potential fecundity values moved from 54,717 to 104,506 oocytes/kg TW with a mean of 79,612 oocytes/kg TW (SD 35,206).

Discussion

Relevance of reproductive traits for sustainable management

Fishing activity during spawning seasons may affect population parameters, specifically composition of the size distribution, mortality rate, sexual structure of the population, size at maturity, and changes in the spawning season. These parameters, in turn, can increase the risk of over-exploitation of a stock.

Fishing during spawning periods may result in targeting a specific size class of the population and thus increasing the chance of catching the older (and larger) age classes and making the stock vulnerable to reproductive collapse (van Overzee and Rijnsdorp, 2015). Because spawning is generally limited to specific areas and times (Cushing, 1990), the conservation of resources can be enhanced by limiting fishing activity in a spatiotemporal frame. Furthermore, fishing pressure has been documented to have reduced initial size at maturity—an issue that is a concern particularly for late-maturing species (Stewart et al., 2010). If size of capture is below the size at first maturity, there is a genuine risk of recruitment overfishing. Therefore, knowledge of the spawning season and the size at maturity can help managers establish closed seasons and prevent fishing at this vulnerable time in the life cycle

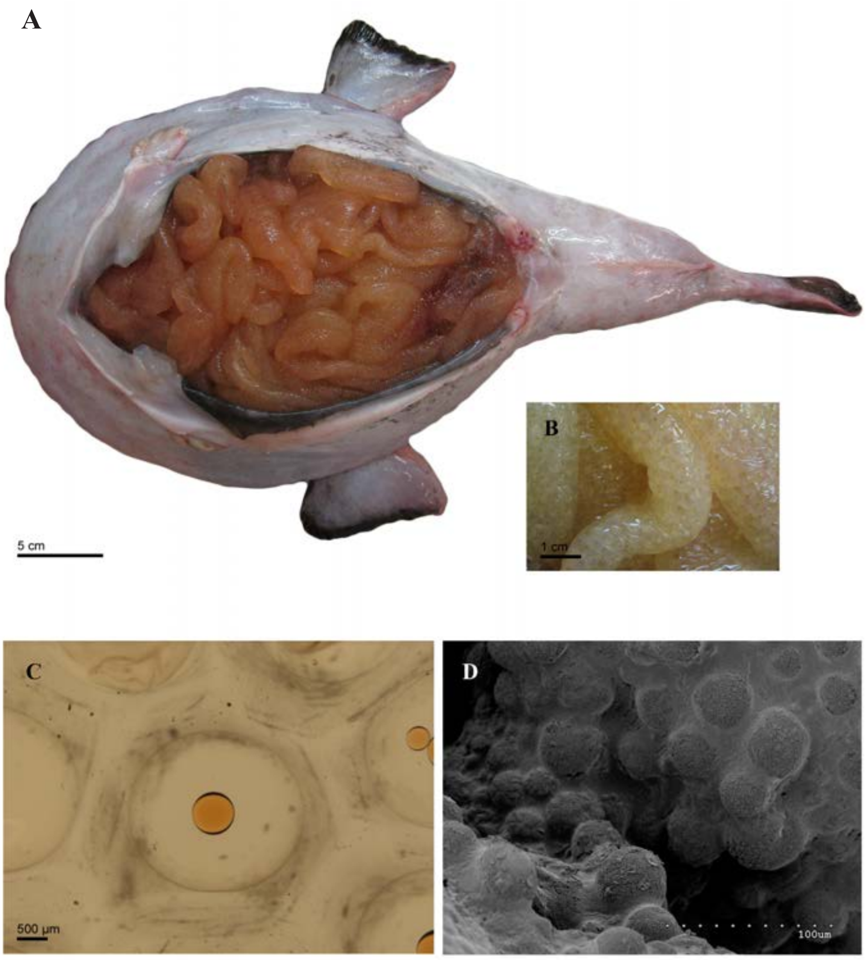

Figure 3

Images of a female of the species *Lophius* showing (**A**) an ovary that occupies the entire abdominal cavity; (**B**) a close-up of the female gonad; and (**C**) a hexagonal chamber of the mucoid veil, which contains an unfertilized egg of white anglerfish (*Lophius piscatorius*) and (**D**) a scanning electron micrograph of yolk vesicles from the inside of the ovum of a white anglerfish.

of fish species by preserving breeding individuals and establishing a legal minimum size.

The results of our study of white anglerfish in the northwestern Mediterranean Sea indicate that a long spawning period occurs during mid-winter and late spring, from February through June, although a secondary breeding period has been observed in November and December. These results agree with those obtained in studies that were focused on the northeastern Atlantic Ocean, where this species spawns from November through June (Fulton, 1898; Afonso-Dias and Hislop, 1996; Hislop et al., 2001). However, a previous study in the northwestern Mediterranean Sea identified a spawning season during spring–summer (Ungaro et al., 2002). Discrepancies between the latter study and our work may be explained by the differences in sampling periods.

Nevertheless, spawning seasonality, which is associated with environmental conditions and local oceanographic features, varies between species as well as by geographical area. An example of this variability in spawning seasonality can be observed in 2 locations along the Atlantic–Iberian coast: on the Portuguese and western Spanish coasts, spawning of the white anglerfish takes place during winter–spring (Duarte et al., 2001), whereas on the northern Spanish coast (Bay

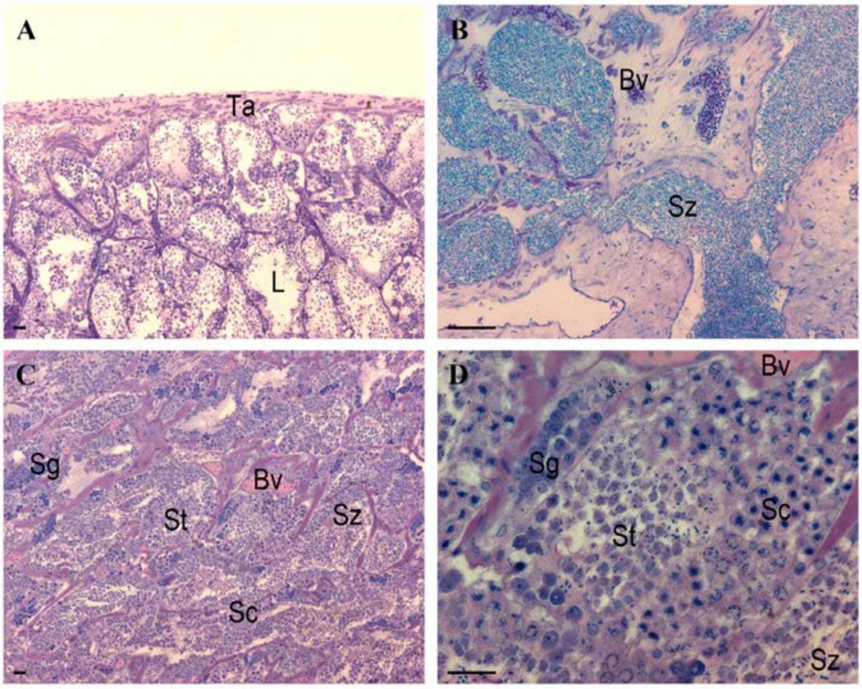

Figure 4

Histological sections of testes from actively spawning male white anglerfish (*Lophius piscatorius*) showing (**A**) lobular organization, (**B**) spermatozoa in the lumen of the seminal lobules and in the sperm duct, (**C**) seminal lobules during spermatogenesis, and (**D**) a close-up of the seminal lobules. Ta=tunica albuginea, L=seminal lobule, Bv=blood vessel, Sz=spermatozoa, Sg=spermatogonia, Sc=spermatocyte, St=spermatid, Scale bars=25 µm.

of Biscay), spawning occurs during summer (Quincoces et al.[4]). In fact, spawning activity for one of its congenerics, the black anglerfish, in the northwestern Mediterranean seems to occur from November through March and a secondary spawning occurs in August and September (Colmenero et al., 2013). Although a little overlap exists between spawning seasons of both of these *Lophius* species in Mediterranean waters, the main period is markedly different, and that difference lessens competition among these species.

Usually, species of *Lophius* have long spawning periods ranging between 4 and 6 months. Black anglerfish off the Spanish-Atlantic coasts spawn from November through February (Duarte et al., 2001), and in the Bay of Biscay the peak spawning period is from May through July (Quincoces et al.[5]). The goosefish off the East Coast of the United States has its reproductive period from May through June (Armstrong et al., 1992), spawning for the blackfin goosefish off the Brazilian coasts takes place during spring and summer (Valentim et al., 2007), and the yellow goosefish spawns between February and May in the East China Sea and the Yellow Sea (Yoneda et al., 2001). The devil anglerfish off the coast of South Africa has a well-defined summer breeding season (Griffiths and Hecht, 1986), and individuals of this species off the coast of Namibia spawn throughout the year with a slight increase between autumn and spring (Maartens and Booth, 2005).

Most deep-sea fish species reach sexual maturity at sizes larger than those of species that inhabit the continental shelf reach maturity, and, in some cases, males mature at smaller sizes than females (Rotllant et al., 2002; Pajuelo et al., 2008). A similar pattern was observed for white anglerfish—one in which females mature sexually at larger sizes (59.9 cm TL) than those recorded for males (48.4 cm TL). This pattern has also

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[4] Quincoces, I., M. Santurtún, and P. Lucio. 1998. Biological aspects of white anglerfish (*Lophius piscatorius*) in the Bay of Biscay (ICES Division VIIIa, b, d), in 1996–1997. ICES Council Meeting (C.M.) Documents 1998/O:48, 29 p.

[5] Quincoces, I., P. Lucio, and M. Santurtún. 1998. Biology of black anglerfish *Lophius budegassa* in the Bay of Biscay waters, during 1996–1997. ICES Council Meeting (C.M.) Documents 1998/O:47, 28 p.

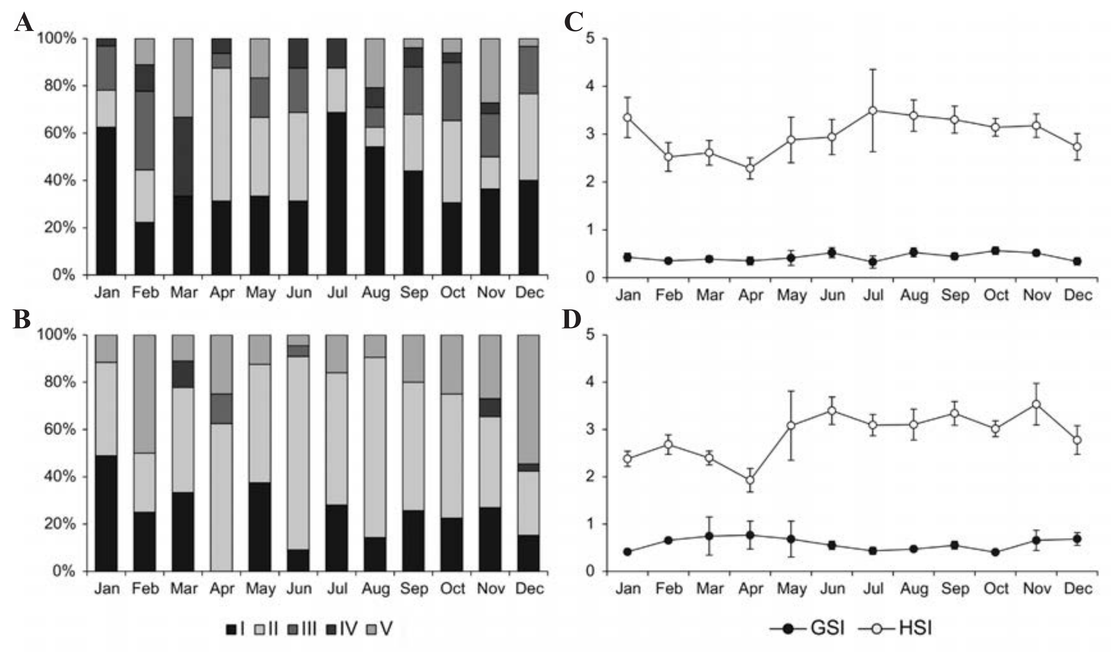

Figure 5

Monthly distribution of maturity phases of gonads for (**A**) males (n=251) and (**B**) females (n=278) and monthly changes in the mean gonadosomatic (GSI) and hepatosomatic (HSI) indices for (**C**) males (n=135) and (**D**) females (n=202) of white anglerfish (*Lophius piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. On the basis of macroscopic examination, specimens were assigned to the following 5 phases: immature (phase I), developing or regenerating (phase II), spawning capable (phase III), actively spawning (phase IV), and regressing (phase V). Error bars indicate standard error of the mean.

been found for white anglerfish in other areas (Ofstad and Laurenson[6]) and for other species of anglerfish. Female black anglerfish, for example, mature at 48.2 cm TL, whereas males attain first maturity at 33.4 cm TL (Colmenero et al., 2013), and female devil anglerfish reach sexual maturity at 58.2 cm TL, whereas males of this species mature at 39.9 cm TL (Maartens and Booth, 2005). For the goosefish, L_{50} for females and males was estimated at 48.5 and 36.9 cm TL, respectively (Armstrong et al., 1992), and female yellow goosefish mature at 56.7 cm TL and males of this species mature at 36.2 cm (Yoneda et al., 2001). This dissimilarity in size at maturity is usually associated with a trade-off between life history traits, where early maturity involves a larger size but a slower growth (Stearns and Koella, 1986; Charnov, 2008).

Reproductive strategy

The reproductive strategy of white anglerfish is one of discontinuous oogenesis with synchronous development of vitellogenic oocytes and is, therefore, this species is considered a total spawner (Afonso-Dias and Hislop, 1996). The oocytes ovulate at once, and the eggs are released in either a unique event or over a short period of time, as part of a single episode during the spawning season (Murua and Saborido-Rey, 2003; Pavlov et al., 2009). This pattern of oocyte development and spawning patterns is also found in other species of *Lophius* (Leslie and Grant, 1990; Armstrong et al., 1992; Colmenero et al., 2013). Yoneda et al. (2001) suggested that yellow goosefish may have the potential to spawn more than once a year, on the basis of the observation of a captive specimen that released several infertile egg masses. However, this spawning behavior cannot be considered normal.

Female anglerfish spawn their eggs in a mucoid veil that floats near the surface. The veil consists of individual chambers that contain 1–3 eggs and has an opening that provides water circulation. In our study, we recognized in some chambers the presence of 2 eggs sharing the same chamber. Although this way of releasing eggs is not common among fish species, some Scorpaeniformes, such as the shortfin turkeyfish (*Dendrochirus brachypterus*) (Fishelson, 1978) or the short-

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[6] Ofstad, L. H., and C. Laurenson. 2007. Biology of anglerfish *Lophius piscatorius* in Faroese waters. ICES Council Meeting (C.M.) Documents 2007/K:07, 16 p.

Table 1

Gonadosomatic (GSI) and hepatosomatic (HSI) indices at each maturity phase for male and female white anglerfish (*Lophius piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010. SE=standard error.

Sex	Maturity phase	GSI range	Mean GSI (SE)	HSI range	Mean HSI (SE)	*n*
Male	I	0.01–0.41	0.10 (0.01)	1.04–4.65	2.37 (0.07)	106
	II	0.06–1.07	0.25 (0.03)	0.27–5.11	2.67 (0.14)	54
	III	0.21–1.30	0.61 (0.05)	1.92–6.72	3.20 (0.18)	35
	IV	0.30–1.70	0.70 (0.09)	0.40–5.39	3.34 (0.28)	17
	V	0.19–1.11	0.50 (0.06)	2.04–5.10	3.35 (0.22)	17
Female	I	0.01–0.86	0.23 (0.02)	0.92–5.33	2.37 (0.11)	66
	II	0.04–1.22	0.40 (0.02)	0.42–7.79	2.87 (0.10)	133
	III	0.61–1.65	1.13 (0.52)	2.15–5.83	3.99 (1.85)	2
	IV	1.59–3.86	2.81 (0.52)	2.68–8.50	5.80 (1.20)	4
	V	0.18–2.44	0.66 (0.06)	0.36–8.59	3.03 (0.17)	60

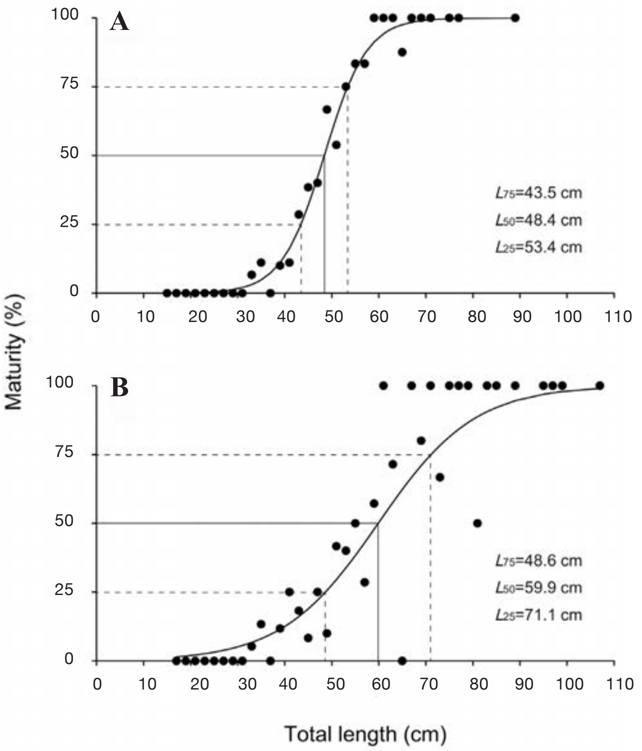

Figure 6

Maturity ogives for (**A**) male and (**B**) female white anglerfish (*Lophius piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010.

spine thornyhead (*Sebastolobus alascanus*) (Erickson and Pikitch, 1993), also spawn buoyant gelatinous egg masses. It has been proposed that the advantages of releasing eggs in these veils facilitate their dispersal; the egg veil floats near the surface and is subject to the actions of wind, currents, and waves. The veil also serves as protection for eggs against predation because of the presence of obnoxious or toxic substances in the veils (Armstrong et al., 1992). Moreover, the veil may help with the fertilization of eggs. When males are present and the egg ribbon is laid, the ribbon keeps the eggs together and prevents their dispersion through the water. The males then eject milt near the veil to guarantee fertilization of all the eggs (Dahlgren, 1928). Armstrong et al. (1992) suggested that sperm reach oocyte chambers through the pores that connect the chambers when the ribbon is extruded from the female and starts to absorb water.

Another feature of the reproduction of the white anglerfish to highlight is its type of spermatogenesis, which is known to be semicystic. Spermatogenesis starts inside the cysts that contain germinal cells in different stages of development from spermatogonia to spermatids, but it is not completed within the cyst. During spermatogenesis, the cyst breaks and spermatids are released from the cyst into the lumen of the lobule, where they become spermatozoa. This kind of spermatogenesis has been described previously in the family Lophiidae only in the blackmouth angler (*Lophiomus setigerus*) (Yoneda et al., 1998) and in black anglerfish

Table 2

Oocyte diameters, ranges and means with standard errors (SEs), and histological characteristics of ovarian follicles in white anglerfish (*Lophius piscatorius*), collected from the northwestern Mediterranean Sea between June 2007 and December 2010. SE=standard error, at each stage of oocyte development.

Stages of oocyte development	Mean oocyte diameter (µm) (SE)	Oocyte diameter (µm) range	Histological characteristics
Primary growth stage	82.79 (2.34)	12–203	Nucleus contains a large nucleolus and some peripheral nucleoli. Yolk granules are not present in the cytoplasm.
Cortical alveolar stage	256.10 (3.08)	207–316	Cortical alveolar vesicles and oil droplets appear in the cytoplasm. Yolk granules are not yet present in the cytoplasm. Nucleus is central within the yolk
Vitellogenesis	729.31 (17.58)	324–876	Yolk granules appear between cortical alveolar vesicles. As vitellogenesis advances, yolk granules fill the cytoplasm until they are in contact with the nucleus, which remains in a central position.
Migratory nucleus	939.31 (7.77)	902–1008	Yolk granules and oil droplets start to fuse. Nucleus migrates to one pole of the oocyte.
Hydration	1672.50 (4.77)	1523–1750	Yolk granules form a single mass. Nucleus is not present in the cytoplasm.

(Colmenero et al., 2013). This specialized spermatogenesis also has been found in other deep-sea species of Neoceratiidae (Jespersen, 1984) and of Macruridae (Fernandez-Arcaya et al., 2013), in the shore clingfish (*Lepadogaster lepadogaster*) (Mattei and Mattei, 1978), in species of Blennidae (Lahnsteiner and Patzner, 1990), in a species of *Ophidion* (Mattei et al., 1993), in the dusky jawfish (*Opistognathus whitehursti*) (Manni and Rasotto, 1997), and in species of *Scorpaena* (Muñoz et al., 2002; Sàbat et al., 2009), which also release their eggs in gelatinous substances.

Fecundity

Because of their particular reproduction behavior, which includes a high parental investment in the offspring, white anglerfish are likely to spawn once a year, and the population dynamics of this species are expected to be highly sensitive to external biological and ecosystem factors (ICES[7]). Spawning occurs in deep waters because mature white anglerfish have been described by Hislop et al. (2001) as migrating to deeper water before spawning. The same behavior is seen in yellow goosefish: adult fish migrate to deeper waters in response to seasonal changes in water temperature and gonadal maturation (Yoneda et al., 2002). These vertical migrations into deeper waters where commercial fishing and scientific surveys cannot reach could be the reason that very few mature females were captured during our study—a trend that is common in other studies of *Lophius* species (Ofstad and Laurenson[6]). Clearly, this low number of mature females will affect the estimation of such reproductive parameters as fecundity.

Generally, deep-sea species have low fecundity and large egg sizes (Gage and Tyler, 1991; Herring, 2002). The white anglerfish has determinate fecundity with values between 661,647 to 885,214 oocytes—levels that are high in comparison with other deep-sea species that inhabit the same depth strata but that are similar to the mean potential fecundity of its Mediterranean congeneric, the black anglerfish (Colmenero et al., 2013). Fecundity values vary among populations as a result of adaptations to local environmental conditions, and they are related to abiotic factors, such as temperature and salinity (Nissling and Dahlman, 2010; Thorsen et al., 2010), and to biotic factors, such as food supply, population density, allocation of energy to reproduction, and fish size (Treasurer, 1981; Merrett, 1994; Nash et al., 2000).

In this study, we were not able to determine correlations between fecundity and these factors because only 2 actively spawning females were collected. Eggs of white anglerfish have been reported to have a mean diameter of 2.72 mm (SD 0.08) (Colmenero et al., 2015b), a size that is considered large for pelagic eggs, which typically range from 0.5 to 5.5 mm in diameter (Ahlstrom and Moser, 1980). Larger eggs have more

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[7] ICES (International Council for the Exploration of the Sea). 2012. Report of the working group on the assessment of southern shelf stocks of hake, monk and megrim (WGHMM), 10–16 May 2012, ICES Headquaters, Copenhagen, Denmark. ICES CM 2012/ACOM:11, 617 p.

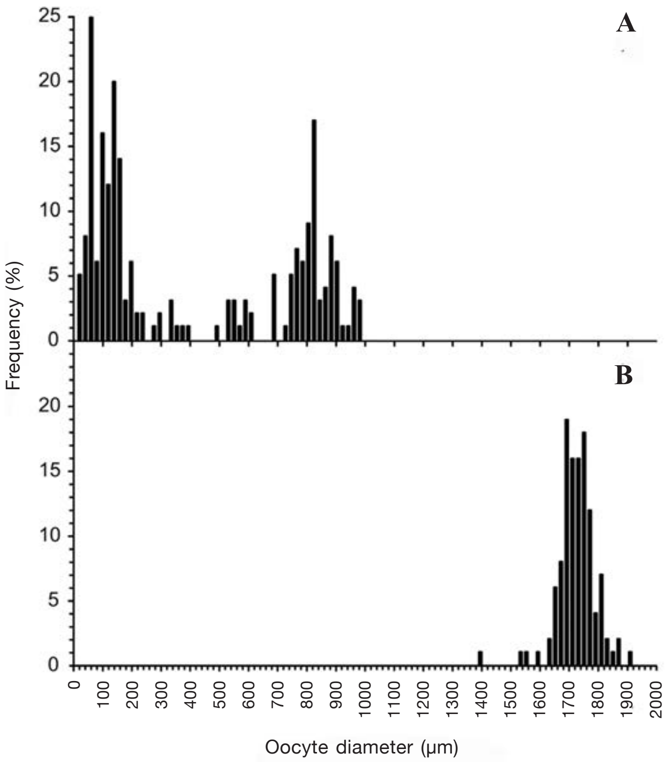

Figure 7

Distribution of oocyte diameters in (**A**) spawning capable and (**B**) actively spawning female white anglerfish (*Lophius piscatorius*) collected from the northwestern Mediterranean Sea between June 2007 and December 2010.

yolk, which increases the potential for larval survival (Duarte and Alcaraz, 1989). The only information available about egg diameters for other species of *Lophius* is for yellow goosefish, which occupy a bathymetric range that is similar to that occupied by white anglerfish and have a similar egg size (Yoneda et al., 2001). In contrast, the black anglerfish has an egg diameter of 1.88 mm (SD 0.12), a size that is nearly 1.5 times smaller than the diameters reported for the white anglerfish and yellow goosefish, and inhabits shallower waters than those inhabited by the other 2 species (Colmenero et al., 2015b). A comparative study of egg sizes in deep-sea species found that egg size increased significantly with depth (Fernandez-Arcaya[8]). Egg size is important to offspring survival in many organisms, and large eggs survive better than small ones in environments where dissolved oxygen is low (Hendry and Day, 2003).

General remarks

In this study, we estimated the spawning season, size at sexual maturity, and fecundity of white anglerfish. Considering the parameter values that we obtained, we can conclude that this species is one that employs a *K* reproductive strategy. In general, this strategy is defined by a large body size, longevity, late maturation, and low fecundity (Pianka, 1970, 1974). A wide range of deep-sea demersal fish species generally display life history characteristics consistent with *K*-selection (Adams, 1980; Gage and Tyler, 1991). These traits make deep-sea fish stocks highly vulnerable to fishing and capable of little resilience to over-exploitation, increasing the urgency for the conservation and management of this group of animals (Koslow et al., 2000; Morato et al., 2006; Norse et al., 2012).

Theoretically, the *K*-strategy for deep-sea fish species should imply a low fecundity; however, some species, such as the North Pacific armorhead (*Pseudopentaceros wheeleri*), wreckfish (*Polyprion americanus*), and splendid alfonsino (*Beryx splendens*), have high fecundities (Sedberry et al., 1996; Lehodey et al., 1997; Humphreys, 2000). White angelfish and species of *Lophius* in general also should be included in this group because of their high fecundity (Afonso-Dias and Hislop, 1996; Colmenero et al., 2013). This variability in reproductive strategy is the result of adaptation to environmental changes, such as temperature, bathymetric pressure, light, and food availability (Herring, 2002; Brown-Peterson et al., 2011). Likely, the high fecundity and the low economic value of the white anglerfish, at least until the last decades of the 20th century, has allowed the stock to be sustainable within acceptable limits. With the recent expansion of anglerfish fisheries, sustainability is in question, and our study is the first step toward an informed assessment of this deep-sea resource and its management with an ecosystem perspective.

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[8] Fernandez-Arcaya, U. 2015. Personal commun. Departamento de Recursos Marinos Renovables, Institut de Ciències del Mar, Passeig Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain.

Martínez for helping with data collection, A. Ospina for assisting with the map of the study area, and C. Barría, N. Amat, and R. Alarcon for their valuable comments. We offer special thanks to K. Denning for revising the English of the early draft. This study was part of the project Monitoratge del recursos pesquers i marisquers al litoral català of the Directorate of Fishing and Maritime Affairs, Government of Catalonia.

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The chorion ultrastructure of ova of *Lophius* spp.

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The chorion surface ultrastructure of unfertilized eggs of black anglerfish *Lophius budegassa* and white anglerfish *Lophius piscatorius* was examined by scanning electron microscopy. Species-specific differences were observed.

Key words: anglerfish; egg identification; *Lophius budegassa*; *Lophius piscatorius*; micropyle; monkfish.

Identification of fish eggs is one of the most difficult problems faced when working with ichthyoplankton (Olivar, 1987). Some characteristics observed under light microscopy, which include egg shape, egg size, chorion structure, yolk structure, presence or absence of oil globule and perivitelline space, have been proposed by several authors in order to solve this problem (Ahlstrom & Moser, 1980; Markle & Frost, 1985). Many of these characteristics are the same over large groups of fishes and the use of a scanning electron microscope (SEM) helps to discern differences that then aid in identification of eggs. In marine teleosts, the structure of the egg chorion has been considered as an important taxonomic characteristic for identifying eggs of different species because it is an indicator of the systematic relationships of the species (Ivankov & Kurdyayeva, 1973; Chen *et al.*, 2007) and it has a morphology that is species specific (Hagström & Lönning, 1968). Although there are major differences in the structure of the chorion in different species of teleosts with respect to thickness, number of lamellae and the presence or absence of pores due to the diversity in habitat and breeding habits of teleosts (Kuchnow & Scott, 1977), geographic differences in chorion structure were found between eggs from the same species (Lönning & Solemdal, 1972). These variations may be correlated with differences in salinity, temperature and viscosity of the seawater, necessitating adjustment in the structure of pelagic eggs to secure adequate buoyancy (Lönning, 1972). For this reason, it is important to characterize eggs of different species from different geographic locations. The chorion is perforated by the micropyle, through which a spermatozoan can penetrate the chorion for fertilization.

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TABLE I. Characteristics of fresh ova examined under light microscope in Mediterranean *Lophius* spp.

	Ova diameter (mm)		Oil globule diameter (mm)	
Species	Mean ± S.D.	Range	Mean ± S.D.	Range
Lophius budegassa	1·88 ± 0·12	1·52–2·05	0·48 ± 0·03	0·41–0·56
Lophius piscatorius	2·72 ± 0·08	2·45–2·90	0·63 ± 0·02	0·59–0·68

The microstructure of the micropyle in unfertilized eggs is an important characteristic in fish identification (Chen *et al.*, 1999; Li *et al.*, 2000). The aim of the present work is to examine morphological characteristics of ova in the two species of *Lophius* inhabiting the Mediterranean Sea by means of light microscopy and SEM, provide some information about their chorion ultrastructure and to use these findings for aid in taxonomic classification from ichthyoplankton samples.

In this study, specimens of black anglerfish *Lophius budegassa* Spinola 1807 and white anglerfish *Lophius piscatorius* L. 1758 were collected on board commercial trawler fishing vessels in the north-western Mediterranean Sea at depths of 20–600 m and identified following Caruso (1986). Unfertilized eggs were removed from gonads, measured under a light microscope and prepared for SEM using two different techniques: cryo-SEM (Klein *et al.*, 1992; Sempere & Santamarina, 2011) and critical-point drying (Boehlert, 1984). For cryo-SEM, unfixed fresh ova were placed on a polycarbonate filter and mounted on aluminium stubs by means of conducting gel. Ova were then immediately plunged into liquid nitrogen slush. Once samples were frozen, they were introduced into the cryo preparation chamber Quorum PP3000T (www.quorumtech.com) where water sublimed at −90° C within 5 min, leaving ova free of impurities. After ice sublimation, ova were sputter coated with platinum and transferred inside the chamber of the Hitachi S-3500N SEM (www.hitchi-hitec.com) maintained at −130° C, where samples were examined and photographed with an acceleration voltage of 5 kV or less. Ova shrinkage of *c.* 4 % was observed using this method. For critical-point drying, samples used were fixed in 10% formalin. Before observation under SEM, ova were rinsed with filtered seawater and prepared following Olivar (1987) protocol: (1) post-fixed in 2% osmium tetroxide, (2) dehydrated in a graded series of ethanol, (3) critical-point dried (Bal-Tec CPD 030; www.leica-microsystems.com) and (4) coated with a layer of gold-palladium prior to viewing. This method showed shrinkage of *c.* 40%. Ova and oil globule diameter of fresh unfertilized eggs were measured under a light microscope (Table I). Measurements of chorion characteristics including thickness, ova size, pore diameter, minimum distance between pores, pore density and micropyle diameter of fixed ova were obtained using photographs from both SEM techniques (Table II). All measurements were performed using Image-Pro Plus software (www.mediacy.com). Differences in these characteristic values were analysed statistically by using a Mann–Whitney *U*-test. Statistical analyses were performed using SPSS Statistics software (www-01.ibm.com/software/analytics/spss/products/statistics/downloads.html) with statistical significance level set at $P < 0·05$. All data presented are mean ± S.D.

The unfertilized eggs of both species of *Lophius* are transparent and spherical in shape [Fig. 1(a)]. Ova of both species have a single oil globule, yellow-ochre in colour

TABLE II. Characteristics of fixed ova examined under scanning electron microscope in Mediterranean *Lophius* spp.

	Ova diameter (mm)		Pore diameter (µm)		Minimum distance (µm)		Density (number of pores per 100 µm^2)	
Species	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.	Range
Lophius budegassa	1·11 ± 0·10	1·01–1·29	0·12 ± 0·03	0·06–0·22	1·39 ± 0·22	0·93–1·98	56 ± 14	40–70
Lophius piscatorius	1·67 ± 0·08	1·52–1·75	0·10 ± 0·02	0·06–0·16	1·09 ± 0·17	0·62–1·48	91 ± 7	81–100

and located slightly off-centre position [Fig. 1(b)]. Some eggs of the species of *Lophius* hold multiple oil globules because before fertilization there is a coalescence of many oil globules into a single one and some of the studied ova might be in the middle of this process. Another reason could be the easy rupture of this structure during gonad manipulation. Both ova and oil globules of *L. piscatorius* are significantly larger than *L. budegassa* (Mann–Whitney *U*-test, $P < 0{\cdot}05$). The chorion of *L. budegassa* and *L. piscatorius* are smooth and transparent [Fig. 2(a), (b)], with oval pores distributed evenly over the surface. Both species have similar pore patterns although *L. piscatorius* had more pores per unit area [Fig. 2(c), (d)]. The pore diameter and minimum distance between pores are significantly less in *L. budegassa* (Mann–Whitney *U*-test, $P < 0{\cdot}05$). The granular matter observed on the surface of the chorion might be remains of its mucous layer dissolved by the fixation methods used in SEM. In *L. piscatorius*, the chorion consists of a thin outer layer and a thicker lamellated inner layer (1·15 ± 0·08 µm) with five lamellae [Fig. 3(a)]. The micropyle observed on an ovum prepared for cryo-SEM was funnel-shaped with an aperture diameter of 74·87 ± 0·68 µm that leads to the micropyle canal which traverses the entire chorion layer [Fig. 3(b)].

These results show that ova size, oil globule diameter, pore diameter, minimum distance between pores and pore density are useful characteristics for distinguishing the ova of *L. budegassa* and *L. piscatorius*. *Lophius piscatorius* has the largest ova,

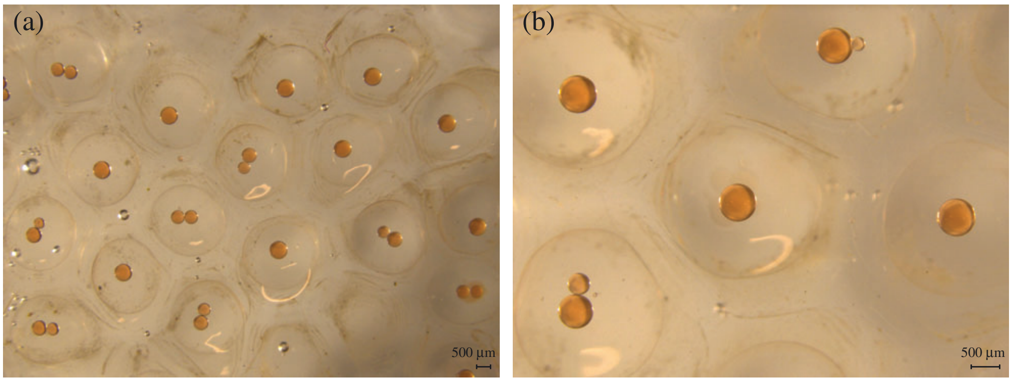

FIG. 1. Unfertilized eggs of *Lophius piscatorius*. (a) General view and (b) closed-up of ova and oil globules.

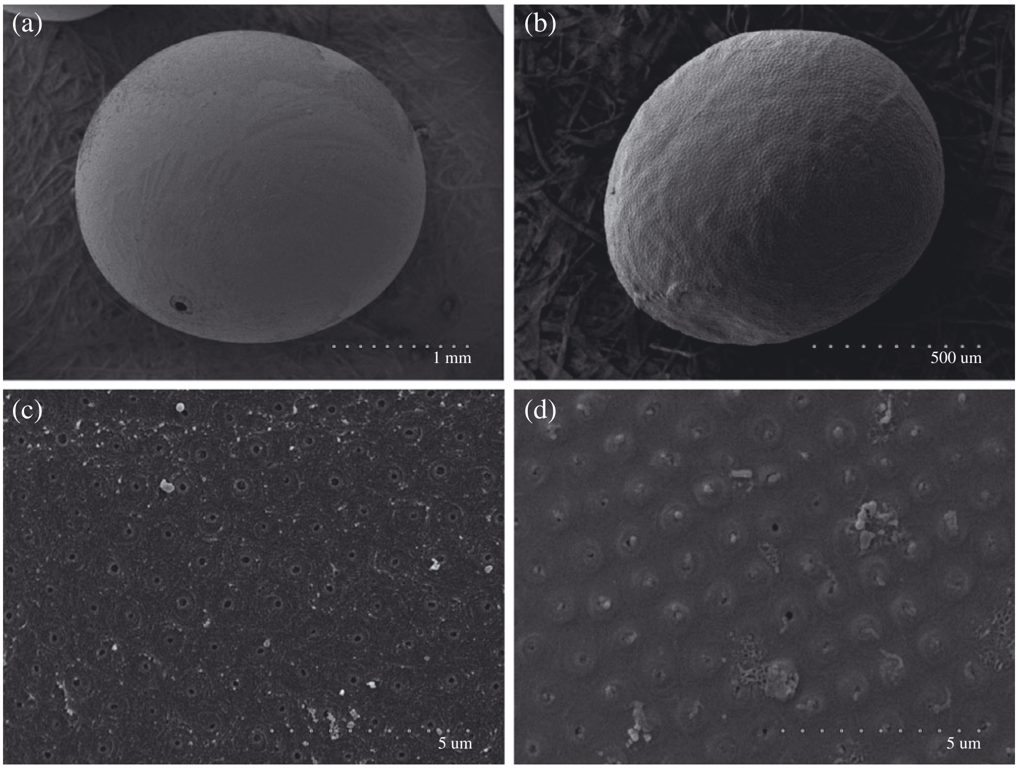

FIG. 2. Scanning electron micrographs of (a) ovum of *Lophius piscatorius* and (b) ovum of *Lophius budegassa*; chorion surface with pores of (c) *L. piscatorius* and (d) *L. budegassa*.

which measures 2·72 ± 0·08 mm in diameter, being within the range given by Bowman (1920) for Atlantic specimens, whilst ova diameter of *L. budegassa* was measured in this study as 1·88 ± 0·12 mm, which is the first available data from non-fixed ova. Egg size is important to offspring survival in many organisms where a positive correlation between adult female size and egg size is common (Hendry & Day, 2003). This

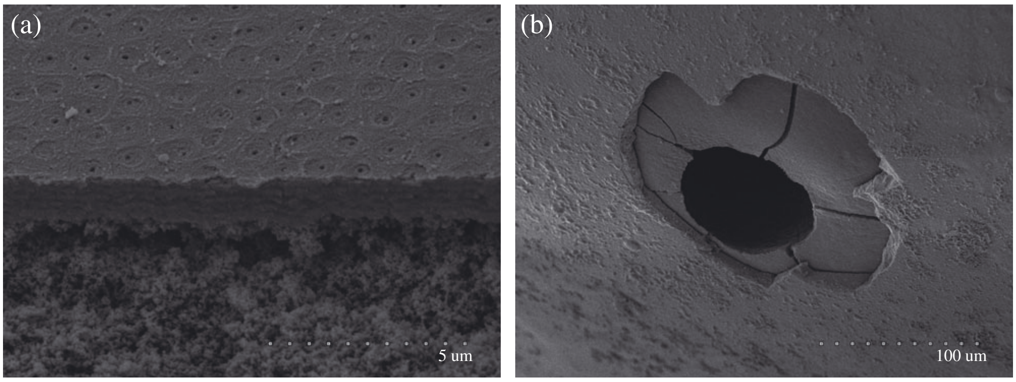

FIG. 3. Scanning electron micrographs of (a) chorion lamellar layer and (b) micropyle aperture of ovum of *Lophius piscatorius*.

ecological premise appears to be appropriate in the two species studied here, because the maximum size of *L. piscatorius* (200 cm) is double that of *L. budegassa* (100 cm) (Caruso, 1986) and their ova are 70% larger. The ova of these species can be considered large for pelagic eggs, which range from 0·5 to 5·5 mm in diameter (Ahlstrom & Moser, 1980). Not surprisingly, larger ova also have larger oil globule diameters (*L. piscatorius*: 0·63 ± 0·02 mm; *L budegassa*: 0·48 ± 0·03 mm), both for larval buoyancy and as a concentrated energy source (Eldridge *et al.*, 1977; Markle & Frost, 1985). In addition, the chorion of *L. piscatorius*, which protects the embryo from the external environment (Stehr & Hawkes, 1979; Olivar, 1987), has more pores per unit area. In the ovary, the pore canals distributed throughout the chorion contribute to the transportation of nutrients from the follicle cell to the developing oocyte (Nagahama, 1983; Groot & Alderdice, 1985) and hence the larger eggs of species of *Lophius* could need more pores in order to satisfy this nutritional intake. It is still unknown if the pores remain open after fertilization but, if this occurs, they could expose the embryo to the natural environment or contaminants (Stehr & Hawkes, 1979). In conclusion, although both species occupy the same habitat, differences noted in the characteristics of their ova support the hypothesis that both species of *Lophius* have different ecological strategies, as was previously observed in Colmenero *et al.*'s (2010) study of the species' biorhythms.

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SHORT COMMUNICATION

Observations on the occurrence of *Spraguea lophii* in Mediterranean lophiids

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Abstract In the Mediterranean Sea, anglerfish *Lophius budegassa* and *Lophius piscatorius* support an important fishery, and landings have increased in recent years. These species are infected by the microsporean *Spraguea lophii*, a parasite that infects their nervous system giving rise to multiple cysts. Due to the high value of these fish and conspicuous nature of the infection, we determined the apparent prevalence of this parasite in both *Lophius* spp. Specimens were obtained from commercial catches along the NW Mediterranean Sea during 2013. The parasite was observed in the nerves from the peripheral nervous system of the individuals and was detected at the prevalence of 69.2 % (202/292) and 100 % (58/58) in *L. budegassa* and *L. piscatorius*, respectively. Non-significant differences were noted between sexes of *L. budegassa* (χ^2=0.683; p=0.409), although a positive correlation was noted between host size and prevalence (χ^2= 6.134; p=0.013). During the sampling, two specimens of *L. budegassa* with atypical morphological characteristics, pigment anomalies and blindness and infected with *S. lophii* xenomas were described.

Keywords *Lophius budegassa* · *Lophius piscatorius* · Microsporidia parasite · Morphological abnormalities · Blindness · Pigment anomalies

Introduction

Microsporidia are intracellular parasites related to fungi whose spores infect a broad range of hosts, such as arthropods, molluscs and vertebrates including immunocompromised humans (Hinkle et al. 1997; Lom and Dyková 2005; Williams 2009; Abdel-Ghaffar et al. 2012; Morsy et al. 2012; Stentiford et al. 2013). Some microsporidians parasitize a variety of fish species and are capable of causing serious disease affecting various tissues and organs and resulting mortality in both wild stocks and aquaculture (Antonio and Hedrick 1995; Maíllo et al. 1998). Spores are released into the environment in faeces (from infected hosts or their predators), via the urinary tract or post-mortem (Kramer 1976; Maíllo et al. 1998). They are the only microsporidian stage that can survive for extended periods in the environment and are responsible for its dissemination (Vávra and Lukes 2013). Although horizontal transmission by direct ingestion of the spores is the main transmission route among the aquatic microsporidia (Stentiford et al. 2013), many species infect germinal cells (oocytes, sperm) and are vertically transmitted to offspring (Galbreath et al. 2004) and autoinfection may occur. In humans, the sources of microsporidian infections and the modes of transmission remain unknown, but may be the result of the ingestion of poorly cooked infected fish (Curry 1999), as well as by the use of well water and groundwater containing microsporidian spores (Dowd et al. 1998). Inactivation of spores in fish can be achieved by freezing the fish at −20 °C for 48 h, treatment at 60 °C for 15 min and microwaving at 750 W for more than 60 s (Leiro et al. (2012).

Spraguea lophii (Doflein, 1898) Vávra & Sprague, 1976 is a microsporidian that infect the brain and ganglion cells in the peripheral nervous system of black anglerfish *Lophius budegassa* Spinola, 1807 and white anglerfish *Lophius piscatorius* Linnaeus, 1758, inhabiting the North Atlantic and Mediterranean regions (Thelohan 1895; Döflein 1898;

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Campbell et al. 2013). Once the spores of *S. lophii* reach the host digestive tract, they enter the intestine and insert their sporoplasms into neurofibrils in the epithelial layer, intestinal connective tissue or muscular layers. If the neurofibrils become assembled into a nerve fibre, sporoplasms may migrate up to the central nervous system and finally reach its ganglion cells where sporogony occurs (Weissenberg 1976; Lom and Dyková 1992). The infection of *S. lophii* is manifested as cystic structures called xenoparasitic complexes (or xenomas) located in the nerve ganglia running along the spine into the head. These cysts sometimes measure several centimetres cross and each contains large numbers of xenomas harbouring thousands of spores (Amigó et al. 1995; Freeman et al. 2004; Casal et al. 2012; Mansour et al. 2013). The host cell and the parasite are morphologically and physiologically integrated in the xenomas (Vávra and Lukes 2013) but the cell biology of this reaction is not known, nor is it understood whether the xenoparasitic complex occurs as fish tissue encapsulates the infection for its own defence or if the microsporidian transforms the host cell for its own shelter (Williams 2009). After the initial infection, other nerves may become heavily infected causing serious pathogenicity as pigment anomalies and blindness (Bucke et al. 1994; Landa et al. 1998; Bañón and Armesto 2004; Ragonese and Giusto 2006; Arculeo et al. 2010). However, no data exist to suggest that *S. lophii* infections cause mortalities in anglerfishes (Freeman et al. 2011).

The *Lophius* spp. of the Mediterranean Sea are demersal fishes living in sandy, muddy and rocky bottoms, cohabiting on the continental shelf. Despite the overlapping distribution of both species, no ecological competition exists between them due to a temporal segregation of their biorhythms (Colmenero et al. 2010). The demand for anglerfish for human consumption has increased in the last two decades, with more than 27,500 t of *L. budegassa* and *L. piscatorius* captured in 2012 (FAO 2008). In the NW Mediterranean Sea, 5,450 t were caught in the last 10 years, with a value of 37 million of Euros (unpublished data from the Directorate of Fishing and Maritime Affaires; Government of Catalonia). Due to the relevance of these species for the humans, the goals of the present study were (i) to evaluate the current presence of the parasite *S. lophii* in individuals of *L. budegassa* and *L. piscatorius* from the NW Mediterranean Sea, (ii) to compare the data with previous studies in *L. budegassa* inferring temporal changes and (iii) to hypothesize about possible side effects of severe infections on morphology of *Lophius* spp.

Materials and methods

Individuals of *L. budegassa*, with a size range between 7.0 to 52.5 cm in total length (TL) (n=292), and *L. piscatorius*, from 17.0 to 53.0 cm (TL) (n=58), were randomly selected from commercial catches along the NW Mediterranean Sea during 2013. Each fish was measured, dissected, sex determined (male, female and indeterminate) and examined macroscopically for the presence of microsporidia *S. lophii*. The prevalence was the only quantitative descriptor of parasite populations calculated due to the difficulty in quantifying the clustered individuals in the xenomas. For that, the spinal cord, the medulla oblongata and the nerves from the peripheral nervous system of each specimen were inspected. Prevalence was estimated according to Bush et al. (1997):

$$\text{Prevalence} = \frac{\text{number of hosts infected}}{\text{number of hosts examined}} \times 100$$

To assess the independence of prevalence of the parasite in relation to the fish sex and size, a chi-squared test ($p<0.05$) was performed. Finally, results were compared with a previous study conducted by Maíllo et al. (1998) to analyse the temporal variation. Statistical analysis was performed in XLStat 2012, a statistical plug-in for MS Excel 2011 spreadsheet program.

Two specimens of *L. budegassa* were obtained from commercial catches and scientific surveys presenting atypical characteristics (blindness and orange colouration, respectively) and both with several *S. lophii* xenomas present.

Results and discussion

Of the 292 *L. budegassa* analysed, 202 (69.2 %) were infected by the parasite *S. lophii*; whilst all specimens of *L. piscatorius* (n=58) presented parasitism. The occurrence of this parasite in *L. budegassa* had been reported in the Mediterranean Sea (Maíllo et al. 1998), but this is the first report of its occurrence in *L. piscatorius* in this location. However, spores had been noted in both species in the Atlantic waters (Canning and Lom 1986; Cañás et al. 2010). Currently, five (*L. piscatorius*, *L. budegassa*, *Lophius americanus*, *Lophius litulon* and *Lophius gastrophysus*) of seven lophiid species have their nervous tissues parasitized by microsporidia of this genus (Weissenberg 1976; Takvorian and Cali 1986; Freeman et al. 2004; Casal et al. 2012; Campbell et al. 2013).

In the current investigation, infection was always observed in the vagus nerve situated near the kidneys. In some cases, microsporidian xenomas were also present in the spinal cord and the medulla oblongata and in the glossopharyngeal and trigeminal nerves (Fig. 1). Occurrence in these locations is in agreement with previous descriptions for these and other lophiids (Mansour et al. 2013). Members of the genus *Spraguea* normally infect nervous tissues and rarely other tissues of lophiid hosts (Casal et al. 2012).

Non-significant differences were noted between males and females of *L. budegassa* (χ^2=0.683; p=0.409) (Fig. 2a).

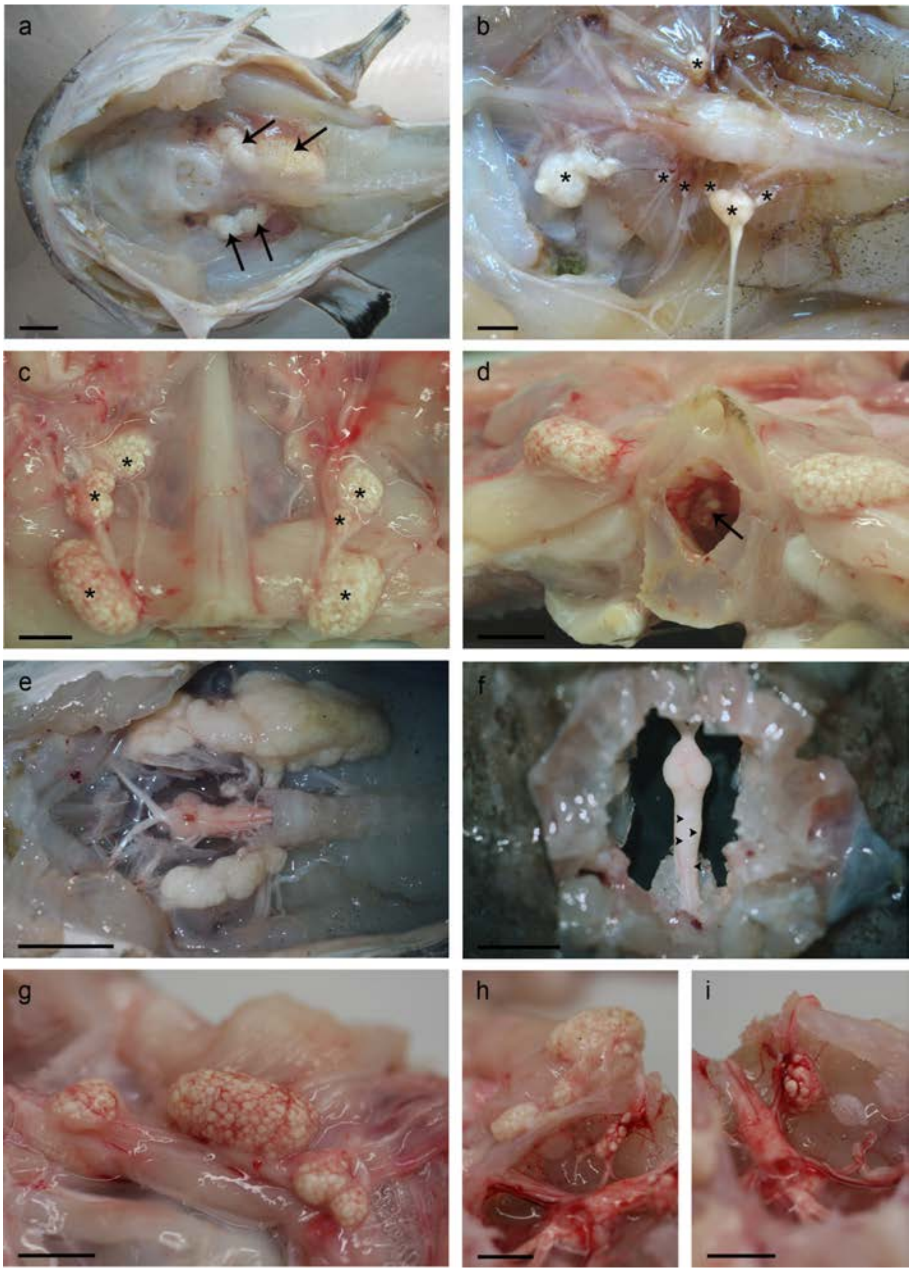

Fig. 1 *Spraguea lophii* infection in *Lophius* spp. showing the organization of xenomas and spores on nervous tissues. **a** Ventral view of a dissected *Lophius piscatorius* showing the microsporidian cysts situated near the kidneys (*arrows*). **b**, **c** Nerves exiting the central nervous system enlarged with microsporidian infection (*asterisks*). **d** Xenomas developing in the spinal nerves inside the vertebral column (*arrow*). **e** Ventral view of the brain showing heavily infected vagus and glossopharyngeal nerves. **f** Dorsal view of the brain showing spores of *S. lophii* in the medulla oblongata (*arrowheads*). **g**, **h**, **i** Groups of xenomas that run into the brain cavity. *Scale bars*= 1 cm

However, a positive relationship was observed between host size and prevalence (χ^2=6.134; p=0.013) increasing the presence of the parasite in individuals larger than 30 cm (Fig. 2b). Although both species feeds mainly on fishes and crustaceans (Crozier 1985; Negzaoui-Garali et al. 2008), cannibalism is also common in larger specimens (Gordoa and Macpherson 1990; Armstrong et al. 1996; Laurenson and Priede 2005). Since one of the transmission pathways of the parasite is by direct ingestion of the spores, feeding habits could explain the increased severity of infection in larger individuals (Gibson and Jones 1993). The possibility of autoinfection may also be a factor influencing severity in larger fish. However, gender does not appear to be an influential factor since male and female anglerfish select the same prey items (Cañás et al. 2010). The difference in parasite prevalence between the two species is difficult to explain since both species have similar depth distribution and also feed on the same prey (Laurenson and Priede 2005; Preciado et al. 2006).

The 69.2 % of individuals of *L. budegassa* analysed in the present study were infected with *S. lophii* (Fig. 2c) compared

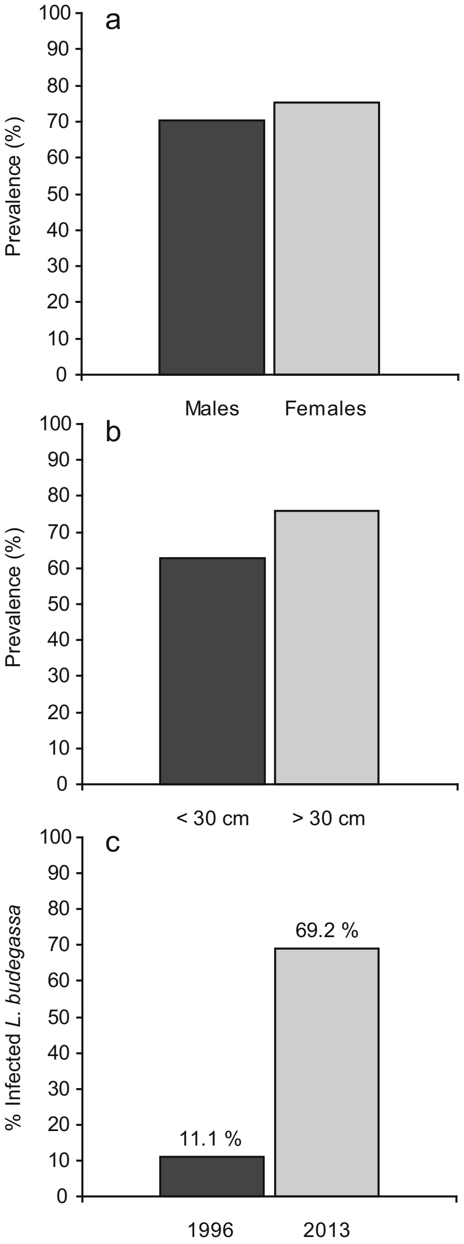

Fig. 2 Prevalence of *Spraguea lophii* in *Lophius budegassa* from the NW Mediterranean Sea by sex (**a**) and by size (**b**) and increase of parasitism in the last 18 years (**c**)

with 11.1 % prevalence reported by Maíllo et al. (1998). Some studies had demonstrated an increase of the temperature average on the Mediterranean Sea, associated with the accelerating trend in global warming from the early 1990s (Carillo et al. 2012; Skliris et al. 2012). It is hypothesized that increase in water temperatures could significantly impact prevalence and intensity of parasitism through thermal stress leading to reduced immunocompetence and increasing the susceptibility of the host to the parasite (Antonio and Hedrick 1995; Marcogliese 2008).

Although parasite impact on host population level dynamics is unknown, severe infections may be associated with anatomical anomalies such as eye deformities (Bucke et al. 1994). However, in the study of Bucke et al. (1994), histological assessment of the eye and optic nerve did not reveal the presence of *Spraguea* (unpublished data). In the current study, we found two specimens of *L. budegassa* with morphological anomalies. A blind specimen of 23.3 cm in length was apparently lacking an eye on gross examination (Fig. 3a, b). However, a rudimentary and deformed sclera with degenerate retinal tissue under the dermis and reduced optic nerve was found (Fig. 3c). Body colouration was dark brown similar to other *Lophius* spp. This specimen was infected with microsporidian xenomas of *S. lophii* in the vagus nerve near the kidneys as well as the spinal cord and medulla oblongata region of the hind brain. Xenomas were also observed in the trigeminal nerve. Bucke et al. (1994) considered that this anomaly was caused by the parasite *S. lophii* affecting the nervous system and did not consider genetic alterations or

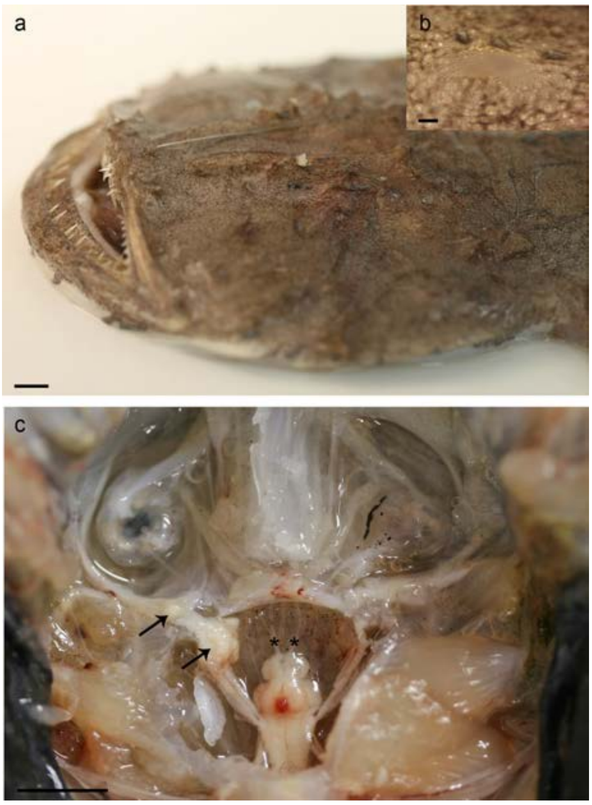

Fig. 3 Blind specimen of *Lophius budegassa*. **a** General view (*scale bar*=1 cm). **b** Close-up of the ocular region showing a depigmented area where the eye should be present (*scale bar*=1 mm). **c** Ventral view revealing microsporidian xenomas in the trigeminal nerve (*arrows*). A deformed sclera and degenerate retinal tissue are also visible. Optic nerves are reduced (*asterisks*) (*scale bar*=1 cm)

pollution effects as likely aetiologies. The other atypical specimen of *L. budegassa* (Fig. 4) was 33.6 cm in length, bright orange in colour and without the ocular anomalies in the previous specimen. Similar cases have been reported previously (Cendrero and de Cárdenas 1979; Fariña and Fernández 1981; Alonso-Allende 1983; Allué and Sánchez 1986; Bucke et al. 1994; Pereda and Gancedo 1994; Landa et al. 1998; Bañón and Armesto 2004; Ragonese and Giusto 2006; Landa et al. 2007; Arculeo et al. 2010). In teleosts, colour changes are produced by both endocrine and neural systems and depend to chromatophoral responses to ecological, physiological or ethological conditions (Fujii 2000). Consequently, the cause of this anomaly may be multifactorial and therefore difficult to determine. Some studies attribute alterations in skin pigmentation to parasites (Roberts and Bullock 1980; Oetinger and Nickol 1981). In lophiids, the cutaneous mucous glands, where the peripheral nervous system are known to extend, are one of the points of infection of *S. lophii* (Freeman et al. 2011) which may produce changes in skin colouration.

Although human infections with microsporidians from several genera are known to occur in immunocompromised human patients, there are no records of infection with *Spraguea* spp. (Leiro et al. 2012). However, some authors suggest a possible fish-human transmission route for infections by other species of microsporidia (Cali et al. 2005). Since there is evidence that suggest an increase of parasites, disease transmission and possibly virulence, associated with global warming (Marcogliese 2008), there is a need for better understanding of microsporidian ecology in the marine environment, including *Spraguea* spp. and others infecting commercial fish species that enter the human food chain.

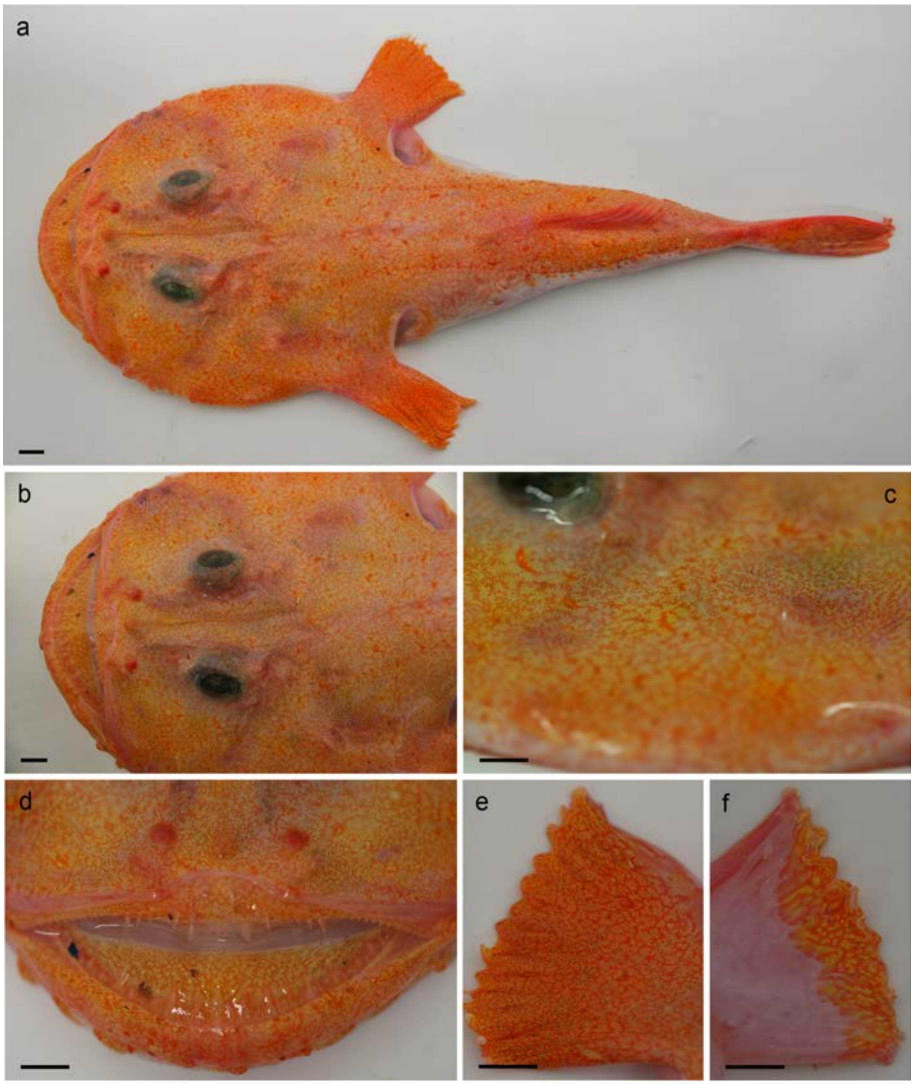

Fig. 4 Specimen of *Lophius budegassa* with atypical colouration**. a** Dorsal view. **b** Head region. **c** Detail of the skin showing the orange colouration. **d** Close-up of the mouth. Dorsal (**e**) and ventral (**f**) part of the pectoral fin. *Scale bars*=1 cm

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