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The Life History and Ecology of Black Scabbardfish (*Aphanopus carbo* Lowe 1839) in the North-east Atlantic

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Thesis submitted to the National University of Ireland in fulfilment of the requirements for the degree of Doctor of Philosophy

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4th January 2013

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Dedicated to my mother,

Mariana dos Santos Aranha

Contents

Declaration

The thesis submitted here is my own work and has not been submitted for another degree, either at University College Cork or elsewhere unless otherwise stated.

Ana Ribeiro Santos

Acknowledgments

This research was carried out under the Sea Change strategy with the support of the Marine Institute and the Marine Research Sub-programme of the National Development Plan 2007–2013.

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Finally, I would like to thank to my family for their support, patience, comprehension and especially to my dad for his supportive and encouragement words during all these years, particularly in times when was so hard to be far away.

General abstract

The black scabbardfish is a deep water species that supports commercial fisheries across a large area of the NE Atlantic shelf. The life history of black scabbardfish is poorly understood and a major unresolved issue is population structure. In this study it was used a combination of methodologies to get further knowledge in the life history and population structure of *A. carbo* over its wide distribution range in the Northeast Atlantic. The new knowledge acquired during this study, will increase our ability to better manage this species in the NE Atlantic.

It has been postulated that fish caught to the west of the British Isles are pre-adults that migrate further south (to Madeira) for spawning, implying a single panmictic population. In this study, specimens of *Aphanopus carbo* were sampled between September 2008 and May 2010 from two different areas: NW Scotland (French trawlers and deep water surveys) and Madeira Islands (longliners commercial landings). Geographical differences in reproductive state of scabbardfish were evident, supportive of a north-south migration theory. In the northern area, all specimens found were immature, while in Madeira all maturity stages were observed. In Madeira, spawning occurred during the fourth quarter, with peak maturity in October (males) and in November (females).

The age of this species has proven difficult and has led to different and contradictory age and growth estimates. For this study, we used two reading interpretations to determine age and estimate the growth parameters. To the west of the British Isles, specimens reached a lower maximum age and had a higher growth rate than those caught off Madeira. These differences are consistent with the theory of a single population of black scabbardfish in the NE Atlantic, highly segregate, with smaller, immature and younger fish caught to the west of the British Isles and bigger and mature caught in Madeira Islands.

The feeding ecology showed strong evidence that the diet of black scabbardfish is associated with the spawning migration of blue whiting, which may support a northerly feeding migration theory for black scabbardfish. The stable isotope analyses in the muscle of black scabbardfish identified that black scabbardfish feeds on species with epipelagic and benthopelagic affinities. Comparison with stable isotope analysis in Madeira samples indicated that black scabbardfish

feed at a similar trophic level and has the same trophic niche width in both areas, assuming similar baseline isotope compositions.

Otolith stable isotopes (oxygen - $\delta^{18}O$ and nitrogen - $\delta^{15}N$) analyses were used as a tool to clarify migratory behaviour. Otolith isotope ratios can provide insight into whether adults caught around Madeira fed in an isotopically depleted northerly ecosystem (NW Scotland) during their pre-adult period and then migrate towards south to spawn. Overall, the results support a south-north migration of pre adult fish from spawning areas around Madeira and a north-south migration from the west of Scotland to the spawning areas.

Given its life cycle there is an urgent need that the management process recognizes the existence of a continuous widely distributed stock of black scabbardfish between the west of the British Isles and Madeira. The results highlight large scale dispersal in this species which needs to be treated as a highly migratory species and be managed as a single population.

Chapter 1

General Introduction

Contents

1.1 Deep sea fisheries

Continental shelves have supported the great fisheries of the world for more than 500 years (Haedrich *et al.*, 2001), but an overly aggressive fishing effort during the 1980s led to the decline and collapse of continental shelf fisheries in many places. Some populations in the Northwest Atlantic, such as the northern cod (*Gadus morhua*) reached such low levels that the fishery totally closed in 1992 (Kurlansky, 1997).

In the last 50 years, as traditional fisheries in continental shelf waters declined, distant water fleets developed to exploit less accessible deep water species (Morato *et al.*, 2006). More powerful and more sophisticated fishing and navigation gear were developed to reach and exploit the deep water resources (Haedrich *et al.*, 2001; Roberts, 2012). The global expansion of fisheries, particularly by the Soviets in the late 1960s, soon uncovered deep-water habitats, such as seamounts, with substantial aggregations of benthopelagic fishes (Koslow *et al.*, 2000). Thereafter, deep water fisheries quickly expanded, partly because of the improving markets, but mostly due to overfishing and increased management restrictions on the continental shelf fish stocks (Gordon, 2001; Large *et al.*, 2003). In contrast to the continental shelf fisheries, the deep water fisheries were largely unexploited and unregulated.

After the first years of rapid expansion and high catch rates, several deep water fisheries displayed patterns of steep decline (Lorance and Dupouy, 2001). Concerns over the vulnerability and sustainability of deep water stocks arose because they are generally perceived as long lived, slow growing, with a high age at maturity and low fecundity (Koslow *et al.*, 2000; Gordon, 2001; Andrews *et al.*, 2009). The ecological characteristics of these fish make them vulnerable to over-exploitation and slow to recover from it (Clark, 2001; Morato *et al.*, 2006).

During decades, the deep water fisheries developed and increased without programmes in place to collect biological and fisheries data. Although biological studies on deep water species have increased during the last decade (Kelly *et al.*, 1997; Allain and Lorance, 2000; Allain, 2001; Neat and Burns, 2010), our understanding of the population dynamics of deep sea species and the impacts of fishing on these resources are still considerably behind exploitation (Large *et al.*, 2003).

Although biological studies of deep-water species have increased during the last decade, knowledge of biological processes such as growth, feeding, maturation and fecundity still lags behind that of commercially exploited shelf-based species. Also, little is known about recruitment processes, stock identity, fish migration (Large *et al.,* 2003) and the long term ecological implications of deep water fisheries are still very unclear (Koslow *et al.,* 2000). Gordon *et al*. (1995) pointed out that, although the continental slopes only comprise something like 8.8% of the ocean bottom, they are among the most complex and dynamic parts of the deep sea. Because so many species on the slope have over-lapping depth distributions and because fishermen will target certain depths to maximize catch rates of target species, fishing is likely to impact on the entire fish community (Gordon *et al.,* 1995). Therefore considerable biological and ecological knowledge is required in order to determine which species are sustainable to commercial fisheries and how they can be managed properly (Merrett and Haedrich, 1997).

Black scabbardfish is a charismatic inhabitant of the continental slope and one of the main deep water commercial species in Europe, where it is captured by multi-species trawlers in the North of Europe and small scale longliners in Portugal and the Madeira Islands. Despite the commercial interest in black scabbardfish, biological studies are relatively sparse and spatially confined to the southern Northeast Atlantic. Since the effects of fishing on black scabbardfish are unknown, more research into the biology, population structure it is required. It is the purpose of this study to further the knowledge of the biology and life history of black scabbard in the wide range of its distribution in NE Atlantic. The new knowledge acquired during this study, will thus increase our ability to better manage this species in the NE Atlantic.

1.2 Black Scabbardfish, *Aphanopus carbo* Lowe 1839

The black scabbardfish, *Aphanopus carbo* Lowe, 1839 is a deep sea species belonging to the order Perciformes and family Trichiuridae (Nakamura and Parin, 1993). It has an extremely elongate and compressed body. The coloration of its body is black with a metallic lustre. The mouth is large and armed with rows of very sharp teeth. The large black eyes comprise almost one-half of the length of the snout. The dorsal fin is low, but long. Behind the anal opening there are two spines, the posterior one is represented as a hard bone spike. The pectoral fins are relatively small and the lateral line is well marked (Zilanov and Shepel, 1975; Nakamura and Parin, 1993) (Fig. 1).

Fig. 1 Black scabbardfish, *Aphanopus carbo.*

1.3 Distribution

In the North Atlantic, black scabbard has been reported from Iceland (Magnússon and Magnússon, 1995) to the Canary Islands and Bojador Cape (Uiblein *et al.*, 1996) including the mid-Atlantic Ridge, the islands of Madeira, Azores and numerous submarine banks and seamounts (Zilanov and Shepel, 1975; Nakamura and Parin, 1993; Uiblein *et al.*, 1996; Vinnichenko, 2002; Vinnichenko and Bokhanov, 2006) (Fig. 2). There are also occurrences of the species reported from the Indian and Pacific Oceans, although these reports need to be confirmed (Piotrovskiy, 1981).

Black scabbardfish belongs to the benthopelagic category of deep-water fishes, in which the fish are more proximate to the demersal fishes of the continental shelf and live close to the bottom (Nakamura and Parin, 1993; Gordon, 2001; Bordalo-Machado and Figueiredo, 2008). In the study carried out by Menezes *et al*. (2006) in the Azores Archipelago, *A. carbo* is one of the typical species in the deep mid-slope assemblage.

It has been recorded at a variety of depths, depending on geographical locations; from 200 m around the British Isles (Tucker, 1956; Bordalo-Machado *et al.*, 2001) to 1800m south of Madeira (Bordalo-Machado *et al.*, 2001). In a recent study by Pajuelo *et al*. (2008) in the Canary Islands, *A. carbo* has been located at 2300m deep. In the north of Europe, it is more commonly found from 500 to 800 m (Mauchline and Gordon, 1984); between 800 and 1200 m off the Portuguese shelf (Anon., 2008) and from 800 to 1300 m in the waters around Madeira (Morales-Nin and Sena-Carvalho, 1996).

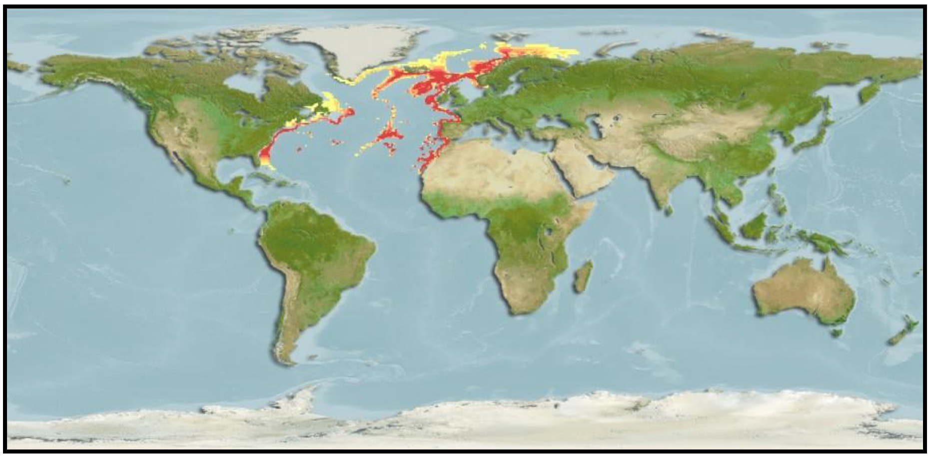


Fig. 2 Distribution map for *Aphanopus carbo* (Black scabbardfish) (Adapted from Fishbase (Froese and Pauly, 2006).

1.4 The fishery of *Aphanopus carbo* in the NE Atlantic

1.4.1 Madeira Island

The fishery for black scabbardfish in Madeira is an ancient fishery, being one of the oldest deep water fisheries in the world. The first historical record dates back to the seventeenth century. For centuries this fishery, which only supplied the local markets, seemed to be sustainable. However, since the onset of exports, the fishery expanded and the landings have decreased considerably (Haedrich *et al.*, 2001; Alves, 2003). For several decades, this was the only fishery targeting black scabbardfish in NE Atlantic (Bordalo-Machado and Figueiredo, 2008) and to this day, it has great economic and social importance to Madeira since it represents an important component in the local diet (Bebianno *et al.*, 2007).

From 1980 to 1986, several prospective surveys were carried out by the Instituto Português de Investigação das Pescas e do Mar (IPIMAR) to prospect new fishing grounds in the eastern Atlantic for black scabbardfish and to introduce new fishing gear and technology (Martins and Ferreira, 1995; Pajuelo *et al.*, 2008). The improvements in the fishing gear and methods during the 1980's, resulted in the replacement of the vertical drop-lines (Fig. 3a) by the mid-water horizontal drifting longlines (Fig. 3b), which is set in the water column usually at depths of 1000m. This method is still used today and the changes are mainly related with the increase in the number of hooks per set. The bait used to catch black scabbardfish is squid (*Omnastrephes* sp.) or horse mackerel (*Trachurus picturatus*) and mackerel (*Scomber japonicus*).

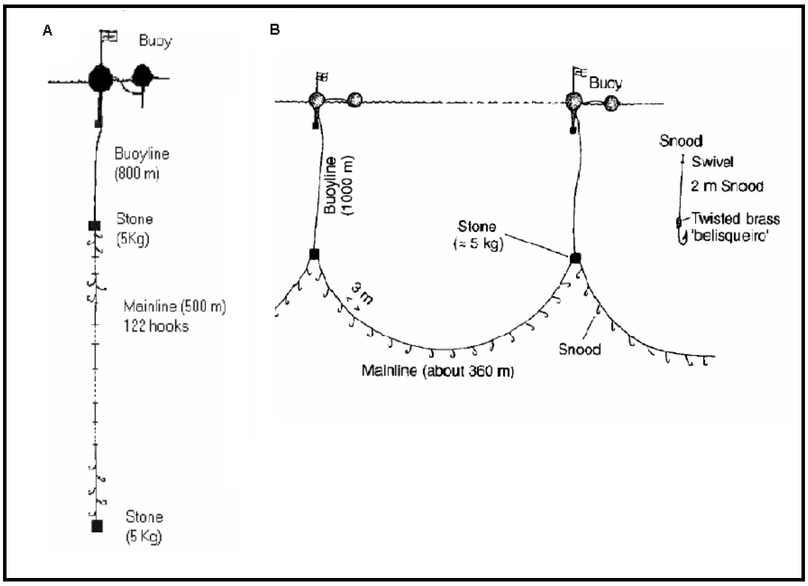


Fig. 3 a) Drifting vertical longline used until 1980's; B) Drifting horizontal longline used nowadays to capture *A. carbo* in Madeira (adapted from Reis *et al*.(2001))

Between 1998-2000, the Madeiran black scabbardfish fleet comprised *ca.* 40 boats with artisanal characteristics, on average 13m in length and with a low engine power (Reis *et al.*, 2001) (Fig. 4). In more recent years, the fleet size decreased to around 30 boats, with no significant difference in their technical characteristics (Bordalo-Machado *et al.*, 2009). The number of fishing days per trip is around 5 to 7 days and the vessels conduct several hauls during each trip. The number of days per fishing trip increased in the last few years because fishermen are fishing new fishing grounds located further south of Madeira, near the Canary Islands (Bordalo-Machado *et al.*, 2009).

Fig. 4 Some of the Madeira black scabbardfish longliner fleet.

During the 1980's, due to the improvements in the fishing gear and in the fleet, a dramatic increase in the landings of black scabbardfish in Madeira was observed. In 1998, the landings reached a maximum value of 4430 tonnes and steadily decreased to 2922 tonnes in 2007, corresponding to €5,351,000 in 1998 to €7,715,000 in 2007 (Reis *et al.,* 2001, Bordalo-Machado *et al*., 2009).

It is known that the black scabbardfish fishery in Madeira is composed of two sympatric species, *A. carbo* and *A. intermedius* (Biscoito *et al.*, 2011). However, the proportion of each species in the total landings is unknown. In the last 4 years, an effort has been made to discriminate both species in monthly sampling programme (Sara Reis, *pers. comm*.)

1.4.2 Portuguese continental slope (ICES Areas IX)

In Portuguese continental waters, the commercial exploitation of black scabbardfish started in 1983. This fishery involves an artisanal fleet, composed of small vessels, with length generally below 12 m and an engine power *ca.* 96kw. The number of vessels has been decreasing since 1986, from 28 vessels to 20 vessels in 2008 (Bordalo-Machado *et al.*, 2009).

The fishing method and gear used by the continental fishermen was modified from the Madeiran traditional drifting longline in order to catch the species in continental shelf waters – setting a horizontal bottom longline, where alternating floats and weights occur at constant intervals on the main line (Bordalo-Machado and Figueiredo, 2008). The bait normally used is the sardine (*Sardina pilchardus*) (Martins *et al.*, 1989; Bordalo-Machado and Figueiredo, 2008).

The vessels leave the port at dusk and at the fishing ground two activities take place: 1) a newly baited longline is deployed into to sea and 2) the longline previously deployed (usually the day before), is recovered with the aid of a hauling winch. The fishing activity takes place on hard bottom substrate along the Portuguese slope (with depth ranging from 800 to 1450 m) around the centre of the mainland Portugal and landed into Sesimbra port (Martins *et al.*, 1989; Bordalo-Machado and Figueiredo, 2008).

More than 95% of the landings of black scabbardfish from the continental slope are into Sesimbra port. The first landing records of the black scabbardfish longline fleet in mainland Portugal was a total of 69 t at the end of 1983 (Martins *et al.*, 1989). During 1984 – 1989, landings rapidly increased from 676 to 3828 t. Between 1990 and 1993, landings showed the largest increase, up to 4520 t, but decreased to nearly 3400 t in 1994. Between 1995 and 2000, the landings showed a decreasing tendency, from more than 4000 t to 2500 t (Fig. 5). Since then, the catches of black scabbardfish from the Portuguese coast are fairly constant, around 2500 t (Bordalo-Machado and Figueiredo, 2008).

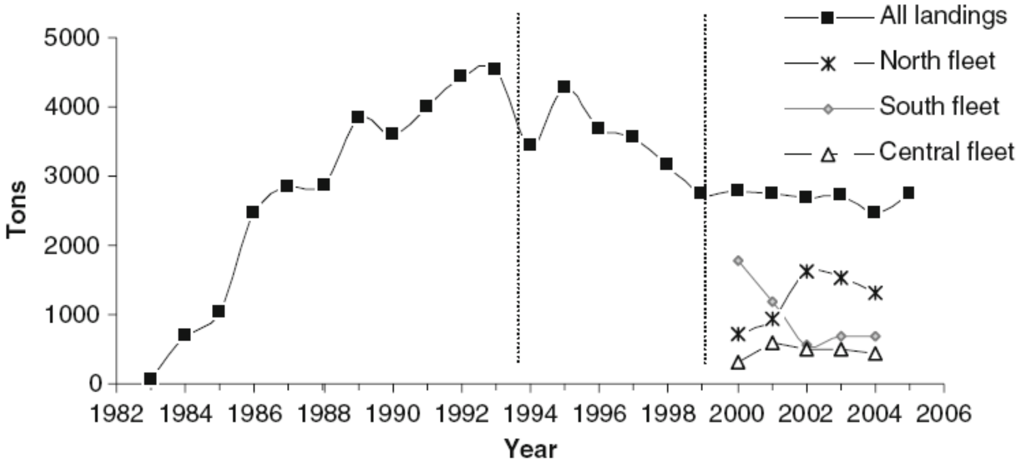


Fig. 5 Annual landings of black scabbardfish into mainland Portugal since the beginning of the fishery (adapted from Bordalo-Machado and Figueiredo, 2008).

Despite the differences in the fleet size and the fishing regime, the annual landings of black scabbardfish have been similar in Madeira and the Portuguese mainland in the last years 4 years (since 2008), with figures around 3000 tonnes.

1.4.3 North and west of Scotland and Ireland (ICES Areas VI, VII, Vb and XIIb)

In the north of Europe, the main fishing grounds for black scabbardfish are north and west off the British Isles (ICES Subareas V, VI and VII) and around Iceland (ICES Subarea Va) (See Fig.6). The species has been mainly captured by French, Faroese, Spanish and Icelandic trawlers since the early 1990’s (Bordalo-Machado and Figueiredo, 2008; ICES, 2008) and is one of the main deep water species captured by the mixed trawl fishery. In addition to black scabbard, this fishery also captures roundnose grenadier (*Coryphaenoides rupestris*), deep-sea sharks (*C. coelolepis, C. squamosus*), blue ling (*Molva dypterigea*), ling (*Mova molva*), saite (*Pollachius virens)* and monkfish (*Lophius piscatorius*).

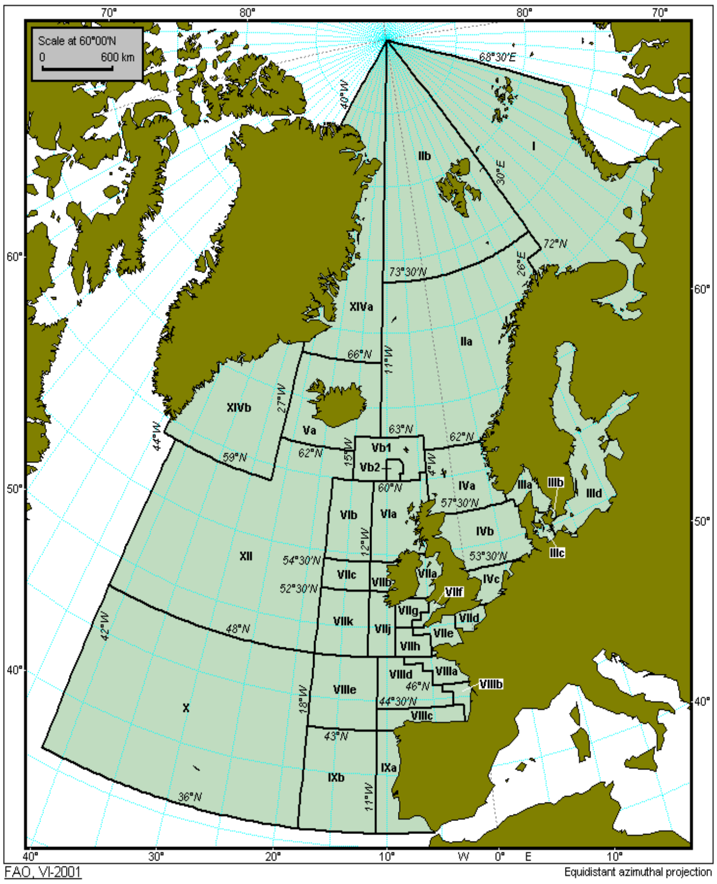


Fig. 6 Fishing areas used by the International Council for the Exploration of the Sea (ICES).

In the early years (1990s') the French trawlers landings represented more than 75% of the total landings from the north of Europe, but since 2006 the Faroese and Spanish fleets have increased their relative contribution for the total landings (Fig. 7).

The French deepwater mixed trawl fisheries (Fig. 8) operates mainly in sub-areas VI and VII targeting roundnose grenadier, black scabbardfish, blue ling and deepwater sharks. In the early 80's the French fishery started to operate in these areas targeting the blue ling (*Molva dypterygia*) and the by catch of species such *C. rupestris, A. carbo* and deep water sharks were discarded (Gordon, 2001; Lorance and Dupouy, 2001). It was only in 1989 that these species began to be landed as a result of a marketing initiative by the French industry (Gordon, 2001).

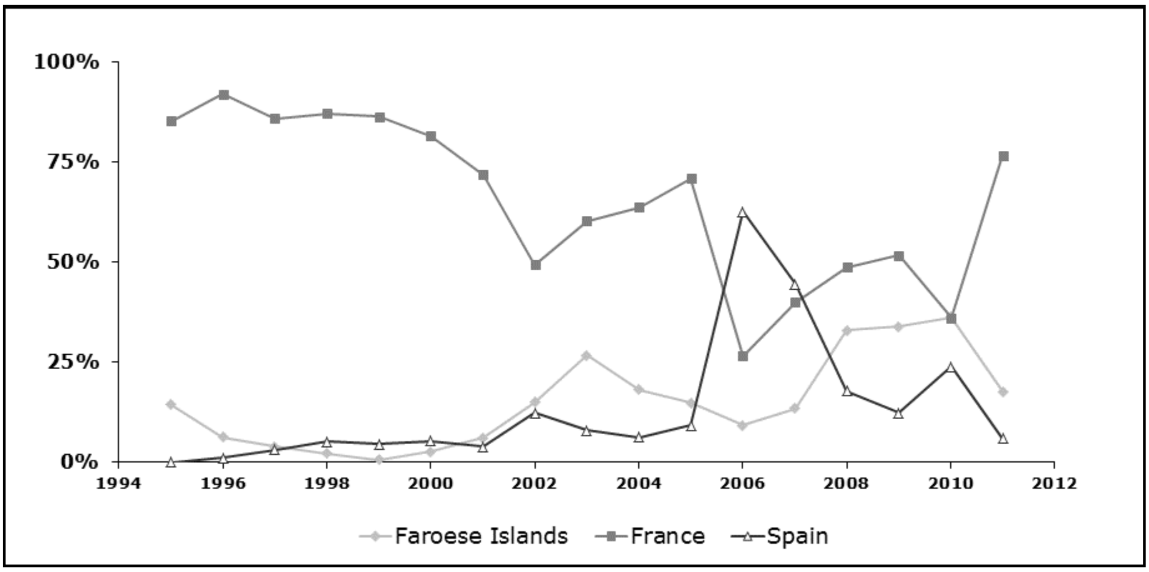


Fig. 7 Relative contribution by France, Spain and the Faroes to the annual landings for the ICES subareas (Vb, XIIb, VI and VIII) (adapted from ICES, 2012).

The Faroese fleet mainly operates on the slope around the Faroe Bank (ICES subarea Vb) and in recent years there has been an increased effort in this ICES subarea, with a corresponding increase in landings (ICES, 2012).

The ICES division with the highest landings is division VI (Scottish and Irish slope), accounting for 72% of the total landings from the north of Europe. The French multi-species trawlers account for almost 90% of the combined landings from this area (Fig. 9). From the beginning of

1990s' until 2001, the landings of black scabbardfish from the ICES division VI showed an accentuated increase, from 1023 tonnes in 1990 to 5057 tonnes in 2001. Since then until 2005 the landings suffered a reduction to 2850 tonnes. In 2006, there was a peak in landings (6527 tonnes) followed by a subsequent decrease to around 2270 tonnes (ICES, 2012) (Fig. 9).

Over recent years, the landings of black scabbardfish from the north of Europe have declined, but landings of other deepwater species (roundnose grenadier, orange roughy, and deepwater sharks) have declined to a greater extent. As a result, black scabbardfish is now landed in comparable quantities to blue ling and roundnose grenadier and more than deepwater sharks and grenadier (ICES, 2008) and is one of the most important deep water species landed in Europe.

Fig. 8 French deepwater trawlers at Lochinver port, Scotland.

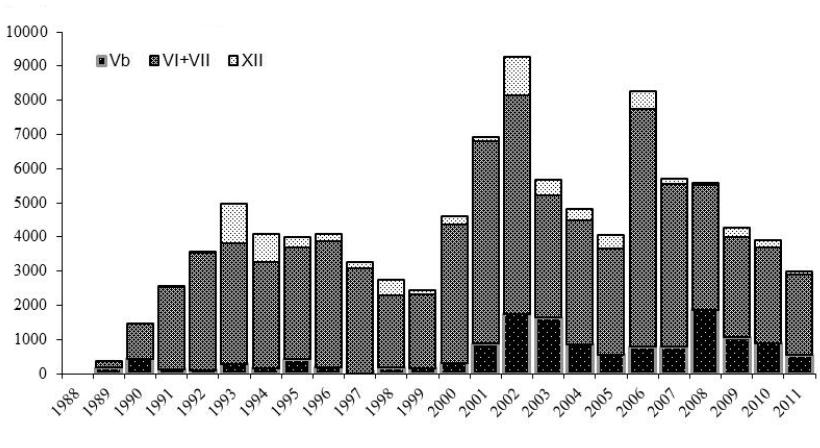


Fig. 9 Total landings (tonnes) of black scabbardfish in ICES areas Vb, VI, VII and XII. (ICES, 2012).

1.4.4 Other Areas including Azores (ICES Areas; I, II, X, XIV, IIIa and Va)

The black scabbardfish fishery in these areas has occurred sporadically or at very low levels, which may be related with low abundances in those areas (ICES, 2012).

In the islands of the Azores (ICES Subarea X), an experimental fishery targeting black scabbardfish started in 1998 using just one vessel and the fishery was closely monitored by on-board observers. Since then, the number of vessels has increased and the landings have increased significantly in the last years (Machete *et al.*, 2011).

Between 1991 and 2001, the landings in this subarea have been very variable, mainly as a result of exploratory fishery surveys carried out in this area during those years and between 2008 and 2012, an increasing commercial interest for the exploitation of this species has been observed. The fishing fleet is similar to the Madeira fleet, predominated by small vessels, <12 m, using mainly traditional bottom longlines (Machete *et al.*, 2011; ICES, 2012). The catches from Subarea X have fluctuated greatly over the years, mainly as a result of Portuguese exploratory surveys carried out in this area (ICES, 2006) (Fig 10).

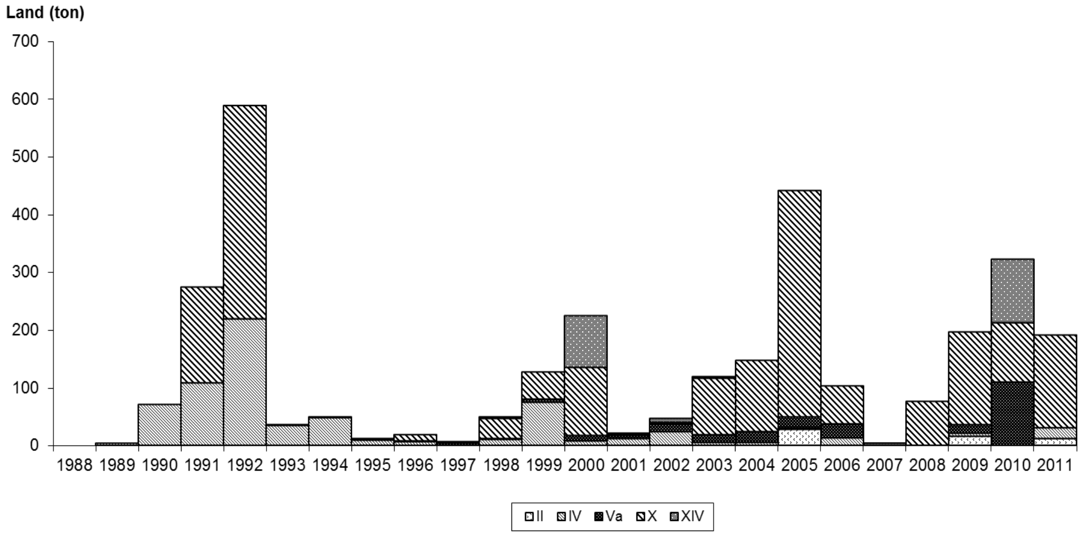


Fig. 10 Total landings (tonnes) of black scabbardfish in ICES areas I, II, IV, X and XIV. (ICES, 2012).

Figure 11 shows the overall landings (tonnes) reported in Portugal (mainland, Madeira and Azores) and northern Europe (ICES subareas II, IV, V, VI and VII combined) (Gordo, 2009). In Portuguese slope waters, an increase in landings occurred between 1988 and 1993, followed by a slight decrease until 2000. Since then the landings are stable around 2700 tonnes. The

Madeira landings were marked by an increase in the first decade, reaching 4430 tonnes in 1998, followed by a decrease, reaching 2900 tonnes in 2007, and at the same levels until 2011. In the north of Europe, the landings showed an accentuated increase until 2002, followed by a decrease and a new increase in 2006. Since then, the landings have been decreasing reaching the 3000 tonnes in 2011 (Gordo, 2009, ICES, 2012).

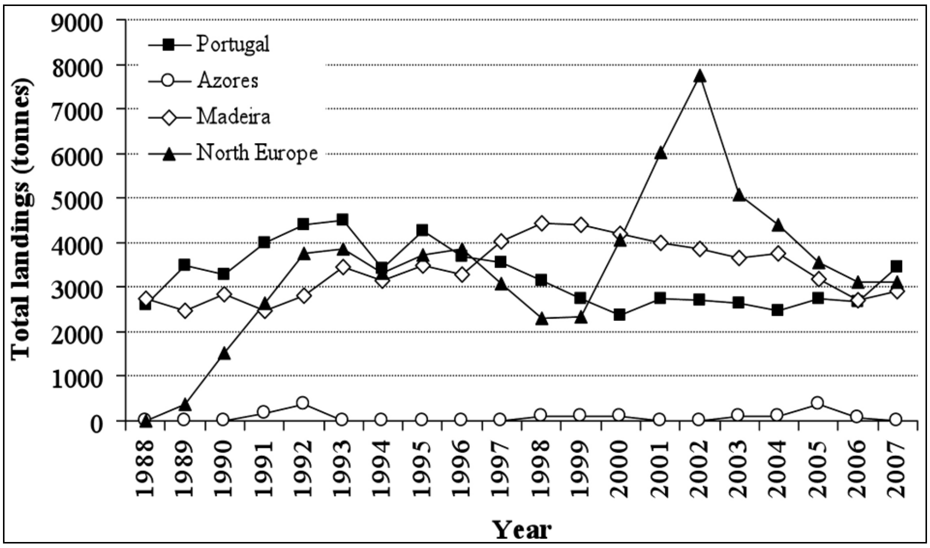


Fig. 11 Total landings (tonnes) reported in Portugal and north Europe between 1988 and 2007 (from Gordo, 2009).

1.5 Assessment, Management and Advice

Black scabbardfish has been one the main commercial deep-water species landed in Europe over the last 10 years, which makes it one of the most important among the various deep-water species that are covered by the ICES Working Group on the Biology and Assessment of Deep-sea Fisheries Resources (WGDEEP). The stability of the landings in the last decade in ICES Division IXa (Portugal slope) and in Madeira, the decrease in the northern Europe areas and the lack of information on black scabbardfish led ICES to establish (arbitrarily) the existence of a single stock in the NE Atlantic (Gordo *et al.*, 2009). However, because of the different nature of the fisheries in the northern and southern areas and the lack of information on migration, the stock has traditionally been divided into three management units: a “northern component”, a “southern component” and “other areas” for management purposes. The northern component comprises fish exploited by trawl fisheries in Subareas V, VI, VII and XII, the southern component being exploited by longline fishery in Subarea IX and VIII and the “Other areas” comprises subareas I, II, IIIa, IV, X, Va and XIV where only small catches have been taken (ICES, 2012).

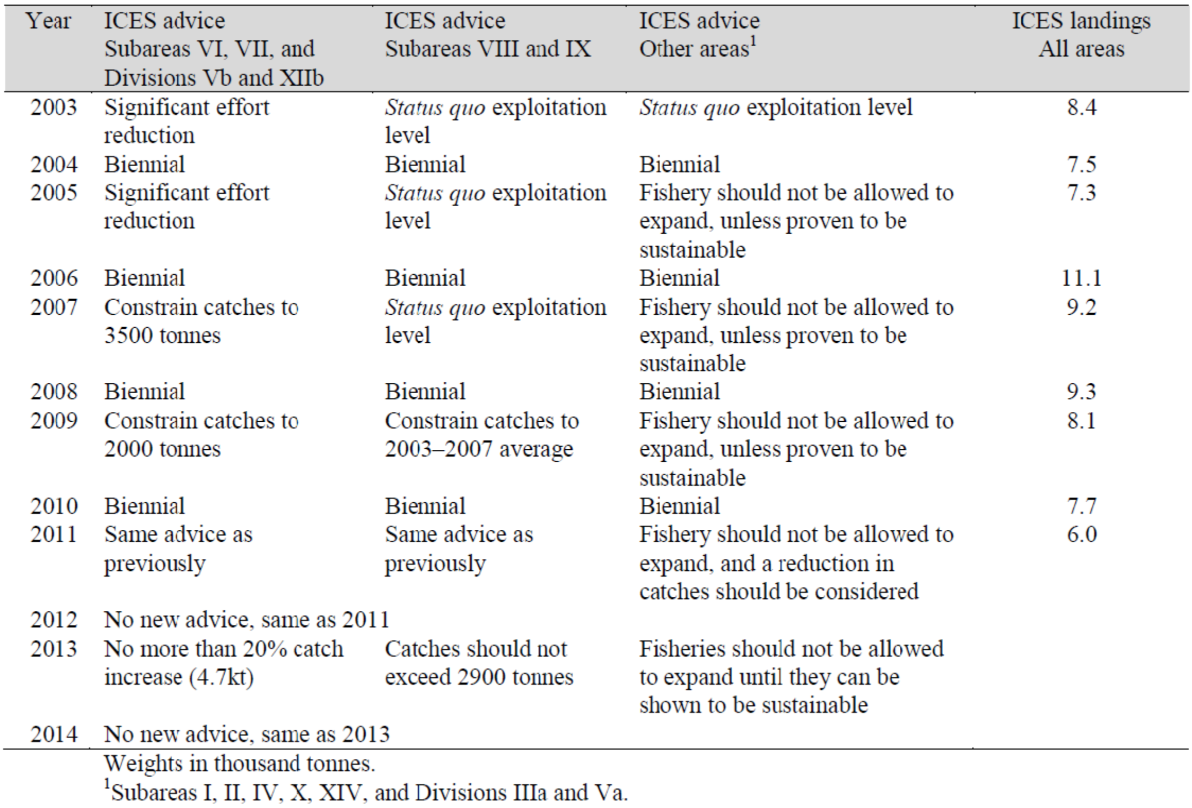
The scientific advice produced by ICES on the exploitation status of black scabbardfish has been used as the key source for establishing member states' biannual quota regimes for this species (Bordalo-Machado and Figueiredo, 2008).

The assessment of black scabbardfish is based on catch trend-based assessments, using landings data from the assessment unit distribution area. The available information is inadequate to evaluate spawning stock or fishing mortality, so the state of the stock is unknown.

Since 2003, management of black scabbardfish by EU vessels fishing in EU and international waters includes a combination of Total Allowable Catch (TAC) and a licensing system. From 2008 the TAC has been decreasing in all ICES areas and restrictions in fishing effort have been increasing (Table 1). The TAC in 2012 for the northern, southern and "other areas" components are: 2179, 3348 and 9 tonnes, respectively. The Irish TAC for black scabbardfish has also decreased and for 2012 the TAC is 62 tonnes (Anon., 2011). In the southern areas (subareas IX) the CPUE evolution does not indicate any clear trends, and ICES recommendations changed, from a *status quo* exploitation level in 2006 to a constrain on the landings to 3348 tonnes in 2012 (ICES, 2012).

Currently, the black scabbardfish fishery in Madeira and the Canary Islands is managed based on the biennial CECAF (Fishery Committee for the Eastern Central Atlantic) scientific advice, which in turn is based on the ICES advice for the ICES Division IX (Sara Reis *pers comm*.).

Table 1 Summary of advice for different assessment units of black scabbardfish in the Northeast Atlantic (adapted from ICES, 2012)



Year	ICES advice Subareas VI, VII, and Divisions Vb and XIIb	ICES advice Subareas VIII and IX	ICES advice Other areas[1]	ICES landings All areas
2003	Significant effort reduction	*Status quo* exploitation level	*Status quo* exploitation level	8.4
2004	Biennial	Biennial	Biennial	7.5
2005	Significant effort reduction	*Status quo* exploitation level	Fishery should not be allowed to expand, unless proven to be sustainable	7.3
2006	Biennial	Biennial	Biennial	11.1
2007	Constrain catches to 3500 tonnes	*Status quo* exploitation level	Fishery should not be allowed to expand, unless proven to be sustainable	9.2
2008	Biennial	Biennial	Biennial	9.3
2009	Constrain catches to 2000 tonnes	Constrain catches to 2003–2007 average	Fishery should not be allowed to expand, unless proven to be sustainable	8.1
2010	Biennial	Biennial	Biennial	7.7
2011	Same advice as previously	Same advice as previously	Fishery should not be allowed to expand, and a reduction in catches should be considered	6.0
2012	No new advice, same as 2011			
2013	No more than 20% catch increase (4.7kt)	Catches should not exceed 2900 tonnes	Fisheries should not be allowed to expand until they can be shown to be sustainable	
2014	No new advice, same as 2013			

Weights in thousand tonnes.

[1] Subareas I, II, IV, X, XIV, and Divisions IIIa and Va.

1.6 Stock discrimination

The concept of the "stock" is fundamental to fisheries management. Stocks are arbitrary groups of fish large enough to be essentially self-reproducing, with members of each group having similar life history characteristics and are available for exploitation in a given area. To manage a fishery effectively, it is important to understand the stock structure of a species and how fishing effort and mortality is distributed. There are many techniques which can be used for stock identification and stock discrimination, e.g., catch data, tag recoveries, meristics, morphometrics, parasites, mitochondrial DNA, elemental composition of otoliths, stable isotope measurements, otolith microstructure (Begg and Waldman, 1999). The uncertainties in stock structure led researchers to carry out several studies, using different approaches to identify the stock structure of black scabbardfish in the NE Atlantic.

1.6.1 Otolith microchemistry

The advances in analytical techniques have led to the use of otolith microchemistry as an aid to discriminate stocks. Its use relies on the assumption that otoliths incorporate elements from the environment throughout the life of the fish and may provide a useful record of the environment to which the fish was exposed (Campana, 1999). If black scabbardfish carries out large scale migrations, it would pass through and reside in several different water masses. The chemical signature of the otolith would reflect these differing phases of the life cycle (Santos, 2000; Swan *et al.*, 2003). If the fish in a stock are all derived from the same spawning area and have shared a common nursery ground, differences in chemical composition of the whole otolith associated with the migration to other areas might be relatively small.

Based on these principles, Swan *et al*., 2003 collected otoliths from six different locations throughout the NE Atlantic: Reykjanes Ridge, Hatton Bank, Rockall Bank, Mid-Atlantic Ridge, Madeira and the Portuguese mainland and the elemental concentrations were determined. The hypothesis tested was that there is a single stock of *A. carbo* in the northeast Atlantic and that spawning occurs in the Southern areas, especially in Madeira. The results showed that there were only small differences between overall chemical signatures of the otoliths from the different locations, consistent with the single stock hypothesis. However, the authors considered the results inconclusive. Despite being a useful tool, microchemical analysis of otoliths needs to be developed in conjunction with others methods used for stock discrimination, such as genetics, morphometrics and otolith shape analysis (Santos, 2000; Swan *et al.*, 2003).

1.6.2 Genetics

In order to investigate the population structure of *A. carbo* in NE Atlantic, different genetic techniques can be used. In the study carried out by Quinta *et al*., (2004) mitochondrial DNA variation in part of the cytochrome *b* gene was examined. The results obtained in this study suggest that the black scabbardfish population in the NE Atlantic is genetically structured and can be divided into two groups, one from the eastern Atlantic (Portuguese slope and Hatton Bank) and the other from around the Madeira Archipelago (Quinta *et al*., 2004).

The study carried out by Stefanni and Knutsen (2007) used a phylogeographical approach using molecular markers to investigate the history and structure of *A. carbo*. Two distinct groups were identified from complete sequences of the control region and partial sequences of cytochrome *b.* In one of these groups, all sequences from the Mid-Atlantic Ridge, Portuguese slope and Madeira were clustered together. The other group constituted all the sequences from the southern Pico Island (Azores). The rest of the samples (Sedlo and Seine Seamounts, Azores islands, Condor and Princess Alice banks) had sequences represented in both groups. These results suggest that the divergence between the two groups coincides with geological events that might cause a split in the original population of black scabbardfish. Once the climate conditions and sea level were restored, the two separate populations came into contact again, leaving traces of the historical events in the non-recombinant mtDNA genes. An alternative hypothesis suggested is that two species of scabbardfish are present. The outcome from the comparison of the same mtDNA regions of the closely related *A. intermedius* from Angola clustered with one of the groups (from the southern coast of Pico Island). Therefore, these two species may have overlapping distribution ranges and are found sympatrically in the Azores (Stefanni and Knutsen, 2007).

1.6.3 Other techniques for stock discrimination

Besides molecular techniques and otolith microchemistry there are other methods that can be applied for population discrimination. During the project APHACARBO the stock structure of black scabbardfish in southern northeast Atlantic (Madeira, Azores and Portugal mainland) was investigated using a holistic approach. The factors examined included life history parameters, otolith shape analysis, parasites, landing patterns and contaminants (mercury and cadmium). The majority of results revealed the existence of different stocks of black scabbardfish in the study area, but not in a consistent way.

The age and growth study by Vieira *et al*. (2009) and the landings analysis (Bordalo-Machado *et al.*, 2009), concluded that there were two separate stocks between the Madeira Islands and Portugal. The first based on the differences of the mean length per age group and the latter based on differences of the landings times series analysis between the two areas. However, the authors did not exclude the hypothesis that the differences found are due to horizontal migrations of the species to the spawning grounds.

On the other hand, otolith shape analysis (Farias *et al.*, 2009), parasites (Santos *et al.*, 2009) and mercury level (Costa *et al.*, 2009) results revealed the existence of three separate stocks: Madeira, Azores and the Portuguese mainland. The otolith shape variation analysis showed significant differences between the three areas. The parasite analysis revealed that the fish from Madeira had a higher number of metazoan species, followed by those caught in Portuguese slope and the Azores (Santos *et al*., 2009). Some of the parasite species found were exclusive to each area and this technique can be used to discriminate black scabbardfish from the three areas (Gordo *et al*., 2009). Finally, the mercury level also suggested the existence of three stocks, showing significant differences on the mean mercury level on the tissues (gonads, liver and muscle) among the areas under study.

1.7 Biology of *Aphanopus carbo*

The knowledge on the biological aspects of black scabbardfish in the NE Atlantic and on the connectivity between the north and south components is very limited and the most comprehensive studies have been spatially confined to the southern Northeast Atlantic.

1.7.1 Size structure

The studies carried out so far on black scabbardfish showed that the individual size of black scabbardfish ranged from 60 cm (in Rockall Trough) to 150 cm (Madeira waters). Small individuals are caught in northern regions (Rockall Trough, Hatton Bank), intermediate size fish in the Azores and Sesimbra regions (mid latitudes), with a mean length around 106 cm (Martins *et al.*, 1989; ICES, 2008), and the larger ones are caught in southern regions (Canary and Madeira Islands) (Anon., 2000; Santos, 2000; Reis *et al.*, 2001; Pajuelo *et al.*, 2008). The spatial analysis of length data from different geographical areas showed that significant differences between the north and the south length distributions occurred and those could be caused by two distinct phenomena acting alone or together: 1) the two corresponding populations are different; the southern one is larger than the northern one and, 2) the two fishing gears exploit different parts of the population: the bottom longline, the larger individuals and the bottom trawl, the smaller ones (Santos, 2000).

1.7.2 Reproduction

Since the 1950's the Soviet research vessels made several deep water surveys in the North East Atlantic, mainly in the seamounts around the Azores. During these years a lot of biological information was collected from several deep water fishes (Vinnichenko, 2002). Regarding the black scabbardfish, they observed that spawning is intermittent, in the North Azores area it is protracted from November to April, on the South Azores banks, from March to August (Zilanov and Shepel, 1975; Vinnichenko, 2002).

Despite the increasing commercial interest in the black scabbardfish, little is known about its life cycle. The existing contributions on the reproduction usually allude to a short analysis of maturity and to the size range of captured specimens. In the waters to the north of the British Isles, the majority of caught specimens were immature or in an intermediate stage of maturity (Kelly *et al.*, 1998) and there is only a reference to two individuals caught at the Porcupine Bank in January with ripe gonads (Enrich, 1983). However, Nakamura and Parin (1993) observed specimens in the spawning condition west of the British Isles from November to April at depths from 700 to 900 m. Specimens in a spent condition were found in Icelandic waters between January and March (Magnússon and Magnússon, 1995), suggesting that the species may also reproduce in northern areas. No spawners were ever observed off the Portuguese coast (Machado *et al.*, 1998; Anon., 2000; Bordalo-Machado *et al.*, 2001; Figueiredo *et al.*, 2003).

During a 3-year project (1998- 2000; *BASBLACK)* the sexual maturity was examined in different areas of the NE Atlantic (Portugal mainland, Madeira, Azores, NW Scotland, and Rockall Trough). In the NW of Scotland, samples were only collected in September, and individuals were either in maturity stages I or II (see Table 2). The majority of males were immature, while most of the females were developing (stage II). In Portugal, samples were taken throughout the year and the majority of individuals from both sexes were also in immature and developing stages. From July onwards, most individuals began their gonadal development reaching maturity stage II in August. However, between December and April, the majority of females in developing stage showed a clear increase in the incidence of atresia in early-developed oocytes. This suggests that although the specimens are potentially capable of reproducing, they do not enter into a spawning process and remain in a resting phase. Possible reasons for this could be related to insufficient energy reserves for a successful reproduction (Figueiredo *et al.*, 2003).

In contrast to the other areas, all the maturity stages were found in the Madeira archipelago for both sexes. Developing specimens were found all year around, being more common between March and April. Pre-spawning males appeared mostly in May, while pre-spawning females appeared later in July. Spawning fish occurred mainly from September to December (females) and from August to December (males) (Anon., 2000; Bordalo-Machado *et al.*, 2001; Figueiredo *et al.*, 2003). The estimated length at first maturity for females was about 1028 mm (Figueiredo *et al*., 2003).

Table 2. Maturity stages for *Aphanopus carbo* (Adapted from (Gordo *et al.*, 2000)).

	Reproductive stage	Stage description
	FEMALE	
I	Immature / Resting	Ovaries small, transparent or translucid. No oocytes can be seen the naked eye
II	Developing	Ovaries larger and thicker, whitish or pinkish in colour. Small opaque oocytes can be seen with the naked eyes
III	Pre-spawning	Ovaries thicker, ocupying almost the whole body cavity. Large opaque oocytes fill the whole ovary
IV	Spawning	Ovaries occupy the whole body cavity. Hydrated oocytes dominate and will be easily extrude under a sligth pressure on the abdomen
V	Post-spwaning	Ovaries reduced in size and reddish in colour. Residual eggs can be seen with the naked eye.
	MALE	
I	Immature / Resting	Testes very small, firm and pinkish in colour
II	Developing	Testes white-pinkish in colour and larger than in previous stage
III	Pre-spawning	Testes white in colour and ocupying a large part of the body cavity. Sperm can be extruded after a pressure on the abdomen
IV	Spawning	Testes white in colour. Sperm can be extruded very easily after a sligth pressure on the abdomen
V	Post-spwaning	Testes reddish in colour. Residual sperm can be observed especially in the sperm duct

Pajuelo *et al.* (2008) studied the reproductive biology of black scabbardfish in the Canary Islands and the results of the spawning season were the same as in Madeira, that is, in the third and fourth quarter of the year. Size at 50% maturity is reached when males are 1095 mm and females 1144 mm. However, in this study, the authors did not distinguish between *A. carbo* and *A. intermedius*, that exist sympatrically in Canary Islands.

As part of a latter project – *APHACARBO*-, a comprehensive study on reproductive strategies of black scabbardfish have been carried out in the southern Northeast Atlantic: Portuguese slope, and Madeira, where the authors investigated the reproductive cycle and fecundity of black scabbard (Neves et al., 2009). This study confirmed the non-existence of spawners in the

Portuguese continental slope and the only spawning grounds for black scabbardfish were found in Madeira. The study also revealed that this species had a determinant fecundity, which means that the total fecundity prior to the onset of spawning is considered equivalent to the potential annual fecundity, after correcting for atretic losses (Murua and Saborido-Rey, 2003). The authors also hypothesized that the fact that there were no spawners in the Portuguese slope may be due to continuously poor nutrition condition that prevents the continuity of gametogenesis (Neves *et al*., 2009).

1.7.3 Growth

The knowledge of the growth pattern of this species is not well known and age determination presents big difficulties and contradictions.

The first study concerning the ageing and growth of black scabbardfish was made by Morales-Nin and Sena-Carvalho (1996). The sampling took place between 1986 and 1988 from landings of the commercial long-line fishery in Madeira. Black scabbardfish was considered to have a fast growth rate and attained a maximum age of 8 years. The estimated von Bertalanffy growth parameters for males were: L_∞= 1553 mm; K= 0.155; t0= -3.265 and for females were: L_∞= 1420 mm; K= 0.269; t_0= -2.079. The males grow at a slightly slower rate than females and consequently have a lower growth coefficient and a higher asymptotic length.

The occurrence of opaque margins, corresponding to periods of fast growth, in all the otoliths read was greatest during October, decreasing from November to January. This seems to be related to the spawning period, which in this species is from November to December.

The study carried out by Kelly *et al*. (1998), with sectioned otoliths, revealed that black scabbardfish had a much slower growth rate and attained a maximum age of 32 years. In an attempt to overcome the discrepancies found in the previous study, Morales-Nin *et al*. (2002) study had the aim to calibrate the age determinations, establish common otolith reading methodologies and attempt to validate the age readings exploring the feasibility of semi-direct methods. Different methodologies on preparation of the otoliths were tested: whole otoliths; burned otoliths and sectioned otoliths. From the three techniques, the authors stated that the best method was to use whole otoliths, since in the sectioned otoliths it was difficult to define the true increments, because the false rings became more evident. The maximum age was determined to be 12 years. One of the main problems as evidenced by these authors was the interpretation of the first increment due to the variability in the morphology of the nucleus.

In the study with black scabbardfish from the Canary Islands, Pajuelo *et al.* (2008), using burned whole otoliths, estimated age ranged between 2 and 8 years for males and between 2 and 12 years for females (no significant differences were found between sexes). The growth parameters obtained in this study were for males: $L\infty$= 1410 mm; K= 0.263; t_0 = -3.507 and for females: $L\infty$= 1483 mm; K= 0.196; t_0 = -4.647.

The latest study on age and growth of black scabbardfish from Madeira, Azores and Portugal, the maximum recorded age was 15 years, and the sectioned otoliths proved to be the best method for ageing (Vieira *et al.*, 2009). The differences among the published age estimates are the result of the features of the black scabbardfish otoliths, which have poor contrast between the alternating dark and light zones, and a confusing sequence of narrow zones, which can either be counted singly or grouped. But also on the preparation and interpretation of the periodic features in the calcified structures, which can vary markedly among readers and laboratories (Campana, 2001).

1.7.4 Feeding

The available information on the diet of black scabbardfish is confined to general comments on the stomachs contents from specimens collected from the Hatton Bank (Du Buit, 1978), the Rockall Trough (Mauchline and Gordon, 1984), west of the British isles (Zilanov and Shepel, 1975) and Portugal (Santos, 2000), but without any detailed description or interpretation. The lack of studies is due to the difficulty to obtain samples, since the majority of individuals captured have everted or empty stomachs due to varying pressure.

Mauchline and Gordon (1984) examined the stomachs from specimens caught in the Rockall Trough and on the Hatton Bank. From the 148 stomachs examined, only 48 contained food. The diet of *A. carbo* was dominated by fish and the only other organisms presented were remains of squid. A high proportion of the fish was in the form of unidentified fragments but blue whiting (*Microsmesistius poutassou*), deep-water rockling (*Antonogadus macrphtalmus*), argentine (*Argentina* silus) and unidentified scombrids were identified as prey items.

In Madeira, the few stomachs with food content, the prey items identified were described as a meso- and bathypelagic oceanic species. The cephalopod group was the best represented, followed by crustaceans and teleost fish (Santos, 2000).

1.8 Bioaccumulation studies

The black scabbardfish as a top predator, can accumulate some toxic metals (mercury, cadmium, lead, zinc and copper) in its tissues through its diet (Afonso *et al.*, 2007). This species represents an important component of local diet and is one of the most important species caught in Madeira Archipelago and because of that this species has been thoroughly examined, due to human health concerns (Bebianno *et al.*, 2007).

In the studies carried out by Afonso *et al*. (2007) and Costa *et al.* (2009), levels of mercury, cadmium and lead in some tissues (muscle, liver and skin) of black scabbardfish caught in Madeira, Azores and Portuguese slope were quantified. The general results suggested that this species has high levels of mercury, cadmium and lead, especially in the liver and gonads (Costa *et al*., 2009). However, the levels of these metals in the muscle do not represent a risk for human consumption if the liver is excluded and the edible part is consumed with moderation (Bebianno *et al.*, 2007).

General objectives of this study, with notes on the thesis style

Despite the high commercial importance of black scabbardfish in the Madeira Islands and northern Europe, there is still a lack of knowledge on the life history and stock structure of this species in NE Atlantic, especially in understanding the connectivity between the individuals captured in the northern waters (ICES subarea VI) and Madeira. The general concerns about the sustainability of deep water resources and the urgent need to enhance our understanding of the life history and ecology of *Aphanopus carbo* in NE Atlantic constitute the basis for this study.

This study combines for the first time data collected throughout the year from the west of Ireland and Scotland and the Madeira Islands and uses a combination of methodologies to get further knowledge in the life history and population structure of *A. carbo* over its wide distribution range in the Northeast Atlantic.

The objectives of the present study were:

1) Study the **reproductive dynamics** and, thus, the life cycle of black scabbardfish from the west of British Isles and Madeira Islands and discuss the management implications of the findings (Chapter 2);

2) Investigate the **age and growth** of black scabbardfish from the west of the British Isles and Madeira using two reading interpretations. The growth for each interpretation was analysed by fitting to the von Bertalanffy growth model and the difference and the effects on the estimated growth parameters were examined taking into account the bias and precision between the readings. The growth model parameters were also compared between the fish caught west of the British Isles and the specimens from Madeira (Chapter 3);

3) Examine the **diet composition, feeding strategy** and, thereby the **trophic ecology** of black scabbardfish in two areas of NE Atlantic. Classic stomach examination was augmented by stable isotope analysis. While the stomach contents provide information on the composition of recent meals, stable isotope compositions integrate the

signatures of different prey consumed over a longer period, and can be used to infer trophic level and discriminate sources of food between the two areas (Chapter 4).

4) Investigate whether fish from the two different areas are likely to form independent populations, or whether some degree of **population connectivity** exists at some stage of the life cycle. By examining the ratios of **stable isotopes** (oxygen and carbon) in three different regions of the otolith (core, middle and edge) fish movements and metabolic activity during these three life stages were tracked (Chapter 5).

5) Investigate the trends in **distribution, abundance and size structure** of black scabbardfish off the west of the British Isles, compiling data from the Scottish and Irish time series deep water trawl surveys (Chapter 6);

Each chapter of this thesis has been written in a paper-style format, suitable and appropriate to be published in a scientific journal. Each chapter constitutes a complete study (although references to other chapters are included), and can be read independently of others. At the beginning of each chapter, information is given regarding the publication status, list of co-authors and the complete reference to the journal where it has been published or submitted. Some of the chapters are still being prepared for submission to scientific journals and therefore are classified as “in preparation”. Tables and figures appear in the text inside each chapter.

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CHAPTER 2

Oocyte dynamics and reproductive strategy of *Aphanopus carbo* in the NE Atlantic – implications for fisheries management

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Contents

Abstract

Black scabbardfish is a deep water species of high commercial interest in the NE Atlantic; however the life history and stock structure is poorly understood. For this study, specimens were collected from commercial trawls off NW Scotland and longliners from the Madeira Islands, between September 2008 and May 2010. Geographical differences in the reproductive state of scabbardfish were evident and are consistent with the theory of a north-south migration. Only immature specimens were found in the northern area, while in Madeira all maturity stages were observed, with the peak spawning in October-November. Consistently, the gonadosomatic index (GSI) showed an increase throughout the year, reaching a maximum in October and November for males and females, respectively; while for the northern area the GSI values had low variability. Oocyte development was described and characterized for each maturity stage. Histology revealed that black scabbardfish is total spawner, has a determinate fecundity and the oocytes show a group-synchronous development. Distinguishing resting from developing females was resolved by measuring ovarian wall thickness. The geographical quasi-complete separation of the immature and mature individuals necessitated the use of a novel bias-reduction GLM in the estimation of LC50 when using samples from Madeira. Estimated length at maturity (LC50) for both sexes was significantly higher when data from both areas were combined (Females = 1156mm, Males = 1098mm) than just using the Madeira dataset (Females = 1110mm, Males = 1010mm). The results highlight large scale dispersal in this species which needs to be treated as a highly migratory species and be managed as a single population.

1. Introduction

Understanding the reproductive biology and the life cycle of black scabbardfish (*Aphanopus carbo* Lowe, 1839) is intricate due to the wide distribution of this species in the NE Atlantic. It is distributed from Iceland (1995) to the Canary Islands and Bojador Cape in the Western Sahara (Pajuelo *et al*., 2008; Uiblein *et al*., 1996) including the islands of Madeira, the Azores and numerous submarine banks and seamounts (Nakamura and Parin, 1993; Zilanov and Shepel, 1975). Black scabbardfish belongs to the benthopelagic category of deep-water fishes, living close to the bottom along the continental slope and occurs mainly at depths from 700 to 1300m (Bordalo-Machado *et al.*, 2001; Bridger, 1978; Enrich, 1983; Figueiredo *et al*., 2003).

Previous preliminary studies in the NW of Scotland comprise a short analysis of maturity and the size range of captured specimens, showing that the majority the specimens were immature (Kelly *et al*., 1998; Figueiredro *et al*., 2003). There is reference to only two individuals caught on the Porcupine Bank in “ripe” condition (Enrich, 1983) and individuals in spent condition were found in the Reykjanes Ridge between January and March (Magnússon and Magnússon, 1995) suggesting that this species may also reproduce in the northern areas. However all of these studies lack temporal replication and histological validation of the maturation process.

The most comprehensive studies on reproductive strategies of black scabbardfish have been spatially confined to the southern Northeast Atlantic: Portugal, Madeira and Canary Islands, where the authors investigated the reproductive cycle (Bordalo-Machado *et al*., 2001; Figueiredo *et al*., 2003; Pajuelo *et al*., 2008), fecundity (Neves *et al*., 2009) and the maturity ogive for females of black scabbardfish was estimated (Figueiredo *et al.*, 2003). In these studies, no spawners were found off the Portuguese continental slope (Figueiredo *et al.*, 2003) and the only spawning grounds for black scabbardfish were found in Madeira and Canary Islands (Figueiredo *et al*., 2003; Neves *et al*., 2009; Pajuelo *et al*., 2008).

It has been postulated that black scabbardfish does not complete its life cycle in one geographical area, large-scale migrations occur and the fish caught to the west of the British Isles are pre-adults that migrate further south (possibly to Madeira) as they reach maturity and spawn (ICES, 2011). However due to the lack of information on some aspects of the biology, there is still a lack of scientific evidence to support this theory of a single stock. One of the main limitations of previous studies on reproduction of black scabbardfish is the lack of understanding of the connectivity between the specimens caught to the West of the British Isles and the ones from the Madeira Islands.

Black scabbardfish is an economically important deep water species that has been traditionally exploited in Portuguese waters by longliners. The first known artisanal handline fishery for this species started around the Madeira Islands (Haedrich *et al.*, 2001) and since 1983, the exploitation of black scabbardfish expanded to the Portuguese continental waters *(Gordon et al.*, 2003; Gordon, 2001). Black scabbardfish is the most important fishery resource in Madeira and one of the most valuable deep water species landed in Portugal (Bordalo-Machado *et al.*, 2009). In the North of Europe, the species has been mostly captured around the British Isles (ICES Subareas V, VI and VII) and Iceland (ICES Subarea Va), mainly by French, Icelandic and Spanish trawlers (ICES, 2011) since the early 1990's, following the development of a multi-species deep-water fishery (ICES, 2008). In the early years of these fisheries, black scabbardfish was mostly discarded as no market had developed for the species, but it subsequently became one of the main target deep-water species (Bordalo-Machado and Figueiredo, 2008). The total landings of black scabbardfish for the ICES Subareas V, VI and VII showed a peak in 2006, with landings reaching 8,000 tonnes, decreasing afterwards to levels around 3,000 tonnes (ICES, 2012). The reduction of catch limitations (TACs) and fishing effort since 2006 might have also contributed for the decreasing landing trends (Neat and Burns, 2010).

Currently the black scabbardfish fishery is managed following biennial advice from ICES providing advice for the NE Atlantic fishery - and CECAF (Fishery Committee for the Eastern Central Atlantic) providing advice for the Madeira longline fishery. However, there is still a lack of knowledge on stock structure of black scabbardfish over its geographical distribution, so the state of the stock is unknown (ICES, 2012).

The aim of this paper is to increase understanding of the connectivity, the reproductive dynamics and, thus, the life cycle of black scabbardfish in the NE Atlantic, combining for the first time, data collected throughout the year from West of the British Isles and the Madeira Islands.

In this context we will investigate 1) the reproductive cycle of black scabbardfish in both areas, 2) characterize the oocyte development and dynamics throughout gonadal development, 3) determine the intensity and prevalence of atretic oocytes throughout the year in both areas, 4) the variations of gonad and liver weight with season and maturity stages and 5) determine the size at maturity for males and females. The management implications of these findings are then discussed.

2. Material and Methods

2.1 Sampling

Samples were collected from a monthly sampling programme, conducted between June 2009 and May 2010, from commercial French trawlers operating to the West of the British Isles and on a fortnightly sampling programme, between April 2009 and February 2010, from landings of the commercial longline fishery in Madeira Archipelago (Portugal) (See Appendix I, Table 1). Additional samples were obtained from scientific deepwater bottom trawl surveys: Marine Institute Deep water Survey, carried out on board *R/V Celtic Explorer*, in September 2008 and December 2009; Marine Scotland Deep water survey, on board *R/V Scotia*, in September 2009 and the French IBTS (International Groundfish Survey - EVHOE 2009) survey, conducted by IFREMER, on board R/V *Thalassa* off the Biscay Bay, in October 2009 (Fig.1). Details of data acquisition are provided in Appendix I - Table 2.

Since the early 1990’s a second species of the genus *Aphanopus* - *A. intermedius* – has been recognised in the southern NE Atlantic (Madeira and Azores). This species is morphologically similar to *A. carbo* and can only be differentiated by counting the vertebrae and the dorsal fin spines (Nakamura and Parin, 1993). To ensure that only *A. carbo* was sampled, all the specimens from Madeira were morphologically analysed to discriminate both species, following the study carried out by (Biscoito *et al.*, 2011). To determine the presence or absence of both species to the West of the British Isles and Bay of Biscay samples, a preliminary meristics study was carried out on 250 specimens, and the results indicated that in these areas only *Aphanopus carbo* was present.

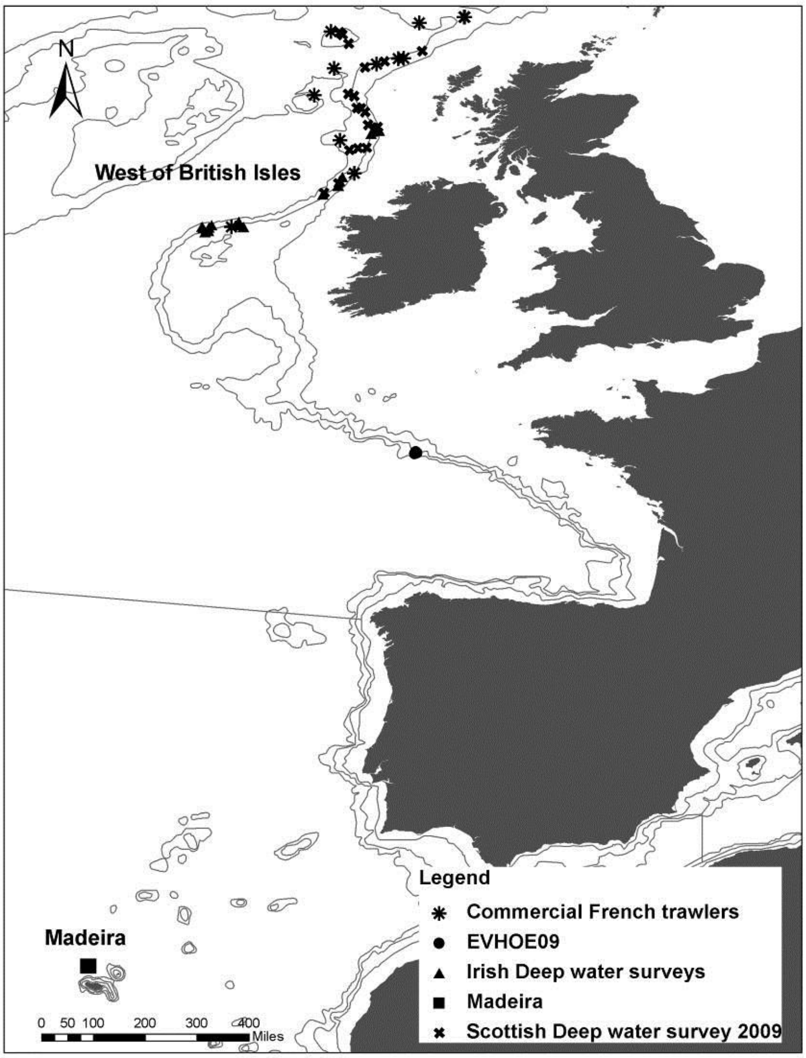


Fig 1. Map with the locations where the samples of black scabbardfish used for this study were collected.

For the purpose of the study of the reproductive cycle, a total of 2145 fish were sampled from the area to the West of the British Isles, and 409 specimens from Madeira were analysed. Each specimen was measured - total length (L_T, mm), weighed – total and gutted weights (g) and sexed (Table 2). The liver and gonads were weighed to 0.1g using an electronic balance and the maturity stages were assigned by macroscopic examination using a modified maturity scale adapted by Gordo *et al*. (2000) (Table 1).

Table 1. Description of females and males maturity stages for black scabbardfish (adapted from Gordo *et al*., 2000)

Maturity stage		**Stage description**
	Female	
I	Immature	Ovaries small, transparent. No oocytes can be seen with naked eye. Previtellogenic oocytes in different stages of development
IIa	Developing	Ovaries larger and thicker, whitish or pinkish in coulour. Small opaque oocyte can be seen with naked eye. Appearance of cortical alveoli in the oocytes.
IIb	Resting	Ovaries pinkish, with thick wall. Early-vitellogenic and atretic oocytes present.
III	Pre-spawning	Ovaries thicker, yellowish in colour, occupying alomost the whole abdominal cavity. Large opaque oocytes are easily distinguisable. Oocytes characterize by the presence of yolk globules
IV	Spawning	Ovaries occupy the whole abdominal cavity, with the hyaline oocytes easily extruding under pressure. Oocytes up to 1500µm, completely hidrated.
V	Post-spawning	Ovaries flaccid and reddish in colour with residual eggs. Post ovulatory follicles present
	Males	
I	Immature	Firm testes with laminar aspect, translucent. Spermatogonia dominate.
IIa	Developing	Testes white-pinkish in colour and larger than previous stage. Seminiferous tubules become distinguishable. Spermatocytes predominate
III	Pre-spawning	Testes hite in colour. Sperm can be extruded after pressure on the abodomen. Spermatids predominate, spermatozoa already present.
IV	Spawning	Testes white and big Sperm is easily extruded after silght pressure. Collecting and sperm ducts full with spermatozoa.
V	Post-spawning	Testes flaccid with haemotthagic aspect. No spermatogenesis; some residual spermatozoa.

2.2 Histological procedures

Of the total fish sampled, a sub-sample of 650 gonads was used for histological analysis, 350 were female (250 from specimens captured to the West of the British Isles and 150 from Madeira) and 250 were male (150 from West of the British Isles and 100 from Madeira). The gonads were fixed in Davidson's Solution for 48-72 hours, depending on their size and thickness, and preserved in 70% ethanol prior to histological analysis.

Transverse slices of about 1cm thick were taken from the middle, anterior and posterior regions of the gonads, dehydrated through a series of ethanols and embedded in wax using a Shandon Citadell 1000 and Tissues-Tek® TEC. At least three sections, 5µm thick, were cut from each region using a Leica RM 2235 microtom and stained with Haematoxylin and Eosin by a Leica Autostainer XL.

To determine whether the development in the middle region of the gonads was representative of the whole gonad, 50 additional sections from the anterior and posterior ends were analysed and compared with the middle section. Since no differences were observed between regions, the analysis continued using only the middle region of the gonads. Each slide was examined under a Nikon Eclipse 80 at x40, x100 and x200 magnification. The sex and the maturity stage were determined without prior knowledge of the length and macroscopic stage of the specimen. The criteria for the microscopic identification of different maturity stages and post ovalutory follicles (POFs) were adapted from Gordo et al. (2000).

A minimum of 1,000 oocytes were randomly selected from section and the diameter was measured on the horizontal axis. Only those oocytes clearly sectioned through the nucleus were measured. To distinguish between immature and resting females, the thickness of the ovarian wall was measured in three places in each section using the NIS Element BR 2.10 software.

2.3 Data analysis

Sex ratio

The sex ratio was calculated for each depth strata and length class (50 mm length classes). Chi-squared tests were used to examine the differences between observed sex ratios and the expected ratio of equal numbers of each sex along depth strata and length class (Zar, 2010).

Reproductive cycle

The reproductive cycle was examined based on the monthly evolution in the percent frequency of the maturity stages for both sexes in each area.

To study the oocyte dynamics at each maturity stage, the percentage of previtellogenic, early vitellogenic, vitellogenic, mature, atretic oocytes and post ovalutory follicles (POFs) were calculated by counting 300 - 350 total ovarian follicles in each ovary section. Oocyte development was described and characterized for each maturity stage using a modified maturity scale developed by Gordo *et al*. (2000).

Atretic oocytes and the postovulatory follicles were classified according to Hunter and Macewicz (1984) and Ganias (2011), respectively. The prevalence of atretic oocytes was determined as the number of female fish with atretic oocytes as a proportion of the total female fish. The relative intensity of atresia was calculated, for each month and maturity stage, as the percentage of atretic oocytes in the total number of oocytes present in an individual ovary section.

2.4 Wall thickness

The ovarian wall thickness was compared between maturity stages using the non-parametric Tuckey-Type pairwise test (Zar, 2010). To investigate the variation of the ovarian wall thickness (WT_{ij}) with total length (TL_i) and maturity stage (M_i), the data were analysed using generalized least squares (gls), with "nmle" package within statistical software R 2.9.2.(R Development Core Team, 2011).

The initial model was:

$$WT_{ij} = \alpha \times M_j + TL_i \times M_j + \varepsilon_{ij} \text{, (Eq. 1)}$$

where WT_{ij} is the wall thickness of the *i*th observation in maturity stage *j*; α is as intercept; TL is total length (mm); M is the maturity stage and *ε* are the random residuals, which are normally distributed. Exploratory analysis indicated that the residuals plots for constant variance models showed violations of homogeneity, requiring the use of different variance structures that allow the residual spread to vary with respect to total length and maturity stage (Zuur *et al*., 2007).

The model was optimized by first looking for the optimal random structure (among candidate variance structures including: homoscedastic errors, by-group heteroscedasticity, power function of total length and a combination of by-group and power function of total length), and then for the optimal fixed structure, using Akaike Information Criterion, AICc. Once the optimal model was found, in terms of the random structure, further selection was applied by rejecting any remaining non-significant explanatory variables (Zuur *et al*., 2007).

2.5 Hepatosomatic and Gonadosomatic indexes

To assess temporal changes in female and male reproductive condition, the gonadosomatic (GSI) and hepatosomatic (HSI) indexes were calculated in each area. The GSI was calculated as, $GSI = (G_W \times 100) / Gut_W$,

where, G_W is the gonadal weight (g) and Gut_W is the gutted weight (g).

The hepatosomatic index (HSI) calculated as: $HSI = (L_W \times 100) / Gut_W$, where L_W is the liver weight (g) and Gut_W is the gutted weight (g).

The variation of these indexes was analyzed amongst months and maturity stages using non-parametric Kruskal-Wallis tests (*H*) followed by a non-parametric post-hoc Nemenyi test. Mann-Whitney (*U*) test was used to test for differences between sexes.

2.6 Size at maturity

The Kolmogorov-Smirnov (KS) two sample test was used to test for significant differences in the length frequencies by sex and area (W British Isles and Madeira) (H_0: No difference in length frequencies between males and females *or* the W British Isles and Madeira).

Based on the fraction of mature specimens by length class (10 mm), the maturity ogive and length-at-first maturity ($LC_{50\%}$) were estimated. Specimens in stages I and II-a were considered immature and individuals in subsequent stages (III, IV, V and II-b) were considered mature (see Table 3).

Due to the nature of the data in each area – West of the British Isles and Madeira – it was necessary to have a different approach to the estimation of the maturity ogive for black scabbardfish. Data revealed that the Northern area specimens were all immature (Stages I and II-a) and the Madeira specimens were mostly mature (only 9 and 14 immature females and males were sampled, respectively). First, the maturity ogive was fitted for both sexes separately, using only the Madeira dataset (Females *n* = 200 and Males *n* = 198) using a bias-reduction GLM (BRGLM, (Kosmidis, 2007) with the binomial family and a logit link. BRGLM penalizes the maximum likelihood and is useful in cases of complete or quasi-complete separation in the data (Firth, 1993). Complete separation arose in the Madeira female data as the largest immature fish was 1085mm and the smallest mature fish was 1107mm. Such separated data cannot be fit within a regular GLM framework (Heinze and Schemper, 2002).

A second maturity ogive for each sex was fitted with the combined datasets – West of the British Isles and Madeira (Females *n* = 1411 and Males *n* = 1114) – using a binomial GLM. Both maturity ogives and length-at-first maturity estimated were compared visually by inspecting the overlap of the confidence intervals over total length. The models mentioned above were estimated using the software R 2.9.2 (R Development Core Team, 2011).

3. Results

3.1 Sex ratio

The sex ratio in the northern area was biased towards females ($\chi^2 = 41.33$, df = 1, $p < 0.05$), with a sex ratio of 1: 1.32 (M: F). Females predominated throughout the year, except in July. It was only possible to examine the variation of sex ratio per depth strata for the specimens sampled during the deep water trawl surveys in the northern area. Females significantly outnumbered males in all depth strata except the deepest (>1200m depth) where the sex ratio was 2.2:1 (M: F), but sample size was very small (Table 2).

For the Madeira specimens, the overall sex ratio was not significantly different from 1: 1 (M: F) ($\chi^2 = 0.02$, df = 1, $p > 0.05$). However, the sex ratio of black scabbardfish commercially sampled in Madeira varied seasonally. Males outnumbered females throughout the year, except in April and November.

Table 2. Sex ratio per depth strata in the black scabbardfish captured during the deep-water trawl surveys off the West of the British Isles

Depth strata	Females	Males	Sex ratio	Chi-squared
<700	147	71	1: 0.48	26.50*
800 - 900	237	187	1: 0.79	5.90*
901-1099	651	547	1: 0.84	9.03*
1100 - 1199	167	106	1: 0.63	13.63*
>1200	11	5	1: 0.45	2.25

* $X^2 > X^2_{(0.05,\ 1)} = 3.84$

3.2 Reproductive cycle

All the specimens captured to the West of the British Isles were immature. Only the first two maturity stages (I and II-a) were observed (Fig. 2). Males and females were predominantly in stage I throughout the year, except in April, when 70% of females sampled were developing (stage II-a).

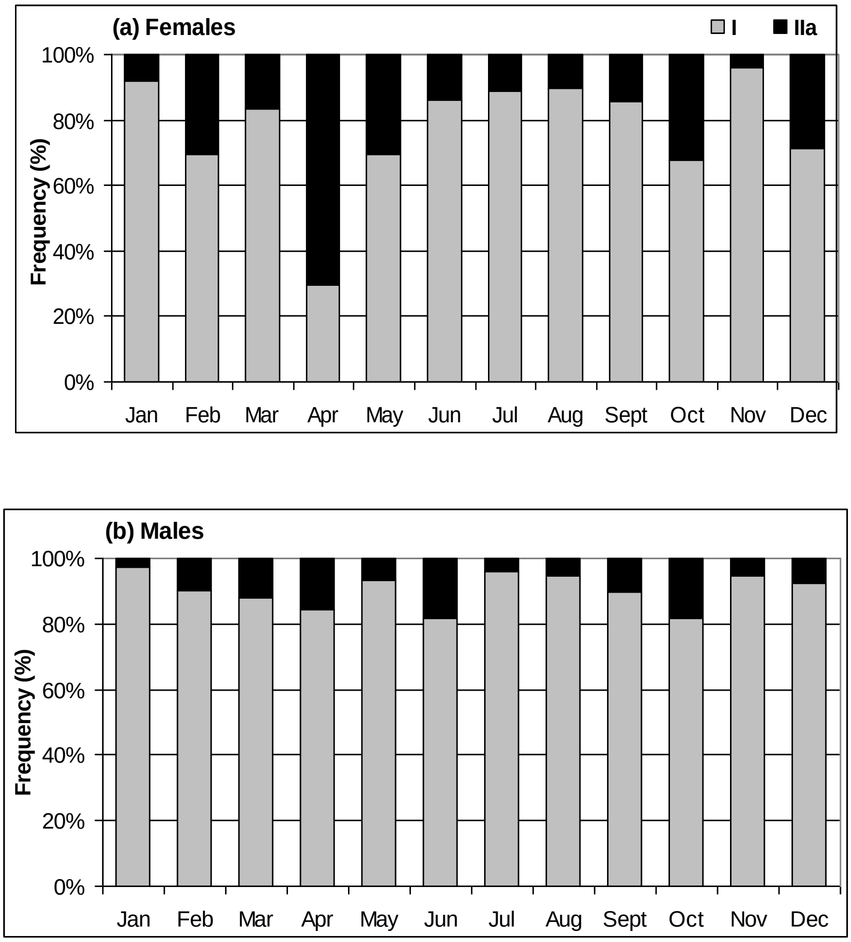
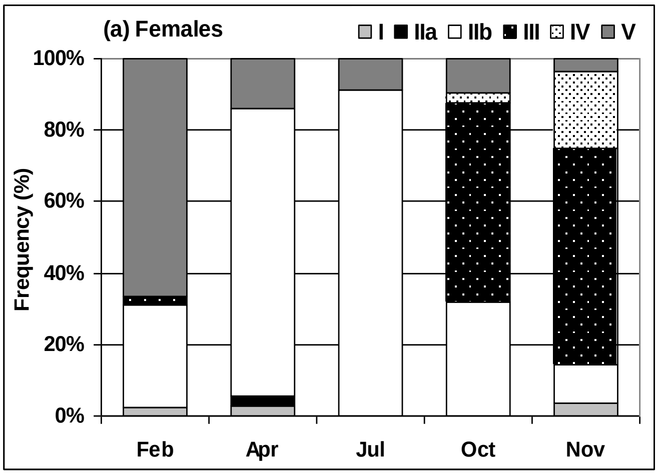


Fig 2. Monthly percentages of maturity stages of black scabbardfish from West of British Isles: females (a) and males (b).

In Madeira, all maturity stages were observed in both sexes (Fig. 3), although immature specimens were recorded very rarely (only 1.7% of the fish captured was immature). There was a clear reproductive cycle, with the majority of the females (95%) in the post-spawning (Stage V) or resting stage (Stage II-b) in February. Between April and July, most of the females were in the maturing / resting stage (stage II-b) and the main spawning period was determined to be between October and November, with pre-spawning (Stage III) and spawning (Stage IV) females prevailing (Fig. 3a). In males, the pre-spawning stage (III) occurred throughout the year, with a clear prevalence in February and between July and October. Spawning males (stage IV) started to occur in July and become more abundant in October and November, whereas post-spawning males mainly occurred between November and February (Fig. 3b).



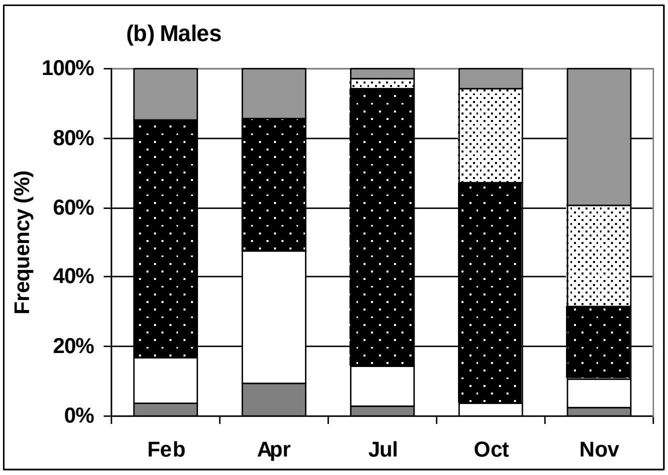


Fig 3. Monthly percentages of maturity stages of black scabbardfish from Madeira Islands: females (a) and males (b).

3.3 Gonad microscopic development

Females

Stage I – Immature female

This stage is characterized by the existence of previtellogenic oocytes in different growth phases, with chromatin nucleolar and/or perinucleolar, creating a mosaic appearance (Fig. 4a). In this stage, the ovary was formed exclusively with previtellogenic oocytes (Fig. 5). These oocytes appeared gathered in nests, set in the ovarian lamella and presented a single big nucleus in a central position (Fig. 4a). As the development progresses, some of theses oocytes presented an increase of volume and the nucleoli became more numerous and migrated to the edge of the nucleus. The diameter of the previtellogenic oocytes varied between 33 and 189 µm (diameter average = 93 µm, S.E. = 0.63) (Fig. 6). In the most advanced phase of development some vitellogenic oocytes started to appear, but in very low frequencies to assign the ovaries to maturity stage IIa.

Stage IIa - Developing, immature female

The ovaries presented a higher proportion of vitellogenic oocytes, among the typical stage I oocytes (Fig. 4b- photo). Previtellogenic oocytes were still predominant (75% of the total oocytes), but in a lower proportion than the stage I (Fig. 5). The early-vitellogenic oocytes were characterized by the appearance of the cortical alveoli in the cytoplasm which made an increase of volume. The oocytes diameter ranged from 100 to 370µm (diameter average = 200µm, S.E. = 1.61) (Fig. 6) and the nucleus/cell ratio decreased. The follicular layer of the oocytes became more conspicuous, with the three layers (theca, granulosa and radiate) already visible.

The following stages were only encountered in females caught off Madeira Islands.

Stage III – Pre spawning female

The general appearance of the ovary revealed the formation of big vitellogenic oocytes, but previtellogenic oocytes still occurred in great numbers (Fig. 5). The ovarian lamella lost their conspicuous contour. The vitellogenic oocytes increased remarkably in size, attaining diameters ranging between 250 and 1000µm (diameter average = 700µm, S.E. = 7.5) (Fig. 6). The oocytes in this stage were characterized by the presence of lipid vesicles and deposit of protein granules in the cytoplasm. These round structures were small in the beginning giving the cytoplasm the appearance of a mosaic. The nucleus lost the round shape and decreased in size. The follicular layer became thicker and very distinctive (Fig. 4c).

Stage IV – Spawning female

The ovaries in this stage were very large, occupying almost all the abdominal cavity. The prevailed oocytes were fully mature and completely hydrated (Fig. 5). The yolk droplets fused with the lipid vesicles, forming a homogeneous layer. The diameter of the mature oocytes varied between 520 and 1250μm (average diameter = 972μm, S.E. = 7.5) (Fig. 6). The nucleus migrated towards the animal pole and in some cases had already disintegrated. In some samples was observed some atretic oocytes and postovulatory follicles (Fig. 4d).

Stage V – Spent

The ovaries presented different levels of flaccidity and very thick ovarian walls. In this stage, the ovaries were highly disorganised and cordon-like structures among new oocytes were observed, which correspond to postovulatory follicles. It was observed a high percentage of atretic oocytes (Fig. 4e). Most of oocytes were in pre-vitellogenic and early-vitellogenic stage (Fig. 5).

The stage IIb – Mature, resting female

This is the later stage of development after a spawning episode. The ovaries reabsorbed most of POFs and recommence the development of new oocytes for the next reproductive season (Fig. 4f). The internal structure of the ovary is more organized than the previous stage; the ovarian lamellae are again noticeable and most of the oocytes are in a pre-vitellogenic stage (Fig. 5). This stage is distinguishable from the stage II-a due to the general internal structure of the ovary, the presence of a higher percentage of atretic oocytes, and a much thicker ovarian wall.

Note: For detailed description on the male gonad development of *Aphanopus carbo* see Appendix II.

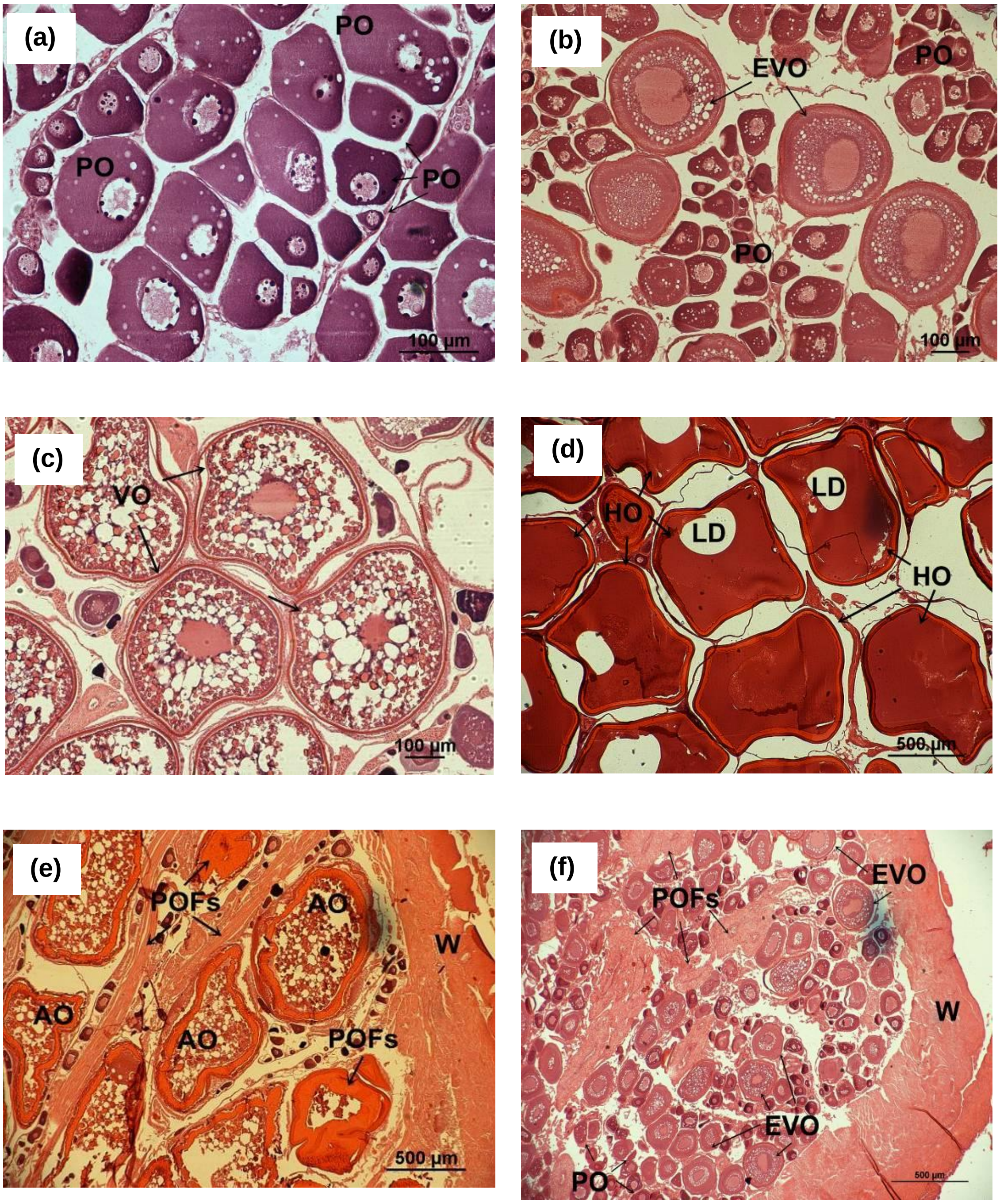


Fig 4 – Ovary development stages of *Aphanopus carbo*: (a) immature – stage I; (b) developing female – stage IIa; (c) Pre-spawning female – stage III; (d) Spawning female – stage IV; (e) Post-spawning female – stage V; (f) Resting female – stage IIb. PO, previtellogenic oocytes; EVO, early-vitellogenic oocytes; VO, vitellogenic oocytes; HO, hydrated oocyte; LD, Lipid droplet; POFs, postovulatory follicles; AO, atretic oocytes; W, ovarian wall.

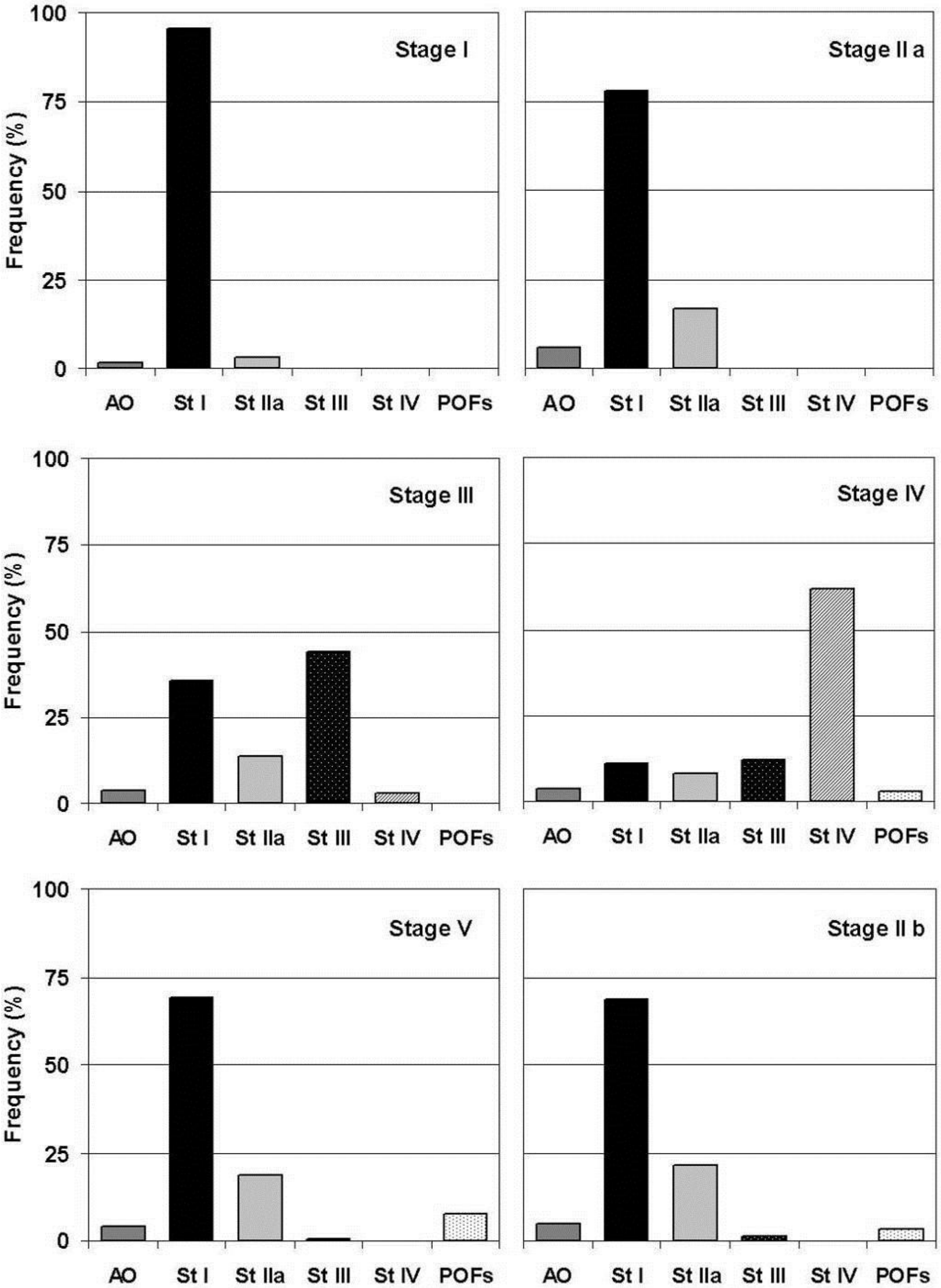


Fig 5 . Frequency of ovarian follicles in the ovaries at each ovarian maturity stage. AO- Atretic oocytes; StI – previtellogenic oocytes; St IIa – early-vitellogenic oocytes; St III – vitellogenic oocytes; St IV – hydrated oocytes; POFs - and post ovalutory follicles

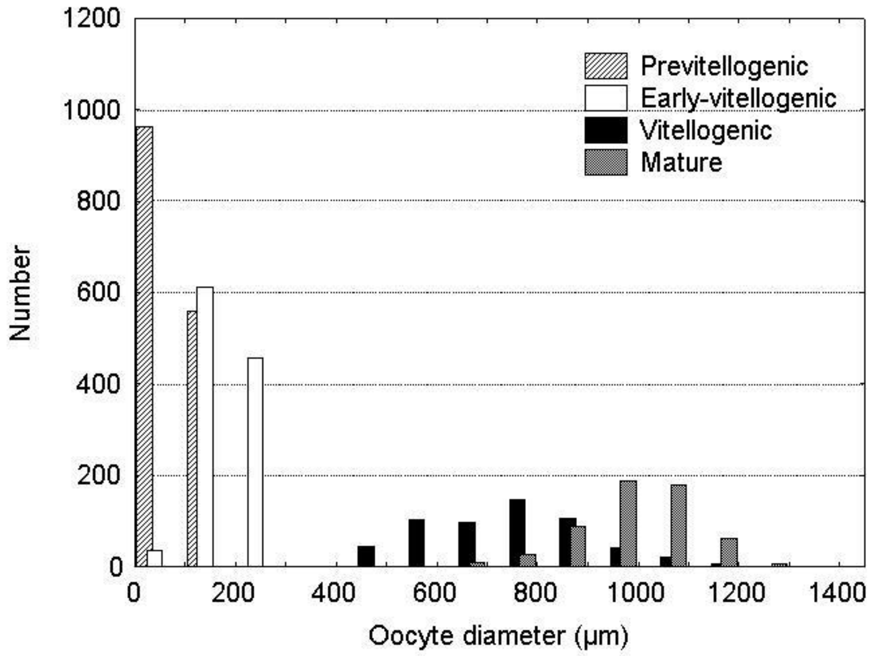


Fig 6. Frequency distribution of oocyte size (in µm) of black scabbardfish, in each maturity stage

3.4 Atresia

The specimens caught to the West of the British Isles showed atretic oocytes among the previtellogenic and early vitellogenic oocytes. Atretic oocytes were only identified in the early-vitellogenic oocytes and the prevalence of atresia was higher in stage II-a females, where the majority of ovaries in this stage showed atretic oocytes (65%).

Throughout the year the females in stage I presented low relative intensity of atretic oocytes, with an average of 1.7% of atresia in most of the months (ranging from 0.3% to 3.4%). The stage II-a females showed higher relative intensity of atresia along the year, with higher values observed in April, ranging between 4.2% and 16.2% of atretic oocytes (average = 8.0%) (Fig.7).

For the specimens sampled in Madeira atresia was present in all maturity stages. The mean relative intensity of atresia presented higher values in the stage V - Spent (average intensity of atresia = 6.3%), ranging between 2.2% and 16.1%. The pre-spawning (III) and spawning (IV) females showed atresia oocytes also, varying between 2.1% and 8.8% in pre-spawning ovaries and between 1.8% and 6.5% for spawning females (Fig. 8).

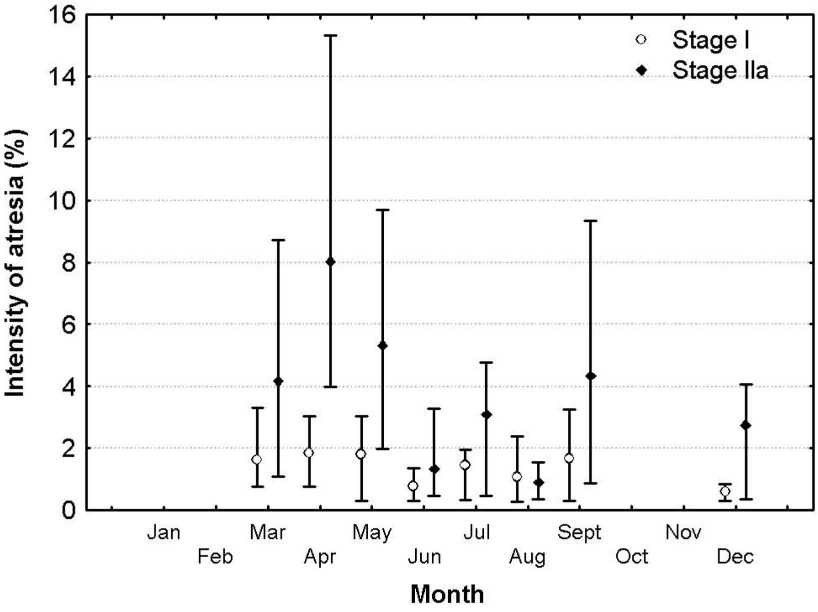


Fig 7. Intensity (%) of atresia (Mean, minimum and maximum) in stages I and IIa females caught off West of British Isles.

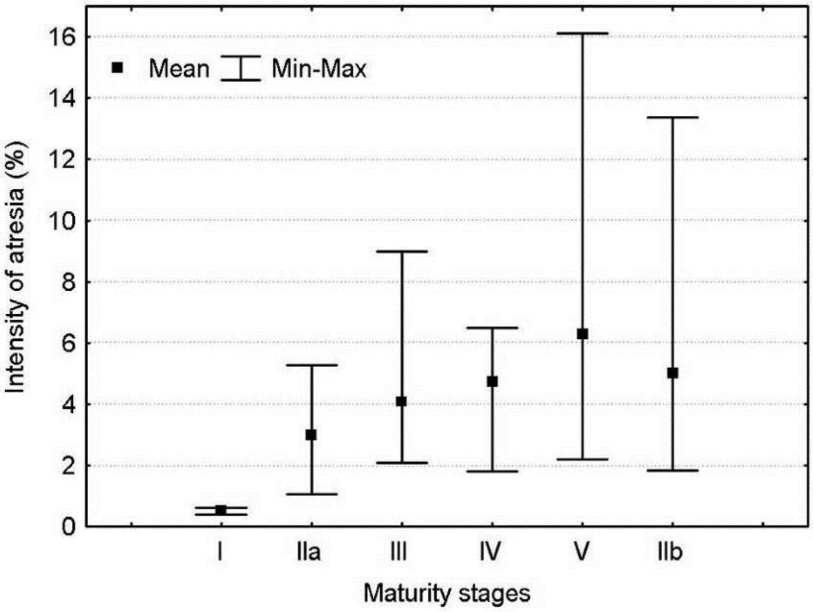


Fig 8. Intensity of atresia (%) in each maturity stage from females caught off Madeira islands

3.5 Wall thickness

Ovarian wall thickness increased with maturation. Females in stages I and IIa had a very thin ovarian wall, with no significant differences between them (Tukey-type pairwise comparisons, *p* = 0.523), varying between 33 and 200µm. The pre-spawning and spawning females showed a thicker wall relative to the immature fish, attaining 270µm in some cases, but the difference was not significant (Tukey-type pairwise comparisons, *p* = 0.876). In the last two maturity stages, V and IIb, the ovarian wall was typically thicker. In stage V, the wall thickness ranged between 498 and 1050µm and in the stage IIb the wall decreased in thickness, varying between 261 and 888µm (Fig. 9). Females in developing (Stage IIa) had significantly thinner ovarian walls than females in the resting stage (Stage IIb) (Tukey-type pairwise comparisons, *p*<0.05).

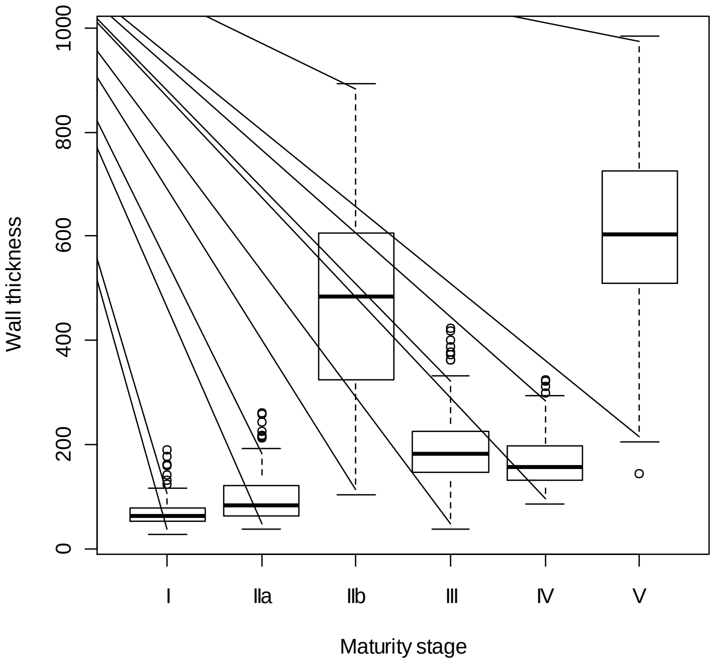


Fig. 9. Wall thickness (in µm) in each maturity stage. Middle line: mean; box: Standard error; Whisker: Min-Max.

The final model that allowed the residuals to vary with respect to total length and maturity stage was:

$$\text{Wall\_Thickness} = \alpha \times M_j + TL_i \times \text{Matstage}_j + \varepsilon_{ij}, \text{weights} = \text{varPower}(\text{form} =\sim TL \mid \text{fMatstage}) \quad \text{Eq. (2),}$$

where $\varepsilon_{ij} \sim N(0, \sigma^2 \times |TL_i|^{2\delta_j})$

This model had the lowest AIC and is therefore selected as the optimal model (Table 3). Table 4 lists the estimates of the fixed effects obtained by fitting the wall thickness data using Eq. (2).

Table 3 Residual standard errors and AIC values for the linear model and the extended GLS models using various variance structures to select the optimal model for the variance of the wall thickness. GLS, Generalized Linear Squares model; TL, Total length; MatStage, maturity stage

Model	Residual stantard error	AIC
Linear model	93.213	9851.54
GLS, VarFixed (~TL)	2.642	9746.28
GLS, VarIdent (~MatStage)	22.557	8885.92
GLS,VarExp (form= ~TL)	0.303	9170.06
GLS, VarPower (form=~TL|fMatStage)	**0.295**	**8883.13**
GLS, VarComb (varIdent (form=~1|MatStage), varExp (form= ~TL)	11.956	8884.13

Table 4 The estimates of the of the fixed effects parameters by fitting Eq. (12) to the wall thickness data for black scabbardfish. * significant differences, *p* < 0.05

Parameters	Value	S.E.	*t*-value	*p* - value
Stage I	39.637	16.054	2.469	0.014*
TL	0.03	0.017	1.798	0.073
Stage IIa	-82.238	37.101	-2.216	0.027*
Stage IIb	-542.994	410.832	-1.321	0.186
Stage III	-156.074	115.555	-1.350	0.177
Stage IV	-442.162	162.889	-2.714	0.007*
Stage V	1366.521	341.591	4.000	0.0001*
TL: Stage IIa	0.102	0.036	2.842	0.004*
TL: Stage IIa	0.726	0.324	2.240	0.025*
TL: Stage III	0.216	0.091	2.346	0.019*
TL: Stage IV	0.435	0.131	3.310	0.001*
TL: Stage V	-0.659	0.266	-2.476	0.013*

3.6 Hepatosomatic and Gonadosomatic index

The GSI for the specimens captured off the West of the British Isles were very low and with small variations for both sexes throughout the year, showing no evidence of reproductive behaviour. Female GSI attained higher values (*U* = 432.34, $p < 0.05$) ranging from 0.02 to 0.99 (mean GSI = 0.35, S.D = 0.11) than males and with higher values in April and September. The male GSI values ranged from 0.01 to 0.79 (mean GSI = 0.08, S.D. = 0.07), with a very small variation throughout the year (Fig.10). Although the GSI values for both sexes did not show evident variations throughout the year, significant differences among months were found ($H_{(11,792)} = 97.54$, $p<0.05$ for females and $H_{(11,524)} = 36.88$, $p < 0.05$ for males).

The HSI values from the northern area specimens had the same trend throughout the year for both sexes, with no significant differences between males and females (*U* = 345.78, $p = 0.79$). However, the monthly evolution of the HSI values presented significant differences ($H_{(11,794)} = 97.54$, $p < 0.05$) among the months, with higher values being observed between January and May (values ranging from 1.2 to 1.9) (Fig. 10).

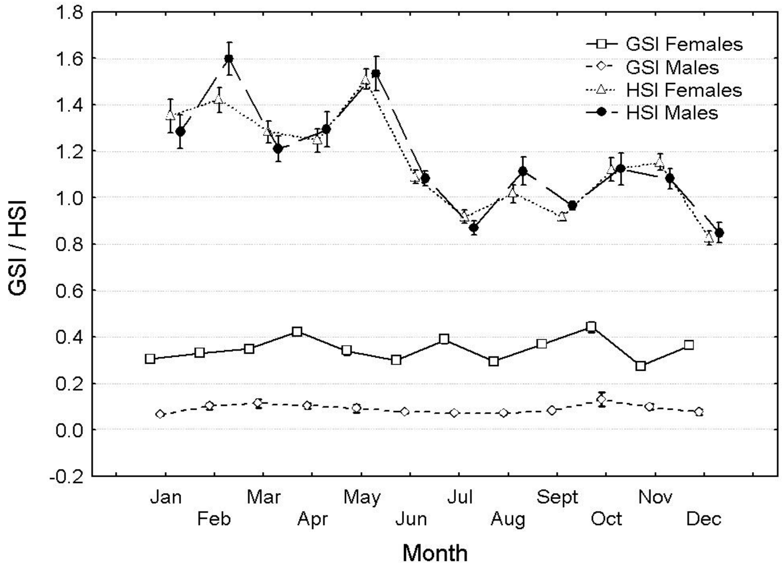


Fig. 10 Monthly changes in the GSI and HSI values for males and females of black scabbardfish caught in the west of the British isles. Each GSI and HSI value represented by the mean ± SE (error bars)

The GSI values for females sampled in Madeira showed low values between February and July (mean GSI < 2), progressively increasing in the following months, peaking in November (mean GSI =8.53, S.D. = 5.22), indicating that the reproductive activity takes place in the last quarter of the year (Fig. 11) ($H_{(4,191)} = 6.20$, $p < 0.001$). The male GSI values were lower, ranging from 1.35 to 2.75. The highest GSI values in males were recorded in October (mean GSI = 2.75, S.D = 1.51), indicating that males are reproductively active earlier in the year than females. The HSI values from the Madeira specimens showed the same increasing trend as HSI throughout the year for both sexes, with females having significantly higher values than males ($U = 234.67$, $p < 0.05$) (Fig. 11). The average female HSI values varied between 0.83 in February and 1.91 in November, with significant difference among months ($H_{(4,205)}= 32.73$, $p<0.05$). The average male HSI values ranged from 0.65 in February and 0.87 in October ($H_{(4,191)} = 27.71$, $p <0.05$).

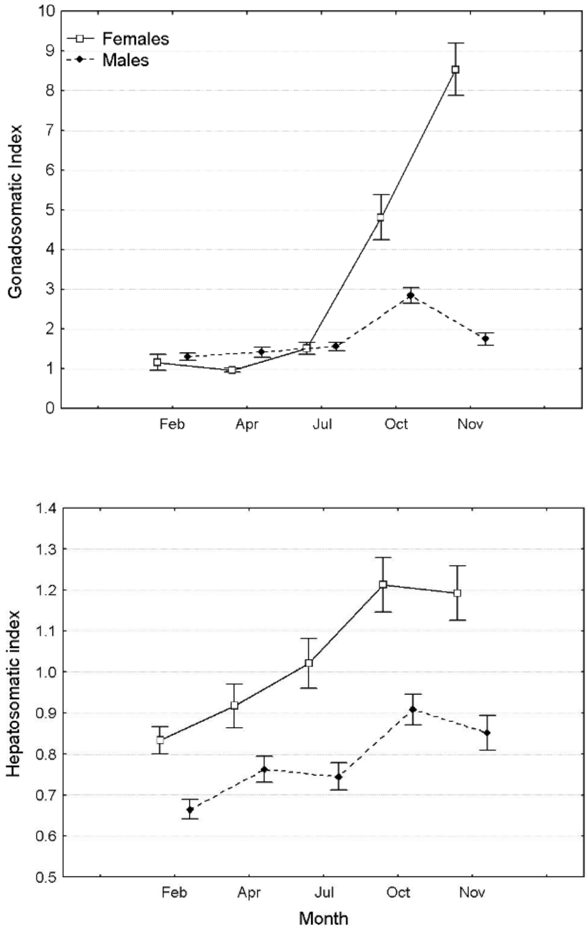


Fig. 11 Monthly changes in the GSI and HSI values for males and females of black scabbardfish caught in Madeira Islands. Each GSI and HSI value represented by the mean ± SE (error bars)

Significant differences were observed between maturity stages ($H_{(5,200)}$= 74.09, $p < 0.001$). HSI increased from immature individuals (0.57 ± 0.13) to pre-spawning ones (1.42 ± 0.34), decreasing in the following stages (Fig. 12).

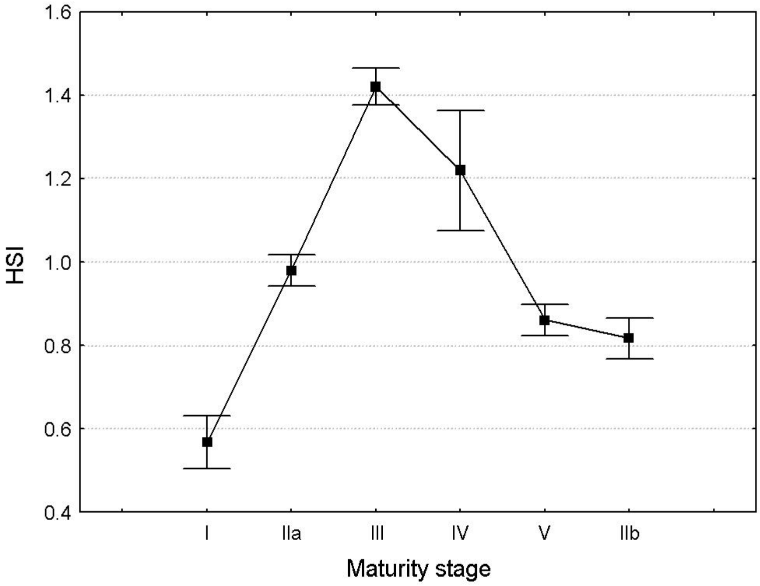


Fig. 12 Mean values ± standard error of HSI in relation to ovary developmental stages of black scabbardfish caught off Madeira Islands

3.7 Size structure

Length data were collected from 2126 and 406 specimens from west of the British Isles and Madeira, respectively. In the northern region, the length range for females was 710 – 1300 mm (meanTL = 955 cm; S.E. = 2.51) and for males was from 620 to 1170 mm (mean TL = 923 mm; S. E. = 2.54). Overall, the specimens captured off Madeira Islands were bigger than the ones caught in the northern area. The total length for females ranged from 1060 to 1410 mm (mean TL = 1210mm; S.E. = 4.74) with males ranging from 630 to 1270 mm (mean TL = 1150 mm; S.E. = 3.95). Results of the Kolmogorov-Smirnov two sample test indicated a significant difference ($p < 0.05$) in length frequency distributions between sexes and areas (Fig. 13). The length frequencies showed that females in both areas attained greater total lengths than males.

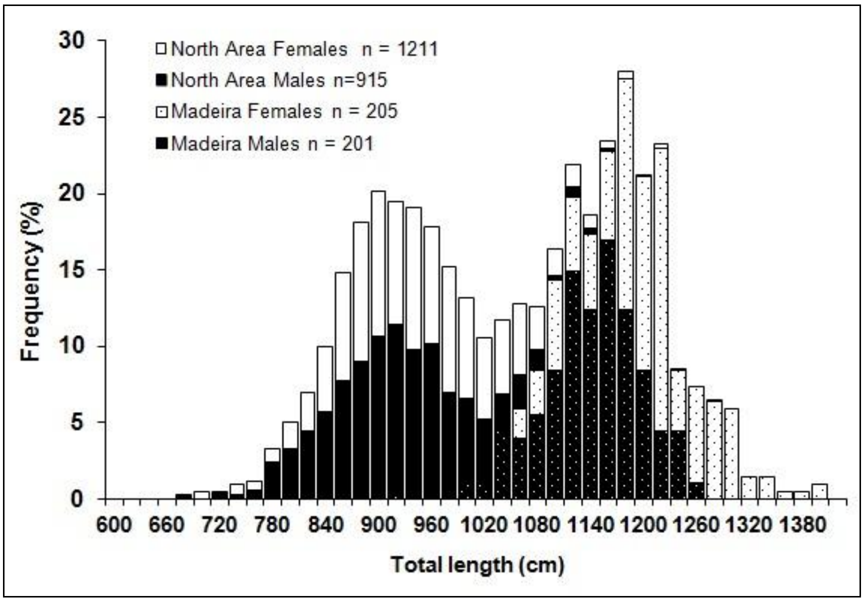


Fig. 13 Length-frequency distribution of males and females of *Aphanopus carbo* sampled off western of the British Isles and Madeira

3.8 Size at maturity

Based on the estimates obtained with the bias-reduction GLM (BRGLM, binomial family) approach using only the Madeira dataset, the estimated size at 50% maturity ($LC_{50\%}$), was 1110mm for females and 1010mm for males (Fig. 14 a and b).

When we combined both datasets - West of the British Isles and Madeira – the $LC_{50\%}$ estimated based on the logistic regression (GLM, binomial family) was significantly higher than the previous estimations, 1156mm females and 1098mm for males (Fig. 14 a and b).The maturity ogive parameters for each sex and model are presented in Table 7.

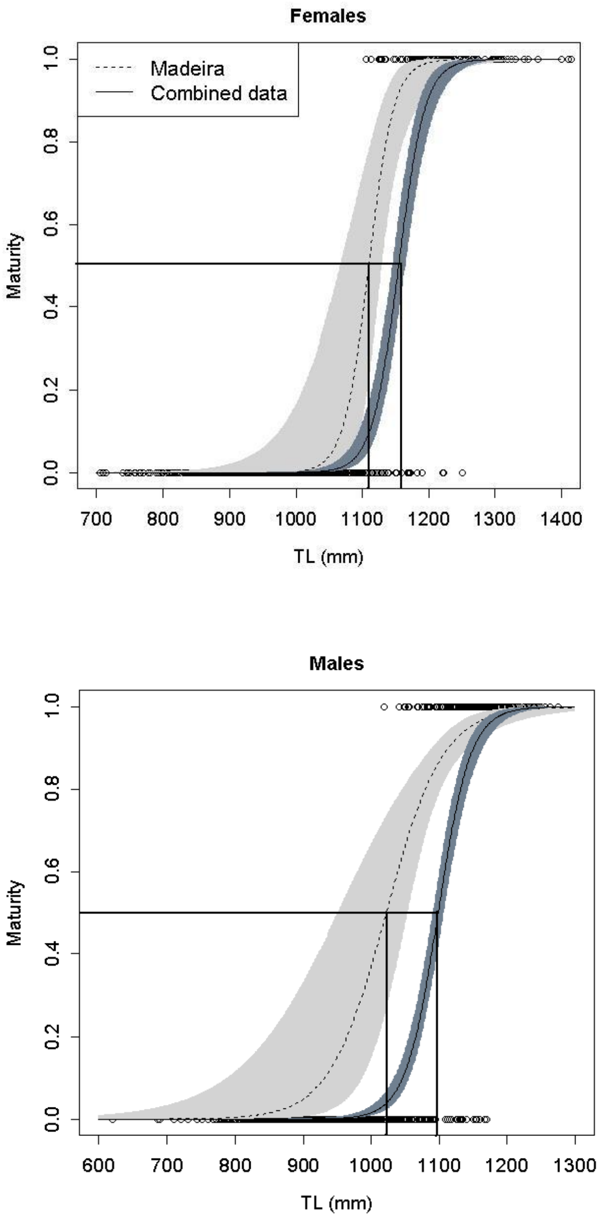


Fig. 14 Estimated proportion mature at length for a) female and b) male black scabbardfish ($P[mature] = 1 / 1 + \exp[(a - b \times TL)]^{-1}$) using a regression fit (binomial GLM, *logit* link function) for combined (West of British Isles and Madeira) data– thick line- and 95% confidence intervals – dark grey shadow; and the bias reduction method (binomial BRGLM, *logit* link function) for Madeira data – dashed line- and 95% confidence intervals – light grey shadow

Table 5 Parameters of binomial GLM (for combined data) and Bias-reduction GLM model (for Madeira data) of length-at 50% maturity for black scabbardfish

Ogive type	Males					Females				
	LC$_{50\%}$ (mm)	Ogive parameters				LC$_{50\%}$ (mm)	Ogive parameters			
		a (S.E.)	b (S.E.)	df	Res. Var.		a (S.E.)	b (S.E.)	df	Res. Var.
Combined data	1098	-46.51 (3.88)	0.04 (0.004)	1113	230.61	1156	-53.66 (5.73)	0.04 (0.005)	1149	128.17
Madeira	1010	-24.81 (6.19)	0.02 (0.005)	199	72.14	1110	-59.55 (18.15)	0.05 (0.016)	125	10.27

4 Discussion

The results obtained in this study on the reproductive cycle and oocyte development are consistent with the existence of a wide ranging, likely panmitic population of black scabbardfish in the NE Atlantic. The analysis of the data from two distinct geographic regions corroborates that the fish caught off the west of the British Isles are pre-adults that undertake large scale north - south migrations towards to the Madeira and Canaries Islands where they reach maturity and spawn (Figueiredo *et al.*, 2003; Pajuelo *et al.*, 2008). This study highlights the importance of understanding the life cycle and the migratory pattern of *A. carbo* in the NE Atlantic and emphasizes the need for a deeper look at this species to understand the migration and stock structure using other tools, such stable isotope analysis from otoliths and genetics.

Based on our macroscopic and histological results, the specimens caught to the West of Scotland were immature throughout the year. Previous studies in the same region also found only immature specimens (Figueiredo *et al*., 2003; Kelly *et al.*, 1998), but those studies did not carry out systematic annual monthly sampling. However, Magnússon and Magnússon (1995) observed spent specimens and one spawning fish around Icelandic waters in March 1993. In that study, the maturity scale used was not presented, the observations were not histologically validated and the maturity stages could have been wrongly assigned.

The lack of gonadal development throughout the year, confirmed by the constant values of GSI, suggest that the fish leave the northern region prior to/once gonads start to develop into the early-vitellogenic stage and likely migrate southwards to proceed with maturation and spawning.

The high level of atresia in the pre-vitellogenic oocytes, especially in April, confirms the lack of appropriate conditions; environmental, physiological and/or ecological to proceed with the maturation process (Rideout *et al.*, 2005; Jørgensen *et al*., 2006).

The “decision” of migrate has to be a trade-off between the potential benefit of reproduction and the costs of migration and natural and fisheries-induced mortalities (Jørgensen *et al.*, 2006).

A factor that might trigger the migration and affect the intensity of atresia is the shift in the diet composition throughout the year. The diet of black scabbardfish is predominated by blue whiting (*Micromesistius poutassou*) in the first quarter of the year, and then it changes to a less nutritional diet, composed of cephalopods and crustaceans from the second quarter onwards (Ribeiro Santos *et al*., *in review* Chapter 3). The change in the diet composition of black scabbardfish is linked to blue whiting migration pattern, which undertakes long distance migrations from the spawning grounds to the west of the British Isles to the feeding grounds in the Norwegian Sea by the end of April (Bailey, 1982; Was *et al.*, 2008). This change in the diet composition may result in a decrease in the black scabbardfish’s condition to proceed with maturation and trigger the migration, but the specimens with poorer condition remain in the northern area and eventually enter into an atretic process and do not spawn, at least that year. Spawning “omissions” due to scarce prey availability have been suggested for other species, such as cod *Gadus morhua* (Oganesyan, 1993) and orange roughy *Hoplostethus atlanticus* (Bell *et al.*, 1992). The study from Jørgensen *et al*. (2006) stated that a long and energy-costly migration makes skipped spawning an attractive option, because the saved energy if invested in growth, leads to a large increase in future fecundity. It seems that black scabbardfish goes through an intense feeding activity on blue whiting between January and April, to prepare for their migration, and the fish with better nutritional conditions migrate towards the south to progress with maturation and spawning.

Other factors that might interrupt the gamete development in the northern area are environmental (e.g. cold water temperature) and ecological and physiological (Jørgensen *et al.*, 2006). Although the distribution of eggs and larvae of black scabbardfish is unknown, it is postulated that they have a pelagic distribution (Vinnichenko, 2002; Quinta *et al*., 2004). The migration towards southern waters could increase reproductive success, due to warmer surface waters, lower productivity and consequently a lower density of pelagic planktivores than the northern waters. Finding the location of the occurrence of the larvae will facilitate the understanding of the migratory pattern and spawning ecology of black scabbardfish in the NE Atlantic.

Long scale migrations between the feeding and spawning grounds are commonly found among several fish species, such as Atlantic cod (Robichaud and Rose, 2001), North Sea plaice *Pleuronectes platessa* (Hunter *et al.*, 2003), blue whiting *M. poutassou* (Was *et al*., 2008), Atlantic bluefin tuna *Thunnus thynnus* (Fromentin and Powers, 2005), Greenland halibut *Reinhardtius hippoglossoides* (Walsh and Bowering, 1981). However, the absence of bigger sizes and spent and/or mature fish in the northern samples suggest that black scabbardfish migration is a single life event and that they do not return to the feeding grounds off the west of Scotland after they spawn. This kind of migration is very common for diadromous semelparous species (e.g. salmon), but not for oceanic iteroparous species. The fact that black scabbardfish do not migrate back to the feeding grounds to the West of the British Isles may be related with the energetic costs of migration and, as part of the individuals growth trajectory, once they reach a certain size most of the energy is allocated to reproduction, as observed from an energy-allocation life history model on the Atlantic cod (Jørgensen *et al*., 2006).

Previous studies on reproduction of black scabbardfish in Portugal and Madeira (Bordalo-Machado *et al*., 2001; Figueiredo *et al.*, 2003; Neves *et al.*, 2009) revealed a total absence of mature specimens on the Portuguese shelf, and it is postulated that the individuals with better condition migrate towards southern areas (Madeira and Canary Islands) to spawn and the fish in poorer condition remain off the Portuguese coast; the reproductive development is interrupted, as they increase in length but never spawn, remaining immature (Neves *et al*., 2009).

In the waters off Madeira, all maturity stages were observed, although very few immature specimens were caught. Possible explanations for the low number of immature specimens in the catches could be that they are not selected by the long-line gear, or they have a different vertical distribution where the fishing effort is applied or that, in fact there are very few immature black scabbardfish in Madeiran waters. The few immature fish might suggest that these fish newly arrived back to Madeira or possibly some fish do not migrate and spend their whole life cycle in Madeira waters.

The spawning season appears to be well defined, the reproductive cycle and GSI monthly variation indicate that the spawning season starts between September and October and finishes in December, which is in agreement with the findings of previous studies (Bordalo-Machado *et al*., 2001; Figueiredo *et al*., 2003; Neves *et al*., 2009). A decrease in monthly HSI values might be expected during the spawning season, as energy is redeployed into the gonad maturation. However, the converse was observed with a HSI peak at the beginning of the spawning season, in October, followed by a slight decrease in November, for both males and females. This could indicate that before the spawning season, the intake of energy from feeding is allocated and accumulated in the liver to be used in vitellogenesis and spermatogenesis during the spawning season as suggested by Sequeira *et al* (2012) for bluemouth *Helicolenus dactylopterus*. The HSI increased progressively from immature females to pre-spawning females (stage III), decreasing in the following maturity stages, also indicating that the hepatic reserves play an important role for the maturation process (Domínguez-Petit and Saborido-Rey, 2009).

Regarding the oocyte dynamics, the existence of a hiatus in the oocyte diameter frequency distribution between pre / early vitellogenic and mature oocytes indicates that black scabbardfish has a determinate fecundity, which means that the total fecundity prior to the onset of spawning is considered equivalent to the potential annual fecundity, after correcting for atretic losses (Murua and Saborido-Rey, 2003).

There are three types of oocyte development, i.e. synchronous, asynchronous and group-synchronous, with the latter being the most common among teleosts. According to Wallace and Selman (1981) in the synchronous group type: “at least two populations of oocytes can be distinguished at some time: a fairly synchronous population of larger oocytes (clutch) and a more heterogeneous population of smaller oocytes from which the clutch is recruited” (Murua and Saborido-Rey, 2003; Wallace and Selman, 1981). According to our observations, black scabbardfish conforms to the group-synchronous pattern, as a clutch of oocytes develop and mature synchronously, which are clearly distinguished from the stock of smaller oocytes from which they recruit.

Tyler and Sumpter (1996) described two types of spawning patterns: total spawners, which refers to species where the whole clutch of yolk oocytes ovulates at once and the eggs are shed in a single event or short period; and batch spawners, where the yolk oocytes ovulate in several batches over a protracted period during the spawning season. According to our observations black scabbardfish is a total spawner, since the duration of the spawning stage (stage IV) is very short, supported by the fact that even at the peak of the spawning season (November) the proportion of females in stage IV did not exceed 20% of the total females sampled. In a comprehensive study on fecundity of black scabbardfish in Portugal and Madeira, Neves *et al*. (2009) reached to same conclusions.

The reproductive strategy of black scabbardfish is different from some other deep water species, which in general present a protracted reproductive season, asynchrony and have indeterminate fecundity, such as *Coryphaenoides rupestris* (Allain, 2001; Kelly *et al*., 1996), *Alepocephalus bardii* (Allain, 2001; Morales-Nin *et al.*, 1996), *Lepidion eques* (Rotllant *et al.*, 2002), *Hymenocephalus italicus* and *Nezumia sclerohynchus* (D'Onghia *et al.*, 1999). These differences could be related with the necessity to synchronize the reproductive cycle of black scabbardfish with the surface primary production, so that the developing eggs float upwards and larvae are produce in food-rich waters, as was observed for other deep water fishes reproductive cycle (D'Onghia *et al*., 1999).

The macroscopic assignment of maturity stages was sometimes difficult, because the differentiation between maturity stages is not always clear, especially between females in spent (stage V) and resting (stage II-b) stages and between females in developing (stage II-a) and resting condition (stage II-b). Microscopic analysis of gonads proved to be very useful in clarifying macroscopic issues, thus reducing the errors on maturity stage assignments. Previous studies (e.g. Figueiredo *et al.*, 2003; Neves *et al.*, 2009; Pajuelo *et al*., 2008) on the reproductive cycle of black scabbardfish failed to distinguish developing females that never spawned from resting females. From our perspective this differentiation is not just essential for better understanding the reproductive cycle, but in a stock management perspective, the lack of this differentiation can result in erroneous estimates of length at first maturity ($LC_{50\%}$) and have serious consequences for the larger part of the reproductive stock, as was demonstrated by

Honji *et al*. (2006) for the Argentine hake (*Merluccius hubbsi*) and by Vitale *et al.* (2006) for cod. To distinguish resting/developing females it is necessary to validate the macroscopic observations with histological analysis. The measurement of the wall thickness could be a good mechanism to differentiate between immature and non-reproductive mature females, since the ovarian wall thickness was greater for resting females (stage II-b) than for developing immature females (stage II-a). This technique has been successfully used for other species, such as Atlantic cod (Rideout *et al.,* 2000) and the winter flounder (*Pleuronectes americanus*) (Burton and Idler, 1984).

Size segregation was observed between the specimens captured to the West of the British Isles and Madeira. The largest specimens were caught off the Madeira Islands (attaining 1450mm), while the smallest were captured to the West of the British Isles (~620mm). This was previously observed by other authors (Bordalo-Machado *et al.*, 2001; Figueiredo *et al.,* 2003; Santos, 2000). The difference in the length- frequency distributions between the south and north might be related to the different fishing gear used in the different areas. The bottom longline fishery in Madeira exploits larger fish and the bottom trawl in northern waters exploits the smaller fish of the population. However, in the northern area, a large number of fish from the trawls were examined in this study; experimental fishing and surveys that operated over a wide depth range never recorded a fish bigger than 1250mm in length in this area, suggesting that there is geographical size segregation. This hypothesis is difficult to definitely prove without directed fishing using the same gear types in both areas. We infer, based on the length-frequency distribution found and the reproductive cycle previously discussed, that this difference is more likely related with the life cycle and migration pattern of this species

The present study revealed that black scabbardfish possesses a very particular maturation process, with a geographical quasi-complete separation of the immature and mature individuals. While in the northern area only immature specimens were sampled, in the Madeira Islands a very low number of immature specimens was sampled, with a low overlap along the size range, making it impossible to fit a binomial GLM to each dataset separately. To overcome this data structure, we choose to use the BRGLM function in the Madeira dataset, that penalizes the maximum likelihood estimate for cases of complete or quasi-complete separation of the response variable (immature/mature) over the explanatory variable (total length) (Kosmidis and Firth, 2008). This function has been used in other ecological and ecotoxicology studies where there are binomial responses (Denton *et al*., 2009; McClellan *et al.*, 2009; Senior and Nakagawa, 2011), but never, to our knowledge, in the estimation of maturity ogives. The length-at-maturity estimated for females from Madeira using this function was 1110mm, and is larger than the estimations by Figueiredo *et al*. (~1028mm) (2003). This difference could be due to various factors: low numbers of immatures in the samples in the present study, or time of sampling, since in the previous study only samples from the reproductive season (between September and February) were used. Pajuelo *et al*. (2008) estimated $LC_{50\%}$ for black scabbardfish in Canary Islands as 1114mm, however, the lack of differentiation between the two species of *Aphanopus, A. intermedius* and *A. carbo*, present in the waters around the Canary islands precludes any meaningful comparisons.

When we added the immature specimens from the West of Scotland and estimated the maturity ogive with the combined data (Madeira and West of Scotland), the length-at-maturity was significantly higher than the ones calculated using only the Madeira dataset, for both sexes. The fact that we are introducing immature specimens from the northern area with the same lengths as some of the mature specimens in Madeira caused a shift of the curve to the right. Notwithstanding the geographical distance between the west of Scotland and Madeira, we consider it important to incorporate the immature species from the former location into the maturity ogive estimations, since our data strongly suggests that the life cycle of black scabbardfish is not completed in just one area.

Currently, black scabbardfish is managed based on the biennial ICES and CECAF scientific advice According to the ICES scientific advice, the available information is inadequate to evaluate the spawning stock and fishing mortality, so the state of the stock is unknown (ICES, 2010). From our perspective this lack of knowledge is because the only known spawning grounds for black scabbardfish – the Madeira and Canary islands, are outside the “jurisdiction” of ICES and the data from these areas are not considered nor integrated for the ICES assessment and advice. Species that transpose the barriers of the Regional Fishery Bodies need to be treated as highly migratory and effective management requires cooperation between the States and/or the Regional Fishery Bodies where black scabbardfish is exploited. This cooperation involves *inter* alia, the standardisation of data collection, including reproductive material, exchange of biological, distribution and fishery (catches, landings, effort, etc) information. Understanding the logical connection between juveniles and spawning biomass and the effect of the migration behaviour within the distribution area is vital for the maintenance of the population (Secor, 1999; Trippel, 1999). It is important that fish are able to grow to a reproductive size and are able to spawn before they are harvested. Harvesting of juveniles ultimately reduces the number of individuals that contribute to the spawning stock. It is vital to allow potential spawners to reproduce and produce viable offspring in order to maintain long term sustainable population's levels. It is important to understand, not only the large scale distribution and migration, but the fine-scale dynamics of black scabbardfish in the NE Atlantic.

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Appendix I

Table 1. Overview of the data acquisition for the study on reproduction of black scabbardfish, *Aphanopus carbo* in the NE Atlantic

Source	Date	Area	ICES Area	Depth range (m)	Bottom time
Commercial French Trawlers	June 09 - July 2010	Continental slope West of Scotland	Area Via	750 - 1100	3 - 6 h
Irish Deep water survey 2008	September 2008	North and western Slopes of the Porcupine bank	Area VII	500 - 1800	2 h
Irish Deep water survey 2009	December 2009	North and western Slopes of the Porcupine bank	Area VII	500 - 1800	1 h
Scottish Deep water survey 2009	September 2009	Continental slope West of Scotland and Rosemary bank	Area VIa	500 - 1800	1 h
French IBTS (EVHOE)2 2009	October 2009	Continental slope of Biscay Bay	Area VIIIa	950 - 1400	1 h
Madeira longline landings	April 09 - February 2010	Madeira Island		~ 1000	~ 24 h

Table 2. Overview of data (no. of specimens) used for the study

Source	Date	No fish sampled	Total length (mm)		Total weight (g)		Maturity		Gonad weight (g)		Liver weight (g)	
			M	F	M	F	M	F	M	F	M	F
French Trawlers	June 09 - May 2010	1053	447	600	448	596	447	600	399	549	558	409
Irish Deep water survey 08	Sept 2008	401	169	228	168	228	169	228	70	106	67	103
Irish Deep water survey 09	Dec 2009	189	89	99	89	99	89	99	1	15	7	16
Scottish Deep water survey 09	Sept 2009	440	164	253	181	253	181	253	74	160	158	111
French IBTS	Oct 2009	62	30	32	30	32	30	32	16	27	29	31
Madeira longline landings	May 2009 - Feb 2010	409	205	202	205	202	205	202	205	202	200	193
TOTAL		**2554**	**1104**	**1414**	**1121**	**1410**	**1121**	**1414**	**765**	**1059**	**1019**	**863**

Appendix II

This appendix contains additional information on the male gonad development of *Aphanopus carbo.* Photographs of the testes development stages are also included.

Male gonad microscopic development

Stage I – Immature

The testes in this stage are very small and have a laminar aspect, transparent or slightly pink. Microscopically they are characterized by the existence of spermatogonia cells with a prominent nucleus in the seminiferous tubules and a central empty lumen. In some cases, the seminiferous tubules are not clearly defined. Cells in a more advanced state of development can also be found: primary and secondary spermatocytes (Figure 11a).

In stage II – Developing

The testis are firm and with a whitish colour. The seminiferous tubules and the collecting ducts become more conspicuous. Cells in different stages of spermatogenesis are present in the seminiferous tubules: primary and secondary spermatocytes and spermatids (Figure 11b). In some cases is possible to observe the presence of spermatids and spermatozoa in the collecting ducts.

As occurred with females, the males captured in West of British Isles were only in stages I and II of development. The following stages were only encountered in the specimens sampled in Madeira.

The stage III – Maturing

This stage is characterized by an increase in volume of the testis with a white / pink colour. The sperm duct is very conspicuous and full with sperm. The seminiferous tubules showed an intense spermatogenesis, with the presence of cells in different stages, but mainly spermatozoa cells (Figure 11c).

Stage IV – Mature

At this stage the testes are white and the sperm is expelled with a slight pressure. The seminiferous tubules and the collecting ducts are completely full of spermatozoa (Figure 11d).

Stage V – Spent

The testes have a flaccid and haemorrhagic aspect. At this stage the testes revealed structural disorganization of the seminiferous tubules, with an empty appearance but with some residual spermatozoa. The remaining spermatozoa enter into reabsorption and spermatogonia were also observed (Figure 11e).

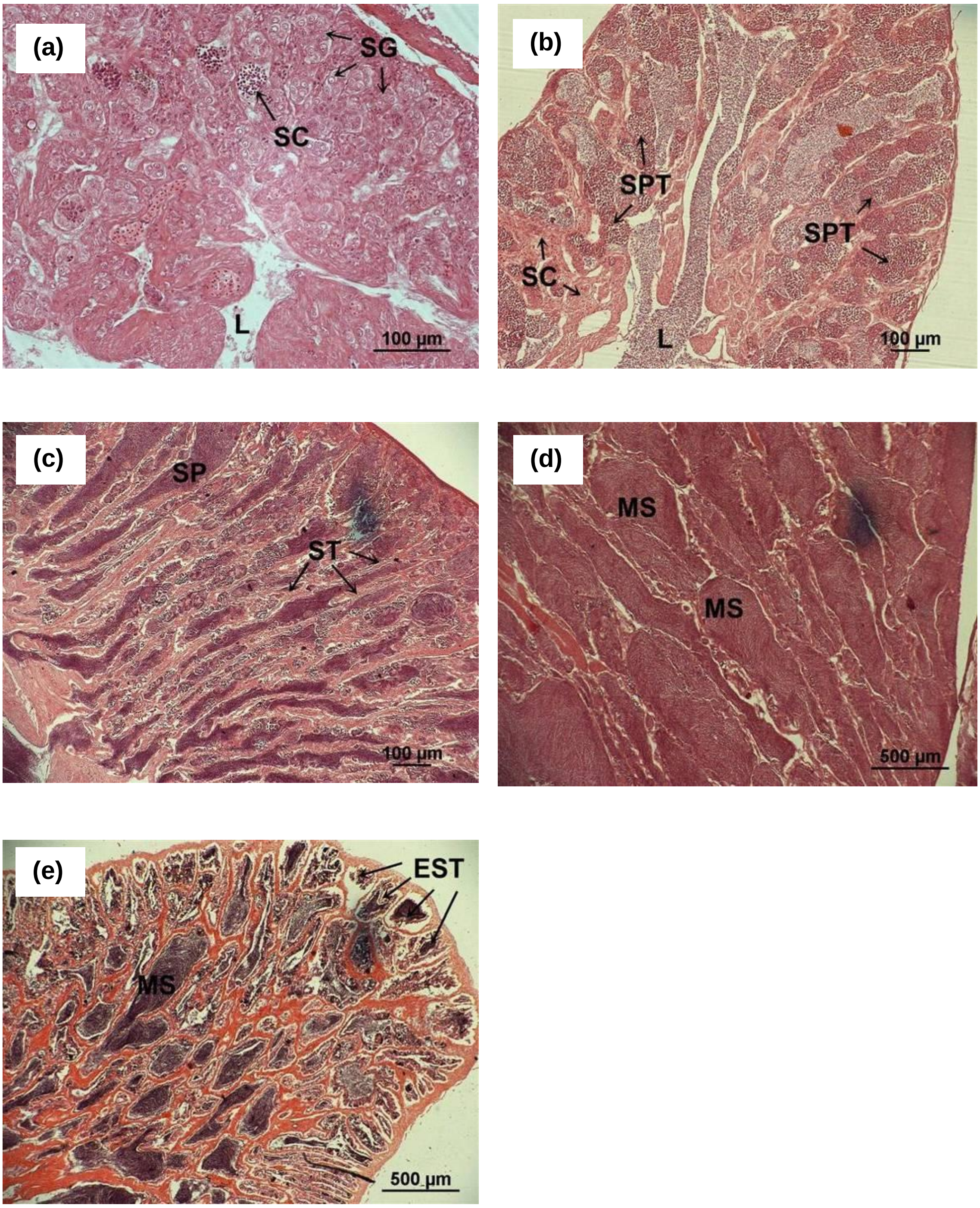

Figure A1 Testes development stages: (a) immature male – stage I; (b) developing male – stage II; (c) pre-spawning males – stage III; (d) post-spawning male – stage V. SC, spermatocytes; SG, spermatogonia cells; L, lumen; SPT, spermatids cells; ST, seminiferous tubules; SP, spermatozoa cells; MS, mature sperm; EST, empty seminiferous tubules.

Ribeiro Santos, A. 2013. *The life history and ecology of black scabbardfish (Aphanopus carbo Lowe 1839) in the North-East Atlantic.* PhD Thesis, University College Cork.

CHAPTER 4

Trophic ecology of Black Scabbardfish, *Aphanopus carbo* in the NE Atlantic – Assessment through stomach content and stable isotope analyses

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Contents

Abstract

The black scabbardfish is a deep water species of high commercial interest in the NE Atlantic. In conjunction with stomach content data these methods made it possible to investigate the feeding strategy of black scabbardfish. Specimens were collected from commercial trawls to the West of the British Isles and from longliners operating near Madeira between September 2008 and May 2010. Stomach content analysis was confined to samples from the northern area, because of a high number of empty stomachs from Madeira. Stable isotope analyses identified that black scabbardfish feeds on species with epipelagic and benthopelagic affinities. For the west of British Isles, the δN values were significantly different between seasons suggesting a change in the diet throughout the year. Black scabbardfish have higher δN and δC values compared with other co-occurring benthopelagic feeders and lower nitrogen values than the true benthic predators and/or scavengers. Comparison with stable isotope analysis in Madeira samples indicated that black scabbardfish feed at a similar trophic level and has the same trophic niche width in both areas, assuming similar baseline isotope compositions. The diet in the northern area comprised fish (68% N), crustaceans (22% N) and cephalopods (15% N) with blue whiting (*Micromesistius poutassou*) constituting 40% of the prey. Seasonal shifts in diet were observed, with a predominance of blue whiting (70%) in the first quarter of the year, shifting to a more diverse diet in the remainder of the year. These results indicate that the diet of black scabbardfish is closely linked with the seasonal migration of blue whiting and that they likely select prey in proportion to availability.

This study demonstrates that the combined used of both methods can elucidate the trophic ecology of black scabbardfish, in situations where conventional methods alone provide insufficient data.

1. Introduction

The Black scabbardfish (*Aphanopus carbo* Lowe, 1839) is a deep water fish, belonging to the family Trichiuridae. This species has a world-wide distribution, with records in the Atlantic from Iceland (Magnússon and Magnússon, 1995) to the Canary Islands (Uiblein et al., 1996), including the islands of Madeira, Azores and numerous submarine banks and seamounts (Zilanov and Shepel, 1975; Nakamura and Parin, 1993; Morales-Nin and Sena-Carvalho, 1996; Vinnichenko *et al.*, 2005; Pajuelo *et al.*, 2008). It belongs to the benthopelagic category of deep-water fishes, living close to the bottom along the continental slope (Nakamura and Parin, 1993; Gordon, 2001; Bordalo-Machado and Figueiredo, 2008) and occurs at depths between 200m in the British Isles (Tucker, 1956; Bordalo-Machado *et al.*, 2001; Bordalo-Machado and Figueiredo, 2008) to 1800m in the south of Madeira, being more commonly found between 800 to 1200m (Bordalo-Machado *et al.*, 2001).

Black scabbardfish is an economically important deep water species that has been exploited in the eastern Atlantic, off the Madeira Islands, for centuries (Haedrich *et al.*, 2001; Alves, 2003). Since 1983, the exploitation of black scabbardfish expanded to the Portuguese continental waters (Martins *et al.*, 1989; Bordalo-Machado and Figueiredo, 2008). In the North of Europe, the species has been captured around the British Isles (ICES Subareas V, VI and VII) and Iceland (ICES Subarea Va), mainly by French, Icelandic and Spanish trawlers (ICES, 2011) since the early 1990's (ICES, 2008).

Despite the wide distribution and commercial interest in black scabbardfish, biological studies are relatively sparse and have concentrated on distribution (Zilanov and Shepel, 1975; Piotrovskiy, 1981; Mauchline and Gordon, 1984c; Nakamura and Parin, 1993; Magnússon and Magnússon, 1995; Uiblein *et al.*, 1996; Vinnichenko *et al.*, 2005), anatomy (Bone, 1971), age and growth (Morales-Nin and Sena-Carvalho, 1996; Morales-Nin *et al.*, 2002; Pajuelo *et al.*, 2008; Vieira *et al.*, 2009) and reproduction (Bordalo-Machado *et al.*, 2001; Figueiredo *et al.*, 2003; Pajuelo *et al.*, 2008). The available information on the diet of black scabbardfish is confined to general comments on the stomachs contents from specimens collected from the Hatton Bank (Du Buit, 1978), the Rockall Trough (Mauchline and Gordon, 1984c), west of the

British isles (Zilanov and Shepel, 1975) and Portugal (Santos, 2000), but without any detailed description or interpretation. Overall, detailed diet studies of deep water species are very limited and scarce due to the difficulty of collecting samples and high rate of stomach eversion.

Stable isotope analysis offers a complementary perspective to investigate the long term view of feeding relationships by accounting for all the sources of energy assimilated during a feeding season and the trophic position of an organism (Iken *et al.*, 2001). The stable nitrogen isotope ratio ($^{15}N/^{14}N$) increases at every step in the food chain, thus indicating trophic level of a species (DeNiro and Epstein, 1981), while the carbon isotope ratio ($^{13}C/^{12}C$) may provide information on nutrients sources (DeNiro and Epstein, 1978; Vander Zanden and Rasmussen, 2001). Benthic and benthopelagic fish may derive nutrients directly from the pelagic food web, or via the benthic food web. The benthic food web pathway contains more trophic steps between the primary production and fish production, thus fish supplied with nutrient from the benthic food sources will be relatively isotopically enriched (Iken *et al.*, 2001; Drazen *et al.*, 2008; Doyle *et al.*, 2012)

Although detailed interpretation of stable isotope data to infer diet composition is dependant on the knowledge of isotope signatures of the prey species, some inferences about patterns of variation in diet (e.g. ontogenetic variations) can be made in the absence of such information (Stowasser *et al.*, 2009). Stomach content and stable isotopes analysis have been rarely applied to other deep water fish. The existing studies using both analyses are mainly focused on the dominant families of the deep sea: Macrouridae and Moridae (Mauchline and Gordon, 1984a; Iken *et al.*, 2001; Polunin *et al.*, 2001; Drazen *et al.*, 2008; Stowasser *et al.*, 2009; Bergstad *et al.*, 2010).

In the present study, stable isotope and stomach contents analyses, were used to investigate the trophic ecology of black scabbardfish in two areas of NE Atlantic: West of British Isles and Madeira. As a consequence of limited recovery of stomach contents, especially from Madeira, classic stomach examination was augmented by stable isotope analysis. While the stomach contents provide information on the composition of recent meals, stable isotope compositions integrate the signatures of different prey consumed over a longer period, and can be used to infer trophic level and discriminate sources of food between the two areas.

2. Material and Methods

2.1 Data acquisition

The specimens of black scabbardfish used in this study were obtained from a monthly sampling programme, conducted between June 2009 and May 2010, from the commercial French trawlers operating off West of British Isles and from a quarterly sampling programme, between April 2009 and February 2010, from the longliners landings of the commercial catch in Madeira Archipelago (Portugal). Additional samples were obtained from scientific deepwater bottom trawl surveys: Marine Institute Deep water Survey, carried out on board *R/V Celtic Explorer*, in September 2008 and December 2009; Marine Scotland Deep water survey, on board *R/V Scotia*, in September 2009 and the French IBTS (International Groundfish Survey - EVHOE 2009) survey, conducted by IFREMER, on board R/V *Thalassa* off the Biscay Bay, in October 2009 (Fig. 1).

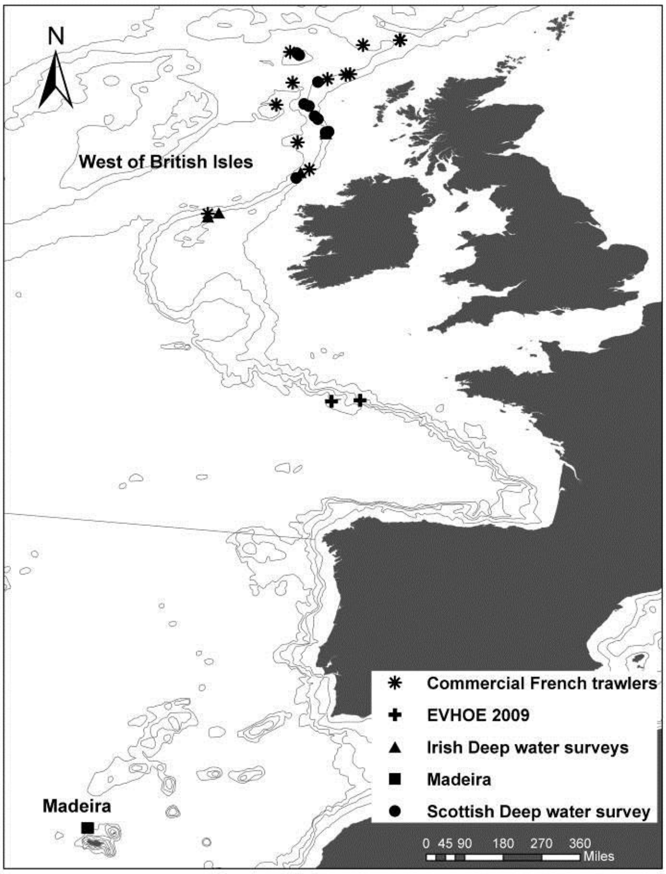

Fig. 1. Map with the locations where the samples of black scabbardfish used for this study were collected.

Since the early 1990's a second species of the genus *Aphanopus*- *A. intermedius* - has been recognised in the southern northeast Atlantic (Madeira and Azores) (Nakamura and Parin, 1993). This species is morphologically similar to *A. carbo* and can only be differentiated by counting the vertebrae and the dorsal fin spines. To ensure that only *A. carbo* was sampled, all the specimens from Madeira were morphologically analysed to discriminate both species. To determine the presence of both species to the west of the British Isles and Bay of Biscay, a preliminary meristics study was carried out on 250 specimens, and the results indicated that in these areas only *Aphanopus carbo* was present.

2.2 Stable isotope analysis

For stable isotope analysis, samples of white muscle tissue were collected from the dorso-lateral region of fish sampled of the west of the British Isles (n = 30) and in Madeira (n = 40), and frozen prior to freeze drying. The samples were randomly selected from the total muscle samples collected from the commercial French trawlers and Scottish and Irish surveys conducted in 2009. Dried samples were powered with a pestle and mortar and a 0.8-1.0 mg sample was weighed into a tin capsule for the simultaneous determination of carbon and nitrogen ratios. C and N isotope ratios were measured by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Costech (model ECS 4010) elemental analyser (EA) combined with a ThermoFinnigan Delta Plus XP mass spectrometer at the National Oceanography Centre, University of Southampton. Three laboratory standards were analysed for every 8–10 unknown samples in each analytical sequence, allowing instrument drift to be corrected if required. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviation from the international standards V-Pee dee belemnite (carbon) and AIR (nitrogen), according to the equation: $\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The measurement precision of both $\delta^{15}N$ and $\delta^{13}C$ was estimated to be ≤0.3‰.

$\delta^{13}C$ ratios were corrected for lipid contents in the tissue using the equation developed by Hoffman and Sutton (2010): $\delta^{13}C_{protein} = \delta^{13}C_{bulk} + \left(-6.39‰ \times \left(3.76 - C:N_{bulk}\right)\right)/C:N_{bulk}$

Stable isotopes differences between the two areas were examined using a linear regression model (LM) in R, using total weight (in grams) as a covariate. The total weight was $\log_2$-transformed.

Factors affecting $\delta^{15}N$ and $\delta^{13}C$ values in the muscle of black scabbardfish were analysed for each area separately, using LMs, since the two response variables were continuous and had a normal distribution. The explanatory variables considered were $\log_2$ (total weight), sex, and semester (1 – January to June; 2 – July to December). Effects of adding interaction terms were also considered. Variance in $\delta^{15}N$ values was taken as a measure of trophic niche width (Bearhop *et al.*, 2004) and homogeneity of variance was compared between the two areas: West of British Isles and Madeira using a variance ratio test (*F*-test). We recognize that this test assumes a constant variance in baseline $\delta^{15}N$ values, and this assumption cannot be validated in this study.

2.3 Diet analyses

A total of 1994 *A. carbo* specimens were analysed for the purpose of this study (Table 1). Each specimen was measured - total length (TL, cm), weighed (total weight (TW, g)), sexed and maturity stage assessed by macroscopic examination using the maturity scale proposed by Gordo *et al.* (2000). The stomachs were classified as either everted, empty or with food contents. The stomachs with food contents were weighed (g), placed in a plastic bag and frozen for subsequent analysis

Table 1. Summary of the data (no. of specimens) used for the feeding ecology study of black scabbardfish

Source	**Date**	**No fish sampled**	**Stomachs analysed**			
			Empty	**Everted**	**With food contents**	**Total**
Commercial French Trawlers	June 09 - May 2010	1053	329	629	95	1053
Irish Deep water survey 08	Sept 2008	401	*	*	10	10
Irish Deep water survey 09	Dec 2009	189	13	81	6	100
Scottish Deep water survey 09	Sept 2009	440	38	358	22	418
French IBTS (EVHOE) 2009	Oct 2009	62	*	*	4	4
Madeira longline landings	May 09 - Feb 2010	409	402	0	7	409
TOTAL		2554	782	1068	144	1994

In the laboratory, the prey items were careful separated, counted, weighed and whenever possible measured (Total length for fish (TL, mm), cephalothorax length (CL, mm) for shrimps and mantle length for squids (DML, mm)). Otoliths and bones were cleaned and stored dry and cephalopods beaks stored in 70% alcohol. The prey remains were identified to the lowest possible taxonomic level. The identification of fish, based on the hard structures (otoliths, premaxillae and vertebrae), followed the published guides (Härkönen, 1986; Watt *et al.*, 1997; Moller, 2001; Campana, 2004; Girone *et al.*, 2006; Tuset *et al.*, 2008). The identification of cephalopods was based on the lower beaks, following Clarke (1986) and the shrimps were identified following Pohle (1988).

The importance of each prey category was evaluated using the following quantitative indices: the percentage by number (%N):

$$\%N = \frac{S_i}{S_t} \times 100,$$

where S_i is the number of prey from a specific category *i* and S_t is the total number of prey found in all the stomachs.

The proportion in terms of weight (%W) of each prey category was calculated as:

$$\%W = \frac{W_i}{Wt} \times 100,$$

where W_i is the weight from a specific prey category *i* and W_t is the total weight of prey found in all the stomachs.

The frequency of occurrence (%O) was calculated as

$$\%O = \frac{N_i}{N_t} \times 100,$$

where N_i is the number of stomachs with specific prey type *i*, N_t is the total number of stomachs with prey.

The index of relative importance (IRI) and its standardized value (%IRI) were calculated as

$$\mathrm{IRI} = (\%\mathrm{N} + \%\mathrm{W}) \times \%\mathrm{O}$$
$$\%\mathrm{IRI} = 100 \times \mathrm{IRI}_i \Big/ \sum_{i=1}^{n} \mathrm{IRI}_i ,$$

where IRI_i is the IRI value for each prey category *i* (Hyslop, 1980).

When fragments were found, the number of individuals was determined as the lowest possible number of individuals from which fragments could have originated. When only hard structures were present in the stomachs, the numbers of fish and cephalopods were estimated from the number of otoliths and beaks, respectively.

The %O, %N and %W values of each prey category were plotted following the method proposed by Cortés (1997), which allows an easy and adequate interpretation of prey importance in the diet predators.

2.3.1 Estimation of prey size and weight

To estimate original prey sizes, the length (from the rostrum to the posterior edge of the otolith, parallel to the sulcus) and width of otoliths, and the lower rostral length of cephalopod beaks were measured using a graticule under a binocular microscope or a vernier calliper. Otoliths were separated into lefts and rights, paired when possible and the average otolith length was calculated. For each prey species the length and weights were estimated for which regressions equations were available in the literature (Härkönen, 1986; Harvey *et al.*, 2000; Magnússon, 2001; Campana, 2004; Rosa *et al.*, 2006; Santos *et al.*, 2007; Tuset *et al.*, 2008; Fock and Ehrich, 2010). Cephalopod mantle lengths (DML, mm) and weight were estimated using regressions from Clarke (See Appendix I). No corrections were applied for possible otolith erosion.

2.3.2 Accumulation and Rarefaction curves

Since the diversity of prey increases with the number of sampled stomachs, sample size sufficiency was assessed by constructing prey species accumulation and rarefaction curves, using the freeware program Estimate S, version 8.0 (Colwell, 2005). The species accumulation curve plots the total number of prey species revealed during the process of data collection, as sample units are added to the pool of all previously collected samples. In these curves the order in which samples are added affects the shape of the curve. To eliminate this arbitrariness, the sample order has to be randomized. The rarefaction curve is produced by repeatedly re-sampling the pool of N samples, at random, plotting the average number of species represented by 1, 2,…*N* individuals or samples (Gotelli and Colwell, 2001).

2.3.3 Trophic diversity

Trophic diversity of the prey was assessed for each quarter of the year, with the Shannon-Wiener diversity index, $(H'): H' = -\sum_{i=1}^{n} p_i \times \ln p_i$,

where p_i is the numeric proportion of prey *i* in the diet (Krebs, 1999).

For dietary analysis, the prey items were grouped into the following categories: *Micromesistius poutassou,* Mesopelagic fish, other teleost fish, *Acanthephyra* sp, *Pasiphaea* sp, other shrimps, *Gonatus* sp, *Branchioteuthis* sp, *Histioteuthis* sp and other cephalopods.

The diet composition was analysed by length class, sex and seasonal variation (Jan-Mar, Apr-Jun, Jul-Sept and Oct-Dec). Differences in the ranking of %N values for prey categories between three or more groups (e.g. four year seasons) were tested for significance with Kendall's Coefficient of Concordance (*Wc*) (H_0: The diet composition is different among groups). For paired groups (e.g males and females), the Spearman rank correlation (r_T) was used (Zar, 2010).

3. Results

The specimens of black scabbardfish used in this study were caught over a bathymetric range of 500 to 1500m, but mostly between 900 and 1100m depth (Fig. 1).

3.1 Stable isotopes

Isotopic information was obtained from two geographical areas: west of the British Isles and Madeira. Overall, samples from Madeira had higher mean δ^{15}N and δ^{13}C values than fish from the west of British Isles (Table 2)

Table 2. Summary of biological and stable isotope values obtained from West of British Isles and Madeira Islands

Area	n	Total length (mm)	Total weight (g)	$\delta^{15}N_{Air}$ (‰)	$\delta^{13}C_{V\text{-}PDB}$ (‰)
W Scotland	30	966 ± 73	1175 ± 362	13.4 ± 0.44	-18.52 ± 0.25
Madeira	40	1193 ± 82	2258 ± 431	14.1 ± 0.50	-18.01 ± 0.22

However, significant correlations were found between fish weight and isotope values (δ^{15}N; r^2 = 0.61, $p < 0.01$; δ^{13}C; r^2 =0.55, $p < 0.01$), and fish caught west of the British Isles were significantly smaller than those caught in Madeira (Tukey's HSD, $p < 0.05$). Therefore, all the subsequent analyses used total weight as a covariate.

Collectively, samples from west of the British Isles and Madeira were well separated isotopically, as illustrated in the bivariate plot of δ^{15}N vs. δ^{13}C values (Fig. 2). Although the mean δ^{15}N value was significantly different between areas, this is likely because of the difference in mean length between the areas. After adjusting for size, linear model (LM) results showed that δ^{13}C signatures were significantly different between samples from both areas ($F_{1,68}$ = 7.21, $p < 0.01$), δ^{15}N values were not significantly different between regions ($F_{1,68}$ = 2.35 p = 1.30) (Table 3). The variance in δ^{15}N values, which provides a comparable estimate of trophic niche width, was not significantly different between the two areas ($F_{29,39}$ = 0.76, p = 0.785), suggesting that the trophic niche width was the same in both areas, assuming a comparable variance in isotopic baselines.

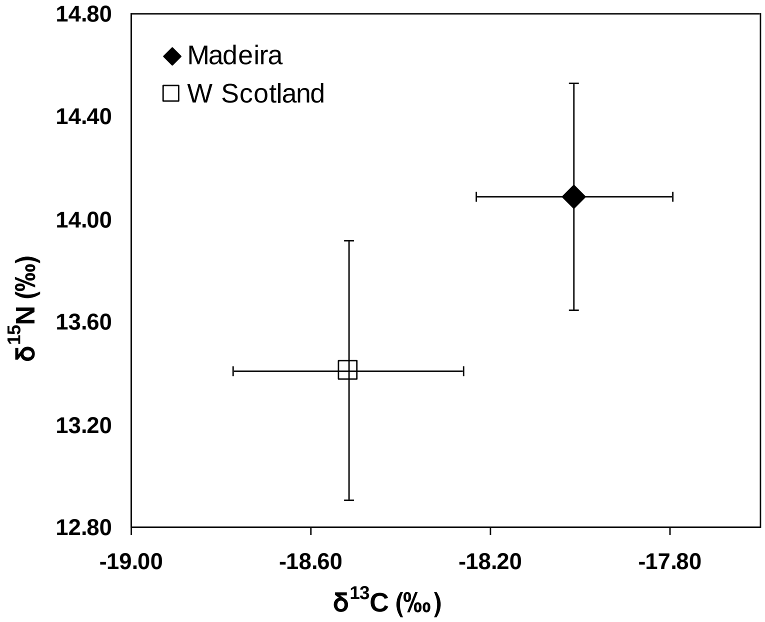

Fig. 2. Bivariate plot of $\delta^{15}N$ against $\delta^{13}C$ values (mean ± s.d.) of West of British Isles and Madeira samples.

Table 3. Results of the linear model (with total weight as covariate) comparing isotope composition among black scabbardfish from West of British Isles and Madeira

Variable	Model d.f.	Error d.f.	Model R^2	Model MS	Error MS	*F*	*p*-value
$\delta^{15}N$	1	68	0.37	8.48	0.21	2.35	1.299
$\delta^{13}C$	1	68	0.55	4.46	0.05	7.2	<0.01

3.1.1 Analysis of stable isotope composition and variability

For the northern area samples, $\delta^{15}N$ values was not significantly correlated with body mass or sex, but showed a significant depletion between the first and second semester ($F_{(1,26)}$ =9.798*, p* = 0.004). The $\delta^{13}C$ values showed a significant enrichment with body weight, but no differences between sex and semester (Table 4).

For the Madeira samples, the $\delta^{15}N$ values showed a significant linear enrichment with body weight ($F_{(1, 36)}$ = 9.165, p = 0.005) and a significant depletion in males ($F_{(1,36)}$= 4.131, p = 0.049). LMs showed that $\delta^{13}C$ values were only significantly different between semesters ($F_{(1,36)}$= 4.496, p = 0.041) (Table 4).

Table 4. Analysis of variance table for the linear model fitted to $\delta^{15}N$ and $\delta^{13}C$ data to the total weight (Log_2TW), semester and sex, for black scabbardfish from West of British Isles and Madeira. * p <0.05

Area	Variable	Source of variation	Df	Sum of Squares	*F* value	*p* -value
West of British Isles	$\delta^{15}N$	Log_2TW	1	0.234	1.210	0.281
		Semester	1	1.895	9.798	0.004*
		Sex	1	0.284	1.471	0.235
		Residuals	26	5.027		
	$\delta^{13}C$	Log_2TW	1	0.514	10.101	0.004*
		Semester	1	0.008	0.167	0.685
		Sex	1	0.067	1.330	0.259
		Residuals	26	1.324		
Madeira	$\delta^{15}N$	Log_2TW	1	1.348	9.166	0.004*
		Semester	1	0.42	2.857	0.099
		Sex	1	0.607	4.131	0.049
		Residuals	36	5.294		
	$\delta^{13}C$	Log_2TW	1	0.154	3.587	0.066
		Semester	1	0.192	4.496	0.040*
		Sex	1	0.0004	0.009	0.922
		Residuals	36	1.541		

3.2 Diet Analyses

Of the 1994 stomachs examined, 1068 (53.6%) were inverted, 782 (39.2%) were empty and 144 (7.2%) had food contents. From the 409 specimens caught by longliners in Madeira, only 7 stomachs (1.7%) had food contents; three had bait remains and four had food contents (Table 1). These stomachs were excluded for any further statistical analyses but will be mentioned for the diet composition.

In total, 197 prey items were found in the stomachs of black scabbardfish, belonging to 35 prey categories. Table 5 provides details of the prey composition of the pooled stomach contents. The diet of black scabbardfish consisted of a wide variety of organisms, dominated by fishes (68.4%, %N), followed by crustaceans (21.6%) and cephalopods (15.3%). Blue whiting (*Micromesistius poutassou*) was by far the predominant fish prey item, contributing 37.6% to the total number of prey (%N) and 63.7% by weight. The mesopelagic fish species (*Nemichthys scolopaceus,* Synaphobranchidae, *Notoscopelus* sp, *Centrolophus* sp, *Lycodes* sp, Alepocephalidae, *Bathylagus greyae, Stomias boa* and unidentified mesopelagic species) were the second largest group, representing 15.3% of the total number of preys.

Black scabbardfish also fed upon several species of crustaceans and cephalopods. Among the cephalopods species, the most frequent were *Gonatus* sp. and *Branchioteuthis reesei*, which represented 3.7% and 2.67% of all food items counted, respectively. Numerically, crustaceans species were more important than the cephalopods, with *Acanthephyra* sp. and *Pasiphaea* sp. representing 7.6% and 6.6% of the diet.

The use of three-dimensional graphical representation of diet provides a good depiction of prey importance (dominant or rare) and predator feeding strategy (specialized or generalist). The graph From this, the diet of black scabbardfish feeding predominantly on blue whiting, *M. poutassou* (Figure 3). Only seven specimens caught in Madeira had food contents; three had bait, identified as scombrids and squid. The other four stomachs contained two species of cephalopods (*Gonatus* sp. and *Histioteuthis arcturi*), one crustacean decapoda (*Acanthephyra purpurea*) and one unidentified mesopelagic fish. Overall, the diet composition presented a high trophic diversity (H’ = 3.35).

Table 5. Diet composition of *Aphanopus carbo* caught to the west of the British Isles and Madeira between June 2009 and May 2010. (Si) number of prey items, (%N) Percentage by number, weight (%W), occurrence (%O) and Index of relative importance (IRI and %IRI) for each prey item observed.

Prey items	**Si**	**%N**	**%O**	**%W**	**IRI**	**%IRI**
CEPHALOPODA	**29**	**15.32**	**16.06**	**24.43**	**638.25**	**5.87**
Ancistroteuthis lichtensteini	3	1.60	2.13	7.21	18.76	0.49
Branchioteuthis reesei	5	2.67	2.84	0.14	7.99	0.21
Gonatus sp	7	3.74	2.84	3.43	20.34	0.53
Histioteuthis arcturi	1	0.53	0.71	2.22	1.96	0.05
Histioteuthis bonnelli	1	0.53	0.71	0.24	0.55	0.01
Histioteuthis reversa	3	1.60	2.13	4.40	12.78	0.33
Toraropsis eblanae	2	1.07	1.42	0.64	2.42	0.06
Cephalopod NI	7	3.55	5.11	6.15	49.56	1.29
CRUSTACEA	**40**	**21.62**	**23.36**	**1.05**	**529.57**	**4.87**
Decapoda						
Acanthephyra pelagica	4	2.16	1.92	0.09	4.34	0.11
Acanthephyra purpurea	4	2.16	2.56	0.08	5.76	0.15
Acanthephyra sp	7	3.78	3.85	0.15	15.13	0.39
Pasiphaea multidentada	2	1.08	1.28	0.18	1.62	0.04
Pasiphaea tarda	3	1.62	1.92	0.08	3.27	0.09
Pasiphaea sp	8	4.32	4.49	0.32	20.83	0.54
Lophogastridae						
Gnathophausia zoea	7	3.78	4.49	0.07	17.30	0.45
Undentified Crustacea	5	2.70	3.21	0.07	8.89	0.23
PISCES	**107**	**68.37**	**67.88**	**74.52**	**9010.53**	**88.53**
Anguilliformes						
Nemichthydae						
Nemichthys scolopaceus	2	1.08	1.28	0.22	1.67	0.04
Synaphobranchidae						
Synaphobranchus kaupii	3	1.62	1.92	0.47	4.01	0.10
Synaphobranchidae unid.	1	0.54	0.64	0.18	0.46	0.01
Gadiformes						
Gadidae						
Ciliata septentrionalis	1	0.54	0.64	0.19	0.47	0.01
Gadiculus argenteus	2	1.08	1.28	0.27	1.73	0.05
Micromesistius poutassou	74	37.56	40.30	63.75	4082.87	94.31
Gadidae unid.	5	2.54	3.65	4.47	25.59	0.67
Macrouridae						
Nezumia aequalis	1	0.54	0.64	0.11	0.41	0.01
Moridae						
Halargyreus johnsoni	3	1.62	1.92	1.37	5.76	0.15
Lepidion eques	1	0.54	0.64	0.12	0.43	0.01
Myctophiformes						
Myctophidae						
Notoscopelus sp	1	0.54	0.64	0.14	0.44	0.01
Perciformes						
Centrolophidae						
Centrolophus sp	1	0.54	0.64	*	*	*
Scombridae						0.00
Scomber scombrus	1	0.54	0.64	0.64	0.76	0.02
Zoarcidae						
Lycodes sp	1	0.54	0.64	0.34	0.57	0.01
Osmeriformes						
Alepocephalidae						
Alepocephalus bardii	1	0.54	0.64	0.36	0.58	0.02
Alepocephalus sp	3	1.62	1.92	1.08	5.20	0.14
Xenodermichthys sp	2	1.08	1.28	0.30	1.77	0.05
Bathylagidae						
Bathylagus greyae	2	1.08	1.28	0.38	1.87	0.05
Stomiiformes						
Stomiidae						
Stomias boa	3	1.62	1.92	0.13	3.36	0.09
Mesopelagic unid.	11	5.58	8.03	*	*	*
Teleost unid.	9	4.57	5.11	*	*	*

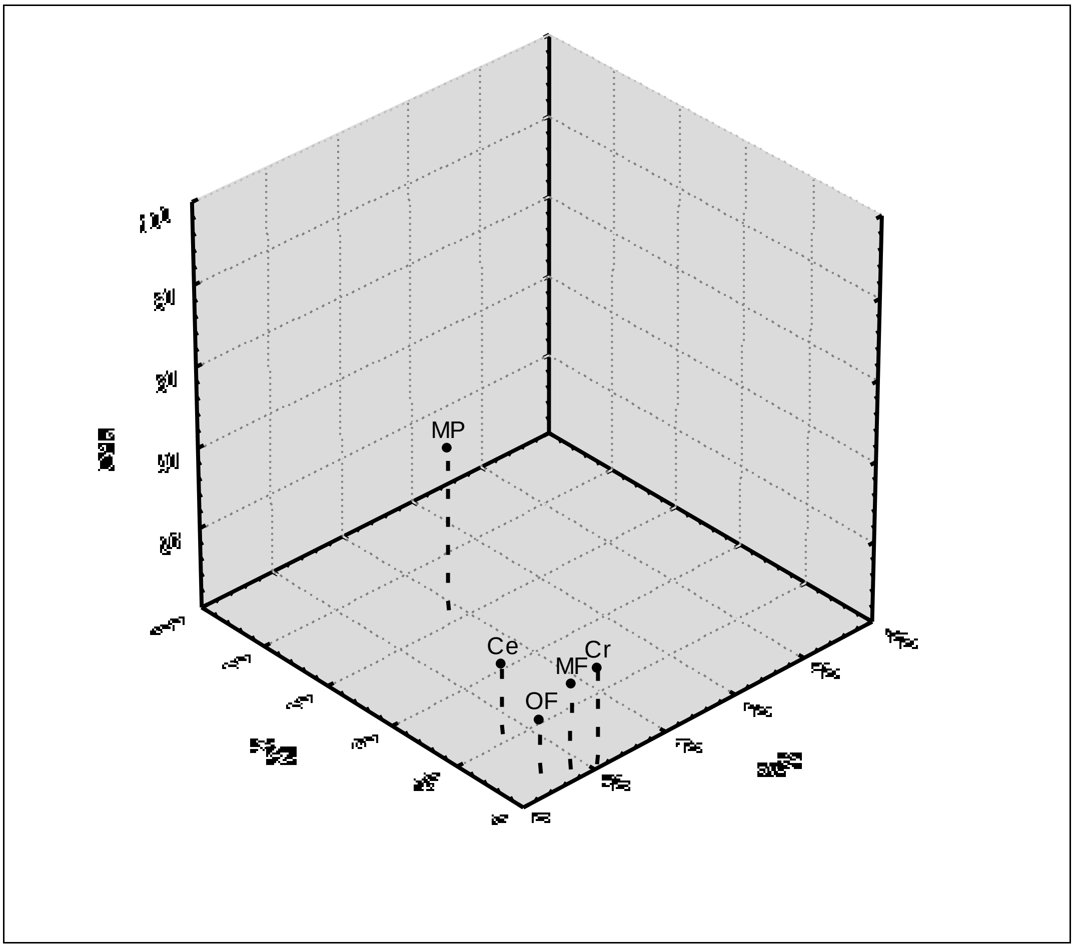

Fig. 3. Three dimensional graphical representation of the relative importance of prey in the diet of black scabbardfish: percentage by number (%N), percentage by weight (%W) and frequency of occurrence (%O). Prey items are: MP) *Micromesistius poutassou*; Ce) Cephalopods; Cr) Crustaceans; OF) Other fish; MF) Mesopelagic fish.

Rarefaction and accumulation curves were used to assess if the size of the sample of stomachs was adequate to describe the diet of black scabbardfish in the NE Atlantic. The accumulation curve does not appear to have reached a prolonged asymptote, which may indicate that the number of stomachs samples were insufficient to identify all prey consumed (Fig. 4) and/or that they are opportunistic feeders.

The Spearman rank correlation (r_T) and Kendall's Coefficient of Concordance (W_c) showed no differences in the diet between sexes ($r_T = 0.896$, $p < 0.01$) nor among length classes ($W_c = 0.65$, $p < 0.01$), respectively.

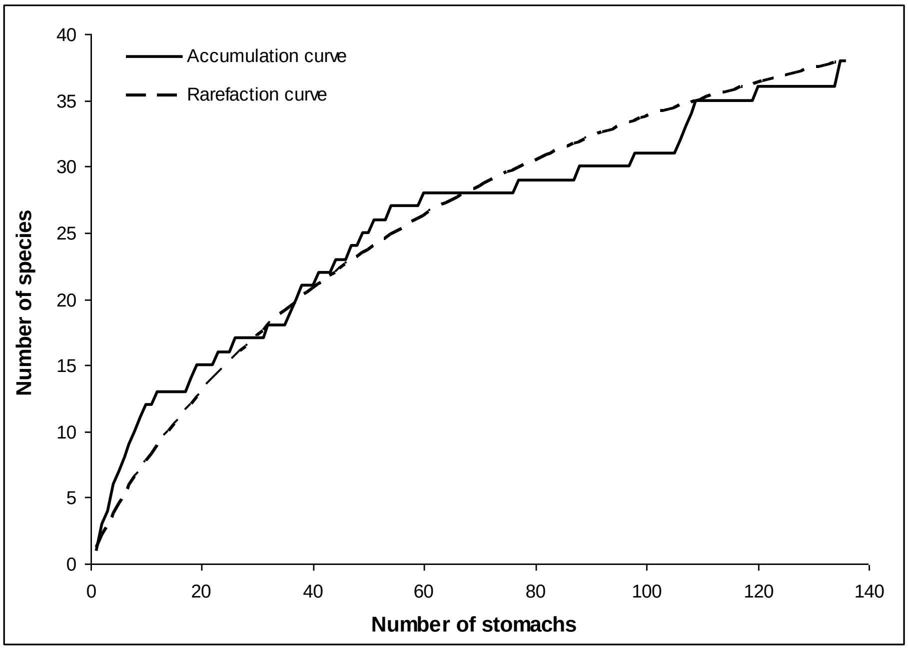

Fig. 4. Species accumulation curve and species rarefaction curve for black scabbardfish with sample size.

3.3 Diet composition by season

The diet analysis by season was only examined for the samples from west of the British Isles.

Examination of season-related differences in the black scabbardfish diet indicated that the ranking of %N values for each season were not significantly correlated (*Wc* = 0.05, *p*> 0.05) indicating that overall the diet of black scabbard was different throughout the year (Table 6). Between January and March, *M. poutassou* was by far the most abundant prey in the black scabbard diet (72.3% N). In the second quarter of the year (Apr-Jun) the abundance of blue whiting decreased and the consumption of mesopelagic fish increased to 31.8% (%N). In the third quarter (Jul- Sept) crustaceans were the most important prey category (39.4% N) and finally, in the last quarter of the year the diet of black scabbard appear to be more evenly dispersed among cephalopods (27.5% N), crustaceans (25% N), blue whiting (22.5% N) and mesopelagic fish (17.5% N) (Table 6). The diet trophic diversity (H') of black scabbardfish increased throughout the year.

Table 6. Percentage number (%N) of prey categories of *Aphanopus carbo* caught to the west of the British Isles, by quarter of the year. Prey items occurring in less than 3 stomachs were grouped in higher taxonomic levels. *Wc* Kendall's coefficient of concordance. **P<0.01.* Trophic diversity (H') is also presented.

Prey category	**Year quarter**			
	Jan-Mar	**Apr-Jun**	**Jul-Sept**	**Oct-Dec**
Acanthephyra sp	1.79	4.67	16.28	5.41
Pasiphae sp	0.00	9.34	10.60	10.81
Gnathophausia zoea	0.00	0.00	9.82	2.70
Shrimp NI	0.00	0.00	3.27	8.11
Histioteuthis sp	0.00	6.60	0.00	8.11
Brachioteuthis sp	1.79	0.00	2.31	5.41
Gonatus sp	5.66	0.00	1.64	2.70
Other cephalopods	0.00	4.67	9.82	13.51
Micromesistius poutass	71.57	28.02	16.03	16.22
Mesopelagic fish	5.37	32.69	19.63	18.92
Other Fish	13.40	14.01	10.60	8.11
	Wc = 0.05*			
Trophic diversity (H')	1.14	2.62	3.14	3.49
No Stomachs	45	24	45	23

3.4 Reconstruction of prey lengths and weights

Length frequency distribution was constructed to allow examination of the lengths of the most important species eaten by black scabbardfish, *Micromesistius poutassou* (Fig. 5). The total length of blue whiting consumed ranged from 13.6 to 34 cm, with a mean length of 26.3 cm (S.E.= 0.55, n = 74). The modal size of blue whiting was between 26 and 30 cm, but the distribution was skewed towards smaller fish. No correlation was found between the predator length and prey length (*r* = 0.01). The estimated weight of blue whiting collected from the stomachs ranged from 12.2 g (TL = 13.6 cm) to 256.7 g (TL = 340 cm).

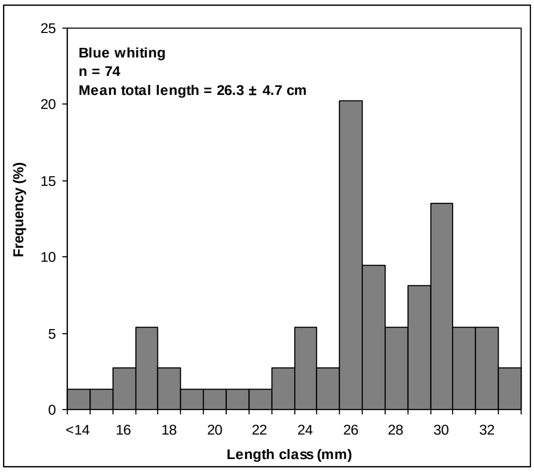

Fig. 5. Length frequency distribution of reconstructed size (Total length, cm) of *Micromesistius poutassou* (from measurements of 74 otoliths) in the stomachs of black scabbardfish.

4. Discussion

Unravelling the feeding ecology of a deep-water fish species can be exceedingly complicated due to the difficulties in obtaining samples, compounded by the high rate of stomach eversion and regurgitation. As with all methods, there is inherent bias in reconstructing diet from stomach content analysis due to differences in detectability, quantification and digestibility of the prey remains (Bergstad *et al.*, 2010). Different prey categories have different digestion rates, and prey with hard parts (e.g. fish, cephalopods, crustaceans) would be overrepresented compared to soft-tissue prey (e.g. gelatinous prey). However, direct stomach content analysis offer great taxonomic resolution and size composition of the diet, but provides only a snapshot of recent diet items (Pinnegar and Polunin, 1999). Stable isotopes, in contrast, offer poor taxonomic resolution in relation to prey items, but provide temporally integrated information regarding what an organism has consumed and assimilated over a period of time (Hesslein *et al.*, 1991), and provide evidence of nutrient pathways between primary production and subsequent higher trophic levels. The combined use of both analyses can provide valuable information on feeding ecology within and among populations (Reñones *et al.*, 2002), but this approach has rarely been used for deep water species (Stowasser *et al.*, 2009). For black scabbardfish this is particularly useful because the stomach vacuity and eversion rates are very high, principally in the samples collected in Madeira, where the stomach vacuity was nearly 100%. In fact, for the Madeira samples, the stable isotope analysis was the only method that could be used due to the extremely reduced number of stomachs with contents (only 4 stomachs with food contents). This is likely attributed to the method of capture. Longlining is a passive fishing method, which suggests that fish with empty stomachs or partial stomach fullness respond to bait odour and get caught (Løkkeborg *et al.*, 1995).

The diet of fish to the west of the British Isles consisted of a wide variety of prey species including fish, crustaceans and cephalopods. Although blue whiting (*Micromesistius poutassou*) was the predominant prey species, especially in the first quarter of the year, black scabbard seems to feed upon a wide variety of organisms, consistent with the scarce information from previous studies carried out in the same geographical area (Zilanov and Shepel, 1975; Mauchline and Gordon, 1984c). However, those studies were limited by the low number of stomachs examined and only referred to in general comments, with no diet analysis performed, which preclude any meaningful comparisons.

Although we analysed a much higher number of stomachs than the previous studies and we sampled throughout the year, the common concerns about the limited number of stomachs with contents are also applied to this study. The rarefaction analysis may indicate that the number of stomachs sampled were not sufficient to have a complete knowledge of the dietary composition of black scabbardfish. But this can also inform us about the feeding strategy of black scabbard. According to Mauchline and Gordon (1985) assessing the trophic diversity within diets of fish is very difficult unless the species is a specialist feeder. In a generalist or opportunistic species like black scabbardfish, the asymptote is achieved much more slowly and the prey species composition is more difficult to define comprehensively, since many of the prey species are rare components consumed opportunistically. While analysis of additional specimens would allow for a more robust quantitative estimation, it is unlikely that this would change the overall conclusion about the general diet composition of black scabbardfish.

Blue whiting (*Micromesistius poutassou*) was by far the most abundant fish prey item, occurring in 34.6% of all stomachs and representing 63.7% by weight. Blue whiting is a small mesopelagic gadoid that is widely distributed in the eastern part of the North Atlantic. The highest abundance of blue whiting occurs along the edge of the continental shelf in areas west of the British Isles and on the Rockall Bank where it occurs in large spawning schools between the 300 and 400 m deep, between January and April (ICES, 2010), which makes it a highly available prey to black scabbardfish. Blue whiting, like other mesopelagic species, is a particularly important fish prey in the diet of other fish species, such cod *Gadus morhua* (Du Buit, 1995; Dolgov *et al.*, 2009), whiting *Merlangius merlangus* (Pinnegar et al., 2003), hake *Merluccius merluccius* (Du Buit, 1996) and saithe *Pollachius virens* (Du Buit, 1991). It is also an important species in the diet of some marine mammal species, such as bottlenose dolphins (*Tursiops truncatus*) (Fernándes *et al.*, 2011) and common dolphin (*Delphinus delphis*) (Silva, 1999). However, in the series of diet studies carried out by Mauchline and Gordon (1983; 1984a; 1984b; 1984c; 1991) with deep water species in the Rockall Trough, only the morid *Antimora rostrata* (Mauchline and Gordon, 1984b) and the deep water sharks, *Apristurus* sp and

Centroscymnus coelolepis (Mauchline and Gordon, 1983), prey on blue whiting, and in low quantities.

Most samples used for the stomach content analysis (80%) were caught at 1000-1200m depth. The small number of samples collected in different depth strata precluded a comparative analysis with depth. The presence of prey species with pelagic affinities, such as blue whiting (300 – 400m), and vertically migrating species (e.g. *Pasiphaea* sp., *Acanthephyra* sp., cephalopods species) (Cartes, 1993; Cartes *et al.*, 1993; Bower and Takagi, 2004; Watanabe *et al.*, 2006) in the diet of black scabbard, corroborates the importance of these organisms in the diet of deep-water fish and on the transfer of energy from the epipelagic and mesopelagic zones to the near bottom zone (Vinogradov, 1997). The most important process to facilitate this energy transfer is the diel vertical downward migration of the pelagic nekton to depths where the black scabbard occurs (~700 – 1000m), but also, potential upward migration of black scabbardfish into the water column to intercept diurnally migrating pelagic species (Vinogradov, 1997; Bergstad *et al.*, 2003). The presence of epipelagic and mesopelagic food sources has been shown to be important for other deep water species such as *Coryphaenoides rupestris*, *Alepocephalus bairdii, Antimora rostrata, Synaphobranchus kaupii*, and their occurrence have been associated with scavenging behaviour and vertical migration of the prey and predator species (Mauchline and Gordon, 1991; Gordon *et al.*, 1995; Martin and Christiansen, 1997; Gordon, 2001; Bergstad *et al.*, 2003; Bergstad *et al.*, 2010). Although there is little doubt that the success of benthopelagic fishes results from the energy transfer from the surface downwards (Gordon, 2001), it is not known how frequently upward interception migrations occur in benthopelagic species (Mauchline and Gordon, 1991). To fully understand the daily feeding activity of black scabbardfish it would be necessary to run a sampling program over a 24h period.

The ability to catch pelagic, highly mobile prey provides evidence of black scabbardfish's fine swimming and sensory adaptations for predation (Bone, 1971; Martin and Christiansen, 1997). Blue whiting was found within stomachs bitten, folded over in half and swallowed whole- this together with the presence of two big fish tails, belonging to *Centrolophus* sp and *Alepocephalus* sp. is consistent with the observations by Bone (1971) who suggested that this is indicative of a stalking from behind and striking behaviour as a hunting strategy for black scabbard.

The feeding strategy of black scabbard off the west of British Isles, which seems to be more or less opportunistic, feeding upon species with epipelagic and benthopelagic affinities, was also confirmed by the stable isotope data. The $\delta^{15}N$ values in the tissue of consumers is typically enriched by c. 3‰ in relation to their prey and thus the ^{15}N values reflect the trophic level of an organism (Jennings *et al.*, 2002a; Hoffman and Sutton, 2010), while $\delta^{13}C$ values are more weakly enriched with the increasing trophic level, and may act as a good indicator of sources of production (Jennings *et al.*, 1997; Vander Zanden and Rasmussen, 2001). The mean $\delta^{15}N$ (~13.4‰) and $\delta^{13}C$ (-18.50‰) values support the diet composition encountered in the stomachs – several species with pelagic affinities (e.g. blue whiting, mackerel, *A. pelagica, P. multidentada*, squids) (Cartes and Carrassón, 2004) and species with strong benthopelagic affinities (e.g. the crustaceans: *A. purpurea* and *G. zoea* and fish: *L. eques*, *S. kaupii*) (Iken *et al.*, 2001; Cartes and Carrassón, 2004) that black scabbardfish forms a link between the pelagic and the benthopelagic food webs. The stable isotope composition of range of deep water species sampled in the Rockall Trough and Porcupine bank was determined by Trueman et al. (*in review*). Black scabbardfish has high (enriched) $\delta^{15}N$ and $\delta^{13}C$ values compared with the benthopelagic feeders – *Xenodermichthys copei*, *Argentina silus, Alepocephalus bardii* - and low (depleted) nitrogen and carbon isotope ratios compared to the true benthic predators and/or scavengers – *Deania calceus, Centroselachus crepidater, Chimaera monstruosa*, *Hydrolagus mirabilis* (See Fig. 6). The $\delta^{15}N$ and $\delta^{13}C$ values for black scabbardfish are close to the values found for the other slope dwelling species, such *Lepidion eques, Nezumia aequalis* and large individuals of *C. rupestris*, which where classified as species that link the benthic and benthopelagic food webs (Stowasser *et al.*, 2009; Trueman *et al.*, *in review*) .

The estimated mean $\delta^{15}N$ values in the present study were more enriched than the estimates in Trueman et al. (*in review*). This variability between 2006 and 2009 in $\delta^{15}N$ and $\delta^{13}C$ may not necessarily reflect changes in food web structure and carbon flow, but just a temporal variation in the isotopic baseline, which is not possible to determine without suitable estimates of $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ in each year (Post, 2002). The high variability of the δ^{15} N values (12.30-14.50 ‰)

reflects the stomach content data that shows that black scabbardfish feeds upon a wide variety of species and may also reflect changes in diet seasonally.

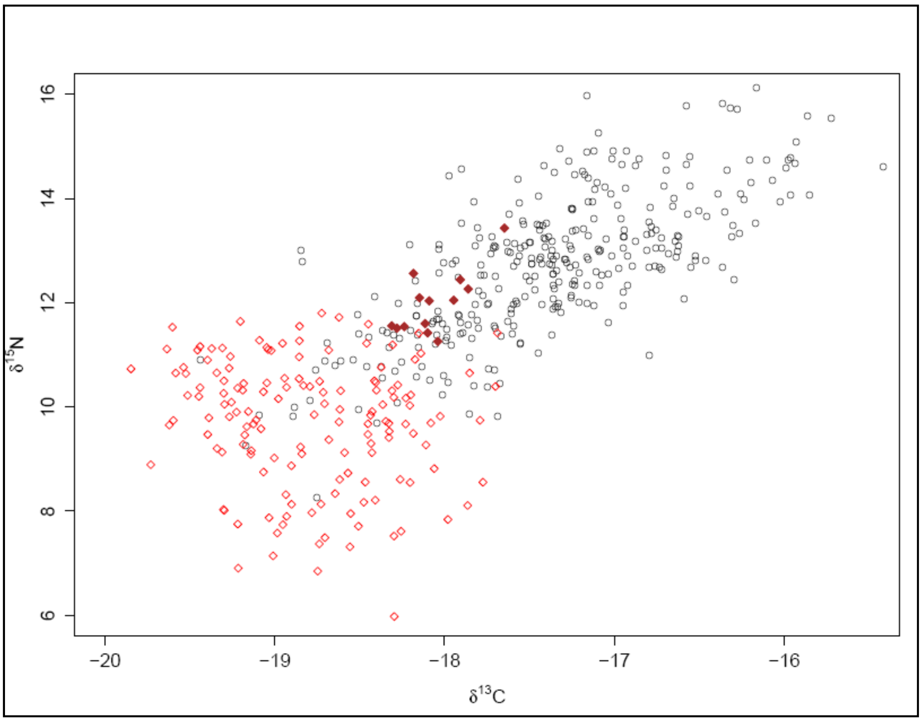

Fig. 6. Relationship between δ^{15}N and δ^{13}C values for (◊) benthopelagic feeders; (○) benthic feeders and (♦) *Aphanopus carbo* in the Rockall Through and Porcupine Bank. Samples collected in 2006 (Adapted from Trueman *et al.* (*in prep*)).

This study showed seasonal changes in the diet composition of black scabbardfish in the northern area. In the first quarter of the year, the diet is predominated by blue whiting and throughout the year there was an accentuated decrease of this species and an increased contribution of cephalopods and crustaceans. These changes are clearly related with the migration pattern of blue whiting which undertakes long annual movements from feeding grounds in the Norwegian Sea to spawning grounds, west of the British Isles, and back again. Between January and April, most NE Atlantic blue whiting aggregate to spawn in the region around the Porcupine Bank and by the end of April / May migrate back to Norwegian Sea (Bailey, 1982; Was *et al.*, 2008). This migration pattern supports the results obtained in this study, which could indicate that black scabbard selects prey in proportion to its availability but can adapt its diet according to changes in abundance of the main prey, feeding on locally abundant or more available prey. Seasonal changes in the diet composition were also detected in the δ^{15}N values in the muscle, which became more depleted in the second time period, confirming a shift to a diet composed of prey from a lower trophic level. The temporal offset between prey consumption and expression in muscle tissue isotopes is unknown, complicating the interpretation of seasonal changes in tissue isotopes, but it is likely to be on the order of weeks-months depending on the growth rate of the individual fish and the isotopic separation between different diets (Martinez del Rio *et al.*, 2009).

In contrast, the samples from Madeira showed seasonal differences only in δ^{13}C values. The significant depletion in δ^{13}C values in November could be related to changes in the nutritional status of black scabbardfish during the reproductive season (between September and December, with a spawning peak in November) (Neves *et al.*, 2009) or a change in habitat. The variation in stable isotopes signatures, which is often assumed to be only a reflection of the diet and foraging location, are often obscured by the individual's physiology (Gannes *et al.*, 1998). However, how these intrinsic factors affect the isotopic signal is still poorly understood (Williams *et al.*, 2007).

The extreme reduced numbers of stomachs with food contents from Madeira Islands (only 4 with prey items) obviated any prey composition analysis and meaningful comparisons between the two areas. However, stable isotope analysis isa useful tool to compare the trophic ecology of black scabbardfish between both areas. After accounting for the effect of fish, differences were found in the mean δ^{13}C values, between the two regions, but not in δ^{15}N values, indicating that there is no isotopic evidence for difference in diet or feeding strategy of black scabbard between the two areas (assuming a constant δ^{15}N baseline value). The difference in the mean δ^{13}C values between areas is likely due to the gradient in the degree of isotopic fractionation during photosynthetic primary production in these regions (Tagliable and Bopp, 2008; Graham *et al.*, 2010), where primary production at high latitudes typically has more depleted δ^{13}C values due to relatively low plankton growth rates, large cells and high levels of dissolved CO_2 .

When both areas are considered together, there is a positive relationship between the body mass and δ^{15}N values, consistent with a size-structured food web (Jennings *et al.*, 2002b), assuming that the baseline of N is the same in two areas. However, when the relationships between body mass and δ^{15}N values are analysed in the different areas separately, the relationship was significant only for the Madeira samples. The lack of significance of any relationship between trophic level and body size in black scabbardfish from the west of the British Isles samples may reflect the high levels of prey variability between seasons, and possibli differential rates of isotopic assimilation between large and small individuals.

The size range of blue whiting recovered from the black scabbardfish stomachs was biased towards smaller fish compared to fish caught in the Irish blue whiting surveys (mean TL of prey blue whiting = 26.3 cm, mean TL of trawled blue whiting =28.5cm) (ICES, 2010; O'Donnell *et al.*, 2011). The biggest blue whiting found in a stomach of a black scabbard was much smaller (34 cm) than the biggest captured by the fishery (48 cm), which may reflect the mouth dimensions and stomach storage capacity of *A. carbo*. The lack of any significance correlation between the length blue whiting in stomachs and black scabbard lengths (r = 0.01) suggest that black scabbard feed on all specimens irrespective of size, up to a threshold size (~ 34cm) and potentially also explains the limited correlation between size and $\delta^{15}N$ values in black scabbardfish within regions. Previous studies have demonstrated that fishes have an “optimal” prey size, which should be the largest size that a predator can handle (Pinnegar *et al.*, 2003). However in the present study, we observed small prey in larger predators, which could be related to a combination of relatively high abundance and a higher vulnerability of smaller prey to predation.

The relationship between the black scabbardfish preferences and prey availability should be considered as being of the upmost importance, particularly in the northern area, since the fishing pressure exerted on the main prey species (blue whiting) might have an indirect impact on the predator stocks. It is becoming increasingly evident that stocks can not be managed in isolation and fisheries managers should focus more on a multi-species assessment and an ecosystem approach to fisheries management, where interactions between predators and prey should be taken into account.

Conclusions

This study has shown that the use of stable isotope analysis in conjunction with stomach content data can provide both taxonomic specificity and integrative information on assimilation in species and environments where conventional methods alone offer limited data. Stable isotope analysis proved to be particularly useful when stomach contents were difficult to obtain, as in the samples from the longline fishery in Madeira. To the west of the British Isles, stomach content and stable isotope analysis indicated that *A. carbo* is a top benthopelagic predator and its diet is associated with prey with both pelagic and benthopelagic affinities. Black scabbardfish thus form a critical link between the pelagic and the benthopelagic food webs. Comparisons of the stable isotope ratios between the west of British Isles and Madeira showed that although the black scabbardfish feed upon preys with different C isotope ratios, depending on the local availability of prey, the feeding strategy does not change spatially and they feed at a similar trophic level, as a top predator in the different areas. Together these methods characterize the diet of black scabbardfish more comprehensively and can be a greater benefit to resource managers and ecosystem modellers.

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Appendix I

Table I. Regression equations used to estimate fish and cephalopod size: DML, Mantle length (mm); LRL, Lower rostral length (mm); TW, total length (g); OL, otolith length (mm); TL, total length (mm); FL, fork length (mm).

Prey items	Estimated prey length	Source	Estimated prey weight	Source
CEPHALOPODA				
Ancistroteuthis lichtensteini	DML = -41.3 + 40.75 x LRL	Clarke (1986)	ln (W)= -0.194 + 3.56 x ln (LRL)	Clarke (1986)
Branchioteuthis reesei	DML = 16.31 + 20.18 x LRL	Clarke (1986)	ln (W)= 0.550 + 1.41 x ln (LRL)	Clarke (1986)
Gonatus sp	DML = -43.4 + 42.87 x LRL	Clarke (1986)	ln (W)= -0.655 + 3.33 x ln (LRL)	Clarke (1986)
Histioteuthis arcturi	DML = -13.60 + 22.21 x LRL	Clarke (1986)	ln (W)= 1.594 + 2.31 x ln (LRL)	Clarke (1986)
Histioteuthis bonnelli	DML = -13.60 + 22.21 x LRL	Clarke (1986)	ln (W)= 1.594 + 2.31 x ln (LRL)	Clarke (1986)
Histioteuthis reversa	DML = -13.60 + 22.21 x LRL	Clarke (1986)	ln (W)= 1.594 + 2.31 x ln (LRL)	Clarke (1986)
Toraropsis eblanae	DML = -10.32 + 35.04 x LRL	Clarke (1986)	ln (W)= 0.590 + 3.17 x ln (LRL)	Clarke (1986)
PISCES				
Anguilliformes				
Nemichthydae				
Nemichthys scolopaceus	OL / TL = 1.9	Tuset *et al.* (2008)	TW = 0.0041 x $TL^{3.000}$	Pauly *et al*. (1998)
Synaphobranchidae				
Synaphobranchus kaupii	OL / TL = 0.5	Tuset *et al.* (2008)	TW = 0.0003 x $TL^{3.315}$	Rosa *et al*. (2006)
Gadiformes				
Gadidae				
Gadiculus argenteus	FL = 19.449 x $OL^{1.053}$	Härkönen (1986)	TW = 0.0207 x $TL^{3.7981}$	Härkönen (1986)
Micromesistius poutassou	TL = -2.140 + OL x 22.090	Santos *et al.* (2007)	TW = 0.019350 x $(TL/10)^{3.34372}$	Santos *et al.* (2007)
Gadidae unid.	TL = -2.140 + OL x 22.090	Santos *et al.* (2007)	TW = 0.019350 x $(TL/10)^{3.34372}$	Santos *et al.* (2007)
Moridae				
Halargyreus johnsoni	OL / TL = 0.47	Campana (2004)	TW = 0.0117 x $TL^{3.000}$	Fock and Elrich (2010)
Lepidion eques	OL / TL = 0.60	Tuset *et al.* (2008)	TW = 0.001 x $TL^{3.498}$	Magnússon (2001)
Myctophiformes				
Myctophidae				
Notoscopelus sp	OL / TL = 3.9	Tuset *et al.* (2008)	TW = 0.00521 x $TL^{3.260}$	Fock and Elrich (2010)
Zoarcidae				
Lycodes sp	FL = 3.47 x OL + 0.48	Harvey *et al.* (2000)	TW = 0.0195 x $FL^{2.522}$	Harvey *et al.* (2000)
Osmeriformes				
Alepocephalidae				
Alepocephalus bardii	OL / TL = 0.20	Campana (2004)	TW = 0.003 x $TL^{3.210}$	Fock and Elrich (2010)
Xenodermichthys sp	OL / TL = 0.12	Campana (2004)	TW = 0.00736 x $TL^{2.984}$	Fock and Elrich (2010)

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Chapter 7

General Discussion

Contents:

General discussion

Black scabbardfish is an enigmatic inhabitant of the continental slope and one of the main deep water commercial species in Europe, where it is captured by multi-species trawlers in the North Europe and small scale longliners in Portugal and the Madeira Islands (Gordon, 2001). As a deep water species, there is much concern about whether it can be sustainably exploited (Haedrich *et al.*, 2001; Roberts, 2012) given that it is generally accepted that deep-water species are generally long-lived, slow- growing, mature late and have low fecundity. The ecological characteristics of these fish make them vulnerable to over-exploitation and slow to recover from it (Clark, 2001; Morato *et al.*, 2006).

Although black scabbardfish is widely distributed in the NE Atlantic and of commercial interest, biological studies over its entire distributional range are very sparse. Before this study, the most comprehensive biological studies were spatially confined to the southern regions (Portuguese slope and Madeira Islands), specifically on reproduction (Figueiredo *et al.*, 2003; Pajuelo *et al.*, 2008; Neves *et al.*, 2009) and age (Morales-Nin and Sena-Carvalho, 1996; Morales-Nin *et al.*, 2002; Vieira *et al.*, 2009). In northern Europe, previous studies had focus only on distribution (Zilanov and Shepel, 1975; Piotrovskiy, 1981; Mauchline and Gordon, 1984; Nakamura and Parin, 1993; Magnússon and Magnússon, 1995; Vinnichenko *et al.*, 2005) and general comments on reproduction (Magnússon and Magnússon, 1995) and on diet composition of black scabbardfish (Mauchline and Gordon, 1984). No complete study focusing and integrating all life history aspects, from specimens caught off western British Isles and Madeira, had ever been carried out. Given that the life history parameters are the basic data needed for population modelling purposes, there was a need to perform a complete life history study, including age, growth, maturity and reproductive seasonality on black scabbard. Only with these data, can further population dynamic assessments be carried out in order to determine, for example, if the current fishing pressures are sustainable and how to manage the species.

The general concerns about the sustainability of deep water resources and the urgent need to enhance our understanding of the life history, ecology and stock structure of *Aphanopus carbo* over its wide geographical distribution in Northeast Atlantic constitute the basis for this study. A combination of methodologies of traditional biological tools was used to describe the reproductive cycle (Chapter 2), age and growth (Chapter 3) and trophic ecology (Chapter 4) of black scabbardfish off western British Isles and Madeira islands. For the first time the recent technique of otolith stable isotope analysis to investigate the migration pattern of black scabbardfish between the two sampling locations was carried out to test whether the fish from the two different locations present some degree of connectivity at different stages of the life cycle (Chapter 5). To investigate the spatial distribution and provide some information on the effects of the fishing pressure, a 10 year time-series was used. These data were collected during Scottish and Irish deep-water surveys and trends in abundance and size structure of black scabbardfish on the slope off the western British Isles (Chapter 6) were analysed. All the objectives proposed at the beginning of this study were achieved and the new knowledge acquired on life history and stock structure of black scabbard will increase our ability to assess the current impacts of commercial fisheries and to better manage this species in the NE Atlantic.

The overall results of this study suggest and support the existence of wide ranging, likely panmitic population of black scabbardfish in the NE Atlantic. The combined analyses of life history characteristics throughout the year of specimens from two distinct geographical areas, west of the British Isles and Madeira, corroborates that the fish caught in the former are pre-adults that likely undertake large scale north - south migrations towards Madeira and the Canaries Islands where they reach maturity and spawn.

This final chapter presents a general discussion, a summary of the key findings and conclusions presented in the various sections of this thesis.

Spatial and depth distribution

In the NE Atlantic, black scabbardfish has its northerly distribution limit in Iceland (Magnússon and Magnússon, 1995) and its southerly limit in Canary islands (Nakamura and Parin, 1993; Pajuelo *et al.*, 2008). The geographical distribution of black scabbardfish might be directly associated with the reproductive (Chapter 3) and feeding (Chapter 5) behaviours.

According to the Scottish and Irish deep water surveys, the bathymetric distribution of black scabbardfish west of the British Isles ranged between 500 and 1500m. The distribution at depth fits a unimodal function, with maximum abundance at the centre of the bathymetric distribution, between 800 and 1000 m. The peak of abundance for black scabbard is in agreement with previous studies made in the Rockall Trough (Mauchline and Gordon, 1984). However, when compared with the surveys conducted in the 1970s (Bridger, 1978), it seems likely that there was a slight change in core distribution of the species, from 600-800 m (Bridger, 1978) in 1970s, to 800-1000 m in 2000s. If this shift is real, it could be related to a change in community structure on the continental slope caused by fishing pressure (Jennings *et al.*, 1999) or perhaps to spatial and temporal variations in the underlying productivity and abundance of other fish species, particularly potential prey (Bailey *et al.*, 2006). The current biomass peak depth for black scabbard is one of most critical characteristics of deep-sea assemblages. Demersal biomass is at its highest between 800 and 1550 m deep, with a pronounced maximum at 1000 m on the slope of the Rockall Trough (Gordon *et al.*, 2003) and is where most deep-water fisheries tend to be concentrated (Hopper, 1995).

Different length-frequency distributions were observed between the specimens captured to the west of the British Isles and Madeira. The largest specimens were caught off the Madeira Islands (attaining 1450mm), while the smallest were captured to the West of the British Isles (~620mm), suggesting that there is geographical size segregation. This was previously observed by other authors (Santos, 2000; Bordalo-Machado *et al.*, 2001; Figueiredo *et al.*, 2003). Although the geographical size segregation is difficult to definitively prove without directed fishing using the same gear types in both areas, the size distributions suggest that black scabbard does not complete its life cycle in one area.

Reproduction

Understanding the reproductive cycle of *A. carbo* across the NE Atlantic is a fundamental first step to identifying and understanding stock structure (Begg *et al.*, 1999). The combined simultaneous analysis throughout the year of specimens from two distinct geographical areas, West of the British Isles and Madeira, corroborates that the fish caught in the former are pre-adults that must undertake large scale north - south migrations towards Madeira and the Canaries Islands where they are known to reach maturity and spawn (Figueiredo *et al.*, 2003; Pajuelo *et al.*, 2008). Therefore the results obtained on the reproductive biology suggest and support the existence of wide ranging population of black scabbardfish in the NE Atlantic.

Based on the macroscopic and histological results, the specimens from west of the British Isles were all immature throughout the year, while in Madeira all maturity stages were observed. The lack of gonadal evolution throughout the year was confirmed by the constant values of GSI and suggests that the fish leave the northern region prior to/once the gonads start to develop to the early-vitellogenic stage and likely migrate southwards to proceed with maturation and spawn. The factors that trigger the “decision” to migrate are difficult to disentangle and most probably are a combination of environmental, ecological and physiological factors (Jørgensen et al., 2006). One factor that might contribute to trigger the migration is the change in the diet composition throughout the year. The diet composition analysis from this study (Chapter 4) showed that the diet of black scabbardfish changed throughout the year, resulting in a shift from a diet predominated by blue whiting in the first quarter of the year to a less energetically nutritional diet, composed of cephalopod and crustaceans in the following quarters. This change in the diet composition may result in a decrease in black scabbard’s condition to proceed with maturation and trigger the migration towards the south. The diet shift in the end of the first quarter may also contribute to the increase in the levels of atresia observed in April, which suggest that the specimens with poorer conditions may remain in the northern area and enter into an atretic process. The decision to migrate has to be a trade-off between the potential benefit of reproduction and the costs of migration and natural and fisheries-induced mortalities (Jørgensen *et al.*, 2006).

In the Madeira Islands all maturity stage were observed during the year, although very few immature fish were observed. The low numbers of immature specimens corroborates the migration pattern of black scabbardfish previously discussed and they are present in Madeiran waters in very low numbers. Notwithstanding this, the low numbers of immature fish caught off Madeira could also be as a result of differential gear selectivity, avoiding being caught by longline gear, or a different vertical distribution. The spawning season was well defined, between September and December, which is in agreement with previous studies from Madeira (Bordalo-Machado et al., 2001; Figueiredo et al., 2003; Neves et al., 2009).

This was the first study to differentiate females in developing stage (IIa) from the ones in resting stage (IIb) (See Table 3, Chapter 2). This differentiation is not just essential for a better understanding of the reproductive cycle, but from a stock management perspective, the lack of differentiation can result in erroneous estimates of length at first maturity ($LC_{50\%}$) and have serious consequences for the larger part of the reproductive stock.

The present study revealed that black scabbardfish has a very particular maturation process, with a geographical quasi-complete separation of the immature and mature individuals. While in the northern area only immature specimens were sampled, in the Madeira Islands a very low number of immature specimens were sampled, with a low overlap along the size range. Notwithstanding the geographical distance between the west of Scotland and Madeira, it is important to incorporate the immature species from the former location into the maturity ogive estimations, since our data strongly suggests that the life cycle of black scabbardfish is not completed in just one area. When the data from the two locations were combined, the length-at-maturity estimated for females was 1156mm, which is much larger than the estimations by Figueiredo *et al*. (~1028mm) (2003).

Age and Growth

The previous studies on age and growth of black scabbardfish presented contradictory results. In the first growth study, Morales-Nin and Sena-Carvalho (1996), considered black scabbard to be fast growing, reaching a maximum age of 8 years, whereas the study by Kelly *et al.* (1998), showed that the growth rate was much slower, with a maximum age of 32 years. In more recent studies (Morales-Nin *et al.*, 2002; Vieira *et al.*, 2009), the maximum age recorded was 12 and 15 years, respectively. The differences among the publish studies are the result of the features of the black scabbardfish otoliths, which have poor contrast between the alternating dark and light zones, and a confusing sequence of narrow zones, which can either be counted singly or grouped. In order to address this problem, two reading interpretations methodologies were employed: a conservative interpretation and a non-conservative interpretation. Taken together, these two methodologies gave an indication of the likely range of the growth rates and maximum ages that could plausibly be ascribed to black scabbardfish. The results indicated that the conservative reading interpretation should be the correct one for ageing black scabbard and the bands counted as true rings were in fact false rings, resulting in overestimated age and underestimated growth (See Chapter 3).

The growth parameters obtained in this study, using the conservative reading interpretation, showed that black scabbardfish from the west of the British Isles is faster growing than estimated by Kelly *et al.* (1998), but slower growing in Madeira than previously stated by Vieira *et al.* (2009), Morales-Nin *et al*. (2002) and Morales-Nin and Sena-Carvalho (1996). These differences result most likely from different interpretations of the growth increments. The results of this study raise concern over the inaccurate age estimations which result in erroneous growth rate estimations (Beamish and McFarlane, 1983) and may cause problems for the assessment and management of the stock. The ageing errors affects directly the catch-at-age data, but also other input data, including maturity at age, age-structured catch per unit effort (CPUE). Hence, age-reading problems may influence virtually all the assessment inputs (Reeves, 2003). Consequently ageing error will affect the estimations of fishing mortality and spawning stock biomass, essential indexes for stock predictions. It is essential otoliths exchange programmes and workshops for otolith interpretation standardization among experts and laboratories.

The results showed area specific differences in the growth parameters and population age structure. To the west of the British Isles, specimens reached a lower maximum age and had a higher growth rate than those caught off Madeira. These differences are consistent with the theory of a single population of black scabbardfish in the NE Atlantic, highly segregate, with smaller, immature and younger fish caught to the west of the British Isles that migrate further south (possibly to Madeira) as they grow, reach maturity and spawn.

Although this study showed that there are rather serious interpretation challenges, the overall precision of these age estimates is acceptable and repeated counts of the same otolith section do not vary much once a certain interpretation of zonation is defined. The age validation of black scabbardfish proved to be difficult and the analysis of the nature of the otolith edge throughout the year was different from previous study by Morales-Nin and Sena-Carvalho (1996) and might not be the best validation method for this species. One of the most promising age validation techniques for long-lived deep water fish is the lead-radium dating of otoliths, which utilizes a known radioactive decay series in the cores of previously aged fish otoliths to provide an independent age estimate of bony fishes (Cailliet *et al.*, 2001; Andrews *et al.*, 2009). This technique relies on the decay of the radioisotope radium-226 to a short-lived product lead-

210 and it is best suited to discriminate age where the candidate species has age interpretations that are widely divergent, as in the case of black scabbardfish.

Trophic ecology

Unravelling the feeding ecology of deep sea fish is very complicated due to the difficulty in obtaining samples and the high rate of stomach eversion. This was particularly true for black scabbardfish, where relatively low numbers of stomachs with food contents were recovered from the west of the British Isles and an insignificant number from Madeira. To augment the limited recovery of stomach contents, the study on trophic ecology of black scabbardfish was augmented by stable isotope analysis. The combined use of both analyses can provide valuable information on feeding ecology within and among populations (Reñones *et al.*, 2002), but this approach has rarely been used for deep water species.

The feeding strategy of black scabbardfish from the west of the British Isles seems to be more or less opportunist, consisting of a wide variety of fish, cephalopods and crustaceans, with epipelagic and benthopelagic affinities and this was confirmed by the stable isotope data. The mean $\delta^{15}N$ and $\delta^{13}C$ values support the diet composition encountered in the stomachs and that black scabbardfish forms a link between the pelagic and the benthopelagic food webs. Although there is no food content information from Madeira, the stable isotope analyses showed that although the black scabbardfish feed upon prey with different C isotope ratios, depending on the local availability of prey, the feeding strategy does not change spatially and they feed at a similar trophic level, as a top benthopelagic predator in the different areas.

Despite the diverse diet, blue whiting was by far the most abundant fish prey item. Blue whiting is a mesopelagic species occurring in the highest abundances between the 300 and 400 m off west of the British Isles (ICES, 2010). Their occurrence might be associated with scavenging behaviour and vertical migration of the prey and predator species. Although there is little doubt that the success of benthopelagic fishes results from the energy transfer from the surface downwards (Gordon, 2001), it is not known how interception migrations occur in benthopelagic species (Mauchline and Gordon, 1991).

This study showed seasonal changes in the diet composition of black scabbardfish in the northern area. In the first quarter of the year, the diet is predominated by blue whiting and throughout the year there was an accentuated decrease of this species and an increased contribution of cephalopods and crustaceans. These changes are clearly related with the migration pattern of blue whiting which undertakes long annual movements from feeding grounds in the Norwegian Sea to spawning grounds, west of the British Isles, and back again

Otolith stable Isotope Analysis

The stable isotope analyses in different regions of the otoliths, provided information on the metabolic and environmental conditions that individual black scabbard fish experienced in the northeast Atlantic throughout its life history. The ontogenetic variations of $\delta^{18}O$ and $\delta^{13}C$ values corroborate the large scale migration of black scabbardfish, described in the previous chapters. The similar values of δ18O during the larval and juvenile phases in both sampling locations, suggest that the ambient environmental conditions experienced by the fish during these life phases were identical. The extreme low values of $\delta^{18}O$ in the core of the otolith indicated that the specimens of black scabbardfish captured off the west of Scotland spent its larval phase in warmer, southerly waters (e.g. Madeira islands) migrating afterwards to deeper and northern waters. On the other hand, the values of $\delta^{18}O$ obtained for the juvenile phase in the otoliths from Madeira presented identical values to their counterparts from west of Scotland that might suggest that the fish caught in Madeira spent their juvenile phase in waters with identical oceanographic features.

The $\delta^{13}C$ composition in the black scabbardfish's otoliths became more enriched with age, indicating a decline in metabolic rate and feeding at a higher trophic level as they get older (Begg and Weidman, 2001; Sherwood and Rose, 2003; Longmore *et al.*, 2011) and no differences were found between west of Scotland and Madeira. This might suggest a similar metabolic rate and feeding strategy in each location. The identical $\delta^{13}C$ values during the juvenile phase in both locations also support the hypothesis that black scabbardfish spend their juvenile phase off the west of the British Isles, feeding.

Biomass and size structure trends

The analysis of the time series data from the Scottish and Irish deep water surveys, has demonstrated that overall, the black scabbardfish stock west of the British Isles declined significantly between 1998 and 2004 and has remained at stable but low levels since. Declines in abundance are an inevitable consequence of exploitation (Hilborn and Walters, 1992; Pauly *et al.*, 1998; Hilborn *et al.*, 2003), and a reduction in biomass may be required for maximum productivity to be attained (Schnute and Richards, 2002). The same trend has been recorded for other deep-water species, particularly for grenadiers (Neat and Burns, 2010). The relative stability of abundance after 2005 may reflect the introduction of stricter management tools, including TACs for commercial deepwater species. Hence fishing pressure has been alleviated, which might suggest that the restriction has been sufficient to prevent further decline of the black scabbardfish stock. However, a lack of knowledge of the population status prior to the fishery starting and regrettably even at the present time makes it difficult to assess accurately the real consequences of fishing pressure and of the decline in abundance of deep-water species over years.

Over ten years, there was no obvious change in the size structure of black scabbardfish west of the British Isles, suggesting that this species has greater resilience to fishing pressure than some other deep-water species (Clark, 2001; Lorance and Dupouy, 2001; Neat and Burns, 2010). This may also be attributed to a large scale movement and size segregation as described in the previous chapters (Chapters 2, 3 and 5). Black scabbardfish undertakes large-scale southward migrations to spawn and then, the juveniles migrate towards the north where an intense feeding activity takes place. With this migration pattern, it means that the spawning stock in Madeira does not suffer the same fishing pressure as the specimens west of the British Isles, allowing this species to reproduce and produce viable offspring in order to maintain long term population levels. Although they are fished in Madeira and the Canary Islands, the fishing pressure level is much lower than in northern Europe, being fished by small scale longliners (Bordalo-Machado *et al.*, 2009).

Final considerations and future research

This study has presented comprehensive and new information on the life history and population structure of *A. carbo* over its wide distribution range and clearly demonstrated the existence of a widely distributed population in the Northeast Atlantic, with a distinct migration pattern.

Given its life cycle there is an urgent need that the management process recognizes the existence of a continuous widely distributed stock of black scabbardfish between the west of the British Isles and Madeira. Currently, black scabbardfish is managed based on the separate biennial ICES and CECAF scientific advice and as species that transpose the barriers of the Regional Fishery Bodies should be treated as highly migratory and managed collectively. An effective management requires cooperation between the States and/or the Regional Fishery Bodies where black scabbardfish is exploited. The inadequacy of the available information to evaluate the state of the spawning stock stated in ICES advice (ICES, 2012), is the outcome of the lack of cooperation and interchange between ICES and CECAF. Understanding the logical connection between juveniles and spawning biomass and the effect of the migration behaviour within the distribution area is vital for the maintenance of the population (Secor, 1999; Trippel, 1999). It is important that fish are able to grow to a reproductive size and are able to spawn before they are harvested. Harvesting of juveniles ultimately reduces the number of individuals that contribute to the spawning stock. It is vital to allow potential spawners to reproduce and produce viable offspring in order to maintain long term sustainable population's levels.

In this study two areas were sampled and analysed; west of the British Isles and Madeira, which corresponds to the known feeding and reproductive grounds, respectively. However, black scabbardfish has a wider geographical distribution, including Iceland, Mid-Atlantic Ridge, the Azores and Canary Islands. It is essential to obtain more biological information from these areas, to understand the population structure and dynamics across a wider geographical area.

While the migration between the northern and southern component was demonstrated, there are still questions about how and when the migration occurs, that should be addressed by further research. The south-north migration should be addressed to find out how long the juveniles take to move between the spawning grounds in the south to the northern waters. Further analysis on the monthly length frequency data collected by the deep water French observer program, possibly could give us insights on recruitment pulses throughout the year or between years. Furthermore, is important to know how long the juveniles remain in northern waters and how do they migrate to the southerly waters where they mature and spawn.

During the present study, it was observed that some of the fish from northern Europe had high levels of atresia in their ovaries and might not migrate southwards to spawn. If only a proportion migrates, it is also very important to assess what proportion of the population migrates and the impacts that might have on the spawning stock. This could be achieved with genetic data and using otolith microchemistry analyses over a wider distribution area and with a large sample size

The egg and larval stages of black scabbardfish are still unknown, and finding them would improve significantly our knowledge on the population structure and on how the migration proceeds. A possible approach for accomplishing this would be by carrying out pelagic scientific surveys in the known spawning grounds (e.g. Madeira Islands).

Despite the great effort to estimate accurately the age and the growth parameters of black scabbardfish, it was found to be very difficult to interpret the ring patterns, due the complexity in the otoliths structure. Age validation studies are urgently required to access the accuracy and the frequency of formation of a growth increment (Campana, 2001). Since some of the validation methods are impossible to apply in deep water species (e.g. mark-recapture), one of the most promising age validation technique that could be used in black scabbardfish is the radiochemical dating of otoliths (Andrews *et al*., 2009). The application of lead-radium dating may provide an independent estimate of the age, differentiate between different age scenarios and provide a reliable age validation. This technique could also be applied to other deep water species that are commercially exploited and where age validation is absent.

The complexity of the structural patterns of the black scabbardfish otoliths raised concerns about whether the deposition of the otolith macrostructures is meaningful for age estimation. Further investigation should be carried out to evaluate if the otolith ring patterns are stable over time or if they are randomly deposited, as a response to environmental or endogenous factors.

It has been known for some time that there are two very similar species in Madeira and Canary Islands, *A. carbo* and *A. intermedius*. It was only recently that these two species have been distinguished in the monthly sampling program and the contribution of each species in the total landings from Madeira is unknown. It is important to know the proportion of each species in the landings, but also important to know the level of interaction of these sympatric species (Stefanni and Knutsen, 2007).

Since there is strong connectivity between the north and south population components, it is vital to understand how the reductions on the biomass and abundance of black scabbardfish west of the British Isles affects the spawning biomass and the fishing regime in the southerly waters of Madeira Islands that could be achieved, as discussed previously, with a change on how black scabbardfish is actually managed.

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