

## Declaration

I hereby declare that the work presented in this thesis is my own and that it has not been used to obtain a degree in this Institute of Technology or elsewhere.



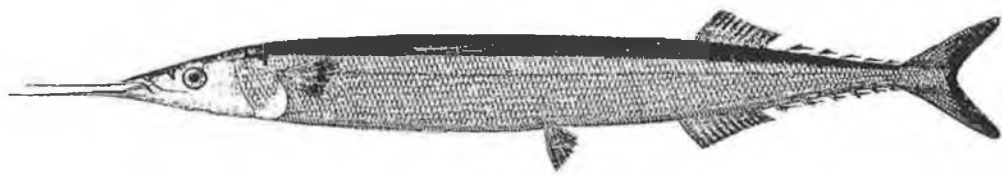
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7/09/2011

Date:

**Data for the sustainable management of an  
emerging fishery: Age, growth and stock structure  
of Atlantic saury *Scomberesox saurus saurus*  
(Walbaum), in the Northeastern Atlantic Ocean**



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# Abstract

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Almost half of Ireland's commercial stocks face overexploitation. As traditional species decrease in abundance and become less profitable, the industry is increasingly turning to alternate species. Atlantic saury (*Scomberesox saurus saurus* (Walbaum)) has been identified as a potential species for exploitation. Very little information is available on its biology or population dynamics, especially for Irish waters. This thesis aims to obtain sound scientific data, which will help to ensure that a future Atlantic saury fishery can be sustainably managed. The research has produced valuable data, some of which contradicts previous studies. Growth of Atlantic saury measured using otolith microstructure is found to be more than twice that previously calculated from annual structures on scales and otoliths. This results in a significant reduction of the expected life span from five to about two years. Investigation of maturity stage at age indicates that Atlantic saury will reproduce for the first time at age one and will survive for one or at most two reproduction seasons. It is concluded that a future Irish fishery will target mostly fish prior to their first reproduction. Finally the thesis gives some insights into the population structure of Atlantic saury, by analysis of otolith morphometric. Significant differences are detected between Northeastern Atlantic and western Mediterranean Sea specimens of the 0+ age class (less than one year old). The implications of these results for the management of an emerging fishery are discussed

# Introduction

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Two thirds of European fish stocks are estimated to be overexploited (FAO 2008). This presents a significant challenge to the commercial fishing industry which generates a significant income through fishing, processing, aquaculture and marketing and employs more than 400.000 people in the European Union (Salz *et al.* 2006).

As traditional commercial species decrease in abundance, their exploitation becomes less economically viable and the demand for fish increases. Consequently, the fishing industry is increasingly turning to novel and emerging species (Salz *et al.* 2006; FAO 2008). Atlantic saury, *Scorpaenopsis scorpaenopsis* (Walbaum) is one of those species.

Atlantic saury is an oily migratory epipelagic fish species, typically oceanic, but that is known to move into coastal waters seasonally. They are one of the most abundant pelagic fish in the temperate areas of the Atlantic Ocean (Sauskan and Semenov 1968). Sauries are common prey items in a wide number of marine predators (Nesterov 1981; Hassani *et al.* 1997; Karakulak *et al.* 2009; Chaput and Hurlbut 2010). There is currently no substantial fishery for saury, but this species has an enormous fishing potential and thus has attracted both fishermen and researchers that see it as an important resource. Saury are considered as a potentially exploitable species by Canadian (Pohle *et al.* 1992; Chaput and Hurlbut 2010) and Irish authorities. Scientists from the former U.S.S.R. undertook extensive research on the species in the northwestern Atlantic and developed a scientific fishery that was abandoned by the mid eighties (Pohle *et al.* 1992). They estimated biomass of about 900,000 tonnes, leading to an annual yield estimate of 350,000 tonnes (Nesterov 1979). No biomass data is available for the northeastern Atlantic. Saury have traditionally been targeted by an artisanal fishing fleet in south Italy (Potoschi 1996) and have also supported a small scale industrial fishery on the Mediterranean coast of southern Spain (Giraldez and Abad 1991) and in the Bay of Biscay with an increase in catches in the last few years (Figure 1). Saury fisheries are seasonal due to the migratory character of the species with the timing of the fishing season varying geographically.

In comparison a very similar species, the Pacific saury, *Cololabis saira* (Brevoort), supports a fishery that ranged in the last 20 years from 180,000 to 470,000 tonnes (FAO Web, <http://www.fao.org/fishery/statistics/global-capture-production/en>).

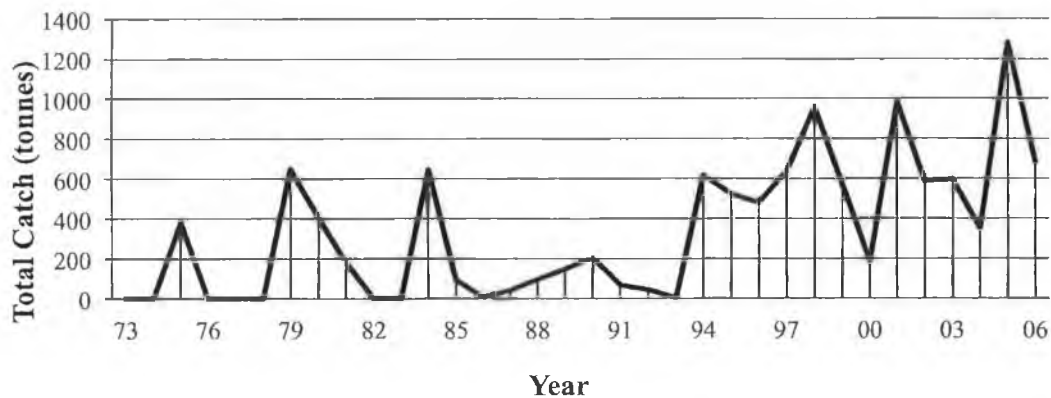


Figure 1. Yearly Catches of Atlantic Saury in northeastern Atlantic and Mediterranean Sea. Data from Italy is not available and thus not included in this figure (<http://www.fao.org/fishery/statistics/global-capture-production/en>)

Similar to the Pacific saury, Atlantic saury is mainly fished for human consumption, fresh and tinned and is also used for bait. Sauries have all the characteristics necessary to be of importance to the food industry, being an oily fish and with a large available biomass to exploit. If large catches are achieved relatively easily it could provide a good alternative or a supplement to other species such as Peruvian anchovies or menhaden for the European fish food industry. To ensure a sustainable exploitation of these fisheries, it is necessary that their development is accompanied by the collection of relevant scientific data on the life history and population dynamics.

The role of Atlantic saury in the ecosystem its importance as a prey of other marine organisms is uncertain. Given its likely importance as a forage species it is important to consider the ecological implications of any exploitation. The collection of sound scientific data on its general biology and ecology is a crucial prerequisite to the development of a commercial fishery on Atlantic saury in the northwestern Atlantic.

Almost all the previous research available on north Atlantic saury is the result of the U.S.S.R. scientific fishery in the 70's and early 80's. They produced a considerable amount of information on age, migrations, behaviour, fisheries, feeding, reproduction, and also a small insight into the population structure. It is mostly focused in the northwestern Atlantic with some references to the northeastern Atlantic (Zilanov and Bogdanov 1968; Dudnik *et al.* 1981). There is little or no information in the literature about the fish that can be found in Irish waters during autumn.

Fisheries research has been greatly advanced by developments in microscopy and computation. The integration of microscopy and computers and the availability of digital image analysis and data processing introduce a new range of possibilities for age and growth estimation, stock identification and assessment of reproduction.

The application of otolith microstructure analysis has improved age estimation particularly in cases where no readable annual structures on otoliths, scales or other hard parts were present (Campana and Neilson 1985) and had obtained results that in some cases have been of vital importance in fisheries management (Campana 1992); while research into population structuring have found in shape analysis a powerful, and of very common use, tool for differentiating stocks (Campana and Casselman 1993).

The present study uses otolith microstructure analysis to give new and reliable data on age and growth of Atlantic saury in the northeastern Atlantic and to calculate growth parameters for the population to the south of Ireland. The study also aims to assess the reproductive state of the fish targeted by the fishery using histological techniques. Spatial and temporal variability in these life history parameters are investigated. Finally, this research aims to give some insight into the population structure of this highly migratory species, and to assess the importance of that knowledge for the sustainable management of an emerging Atlantic saury fishery.



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# Chapter 1:

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## Growth and Age of Atlantic saury, *Scomberesox saurus saurus* (Walbaum), in the Northeastern Atlantic Ocean

A. Agüera and D. Brophy, in review.

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## Introduction

The Atlantic saury, *Scomberesox saurus saurus* (Walbaum), is one of the dominant epipelagic nekton species in the North Atlantic. This species is widely distributed in the subtropical and temperate areas of the north Atlantic including the Mediterranean Sea (Parin 1968; Sauskan and Semenov 1968). It is the object of a small scale fishery in the Bay of Biscay and in the Mediterranean Sea with catches up to 2000 metric tonnes per year<sup>1</sup>. It is also recognized as a potential fishery resource in northwestern Atlantic (Pohle *et al.* 1992; Chaput and Hurlbut 2010).

Previous investigations on Atlantic saury were linked to a scientific fishery carried out by the former USSR in the 70's in the northwestern Atlantic and research surveys carried out in the northeastern Atlantic in the late 60's (Dudnik *et al.*, 1981, Sauskan and Semenov, 1968, Zilanov and Bogdanov, 1968). These studies provided information on distribution, migration patterns, behaviour, growth, feeding and other biological aspects of saury populations in the north Atlantic, with specific focus on the northwestern populations but with some references to the northeastern Atlantic fish. (Dudnik *et al.*, 1981, Nesterov, 1974, Zilanov, 1968, Zilanov, 1970, Zilanov, 1977). Stock structure is still unclear, however previous studies suggest the existence of two different populations in Northeast and Northwest Atlantic (Dudnik *et al.*, 1981), and a separate stock, at least before reproduction, for the Mediterranean Sea (Agüera and Brophy, 2011).

Ageing of Atlantic saury is difficult; annual marks are absent from fin rays and vertebrae (Nesterov 1974); and are also often not visible on scales and otoliths (Sauskan and Semenov 1968). Studies of length distributions for the north Atlantic identified three size classes (Zilanov and Bogdanov 1968); while studies using scales indicated the presence of four age classes for north Atlantic saury (Nesterov 1974; Dudnik *et al.* 1981). However, no age estimation method has been fully validated for the species.

The discovery of daily increments in the otolith of fishes (Panella 1971) has made it possible to estimate age and growth of juvenile fish that do not show annual marks.

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<sup>1</sup> FAO, FISHStat+, Total Fishery Production 1950-2007 Global dataset

Growth increments are bipartite structures, formed due to the differential deposition of calcium carbonate and protein over a 24 hour period (Campana and Neilson 1985). The daily formation of growth increments has been validated in a wide number of species (Geffen 1992).

Of special interest is the use of growth increments in Pacific saury, *Cololabis saira* (Brevoort), another member of the Scomberesocidae family, very similar to Atlantic saury in most biological and ecological aspects (Hubbs and Wisner 1979). It was initially estimated that Pacific sauries could reach five years of age, based on studies of scales, otoliths and length frequencies distributions (Hatanaka 1955; Sunada 1974). Watanabe *et al.* (1988) used daily increments for ageing Pacific saury and presented the first evidence that this species is short lived, a fact that was not clear from examination of length-frequency data (Hatanaka 1955; Sunada 1974), scales or otolith macrostructure (Hotta 1960). Daily deposition of growth increments for Pacific saury was later validated by rearing larvae under laboratory conditions (Watanabe and Kuji 1991). The results of a recent study, in which Pacific saury were reared from eggs to maturation under laboratory conditions, (Nakaya *et al.* 2010) corroborate those obtained by Watanabe *et al.* (1988) for one year old adults. In Pacific saury, the formation of hyaline structures in otoliths has been documented (Suyama *et al.*, 2006, Suyama *et al.*, 1992, Suyama *et al.*, 1996a, Suyama *et al.*, 1996b, Watanabe *et al.*, 1988); they are usually formed when the fish reach maturity, normally towards the end of the first year of life (Nakaya *et al.*, 2010, Suyama *et al.*, 2006) but they are not present in all the otoliths.

There are no published reports of otolith microstructure analysis for Atlantic saury. The apparent absence of annual rings in Atlantic saury otoliths (Sauskan and Semenov 1968; Nesterov 1974), suggests that it may also be a fast growing short lived species. Given that the Atlantic saury is also a nektonic migrating species the possibility that growth rates are faster than previously estimated should be considered.

Furthermore the role of Atlantic saury in the ecosystem is uncertain. Given its likely importance as a forage species it is important to consider the ecological implications of any exploitation. The collection of sound scientific data on its general biology and

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ecology is a crucial prerequisite to the development of a commercial fishery on Atlantic saury in the northeastern Atlantic.

The purpose of this study is to investigate age and growth of Atlantic saury using otolith microstructure. Reliable information on age and growth rates of fish stocks promotes better management and understanding of fish populations (Beamish and McFarlane 1982).

**Materials and Methods**

Samples of saury were obtained from the Bay of Biscay and South Ireland in the years 2008 and 2009; details of sampling and sampling areas are summarized in Figure 1 and Table 1.

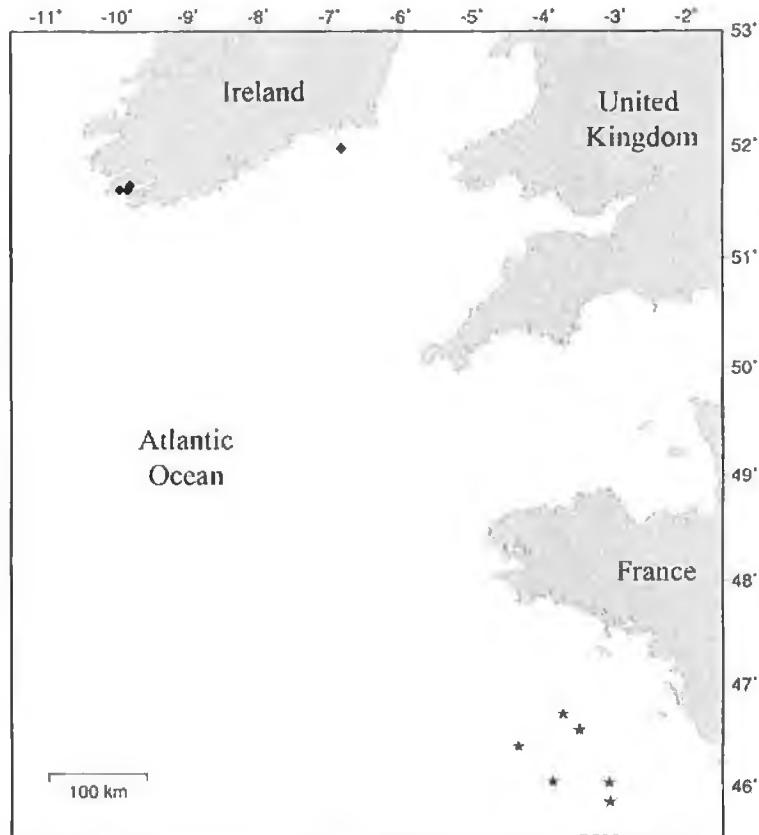


Figure 1. Map showing the sampling area. ♦ South Ireland samples. ★ Bay of Biscay samples.

Samples from Ireland were captured by gill net and small mesh purse seine in fishing trials carried out at Bantry Bay (West Cork, SW Ireland) and by trawling on the RV Celtic Voyager off southeast Ireland coast during a Marine Institute Ireland herring acoustic survey. Those samples were frozen on board and processed later in the laboratory.

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Samples from the Bay of Biscay were captured by small mesh (22mm) purse seine on the anchovy acoustic survey JUVENA'09 using lights. The behaviour of the saury during sampling was noted.

Table 1. Collecting records of saury samples at the northeastern Atlantic. N: specimens in sample, n: otoliths used in this study. G = gill net. T = trawl. S = purse seine. Size range in mm.

Locality	Date	Coordinates		N (n)	Size Range (BS) min-max	Gear
		N	W			
Ireland	16.10.2008	51° 36.6'	009° 58.2'	54 (14)	198 – 280	G
	17.10.2008	51° 58.8'	006° 50.4'	34 (2)	197 – 235	T
	10.09.2009	51° 38.2'	009° 49.3'	27 (8)	187 – 215	S
	29.09.2009	51° 37.5'	009° 48.0'	4 (3)	205 – 231	S
Bay of Biscay	21.09.2009	46° 02.5'	003° 06.5'	22 (5)	150 – 211	S
	21.09.2009	46° 02.9'	003° 53.5'	22 (5)	110 – 188	S
	22.09.2009	46° 24.5'	004° 22.5'	32 (8)	116 – 177	S
	23.09.2009	46° 25.5'	004° 21.5'	5 (1)	120 – 127	S
	24.09.2009	46° 43.1'	003° 44.5'	44 (7)	125 – 205	S
	24.09.2009	46° 33.7'	003° 31.0'	44 (7)	128 – 198	S
	25.09.2009	45° 51.5'	003° 05.6'	14 (6)	161 – 233	S

Samples from the Bay of Biscay were captured by small mesh (22mm) purse seine on the anchovy acoustic survey JUVENA'09 using lights. The behaviour of the saury during sampling was noted.

Captured fish were measured and dissected on board after capture. Because fish usually have damaged beaks (snout, upper and lower jaws) the body size (BS) was measured from the point where the lower jaw begins to elongate into the beak to the posterior end of the muscular knob at the base of the caudal peduncle (Figure 2). Total length from the tip of the snout to the end of the caudal fin was also recorded. Body size as measured here is independent of damaged jaws, and removes the influence of allometric growth of the beak (Hubbs and Wisner, 1979) from growth and condition estimations. In addition



the body size measurement is easier to compare with Pacific saury measurements, as that species is beakless. Precaution is necessary when comparing results with older data as previous studies used total length, different variations of fork length or in some cases did not specify the protocol used to measure the fish. In this study care has been taken when comparing data, and when necessary total length was also included in the comparisons.

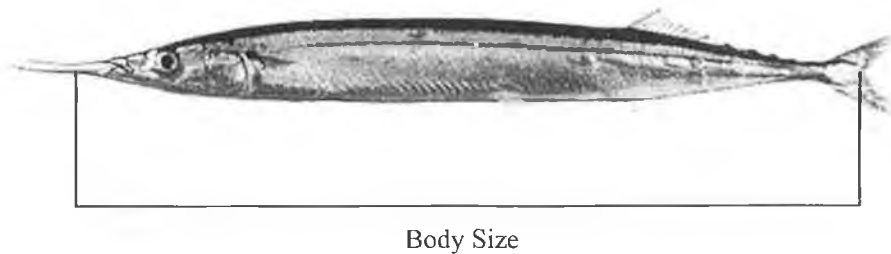


Figure 2. Atlantic saury photograph showing the criteria used for measurements.

Round cycloid scales were taken from the dorsal part of the body of each specimen, according to Nesterov's (1974) description of scales suitable for ageing. Scales were cleaned using a solution of 4% KOH in distilled water, mounted directly between two slides and observed under an Olympus® SZX10 stereomicroscope at a magnification between x2 and x4, to check for the presence of winter rings or annuli.

Sagittal otoliths were dissected from five fish per centimetre length class (68 otoliths in total) and left to dry after removing tissues and membranes. Whole otoliths were observed under an Olympus® SZX10 stereomicroscope to check for the presence of annual rings before processing them for microstructure analysis.

For the observation of microstructure, otoliths were embedded in low viscosity epoxy resin (EPO-THIN®). Both sides of the otoliths were sectioned using Isomet® Low Speed saw with a 0.18 mm thick diamond wafering blade, and polished with wet-dry SiC® sand paper and diamond suspension down to 3µm. A thin section was obtained across the core and parallel to the frontal plane. Otolith microstructure was observed using an Olympus® BX51 compound microscope interfaced with a QImaging-Retiga 2000R digital camera connected to a computer. Images were captured and measurements taken using Image-

Pro® v6.3 software. Otolith increments were clear and easy to interpret according to the criteria given by Campana (1992) and observed increment patterns were similar to previous reports of otolith microstructure in Pacific saury (Nemoto *et al.*, 2001, Watanabe *et al.*, 1988, Watanabe and Kuji, 1991) (Figure 3).

The otolith radius was measured from the core to the posterior edge, and increments were counted following that radius. Growth increments of each otolith were counted three times and an average agreement of 92.6% between counts were achieved. The interpretation and counting was made without any knowledge of length, location and previous counting results of the specimen under observation. Due to the high agreement achieved by a first reader and the clarity of otolith microstructure, no more readers were used.

Number of increments were modelled against body size fitting a Laird-Gompertz curve (Laird *et al.* 1965) and Von Bertalanffy model. The Akaike information criterion (AIC) was used to compare the fit of the models. The Laird-Gompertz curve is well suited to describing larval and juvenile growth and has been previously used to describe growth in Pacific saury (Watanabe *et al.* 1988). Differences in number of increments at body size and growth rates between sampling locations were tested by ANOVA. Growth models were compared between areas following the guidelines given by Ogle (2010) using R v2.10. and the FSA package (Ogle and Spangler 2009). The curves were fit by non-linear least squares method using the FSA package in R v2.10. This method requires the introduction of starting values of the parameters and calculation of new parameters by making consecutive iterations. For the minimum length a value of 6.4 mm was used; this was the minimum size of Atlantic saury larvae captured in the north Atlantic (Nesterov and Shiganova, 1976). The other parameters were taken from the values obtained from Watanabe *et al.* (1988) for the growth of Pacific saury. Separate curves were also fit for each sampling location: South Ireland and Gulf of Biscay, and in the case of South Ireland also for the 2008 and 2009 samples.

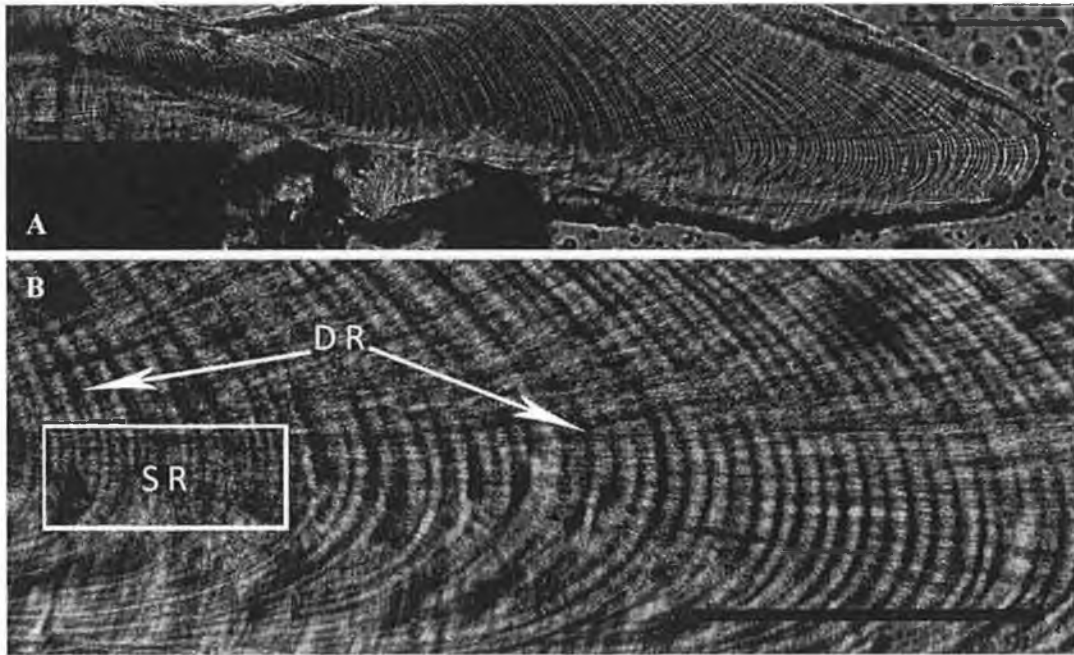


Figure 3. A. Light micrograph of a saury otolith section. Bar = 100 $\mu$ m. B. Detail of microincrements: DR = increments interpreted as deposited daily, SR = subdaily increments Bar = 50  $\mu$ m.

## Results

Observation of mounted scales showed that sclerites are equally spaced, with a slight reduction in the space between sclerites to the edge of the scale. Contrary to the description in Nesterov (1974), no grouping of sclerites which could be interpreted as a winter ring was found.

None of the otoliths used in this study showed a hyaline area and no annual or winter rings were observed.

A number of small dense bodies were observed at the nucleus of most of the otoliths examined (Figure 4A); these appear to be assemblages of calcareous spherules. They are distinct from each other and are usually surrounded by a small concentric ring. In 83% of the otoliths a very distinct ring at about 27  $\mu\text{m}$  from the core centre was detected. Between this ring and the nucleus five or six weak growth rings were detected (Figure 4B).

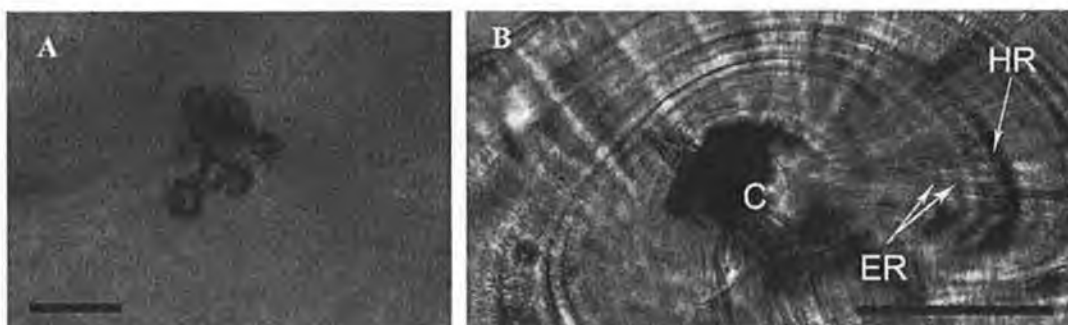


Figure 4. Micrographs of saury otoliths: A. no less than 12 dense bodies forming an otolith core, bar=10 $\mu\text{m}$ . B. frontal plane section through the core, C=core, ER= possible embryonic rings, HR=check identified as hatching ring, bar=25 $\mu\text{m}$ .

The daily periodicity of growth increment formation in the Atlantic saury has not been validated. For that reason the results showed here are labelled as increments and not as days. The average growth rate for northeastern Atlantic saury was 1.04 mm/day, assuming that the rate of increment deposition is daily. The largest specimen examined was captured from the south of Ireland; it measured 280 mm and had 241 increments. The oldest fish, also from the south of Ireland was 230 mm and had 257 increments.

According to AIC the Laird-Gompertz curve attained a slightly better fit than the Von Bertalanffy for Atlantic saury. Only the results by Laird-Gompertz model are presented here.

The resulting growth equation for the northeastern Atlantic saury is (Figure 5):

$$BS=5.7734 \exp \left[ \left( \frac{0.049175}{0.012436} \right) \left( 1 - \exp(-0.012436 (I-5)) \right) \right]$$

Where BS is body size in millimetres and I is the total number of increments observed in an otolith, I-5 indicates that 5 increments (four embryonic and hatching increments) were present at hatching, so these increments were subtracted from the total number of increments counted to derive an estimate total age (I – 5 in above equation).

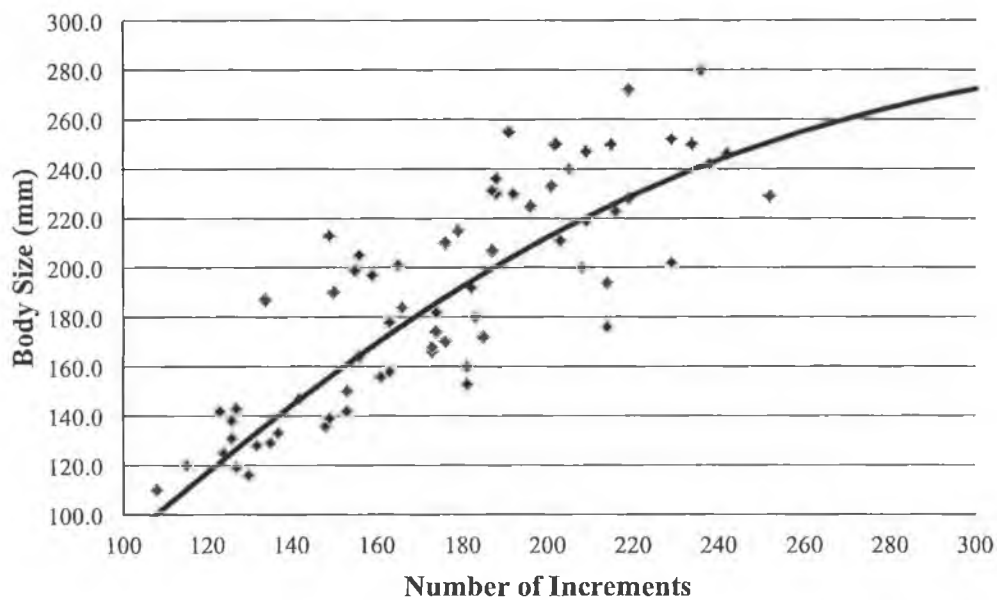


Figure 5. Laird-Gompertz growth curve for Northeastern Atlantic Saury.

When separate Laird-Gompertz curves were fit to the data for each area separately (Figure 6), the size-increment number relationships described by each curve were found to be significantly different (ANOVA,  $F=149.37$ ,  $p$ -value < 0.001). The model fit improved when data for south Ireland and the Bay of Biscay were treated separately

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The spatial variation in growth revealed by the growth model comparison was also apparent when growth rates were compared between locations (ANOVA  $F=41.15$ ,  $p$ -value $<0.001$ ); with average growth rates of 1.13 mm/day for fish from south Ireland and 0.96 mm/day for the Bay of Biscay samples. Significant differences in length were also detected (one way ANOVA  $F=94.93$ ,  $p$ -value  $< 0.001$ ) (Figure 7)

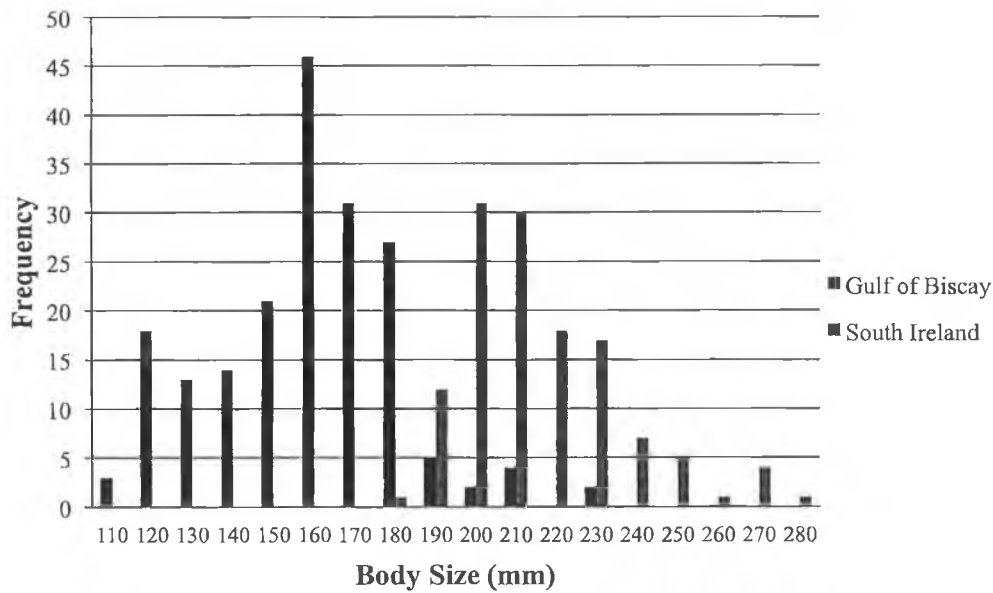


Figure 7. Length frequency distribution by sampling locality

Temporal variation in growth was examined by comparing saury collected in South Ireland during October 2008 and September 2009 were also investigated. Again two curves appeared to fit the data slightly better than one according to the AIC values, and ANOVA results ( $F=4.4726$   $p$ -value= 0.014), however no differences in growth rates (ANOVA  $F=0.00$   $p$ -value=0.956) or increment counts (ANOVA  $F=0.07$ ,  $p$ -value=0.795) were observed.



## Discussion

The microstructure of Atlantic saury saggital otoliths is clear and easy to read as demonstrated by the high level of agreement between counts. Daily rings are regularly spaced and more prominent than subdaily rings which are usually weak or absent in most areas of the otolith (Figure 3B). Examination of the otolith microstructure and the observation of scales and otolith macrostructure suggest that northeast Atlantic saury grow faster than previous studies suggest, reaching a body size of more than 300mm before the end of the first year. The findings of this study are based on the assumption that the observed increments are formed on a daily basis; this has not yet been validated for Atlantic saury.

Otolith growth increments have been shown to be produced at a daily rate in a large number of species (Brothers *et al.* 1976; Campana and Neilson 1985; Geffen 1992; Jones 1992) including Pacific saury (*Cololabis saira*), except during periods of starvation or severe stress (Campana and Neilson 1985). Of special interest are the similarities in the otolith microstructure of Atlantic saury and Pacific saury: the two species share the presence of four embryonic increments and a hatching ring, and the appearance of the daily rings is similar. These structures have all been validated for Pacific saury (Watanabe *et al.* 1988; Watanabe and Kuji 1991; Suyama *et al.* 1992; Suyama *et al.* 1996). It is therefore probable that the increments observed in the otoliths of Atlantic saury are deposited at a daily, or near daily rate. However, further validation is needed, using mark-recapture or laboratory reared fish to confirm this.

Examination of scales and otoliths in this study revealed no annual structure. This is in agreement with the appreciation of Sauskan and Semenov (1968), who noted that annuli in scales are not very easy to interpret and not always present. The absence of annuli from otoliths has also been previously noted (Sauskan and Semenov 1968; Nesterov 1974). It is therefore clear that otolith or scale macrostructures are not useful for age estimation in Atlantic saury in the size range used in this study. However, some previous studies on age and growth of Atlantic saury have been based on observations of annuli on scales (Nesterov 1974; Dudnik *et al.* 1981) and otoliths, in the case of Atlantic saury

from south Italy (Potoschi (1996). Although the fish used in the present study are within the length range used in previous studies, the age estimates obtained differ considerably, with a disparity of 1 to 3 years when compared with Nesterov's (1974) study of saury from the north-east Atlantic. Those works give no reference to any kind of quality test on their age estimation and their claims that saury populations of the northeastern Atlantic are composed of four year classes do not fit with the bimodal length distribution which is typical of Atlantic saury (Zilanov and Bogdanov 1968; Zilanov 1970; Dudnik *et al.* 1981). It is possible that this discrepancy is due to misinterpretation of otolith and scale macrostructure. In any case ageing using otolith and scales macrostructure is at least doubtful and probably subjective.

In this study the average growth rate obtained for Atlantic saury was 1.04 mm/day for fish aged between 4 to 8 months. This is much higher than the growth rate of approximately 0.45mm/day that can be calculated from the length-at-age estimates of Nesterov (1974) obtained from examination of scale annuli in northeast Atlantic saury. A maximum growth rate of 0.33mm/day, 120mm for the first year with a considerable decrease after the third year, can be calculated from observation of otolith annuli reported by Potoschi (1996). Brownell (1983) reared a group of south Atlantic saury (*Scomberesox saurus scombroides*) and obtained growth rates up to 0.75mm/day for the first 45 days of life. In that experiment the fish were exposed to an excess of food, but very high death rates were registered. Despite this, growth rates were considerably higher than those yielded from age estimates derived from counts of otolith and scale annuli. It is possible than when Atlantic saury grow and change their feeding behaviour they can reach higher growth rates. For Pacific saury the growth rate is slower in very young fish and increases with age (Watanabe *et al.* 1988; Nakaya *et al.* 2010) to slow down when they reach a mid size (241-280mm knob length) (Nakaya *et al.* 2010).

To support the results shown here, it is important to compare with the results obtained for Pacific saury, a species that has been very well studied due to its high fishing value. Results from the study of microstructure for Pacific saury showed a fast growth rate of 1.1mm/day (Watanabe *et al.* 1988), which is much higher than growth rates previously estimated from scales, length distributions, otoliths and number of vertebrae (Hotta 1960;



Sunada 1974) of 0.5 to 0.7mm/day for the first year of life. Results from rearing studies suggest that growth rates of young Pacific saury could be more than 1mm/day when food is available (Hotta 1958). New data obtained by Nakaya *et al.* (2010) from laboratory reared larvae agree with the results of Watanabe *et al.* (1988), showing high growth rates for Pacific saury that mature and spawn for the first time in their first year of life.

No fish smaller than 110mm BS (113 increments) or bigger than 280 mm BS (241 increments) was found in our samples. The length distribution of the samples used in this study show two peaks: one around 160mm (fish from the Bay of Biscay) and another one around 210mm (fish from south Ireland). This corresponds to fish with an average of 150 increments and 190 increments respectively. The length of Northeast Atlantic saury varies with latitude, season and year (Zilanov and Bogdanov 1968). The length distribution of the samples used in this study (Figure 7) differs to the reports in the literature in terms of the peaks and intervals (Sauskan and Semenov 1968; Zilanov and Bogdanov 1968; Nesterov 1974; Dudnik *et al.* 1981), however, the observed bimodal distribution is consistent with previous reports.

It is not known how long Atlantic saury survives after becoming adult. The oldest specimen recorded here was about 8 months old (254 increments) and the largest about 280mm (BS) with 241 increments. Atlantic saury is known to grow up to 450mm total length (Hubbs and Wisner 1979) but specimens longer than 400mm total length are not common and are rarely found. The biggest specimen recorded here (about 320mm of total length) is far from the maximum size. With the estimated growth rates, even considering a slowing in growth when the fish reach a mid size length, which happens with Pacific saury (Nakaya *et al.* 2010), Atlantic saury would probably reach that maximum size in a very short time. Therefore the life span of Atlantic saury is probably less than two years.

The formation of a few embryonic rings and a hatching ring (Figure 4B) has been reported previously in several species (Brothers *et al.* 1976; Alemany and Alvarez 1994) including *Cololabis saira* (Watanabe *et al.* 1988; Watanabe and Kuji 1991). There is no clear reason for the formation of embryonic rings; it has been suggested that is related to a long incubation period (Radke and Dean 1982; Watanabe *et al.* 1988), but they are

present also in species with short incubation periods like *Sardina pilchardus* (Walbaum) (Alemany and Alvarez 1994). Atlantic saury like other Scomberesocids such as *Cololabis saira* has a long incubation period of about two weeks at 15 degrees Celsius (C.S. Brownell, *pers. com.*). Therefore it is assumed in this work that the four faint growth increments present between the core and the first check are embryonic rings formed before hatching and the first check immediately after those rings is the hatching ring; similar structures were described and validated for Pacific saury (Watanabe *et al.* 1988; Watanabe and Kuji 1991). However further observation of otoliths from late developmental stage embryos and newly hatched larvae are necessary to validate those structures in Atlantic saury.

The results of this study show significant growth and age differences between fish from the Bay of Biscay and those from the South of Ireland. When comparing sampling localities is important to consider that two of the samples from southern Ireland were taken in a different year and month and showed differences in growth, body size and age. However those differences were not as pronounced as the differences between fish from the South Ireland and fish from the Bay of Biscay. Changes in the length distribution of saury with latitude were noted before by Zilanov and Bogdanov (1968) for the northeastern Atlantic. The Atlantic saury is a migratory fish that undertakes annual migrations from the spawning grounds (southern area of their distribution) to the feeding areas (northern area of their distribution). During September the Atlantic saury is still undergoing this migration or is present in the feeding areas. The physiological state of saury can be determined by the reaction of it to artificial lights, studies show that fish will exhibit a positive response (attraction) to artificial lights when they are migrating, but not when actively feeding (Zilanov and Bogdanov 1968; Zilanov 1977; Dudnik *et al.* 1981) In this study, saury in the Bay of Biscay were strongly attracted by artificial lights using during fishing indicating that they were still undertaking their migration, whereas in the South of Ireland no response (no attraction) or a negative one was observed, saury exhibited a fleeing behaviour, characterised by jumping out of the water when lights were approaching, suggesting that they were actively feeding. The differences between localities show a differential distribution of size and/or age, with older and bigger fish occupying higher latitude areas. Differences in growth rate could arise from the fact that

south Ireland were already at the feeding grounds and have had access higher quality food (Nesterov 1981; Watanabe *et al.* 1988).

In conclusion, the saury population that is available for exploitation in the northeast Atlantic is dominated by fish that are less than one year old. However those results need to be verified by the validation of the daily deposition rate of growth increments in Atlantic saury. The results obtained in Pacific saury microstructure and growth recent studies (Watanabe and Kuji 1991; Nakaya *et al.* 2010) support that the validation of the growth increments by rearing larvae from hatching or by otolith chemical tagging in laboratory conditions are probably reliable methods to achieve the validation of deposition rate for Atlantic saury.

This new data presents a great challenge to management. Short-lived species, specially those that exhibit an annual life cycle, are subject to large fluctuations in abundance and respond rapidly to many factors such as changes in oceanographic conditions, biological interactions, and fishery exploitation (Huang *et al.* 2007; Huang and Smith 2010). Managing those fisheries is difficult given the variability in stock abundance and the uncertainty concerning factors that may affect the future abundance. Atlantic saury stock available for fishing in Ireland and Bay of Biscay could be considered almost entirely composed by one cohort, and depends of a single recruitment every year. Under those circumstances it is necessary for saury fishery to be managed as other short-lived species, with the stock assessed previous to the fishing season and during the fishing season (Pierce and Guerra 1994). That way the fishing pressure will be adapted to the real available stock.

Sauries are essential elements of marine ecosystems. As other small pelagic, they play an important role in connecting lower and upper trophic levels (Cury *et al.* 2000). The effects of its exploitation on the ecosystem will depend mostly on the existence of species that depend on saury as a food source. Atlantic saury have been found in the diet of numerous species, including dolphins (Hassani *et al.* 1997), very valuable fish species such as swordfish (Chancollon *et al.* 2006), Bluefin tuna (Karakulak *et al.* 2009) and birds (Nesterov 1976; Berruti 1988), making up a considerable proportion of the diet of

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gannets (Chaput and Hurlbut 2010). How much these species depend on this food source is not yet known. It is necessary to assess the importance of saury as a forage species, and the impact of a saury fishery on the species that feed on them. Predators of saury should be determined and observed and the fraction of the diet composed of saury be assessed taking into account the seasonal availability of saury for some predators. Observation on predator behaviour toward saury presence in their foraging areas, shoals and saury migrations are also important in assessing the impact of saury fishing on the foodweb.

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## Chapter 2:

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Reproductive Status of the Atlantic saury,  
(*Scomberesox saurus saurus* (Walbaum))  
stock present in South Irish waters during  
autumn.

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## Introduction

The description of reproductive strategies is a fundamental topic in the study of fish biology (Hunter *et al.* 1992). The assessment of parameters such as size at maturity, fecundity, duration of the reproductive season and spawning behaviour permit the quantification of the reproductive capacity of individual fish. Maturity scales are used to classify individuals as mature (fish that certainly will or have spawned) and immature in relation to age or length. Studies of reproductive status increase the knowledge about the state of a stock and improves standard assessment of many commercially valuable fish species (Murua *et al.* 2003).

Atlantic saury, *Scomberesox saurus saurus* (Walbaum), is a short lived pelagic fish that has been reported on numerous occasions in Irish inshore and offshore waters (Gibson 1949; Wheeler and Mistakidis 1960; Quigley 1986). This species has been identified as a potentially exploitable fishing resource by Irish Sea Fisheries Board. There is very little published on northeastern Atlantic saury and no reference at all to their reproductive status in Irish waters.

Atlantic saury is a batch spawner, spawning several times during the reproductive season. They exhibit an asynchronous ovarian development where reproductive cells in different developmental stage are present at the same time, with the more mature ones determining the reproductive stage of the animal; their fecundity is indeterminate as it cannot be estimated from a single observation of the ovaries previous to the reproduction season (Nesterov 1973; Dudnik *et al.* 1981; Korkosh and Timokhina 1983). In the North Atlantic Ocean they spawn in the southern area of their distribution range (Nesterov and Shiganova 1976; Dudnik *et al.* 1981) between the 16.8C and 23.7C isotherms. Also it is known to spawn in the central Mediterranean Sea (Potoschi 1996). The reproductive period is extended all year long in the spawning areas, with characteristic spawning peaks at different locations (Table 1).

Table 1. Reproductive Periods per area

Reproductive Period	Area	Authors
Winter – Spring	North Atlantic	Nesterov <i>et al.</i> 1976
Winter – Spring	North East Atlantic	Dudnik <i>et al.</i> 1981
November – February	Central Mediterranean	Potoschi 1996

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in South Irish Waters during Autumn*

The objective of this study is to describe the maturity stage of the stock available for exploitation in Irish waters during the autumn months. This data will help the management of the stock and assessment of a seasonal fishery based on this migratory species during its transit through Irish waters.

## Material & Methods

Atlantic saury specimens were captured during September – October 2008 and 2009 during targeted fishing trials and during the 2008 Marine Institute herring survey in the Celtic Sea. Details of sampling are summarised in Figure 1 and Table 2.



Figure 1. Sampling locations

Table 2. Sampling details.

Date	Coordinates		No. of fish
	N	W	
16.10.2008	51° 36.6'	9° 58.2'	32
17.10.2008	51° 58.8'	6° 50.4'	34
10.09.2009	51° 38.2'	9° 49.3'	26
29.09.2009	51° 37.5'	9° 48.0'	4

Saury specimens caught during the south Ireland fishing trials were processed immediately after catching, while specimens captured during the Herring survey were

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frozen on board and processed later in the laboratory. A total of 96 specimens were used in this work.

Fish were measured for body size and total length. Total weight and gutted weight were also recorded. Otoliths were dissected and cleaned prior to dry storing. Gonads were dissected and fixed in 4% formaldehyde. When possible sex was determined by visual examination of the gonads. Preserved gonads were weighed using a precision weighing scale. To correct for changes in weight during fixation, weights were multiplied by 0.96 according to the relationship calculated by Suyama *et al.* (1996).

Gonadosomatic Index (GSI) was calculated as follows:

$$\text{GSI} = \frac{\text{Gonad weight (g.)}}{\text{Total weight (g.)}} \times 100$$

Fulton's condition index (FCI) was calculated as follows:

$$\text{FCI} = \frac{\text{Total weight (g.)}}{\text{Body size}^3} \times 10^3$$

The gonads of all specimens were then processed for sex and stage determination. A piece was dissected from the centre of each gonad, as this is where the most developed cells are more abundant and therefore easier to observe (Nesterov 1973). The gonad pieces were processed prior to wax embedding using a RV6/1 intelsint vacuum tissue processor and the standard protocol. Six micrometer sections were obtained using a SLEE@CUT6062 microtome. Sections were mounted on microscope slides and stained with Harris hematoxylin and yellow eosin in a Leica ST4040 linear stainer according to the manufacturer's standard protocol.

The histological gonad preparations were examined under a light microscopic. The sex of each individual was determined and for the females, the stage of reproductive development was assessed according to the six point maturity scale determined by Dudnik *et al.* (1981), with aid of the figures presented by Korkosh and Timokina (1983) and Suyama *et al.* (1996) for south Pacific Atlantic saury (*Scomberesox saurus scombroides* (Richardson)) and Pacific saury (*Cololabis saira* (Brevoort)) respectively. Stages were assigned based on the appearance of the most developed cells observed in the section.

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Females body size, GSI and condition index were compared by maturity stage and also by sampling months. Also maturity stage distribution was compared by sampling months.

A total of 12 (23%) otoliths from the female fish (six for each maturity stage) were used for ageing the fish using daily increments. The otoliths were processed and aged using the same protocol explained by Agüera & Brophy (under review).



## Results

The sex ratio of the samples examined was 1.13 (female/male); of the 96 fish, a total of 51 were females of maturation stages II and III. In stage II females the most developed cells are in the protoplasmic growth phase with a maximum size ranging from 140 to 170  $\mu\text{m}$  (Figure 2A and 2B). Females at stage III have bigger oocytes that are starting to accumulate yolk (yolk vesicle stage; Figures 2C and 2D).

No significant difference in body size between sexes were found (one way ANOVA  $F=0.55$   $p$ -value = 0.577).

Table 3. Summary of the biological information recorded for Atlantic saury in this study.

	Stage II	Stage III
No. Individuals	32	19
Body Size (cm)	19.0 – 22.8	21.2 – 27.5
Total length (cm)	21.7 – 26.5	24.5 – 32.0
Total Weight (g)	37.20 $\pm$ 1.04*	56.31 $\pm$ 2.84*
Gonad Weight	0.061 $\pm$ 0.001*	0.124 $\pm$ 0.012*
GSI	0.159 $\pm$ 0.001*	0.219 $\pm$ 0.017*
FCI	3.611 $\pm$ 0.052*	3.733 $\pm$ 0.052*
Age (days)	188 $\pm$ 24*	210 $\pm$ 14*

\*mean  $\pm$  standard deviation.

There is a significant difference in GSI between maturity stages (Figure 4), as expected (one way ANOVA:  $F=12.15$   $p$ -value=0.001). The fish in stage III have a slightly higher mean FCI but the differences are not significant (one way ANOVA:  $F=2.45$   $p$ -value = 0.124).

GSI is directly correlated with body size (Pearson's  $\rho = 0.512$   $p$ -value < 0.001;  $R^2=24.6\%$ ) (Figure 5) while FCI is completely independent (Pearson's  $\rho = 0.089$   $p$ -value = 0.533;  $R^2 < 0.01\%$ ).

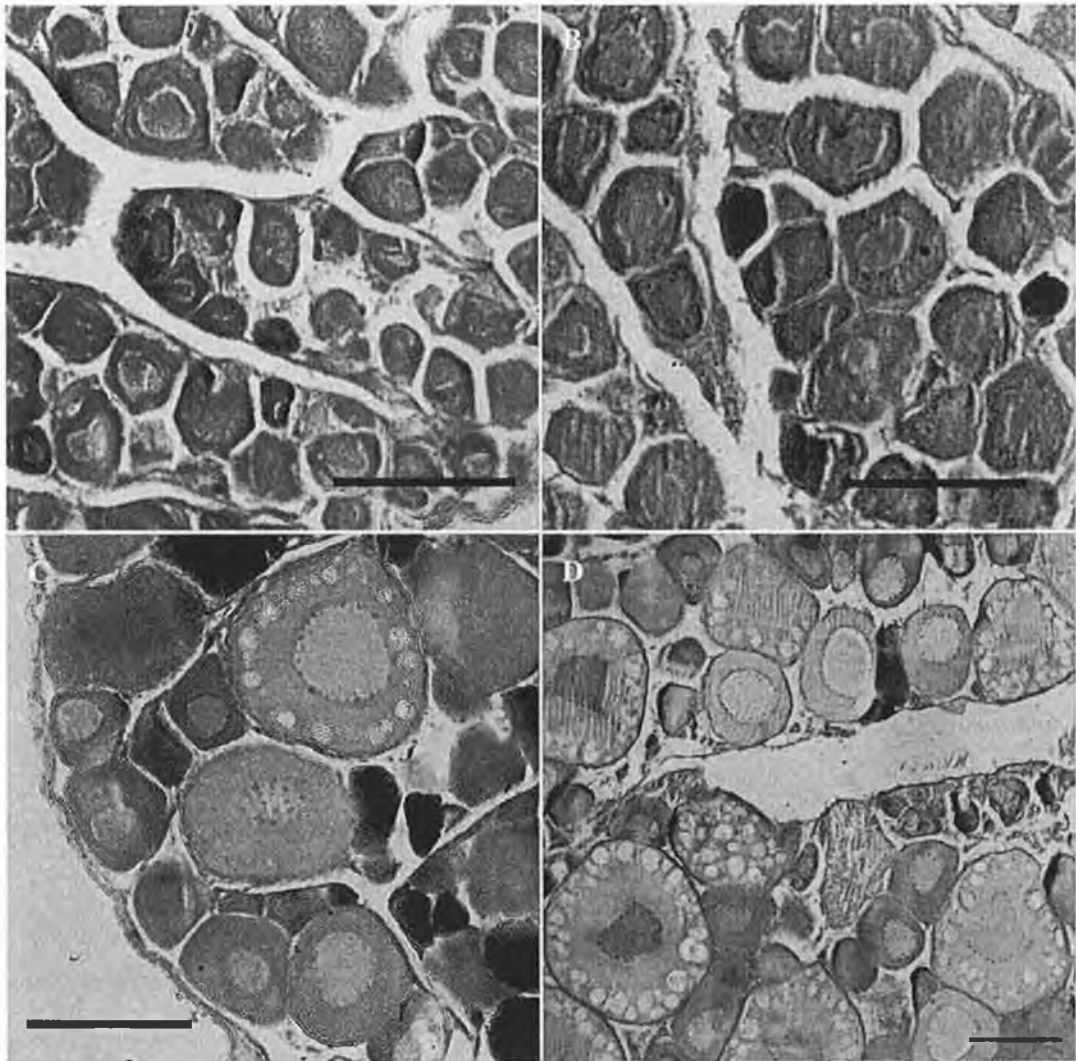


Figure 2. Histological preparation of females gonads. A. Early stage II female; B. Late stage II; C. Early stage III (early yolk vesicle stage); D. Late stage III (late yolk vesicle stage). Black bars = 150 $\mu$ m

GSI and FCI were found to be independent (Pearson's  $\rho = 0.191$   $p$ -value = 0.198;  $R^2=1.50\%$ ).

Significant differences in body length (one way ANOVA  $F=24.44$   $p$ -value < 0.001), GSI (one way ANOVA  $F=13.45$   $p$ -value = 0.001) and FCI (one way ANOVA  $F=8.82$   $p$ -value = 0.005) were found between sampling months (September and October) (Figures 6 to 8), with all parameters increasing between September and October.

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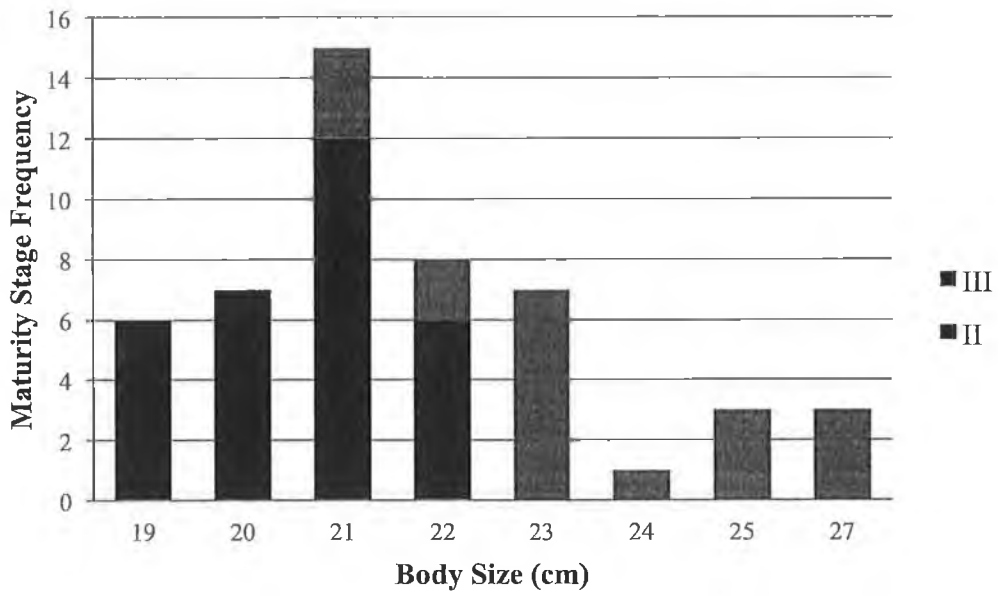


Figure 3. Occurrence of maturity stages per body size (cm) class

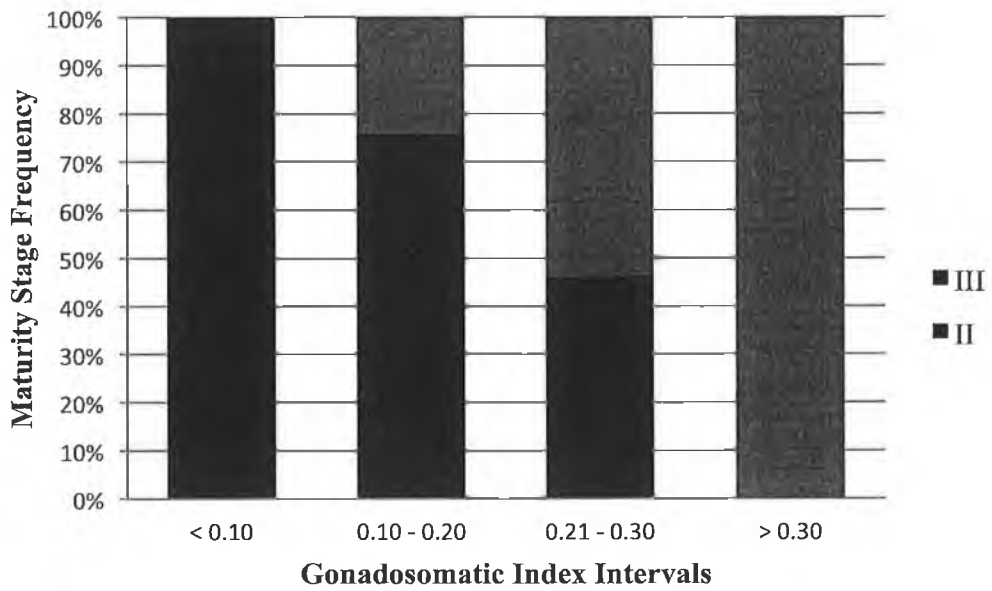


Figure 4. Occurrence of maturity stages in relation to gonadosomatic index intervals

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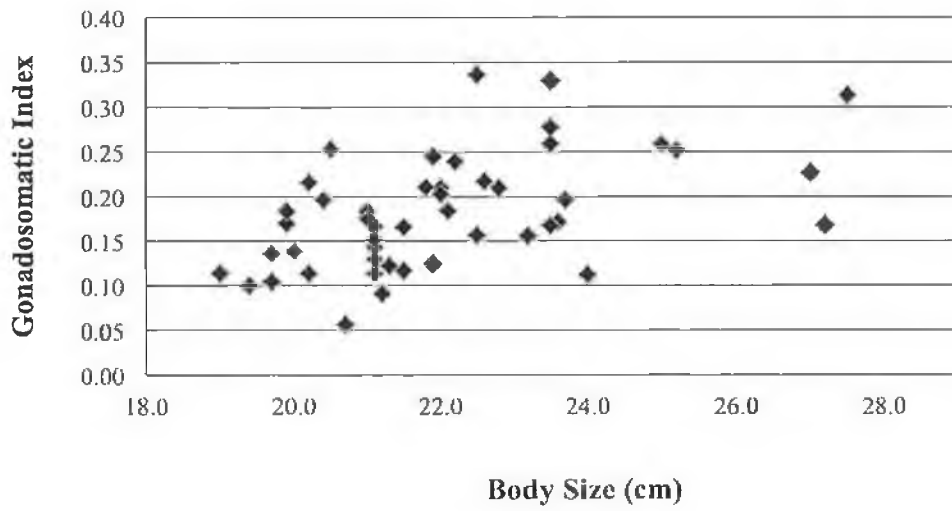


Figure 5. Gonadosomatic Index (GSI) at Body size (cm)

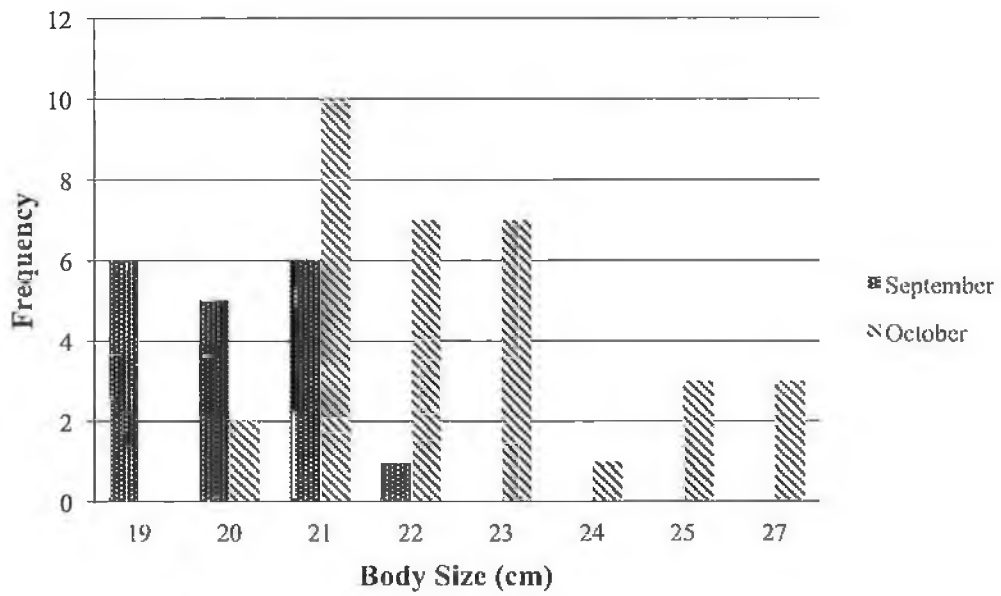


Figure 6. Body sizes frequency distribution for each month of sampling.

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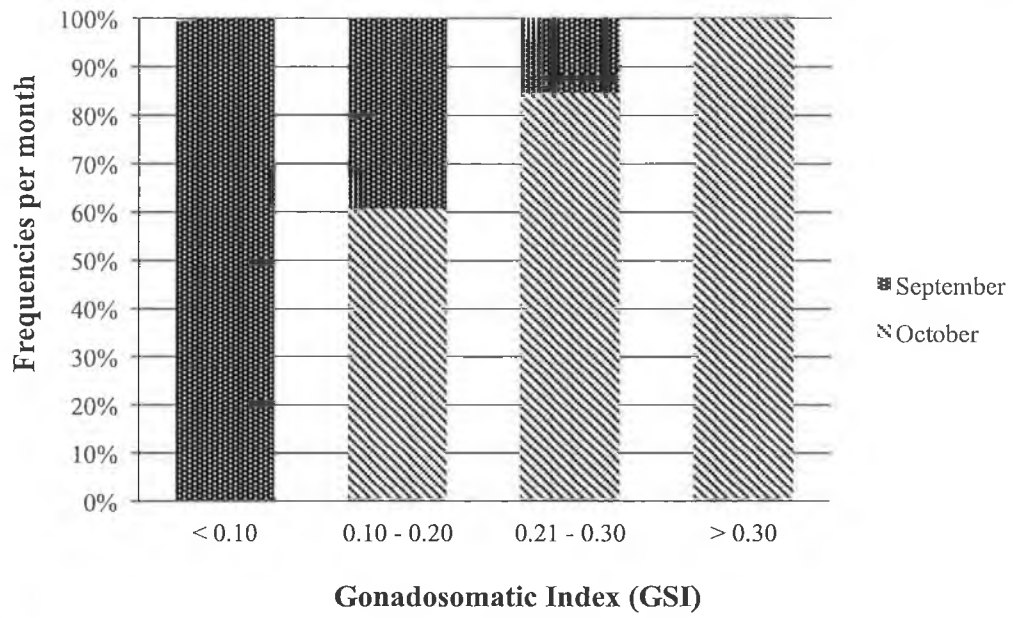


Figure 7. Gonadosomatic index occurrence by month

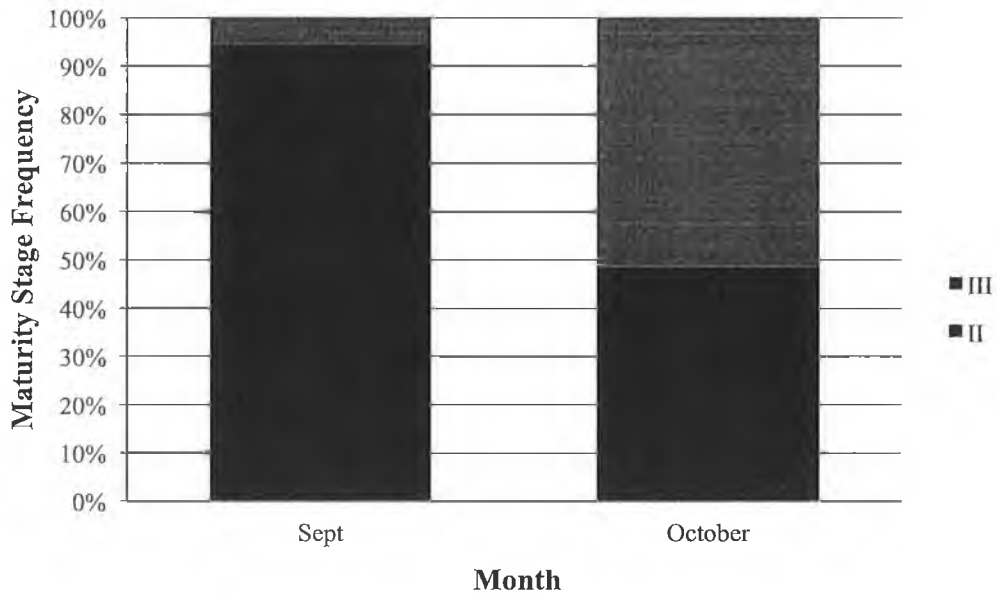


Figure 8. Maturity Stages occurrence by month

## Discussion

This is the first study to present data on the length distribution and reproductive status of Atlantic saury in waters to the North of 45°N. The results show that the bulk of the stock present in Southern Ireland coastal waters during autumn is composed of maturing (Stage II) and early mature (Stage III) fish that have not yet reproduced for the first time. There is a complete absence of ripe (just ready to spawn) or spent (have recently spawned) fish. This is consistent with previous reports of spawning sauries distributed between the surface isotherms of 17 C and 19 C and south of 46° 30'N in September (Zilanov and Bogdanov 1968). However, the possibility that Atlantic saury at a more advanced stage of reproductive development do occur in the waters to the south of Ireland cannot be ruled out as the distribution of spawning saury may have shifted since the 1960's and could be subject to climatic shifts and annual variability in temperature.

Atlantic saury is considered to be mature at stage III (Zilanov and Bogdanov 1968; Korkosh and Timokhina 1983; Guillén 1991) when oocytes are starting to accumulate yolk. The evidence presented here indicates that saury in south Irish waters mature at a minimum total length of 24.5 cm (body size 21.2 cm). This is within the range of the size at maturity reported in the literature of between 23 cm total length for the northeastern Atlantic (Zilanov and Bogdanov 1968) and 28 cm total length for the whole north Atlantic (Zilanov 1970). Between 26 and 27 cm total length (22 to 23 cm body size (Figure 3)) at least the fifty per cent of saury is already mature (L50), in agreement with the lengths given by Dudnik *et al.* (1981) for the northwestern Atlantic and Potoschi (1996) for the central Mediterranean Sea, and close to the 29 cm total length from Zilanov and Bogdanov (1968) for northeastern Atlantic saury.

The difference in maturity stage composition, GSI, length distribution and age between September and October indicate that the reproductive development is progressing during this time. When these results are considered in light of the age estimates from otolith microstructure presented in chapter one, new and important information is revealed; sauries have reached 26 cm and are already mature when they are between 6 and 7 months old, this is much earlier than the estimate of 2 years given by Dudnik *et al.* (1981) and Potoshi (1996). The gonads of Atlantic saury develop while they are at the feeding grounds and the fish start their migration to the spawning areas when they have reached stage III (Zilanov and Bogdanov 1968) and are at least 26 cms in length

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(Dudnik *et al.* 1981). Given their size and stage of reproductive development in autumn, it is likely that the fish that migrate to the spawning areas from southern Ireland, complete their reproductive development and spawn within their first year.

There are few reports in the literature of large fish which are likely to have spawned in the previous spawning season (Zilanov 1970; Dudnik *et al.* 1981). It has been suggested that a considerable number of fish die after their first spawning (Sauskan and Semenov 1968), this is supported by the sharp increment in natural mortality that occurs between fish of 29 and 33 cm total length, as calculated by Dudnik *et al.* (1981). Given that fish are maturing in their first year, it is likely that the bulk of the population of Atlantic saury in the northeastern Atlantic completes the cycle of life between one and two years, with very few fish reaching or surpassing the age of two years old.

It is important to consider that any fishery for saury within the study area would target an annual species with low fecundity in the period prior to spawning (Nesterov 1973; Korkosh and Timokhina 1983; Pohle *et al.* 1992), that may also play an important role as a forage species for many marine predators. The biomass of saury available for exploitation will therefore depend directly on the recruitment success of a single year class. Special care should be taken in the management of any fishery for this species to avoid a recruitment failure and overexploitation that could provoke a collapse of the fishery with as yet unknown effects on interacting species and the entire ecosystem.

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## Chapter 3:

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Use of saggital otoliths shape analysis to  
discriminate Northeast Atlantic and  
Western Mediterranean stocks of Atlantic  
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## Introduction

Sauries are oceanic epipelagic planktivorous. The Atlantic saury, *Scomberesox saurus saurus* (Walbaum), inhabits the North Atlantic and Mediterranean Sea (Sauskan and Semenov 1968). At least part of the population undertakes seasonal migrations (Dudnik *et al.* 1981). Saury migrates for feeding purposes to accumulate reserves (Nesterov 1981), probably for reproduction, which takes place after the migration back from the feeding grounds (Dudnik *et al.* 1981). Atlantic saury has a protracted spawning period, reproducing throughout the year with peaks during winter in the Mediterranean (Potoschi 1996) and winter and spring in the Atlantic (Nesterov and Shiganova 1976). Also, at least in the Atlantic, the spawning ground expands to lower latitudes as water temperatures increase (Nesterov and Shiganova 1976) with spawning taking place along the migration route. Sauries are serial spawners meaning that one female will probably reproduce in different localities as the area suitable for spawning expands. Atlantic saury is a very fast growing, short lived (Agüera & Brophy, in review) species which supports a small scale traditional fishery in southern Italy (Potoschi 1996), as well as a seasonal fishery in south (Mediterranean) (Abad and Giraldez 1990; Giraldez and Abad 1991) and north (Atlantic) Spain. It has been identified as a potentially exploitable species in Ireland (Rihan and Tan 2010) and Canada (Pohle *et al.* 1992; Chaput and Hurlbut 2010).

Most population models assume homogeneity of vital rates and closed life cycles within a stock with young fish produced by previous generations from the same group (Cadrin *et al.* 2005). Stock identification is of crucial importance in modern fisheries stock assessment and also for understanding the population dynamics of a species in an ecological sense. Stock structure of north Atlantic saury is not clear. It has been generally assumed that there are two different stocks in the northeast and northwest Atlantic (Pohle *et al.* 1992). There may be more complexity than this simple two-stock model. Nesterov (1974) suggested that spring and autumn spawned fish can be differentiated based on the observation of different distances between scale annuli in fish from both northwestern and northeastern Atlantic. However, studies carried out by Nesterov (1982) based on the distribution of saury during migrations and a study on morphological differences concluded that saury in the northwestern Atlantic can be considered as a single population. There are no references in the literature to saury that

inhabit the Mediterranean Sea and it has not yet been established whether this forms part of the northeastern Atlantic population, or is a distinct stock.

The Mediterranean Sea is almost closed, with well-defined limits and with physicochemical characteristics very distinct from those in the adjacent Atlantic. The Almeria-Oran front (AOF), which is the phylogeographical break between the Atlantic and Mediterranean (Patarnello *et al.* 2007), is an oceanographic front generated from Almeria to Oran, resulting from the particular water circulation of the Alboran Sea. The OAF exhibits a pronounced step in temperature (1.4 C) and salinity (2 psu) (Tintore *et al.* 1988). Temperature drops at sea fronts have proven to be effective barriers for Atlantic saury, an advantage that has been used in the fishing trials by the USSR in the northwestern Atlantic (Dudnik *et al.* 1981). It is therefore possible that the AOF acts as barrier to Atlantic saury, preventing, at least to some degree, migrations between the Atlantic and the Mediterranean. The Mediterranean Sea is an oligotrophic sea, however there are areas with substantial organic production (e.g. the Catalan coast, northern Aegean sea and Sicily Straits) (Panardi *et al.* 2006), those areas within the Mediterranean Sea could support saury during the feeding season; therefore the complete life cycle of saury could take place in the Mediterranean Sea without any mixing of the Atlantic and Mediterranean stocks.

There are many techniques appropriate for studying stock structure. Geometric morphometrics is one of them. Geometric outline methods quantify boundary shapes so that patterns of shape variation within and among groups can be evaluated (Cadrin 2005; Cadrin and Friedland 2005). The application of this technique to otolith analysis is an important tool in the study of fish populations. Otoliths are recorders of growth and their structure and development are influenced by external environmental conditions as well as the physiological state of individual fish (Campana and Neilson 1985); these characteristics can vary between populations, therefore otoliths may show characteristics that are stock specific. Variation in otolith shape is widely used for discriminating between fish stocks (Bird *et al.* 1986; Campana and Casselman 1993; Torres *et al.* 2000; DeVries *et al.* 2002; Tracey *et al.* 2006; Mériqot *et al.* 2007; Burke *et al.* 2008; Duarte-Neto *et al.* 2008). A few studies have used otolith shape to successfully differentiate between Atlantic and Mediterranean fish; Tuset *et al.* (2003) discriminated between Atlantic and Mediterranean stocks of the comber (*Serranus cabrilla*) while Stransky *et al.* (2008) separated Mediterranean and Atlantic stocks of

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horse mackerel (*Trachurus trachurus*), although these are not genetically isolated stocks (Abaunza *et al.* 2008).

The aim of this study was to analyse otolith shape variability in Atlantic saury from the north east Atlantic and Mediterranean Sea, and to examine the potential use of otolith shape to elucidate stock structure.

## Material and Methods

### 1. Sampling

Samples of saury were obtained from south west Ireland, Bay of Biscay and south east Spain during the year 2009. Sampling details are summarized in Table 1 and Figure 1. Saury samples used in this study were caught in different seasons. Information from otolith microstructure showed that they also hatched in different seasons (Agüera & Brophy, in review): spring in the case of the Atlantic samples and autumn in the case of the Mediterranean samples. All the samples were taken using purse seine nets of 22 mm mesh. In the Bay of Biscay and the Mediterranean, fish were concentrated and attracted to the fishing vessel using lights. Atlantic samples were taken in different nights during the month of September. A single sample was collected from the Mediterranean during one night.

Table 1 Summary of sample data.

Region	Location	Date	n <sup>a</sup>	Body size range	Mean Body size	SD
North East Atlantic	47° 17.5'N 2° 32.4'W	September, 2009	5	160 – 233	190.6	19.27
	46° 02.5'N 3° 06.5'W					
	46° 24.2'N 4° 22.5'W					
	46° 43.1'N 3° 44.5'W					
	46° 33.8'N 3° 31.0'W					
	45° 51.5'N 3° 05.7'W					
	51° 39.0'N 9° 49.2'W					
51° 36.6'N 9° 51.0'W						
West Mediterranean	Off coast Mazarrón (Murcia)	March, 2009	4	165 – 242	194.9	16.99
			6			

<sup>a</sup> number of individuals from each region used in the study

Body size (BS) was measured to the nearest millimetre from the point where the lower jaw begins to elongate into the beak to the posterior end of the muscular knob at the base of the caudal peduncle. The weight of each fish was recorded to the nearest 0.1 g. Saggital otoliths were removed, cleaned of membranes and tissues and stored dry. Only left otoliths that did not show any clear distortion or damage were used. Differences in shape between left and right otoliths have been previously reported (Potoschi 1996), so when the left otolith was damaged or not present the specimen was discarded. A total of

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98 left otoliths were used in this study, 52 from the Atlantic and 46 from the Mediterranean. According to results from otolith increment analysis all the specimens included in this study are in the 0+ age class (i.e. less than one year old) (Agüera & Brophy, in review).

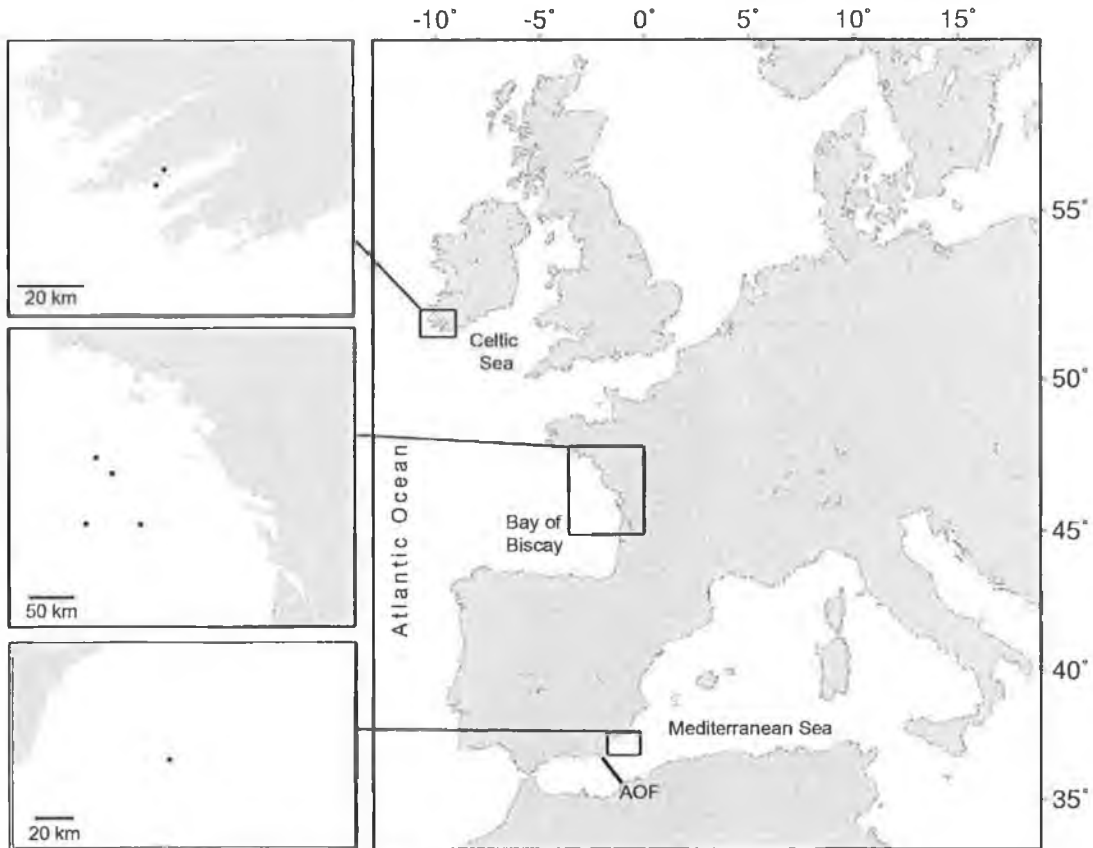


Figure 1. Sampling locations (●). AOF, Almeria-Oran Front.

*2. Image acquisition and feature extraction*

The otoliths were photographed using a QIMAGE Retiga 2000R digital camera attached to an Olympus SZX10 Stereomicroscope. The magnification was kept constant at 4x for all the otoliths photographed. The images were captured and segmented: binarising and partitioning the picture into areas that are easier to analyse, in this case with the objective of differentiating the otolith from the background. Image-Pro v6.3. software was used for segmentation.

Measurements of each otolith as well as the resulting size based shape indices (Table 2) were extracted and calculated using Matlab v2009b and Matlab Image Processing Toolbox function RegionProps on the segmented images. Area (A) is the total number

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of white pixels in the binary otolith image; perimeter (P) is the number of pixels in a 1 pixel wide outline enclosing the white area. Otolith length (OL) and otolith width (OW) are measure as the major axis and minor axis, respectively, of an ellipse with the same normalised second moment as the otolith outline (Gonzalez *et al.* 2004). The use of a constant amplification and image acquisition set-up make it possible to use pixels as measurement units; this avoids the introduction of extra error due to the transformation from pixels to another measurement unit.

Table 1 Size parameters and size based shape indices with calculation formulas

Size parameters	Size base shape indices
Area (A)	Circularity (Cir) = $P/A^2$
Perimeter (P)	Rectangularity (Rec) = $A/(OL*OW)$
Otolith Length (OL)	Form-Factor (FF) = $(4\pi A)/P^2$
Otolith Width (OW)	Roundness (Rnd) = $(4A)/(\pi OL^2)$
	Ellipticity (Ell) = $(OL-OW)/(OL+OW)$
	Aspect Ratio (AR) = $OL/OW$

Ten elliptic Fourier harmonics were calculated using the software SHAPE v1.3. (Iwata and Ukai 2002). Elliptic Fourier Descriptors (EFDs) can delineate any type of shape with a closed two-dimensional contour (Kuhl and Giardina 1982). They use an orthogonal decomposition of a curve into a sum of harmonically related ellipses that can be combined to reconstruct an approximation of the closed curve. EFDs have been effectively applied to the analysis of various biological shapes in animals (Iwata and Ukai 2002), including otoliths (Tracey *et al.* 2006; Burke *et al.* 2008; Duarte-Neto *et al.* 2008). The elliptic method is particularly useful for describing very convoluted or complex shapes that are mathematically impossible for traditional Fourier components (Tracey *et al.* 2006).

Each harmonic is composed of four coefficients, resulting in 40 elliptic Fourier coefficients (eFcs) per individual. Using the same software package each otolith was normalised for size and orientation, causing the degeneration of the first three eFcs of each individual:  $a_1=1$ ,  $b_1=c_1=0$ . As a result each individual was represented by 37 eFcs in the shape analysis.



### 3. Statistical Analysis

A Kolmogorov-Smirnov two sample test was performed to determine if the body size distribution of both localities were significantly different; if present, such differences could introduce bias to the analysis of otolith shape variation due to ontogenic allometry (Simoneau and Casselman 2000; Monteiro *et al.* 2005; Vignon and Morat 2010) Fulton's condition index (FCI) was calculated for each individual and was tested for significant difference between localities.

Shape indices were tested for normality using the Anderson-Darling test (Stephens 1974) and for homogeneity of the variance using Levene's test.

ANCOVAs were used to determine the effect of size (otolith length and body size) on the magnitude of each shape index and to examine regional variability in these relationships. If the 'region\*fish size' interaction term in the ANCOVA was significant, showing that the slope of the size/shape relationship was not consistent between areas, the shape variable in question was excluded from subsequent analysis. Variables that were significantly correlated with size and showed no regional heterogeneity in the size/shape relationship were standardised using the common within-group slope (Tracey *et al.* 2006; Burke *et al.* 2008). Elliptic Fourier coefficients have been already standardised using the software SHAPE v1.3, however they were also tested for correlation with otolith length.

One-way univariate ANOVAs were used to quantify shape variability within and between stocks. Each of the shape indices and eFcs were compared between two areas in the Atlantic, south west Ireland and Bay of Biscay (within-stock) and between the Mediterranean and Atlantic (between-stock). As the Mediterranean was sampled on just one occasion it was not possible to explicitly measure within stock variability for this region. When a variable showed significant within-stock variability, and when the magnitude of this difference was higher than the between-stock variability (as indicated by the R-squared values of the ANOVAs) it was omitted from subsequent analyses.

Principal component analysis (PCA) was used to summarise the variance in otolith shape captured by the shape indices and eFcs. This method has a number of advantages; PCs describe variance in decreasing order, minimising the risk of leaving out an important variable, effectively reducing the number of variables and simplifying

the statistical analysis while summarising the information of the variability contained in the coefficients (Rohlf and Archie 1984). It also avoids problems associated with colinearity in the descriptor variables as the resulting principal components are orthogonal to each other. Other methods such as using only low order harmonics (Campana and Casselman 1993) or using only the harmonics that show significant difference between groups being compared were not used. The former approach could leave out important harmonics whose variability explains significant difference between the groups (Duarte-Neto et al 2008). Burke et al (2008) found significant differences in otolith shape between herring groups based on three high order harmonics. The later approach provides no means of simplifying the analysis when a large number of variables show significant differences, and could also lead to the omission of variables that show only small differences between groups but are still useful for discrimination.

The first PCA was carried out using all the shape indices. This PCA was based on the correlation matrix from the standardised variables; this made the variables independent of the order of magnitude and the scale of the measurements (Torres *et al.* 2000). A second PCA was carried out using the eFc's; this was based on the variance-covariance matrix because coefficients with small variance and covariance values in the matrix are generally not important for explaining morphological variations (Iwata and Ukai 2002). The principal components (PCs) to be used in subsequent analysis were chosen based on the observation of the scree plots (eigenvalues plotted against each principal component, arranged in order of percentage explained variance), excluding the components after the point where the eigenvalues stabilise.

After the generation of the principal components, discriminant function analysis (DFA) was used to discriminate between Atlantic and Mediterranean specimens. Three analyses were performed: one using only the previously selected PCs from the analysis of the shape indices, another using only the selected PCs from the analysis of the eFc's and the last one combining both, DFAs were done using SYSTAT v11 for Windows. Prior to inclusion in the DFA all the variables were tested for normality using the Anderson-Darling test and for homogeneity of the covariance matrices using Box's *M* tests (Norman and Streiner 2008) with the software package PAST v1.99 (Hammer *et al.* 2001). The choice of discriminant function depended on the outcome of this test; linear if the covariance matrices were homogeneous and quadratic in the case that this assumption was not met. Using the software PAST v1.99, one way non-parametric

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MANOVA (Anderson 2001) was applied to test for significant differences between localities in each case, using Euclidean distance and 10,000 permutations.

Except where stated otherwise, statistical tests were performed using Minitab v15.0 for windows.

## Results

No significant differences in body size (ANOVA  $F=1.42$ ,  $P=0.238$ ) and body size distribution between localities were found (Kolmogorov-Smirnov  $P=0.495$ ). There was a significant difference in Fulton's K between localities (ANOVA  $F=19.29$   $P<0.001$ ), with fish from the Mediterranean being in slightly better condition (average 3.86) than fish from the Atlantic (average 3.51).

Form Factor (FF) data were not normally distributed (Anderson-Darling test  $P<0.01$ ), and log transformation of the data did not achieve normality. As FF was highly correlated with the other size variables it was discarded from subsequent analyses. All the other shape indices followed a normal distribution and showed homogeneity of the variance. Univariate ANOVAs revealed significant within-stock variability for Circularity (C) which outweighed any variability between stocks. Therefore this variable was omitted from further analysis.

ANCOVA showed that none of the shape indices or the eFcs was correlated with body size. However, all shape indices with the exception of rectangularity ( $P=0.069$ ) were strongly correlated with otolith length ( $P<0.001$ ). There were no significant interactions between region and otolith length so all shape indices, including rectangularity were standardised using the common within-group slope from the ANCOVA (Table 3) to remove correlations with otolith length. After standardisation for size using the SHAPE software, eFcs were also tested for correlations with otolith size; none of them show a significant correlation. Univariate ANOVAs revealed greater variability within stocks than between stocks for nine eFcs (2.4, 3.3, 4.4, 5.3, 6.1, 7.3, 8.4, 9.3 and 10.3). Therefore, these variables were not used in further analysis

### *1. Shape Indices statistical analysis*

The scree plot of the eigenvalues of the six principal components calculated from the shape indices show the two first principal components explain 99% of the variance, justifying their inclusion in subsequent analysis. Box's  $M$  test ( $P=0.82$ ) showed that the covariance matrices are homogenous, therefore a linear discriminant function was used in the DFA. A mean classification success rate of 66% was achieved after jack-knife cross-validation. Non-parametric MANOVA showed a significant difference between the Mediterranean and Atlantic otoliths ( $P=0.001$ ) (Table 4). PC1, which primarily

expresses variability in AR, Ell and Rnd made the largest contribution to the DFA (Table 5) The second variable in the DFA (PC2), was almost entirely defined by Rec.

### *2. Elliptic Fourier harmonics components*

Only the first five of the principal components calculated from 28 of the original variables were selected for further analysis; these PCs explained 91% of the variance. Box's *M* test of the five principal components showed no significant difference between covariance matrices ( $P=0.060$ ) therefore a linear function was used in the DFA which achieved a classification success rate of 68% after jack-knife cross validation (Table 4). Those results are also confirmed by the non-parametric MANOVA which showed a significant difference between localities ( $P<0.001$ ).

The reconstruction of the shape outline from the average harmonics for each locality show how the otoliths from the Atlantic are slightly more rounded and have a more pronounced anti-rostrum than those from the Mediterranean Sea (Figure 2).



Figure 2. Average outline yielded from the average eFcs of each locality. Superposition of both outlines, grey outline = Mediterranean, white outline = Atlantic

### *3. Shape indices and elliptic Fourier harmonics components Discriminant Function Analysis*

A third discriminant analysis was performed with the significant PCs obtained from the PCA of shape indices and eFcs. Prior to the analysis correlation between variables was

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tested using Pearson's correlation test with no significant results. Box's *M* test showed that the variance matrices were not homogeneous therefore a quadratic function was used in the DFA. The DFA correctly classified 86% after jack-knife cross validation, non-parametric MANOVA support that result with a significant value ( $P < 0.0001$ ) (Table 4).

PC.1 of the eFcs contributes most to the DFA (Table 5). For this component, as in the others, low order eFcs contributed more than high order harmonics.

A higher classification success rate was achieved using eFcs compared to shape indices (Table 4). The canonical discriminant functions from the combined DFA show that the eFcs make a slightly higher contribution to the classification function than the shape indices (Table 5).

Table 2 Results from ANCOVAs of the shape indices with otolith length as the covariate. (*b*) is the within-group slope, used to standardise each variable for otolith length.

Shape Indices	Otolith Length		<i>b</i>
	<i>F</i>	<i>p-value</i>	
Circularity	465.66	<0.001	$1.01 \times 10^{-3}$
Aspect Ratio	19.04	<0.001	$8.40 \times 10^{-4}$
Ellipticity	19.37	<0.001	$2.61 \times 10^{-4}$
Rectangularity	3.37	0.069	$-2.5 \times 10^{-5}$
Roundness	20.13	<0.001	$-3.67 \times 10^{-4}$

Table 4. Results from the three Discriminant Function Analyses and Jackknife classification matrices

Factors	m	% var	Jackknife Allocation success		
			Atlantic	Med	Total
Shape ind.	4	99%	65%	74%	69%
eFcs	5	87.5%	81%	70%	76%
Shape ind. + EFcs	9	n.a.	92%	96%	94%

m: number of variables, principal components used in each discriminant analysis. %var: percentage of the total variance explained by m variables, n.a.: not applicable.

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Table 5. Standardized Canonical Discriminant Functions for the three Discriminant Function Analysis.

	PCs	Canonical Discriminant Functions		
		Shape Indices DFA	eFcs DFA	Shape Indices + eFcs DFA
Shape Indices PCs	PC1	0.846		0.437
	PC2	0.609		0.411
eFcs PCs	PC1		0.909	0.700
	PC2		-0.260	-0.414
	PC3		-0.122	-0.081
	PC4		-0.306	-0.181
	PC5		0.426	0.326

## **Discussion**

The results of this study show that the combination of size based shape descriptors and eFc is a useful tool for discriminating between saury from the western Mediterranean and the Northeast Atlantic. Atlantic saury otoliths are characterised by highly variable shape. This variability is noted between otoliths of fish from the same stock, and even between otoliths from the same fish (Potoschi 1996). Despite this variability, there are consistent differences in otolith shape between stocks which can be captured using principal component analysis of the Fourier coefficients and shape indices generated from otolith outlines. Previous studies have also combined information from elliptic Fourier harmonics and shape indices to discriminate otolith shape (Tracey et al 2006; Burke et al 2008). This approach increases the number of variables available to the analysis and usually results in an improvement in classification success (Tracey et al 2006). Other studies also use principal components to simplify eFc analysis (Iwata and Ukai 2002; Duarte-Neto et al 2008) and Tracey et al (2006) applied a similar approach based on non-parametric classification tools. There is a notable increase in the classification success when combining eFcs and shape indices in the principal components. This is may be due to the different nature of the variables. EFcs primarily describe the outline trajectory (bends, turnings, etc) especially after being standardised by the first ellipse that will eliminate correlations with the dimensions of the object under observation. On the other hand, the shape indices focus on the ratios of the object's dimensions and their approximations to standard polygons (rectangles, ellipses, circles, etc) and even when corrected for otolith length they still contain size ratio information. The method used in the current study to discriminate groups of Atlantic saury may have wider applications to the identification of stock-specific patterns in other species whose otoliths have highly variable shapes, and/or very convoluted outlines, where a large number of Fourier harmonics are needed to fully describe the shape and capture between group variability.

It is important to consider if the observed variation in otolith shape is truly reflective of large scale variation between populations. The samples from the Atlantic population were taken from eight hauls collected over a range of latitudes and on several occasions during the migration of saury to northern waters and is therefore thought to be representative of saury population present in the northeastern Atlantic Ocean. On the other hand western Mediterranean saury is only represented by one sample taken from a



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catch of several hundred kilos also from migrating saury. Univariate comparisons revealed variation in some of the shape variables within the Atlantic samples which was probably related to heterogeneity in size. However, there was far greater variation in shape between the two areas which was shown to be independent of size. This suggests that the discrimination of fish from the Atlantic and Mediterranean based on otolith shape is indicative of large scale differences between the two areas and is not being driven by solely small scale local variation between sites within each area. Nonetheless, more spatially and temporally comprehensive sampling of the saury populations in both areas would help to refine the characterisation of Mediterranean and Atlantic saury using otolith shape.

The observed variation in otolith shape between saury from the Atlantic and the Mediterranean may have a genetic basis, or may reflect environmental differences between the two basins. General otolith shape is determined genetically (Vignon and Morat 2010). However environmental factors that affect somatic and therefore otolith growth also have an effect on the otolith's final shape. For example temperature is proven to change the rate and pattern of deposition during otolith growth producing changes in the otolith length and overall shape (Lombarte and Leonart 1993; Oozeki and Watanabe 2000; Suyama *et al.* 2009); feeding also influences otolith shape, to the point that even recent feeding history creates discernable differences in otolith shape (Gagliano and McCormick 2004).

Differences in growth rates and otolith deposition pattern during part or all of the first year of life may have contributed to the observed variation in otolith shape between the two populations. Such differences may be related to seasonal and/or geographic variation. In this study, the saury samples from the Atlantic were comprised of fish that were hatched in spring whereas the saury from Mediterranean were hatched in autumn. Therefore the fish from the two areas had been exposed to different environmental conditions as larvae, regardless of where they were spawned. The Mediterranean and Atlantic exhibit substantial variation in environmental conditions, such as temperature, salinity and food availability (Patarnello *et al.* 2007) which can influence the growth rates of the fish that reside there and could account for the small but significant difference in fish condition between saury from the two areas.

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Although the underlying causes of the shape differences are not examined here, the results show that at least in their first year saury from the Mediterranean and the Atlantic form distinct stock units that can be distinguished on the basis of otolith shape variation. What is not certain is whether the groups remain distinct for the entire life cycle or mix during spawning. The strong discriminatory power of the otolith shape analysis presented here merits its use for identifying the origin of saury in spawning assemblages and assessing the level of mixing between the Atlantic and the Mediterranean. Further research, using otolith shape and genetic analyses of spawning fish would help to elucidate the population structure of Atlantic saury in the eastern and western Atlantic and the Mediterranean Sea. The possible existence of seasonal subpopulations should also be examined. The evidence of stock heterogeneity in Atlantic saury highlights the need for separate management to avoid overexploitation and collapse of these developing fisheries as the species may in the future be targeted for exploitation by several countries. Saury is a very fast growing species with an almost annual life cycle (Agüera & Brophy, in review). A species displaying such characteristics requires very careful and precise management to avoid over-exploitation, and a clear knowledge of the stock structure is demanded.

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# General Discussion

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The failure of conventional fisheries management is generally recognized (García and Leiva Moreno 2003). Traditionally management efforts are focused on the intrinsic capacities of the species under exploitation. This approach has been impractical, and in some cases not followed or poorly implemented (Cury *et al.* 2005). The result has been the ecological and economic collapse of numerous important fisheries in many parts of the world (Pauly *et al.* 2002).

The fishing industry is now extending the range of exploited species in response to the reduction in profits produced by the collapse of traditional species. To avoid the same thing happening with newly targeted species and to recover and improve the management of already exploited species, it is necessary to move to an Ecosystem Approach to Fisheries (EAF) (Cury *et al.* 2005). Fisheries are deeply embedded in ecosystems and its overall complexity is critical for the sustainability of any exploitation in that ecosystem (Cury *et al.* 2005).

The results obtained here are of value but still insufficient to ensure the sustainable management of Atlantic saury in the future. Atlantic saury is a planktotivorous, epipelagic, oceanic species, with a very large distribution range that undertakes long migrations (Parin 1968). The population structure of Atlantic saury is yet not very well known, but results obtained by otolith shape analysis suggest that at least Mediterranean and Atlantic fish younger than one year could be considered to form different stocks (Agüera and Brophy 2011). Otolith microstructure showed that saury is a very short lived species with a life span of less than two years (Agüera and Brophy, in review) a considerably lower value than that found using other techniques for ageing. Examination of the reproductive state of saury from south Ireland revealed that saury present there have not yet reproduced but are likely to do so before or shortly after reaching one year old (Chapter 2, this thesis). From that information it can be deduced that a fishery in Ireland will focus on immature fish that have not yet reproduced. The same applies to the fishery in the Bay of Biscay where the fish present were even younger (Agüera and Brophy, in review). Considering that about 70% of the saury population may die after first reproduction (Sauskan and Semenov 1968), saury should be considered as an annual species.

This new data presents a great challenge to fisheries management. Short-lived species, especially those that exhibit an annual life cycle, are subject to large fluctuations in

abundance and respond rapidly to many factors such as changes in oceanographic conditions, biological interactions, and fishery exploitation (Huang *et al.* 2007; Hoshino 2010; Huang and Smith 2010). Managing those fisheries is difficult given the variability in stock abundance and the uncertainty concerning factors that may affect the future abundance. The Atlantic saury stock available for fishing in Ireland and the Bay of Biscay is entirely composed of one cohort, and therefore it depends on a single recruitment event every year. Furthermore recruitment does not take place in situ, but it does happens in southern latitudes (Dudnik *et al.* 1981) with recruits being available only during fishing season. Under those circumstances saury fisheries should be to be managed in a similar way to fisheries for other short-lived species such as squid (Basson and Beddington 1993). For example, it may be more appropriate to conduct the assessment of the stock prior to and during the fishing season as suggested by Pierce and Guerra (1994) for cephalopod assessment, rather than carrying out post-season assessment as is typically done with long live species (Hoshino 2010). That way the fishing pressure can be adapted to the available biomass and can be controlled during the fishing season to prevent threatening recruitment. Given the wide distribution of the species and the potential for exploitation by several countries (e.g. Ireland, France, Spain and Portugal), the management and assessment of saury fisheries should be conducted within an international context.

Very little is known of the role of saury in the pelagic ecosystem which it inhabits. As with other small pelagic species, sauries are essential elements of marine ecosystems due to their significant biomass at intermediate levels of the foodweb, playing a considerable role in connecting the lower and upper trophic levels (Cury *et al.* 2000). The effects of its exploitation on the ecosystem will depend mostly on the existence of species which depend on saury as a food source. Atlantic saury have been found in the diet of numerous species, including dolphins (Hassani *et al.* 1997), very valuable fish species such as swordfish (Chancollon *et al.* 2006), bluefin tuna (Karakulak *et al.* 2009) and birds (Nesterov 1976; Berruti 1988), making up a considerable proportion of the diet of gannets (Chaput and Hurlbut 2010). How much these species depend on this food source is not yet known.

This work has initiated the collection of data necessary to ensure the sustainable management of a future fishery on Atlantic saury. There is still a need for further

research and data collection to improve understanding of the role of saury in the ecosystem and the potential impact of its exploitation.

On saury biology, it is important first to validate the ageing structures used in this study. Rearing larvae from eggs has been successfully conducted for Pacific saury (Watanabe and Kuji 1991). Atlantic saury could be relatively easy to maintain and breed in captivity as are Pacific saury (Nakaya *et al.* 2010), making possible otolith marking of captive fish. At the same time data on batch fecundity and annual fecundity could be obtained which is of vital importance for the management of the species. It is necessary to have more information on saury population structure in the north Atlantic. Otolith morphometrics as used here along with genetic analysis will provide a better picture of the degree of mixing/isolation between the Mediterranean Sea, northeastern and northwestern Atlantic populations. A methodology for assessing existing saury biomass should also be developed. This could be achieved through acoustic surveys or scientific fishing trials. Saury migrations and biology are strongly influenced by temperature, therefore climate variability will be a very important factor controlling saury populations movements. Long term monitoring of saury biomass displacements on its distribution range will give an idea of its spatial and temporal variability, giving information on migration patterns and the influence of climate variations on them.

On the other hand, it is necessary to assess the importance of saury as a forage species, and the impact of a saury fishery on the species that feed on them. Predators of saury should be determined and observed and the fraction of the diet composed of saury be assessed taking into account the seasonal availability of saury for some predators. Observation on predator behaviour toward saury presence in their foraging areas, shoals and saury migrations are also important in assessing the impact of saury fishing on the foodweb.

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