UNIVERSIDADE DE LISBOA

FACULDADE DE CIÊNCIAS

Structure and biology of the southern component of blue whiting (*Micromesistius poutassou*) population in Northeast Atlantic

Doutoramento em Ciências do Mar

Patrícia de Jesus Gonçalves

Tese orientada por:

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Documento especialmente elaborado para a obtenção do grau de doutor

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Centro de Ciências do Mar e do Ambiente (MARE)
Faculdade de Ciências, Universidade de Lisboa (FCUL)

Instituto Português do Mar e da Atmosfera (IPMA)
Departamento do Mar e Recursos Marinhos
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Programa Nacional de Amostragem Biológica (PNAB) do IPMA

Fundação para a Ciência e Tecnologia – Bolsa de Doutoramento (SFRH/BD/88092/2012)

"A good traveller has no fixed plans
and is not intent upon arrive.
A good artist lets his intuition
lead him wherever it wants.
A good scientist has freed himself of concepts
and keeps his mind open to what is."

Tao Te Ching (by Lao Tse)

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Abstract

Blue whiting (*Micromesistius poutassou*) is a mesopelagic gadoid widely distributed along the Northeast Atlantic (NEA). Recent studies pointed out the existence of two stock components on their whole area of distribution, with a separation border line at the Porcupine Bank (Ireland western coast). However, the blue whiting continues to be assessed as a single stock, due to the lack of knowledge regarding the biology and structure of their southern component. The present thesis aimed to fill some of these important gaps in the biology and structure of blue whiting at the southern part of stock distribution, the Portuguese coast. Besides, this work also intended contributes to the enlargement of the knowledge on this species assessment. Smaller blue whiting (12cm–24cm) were mostly found in shallow areas down to 200m to 300m while larger individuals (>24cm) were spread deeper down to 400m until 500m. Blue whiting condition (Fulton K) differed significantly between seasons (autumn and winter) in all the three main areas off Portugal (north, southwestern and south) and between sexes. The proportion of females changed between seasons and across depths. The blue whiting life-history parameters analysis shown differences in growth, length, age of first maturity, in the peak spawning and also differences on females spawning stock biomass, between the Portuguese coast and the main spawning ground (west of Ireland Islands and Porcupine Bank), which supports the existence of two components on the blue whiting off NEA. Furthermore, OtoRing, an open source software for otolith age reading, was developed with the principal advantages of: allow the storage and posterior revision of age classifications in an easy way; perform calibrations between readers; and help on the training of new readers. The otolith face orientation (distal *versus* proximal) on image analysis reveal that it must be taken into account during the otoliths images processing for symmetry shape analysis between left and right otolith. On blue whiting the otolith images should be obtained from the concave side (distal surface). Microsatellites' genetic based results showed a lack of differentiation between samples from north to south of the NEA, and even at the Mediterranean coast of Spain. However, was verified a clear differentiation between the samples from the Adriatic Sea and the rest of the areas. The otolith shape showed differences between Ireland, Portuguese coast and Mediterranean. Small scale differences in the otolith shape of immature individuals were identified associated with different depth distributions along the Portuguese coast, but those differences have not been reflected in the comparison between the Portuguese coast and the adjacent areas (Ireland coast and Mediterranean). The main conclusion was that blue whiting presents a unique population structure along the Portuguese coast which is different from the populations at north (Ireland coast) and at the Mediterranean.The basis, that will allow in a near future assess blue whiting considering two stock components, in a more sustainable and conservative based-approach for their effective management across the Northeast Atlantic, have started being created.

Keywords: blue whiting, biology, fishery resource, stock structure, Southern component.

Resumo

O verdinho (*Micromesistius poutassou*) é um gadídeo mesopelágico amplamente distribuído no Nordeste Atlântico (NEA). Estudos recentes apontam para a existência de dois componentes populacionais distintos ao longo de toda a área de distribuição, cuja linha de separação se situa na latitude do Banco de Porcupine (costa Oeste da Irlanda). No entanto, para os efeitos da avaliação do estado do manancial de verdinho assume-se a existência de uma única população ao longo do NEA. Este pressuposto mantém-se principalmente devido a importantes falhas no conhecimento da biologia e da estrutura populacional do verdinho na sua componente mais a sul. Deste modo, a presente tese teve como principais objectivos o estudo da biologia e da estrutura populacional do verdinho ao longo da costa Portuguesa, a parte mais a sul da sua distribuição. Para além disso, pretendeu também ampliar o conhecimento usado na avaliação desta espécie. Os verdinhos mais pequenos (12cm - 24cm) foram maioritariamente encontrados a uma profundidade entre os 200m e os 300m, enquanto que os maiores (>24cm) ocorreram em águas mais profundas, entre os 400m e os 500m. Na costa portuguesa (norte, sudoeste e sul), foram observadas diferenças significativas nos valores relativos ao índice de condição (Fulton K) do verdinho entre estações do ano (Outono e Inverno) e entre sexos. A proporção de fêmeas nas amostras variou, quer com as estações do ano, quer com a profundidade. Entre a costa Portuguesa e a área principal de desova (costa da Irlanda, Banco de Porcupine) foram observadas diferenças relativas aos seguintes parâmetros: crescimento, comprimento, idade de primeira maturação, pico da época de desova e biomassa de fêmeas desovantes do manancial. Estas diferenças apoiam a existência de duas unidades populacionais ao longo do NEA. No decorrer deste trabalho, foi desenvolvido um programa informático, de acesso livre, para leitura de idades em otólitos, o OtoRing, cujas principais vantagens são permitir de forma fácil o armazenamento e a posterior revisão das classificações em idades, fazer calibrações entre leitores, e ajudar na formação de investigadores que se iniciam na leitura de otólitos. A superfície através da qual estão orientados os otólitos nas imagens (superfície distal *versus* proximal), deve ser tida em consideração, nomeadamente quando estas são recolhidas e processadas para estudos de simetria entre o otólito esquerdo e o direito. No caso do verdinho, as análises realizadas no decorrer deste trabalho mostraram que as imagens devem ser sempre obtidas pelo lado côncavo do otólito (superfície distal). Os resultados de genética com recurso a microsatélites não revelaram diferenciação entre as amostras recolhidas no NEA de norte para sul, e mesmo entre a costa Espanhola do Mediterrâneo. Contudo, foi verificada uma diferenciação clara entre as amostras recolhidas no mar Adriático e as restantes áreas. Diferenças entre a Irlanda, Portugal e o Mediterrâneo foram observadas através da análise da morfologia dos otólitos. Para os indivíduos imaturos obtidos na costa Portuguesa, foram identificadas pequenas diferenças na morfologia do otólito associadas com a distribuição a diferentes profundidades, mas estas diferenças não tiveram reflexo na análise a uma escala global, no âmbito da comparação entre as amostras de Portugal com as de áreas adjacentes (Irlanda e Mediterrâneo). Como principal conclusão da presente tese, o verdinho apresenta uma estrutura populacional única ao longo de toda a costa Portuguesa, sendo esta distinta da população a norte (costa da Irlanda) e do Mediterrâneo. As bases que irão permitir, num futuro próximo, avaliar estas subpopulações numa abordagem mais sustentável e conservadora para a gestão efectiva do verdinho ao longo do Atlântico Nordeste, já começaram a ser desenvolvidas.

Palavras-chave: verdinho, biologia, recurso pesqueiro, estrutura populacional, componente Sul.

Resumo alargado

O verdinho (*Micromesistius poutassou*) é um gadídeo mesopelágico amplamente distribuído no Nordeste Atlântico (NEA). Estudos recentes apontam para a existência de dois componentes populacionais distintos ao longo de toda a área de distribuição, cuja separação se situa na latitude do Banco de Porcupine (costa Oeste da Irlanda). No entanto, para os efeitos da avaliação do estado do manancial de verdinho assume-se a existência de uma única população ao longo do NEA. Este pressuposto mantém-se principalmente devido a importantes falhas no conhecimento da biologia e da estrutura populacional do verdinho na sua componente mais a sul. Deste modo, a presente tese teve como principais objectivos o estudo da biologia e da estrutura populacional do verdinho ao longo da costa Portuguesa, a parte mais a sul da sua distribuição. Para além disso, pretendeu-se também ampliar o conhecimento existente e utilizado na avaliação desta espécie.

Nos capítulos 2 e 3, são apresentados os resultados relativos a aspectos da biologia do verdinho na costa Portuguesa. Os capítulos 4 e 5 incluem informação essencial para a melhoria da avaliação desta espécie. A estrutura da população do verdinho para a costa de Portugal foi analisada e integrada no contexto populacional do NEA nos capítulos 2 e 6.

Os verdinhos mais pequenos (12cm-24cm) foram maioritariamente encontrados a uma profundidade entre os 200m e os 300m, enquanto que os maiores (>24cm) em águas mais profundas, entre os 400m e os 500m. Verificou-se um aumento da taxa de crescimento de norte para sul no NEA. O crescimento entre sexos é diferenciado, com as fêmeas a atingirem comprimentos maiores. Apesar de numa fase inicial a taxa de crescimento ser superior nos machos em todo o NEA, como única excepção o Mar do Norte. O pico principal de desova é em Fevereiro na costa Portuguesa e em Abril na área de desova principal (costa da Irlanda). Apesar, de o tamanho de primeira maturação ser semelhante entre diferentes áreas de distribuição, a correspondência com a idade varia de área para área, o que confirma diferenças no padrão de crescimento. Para diferentes zonas na costa Portuguesa (norte, sudoeste e sul), foram observadas diferenças significativas nos valores relativos ao índice de condição (Fulton K) do verdinho entre estações do ano (Outono e Inverno) e sexos. A proporção de fêmeas nas amostras variou quer com as estações do ano, quer com a profundidade. De um modo geral, durante a época de desova os machos encontraram-se em maior abundância junto ao fundo. No entanto fora da época de desova, as amostras recolhidas no fundo são maioritariamente compostas por fêmeas. A biomassa de fêmeas desovantes do manancial (FSSB) encontra-se estabilizada com valores perto da média histórica para Portugal desde 2005, ao contrário dos dados disponíveis para o manancial do NEA no qual foi observada uma diminuição desde 2007 até 2010. Entre a costa Portuguesa e a área principal de desova (costa da Irlanda, Banco de Porcupine) foram observadas diferenças relativas aos seguintes parâmetros: crescimento, comprimento, idade de primeira maturação, pico da época de desova e FSSB. Estas diferenças apoiam a existência de duas unidades populacionais ao longo do NEA.

A avaliação pesqueira de verdinho é feita através da modelação da população estruturada por idades, cujos dados base são: capturas-por-idade, índices de abundância por idade e proporção de indivíduos maduros em cada idade. A atribuição de idades tem deste modo um papel fundamental para a correcta compreensão do estado actual deste recurso. Devido à sua importância, os critérios usados na leitura de otólitos e consequente classificação em idades devem ser objectivos, para que por um lado a precisão entre leitores seja elevada e por outro estas classificações sejam exactas. Na prática, a atribuição de idades está muito dependente do nível de experiência do leitor, o que torna os critérios subjectivos. No decorrer deste trabalho, foi desenvolvido um programa informático de acesso livre para leitura de idades em otólitos com base em análise de imagem, o OtoRing, cujo intuito foi o de tornar este processo mais objectivo e facilmente replicável entre leitores. As principais vantagens do OtoRing são: permitir de forma fácil o armazenamento e a posterior revisão das classificações em idades, fazer calibrações entre leitores, e ajudar na formação de investigadores que se iniciam na leitura de otólitos.

A utilização crescente de ferramentas de análise de imagem aplicadas quer à atribuição de idades, quer aos estudos de crescimento implica a padronização do modo como são recolhidas as imagens dos otólitos. Na recolha de imagens, a superfície a partir da qual se orientam os otólitos (superfície distal *versus* proximal) deve ser tida em consideração, nomeadamente quando estas são utilizadas em estudos de simetria entre otólitos do mesmo par (esquerdo e direito). No decorrer deste trabalho, as análises realizadas mostraram que as imagens devem ser sempre obtidas pelo lado côncavo do otólito (superfície distal).

Os resultados de genética com recurso a microsatélites não revelaram diferenciação entre as amostras recolhidas no NEA de norte para sul, e mesmo entre a costa Espanhola do Mediterrâneo. Contudo, foi verificada uma clara diferenciação entre as amostras recolhidas no mar Adriático e as restantes áreas. Diferenças entre a Irlanda, Portugal e o Mediterrâneo foram observadas através da análise da morfologia dos otólitos. Em verdinhos imaturos da costa Portuguesa, foram identificadas pequenas diferenças na morfologia do otólito associadas com a distribuição a diferentes profundidades, mas estas diferenças não tiveram reflexo na análise a uma escala global, no âmbito da comparação entre as amostras de Portugal com as de áreas adjacentes (Irlanda e Mediterrâneo). Os factores ambientais uma vez que actuam ao nível do metabolismo e consequentemente do crescimento do otólito, são geralmente mais determinantes na definição da forma do otólito do que a própria genética. Os resultados obtidos através da aplicação destas duas metodologias (genética e morfologia dos otólitos) sugerem que estas se complementam entre si como ferramentas aplicadas à biologia populacional. As aplicações em otólitos possuem potencial para revolucionar o nosso conhecimento relativamente à integridade de populações de peixes e consequentemente da gestão de recursos pesqueiros.

Como principal conclusão da presente tese, o verdinho apresenta uma estrutura populacional única ao longo de toda a costa Portuguesa, sendo esta distinta da população a norte (costa da Irlanda) e do Mediterrâneo. A avaliação deste recurso pesqueiro tendo em consideração estas duas populações fenótipicas é de extrema importância, devido às suas características demográficas únicas (por exemplo: crescimento, desova) podendo estas apresentar diferentes respostas quando exploradas. As bases que irão permitir, num futuro próximo, avaliar estas subpopulações numa abordagem mais sustentável e conservadora para a gestão efectiva do verdinho ao longo do Atlântico Nordeste, já começaram a ser desenvolvidas.

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List of Acronymus and Abreviations

ACFM	Advisory Committee on Fishery Management
ALKs	Age-length keys
ANOVA	Analysis of variance
APE	Average percent error
B_{lim}	Limit reference point for spawning stock biomass (SSB)
B_{pa}	Precautionary reference point for SSB
B_{MSY}	SSB that results from fishing at F_{MSY} for a long time
$B_{trigger}$	SSB that triggers a specific management action
CFP	Common Fisheries Policy
Ch.	Chapter
CPUE	Catch Per Unit Effort
CPR	Continuous Plankton Recorder
CV	Coefficient of variation
DCF	Data Collection Framework
df	degrees of freedom
EBA	Ecosystem-based-approach stock assessment models
EBM	Ecosystem-based-management
EC	European Commission
EFD	Elliptic Fourier Descriptors

EU	European Union
FAO	Food and Agriculture Organization of the United States
FCT	Fundação para a Ciência e Tecnologia
F	Fishing mortality
F_{pa}	Precautionary reference point for fishing mortality (mean over defined age range)
F_{lim}	Limit reference point for fishing mortality (mean over defined age range)
F_{MP}	Fishing mortality reference point as defined in management plans.
F_{MSY}	Fishing mortality consistent with achieving MSY
FSSB	Female Spawning Stock Biomass
GLM	Generalized linear model
GLMM	Generalized linear mixed model
HCR	Harvest Control Rule
IBWSS	International Blue Whiting Spawning Stock Survey
ICES	International Council for the Exploration of the Sea
IFREMER	L'Institut Français de Recherche pour l'Exploitation de la Mer
IPMA	Instituto Português do Mar e da Atmosfera
K	Fulton's condition factor
L50	Length at which 50% of the population are mature
LO	Landing obligation
LPUE	Landings Per Unit Effort
M	Natural mortality
MAY	Maximum average yield
MLS	Fish minimum landing size

MSY	Maximum sustainable yield
MSY $B_{trigger}$	A biomass reference point that triggers a cautious response within the ICES MSY framework
NEA	Northeast Atlantic
PCA	Principal Components Analysis
PNAB	Plano Nacional de Amostragem Biológica
PBTS	Portuguese Bottom-Trawl Survey
PT	Portugal
R	Recruitment
SAM	State-space fish stock Assessment Model
SCAA	Statistical Catch-at-Age Analysis
SD	Standard deviation
SIMWG	ICES Stock Identification Methods Working Group
SSB	Spawning stock biomass
SR	Sex-ratio, proportion of females
SRR	Stock-recruitment relationship
std.error	standard error
STECF	Scientific, Technical and Economic Committee on Fisheries (EU)
VPA	Virtual Population Analysis
whb	FAO species code for blue whiting
WKPELA	ICES Benchmark Workshop on Pelagic Stocks
XSA	Extended Survivol Analysis
YPR	Yield Per Recruit

Glossary

Age-length keys - The age structure for a large number of fish can be estimated by summarizing the relationship between age and length for a relatively small subsample of fish and then applying this summary to the entire sample of fish, which is named as an age-length key.

Asymptotic growth – Is usually expressed as L_∞, asymptotic fish length at which growth is zero from the von Bertalanffy model. This is not the maximum length of the animal. Rather L_∞ is the asymptote for the model of average length-at-age. As with any average, some individuals will be larger than average; thus, some animals will be larger than L_∞. L_∞ only has meaning in fish populations where mortality is low enough to allow fish to reach an age at which the mean length (virtually) ceases to increase.

Age reading - Age classification has been based in age readings under stereomicroscopic otoliths observation. The otolith grows in size as fish grows; ring bands are formed in the otoliths surface registering periods of rapid and slow growth, opaque bands appear alternating with translucent bands. Age classification was made considering the number of translucent rings in the otolith; one translucent ring was equivalent to one year.

Aquaculture - The farming of aquatic organisms in inland and coastal areas, involving intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators; and the individual or corporate ownership of the stock being cultivated.

Bayesian method - Assessment method that quantifies uncertainties and formulates advice on the basis of probabilities of reaching a limit or target point.

Biomass – Size of a fish stock (usually expressed in number or in weight).

By-catch - Part of a fishing catch taken incidentally in addition to the target species towards which fishing effort is directed. Some or all of it may be returned to the sea as discards.

Catchability - The fraction of a fish stock which is caught by a defined unit of the fishing effort.

CPUE – The quantity of fish caught (in number or in weight) with one standard unit of fishing effort; e.g. number of fish taken per 1000 hooks per day or weight of fish taken per hour of trawling. CPUE is often considered an index of fish biomass (or abundance). Sometimes referred to as catch rate.

Choke species - Species with the lowest quota in a mixed-fishery, which restrict the fishing opportunities for other quota species.

Demersal fish - Fish that spend most of their life with contact with or dependency on the bottom. Usually refers to the adult stage of a species.

Discards - That component of a catch thrown back after capture. Normally, most of the discards can be assumed not to survive. They are typically discarded because they are non-target species, below minimum landing size, above or below the ideal market size (high grading) or because the quota for that species has been exhausted.

Discard-ban – *see* landing obligation (LO).

Ecosystem - An organizational unit consisting of an aggregation of plants, animals (including humans) and micro-organisms, along with the non-living components of the environment.

Ecosystem approach - Ecosystem approach to fisheries management. Management that takes into account the effects of fisheries on the ecosystem, and the effects of the ecosystem on the fish stocks.

Epipelagic zone – Corresponds to the zone of an ocean extending from the surface to a depth of about 200 meters; light penetrates this zone, allowing photosynthesis.

Exploitation pattern - Distribution of fishing mortality over the age composition of the fish population, determined by the type of fishing gear, area and seasonal distribution of fishing, and the growth and migration of the fish.

Exclusive Economic Zone (EEZ) - A zone under national jurisdiction (up to 200-nautical miles wide) declared with the provisions of 1982 United Nations Convention of the Law of the Sea, within which the coastal State has the right to explore and exploit, and the responsibility to conserve and manage, the living and non-living resources.

Fishery – Group of vessel voyages targeting the same (assemblage of) species and/or stocks, using similar gear, during the same period of the year and within the same area.

Fisheries management - The integrated process of information gathering, analysis, planning, decision-making, allocation of resources and formulation and enforcement of fishery regulations by which the fishery management authority controls the present and future behaviour of interested parties in the fisheries, in order to ensure the continued productivity of the living resources.

Fishing fleet - An aggregation of fishing vessels of a particular country (e.g. the European Union fishing fleet) or using a particular gear (a purse seine fleet).

Fishing mortality - The part of the total mortality rate that is due to fishing. Fishing mortality is usually expressed as an instantaneous rate and can range from 0 per year (for no fishing) to high values such as 1.0 or more per year.

$F_{0.1}$ - The fishing mortality rate at which the marginal yield-per-recruit (i.e. the increase in yield-per-recruit in weight for an increase in one unit of fishing mortality) is only 10 percent of the marginal yield-per-recruit on the unexploited stock. The fishing mortality rate at which the slope of the yield-per-recruit curve is only one-tenth the slope of the curve at its origin.

Forage species – Small pelagic species used as prey by a predator for its food.

Fully fished - State of a stock which current catches are close to the Maximum Sustainable Yield (MSY) or Maximum Average Yield (MAY). Increases in fishing effort would not increase significantly the yields and would substantially increase the risk of overfishing. Fully fished stocks are primary targets for effort and capacity controls.

Growth model - Fish growth models can be divided into two categories. The first category includes statistically based models for fish growth. The models of this type often assume that growth is a function of the current body size of the individual, and they ignore or have only a loose connection to the biology behind the actual growth processes. The most used growth model is the von Bertalanffy, but other different statistical models are available, that also fit empirical data quite well: for example, the Logistic, Gompertz and Richards's growth models.

Growth rate – Represents the fish change in size (length and weight) along time. In the von Bertalanffy growth model is denominated as k.

Hardy–Weinberg equilibrium – Is a principle stating that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. When mating is random in a large population with no disruptive circumstances, both genotype and allele frequencies will remain constant because they are in equilibrium.

HCR – An algorithm for pre-agreed management actions as a function of variables related to the status of the stock. For example, a control rule can specify how F or yield should vary as a function of spawning biomass. Also known as 'decision rules' or 'harvest control laws'.

Landings - Weight of what is landed from a fishery at a landing site. It is different from the catch because it does not include discards.

Landing obligation – Article 15 of the new CFP Basic Regulation (Council Regulation No 1380/2013) introduces new rules on discards including: (i) a landing obligation (LO) under which all catches of regulated species must be landed and counted against quotas of each Member State; and (ii) a requirement that catch of species subject to the LO below a minimum conservation reference size (MCRS) be restricted to purposes other than direct human consumption.

Large-scale fisheries – Also denominated as industrial fisheries. Commercial fishery involving large fishing vessels, with capacity for store large amounts of fish and some of those vessels are also equipped with fish processing machines.

LPUE – Landings per unit effort, similar to CPUE, but based on that part of the catches that are landed and reported.

Management plan - A management plan includes the decision-making processes (harvest control rules, tactical decision making) and the sanctions on implementation and the requirements for monitoring and reporting. Management plans may also exist in the form of rebuilding plans or recovery plans.

Management strategy – Management strategies consist of objectives with associated performance criteria, the implementation measures (e.g. input or output control) and what is considered a relevant knowledge base for decisions.

Maturity ogive - A distribution curve with the cumulative proportions of immature and mature individuals by age or by length.

Maximum Sustainable Yield (MSY) - The largest average catch that can be taken continuously (sustained) from a stock under average environmental conditions. It is often used as a management goal.

Mesopelagic fish – Fish that inhabits the intermediate depths of the ocean between 100 and 1000m. Most of mesopelagic species make extensive vertical migrations into the epipelagic zone at night to feed, and thereafter migrate down several hundred meters to their daytime depths.

Metier – Homogeneous sub-division of a fishery by fleet (e.g. the Portuguese bottom-otter trawl crustacean fishery).

Minimum landing size (MLS) - The lowest individual size allowed in landings or markets. Established by fishery management and enforced through control at landing sites or markets, it is intended to minimize the catch of small (undersized) fish or juveniles giving them a better chance to grow before being vulnerable to fishing. Based on yield per recruit considerations and models, it aims at avoiding or correcting growth overfishing.

Moratorium - A mandatory cessation of fishing activities on a species (e.g. the blue whale, cod), in an area (e.g. a sanctuary), with a particular gear (e.g. large scale driftnets), and for a specified period of time (temporary, definitive, seasonal, or related to re-opening criteria).

Natural mortality - Deaths of fish from all causes except fishing (e.g. ageing, predation, cannibalism, disease and perhaps increasingly pollution). It is often expressed as a rate that indicates the percentage of fish dying in a year; for example a natural mortality rate of 0.2 implies that approximately 20% of the population will die in a year from causes other than fishing.

Overfished - A stock is considered overfished when its size falls below a minimum threshold. A rebuilding plan is required for stocks that are overfished.

Overfishing - A generic term used to refer to the state of a stock subject to a level of fishing effort or fishing mortality such that a reduction of effort would lead to an increase in the total catch.

Panmitic population - A population in which mating is entirely random and any two (male and female) individuals are equally likely to mate. Random mating (or *panmixis*) is one of the assumptions of the Hardy–Weinberg equilibrium but is probably uncommon in natural populations, in which spatial structuring and assortative mating are usually evident.

Pelagic fish - Fish that spend most of their life swimming in the water column with little contact with or dependency on the bottom. Usually refers to the adult stage of a species.

Population – A group of fish of one species which shares common ecological and genetic features. The stocks defined for the purposes of stock assessment and management do not necessarily coincide with self-contained populations.

Quota - A share of the total allowable catch (TAC) allocated to an operating unit such as a country, a vessel, a company or an individual fisher (individual quota) depending on the system of allocation. Quotas may or may not be transferable, inheritable and tradable.

Recruits or recruitment - The new age group of the population entering the exploited component of the stock for the first time or young fish growing into or otherwise entering that exploitable component.

Semi-pelagic species – A species that partially lives their life on the bottom and in the water column (pelagic).

Small-scale fisheries – Also denominated as artisanal fisheries. Traditional fisheries involving fishing households (as opposed to commercial companies), using relatively small amount of capital and energy, relatively small fishing vessels (if any), making short fishing trips, close to shore, mainly for local consumption.

Spawning stock biomass – Abbreviation is SSB. The total weight of all fish (both males and females) in the population which contributes to reproduction. Conventionally defined as the biomass of all individuals beyond age at first maturity or size at first maturity, i.e. beyond the age or size class in which 50% of the individuals are mature. Most often used as a proxy for measuring egg production, the SSB depends on the abundance of the various age classes composing the stock and their past exploitation pattern, rate of growth, fishing and natural mortality rates, onset of sexual maturity, and environmental conditions.

Stock - A group of individuals in a species occupying a well-defined spatialrange independent of other stocks of the same species. Random dispersal and directed migrations due to seasonal or reproductive activity can occur. Such a group can be regarded as an entity for management or assessment purposes. The impact of fishing on a species cannot be fully determined without knowledge of the stock structure.

Stock assessment - The process of collecting and analysing biological and statistical information to determine the changes in the abundance of fishery stocks in response to fishing, and, to the extent possible, to predict future trends of stock abundance. Stock assessments are based on resource surveys; knowledge of the habitat requirements, life history, and behaviour of the species; the use of environmental indices to determine impacts on stocks; and catch statistics. Stock assessments are used as a basis to assess and specify the present and probable future condition of a fishery.

Stock collapse - Reduction of a stock abundance by fishing and/or other causes to levels at which the production is negligible compared to historical levels. The word is normally used when the (reduction) process is sudden compared with the likely time scale of recovery, if any, but is sometimes used melodramatically for any case of overfishing.

Stock-recruitment relationship - The relationship between the level of parental biomass (e.g. spawning stock size) and subsequent recruitment level. Determination of this relationship is useful to analyse the sustainability of alternative harvesting regimes and the level of fishing beyond which stock collapse is likely. The relation is usually blurred by environmental variability and difficult to determine with any accuracy. Such a relationship always exists in principle, in that the existence of a parent stock is a prerequisite for the generation of recruitment. However, in many cases there exist regulatory mechanisms such that the number of recruits is not strongly related to the parent stock size over the range of stock sizes observed: this situation is sometimes described as the absence of a stock-recruitment relationship, but is more logically described as a special case of a stock-recruitment relationship. Some stock assessment methods incorporate the estimation of such a relationship directly into the model, either explicitly (e.g. some age-structured assessments) or implicitly (most stock production models).

Target species - Those species that are primarily sought by the fishers in a particular fishery.

Total Allowable Catch (TAC) - It is the total catch allowed to be taken from a stock in a specified period (usually a year), as defined in the management plan. The TAC may be allocated to the stakeholders in the form of quotas as specific quantities or proportions.

Underfished - Characteristic of a stock which may sustain catches higher than current ones.

Year class – All the fish of a stock spawned or hatched in a given year.

Yield per recruit – The expected lifetime yield per fish recruited in the stock at a specific age. Depends on the exploitation pattern (fishing mortality at age) or fishing regime (effort, size at first capture) and on natural mortality.

Preface

The revision of the scientific studies concerning blue whiting (*Micromesistius poutassou*), conducted to the identification of specific important knowledge gaps on the stock structure and biology along the Portuguese Coast, which has arose several questions that span the proposed work.

- Could the life history parameters (growth, maturity) and females spawning stock biomass of the blue whiting from the Portuguese coast show evidences supporting the existence of two stock components?
- Does the blue whiting sex-ratio, size distribution and condition changes along the Portuguese coast?
- Should ImageJ be used as an automatic approach to improve the accuracy and precision of blue whiting ageing?
- Could the otolith surface's (distal *versus* proximal), from which the images were recorded, have influence in the diagnostic of the otolith symmetry shape (between the left and the right) on blue whiting?
- What is the stock structure of blue whiting off Portugal based on microsatellite markers analysis and on otoliths shape analysis?

The answer to these questions, that is the purpose of this work, is presented in this thesis.

Chapter 1

1. General Introduction

1.1 Fisheries

Fisheries provide livelihood to millions of persons and contribute to national economies through employment in fishing, processing and ancillary services, as well as through subsistence based activities at the community level. Fish is one of the most traded food, with consumption in developing and developed countries increasingly derived from imports, while underdeveloped countries, are more heavily reliant on local supply (FAO, 2014).

Fish consumption per capita increased from an average of 9.9kg in the 1960s to 14.4kg in the 1990s and 19.7kg in 2013. In 2014, fish provided more than 3.1 billion people with almost 20% of their average per capita intake of animal protein in developing countries (FAO, 2016), compared with 8% in developed countries (Toye, 2007).

The total fish production in 2014 exceeded the 167 million tons, 55% were supplied by the capture fisheries and 45% by the aquaculture production (Figure 1-1). The same source estimates that in 2025, that ratio should be inverted, with 48% of the fish income from fisheries and 52% from the aquaculture (FAO, 2016). Notwithstanding, in the structure of the global fisheries the supply is still dominated by fishery catch, while in developing countries transition to supply dominated by aquaculture is already taking place (Toufique and Belton, 2014; Thilsted *et al.*, 2016). The emerging aquaculture fish production present several constraints, mainly their impacts concerning the environment (production discharges effluents, spreads aquatic pathogens and invasive species and alters habitats with the related loss of ecosystem services) and wild fish sustainability (Péron, Mittaine and Le Gallic, 2010). Pelagic forage fish (e.g. anchoveta, herring, sardines and blue whiting) are used to fishmeal at aquaculture farms and fish oil production instead of human food consumption (Tacon and Metian, 2009), corresponding to around 30% of the total catches (Naylor *et al.*, 2009). In average, 4kg to 5kg of forage fish are need to produce 1kg of fishmeal and between 20kg to 25kg of forage fish to obtain 1kg of fish oil (Péron, Mittaine and Le Gallic, 2010).

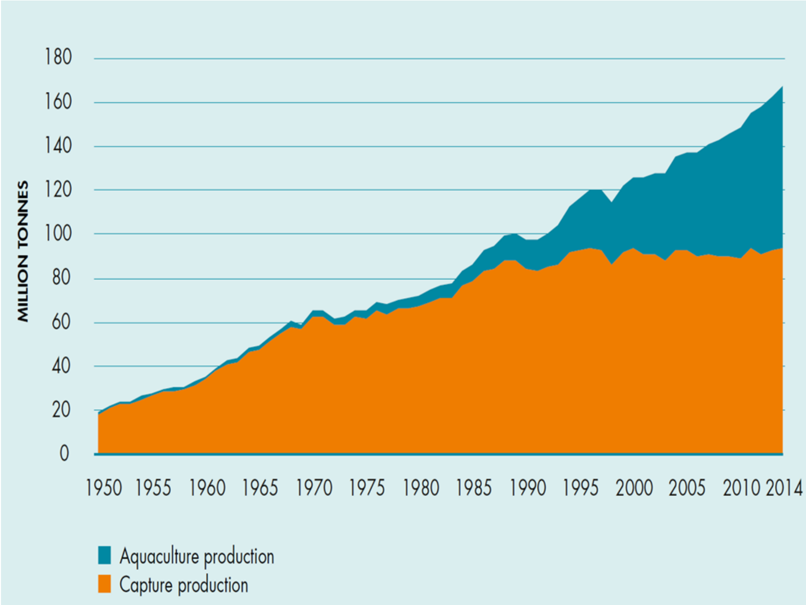

Figure 1-1 World capture fisheries and aquaculture production from 1950 until 2014 (from FAO 2016).

For the majority of coastal populations fish is an important protein source. Portugal appears in the list as one of the countries with higher fish consumption in the European Union (EU) (Bjørndal, Lappo and Ramos, 2015). In 2013, the Portuguese fish consumption per capita was 53.8kg, compared with an average of 20kg within the EU and occupying rank 3 in fish consumption worldwide (FAO, 2014). Consequently, fishing activities play an important socio-economic role on supplying national fish market demand (Leitão *et al.*, 2014).

An overview of the catches over the last decade for all countries of the world, indicate that since 1996, total world catches are declining at a rate of 1.2 million tonnes per year (Zeller *et al.*, 2016). The decrease is more evident in the catches from the industrial fleet, in the small-scale fisheries the annual catch remains almost invariant since 2000 (Figure 1-2).

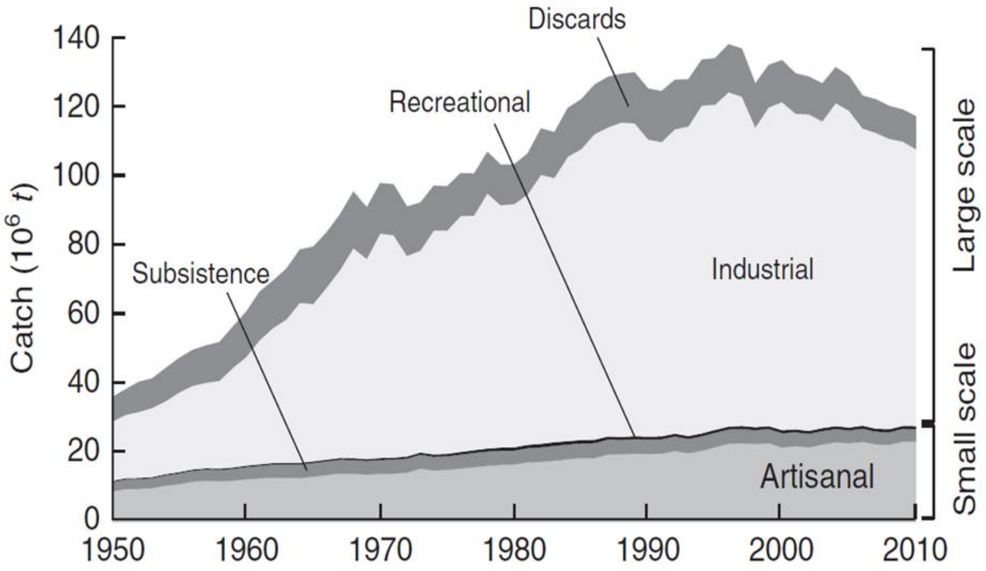

Figure 1-2 Catches for all countries in the world, plus High Seas, by large-scale (industrial) and small-scale sectors (artisanal, subsistence, recreational), with discards (overwhelmingly from industrial fisheries) presented separately (from Pauly and Zeller 2016).

The progressive advances in fishing technology may temporarily maintain high catch rates and mask the decline in stock levels, but eventually the fish abundance diminish and the fishery collapses (Clark, 1996). In the history of fisheries, the decline in fishing resulted from fish stock collapses of many species along the years. The fishery of the pacific sardine (Northern and Southern West Pacific) with an average landing over 600,000tons per year by 1940 began to collapse a few years later. In 1970, the pacific sardine catches were less than 100tons per year (Radovich, 1982). In the same decade (1970), the North Atlantic Icelandic spring and summer spawning herring (Perissi *et al.*, 2017) and the Peruvian anchoveta (Watson and Pauly, 2001) fisheries collapsed. The greatest decline was experienced by what once was one of the world's most abundant cod stocks. The Grand Banks cod stock in Newfoundland, collapsed in the earlier 1990's (Hutchings and Reynolds, 2004). Taking into account that for many, fisheries are critical sources of food and income, alternative employment options are often limited and may not be desirable given traditional and cultural ties to the sea and fishing livelihoods. The impact of fisheries collapse can be devastating; in Iceland, the herring collapse provoked the drastic fall in the number of Herring Salting industries a few years later (Perissi *et al.*, 2017); and in Canada, the collapse of the cod fishery led to changes in the social structure and dynamics of rural communities as the northern cod moratorium led to mass layoff of over 10,000 fishery workers (Schrank, 2005).

In 2004, there were estimated 41million full-time or part-time fishery workers worldwide who were involved directly in the catching and fish farming. A further 123million were involved indirectly in secondary activities (postharvest processing, distribution, and marketing activities) (World Bank 2008). Fisheries employment is unevenly distributed around the world, with a high proportion concentrated in developing countries. This disparity between participation in the fisheries of developed and developing countries lies in the scale of fishing operations. The developed countries fish with sophisticated industrial fleets (Teh and Sumaila, 2011). In contrast, small-scale fisheries are prevalent in many developing countries, where fishing is generally carried out by individuals or household units at a localized scale (Andrew *et al.*, 2007).

Oceans should continue to provide for a substantial portion of the world's protein needs. The past trends of overfishing, wide-scale disruption of coastal habitats and the rapid expansion of non-sustainable aquaculture enterprise, however, threaten the world's food security (Watson and Pauly, 2001). An effective fisheries management is the needed answer to improve the economic situation of fishing communities. Part of the solution is to reduce discards by finding market-based approaches that will increase the value for all by-catch fish.

Discards constitute a substantial part of the worldwide catch that is every year returned to the sea (Catchpole *et al.*, 2017) (Figure 1-2). Reasons for discarding include legal obligations, e.g. minimum landing sizes (MLS) and target species quota exhausted. Discard of by-catch species are usually associated to their economic value (e.g. low or no market value) (Damalas, 2015). By-catch occurs when fishing gear is nonselective, so that both targeted and non-targeted species are caught from the same effort (Melstrom, 2015). High-levels of discards have been considered a problem in European and global fisheries for many years. Discards vary throughout EU fisheries – in some cases representing more than 60% of the catch, while in other cases – including pelagic fisheries – could be very low (STECF, 2015). From these, 11% of the EU total discards are of fish under MLS, also usually from choke species (STECF, 2015).

The European Commission (EC) has identified the 'discard problem' as a driver of poor economic performance and a significant component of marine ecosystem functioning (Commission's green paper on the reform of the CFP-COM 2009/163 final). Towards eradicating this problem the reformed Common Fisheries Policy (CFP—EU regulation1380/2013) introduced the landing obligation (LO) to all catches. The LO regulation was introduced on 1 January 2015 for small pelagic fish which have a total allowable catch (TAC) such as mackerel, herring and sprat, and is being rolled out to all demersal and remaining species which have TACs in a phased manner between 2016 and 2019 (Catchpole *et al.*, 2017).

In addition, numerous technical regulations and associated amendments have been introduced in almost all developed fisheries worldwide in an attempt to improve fishing gear selectivity, reduce discards and enhance the status of fish stocks (Alzorriz *et al.*, 2016 and references therein). Passive gears, such as gillnets, are characterized by low retention probabilities at small length classes, as well as at large length classes, with the catch of medium-sized length classes. In trawls and other active gears, the selective pattern allows smaller fish with specific morphological characteristics of passing through the meshes and escaping, whereas larger fish is being retained in the codend (Stepputtis *et al.*, 2016). The gear size selectivity is an important achievement, since capture and discarding of small immature fish reduces the potential biomass of the exploitable stock and affects subsequent recruitment (Graham, Kynoch and Fryer, 2003). Although, the capture of larger individuals induces early maturation at smaller sizes, leading to reduced fecundity and also fisheries yield reduction (Kuparinen and Merilä, 2007). Thus, gear selectivity can have impact at the community level: not only remove large individuals of the target species, but also decrease the relative abundances of species with the capability to grow to large sizes (Jennings, Greenstreet and Reynolds, 1999). Trawls and active gears have also marked impacts on the ocean substratum. Physical disturbance of the substratum results from direct contact with the fishing gear and the turbulent resuspension of surface sediments. The magnitude of the impact is determined by the speed of towing, physical dimensions and weight of the gear, type of substratum and strength of currents or tides in the area fished. The effects may persist for a few hours in shallow waters with strong tides or for decades in the deep sea (Jennings and Kaiser, 1998). Direct consequences include the modification of the seabed by the physical destruction, the removal of benthic communities and the direct mortality of individuals, while indirect effects include enhanced mortality of damaged individuals, changes in the sediment biogeochemistry and alterations in the food web structure (Jones, 1992; Jennings and Kaiser, 1998; Clark *et al.*, 2016). Therefore, understanding fisheries-induced evolution is important for sustainable management of a particular fish stock, but also for conservation of the ecosystem as a whole.

One of the most important objectives of the Common Fisheries Policy (CFP) in the EU is the implementation of a maximum sustainable yield (MSY) of marine resources based on environmental, social and economic sustainability. In European fisheries, MSY has still not been achieved for all economically valuable fish stocks. According to the Food and Agriculture Organization of the United States (FAO), 31.4% of the assessed fish stocks were considered overexploited, 58.1% fully exploited and just 10.5% of the stocks is underfished (FAO, 2016).

1.2 Stock assessment

Stock assessment involves the use of data from commercial fisheries and scientific surveys to feed mathematical/statistical models and produce estimates of the size and the productivity of a marine population, or one of its geographical/biological components. Then, these estimates were used to simulate the future catch levels corresponding to expected levels of fishing mortality and stock biomass, which illustrate the consequences of alternative management options on the short, medium or long term (Hilborn and Walters, 1992) (Figure 1-3). Finally, the harvesting decision results on an agreed total allowed catch (TAC) which is generally in line with a compromise between conservation, economic and social sustainable objectives. TAC's can either be directly estimated, based on pre-agreed harvest-control rules, or the criteria can vary from year to year.

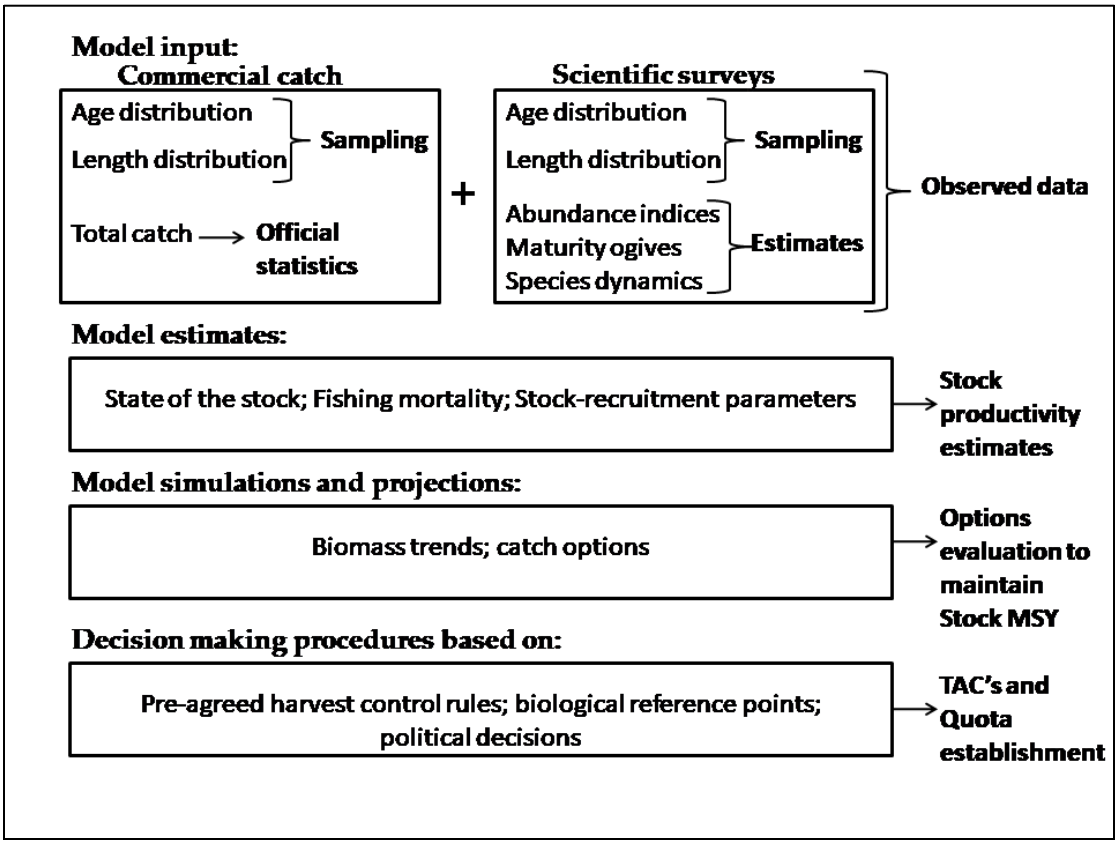

Figure 1-3 Data flow of information in general stock assessment practices.

Modelling of fish population dynamics has advanced and diversified immensely in the past decades. In both theoretical and practical applications, model complexity has increased to account for spatially and temporally disaggregated processes within fish populations, and to account for dynamic interactions between the components of fish ecosystems (Quinn and Deriso, 1999). Sophisticated statistical and computational approaches (e.g. Bayesian and state–space modelling) have been applied to estimate fish population parameters by incorporating more diverse types of data, and by accounting for differing error structures and uncertainty in models and data. Methods of quantitative decision analysis have also been applied to account for more rigorous management advice (Quinn and Deriso, 1999; ICES, 2010). Models and their subsequent hypothesis are subject to several assumptions. The fisheries scientists have always recognize that the results coming out of a stock assessment model are only as good as the data going into it. That is why, apart from including the uncertainty into the models, they also set up standards for collecting information and ensuring data quality (Sullivan *et al.*, 2006).

The management of commercial fisheries clearly requires a good understanding of the characteristics of each stock as a population unit. Notwithstanding, the science that is used to assess commercially exploited species is still dominated by the concept of cohort's dynamics developed by Pope (1972), for single-species assessments (ICES, 2010). A more ecosystem-based approach (EBA) have been voiced in opposition to the classical single-species approach (ICES, 2010) and its application although is already used on a large number of stocks (Pauly and Zeller, 2017) is not wide spread (Figure 1-4). Although the EBA models seems to be promising, the paucity of data and the demands of multiparameterized multispecies models mean that most ecosystem considerations in practical stock assessment still tend to be ad hoc manipulations of the single-species approach (Beddington, Agnew and Clark, 2007).

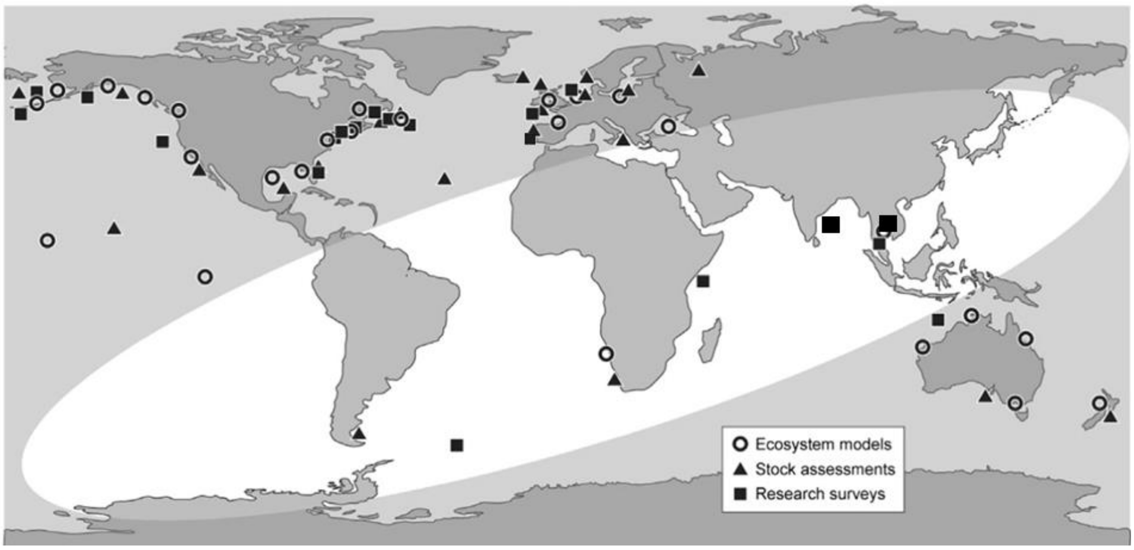

Figure 1-4 An overview of part of the global fisheries assessment, based on ecosystem and stock assessment models and on data collected under scientific research surveys. The white circle represents the developing or underdeveloped countries and the grey area represents the developed countries. Only 7 fish case studies from Asia, Africa and South America (i.e., continents with immense fisheries) and 59 from developed countries in North America, Europe and Oceania, were identified in the map (from Pauly and Zeller, 2017).

The complexity of assessing stock abundance and the main constraints of models application have already been largely addressed and are well known. The majority of the models are age-based, but age and growth could be a problem for some stocks when ageing is inaccurate. Age reading workshops are regularly conducted to calibrate age classification between the stock readers and to maintain the accuracy and precision on the single-stocks data. When age validation studies are not conducted age classification relies on the experts and exchange guidelines are based on unstandardized otolith ring structure interpretation. In the northern and southern hake stocks, tagging data experiments have been conducted and indicated that growth was considerably faster than what would be consistent with the age-length keys (ALKs) used (obtained from otolith readings) and no new otolith reading criteria was agreed. As a consequence, a length-structured model replaced the previous age based model in the hake assessments (ICES, 2010).

Projections of results from stock assessment rely on the assumption that the expected number of recruits (juveniles) is a function of mature female biomass (spawning-stock biomass - SSB) (Quinn and Deriso, 1999). These stock-recruitment functions (SRR) can take the form of a Ricker model, Beverton-Holt model, segmented regression or a constant recruitment (Quinn and Deriso, 1999; Patterson *et al.*, 2001; Cotter *et al.*, 2004). Even though these functions (except constant recruitment) reflect a (biological) parental relation (e.g., Brannstrom and Sumpter, 2005), the choice of the function is typically based on goodness-of-fit between stock biomass results and corresponding recruitment. But despite the caveats the choice of a stock-recruitment relationship is a fundamental tool for the prediction of the future population thresholds under alternative management actions (Hilborn and Walters, 1992; Kuparinen *et al.*, 2012).

The instantaneous rate of natural mortality (M) in exploited fish stocks is defined as the mortality due to, all the other causes than fishing, such as predation, cannibalism, disease, spawning stress, starvation, and senescence. This parameter is one of the most critical on stock assessment models (Quinn and Deriso, 1999) and thus produces significant uncertainties in scientific based management (Zhang and Megrey, 2006). Direct measurements of M are often difficult to obtain (Kuparinen *et al.*, 2012), because is necessary to separate this rate from the instantaneous rate of fishing mortality (F) in non-migrated stocks; and only in non-explored fish stocks is possible to accurately estimate it (Pauly, 1980; Gunderson and Dygert, 1988; Schnute and Richards, 1995; Sinclair, 2001; Zhang and Megrey, 2006). For practical reasons natural mortality is commonly assumed to be independent of the age and (or) size of an individual and not a reflect of environmental variability (Schnute and Richards, 2001), and so most stock assessments treat this parameter counterintuitive as a known constant (Patterson *et al.*, 2001; Cotter *et al.*, 2004; Gislason *et al.*, 2010). Some assessments go beyond this assumption and incorporate an age dependent natural mortality, whereas most advanced studies already account for uncertainty and temporal variability in natural mortality (ICES, 2010).

Maturity is typically assumed to be age dependent, and maturity at age is usually an input of age based assessments (Patterson *et al.*, 2001). For the majority of stocks the maturation process (maturity ogive) refers only to females, relying on macroscopic gonad observation to produce a fixed female maturity ogive at age not accounting to changes across years and areas. However, the sensitivity analysis on some stock assessments do acknowledge variability in the maturation process (ICES, 2010).

1.3 Blue whiting

Blue whiting (*Micromesistius poutassou*) is a migratory mesopelagic gadoid (Figure 1-5) that is widely distributed in the eastern part of the North Atlantic.

Figure 1-5 Blue whiting (*Micromesistius poutassou*) (Risso 1826).

The highest concentrations are found during spawning along the edge of the continental shelf in areas west of the British Isles and on the Rockall Bank plateau where it occurs in large schools at depths ranging between 200m and 600m, but is also present between the Barents Sea and the Strait of Gibraltar, west to the Irminger Sea, on the Mediterranean and Adriatic Seas (Figure 1-6) (Bailey, 1982; Monstad, 1990; ICES, 2016c). The main spawning ground is located west of the British Isles at the shelf edge and at the Porcupine Bank, stretching northwards to the Hebrides (ICES, 2016c). Significant spawning aggregations have also been observed across the Rockall Trough, on Rockall Bank and Hatton Bank (Hátún *et al.*, 2009) and on the continental shelf off Spain and Portugal (Kloppmann *et al.*, 1996; Silva, Pestana, *et al.*, 1996).

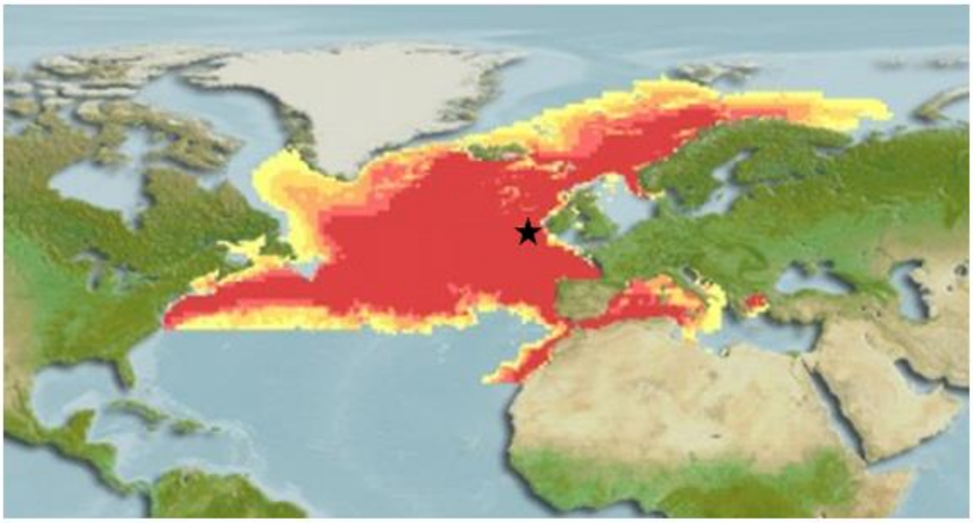

Figure 1-6 Distribution map of blue whiting. Distribution range colours indicate degree of suitability of habitat which can be interpreted as probabilities of occurrence, at light yellow – minimum values (relative probabilities of occurrence between 0.01 and 0.19) and at red – maximum values (relative probabilities of occurrence between 0.80 and 1.00) (from www.aquamaps.org, version of Aug. 2016. Web. Accessed 8 Sep. 2017). ★ - Porcupine Bank.

The major nursery grounds spread along the Norwegian coast (possibly in fjords), to the south west of Iceland, along the continental shelf-edge south of Porcupine Bank (Bailey, 1982), on the continental shelf off Spain and Portugal (Carrera *et al.*, 2001) and in the Bay of Biscay (Ibaibarriaga *et al.*, 2007). On the shelf-edge and slope, the species has a typical pelagic behaviour with diel vertical migrations apparently of trophic nature while on the shelf it remains close to the bottom in a typical demersal behaviour (Bailey, 1982). The average size of blue whiting has been observed to increase with depth (Bailey, 1982).

Feeding grounds extend from the Portuguese coast to the Bay of Biscay, Celtic Sea, and all along the continental slope as far as the Norwegian Sea (Skogen, Monstad and Svendsen, 1999). Large-scale seasonal migrations are described for this species, from the main spawning grounds along the Irish–Scottish shelf edge to the feeding areas after spawning and in the late-winter (January-March) return again to the spawning grounds (Bailey, 1982; Trenkel *et al.*, 2014).

Blue whiting plays an important role in the ecosystem along their whole range of distribution, both by consuming zooplankton and small fish, and by providing a food resource for larger fish and marine mammals (Silva, Azevedo, *et al.*, 1996; Cabral and Murta, 2002; ICES, 2009). Blue whiting diet is mostly composed by crustaceans; the juveniles feed mainly on copepods and Euphausiacea (mostly *Meganicthyphanes norvegica* and *Nicthyphanes couchi*), but with growth, decapods and fish (mainly myctophids) gradually increase in relative importance. Seasonality in the diet is evident, the decapods, *Pasiphaea sivado*, is the most important prey in summer and autumn, being replaced by the euphausid, *Meganyctiphanes norvegica*, in winter (Cabral and Murta, 2002).

In terms of biomass this species is one of the most abundant teleosts in the North Atlantic (ICES, 2016c). Its economic significance increased considerably since their commercial exploitation began in 1970 (Standal, 2006), is also considered one of the largest fishery in the Northeast Atlantic (Bjørndal, 2009).

1.3.1 Biology

In the entire area of distribution spawning starts in January in the southernmost distribution area in the Iberian waters and progresses northwards to the latitudes of the Faroes. In the coast of Portugal, peak spawning is from February to March (Cunha, 1992); March and April is the spawning season in the regions adjacent to the North Sea (Bailey, 1982).

Blue whiting has a long life span (15 years), can reach a total length of 40cm to 45cm and an individual weight of 300g to 500g. Growth is sexually differentiated, females present higher growth rates and larger sizes than males (Monstad, 1990; Trenkel *et al.*, 2015). Length at first maturity is 18cm until 24cm and becomes smaller as one moves south along the distribution range of the species (Bailey, 1982; Cunha, 1992; Mazhirina, 1994). The age of first maturity ranges between 1year and 4years (Raitt, 1966; Cunha, 1992; ICES, 2016c).

Blue whiting otoliths have proven to be quite difficult to age, and though guidelines have been constructed, the experience of the reader determines the interpretation of the otolith structure. In order to overcome those difficulties, age reading calibration exercises and workshops were regularly conducted on blue whiting (see ICES, 2013 for an overview). The last workshop took place in June 2017 and was preceded by an otolith exchange (ICES, 2017b). The overall agreement with modal age of the pre-workshop exchange exercise was 64.1% considering all readers and 70% for the assessment readers. During the workshop 129 otoliths with annotations were discussed in plenary and around 90% agreement was achieved. There were no clear signs of seasonal misinterpretations on otoliths ageing, but the Mediterranean and most northern areas (ICES area 27.14.b and NAFO 1C) proved to be quite difficult, due to their different growth patterns (ICES, 2017b). In the blue whiting age classification, the most reoccurring problems among age readers were still the identification of the position of the first annual growth ring, false rings and interpretation of the otolith edge (ICES, 2013, 2017b).

1.3.2 Stock structure

The blue whiting population composition and dynamics require continued monitoring, due to the large population size, its considerable migratory capabilities and wide spatial distribution (ICES, 2016c). Prior to 1993, for assessment purposes, it was assumed that blue whiting had two components, a northern and a southern component. The northern stock was known to feed in the Norwegian Sea and spawn to the west of the British Isles. The southern stock was found along the continental shelf off the coast of Spain and Portugal with the main spawning areas towards the Porcupine Bank (Figure 1-6). The Porcupine Bank was considered a transitional area between the two main stocks (ICES, 1990). In 1993 it was argued that there was no strong evidence to maintain this division between the two stocks. Results from an otolith age reading workshop at that time showed no significant difference in mean annual ring diameter between northern and southern stocks. The Advisory Committee on Fishery Management (ACFM), based on these results, agreed combine the two stocks for assessment (ICES, 1995). Since then this stock has been assessed by the International Council for the Exploration of the Sea (ICES) as one unit for the whole Northeast Atlantic (NEA), which corresponds to the ICES areas 27.1 to 27.9, 27.12 and 27.14.

Several approaches have been employed to investigate the stock structure of blue whiting. The studies relating to genetics, larval otolith growth patterns, the movements of eggs and larvae and otoliths shape analysis, have been published in recent years. The first genetic work was carried out in the early 1990s. This study included samples from most of the eastern Atlantic but the amount of samples from the southern part of this area was generally low (Mork and Giæver, 1995). Further work revealed significant geographic heterogeneity with reproductive units found at the fringes of the distribution range (Giæver and Stein, 1998). A genetically distinct population was found in the Barents Sea and potential populations identified in the Mediterranean and Romsdalsfjord area of Norway. Samples taken from the area west of the British Isles and from the Norwegian Sea were genetically similar, which suggests a single blue whiting stock throughout the area (Giæver and Stein, 1998). Genetically distinct populations were also found in the Barents Sea and Mediterranean by using one minisatellite and five microsatellite loci (Ryan, Mattiangeli and Mork, 2005). Temporal variation was also seen between samples collected on the main spawning area. In this case there was insufficient data to identify explicitly the geographic range of these possible stocks. A study conducted by Was *et al.* (2008) used a landscape genetics approach which combines spatial and genetic information to detect barriers to gene flow. This microsatellite analysis found that samples collected and analysed from along the south flowing current from the Porcupine Bank, i.e. the Celtic Sea and Bay of Biscay, were genetically different from those in the north-flowing current. Temporal variation was seen in samples collected in the Rockall Bank area but the discussion of possible reasons for this variation was inconclusive.

Oceanographic modelling has been used to examine movements of blue whiting eggs and larvae. Larval drift is considered an important factor in recruitment. A three-dimensional baroclinic model suggested that particles released on the Porcupine Bank drifted southwards with a separation at about 53 and 54ºN (Bartsch and Coombs, 1997). This work gave some additional information about stock separation but suggested that the division might be more southerly. Additional testing of the use of this type of model was recommended by the authors. This hypothesis was put forward by Skogen, Monstad and Svendsen (1999), based that the southern stock will spawn in an area where the eggs and larvae are likely to drift southwards and the northern stock where the eggs and larvae will drift northwards, the modelled drift patterns revealed a possible separation line located at 54.5ºN, but with a significant inter-annual variability found over the twenty years studied.

An investigation of larval growth histories was carried out in 2007 (Brophy and King, 2007). The hypothesis behind was that groups that are spatially or temporally distinct after hatching show measurable differences in the larval portion of the otolith. This study has shown that larvae from the Bay of Biscay grow faster than those from more northerly spawning areas. It also confirmed that fish spawning to the west of Ireland and Scotland, do not form a randomly mixing unit and that subunits within this aggregation have experienced differences during the larval phase. It was also hypothesised that the dispersal of larvae could influence the subsequent dispersal of spawning adults. Thus, the fish that are found in the feeding assemblages throughout the distribution may not contribute equally to the spawning assemblages in the north and south of the spawning grounds.

Results from length-at-age and otolith shape analysis presented at the ICES benchmark workshop on pelagic stocks (WKPELA) in 2012, did not provide evidence of two separate stocks, but rather substantial mixing of individuals on the common spawning grounds (west of British Isles) (ICES, 2012). At the benchmark following the full review of available studies on blue whiting stock structure in the Northeast Atlantic, the conclusion was that no scientific evidence exists in support of multiple stocks with distinct spawning locations or timings on blue whiting. The emerging picture from the stock structure revision in this group was that this species in the NEA is a single stock whose large-scale spatial distribution varies as a function of hydrographic conditions and total abundance; this is commonly described as an abundance-occupancy relationship. There seem to be a number of core nursery and feeding areas with marginal areas being occupied at times of high stock abundance. As a result, it was recommended to keep treating blue whiting as a single stock.

In 2014, a study based on Continuous Plankton Recorder (CPR) data from 1948–2005 using modern statistical techniques indicated a clear spatial separation between a northern spawning area, in the Rockall Trough, and a southern one, off the Porcupine Seabight. In addition, the results showed that spawning started earlier in the southern area (by at least one month), with peak spawning occurring later moving north (Pointin and Payne, 2014).

Otolith-shape analysis has recently shown to be able to reliably identify the stock origin of sampled fish (Keating *et al.*, 2014; Mahe *et al.*, 2016). Those studies revealed distinct morphotypes, from fish occupying distinct geographical distribution areas. This findings support the hypothesis of northern and southern components in the blue whiting population which may overlap to varying degrees in the centre of the spawning distribution.

The ICES Stock Identification Methods Working Group (SIMWG) reviewed the evidence of separate stocks in 2014 (ICES, 2014) and concluded that the perception of blue whiting in the NEA as a single-stock unit is not supported by the best available science. SIMWG have recommended that blue whiting must be considered as two units. However, there is a lack on the information available that can be used as the basis for generating advice on the status of the individual stocks. There is still a need for more information regarding population structure in these stocks, mainly regarding the structure of the southern population unit. In order to have the proper basis to assess this stock separately as two components, growth and reproduction patterns, structure and dynamics of the southern component must be studied and made available.

1.3.3 Stock assessment

For the assessment of the blue whiting combined stock catch data are annually submitted by the member states with fleets exploring this important marine resource in NEA. Since 1988, 19 national fleets have been involved in the blue whiting fisheries (Denmark, Estonia, Faroe Islands, France, Germany, Greenland, Iceland, Ireland, Lithuania, Netherlands, Norway, Poland, Portugal, Spain, Sweden, Russia, UK(England and Wales), UK(Northern Ireland) and UK(Scotland)). The highest concentrations of catches are generally taken along the edge of the continental shelf in the area west of the British Isles (main spawning area), on the Rockall and Hatton Banks and around the Faroe Islands, smaller quantities of blue whiting are caught along the coast of Spain and Portugal (part of the southern area) although an increase has been observed after 2011 (Figure 1-7). Notwithstanding, in 2015 the Portuguese and Spanish blue whiting catches only represented 2% of total catches in the Northeast Atlantic (ICES, 2016c).

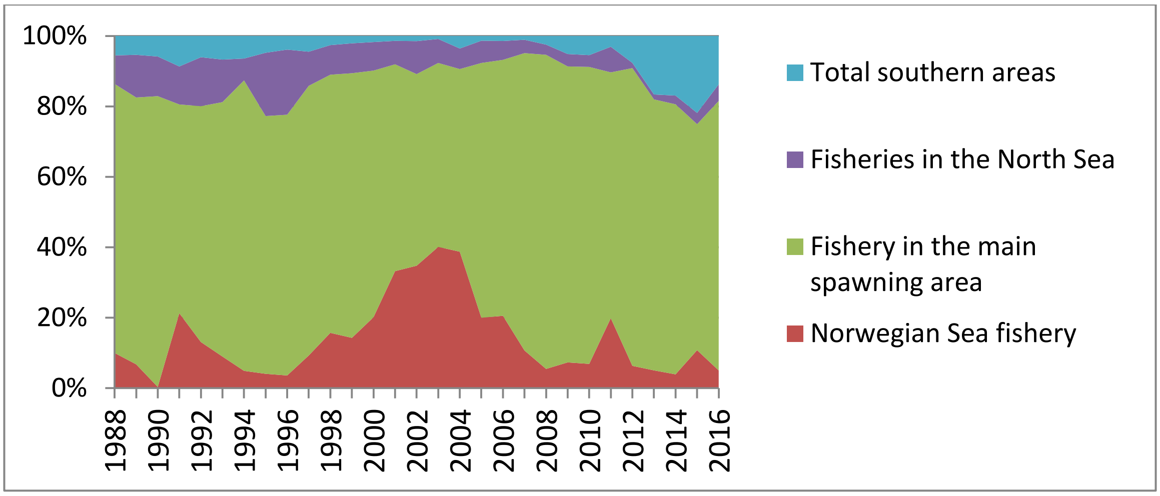

Figure 1-7 The percentage contribution to the overall catch by fishery area (southern and northern: North Sea, main spawning area and Norwegian Sea) from 1988 until 2016 (from ICES, 2017a).

The majority of blue whiting catches are made in the first half of the year (quarters 1 and 2), corresponding to around 90% of the annual catches since 2007 (Figure 1-8) (ICES, 2016b, 2016c).

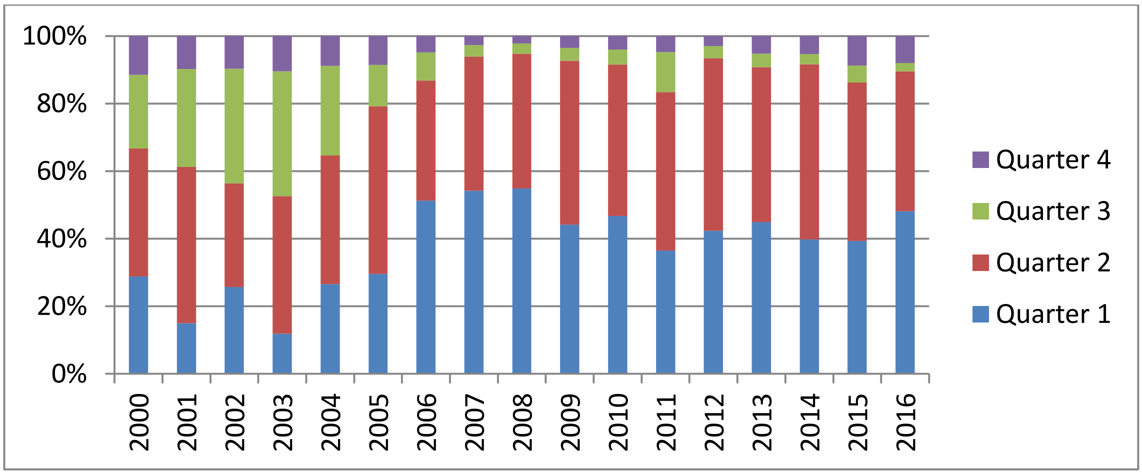

Figure 1-8 Distribution of blue whiting catches (in percentage) by quarter from 2000 until 2016 (from ICES, 2017a).

The total catches considered on assessment comprised apart from the total landings also the total amount of discards since 2014. In general, discards are assumed to be minor in the blue whiting directed fishery from Norway, Iceland, Faroe Islands and Russia. The only exceptions are the discards from Portugal and Spain which constitute respectively around 40% and 20% of the total catches (ICES, 2016c).

The catches are allocated to an age at length distribution (ALKs) and converted into catch-at-age data before submitted to assessment. In the cases where no sampling is available to obtain the ALKs, the allocation is made by fleet (metier), ICES area and quarter, after submission during stock data aggregation as input for the assessment. Besides the catch-at-age data, survey data from the international blue whiting spawning stock survey (IBWSS) conducted during the first quarter (March-April) provides stock abundance and recruitment indices for assessment. Five countries participate annually in the survey; Russia, Norway, Faroes, the Netherlands and Ireland.

A fixed maturity-at-age ogive is used in the assessment since 1994. These values were obtained by combining maturity ogives from the southern and northern areas, weighted by catch in numbers-at-age (ICES, 1995).

The natural mortality (M) is assumed to be 0.2 by year; this value was derived from investigations undertaken in the 1980s that examined the age distribution of the stock before the industrial fishery started. The last attempt to revised M was made during the ICES benchmark workshop (WKPELA) in 2012. The relationship between natural mortality and body weight was then applied to the blue whiting data to determine a variable M by age. The values obtained ranged from around 1.1 at age 0 to 0.7 at age 10, which were considerably higher than the value currently used (0.2) and the group concluded that more studies were still needed (ICES, 2012).

The main data (catch-at-age, survey data, maturity-at-age ogive and M) were used to assess the state of the blue whiting stock, by applying an analytical assessment model, the State–space Assessment Model (SAM) (Nielsen and Berg, 2014; Berg and Nielsen, 2016).

The blue whiting stock was in risk to collapse in the first decade of 2000's, due to the lowest observed year classes between 2006 and 2009 (Figure 1-9 a)). Consequently, as a result of low recruitment, the SSB was also declined for the same period (Figure 1-9 b)).

The adopted measure to recovery the state of the stock, was reducing the fishing pressure (lower fishing mortality) (Figure 1-9 c)), thus the advice catches and consequently the effective catches for the next years were reduced (Figure 1-9 d)) (ICES, 2016c).

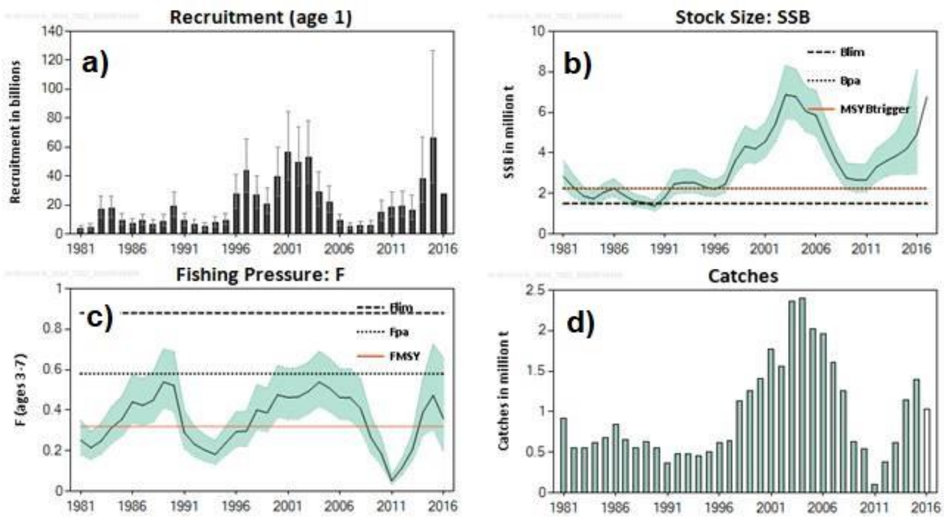

Figure 1-9 Blue whiting stock estimates from 1981 until 2016: a) recruitment estimates (billions); b) spawning stock biomass estimates (million tonnes) for the blue whiting stock from 1981 until 2016. The dashed lines correspond to the established values for B_{lim} and B_{pa} (equally to MSY $B_{trigger}$) for blue whiting stock; c) fishing mortality (for ages between 3 and 7 years). The dashed lines correspond to the established values for F_{lim}, F_{pa} and F_{MSY} for blue whiting stock; d) total catches in million tonnes (from the 2016 advice sheet of whb-comb stock ICES areas 27.1-9, 12 and 14 (ICES, 2016c)).

The SSB of blue whiting increased since 2010 this is mainly due to the large 2014 and 2015 year classes (Figure 1-9 a) and b)). These larger year classes entered into the fishery in 2017, and the ICES advised catches for the recent years 2016 and 2017 has been increasing (ICES, 2016c).

1.3.4 Portuguese coast component

Blue whiting is commonly caught as by-catch by the Portuguese bottom trawl fleet composed by 128 vessels under 30 meters long, of which 25 have licenses for fishing crustaceans and 103 for fin-fish (ICES, 2016c; Bueno-Pardo *et al.*, 2017). Some vessels of the artisanal and purse-seine fishing fleet also catch blue whiting as by-catch, although this is mostly discarded. Blue whiting is rarely used for human consumption in

Portugal and there is some demand for industrial transformation (ICES, 2016c). Most recently, a high percentage of the landed blue whiting is purchased by the Spanish industry for transformation. The total catches (tonnes) for the Portuguese fleet are represented in Figure 1-10. The discard sampling program onboard commercial fleet vessels started in 2004 under the Portuguese data collection framework (DCF - EU program)[1] and the estimates of the total amount of discards for blue whiting were available since then. The coefficient of variation (CV) of the estimated discards varied between 18 and 60%, for the period between 2004 and 2013 (Prista *et al.*, 2014).

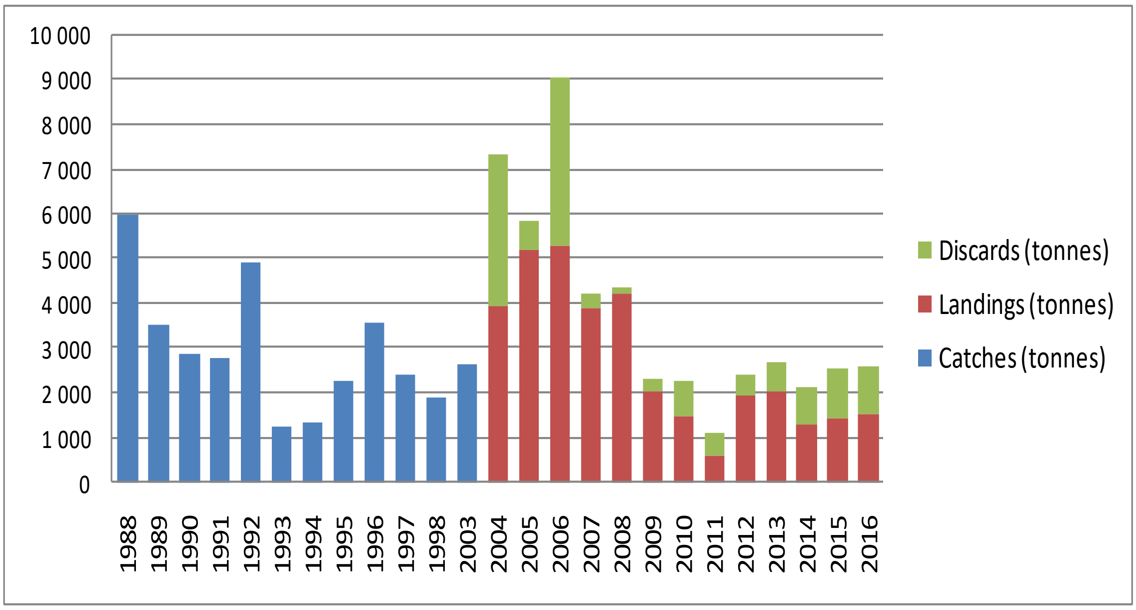

Figure 1-10 Blue whiting total catches (tonnes) from the Portuguese fleet, for the period between 1988 and 2016. For the period 2004 until 2016, the total catches were decomposed in the amount of landings (tonnes) and in the amount of discards (tonnes) (data from DGRM – landings; and IPMA – discards).

In the late 1990's the majority of the blue whiting was landed at the northern part of Portugal (Matosinhos). However, since 2004 the highest percentages of landings are from the southerly ports of Sines and Vila Real de St. António (Figure 1-11). Mainly because an increase of the blue whiting in the total catches from the bottom-otter trawls fishing crustaceans (Bueno-Pardo *et al.*, 2017), a target species on this fleet.

[1] named as Plano Nacional de Amostragem Biológica (PNAB)

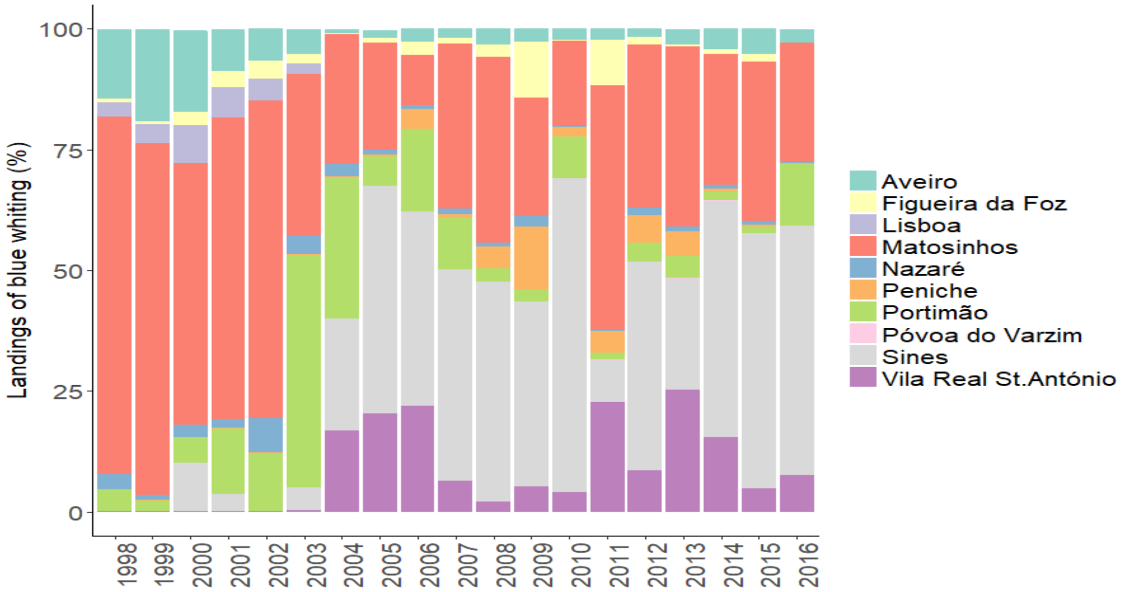

Figure 1-11 Blue whiting landings by port (percentage) in the Portuguese coast, for the period between 1998 and 2016 (data from DGRM).

Part of the landed blue whiting instead of going to auction to be sold, goes directly to the purchaser, owing to a sale contract previously defined with the fishery vessel owner. The most evident difference between the blue whiting sold in auction and by contract is the fish total length, in the sales by contract the fish were generally larger (Figure 1-12).

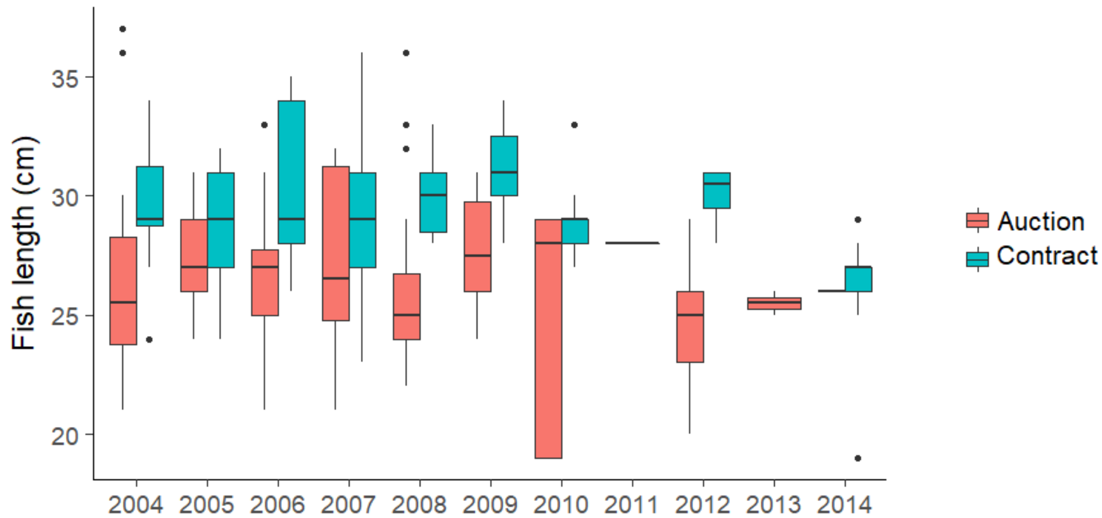

Figure 1-12 Total length (cm) of the landed blue whiting sold at auction and by contracts between 2004 and 2014. This data has been collected under the PNAB discards sampling program. The centre line of each box represents the median; the box limit indicates the 25th and 75th percentiles, the whiskers delimit the non-outliers range and the points represent the outliers (data from IPMA).

In 1998 (May), 1999 (February) and 2000 (April), three acoustic surveys aiming the study of blue whiting, were conducted in the Portuguese coast. The main result of those acoustic surveys were: no significant differences in the fish length distribution were observed in the schools from the same area (station) sampled by bottom or by pelagic trawl. Along the day, some vertical migration was registered at punctual sample locations at 150m, but at deeper waters (400m) this behaviour was not observed. Since these surveys did not cover the whole Portuguese coast didn't provided abundance or recruitment indices, their contribution was on the study of some aspects on the biology and dynamics on this species.

The Portuguese bottom trawl survey (PBTS) has been conducted since 1980, one among other aims is collecting the blue whiting data on abundance and distribution along the Portuguese coast (ICES, 2016a) (Figure 1-13). Although, for the recent years the exploratory pattern (fishery fleet) in Portuguese waters is more intensive at southern areas, in generally the major abundances were observed at the northern coast along the years.

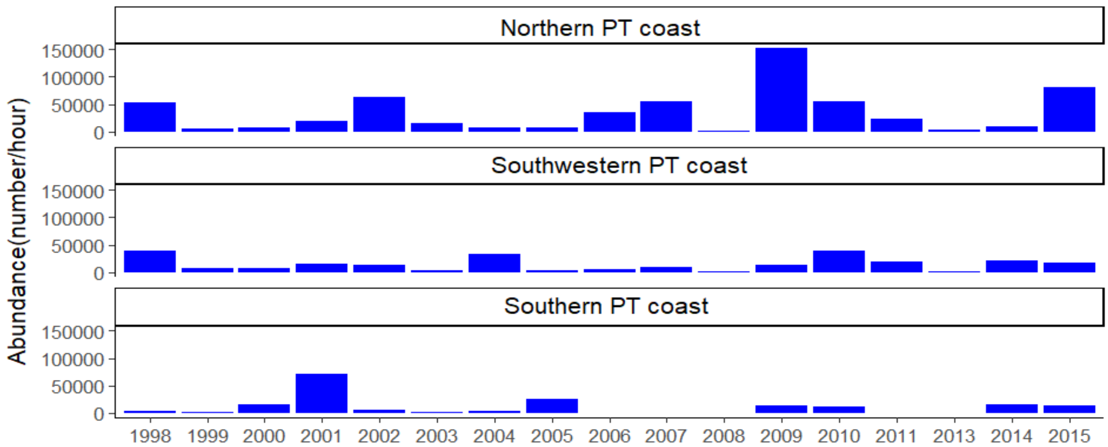

Figure 1-13 Blue whiting abundance (number per hour) for the northern (from Caminha to Lisboa), southwestern (from Lisboa to Sagres) and southern (from Sagres to Vila Real de St. António) off Portugal (PT) between 1998 and 2015, from the PBTS autumn surveys (data from IPMA).

The blue whiting abundance in numbers per hour of trawling by length class (cm) since 1998 is presented in Figure 1-14. Recruitment estimates were also available from this time-series.

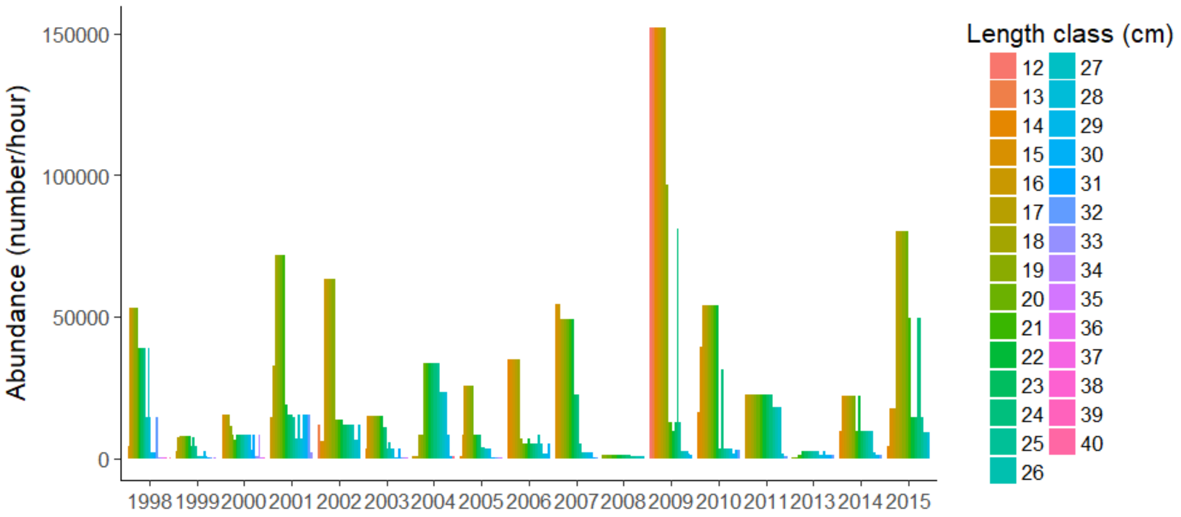

Figure 1-14 Blue whiting abundance (number per hour) by length class (cm) between 1998 and 2015, from the PBTS (data from IPMA).

In the recent years, two strong year classes, 2009 and 2015, were evident on blue whiting off Portugal (Figure 1-14).

The blue whiting data for Portuguese coast were available from the monthly biological sampling (1995-2016) (collected under PNAB) and from the demersal surveys (PBTS) (1990-2016), those time-series constitute valuable inputs to the understanding of this species complexity at their southern border limit of distribution.

1.4 Aims and importance of the thesis

The present work aims to fill important gaps in the biology and structure of blue whiting at the southern part of stock distribution, the Portuguese coast. Besides, this work also intends to contribute to the knowledge enlargement on the blue whiting assessment.

Growth, population size-structure and reproduction, relevant traits to support stock delineation in fisheries assessments, were analysed for the coast of Portugal and compared with the life-history parameters described for blue whiting at northern areas.

The sex ratio is a key parameter for assessing the reproductive potential and stock status of exploited fish populations. Therefore, the knowledge on spatial and temporal sex ratio variation can inform on the capacity of a population to support exploitation and environmental changes. The seasonal patterns in the blue whiting sex distribution, size structure and condition in three areas along the Portuguese coast (north, southwest and south) were studied based on bottom trawl surveys performed in autumn and winter.

The age reading on blue whiting revealed to be a difficult task, dependent on readers guidelines interpretation and on their experience. The assessment model used on this species is age-based. Thus, a misinterpretation on ageing could have impact on the stock status perception. The need to improve the age reading process, becoming more objective and accurate, was the trigger point to develop an automatic image analysis tool to ageing blue whiting otoliths. This system aims to be used as a routine and to be a helpful tool on recording of ageing classification and on training new age readers.

The increasing use of image processing techniques as a tool for age reading and stock identification, demands an enormous number of otolith images to be recorded. The type of symmetry between the left and right otolith has been described as a species characteristic. The influence of the otolith surface (distal *versus* proximal) from which the images were recorded on the results from symmetry studies, was investigated on blue whiting. This study intends to highlight the special attention, which should be made when recording images to be stored in a database for future studies.

Species distribution limits have showed to be important sources of genetic variability since these populations are often isolated by distance and present differentiation from the rest of the populations. Therefore, the Portuguese Coast, as the southern distribution limit of the species in the Northeast Atlantic is of crucial importance to have a realistic scenario of the population dynamics of the blue whiting. Therefore, microsatellite genetic markers and otolith shape methodologies were applied to study the population structure of blue whiting along the coast of Portugal.

1.5 Thesis outline

The present thesis comprises five scientific papers published or in review in peer reviewed international journals. Each paper corresponds to a chapter and with the exception of chapters 1 (General Introduction), 7(General Conclusion) and 8 (Future Research), each one has its own Introduction, Material and Methods, Results, Discussion and References.

In chapter 2, the gaps of knowledge concerning the blue whiting life history parameters, (growth, maturity) and females spawning stock biomass for the Portuguese coast are filled in.

The blue whiting sex-ratio, size distribution and condition patterns off Portugal are studied, based on data from the Portuguese scientific surveys, in chapter 3.

In order to improve the accuracy and precision on age classification of blue whiting otoliths, an automatic image analysis procedure has been developed (named as “OtoRing”), and is presented in chapter 4.

In chapter 5, the otolith surface’s (distal *versus* proximal), from which the images were recorded, and its influence in the diagnostic of the symmetry shape between the left and the right otoliths on blue whiting are examined.

Microsatellite genetic markers and otolith shape analysis of blue whiting are used as an integrative approach to assess population structure off the Portuguese coast in chapter 6.

In chapter 7, the major conclusions are addressed regarding the studies presented here, on the biology and structure of blue whiting along the Portuguese coast.

In the final chapter, the next steps and perspectives for future research on this challenging study are included.

1.6 Thesis structure layout

The thesis structure layout and the interaction between the chapters are presented in the next figure (Figure 1-15):

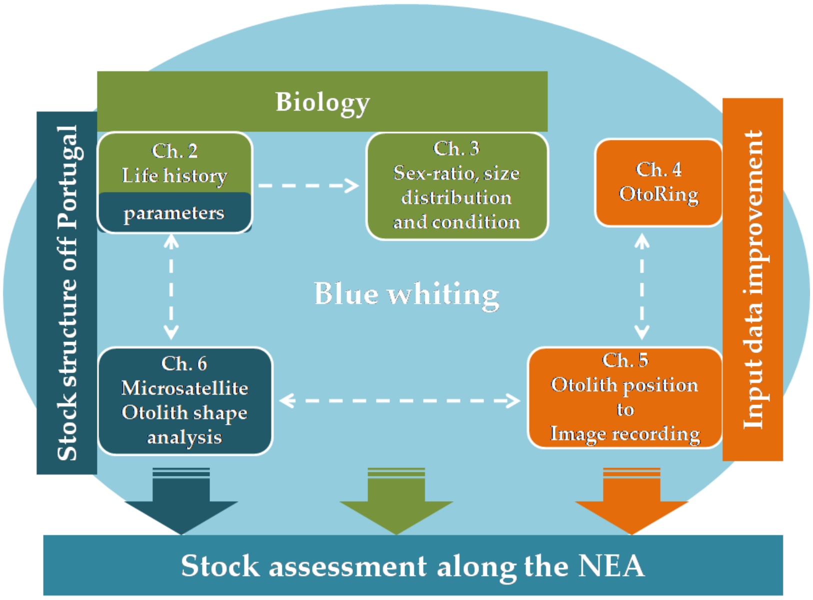

Figure 1-15 Thesis structure and the interaction between chapters.

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Chapter 2

2. Blue whiting (*Micromesistius poutassou*) life history parameters: evidences supporting the stock structure delineation

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In review in Marine Policy

2.1 Abstract

Blue whiting is an abundant teleost widely distributed in the eastern part of the North Atlantic. This species was considered to comprise at least two stock units, but is assessed as a single stock. The gaps on what concerns blue whiting southern distribution need to be fulfilled, to allow this species management as two separate stocks. In this study, blue whiting life history parameters data (growth, maturity) and female spawning stock from Portuguese surveys (1990-2011) were analyzed. Differences were found in the growth rate between the studied area and the northern part of the stock. In the studied area, fishes grow faster and achieve the first sexual maturity at 20 cm with an age until 2 years. Timing of spawning was also different between the Portuguese Coast and the west of British Isles. The female spawning stock for the Portuguese coast seems relatively stable around the mean since 2005, contrarily for the whole stock a decline was observed from 2007 until 2010. A spawning ground on the Portuguese coast, confirms the existence of at least two components of the blue whiting population on the Northeast Atlantic. The definition of these two components should be taken into account in the management of this species which plays an important role at the ecosystem level. The Portuguese time-series for age, length and maturity of blue whiting southern component, constitutes an important data set of this important resource, which may allow to advice separately as two stocks, a southern and a northern.

Keywords: Blue whiting; life history parameters; growth; spawning stock; population structure.

2.2 Introduction

Blue whiting (*Micromesistius poutassou*) is a mesopelagic gadoid fish widely distributed throughout the Northeast Atlantic (Maucorps, 1979; Bailey, 1982). In terms of biomass this species is one of the most abundant teleosts in the North Atlantic, with an estimated total biomass of 1403kt in 2015 (ICES, 2016b). Its economic significance increased considerably since their commercial exploitation began in 1970 (Standal, 2006), being also considered the largest fishery in the Northeast Atlantic (Bjørndal, 2009).

The main spawning ground of blue whiting is located off the British Isles westards until the Porcupine Bank and northwards until the Rockall Basin (Hátún *et al.*, 2009; ICES, 2016b). Northern nursery grounds spread along the Norwegian coast (possibly in fjords), to the south west of Iceland and along the continental shelf-edge south of Porcupine Bank (Bailey, 1982) and on the continental shelf off Spain and Portugal (Carrera *et al.*, 2001) partially overlapping with local spawning areas (Kloppmann *et al.*, 1996; Silva *et al.*, 1996; Ibaibarriaga *et al.*, 2007). Feeding grounds extend along the continental slope, from the Portuguese coast as far as the Norwegian Sea (Skogen, Monstad and Svendsen, 1999). Large-scale seasonal migrations have been documented for this species, from the main spawning grounds west of the British Isles to the feeding areas after spawning and in the late-winter (January-May) returning again to the spawning grounds (Bailey, 1982).

Since 1993, this species has been assessed as a single stock for the entire area of distribution in the Northeast Atlantic (ICES, 2012). The current blue whiting stock assessment model comprises catch-at-age data from commercial catches (1981–2015) and data from the international blue whiting spawning stock survey (IBWSS) (2004–2016) (ICES, 2016b). Although catch data include contributions from all fishing grounds, the only survey used in the assessment covers just a fraction of the northern distribution of blue whiting (Pointin and Payne, 2014; ICES, 2016b). A series of morphological, physiological, and genetic studies developed mainly in the last three decades, suggest that the stock of blue whiting might be composed by several components (e.g. Mork and Giæver, 1995; Brophy and King, 2007; Was *et al.*, 2008). Despite this, in the last revision of the stock status for blue whiting based on all of the available studies, the stock identification methods working group (ICES, 2014) recommended that blue whiting must be assessed as being composed by two stock units, a northern and a southern, with the boundary at north of the Porcupine Bank (at 56ºN). Nonetheless, this species is still assessed as a single stock (ICES, 2016b), mainly due to the evidences that those several components of the stock could mix in the main spawning ground west of the British Isles and due to the difficulty in separating the individuals that composed each one of them (Skogen, Monstad and Svendsen, 1999; Keating *et al.*, 2014), and to the limited survey coverage (Pointin and Payne, 2014).

Although the knowledge on this species benefit from a considerable number of studies, there are still important gaps to be fulfilled (Pointin and Payne, 2014), especially in what concerns blue whiting southern distribution area in the Northeast Atlantic (Trenkel *et al.*, 2014). In this study, we analysed life history parameters data on growth, population size-structure, maturity and female spawning stock biomass, obtained from 36 surveys performed along the Portuguese coast, between 1990 and 2011, in order to answer to the following questions: What does the life-history data analysis reveal in terms of the blue whiting stock structure? Does this data show evidence of the existence of the two stock components?

2.3 Material and Methods

Data were obtained from samples collected during 36 surveys performed (5 in winter, 10 in summer and 21 in fall) along the Portuguese coast between 1990 and 2011, from Vila Real Sto. António (37°11′N, 7°24′W) to Caminha (41°50′N, 8°50′W). The surveys followed a grid of 97 sampling stations performed along the day and spread throughout the shelf between 36m and 710m (Table 2-1). The surveys first aim is to study the demersal fish communities along the Portuguese coast, the primary species are hake, horse mackerel, blue whiting, mackerel, and Spanish mackerel (ICES, 2016a).

Table 2-1 Gear, mesh size, trawls speed and tows duration of the Portuguese surveys (1990 and 2011).

Period	**Gear**	**Mesh size**	**Target speed**	**Tows duration**	**References**
1990-2004	Bottom trawl	20mm	3.5 knots	1 hour	(Cardador *et al.*, 1997)
2005-2011	Bottom trawl	20mm	3.5 knots	30 minutes	(WKPGFS, 2004)

In each survey, total length (cm), total weight (g), sex and macroscopic maturity stage of all fish sampled were recorded. From each haul, the otoliths of 10 fishes (5 males and 5 females) per one centimeter length class were removed. The otoliths were washed and stored dry for later ageing. A total of 33,799 fish were sampled, being composed by 17,283 females, 14,927 males and 1,589 for which sex was undetermined. In total, 15,546 otoliths were used to age reading.

Otoliths were submerged in a 0.1% thymol solution (1g of thymol for 1l of filtered and distiled water) for approximately 24h. The whole otolith was immersed in oil and observed in a binocular stereomicroscope (0.64x), under reflected light against a black background. For age classification only one otolith of the sagittal pair was used. Age classification was made considering the number of translucent rings in the otolith; one translucent ring was equivalent to one year. The results from the last blue whiting age reading workshop show that readers providing the age compositions for stock assessment, presented null or low bias in 3/4 of the samples used (ICES, 2013), allowing comparison of the blue whiting age data from the different sources along the NE Atlantic.

The maturity data available was based on gonads observation and staged according to their macroscopic characteristics. From 1990 until 1998, a seven stages maturity key (ICES, 1979) was applied according to the following stages: 1 – immature; 2 – maturing/recovery; 3 and 4 - development; 5 and 6 – spawning; 7 – post-spawning/resting. After 1998, the blue whiting maturity scale was revised and a new macroscopic stage key with five stages was implemented, classified as: 1 – immature or resting; 2 – development; 3 and 4 – spawning; 5 – post-spawning. The macroscopic gonadal classifications were validated through histology (Cunha, 1992a; Amorim, 2000). In order to guarantee that the maturity stages assignment and their interpretation along the decades was performed avoiding misinterpretations; in the maturity analysis presented here the period (year) when data was collected was taken into account.

The parameters a and b of the weight-length relationship of 3,164 individuals (1,804 females and 1,360 males), were estimated by sex and by semester (according to the survey month) as

$$W_t = a\ L_t^{b}$$

with W_t as fish total weight (g) and L_t fish total length (cm).

Age-length observations were assembled by sex to produce separate age-length keys.

The von Bertalanffy growth model where fitted to length-at-age blue whiting survey data by sex, for the period from 1990 until 1999, through a non linear regression as:

$$L_t = L_\infty - (1- e^{-K(t-t0)})$$

where L_t is length-at-age t (age in years, length in cm), L_∞ is the asymptotic size (in cm), K is the growth coefficient (in year^{-1}) and t_0 is defined as age at which the fishes would have had zero size.

A sex combined maturity ogive curve describing the proportion of fish mature at a certain length class (cm) was fitted. The maturity ogive was represented using a logistic curve, defined as:

$$P_i = \frac{1}{1+e^{-K(L_i-L50)}}$$

where P_i is the proportion mature at length L_i and K and L_{50} are the estimated parameters. K represents the instantaneous rate of maturation, or the slope of the curve. L_{50} represents the length at which 50% of the fish are mature (Chen and Paloheimo, 1994) . The method used to fit the logistic curve was a generalised linear model (GLM) with a binomial error distribution and a logit link function.

Female spawning stock biomass (*FSSB)* was calculated, from 1990 until 2011, as following:

$$FSSB = \sum_{i=k}^{plus\ group} \mathrm{FMO_i} \times \mathrm{FW_i} \times \mathrm{SR_i} \times \mathrm{N_i}$$

where FMO_i is the proportion of mature females at length i relative to the total number of females at length i, FW_i is the female mean weight at length i, SR_i is the sex ratio at length i and N_i is the index of the abundance at length i. The values of *FSSB* were standardized, through $(\frac{Xi-\mu}{\delta})$ (Zar, 1999), taking X_i as the FSSB at each year i, μ the overall mean of *FSSB* and δ as the corresponding standard deviation. Female's maturity ogive was assumed constant. A sex-ratio of 1:1 was assumed for the entire period. In order to allow comparison with the FSSB pattern for the combined stock: the maturity ogive, the mean weight at age and the indices of abundance from the surveys available from the ICES assessment working group were used for the period 2000-2011 (ICES, 2016b). Since the abundance time-series for the IBWSS were available only from 2004, from 2000 until 2003 the data from the International Norwegian Sea ecosystem survey was used. Although, the quality of the 2010 IBWSS was regarded as not satisfactory according to the assessment report; this value was considered for the comparison exercise.

All statistical analyses were conducted using the “fishmethods”, “ggplot2”, “mapdata” and “maps” packages in the statistical environment R (R Core Team, 2017).

2.4 Results

Fish length varied between 10cm and 40cm corresponding to weights from 20g to 450g.

The length-weight relationships did not reveal differences between the two sexes (females: n=3654; a= 0.0025; b= 3.28; R^2=0.97; males: n=2739; a=0.0026; b= 3.27; R^2=0.97) (Figure 2-1). Length-weight by semester revealed significantly differences between the first (a= 0.0072, b= 2.95 and R^2= 0.99) and the second semester (a= 0.0039, b= 3.16 and R^2= 0.99) (Figure 2-2). Those differences in the length-weight of the fishes amongst semesters were just observed above the 20cm.

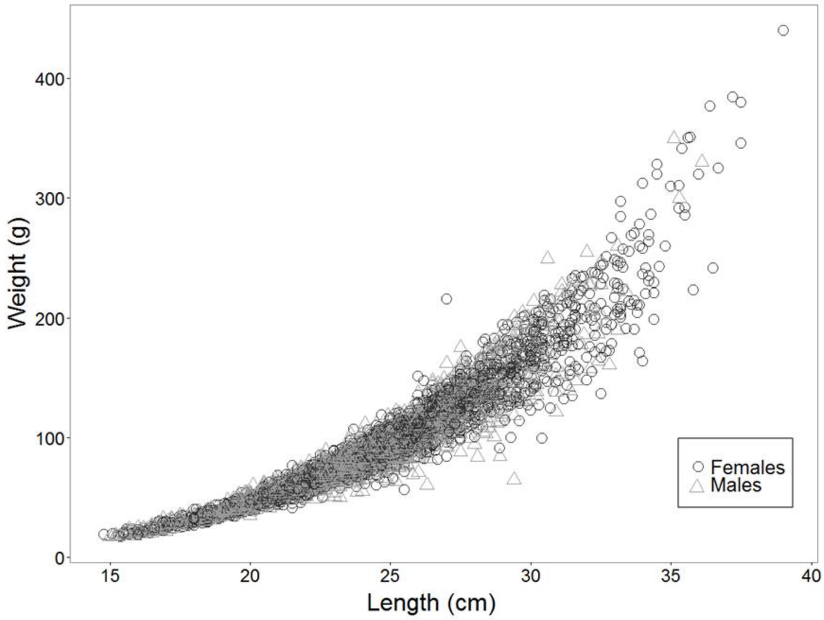

Figure 2-1 Weight-length relationships of blue whiting, from the Portuguese coast (between 1990 and 2011), by sex.

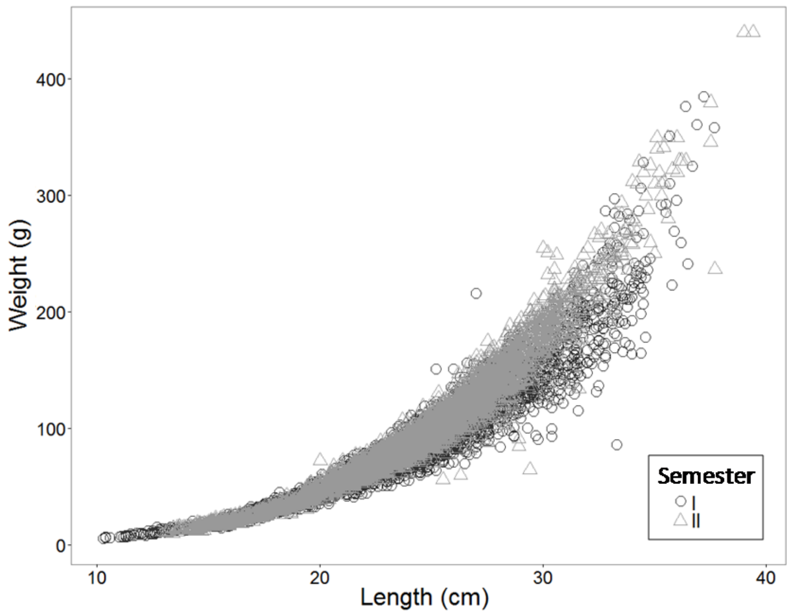

Figure 2-2 Weight-length relationships of blue whiting, from the Portuguese coast (between 1990 and 2011), by semester.

The sex combined maturity ogive, for the Portuguese coast, showed that fifty per cent of the fishes were mature at 20cm length (Figure 2-3).

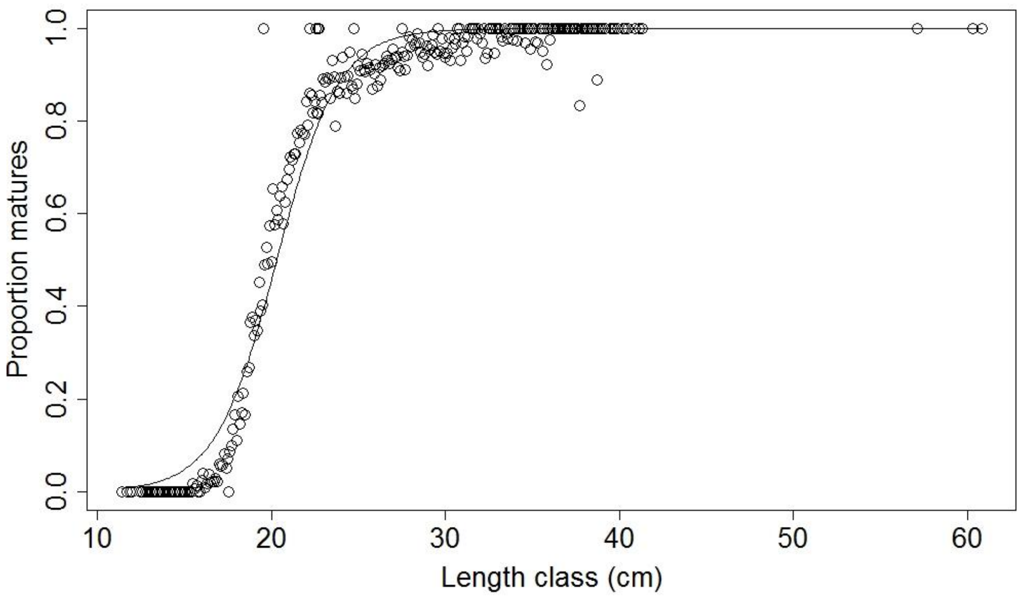

Figure 2-3 Sex combined maturity ogive of blue whiting, from the Portuguese coast (between 1990 and 2011), describing the proportion of matures by length class (cm).

Males grow faster than females, at the Portuguese coast (K♀=0.34; K♂=0.36) (Figure 2-4 a, Figure 2-4 b), but females achieve greater lengths.

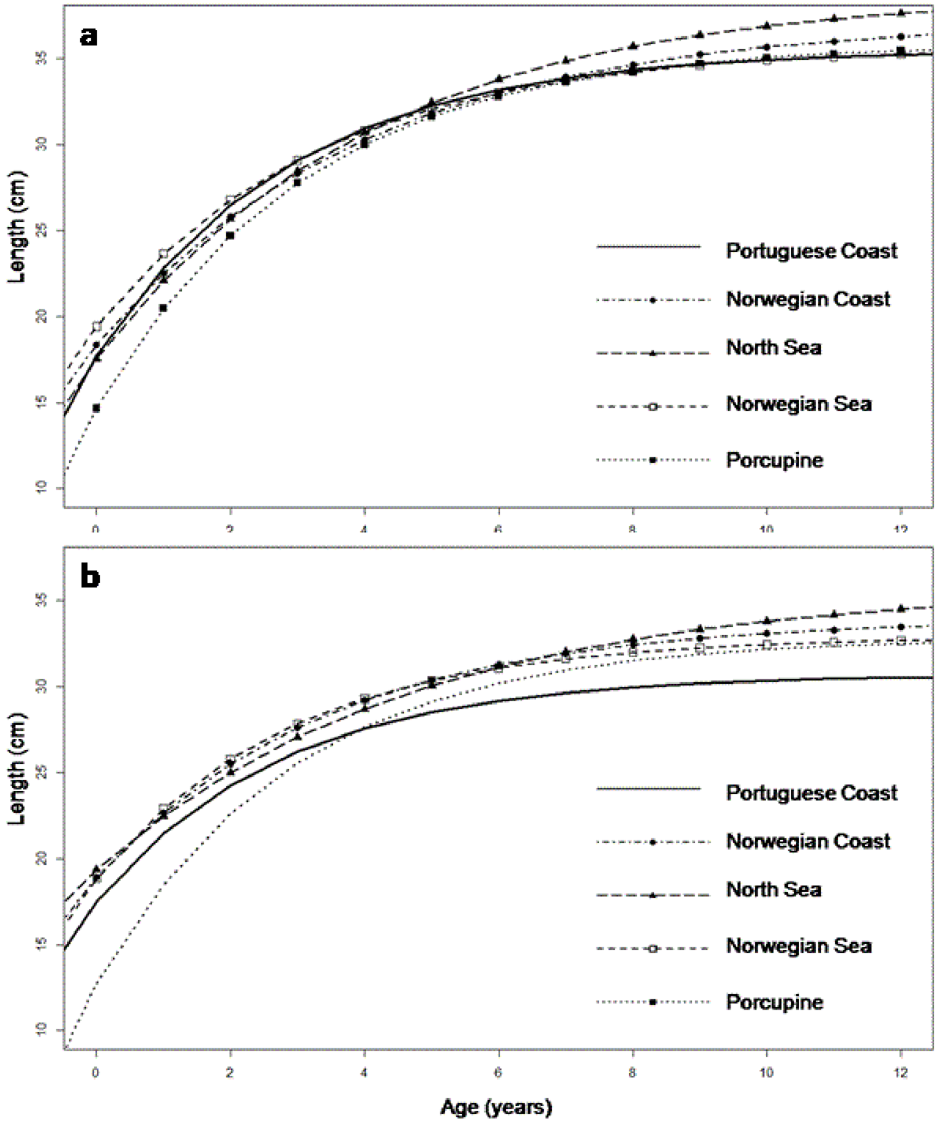

Figure 2-4 The von Bertalanffy growth curves for the Portuguese coast (between 1990 and 1999) (present study), Nowergian coast, North Sea, Norwegian Sea and Porcupine according to Monstad (1990) for females (a) and males (b).

Females spawning stock biomass (FSSB) analysis, along the period considered from 1990 to 2011 (Figure 2-5), revealed that 1990, 2001 and 2004 were the years where FSSB was higher. However, the FSSB observed in 2001, was the highest of the time-series (almost the double of the one reported on 1990). For the period from 1996 to 1999, lower values were observed and, more recently, since 2006, the FSSB has been decreasing. In 2011, FSSB still remained in a value lower than its mean from the entire time period. In the FSSB for the combined stock the highest values where observed for 2003 and 2007. Although, the 2010 survey data was not satisfactory, lower values of FSSB were also observed after 2008 until 2011.

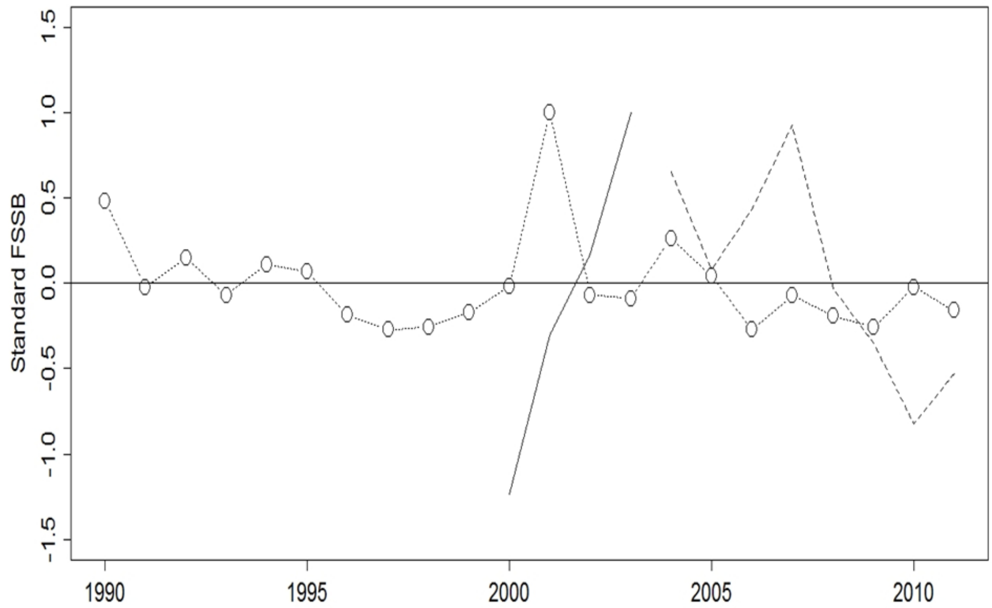

Figure 2-5 Standardized spawning stock biomass of blue whiting females (FSSB) by year (between 1990 and 2011), for the Portuguese coast (o....), combined stock using the abundances indices from the International Ecosystem Survey in the Nordic Seas (IESNS) (2000-2003) (—) and from the IBWSS survey (2004-2010) (-----).

The distribution of mature females along the Portuguese coast was recorded during the fall surveys between 1990 and 2011 (Figure 2-6). For the whole period considered in the present study, all the maturity stages were well represented in the samples. Mature females were mainly registered at depths higher than 200m. Along the northwest part of the Portuguese coast until Peniche (39º21'N, 9º22'W), mature females where concentrated above 200m depth. From Peniche to the southeast part of the Portuguese coast, mature females were found at deeper bottoms near or below 200m, with the exception of a spawning area identified at the 100m bathymetry near Lisbon (38º42'N, 9º08'W). Large concentrations of immature females were found near the edge of the continental shelf on several years (1992, 2006, 2009 and 2010). During 2001, 2004 and 2005 surveys, the majority of females observed were mature and were distributed along and forward the 200m bathymetric line.

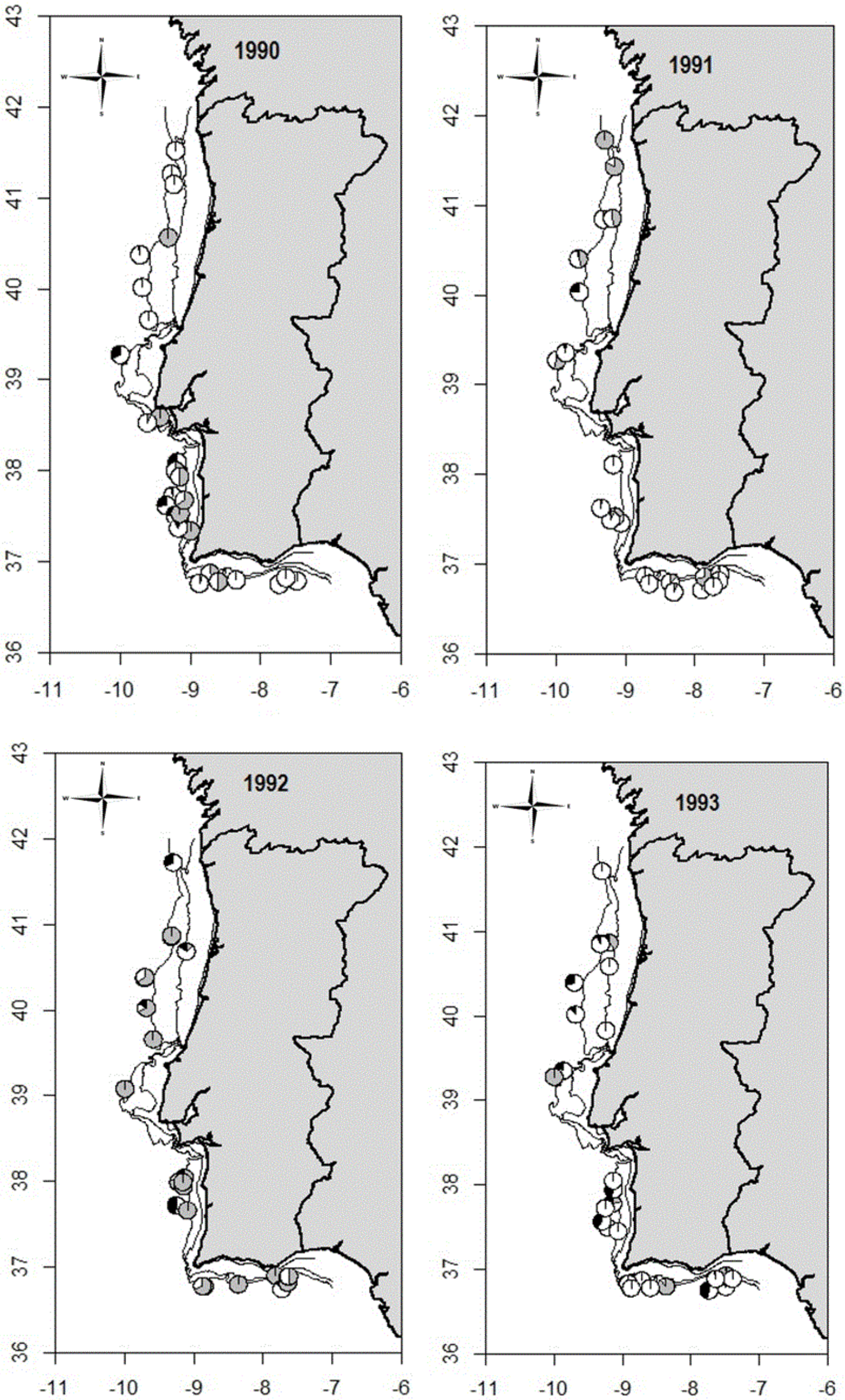

Figure 2-6 Geographical position of the sampling stations in which were represented the proportion of blue whiting immature, mature and spawning females, from the surveys between 1990 and 2011 and 20, 100 and 200m isobaths (contour lines). Pie charts represent the proportion of total number of immature females (grey), of mature females (white) and of spawning females (black). x-axes corresponds to longitude west (degrees) and y-axes to latitude north (degrees). This figure maps continues from this page (50) to page 55.

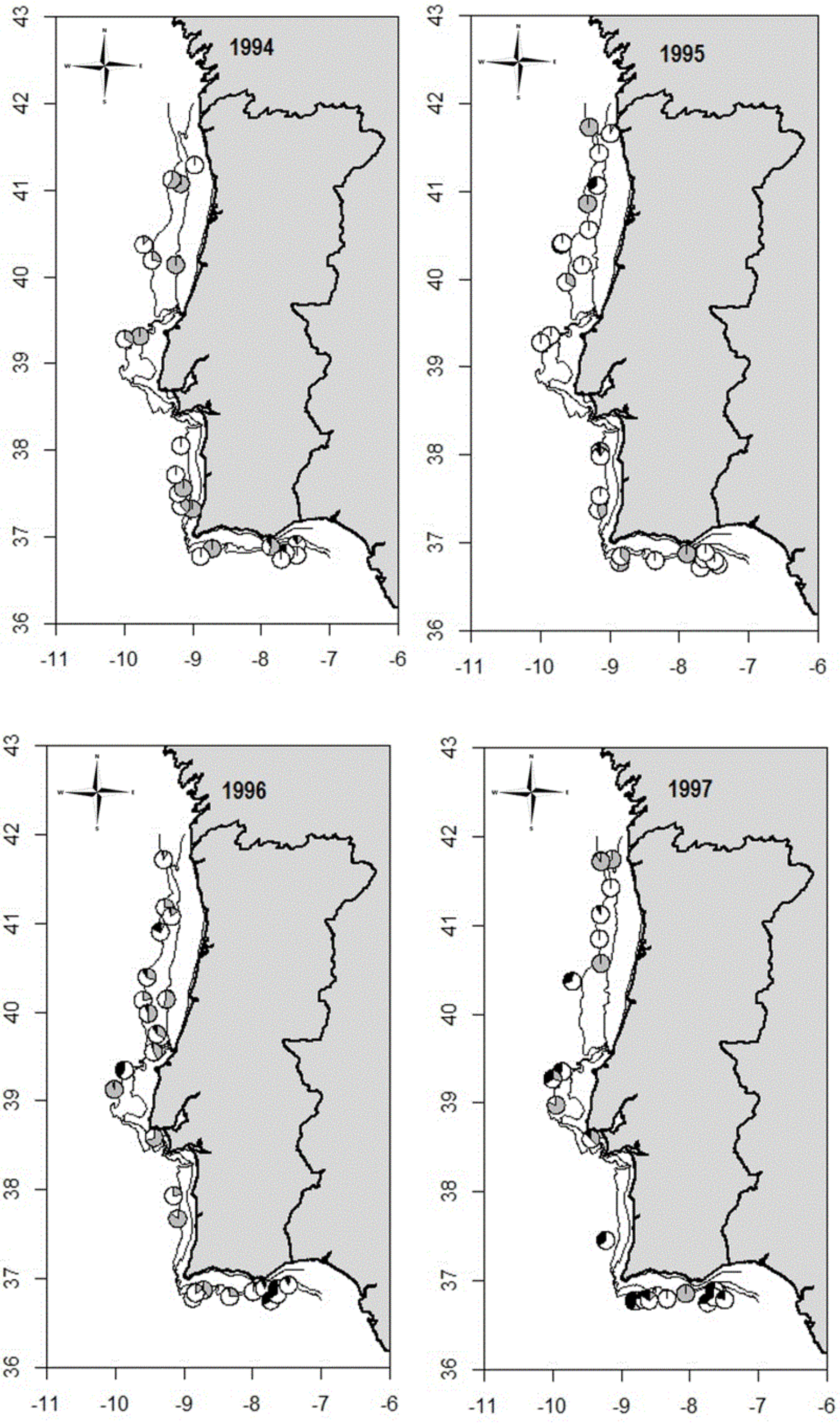

Figure 2-6 (continuation)

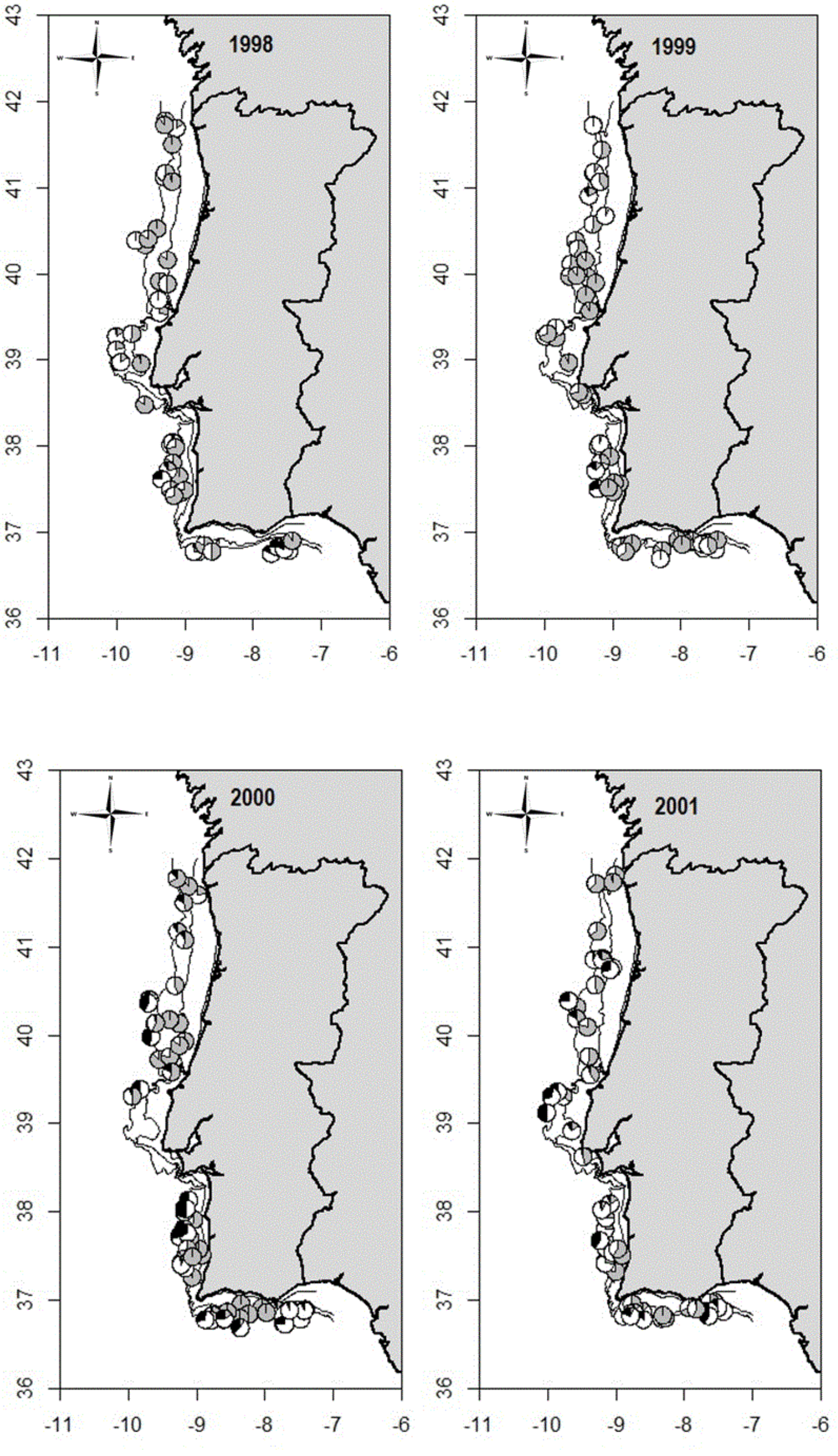

Figure 2-6 (continuation)

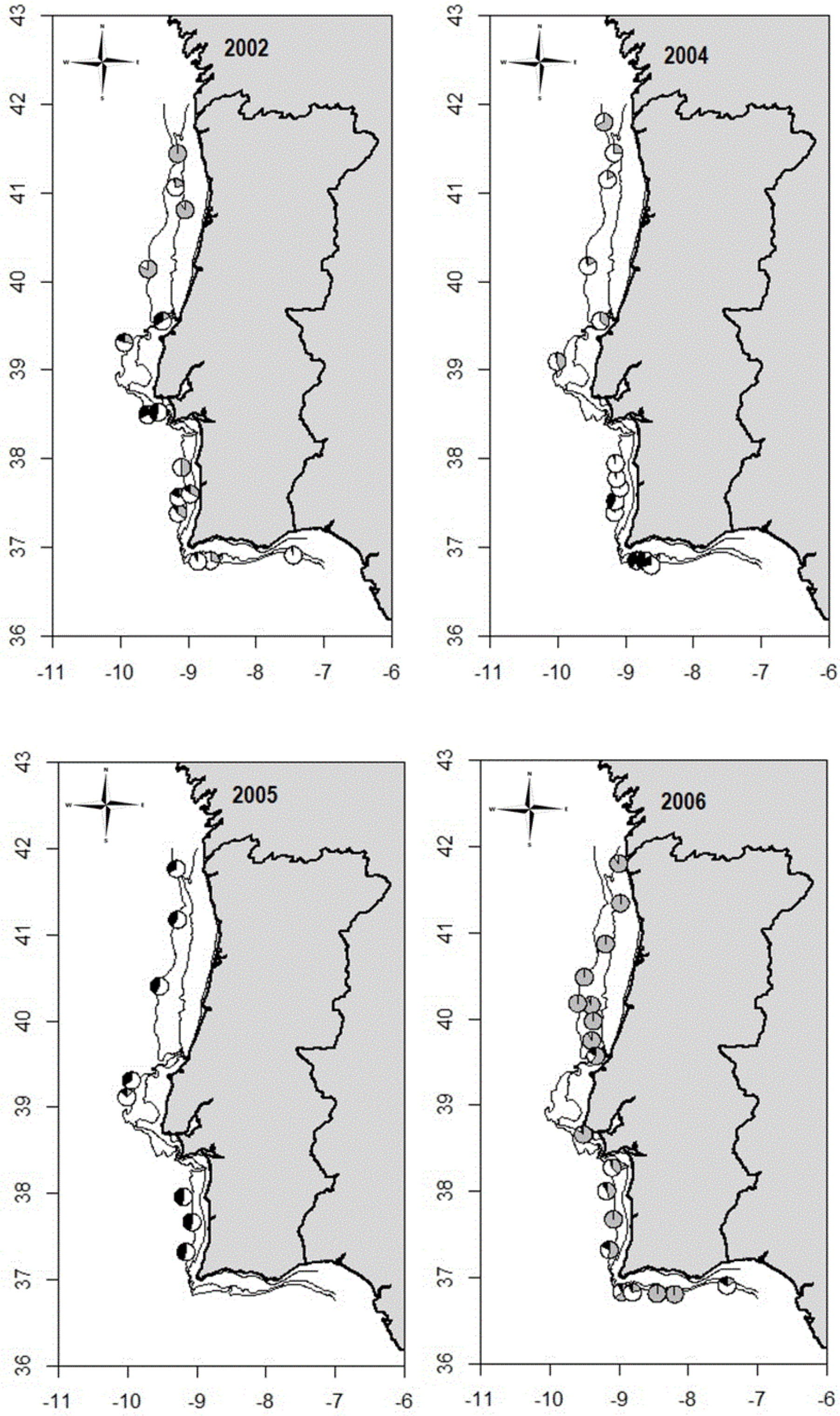

Figure 2-6 (continuation)

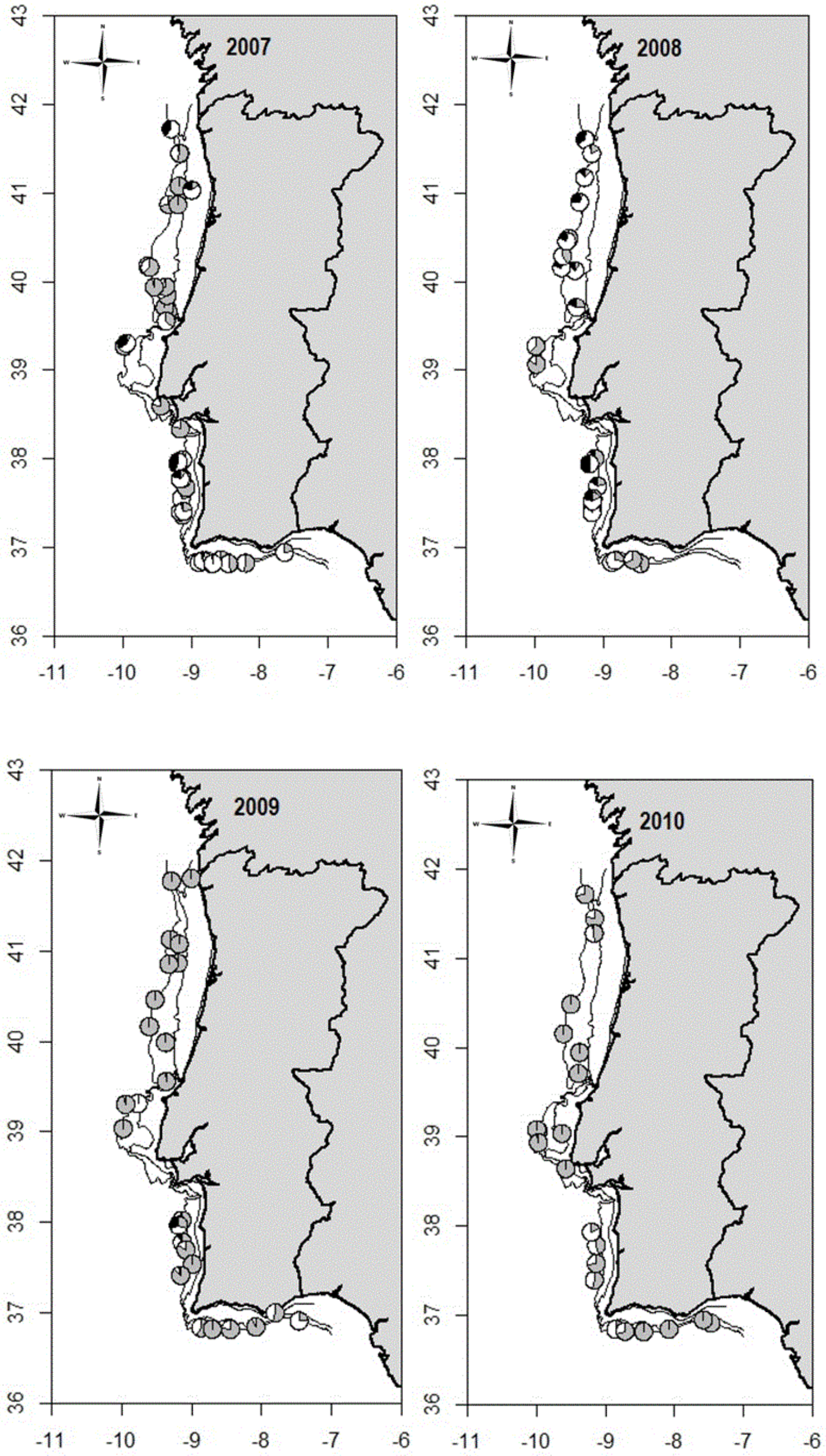

Figure 2-6 (continuation)

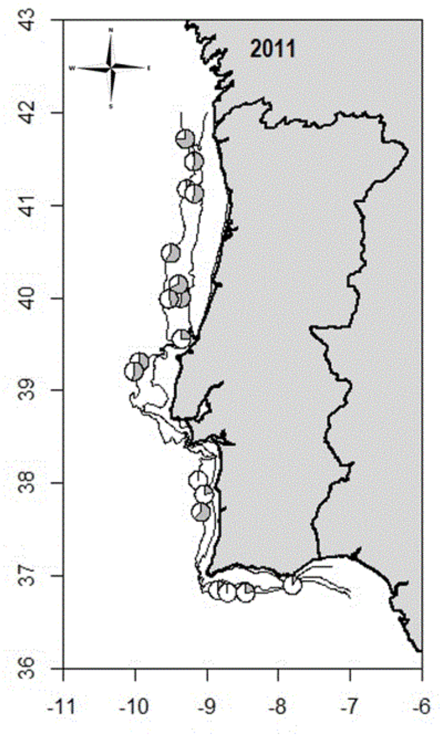

Figure 2-6 (continuation)

2.5 Discussion

Growth is sexually differentiated, with blue whiting females reaching larger sizes than males (Monstad, 1990). Growth rates increase up to two years of age, more in males than in females (Petitgas *et al.*, 2010). Blue whiting is described to reach a maximum length between 45cm (Maucorps, 1979) to 50cm (Petitgas *et al.*, 2010). However, for the great majority of areas including the area of sampling in this study, specimens over 40cm are scarce (Bas, 1959; Bailey, 1982; Petitgas *et al.*, 2010).

Length-weight curves were similar for both sexes and present a positive allometric growth (b>3) which was also been described for this species on the Mediterranean (a=0.0033; b= 3.21) (Papaconstantinou C., 1989) and Adriatic Sea (a=0.0054, b=3.07) (Froglia C., 1981). The length-weight curves revealed that individuals larger that 20cm, where leaner in the first semester. In an earlier study conducted in the southern of Portugal with samples from crustacean trawl fishery, during the second semester of 1994 and 1995, similar results were obtained (a=0.0039, b=3.17 for n= 130) (Mendes, Fonseca and Campos, 2004). The 20cm is the size which more than fifty percent of the fish are mature and is within the L50 range for the Cantabric and Mediterranean waters (ICES, 1983). The main spawning period for the Portuguese coast is between January and March, with a peak in February (Amorim, 2000), also confirmed by the reproduction indicators (females at spawning stage) analysed in the current study. The observed differences in the fish weight between semesters were due to the spawning occurrence at the beginning of the year. Since, blue whiting spawners could cease feeding during spawning (Bailey, 1982). During spawning, feeding occurs exclusively among immature specimens (Bailey, 1982), corresponding to fishes below 20cm where no differences in weight were observed between the semesters. Mature blue whiting start feeding after spawning, increasing in weight until the following spawning season.

The size of first maturity is similar in all the area of distribution (ICES, 1983), although the correspondence to age varies with latitude. Age of first maturity for the Portuguese coast is between 0 and 2 years, which is similar to the Mediterranean where the size at first maturity was 19cm, for both sexes and was reached at age 2 (Bas, 1959). On Faroese-Scottish-west Ireland waters, this species reached the first maturity also at 20cm, but at age between 2 and 4 years old (Raitt, 1966). On the North Sea, 25cm was the length at which fifty percent of the blue whiting's were mature, at an age of 2 or 3 years (Perry *et al.*, 2005), this also corresponds to the described age of first maturity for the areas of the northern component (ICES, 2016b).

Males grow faster than females, at the Portuguese coast, but also at Porcupine ($K_{♀}$=0.32; $K_{♂}$=0.34), at Norwegian coast ($K_{♀}$=0.25; $K_{♂}$=0.29) and Sea ($K_{♀}$=0.26; $K_{♂}$=0.23), except in the North Sea ($K_{♀}$=0.24; $K_{♂}$=0.21), although females achieve greater lengths. The asymptotic length (L_{∞}) of females and males was higher for the Norwegian coast (♀=37.2; ♂=33.9) and the North Sea (♀=38.8; ♂=35.8). The parameter t_0 was lower for fishes at Porcupine (♀=-1.64; ♂=-1.74). There was an evidence of a slower growth rate in blue whiting when moving northern along their area of distribution. In a study of otolith's microstructure, larvae's distributed in the warmer waters shows an individual growth rate significantly greater compared to the larvae distributed in cooler waters (Bailey and Heath, 2001). Similar results were obtained based on otolith increment analysis, which revealed that adult fish captured in the southern part of the distribution range showed a faster growth rate as larvae and juveniles, than those from the northern part (Brophy and King, 2007). The otoliths from the southern component appear to be wider at a given otolith length than the ones from the north (Keating *et al.*, 2014; Mahe *et al.*, 2016). Since growth is directly correlated with water temperature, different temperatures result in different growth rates (Gonçalves, Henriques and Angélico, 2013) and these in turn result in a different age to reach maturity. At the Porcupine Bank, water temperature on the spawning season (between March and May) is around 9º – 10ºC (Mazhirina, 1994; Hátún *et al.*, 2009). At the Portuguese coast, the temperature varies between 12 and 15ºC during spawning and at depths ranged from 150 to 200m, according to CTDF profiling (oceanography instrument which determine the conductivity, temperature, depth and fluorescence of the ocean) registers.

FSSB was higher in 1990, 2001 and in 2004, due to the highest frequencies of mature female with large sizes from those years survey's. In 2001, this highest value also reflects the highest survey abundance observed in the time-series for this species. In 1990 and 2004, the length of the fishes sampled in the survey ranged between 10cm and 60cm. In 2004, although blue whiting occurred on fewer stations, it was found on larger concentrations and mostly composed by mature females. The female distribution in 2006, 2009 and 2010 shows that almost all fish sampled were immature this could explain the lower values of FSSB observed. The increase of mature females in 2011 was noticed in the estimated FSSB. The comparison of the FSSB from the Portuguese coast with the combined stock of blue whiting (ICES, 2016b), revealed a similar trend for the last period from 2008 to 2011. However, the recently decrease of FSSB in relation to the mean of FSSB, was higher in the combined stock. Concerning the present study, the spawning stock for the Portuguese coast seems relatively stable around the mean since 2005, but this was not the case for the whole stock, where a decreased was observed from 2007 until 2010.

Blue whiting in the Portuguese coast, was found mainly distributed between the 100m and 200m bathymetric line and up to a bottom depth below 500m, based on the data from daytime sampling surveys. The sampling strategy and scheme from daytime sampling surveys, though based on bottom trawl, allowed collection of blue whiting throughout its life cycle, from the juvenile to the adult fraction of the population. Blue whiting mostly concentrates in the water column at bottom depths greater than 600m, but at bottom depths less than 500m it remains increasingly closer to the seabed (Johnsen and Godø, 2007). Besides, acoustic recordings show diel migrations in water column, with the highest densities in shallow waters (<350m) at night (Johnsen and Godø, 2007). Thus, considering the area covered by the surveys and the fact that the sampling was conducted during the day, it was expected that the majority of blue whiting was located closer to the bottom, thus the samples collected are representative of blue whiting local population at the Portuguese coast.

Large concentrations of immature females were found on shallow bottoms near the edge of the continental shelf on several years (1992, 2006 and 2009), while concentrations of mature females were found at depths greater than 100m, showing that the fish length increases with depth. This increase in size with depth was first detected on the early 1990's (Cunha, 1992b) for this species at the Portuguese coast. The spawning females were found deeper, extending down to 500m. Similar pattern is described for the main spawning ground area, west of the British Isles, where spawners occur between 300m and 600m depth (Coombs, Pipe and Mitchell, 1981), with larger concentration within 300m and 400m (Monstad, 1990; Villasante, 2012). The larvae of this species were usually found in all depth strata in the main spawning ground, although a decrease in their density with an increase in depth has been reported (Hillgruber *et al.*, 1997). A mathematical model describing the blue whiting eggs and larvae off west of the British Isles' vertical distribution in response to changes in their buoyancy, showed that early egg stages are distributed between 460m and 580m (Ådlandsvik *et al.*, 2001).

The occurrence of immature and females at all maturity stages (from immature, passing from mature to spawning), across the Portuguese coast, is an evidence of spawning. The identification of spawning in females was based on the presence of hydrated oocytes in the ovaries; also in the recent post-spawners remaining hydrated oocytes were observed. For the other indicators of spawning, eggs and larvae, no data was been published or is available in what concerns the Portuguese coast. During these surveys (demersal), also no ichtyoplankton samples were collected.

The evidences of spawning along with the differences in growth, length, age of first maturity, and in the peak spawning season, between the Portuguese coast and the main spawning ground, suggest that spawning on these two areas could be carried out by spawners geographically segregated or at least with a low level of mixing. Similarly to what has been shown for cod (Hutchings *et al.*, 2007), were different life-history traits reflect separate evolutionary paths and may lead to separate assessment and management units, the differences in blue whiting biology between northern and southern North Atlantic grounds should be taken into account for the future assessment of this widely distributed fish.

Migration to and from the spawning ground, in what concerns the southern component, should mostly be composed by the fraction of this component that inhabits the Bay of Biscay area, and even there at a low level. In the acoustic survey, performed from the Bay of Biscay to the north of France, only few young mature specimen (mainly age groups 2 and 3) have been sampled (Carrera *et al.*, 2001). The diel migrations, a blue whiting characteristic, and or the existence of a dead zone in the acoustic echograms which prevents the identification of the fish schools at the bottom (Stensholt, Aglen and Mehl, 2002) could be the reason for the scarce presence of adults in the acoustic surveys. Despite these two reasons, could also be that the migration to the spawning ground of fish from the Bay of Biscay takes place towards the south, for the Western Iberia Basin, but earlier in the year (between January and March).

The Western Iberia Basin in terms of oceanography is known by two major reasons (Peliz *et al.*, 2005): first, it constitutes the northern limit of the Eastern North Atlantic Upwelling Region, with strong and recurrent filament activity (Haynes, Barton and Pilling, 1993); second, it contains the main pathway of circulation of Mediterranean Water into the Atlantic, which tends to follow the contours of the Portuguese southwestern slope, generating mesoscale features called Meddies (e.g. (Armi and Stommel, 1983)). The existence of poleward slope-flows during the winter period was described at the beginning of the 1990s (Peliz *et al.*, 2005). The poleward flows were attributed to the seasonal reversal of the wind regimes, and to the response of the slope waters to meridional density gradients (e.g., (Frouin *et al.*, 1990)). The frontal and eddy activity in the open-sea can: mechanically affect local concentrations of organisms; result in selectivity pattern components of populations or communities (quasi-ordered patchiness); serve as reproduction refuges; and induce or sustain higher local production of organisms (Owen, 1981).

The identification of a reproductive unit for this area, the Portuguese coast, has implications on spawning stock biomass, on recruitment estimates and as a consequence on the assessment of the current status of the blue whiting in the Northeast Atlantic. The description of this spawning ground at south, confirms the existence of at least two components of the blue whiting population on the Northeast Atlantic. The definition of those two components must be taken into account in the management of this species which plays an important rule at the ecosystem level. The Portuguese time-series for age, length and maturity from the blue whiting southern component are an important data set of this important resource which contributes to improve input assessment data to advice taking into account two stock components, a southern and a northern.

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Chapter 3

3. Blue whiting (*Micromesistius poutassou*) sex ratio, size distribution and conditions patterns off Portugal

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3.1 Abstract

The sex ratio is an important trait of natural populations and a key parameter for assessing the reproductive potential and stock status of exploited fish populations. In fisheries research, knowledge on spatial and temporal sex ratio variation can inform on the capacity of a population to support exploitation and environmental changes. Blue whiting (*Micromesistius poutassou*) show sexual growth dimorphism after maturation, with females achieving greater lengths than males. The main goal of this work was to investigate seasonal patterns in the blue whiting sex distribution, size structure and condition in three areas along the Portuguese coast (north, southwest and south), based on bottom trawl surveys performed in autumn and winter between 1998 and 2015. Smaller blue whiting (12cm– 24cm) were found in shallow areas down to 250m-300m while larger individuals (>24cm) were spread deeper down to 400m-500m. Condition factor Fulton K differed significantly between seasons in all the areas ($F=11.72$; p-value < 0.001) and between sexes ($F=6.14$; p-value < 0.05). The proportion of females changed between autumn and winter ($\chi2(1)=4.38$, $p=0.03$) and across depths ($\chi2(1)=4.73$, $p=0.03$). Thus, this study revealed seasonal and depth variations in the blue whiting sex ratio along the Portuguese coast.

Keywords: Blue whiting; *Microsmesistius poutassou*; spawning; sex ratio; Portuguese coast.

3.2 Introduction

The sex ratio is an important trait of natural populations (Charnov, 1982) and is a key parameter for assessing reproductive potential and stock status of exploited fish populations (Vazzoler, 1996). In theory, it is expected that natural selection should maintain a 1:1 sex ratio by continuously favouring the less represented sex, thereby always returning skewed sex ratios to equality (Fisher, 1930). However in nature sex ratios can vary, though neither causes nor implications of this variation are fully understood for most species (Székely, Weissing and Komdeur, 2014).

The sex ratio of exploited populations can be modified by non-random harvest as a consequence of sex specific behaviour, size, or morphology (Schultz, 1996; Marealle *et al.*, 2010). Sex ratios can also be environmentally driven, namely by changes in temperature regime that may trigger changes in food availability (Vicentini and Araújo, 2003) leading to sex specific growth and mortality (Conover and Kynard, 1981; Kappus, 2012). The perception that sex ratios vary, and that males and females often differ in key ecological traits, suggests that sex ratio variation may have effect on communities and ecosystems (Fryxell *et al.*, 2015).

In fish, some populations can exhibit strong deviation from the expected 1:1 sex ratio (E.A. Trippel, 2003), which can vary over space and time (Pawson and Pickett, 1996; Edward A. Trippel, 2003). Knowledge on spatial and temporal sex ratio variation can inform on the capacity of populations to sustain exploitation and environmental variation (Palsbøl, Bérube and Allendorf, 2007; Schwartz, Luikart and Waples, 2007). Many fish species present sexual growth dimorphism; the sex with slower growth rate is more likely to be exposed to extended predation on early life stages, leading to premature depletion before entering into next development phases (Nikolsky, 1963). However, in the majority of fish the dimorphism on growth occurs after fish initiate maturation. Sexual dimorphism has the potential to influence species spatial distributions because predator-prey relationships are size-dependent (Brose *et al.*, 2006), with body size conditioning overall feeding rate (Rall *et al.*, 2012). Males and females can also be dimorphic in behavioural or morphological traits (Shine, 1989). Sexual dimorphisms as well sexual differences in size have been reported for many types of mesopelagic fishes (Clarke, 1983).

Blue whiting (*Micromesistius poutassou*) is a mesopelagic widely distributed species, living generally deeper than 200m (Monstad, 2004; Dolgov *et al.*, 2010). Growth in blue whiting is sexually differentiated, with females achieving greater lengths than males (Monstad, 1990; Trenkel *et al.*, 2015); this differentiation was only visible at sizes above the length of first maturity (20cm). Blue whiting, which are also a commercially important species (Pálsson, 2005), with the major fishing areas located at the spawning grounds west off Ireland and Scotland, the Norwegian Sea and the Bay of Biscay (ICES, 2016b). Although, the most important spawning area is the region to the west of the British Isles (Bailey, 1982), local spawning occurs in many areas, e.g. along the coasts of Iceland, in the Norwegian Sea, in some fjords along the Norwegian coast (Zilanov, 1968; Coombs, Pipe and Mitchell, 1981; Monstad, 1990; Giæver and Mork, 1995) and on the shelf of Portugal and Spain (Silva *et al.*, 1996; Ibaibarriaga *et al.*, 2007).

In the Portuguese part of the stock, blue whiting is mainly caught as a by-catch species by the bottom-trawl fishery. In 2015, the Portuguese blue whiting catches represented 0.2% of total catches in the Northeast Atlantic (ICES, 2016b). The catch-at-age data used in stock assessment is based on sampling landings by countries involved in blue whiting fisheries. The Portuguese blue whiting sampling data collection for length and age distributions is stratified by sex. For estimating catch-at-age based on these data, a sex ratio of 1:1 is subsequently assumed. However, if the sex composition of catches was not as expected 1:1, this should be taken into account when producing the data to be used on assessment, in order to better reflect the stock dynamics in this area.

The aim of this study was to better understand the stock dynamics of blue whiting on the Portuguese shelf in view of improving the design of the commercial sample data collection. For this the seasonal patterns in blue whiting sex distribution, size and condition in three areas along the Portuguese coast (north, southwest and south), based on data from bottom trawl surveys, autumn and winter/spring, performed between 1998 and 2015, were studied.

3.3 Material and Methods

3.3.1 Sample collection

Data were obtained from samples collected during 26 scientific bottom trawl surveys performed between 1998 and 2015 (1998- 2015 in autumn (n=22); and 2005 – 2008 also in winter/spring (n=4)) along the Portuguese coast, between latitudes 41º49.5N and 36º41.0N and between the 20 and 500m isobaths, carried out by IPMA (Instituto Português do Mar e da Atmosfera). Three different areas were considered along the Portuguese coast: northern coast (north); southwestern coast (southwest); and southern coast (south) (Figure 3-1). Most of the surveys were performed on board RV “Noruega” using a Norwegian Campelen bottom-trawl with a 20mm cod end mesh size with a vertical opening of 4.6m and a ground rope with bobbins. The 1999, 2003 and 2004 surveys were conducted aboard RV “Capricórnio” using a bottom-trawl without bobbins in the ground rope. The surveys between 1998 and 2002 followed a fixed grid of 97 one hour tow sampling stations, performed during daytime at a target speed of 3.5knots (Cardador *et al.*, 1997). Between 2002 and 2004 the same grid was covered with 30minutes daytime tows at 3.5knots (WKPGFS, 2004). From 2005, the autumn surveys followed a fixed grid of 66 stations plus 30 random, and in the winter surveys 50 fixed stations plus 25 random. The surveys first aim was to study the demersal fish communities along the Portuguese coast, the primary species were hake, horse mackerel, blue whiting, mackerel, and Spanish mackerel (ICES, 2016a).

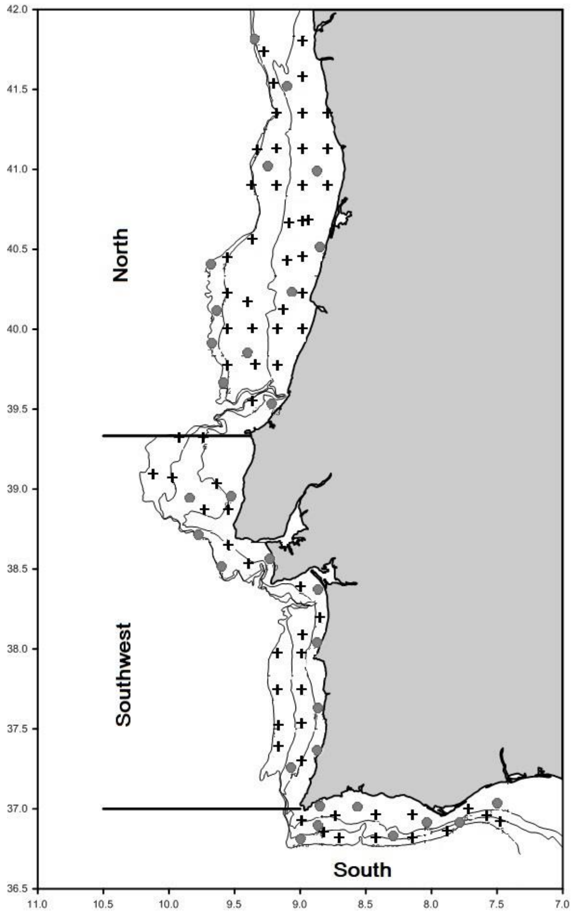

Figure 3-1 Portuguese continental margin showing the sampling stations for bottom trawl surveys conducted between 1990 and 2011, where blue whiting was sampled, and 100m, 200m and 500m isobaths. + Fixed stations; ● random stations. x-axis correspond to longitude west (degrees) and y-axis to latitude north (degrees).

During the surveys the bottom depth (m) of each bottom trawl station was recorded. Total length (cm), total weight (g), sex and the macroscopic maturity stage of all blue whiting sampled were recorded. A five stages maturity key was used, with stages classified as: 1 – immature or resting; 2 – development; 3 and 4 – spawning; 5 – post-spawning. The maturity classification of gonads was based on their main macroscopic characteristics and has been validated through histology (Amorim, 2000). A good agreement was obtained between the maturity classifications made visually at sea and using histology, after the surveys. Immature gonads are smaller and composed of a homogeneous mass, in females without visible oocytes and in males the gonad appearance is like a thin ribbon or a small band. On mature gonads (stage 2 and 5), ovaries present visible opaque oocytes and testes appearance is opaque white with a large band as shape. Females at spawning stage (stage 3 and 4) are classified based on the presence of hydrated oocytes in the ovary which could easily flow/run under some pressure at the abdomen. Males at spawning stage (stage 3 and 4) present bigger gonads in which the sperm could flow under pressure on the abdomen. Fish classified as stages 2 and 5 were considered as mature and fish at stages 3 and 4 as spawners for this study. The proportion of immature, spawning and mature fish in the samples by season (autumn and winter) and sampling area (north, southwest and south) was determined. Similarly, sex ratio dynamics were studied using the proportion of females (*SR*=F/(F+M)) by length class, season and sampling area.

3.3.2 Statistical analysis

Blue whiting abundance, number caught per hour, was determined annually (1998 - 2015) based on sample stratified mean and variance (Cochran, 1977).

Fulton's condition factor (*K*) assesses the condition of mature fish as (Fulton, 1902):

$$K = \frac{W100}{L^3}$$

Where *W* is total fish weight (g) including gonads and *L* is total fish length (cm). *K* was calculated by sex (F and M), season and area. Analysis of variance (ANOVA) was used to examine variation of the Fulton's condition between sexes, areas and seasons.

A generalized linear mixed model was applied to analyse variations in the proportion of females. Year, season, depth and area were considered as fixed effects (main effects only). A random effect was used for station ID. Season, area and station ID were treated as factors. Residual plots were inspected visually for deviations from normality. Likelihood ratio tests were applied to test the effect of each variable in turn by comparing the full model including all variables against the model without the given variable.

Data analyses, including figures and models, were performed in R 3.3.1 (R Core Team, 2017) and using packages “ggplot2” (Wickham, 2009), “ggthemes” (Arnold J. B., 2017) and “lme4” (Bates *et al.*, 2015).

3.4 Results

Depth changes of blue whiting length composition and sex ratio differed between the studied areas along the Portuguese coast. Small blue whiting (12cm–24cm) were found over shallow bottoms down to 250m-300m while larger individuals were spread deeper down to 400m-500m (Figure 3-2).

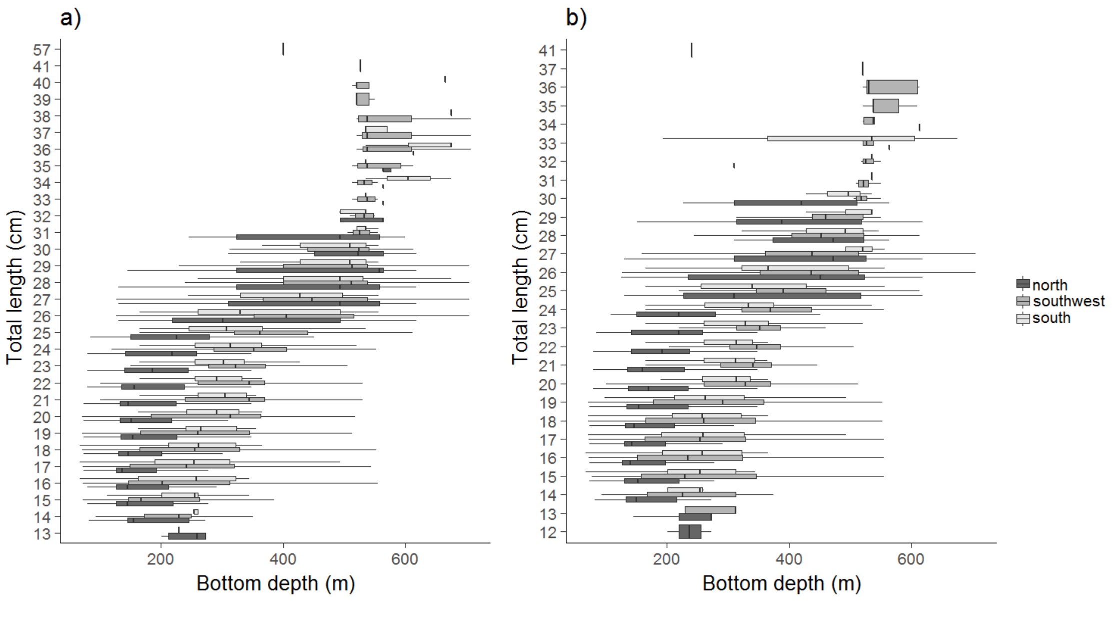

Figure 3-2 Length (cm) distribution of blue whiting individuals by depth. (a) Females and (b) males. Sampling stations covered the depth range 20m-750m (see Figure 3-1). Centre line of each box represents the median; box limit indicates the 25th and 75th percentiles and the whiskers delimit the non-outliers range.

The depth occupied by males and females of the different length classes was similar. In the northern area, the majority of small fish (<25cm) were observed between 100m and

250m while larger individuals (>25cm) were found between 300m and 600m. In the southwestern area, the majority of small fish (14cm-20cm) concentrated around the 200m depth contour while larger fish (>24cm) were mainly found between 300m and 500m. In the south, small fish (<24cm) were spread over a wider depth range (100m-300m) and, similar to the other areas, larger sized individuals were found between 250m and 550m. In general, larger fish were distributed over a smaller depth range compared to smaller fish. Thus, larger fishes were confined to deeper areas (≥500m) in which the sampling intensity was much smaller with 21 stations out of 97.

In winter, the proportion of females (SR) increased from south to north while in autumn it was constant around 0.5 (1:1 sex ratio) in all regions (Figure 3-3 and Table 3-1).

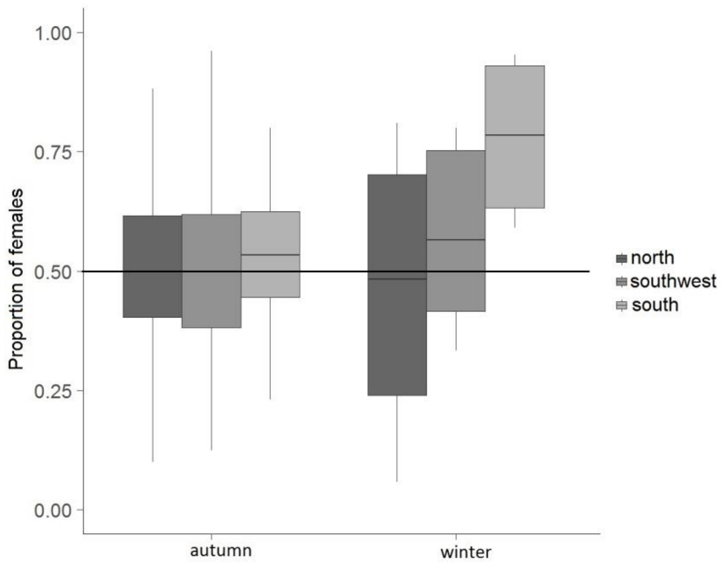

Figure 3-3 Blue whiting proportion of females by season (autumn and winter) and area. Centre line of each box represents the median, box limit indicates the 25^{th} and 75^{th} percentiles and the whisker delimits the non-outliers range. Horizontal line indicates sex ratio 1:1.

Table 3-1 Mean proportion of blue whiting females (standard deviation in brackets) by length class (cm) by survey season and study area on the Portuguese shelf. NA – not available.

	Length class							
	<20 cm		**21-25 cm**		**26-30 cm**		**>31 cm**	
	Autumn	**Winter**	**Autumn**	**Winter**	**Autumn**	**Winter**	**Autumn**	**Winter**
North	0.49(0.16)	0.48(0.27)	0.53 (0.29)	0.35 (0.39)	0.79 (0.20)	0.48 (0.01)	NA	NA
Southwest	0.49(0.16)	0.64(0.01)	0.45 (0.16)	0.50 (0.25)	0.67 (0.24)	0.37 (0.21)	0.93 (0.10)	NA
South	0.51(0.19)	NA	0.54 (0.19)	0.81 (0.14)	0.68 (0.07)	NA	NA	NA

Thus, females were more abundant in winter in the north, with a median SR of around 0.75. In the autumn months, in all areas, the SR of individuals with length size above 25cm was 1:1(Table 3-1). During winter, blue whiting individuals in the surveys were <30cm. The largest differences in SR between areas were observed in winter (Table 3-1). The GLMM results confirmed that season but also year and depth significantly explained variations in SR (Table 3-2; Figure 3-4a). Blue whiting abundance varied over time years, with the greatest abundances observed in 2001, 2009 and 2015 (Figure 3-4b).

Table 3-2 Summary of the generalized mixed model results for blue whiting proportion of females on the Portuguese shelf. df – degrees of freedom; std. error – standard error.

	df	Estimate	std. error	z-value	Probability	Probability(>|z|)
(Intercept)		9.449	1.727	5.472	4.44e-08	**
Year	1	-0.005	0.001	-5.356	8.53e-08	**
Season	1	-0.641	0.303	-2.113	0.0346	*
Depth	1	0.002	0.001	2.239	0.0252	*
Area	2	-0.163	0.224	-0.728	0.4668	

*<0.01.
** <0.001.

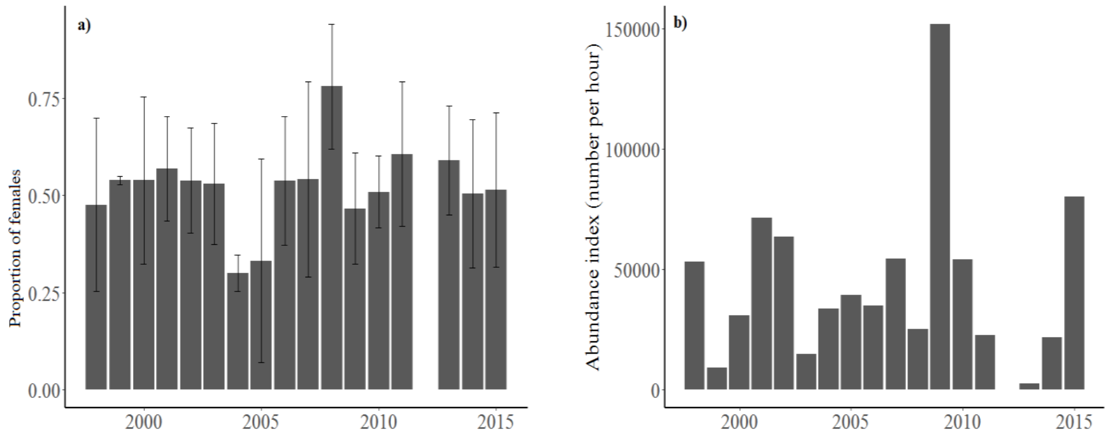

Figure 3-4 The average of (a) proportion of females (mean ± SD) and (b) abundance index (mean number of individuals caught per hour) by year for blue whiting on the Portuguese shelf.

The percentage of immature fish, observed in the samples, was higher in autumn compared to winter and somewhat higher in the north compared to the other two areas (Figure 3-5). Conversely, the percentage of mature fish was higher in winter except for the southwest were it remained constant between autumn and winter at around 50%. The percentage of spawners was higher during winter in all the areas. On the south and southwest coasts, fishes at spawning stage were sampled in both seasons while in the north spawners were mostly observed during winter.

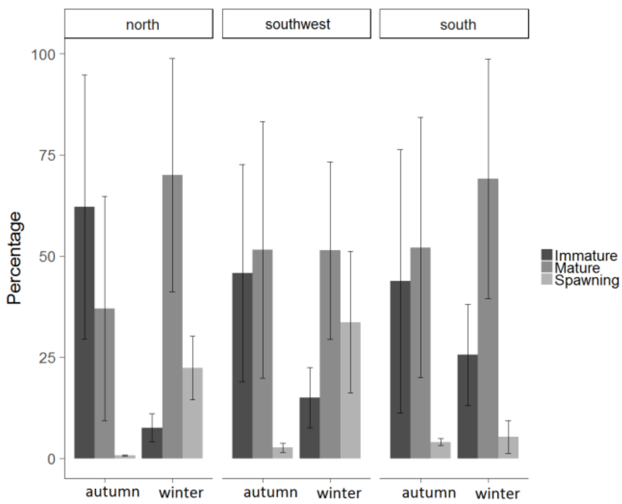

Figure 3-5 The percentage immature, mature and spawning fishes, data combined from both sexes, in the samples by season (autumn and winter) and for each of the three areas (north, southwest and south).

Mature blue whiting had a condition factor (Fulton K) between 0.45 and 0.8 (Figure 3-6). During winter, mature males and females were in slightly lower condition compared to autumn, the difference being more pronounced in the north (Figure 3-6a), than in the other two areas (Figure 3-6b and c). Differences in condition (K) were statistically significant for season combining all individuals (F(4) = 9.76; p-value <0.001) and for sex (F(1) = 6.42; p-value <0.05).

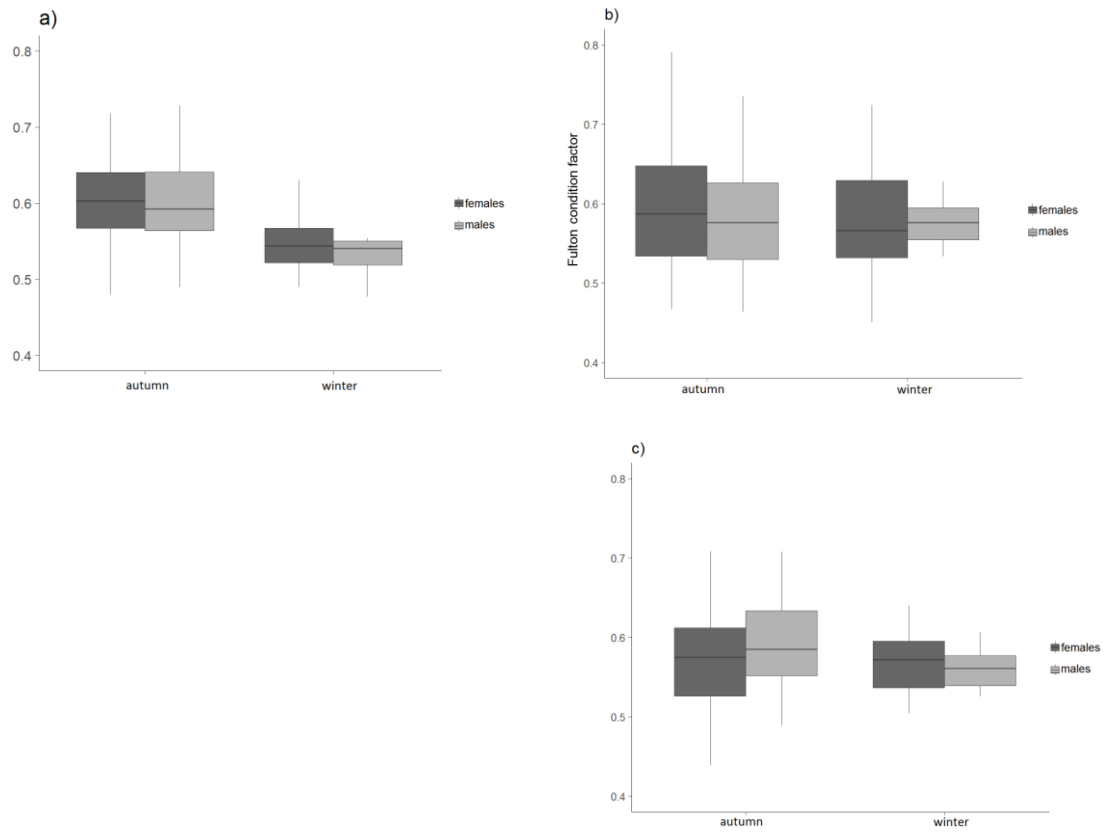

Figure 3-6 Fulton condition factor (K) for mature blue whiting, by sex (F – females (dark grey); M– males (light grey)) and by season (autumn and winter) along the areas of sampling: (a) north, (b) southwest and (c) south. Centre line of each box represents the median, box limit indicates the 25th and 75th percentiles and the whisker delimits the non-outliers range.

3.5 Discussion

In this study, the sex ratio of blue whiting was analysed using bottom trawl survey data from the Portuguese coast. Blue whiting is a mesopelagic species that occupies deeper waters than other pelagic species (Heino and Godø, 2002), but can also be found close to the seafloor (Bailey, 1982). In the Barents Sea, a relatively shallow area with an average depth of 230m, the species has been described to live close to the sea floor, allowing for the possibility of strong ecological interactions with the demersal fish community (Heino and Godø, 2002). The same seems to occur on the Portuguese coast, where in the bottom trawl surveys planned to study demersal communities, blue whiting often composed more than 90% of the biomass in the catches of the shallow (100-200m) and deeper assemblages (>200m) (Gomes, Serrão and Borges, 2001; Sousa, Azevedo and Gomes, 2005). Further, the diel vertical migration of this species occurs at night (Johnsen and Godø, 2007). Thus, although by using the data from bottom trawl sampling an unknown part of the Portuguese blue whiting stock component remained unsampled, the results obtained should still be representative for sex ratio and condition variations. The lack of pelagic sampling during the Portuguese trawl surveys precluded studying SR patterns in the water column. In contrast, data from the annual international acoustic blue whiting survey performed during the spawning period covering the main spawning area and also the annual Spanish acoustic pelagic survey among others could provide understanding and a description of vertical SR patterns.

Differences in blue whiting abundances were observed among years and also found for the proportion of females. These inter-annual differences reflect intrinsic species distribution dynamics off Portugal, rather than methodological challenges or biases linked to the natural behaviour or distribution of blue whiting along the coast. As the survey sampling selectivity was standardized, the results are representative of the true blue whiting distribution in this area, taking into account the species' close association to the sea bottom observed in this study and in previous studies (Gomes, Serrão and Borges, 2001; Sousa, Azevedo and Gomes, 2005).

Differences in the distribution of males and females have been noted for many species, resulting in spatial trends in their sex ratio (Trippel, 2003). Some of these trends were associated with migration behaviour during the spawning season (Stoner *et al.*, 1999). For blue whiting, the observed seasonal pattern could also be related to spawning and to feeding. The majority of blue whiting individuals were at mature stage (>20cm) and at depths between 250 and 500m. At those depths, blue whiting find large concentrations of the main items of their diet composed of zooplanktonic crustaceans, mainly copepods and euphausids (Cabral and Murta, 2002). The spawning period described for this species along the Portuguese coast spans January to March, with a peak in February (Amorim, 2000). Thus, the higher number of spawners during winter in the northern and southwestern areas confirms this peak season at least for these two areas.

The lowest condition indices (Fulton's K) were found in winter while in autumn higher values were observed, except in the southern area where no differences were evident. Similar results have been reported for cod, for which condition values were lowest during the spawning season (winter), increased during post-spawning, to reach the maximum values in autumn (Mello and Rose, 2005). The low condition in winter could result from the high energy spent on spawning which is not sufficiently replaced by food intake; and adult blue whiting may also cease feeding during spawning (Bailey, 1982). In the south during winter, few spawners were observed and the samples were mostly composed of mature females (SR≈0.81) in better condition, in contrast to what was observed in the other areas. These females were between 21 and 25cm total length. The absence of spawners may be explained by horizontal migration or by earlier spawning in warmer temperatures (Relvas *et al.*, 2007). Spawning is induced in gonads at a late stage of development by a combination of changes in temperature and light periodicity. On the Portuguese coast waters become warmer from south to north (Relvas *et al.*, 2007).

This study revealed the existence of a seasonal pattern in the sex ratio of blue whiting off the Portuguese coast. On the northern and southwestern coasts, the sex ratio during spawning was around 0.5 in all length classes, an unequal outside spawning. Sex differentiated seasonal migrations have been described for several species. In lingcod (*Ophiodon elongatus*), seasonal migrations differ by sex, with males aggregating in shallow waters and adult females in deep water during winter, while in summer both sexes are distributed equally over deep and shallow sea bottoms (Okamura *et al.*, 2014). For megrims and other flatfish the opposite has been noticed, the proportion of males increases with increasing bottom depth (Poulard, Peronnet and Rivoalen, 1999). Other studies have reported that in general females were more vulnerable to environmental conditions than males (Vicentini and Araújo, 2003). Nikolsky (1963), described that when food is abundant, females predominate, with the situation inverting in regions where food is limited. Thus, blue whiting females might remain near the bottom at mature stages where food is more abundant and during spawning might migrate to shallower depths, at least during daytime when the samples were collected. This seasonal migration could also be to and from the main spawning grounds to the west off the British Isles, although the presence of spawning individuals indicates the occurrence of spawning events in Portuguese waters. In the south, where the number of spawners was small and samples were composed of smaller fish, the horizontal migration to the main spawning ground seems less plausible. If occurring it should be to and from the southwest or to the neighbouring Mediterranean Sea.

Significantly different body sizes and sex composition have been reported for fish caught by different nets. For example, longlines were found to catch mostly females of black dogfish (*Centroscyllium fabricii*) (Wirtz and Morato, 2001). For blue whiting, the individuals dominating in bottom trawl catches were generally larger than those caught by midwater trawls in a number of areas (Bailey, 1982). During spawning the opposite was observed (Pawson, 1979). The same effect was observed in this study with the absence of larger fish in winter.

Blue whiting is commonly caught as by-catch by Portuguese bottom-trawl fleets targeting finfish and crustaceans (ICES, 2016b). Although these fleets operate to deeper depths and thereby catch fish bigger than the ones caught by the surveys (unpublished data), the survey data are still representative of the Portuguese component of the population. Thus, the results from this study based on bottom trawl sampling revealed that attention must be paid to the area and season where the fleet is operating, because a shift in the 1:1 sex composition, in species with dimorphism in growth, if not taken into account may biased the age-at-length key and subsequently the data produced for assessment. However, sampling during the surveys took place during daytime, while the fishing fleets operate day and night. Thus the sex composition of commercial catches could also change related to the time of capture (day *versus* night), due to diel vertical migrations (Johnsen and Godø, 2007). This suggests that the use of separate age-length keys by season and area reflecting the sex composition in commercial catches should be evaluated.

In conclusion, outside the spawning season blue whiting females occurred predominantly close to the sea bottom during the day. During spawning, males remained in great numbers close to the sea bottom surface. However, the underlying processes remain unknown. Implementing acoustic echosounder recordings during the Portuguese bottom trawl surveys would be a first step to study blue whiting vertical migrations and contribute to improve community and ecosystem studies.

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Chapter 4

4. Image analysis as a tool to age estimations in fishes: an approach using blue whiting in ImageJ

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4.1 Abstract

Otoliths are the fish bones that allow it to hear sounds and achieve balance. The otolith grows in size as fish grows; ring bands are formed in the otoliths' surface registering periods of rapid and slow growth, opaque bands appear alternating with translucent bands. Age classification was made considering the number of translucent rings in the otolith; one translucent ring was equivalent to one year. The modelling of fish species abundance on the majority of fisheries assessment use age based models. The task of ring counting and ageing is time consuming and may introduce errors that can have a strong impact in stock assessment results. Thus, accurate and precise age estimates are crucial for the effective management and understanding of fisheries resources because recruitment dynamics, growth and mortality estimates relies on these data. The main goal of this study is to produce automatic reading procedures to help researchers, ageing blue whiting fish, minimize ring error count and improve accuracy and precision on age estimation.

Keywords: Otoliths, fish ageing, image analysis, image processing, blue whiting and fisheries science.

4.2 Introduction

Otoliths are constituted by three pairs of calcareous structures, the *sagittae*, *lapilli* and *asterisci*, which are found in the inner ear. At the end of the 1960s and the beginning of the 1970s, *sagittae* otoliths started to be used for age determination and to study the feeding relationships between fish predators and their prey (Tuset, Lombarte and Assis, 2008). Since then the modelling of fish species abundance on the majority of fisheries assessment relies on age based models. Accurate and precise age estimates are critical for the effective management and understanding of fisheries resources because dynamic rates of recruitment, growth and mortality depend on these data (Campana, 2001).

Age classification has been based in age readings under stereo-microscopic observation. The interpretation of age reading is a very difficult task and may change among different readers or as a reader becomes more experienced (Doering-Arjes, Cardinale and Mosegaard, 2008). Regularly, calibration age reading workshops are conducted aiming the improvement of accuracy and precision of age estimations between the readers of the same species. Quality assurance in the ageing process guarantees the consistency which is uniquely important to this type of research work (Morison, Robertson and Smith, 1998). The criteria used to count the rings and determine age are made to be objective, but the final classifications are always dependent on the reader's experience, which introduces an interpretation error in the process (Campana and Moksness, 1991). This interpretation error can be either biased or random. In combination, process and interpretation error can result in age estimates that may differ by as much as a factor of three among readers (Donald *et al.*, 1992).

There is an urgent need to validate and calibrate the currently age reading procedure within more effective automatic classification, in order to avoid subjective, and misinterpretation among readers. The image analysis constitutes a valuable tool applied to otoliths ageing and the recorded images allow performing simultaneously other studies, such as age validation. Several image analysis programs had been adapted in order to allow automatic count of daily increments such as RATOC (RATOC System Engineering Inc.), Image Pro Plus (Image Pro Plus) and TNPC (TNPC) and also annually increments like Image Pro Plus, TNPC, CAF (Morison, Robertson and Smith, 1998) and ImageJ (N. I. of Health, ImageJ). Although, the majority of the programs requires a license and is expensive, ImageJ has the advantage of being free software which allows record macros and plugins making it simple to add algorithms to improve species ageing classifications.

The aim of this study is to improve ageing classifications through image analysis making the process based in more objective criteria. In order to achieve that goal a plug-in has been written and added to ImageJ. The otoliths used to test this new approach are from the blue whiting (*Micromesistius poutassou*) and the preliminary results are presented here. This study has been developed in order to know if: ImageJ could be used as an approach to improve the accuracy and precision of blue whiting ageing estimation?

4.3 Contribution to Smart Systems

In fisheries, for stock assessment annually a huge number of otoliths are read and the data for different countries combined. As an example, concerning the blue whiting stock, in 2015, around 102,000 fishes were measured and from these around 30,000 otoliths were processed for age reading, comprising data from 16 countries (ICES, 2016). The consistent application of the ageing method over years, among readers, and even among laboratories (countries), is a particular requirement of stock assessment studies (Campana, 2001). The inter-calibration workshops conducted regularly aim to guarantee that these age data can be combined and all the readers, from the different countries, follow the same criteria. The criteria for age estimation, based on ring counts, is defined to be easy to follow and objective. In practice, the accuracy and precision on age classifications is always dependent on the reader experience which reveal some subjectivity on the criteria. The age reading protocols based on images observations, is time consuming and based on decisions which can differ according to the reader experience.

The increasing demand on fisheries science to produce more data, the time involved and the ageing criteria mostly based on human decision, created the requirements to searching new technologies applied to age reading estimation. This new technologies should be easy to apply and available to a wide range of scientists.

The otolith grows in size as fish grows; ring bands are formed in the otoliths surface registering periods of rapid and slow growth, opaque bands appear alternating with translucent bands. Therefore, the otoliths are constituted by different optically zones according to their structural appearance or light properties, e.g., translucent or opaque and the distinction among them allows to identify and count the rings through stereo-microscope observation (Casselman, 1983).

ImageJ is an open source software for image analysis and processing, and allows java written plugins to be added (N. I. of Health, ImageJ). Thus, new plugins are easily spread and become available to a huge number of scientists with the purpose of improving fish age classification. A smart system for otolith age classification could provide input from different expert readers for the same otolith, and then adaptively learn to classify correctly the ring identification providing further information within each ring that may be useful for the expert readers' further research. The best automatic system should use the output of a camera with optical magnification, identify the otolith and determine its age by providing a value, its confidence intervals, and precision to help the researchers evaluate and decide in a more objective way the age classification. In this study, the automatic system is still in development aiming to improve the age classification on blue whiting.

4.4 Material and Methods

4.4.1 Sample collection

Fish samples of blue whiting were collected along the Portuguese coast during 2013, from January to December. Total length (cm) and sex of all blue whiting sampled were recorded and the otoliths were removed from each fish. After, the otoliths were washed and stored dry. Sixty seven blue whiting otoliths were considered for this study. Otoliths were submerged in a 0.1% thymol solution (1g of thymol for 1l of filtered and distilled water) for approximately 24h. The whole otolith was immersed in oil and observed under reflected light against a black background in a stereo-microscope. The otoliths images acquisition was performed using the TNPC software (version 7.0). For age classification only one otolith of the *sagittae* pair was used. On the current study the left otolith was used. Age classification was made considering the number of translucent rings in the otolith; one translucent ring was equivalent to one year.

4.4.2 Image analysis

A plugin was developed in Java for the ImageJ software which allowed counting the age rings in the otolith image files. On the left otolith of each pair a transect line crossing the otolith longer axis is drawn (Figure 4-1).

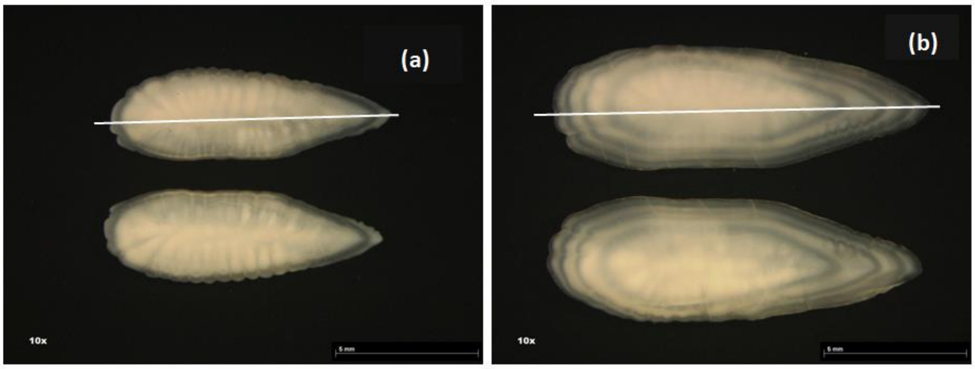

Figure 4-1 Blue whiting left (top) (with the transect line) and right (bottom) otoliths from fishes with: (a) 1 year old and (b) 4 years old.

The automatic reading evaluation is based on the results from the otolith profile plotted along the longer axis (Figure 4-1).

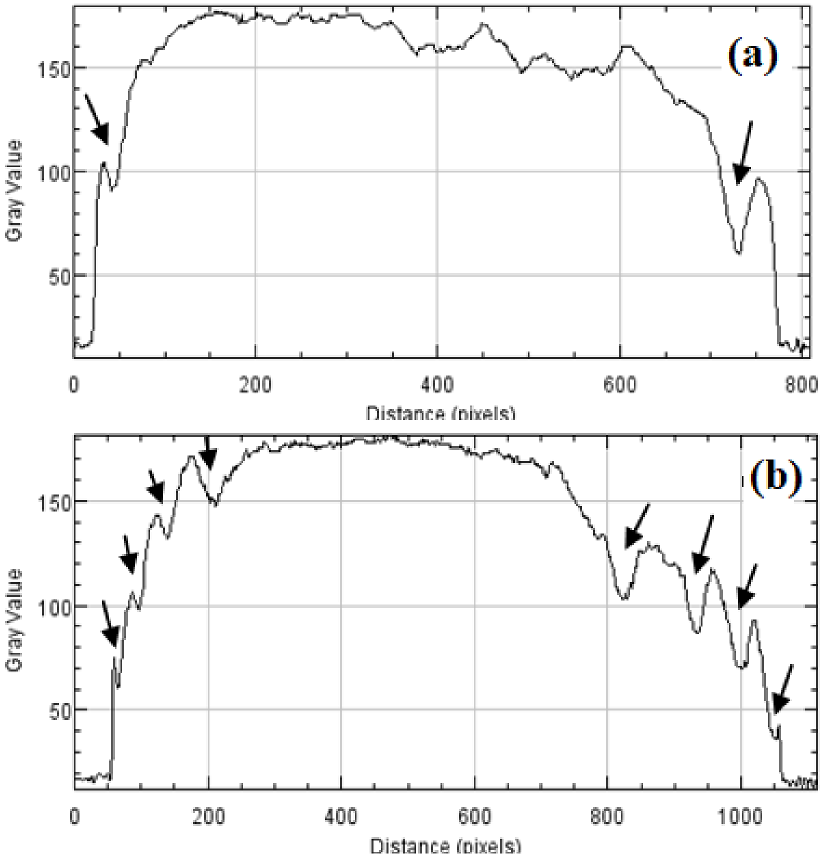

Figure 4-2 Blue whiting left otolith profile along the long axis from fishes with: (a) 1 year old (Figure 4-1a) and (b) 4 year old (Figure 4-1b). The arrows indicate the rings position in the profile density plot.

The results from the automatic reading application were recorded and compared to the reader's age estimation.

4.4.3 Statistical analysis

A test of symmetry (Hoenig, Morgan and Brown, 1995) for determining if significant differences existed between the ages by readers and the ages by ImageJ was applied. The test of symmetry uses a chi-square-type statistical test to determine if the age-agreement table is symmetric or not. If the age-agreement table is determined to be asymmetric then it can be concluded that there is a systematic difference in ages observed between readers and automatic ageing (ImageJ).

Two statistical indicators were used to measure the precision: (i) the average percent error (APE) (Beamish and Fournier, 1981) and (ii) the coefficient of variation (CV). The APE assumes that the standard deviation of the age estimates are proportional to the mean of the age estimates.

All statistical analyses and plots were performed using the packages FSA and ggplot2 from the statistical environment R (R Core Team, 2017).

4.5 Results

The length at age by sex was represented taking into account age classifications by readers (Figure 4-3a) and by ImageJ (Figure 4-3b). There are differences in the growth curves, namely the range of ages is higher in the ImageJ between 0 and 7, while the range by readers varied from 1 to 6. The indeterminate[1] fish were classified as 0 year with the ImageJ, although the readers aged those fish as 1 year old.

[1] Denomination used when fish gonads show an earlier development stage and is not possible to assign a sex, *i.e.*, to distinguish between female or male.

The Hoenig test of symmetry (p = 0.094) indicates that there are systematic differences in the assigned ages between readers and ImageJ. The ages estimation based on the automatic procedure are overestimated compared with the ages attributed from the readers (Figure 4-4). The same age was attributed by the two procedures in 36 otoliths, in a total of 67.

The precision methods reveal a low percentage of agreement (54%) between the two procedures, with CV=21 and APE=16.

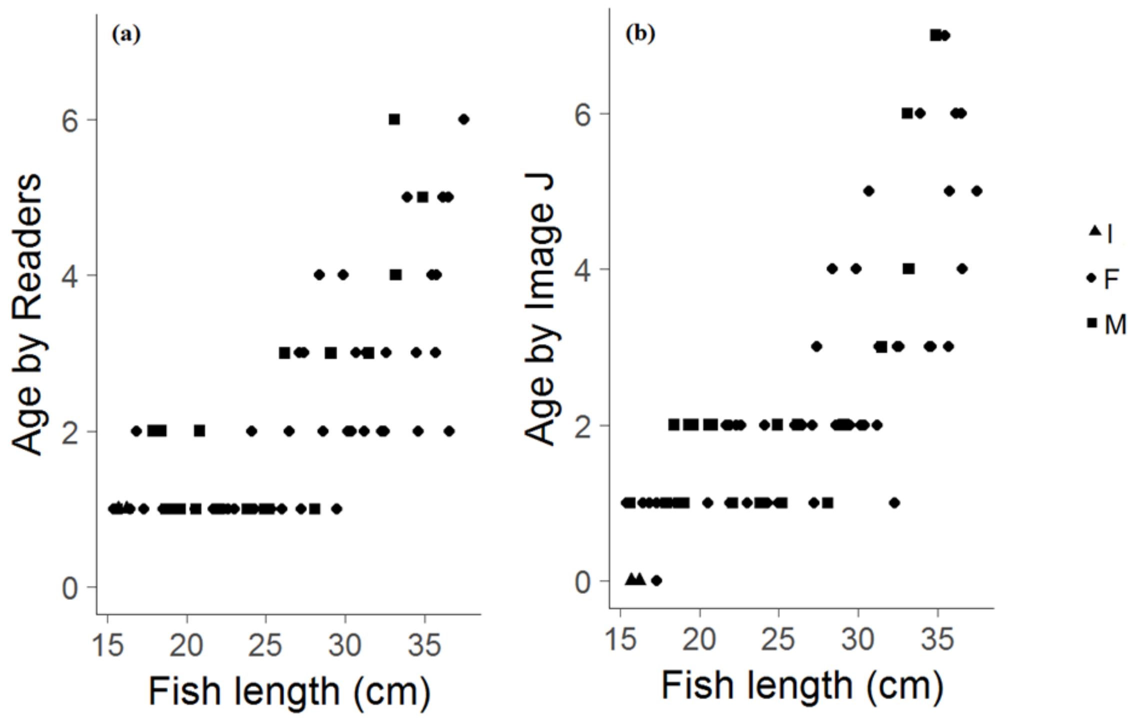

Figure 4-3 Fish length (cm) by age according to classifications by: a) readers and b) ImageJ application. Symbols correspond to fish's sex: ▲indeterminate; ● females; and ■ males.

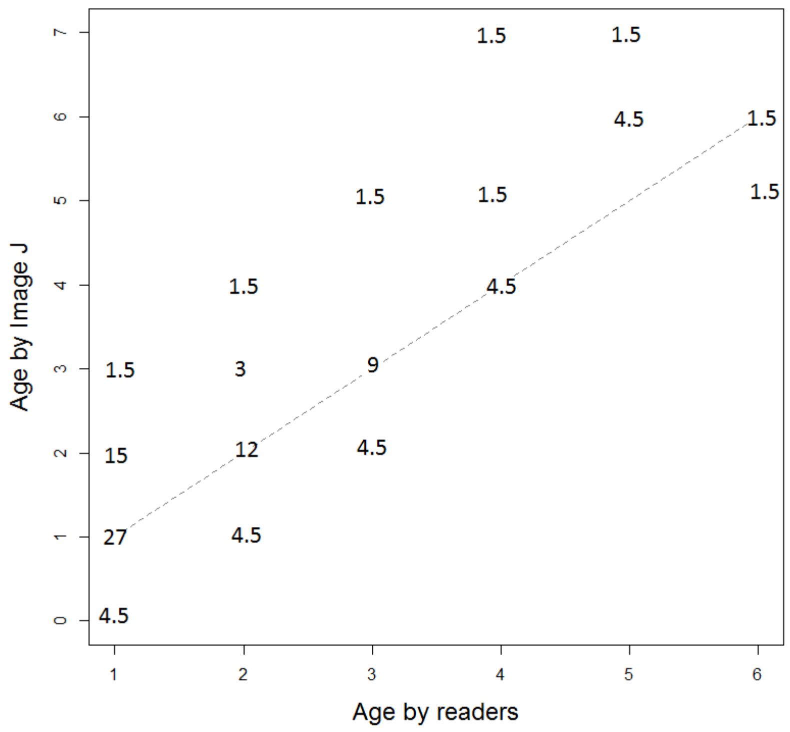

Figure 4-4 Age-bias plot of estimations by readers and by ImageJ application. The dashed line represents the age-agreement between procedures. Values represent number of otoliths in percentage.

4.6 Discussion

The results from this study constitute the first application test performed in blue whiting otoliths from the Portuguese coast. In this test fish from a large length range and both sexes have been used, allowing identification of this procedure applicability in otoliths with different growth patterns. Differences were found in the age estimation from readers and using the ImageJ. Ages from readers take into account the time of the year, the type of otolith border and some subjectivity during ring count based on their experience. Ages are also estimated with the use of auxiliary information, such as fish size or sex which may bias the reader's interpretation (Morison, Robertson and Smith, 1998). There is a certain risk on use the fish size as auxiliary information on ageing, which could result in inability to detect changes on fish growth pattern due to the periods of starvation or to the climate changes, since temperature affects growth. The age estimation from ImageJ was only based on the ring count on the density profile plot which is an advantage. As an example, of this study results, a fish with length higher than 30cm, was classified as 1 by ImageJ and 2 years by the readers, this underestimation by the program could be due to readers based their classifications on the fish length and this fish could present a different growth pattern which was not detected in automatic classification. Notwithstanding this particular case, ImageJ is overestimating the ages in the majority of this study classifications, which could be due to a higher sensitivity of the software into recognizing patterns in the otolith density across the whole otolith section which could not be so evident through direct image observation or the irregular surface of some otoliths or the software counts is considering the denominated false rings[2] as valid rings. False rings are a common issue in blue whiting and a source of bias amongst the readers (ICES, 2013). Although, since the age's estimations comparison were based on reader's classifications and not in validated ages those hypothesis need to be further evaluated. In order to validate the plugin ImageJ estimations, it is important to repeat this test using otoliths from fishes in which the absolute age is known. There are also some basic age validations techniques, such as the relations between fish length and otolith length, fish length and otolith weight (Campana, 2001), which could be applied to the samples used on this study as a way to obtain more precision in estimations. This study constitutes a first approach to the blue whiting ageing validation and classification, the facility of incorporate macros on ImageJ will allow improving and calibrating the results until obtaining a good compromise between the automatic procedures and the fish growth.

The future steps, are applying this plugin and provide the age estimations using the information of the otolith type of border and the time of the year when the fish was collected. Also, applying the ImageJ plugin to otoliths with ages validated or in which the age estimation does not present any doubts. These tools will also be tested by experiment readers on blue whiting, as an exercise where the auxiliary information (fish length and sex) will not be provided, during the next international calibration workshop

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2 Any opaque area similar to an annual band but interrupted or without a "discontinuity" (if in the central area of the section) (Peres and Haimovici, 2004).

which will be held at Lisbon this June. The authors of this study truly believe that this new approach is a valid and useful application to help, the readers from the different countries, producing more precise and accurate fish age estimations on blue whiting.

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Chapter 5

5. Blue whiting otoliths pair's symmetry side effect

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5.1 Abstract

Otoliths are paired calcified structures located in the inner ear of the teleost fishes. Fisheries scientists have been using the otoliths for a variety of applications as fish ageing, species identification and also species interaction. The symmetry between left and right otoliths is considered species dependent. The application of analysis techniques on otoliths images has been increasing in recent years. The diagnosis on otoliths symmetry as a species characteristic is currently being done. It is expected that the otolith surface (distal *versus* proximal) used to collect images should have no influence on the symmetry results. The goal of this study is to investigate if the otolith position (distal *versus* proximal) when analyse images has influence in the diagnostic of the symmetry shape between the left and right otoliths. The results showed that the otolith face orientation should be taken into account during the otoliths images processing for symmetry shape analysis. On blue whiting the otolith images should always be obtained from the concave side, because for symmetry studies the position is important.

Keywords: Otoliths image analysis; morphometry analysis; fish otoliths; marine science application; Elliptic Fourier analysis.

5.2 Introduction

Otoliths are constituted by three pairs of calcified structures, the *sagittae*, *lapilli* and *asterisc*i, located in the inner ear of the fishes, involved in hearing and balance systems (Campana and Casselman, 1993). The *sagittae* otoliths are characterized by high morphological variability between species (Platt and Popper, 1981). The environment and endogenous factors influence both overall otolith shape and growth patterns (Lombarte, Torres and Morales-Nin, 2003; Capoccioni *et al.*, 2011). Otoliths have been used for a variety of purposes, as important tools in taxonomy, for species identification, for determining fish age, to investigate changes in marine populations and to infer the fish diet in marine food webs.

The use of otoliths for age determination has long been recognized by fisheries biologists (Jackson, 2007) and broadly applied to assess the stock abundance. Traditionally the otoliths age classification has been based on a direct observation through stereo-microscope. In recent years, the application of image analysis on otoliths facilitated the construction of image databases. These otoliths image databases allow to save the age classification, which provides an effective tool on the training of new age readers and also to use on international calibration exchanges.

The otoliths images are also used in studies to distinguish between populations of the same species. Taking into account that symmetry is species dependent, thus it is expected that the otolith surface (distal *versus* proximal) used to collect images should have no influence on the results. Previous works studying otoliths asymmetry have been conducted, in round and flatfishes (Mille *et al.*, 2015) and on blue whiting (*Micromesistius poutassou*) (Mahe *et al.*, 2016), but only used images obtained from the otolith distal surface

In fisheries science, currently there is an increase demand to collect even more data, to extend the number of species studied, to study more deeply those species and to annually produce for assessment studies a great quantity of data to be used on complex statistical models. This data also includes the collection of otoliths to achieve species age stock structure annually. The marine institutes involved in blue whiting stock assessment on the Northeast Atlantic, sample by year around 102,000 fishes and from these around 30,000 otoliths were processed for age reading (ICES, 2016).

The aim of this study is to investigate if the otolith surface position on blue whiting could have influence in the diagnostic of the symmetry shape between the left and right otoliths of the same pair.

5.3 Material and Methods

5.3.1 Sample collection

The blue whiting samples were collected on the Portuguese southern coast (around Latitude 36.7ºN, Longitude 7.7ºW), during October of 2015 and March of 2016. Total length (cm), total weight (g) and sex of all blue whiting sampled were recorded and the otoliths were removed from each fish. After, the otoliths were washed and stored dry. Forty pairs of blue whiting otoliths were considered for this study, 17 from October and 23 from March. The left and right *sagittae* otoliths of the same pair were considered for the otolith shape analysis.

5.3.2 Otolith shape analysis

Images of the whole left and right otoliths were scanned (Epson V750) under reflected light and stored with high resolution (3200 dpi). Image processing was performed using the TNPC software (version 7) (TNPC - Numerical Treatment of Calcified Structure) with the proximal surface facing up (concave side in the scanner) (Figure 5-1a) and with the distal surface facing up (convex side in the scanner) (Figure 5-1b).

Otolith length and width were measured and the contour of each otolith was obtained using the automatic threshold in the TNPC software. To describe otolith contours, Elliptic Fourier Analysis (EFA) was carried out. For each otolith, the first 99 elliptical Fourier harmonics (Hi) were extracted and normalised with respect to the first harmonic to be invariant to otolith size, rotation and starting point of the shape measurements. To determine the number of harmonics needed to reconstruct the otolith outline, the Fourier Power (PF) was calculated for each individual otolith k as a measure of the amount of contour rebuilt by each harmonic:

$$\mathrm{PF}(\mathrm{n_k}) = \sum_{\mathrm{HI}=1}^{\mathrm{n_k}} \frac{A_{HI}^2 + B_{HI}^2 + C_{HI}^2 + D_{HI}^2}{2}$$

where A_{HI}, B_{HI}, C_{HI} and D_{HI} are the parameters of the HI^{th} harmonic and n_k is the total number of harmonics included. The value of n_k was chosen such that $\mathrm{PF}(\mathrm{n_k})$ explains 99.99% of variance in contour coordinates or, in other words, such that shape is reconstructed at 99.99%. The PF was calculated for each individual and the maximal number of harmonic was kept to the shape analysis. Consequently, only the first 34 harmonics were included in the statistical analysis.

5.3.3 Statistical analysis

A Principal Component Analysis (PCA) was carried out on the Elliptic Fourier Descriptors (EFD) matrix of the sample (EFDs as columns and individual otoliths as rows) and a sub-set of the resulting principal components were selected as otolith shape descriptors according to the broken stick model. This allowed the number of variables used to describe otolith shape variability to be decreased while ensuring that the main sources of shape variation were kept, and to avoid co-linearity effect between shape descriptors (Rohlf and Archie, 1984). The statistical analysis followed the procedure used and described by Mille *et al.*, 2015. The following shape comparisons were performed: (i) left otolith using images from the distal and proximal surface; (ii) the right otolith using images from the distal and proximal surface; (iii) images from the proximal surface of right and left otoliths; and (iv) images from the distal surface of right and left otoliths.

All plots and statistical analyses were performed using the statistical environment R (R Core Team, 2017).

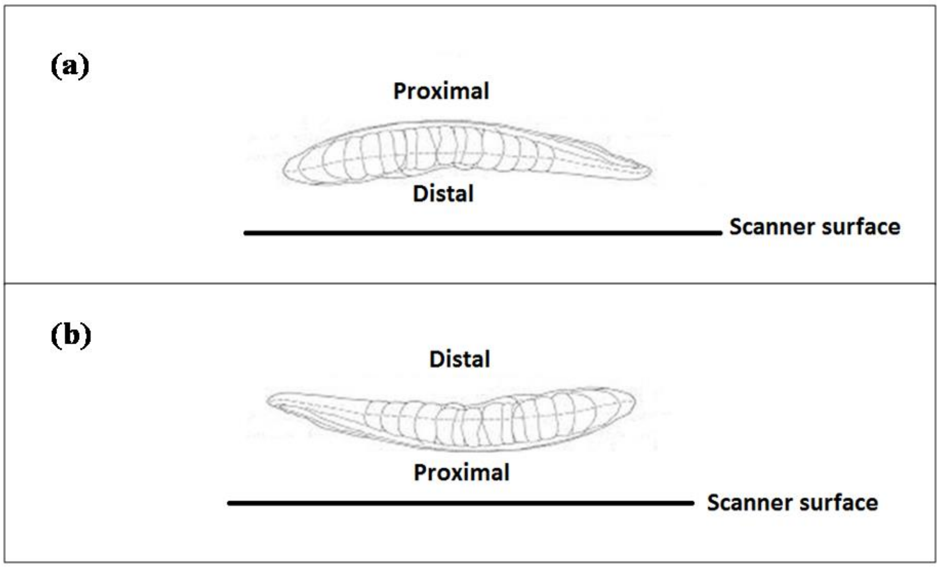

Figure 5-1 A lateral image of the right otolith, showing the otolith position in the scanner surface: (a) the distal surface (concave side); (b) the proximal surface (convex side).

5.4 Results

The length of the fishes collected in October 2015 ranged between 15cm and 17cm and weighted between 15g and 40g (Figure 5-2). The fishes from the March 2016 sample were larger (between 27cm and 30.7cm) and heavier (from 85g to 175g) than those sampled during October 2015.

In the October 2015 sample, the blue whiting were constituted by 7 females, 2 indeterminate and 8 males (Figure 5-3). In the March 2016, sample consisted of only females and males, 15 and 8, respectively with no indeterminate observed.

The samples used comprised of two groups, one constituting the smallest fishes and the other the biggest fishes, and from both sexes. The presence of fishes from both sexes and different sizes allowed studying left and right otoliths symmetry across different blue whiting length ranges.

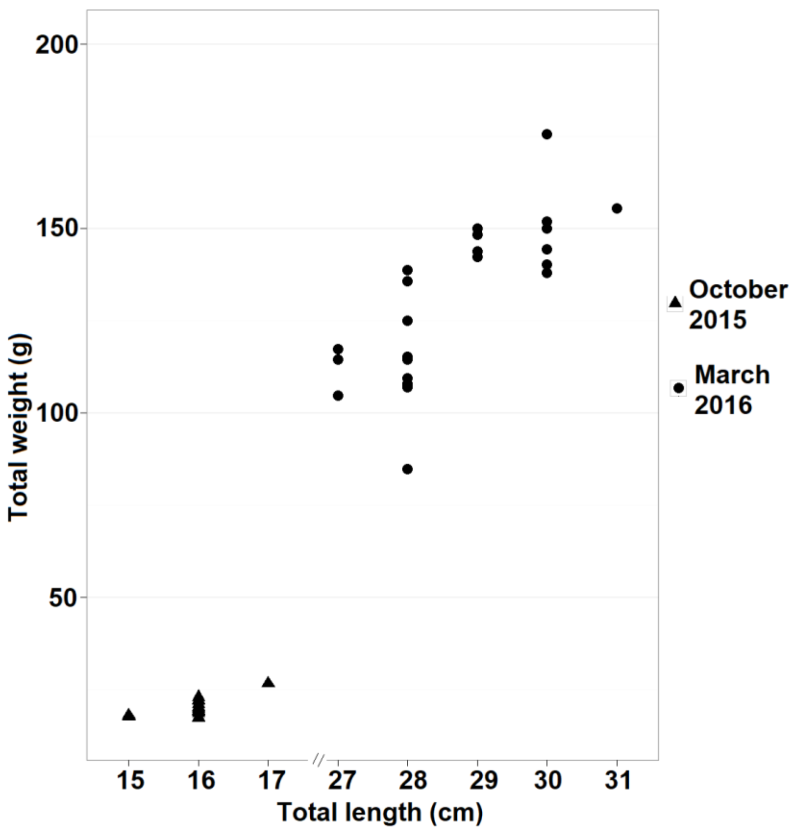

Figure 5-2 Total length (cm) and total weight (g) of the blue whiting samples collected during October 2015 (n=17) and March 2016 (n=23).

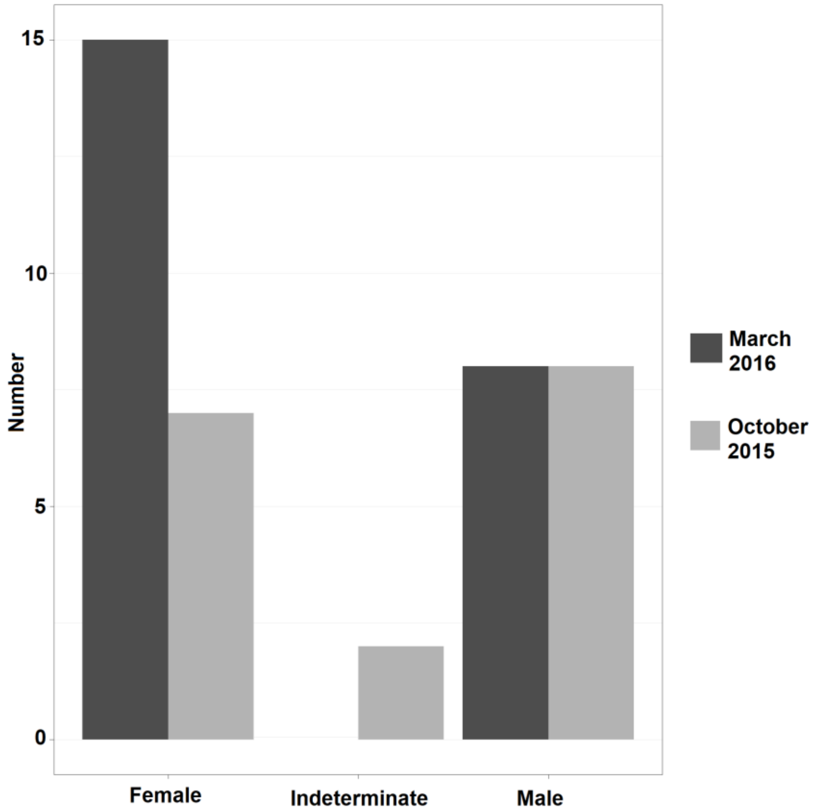

Figure 5-3 Number of females (n=22), indeterminate (n=2) and males (n=16) in the blue whiting samples from March 2016 and October 2015.

The otoliths shape comparisons based on the EFD are shown in Figure 5-4 and Figure 5-5. Differences in the symmetry were observed in: the left otolith images from distal and proximal position (Figure 5-4(i)); the right otolith images from distal and proximal position (Figure 5-4(ii)); the left and right otoliths images taken from the proximal position (Figure 5-5 (iii)); the images taken from left and right otoliths collected from the distal surface were symmetric (Figure 5-5(iv)).

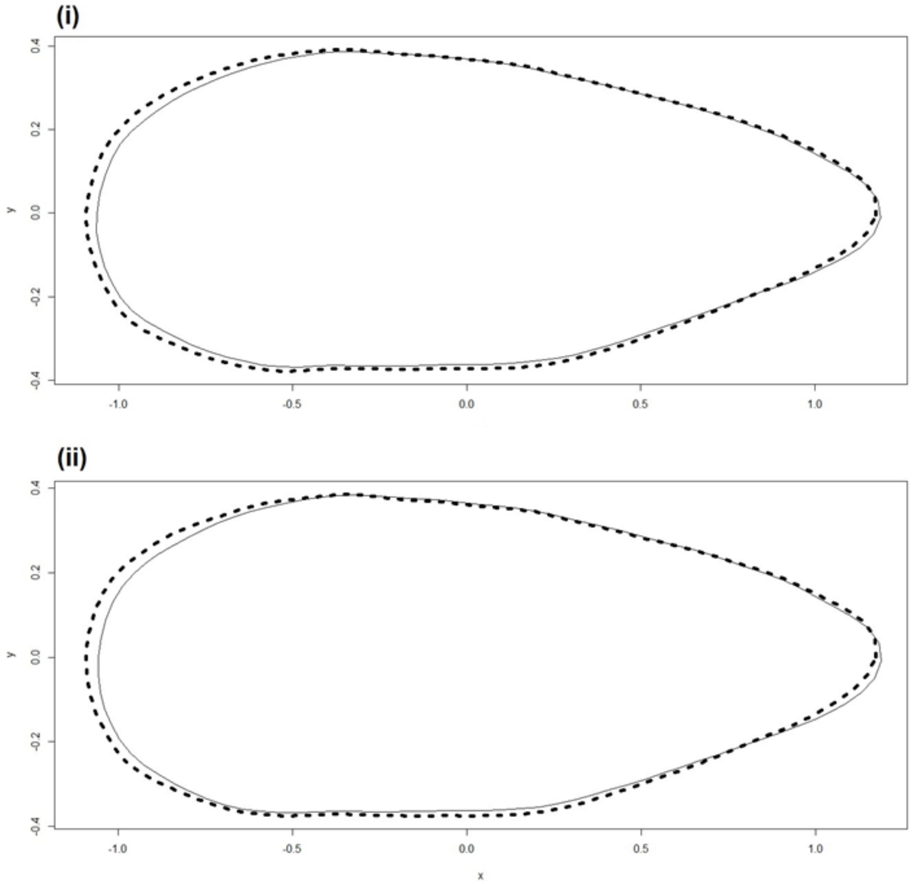

Figure 5-4 Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 34 harmonics showing the overlap and variations between: (i) left from the proximal surface (dark grey dash line) and left from the distal surface (grey solid line); (ii) right from the proximal surface (dark grey dash line) and right from the distal surface (grey solid line).

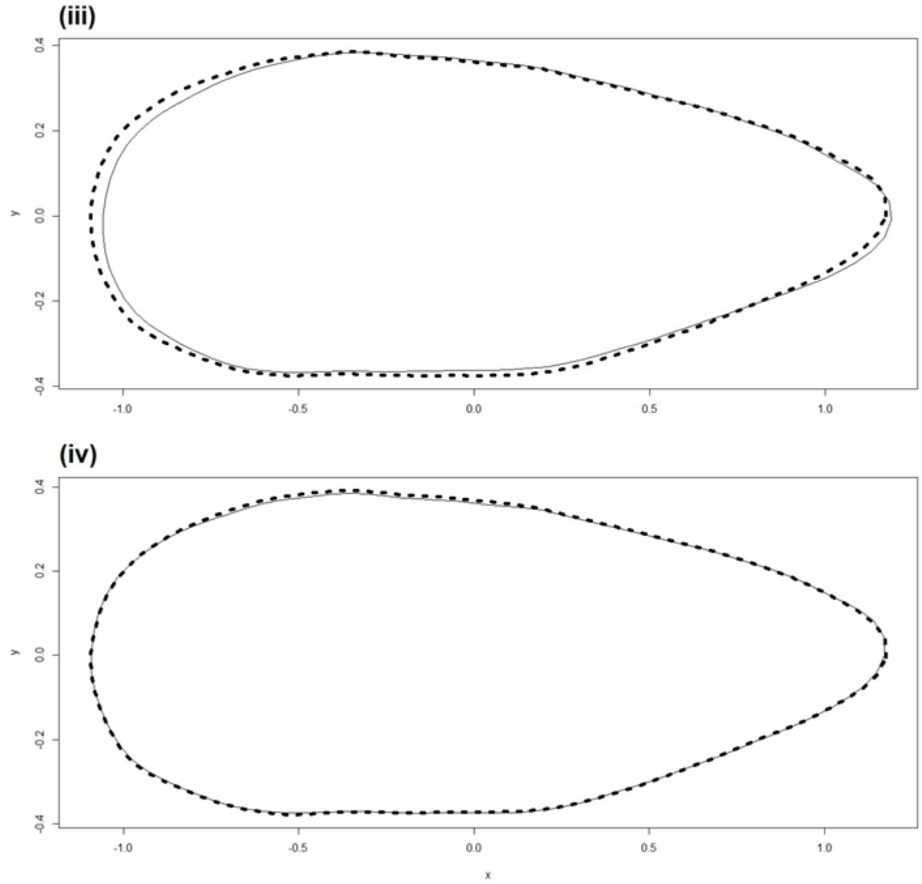

Figure 5-5 Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 34 harmonics showing the overlap and variations between: (iii) right from the proximal surface (dark grey dash line) and left from the proximal surface (grey solid line); (iv) right from the distal surface (dark grey dash line) and left from the distal surface (grey solid line).

5.5 Discussion

The result obtained on this study emphasizes the need to carefully evaluate the differences between the otoliths side, when obtaining images, before performing morphometry on those structures. In the majority of otoliths morphometric studies, the images were recorded with the *sulcus acusticus* facing up to the camera, from the distal face (e.g. Campana & Casselman, 1993; Tracey, Lyle & Duhamel, 2006; Vignon, 2012; Mahe *et al.*, 2016). Notwithstanding, in some works the face were images were captured was not mentioned (e.g Bird and Eppler, 1986; Gagliano and McCormick, 2004). On some otoliths shape analysis studies although the image from left and the right otolith were recorded, just one of the otoliths was used to perform the analysis. As an example for cod, it is assumed a shape symmetry between the left and the right otolith of the same pair, thus in such cases were the chosen otolith (left or right) was damaged or crystalline, the other otolith (right or left) is used in its place (Galley, Wright and Gibb, 2006; Petursdottir, Begg and Marteinsdottir, 2006). Although, for cod, the images were captured with the otolith positioning with the proximal side facing up to the camera (Petursdottir, Begg and Marteinsdottir, 2006) and also with the distal side facing up (Campana and Casselman, 1993). To our knowledge the effect of the otolith position was never been evaluated in other species. According to this study results, this effect should be studied for other species otherwise the images must be obtaining always with the otolith on the same position. The standardization of the otolith side on image along the different works for the same species will avoid some degree of uncertainty between the results and allow a better comparison and interpretation of the results between them.

The results obtained, in the comparison of left and right otoliths shapes from the same otolith pair, showed that the otolith face orientation should be taken into account during the otoliths images processing for symmetry shape analysis. According to a previous study (Mahe *et al.*, 2016), the expected symmetry between the left and the right otoliths, on blue whiting was only observed using the images taken from the distal surface. In conclusion, for blue whiting, the otolith images should always be obtained from the concave side, because for symmetry studies the position real matter.

In the future, a larger blue whiting sample will be used to study if on this species the geographical area, depth and sex could have a significant effect on the otolith’s morphometry.

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Chapter 6

6. Integrating microsatellite and otolith methodologies to assess population structure of blue whiting (*Micromesistius poutasou*) off the Portuguese coast

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Submitted to Fisheries Research

6.1 Abstract

On blue whiting (*Micromesistius poutassou*), a mesopelagic gadoid widely distributed in the Northeast Atlantic, stock definition is still under revision. The geography of the Portuguese coast, the southern border of this stock, with the influence of both the Mediterranean current (south) and the northern current (west) justifies the investigation on local stock structure. The contingence of the blue whiting population off Portugal could be genetically closer to the Mediterranean or to the Celtic Sea, is the research question addressed here. Thereby, the main goal was investigate the blue whiting population structure along the Portuguese coast, by integrating microsatellite genetic markers and otolith shape methodologies. In order to track possible differences between stock areas, samples collected along the Portuguese coast, in the Ireland coast (Porcupine Bank), in the Mediterranean Sea and in the Adriatic Sea were analysed. Microsatellite DNA markers analysis revealed genetically differentiation only between the blue whiting collected in the Adriatic and the other sampling locations (Northeast Atlantic and Mediterranean). But the Elliptic Fourier analysis from the blue whiting otoliths revealed different shapes on Ireland, Portugal and the Mediterranean. Off the Portuguese coast differences were related to samples depth on immature. However, in this area the mostly significant differences were connected to ontogeny. Microsatelite and otolith morphometry results concerning the Portuguese coast, indicates that local blue whiting constitutes a single population unit. Otolith shape analysis highlights a clear separation between two subpopulations along the Northeast Atlantic, one at south ending on the Portuguese coast and another at north on the Ireland coast. There is a need to take into account the identification of phenotypic stocks to ensure sustainable fishery benefits and efficient conservation as they may have unique demographic properties (e.g. growth rates, spawning sites) and therefore, responses to exploitation states.

Keywords: Blue whiting; microsatellite genetic markers: otolith shape analysis; stock structure; Portugal; Northeast Atlantic.

6.2 Introduction

Fish stocks are defined as demographically independent populations whose dynamics depend largely on local birth and death rates rather than on immigration alone (Hilborn and Walters, 1992; Quinn and Deriso, 1999; Palsbøl, Bérube and Alendorf, 2007). Stock identification is of crucial importance in modern fisheries stock assessment for the short-term management and conservation of natural populations since it permits to delineate monitoring entities (Schwartz, Luikart and Waples, 2007) and understand population dynamics of a species on an ecological perspective (Agüera and Brophy, 2011).

There are many techniques that have been traditionally used to study fish stock structure (reviewed in Begg and Waldman, 1999), in particular, molecular and otolith shape analyses. The application of genetic markers to stock identification started in the early 1980s with simple alloenzyme electrophoresis, but since then a series of quick technological advances have significantly enhanced the use of different and more appropriate molecular markers for identification of stocks (Chistiakov, Hellemans and Volckaert, 2006), like microsatellites that are extensively used. Otolith shape is also widely used to discriminate and characterize fish stocks (Bird, Eppler and Checkley, 1986; Campana and Casselman, 1993; Tracey, Lyle and Duhamel, 2006) since fish from different geographic areas can present variations in otolith shape depending on exposure to environmental conditions, representing a phenotypic measure of stock identification (Lecomte-finiger, 1999).

However, the majority of the used tools to differentiate and characterize fishing stocks have proven to be insufficient or misleading in most studies. More recently, it had become clear that only multidisciplinary approaches can lead to accurate distinguish management stocks.

Stock limits of blue whiting (*Micromesistius poutassou*), a mesopelagic gadoid widely distributed throughout the Northeast Atlantic (Bailey, 1982), have long been studied, contested and are still under revision. Prior to the year of 1993 (ICES, 1995), it was assumed that blue whiting had two stock components, a northern and a southern component along the Northeast Atlantic, with the Porcupine Bank latitude as border delimitation. The Northern stock is believed to feed in the Norwegian Sea and spawn west of the British Isles. The Southern stock is found along the continental shelf off the coast of Spain and Portugal. However, after 1993, based on an otolith growth study showing no significant difference in mean annual ring diameter between northern and southern stocks, this division was contested and since then blue whiting stock is considered as a single management unit (ICES, 2016) and fisheries management measures have been applied to the entire stock.

The stock structure, on this species, was investigated using several different approaches like larval otolith growth patterns, the movements of eggs and larvae, genetics and otoliths shape analyses (e.g. Mork and Giæver, 1995; Giæver and Stein, 1998; Ryan, Mattiangeli and Mork, 2005; Pointin and Payne, 2014; Mahe, Oudard, *et al.*, 2016).

The first genetic study on blue whiting used allozymes (Mork and Giæver, 1995), and revealed a clear genetic differentiation of Barents Sea's samples from the rest of the Northeast Atlantic. In 1998, genetically distinct populations were also observed in the Barents Sea and in the Romsdals fjord area of Norway (Giæver and Stein, 1998). Later on, similar results were obtained using one minisatellite and five microsatellite loci, besides a clear genetic differentiation of a Mediterranean population (East of Corsica) (Ryan, Mattiangeli and Mork, 2005). On a smaller scale, a study using a landscape genetics approach, revealed a clear separation between samples from the south flowing current of Porcupine Bank (Celtic Sea and Bay of Biscay) from those of the north flowing current (Was, Gosling, Mccrann, *et al.*, 2008).

On another hand, otolith shape analysis also showed to reliably identify blue whiting stock origin (Keating *et al.*, 2014; Mahe, Oudard, *et al.*, 2016) and two distinct otolith morphotypes were found between fish from distinct geographical distribution areas north and south of the Porcupine Bank.

Despite all these findings supported the hypothesis of a clear population differentiation, i.e., two main fishery stocks, with a separation at the Porcupine Bank latitude, the blue whiting is still treated as a single stock unit.

In general, most existing studies included samples from a wide range of locations of the Northeast Atlantic (northern stock) but there was a low representativity of southern distribution of the species, which have contributed to the non-assessment and consideration of the two separated stocks in blue whiting assessment.

Distribution limits have showed to be important sources of genetic variability since these populations are often isolated by distance and present differentiation from the rest of the populations. Therefore, the Portuguese Coast, as the southern distribution limit of the species in the North Atlantic is of crucial importance to have a realistic scenario of the population dynamics of the blue whiting. However, this area has only been superficially explored in one genetic study (Ryan, Mattiangeli and Mork, 2005) and an otolith shape analysis (Mahe, Oudard, *et al.*, 2016). The Portuguese south coast receives from southeast Mediterranean waters while the colder North Atlantic waters went down the west coast. This oceanography mixed scenario could support the co-existence on Portuguese waters of blue whiting genetically closer to the Mediterranean at south and closer to the Celtic Sea at north.

The main goal of this study is to integrate microsatellite genetic markers and otolith shape methodologies to investigate the blue whiting population structure at the Portuguese coast. For achieving this purpose samples collected in the Portuguese coast, Ireland coast (Porcupine Bank), Mediterranean Sea and Adriatic Sea will be used. Thereby, genetic and otolith shape variability will be determined amongst these areas and across the coast of Portugal.

6.3 Material and Methods

6.3.1 Sample collection

A total of 423 blue whiting samples from the Adriatic, Mediterranean, Ireland (Porcupine Bank), and several locations on the Portuguese coast (Figure 6-1; Table 6-1) were collected. The samples from the Ireland Coast (IRL) and the Portuguese Coast (PT-CAM, PT-LIS, PT-SW2 and PT-VSA) were collected during scientific surveys, the remaining sample locations were covered under commercial fishery sampling (PT-SW1, MED and ADR). On the samples collected at the Portuguese coast from the survey also included the registration of the hauls' depths (Table 6-2).

Figure 6-1 Sampling locations of blue whiting in the Northeast Atlantic, Mediterranean and Adriactic Sea. The samples locations collected for otolith shape analysis and genetic (white), only for otolith shape (orange) and only for genetic (yellow). IRL – Ireland Shelf (Porcupine Bank); PT-CAM – Caminha; PT-LIS – Lisboa; PT-SW- Portuguese Southwest Shelf (Sines); PT-VSA – Vila Real de Santo António; MED – Mediterranean Sea (south of Cape of Palos); ADR – Adriatic Sea. The prefix "PT" denominates the samples collected off Portugal. Map from Google maps.

Table 6-1 Sampling location, date of sampling, sample size (n) according to the type of analysis (OS – otolith shape; G – genetic) and fish total length (Lt) in cm of blue whiting. The samples from IRL, PT-CAM, PT-LIS, PT-SW2 and PT-VSA were collected during scientific surveys. The samples from PT-SW1, MED and ADR were from the commercial fishery.

Sample location	**Abbreviation**	**Date of sampling**	**n**		**Lt (cm) (OS)**		**Lt (cm) (G)**	
			OS	**G**	**Mean**	**SD**	**Mean**	**SD**
Ireland Shelf	IRL	March 2016	40	40	27.51	3.49	27.51	3.49
Portuguese Southwest Shelf (Sines)	PT-SW1	March 2016	38	-	30.3	2.54	-	-
Mediterranean Sea (Cape of Palos)	MED	June 2016	20 20	20 20	27.27 14.37	2.83 0.66	27.27 14.37	2.83 0.66
Adriatic Sea	ADR	July 2015	-	47	-	-	22.97	2.54
Caminha	PT-CAM	November 2015	70	35	17.92	1.72	17.76	1.79
Lisboa	PT-LIS	November 2015	95	45	17.67	1.67	18.45	2.84
Portuguese Southwest Shelf (Sines)	PT-SW2	October 2015	70	-	17.37	0.73	-	-
Vila Real de Santo António	PT-VSA	October 2015	70	38	16.62	0.73	16.59	0.75

Total length (±1cm) was determined, and sagittal otoliths as well as muscle tissue were extracted for each fish. The otoliths were cleaned from the adhering tissue dried and stored in plastic eppendorf tubes (n=423). Muscle tissue was removed and stored in pure ethanol for genetic analysis (n=245). Sex and sexual maturity of the sampled individuals were determined by macroscopic examination of gonads.

Table 6-2 Detailed information of the samples (OS – otolith shape; G – genetic) collected along the Portuguese coast, number of hauls by sample location and the respective depth of sampling (meters).

Sample location	**Abbreviation**	**Number of hauls**		**Depth (m)**	
		OS	**G**	**OS**	**G**
Caminha	PT-CAM	2	1	134 148	134
Lisboa	PT-LIS	2	2	100 259	100 259
Portuguese Southwest Shelf (Sines)	PT-SW2	3	-	147 148 385	-
Vila Real de Santo António	PT-VSA	2	2	180 324	180 324

6.3.2 Microsatellite marker analysis

Total genomic DNA was extracted from a total of 245 blue whiting muscle samples (Figure 1; Table 1) using a standard phenol/chlorophorm extraction method (Sambrook, Fritschi and Maniatis, 1989). Six microsatellite markers (MpouBW07, MpouBW08, MpouBW09, MpouBW13, Mmer-UEAW01, Tch06) previously described for this species (O'Reilly *et al.*, 2000; Was, Gosling, Mccrann, *et al.*, 2008) were amplified following PCR reaction conditions described in Was, Gosling, McCrann, *et al.* (2008). Forward primers of each marker were fluorescently labelled: Tch6 with FAM, MpouBW09 with VIC, Mmer-UEAW01 and MpouBW07 with NED, MpouBW08 and MpouBW13 with PET. GeneMarker V1.97 software (SoftGenetics, LLC) was used to determine allele size and genotype all individuals. Genotypic scores were cross-checked by two independent readers.

6.3.3 Otolith shape

In this study the blue whiting otoliths were analysed considering two distinct groups:

(i) samples from IRL, PT-SW1 and MED;

(ii) samples only from the Portuguese coast (PT-CAM, PT-LIS, PT-SW1, PT-SW2 and PT-VSA).

Those two groups were made to allow the comparison of samples collected on the same period from adult fish, which is the case in (i) of the Portuguese sample chosen to be used on the large scale analysis (PT-SW1). But, in (ii) the main purpose was the analysis of the differences along the Portuguese coast in otoliths from immature fish (PT-CAM, PT-LIS, PT-SW2 and PT-VSA), and the existence of differences in the shape between these and the adults (PT-SW1). Since sexual maturity has been described as a source to the otoliths' shape modification (Campana and Casselman, 1993; Hüssy, 2008; Vignon, 2012).

Blue whiting otoliths are flat with rounded dorsal and ventral margins. The rostrum is large and straight and therefore, the antirostrum is indistinct. Images of the whole left and right sagittal otoliths were scanned (Epson V750) under reflected light and stored with high resolution (3200 dpi). Image processing was performed using the image analysis system TNPC (Digital processing for calcified structures, version 7, www.tnpc.fr) with the *sulcus acusticus* facing up (Gonçalves *et al.*, 2017).

To describe otolith contours, Elliptic Fourier Analysis (EFA) (Lestrel, 2008) was carried out as the last study on the otoliths of blue whiting (Mahe, Oudard, *et al.*, 2016) to compare the results between studies. Otolith shape between right and left sides did not show significant differences (Mahe, Elleboode, *et al.*, 2016; Gonçalves *et al.*, 2017). Consequently, in this study, the right otolith was used and when the right otolith was broken, the mirror image of left otolith was used.

For each otolith, the first 99 elliptical Fourier harmonics (Hi) were extracted and normalised with respect to the first harmonic using the TNPC software and were, thus, invariant to otolith size and rotation and starting point of the shape measurements (Kuhl and Giardina, 1982). To determine the number of harmonics needed to reconstruct the otolith outline, the Fourier Power (PF) was calculated for each individual otolith k as a measure of the amount of contour rebuilt by each harmonic:

$$PF(n_k) = \sum_{HI=1}^{n_k} \frac{A_{HI}^2 + B_{HI}^2 + C_{HI}^2 + D_{HI}^2}{2}$$

where A_{HI}, B_{HI}, C_{HI} and D_{HI} are the parameters of the *HI*th harmonic and n_k is the total number of harmonics included. The value of n_k was chosen such that $PF(n_k)$ explains 99.99% of variance in contour coordinates or, in other words, such that shape is reconstructed at 99.99% (Lestrel, 2008).

6.3.4 Statistical analysis

6.3.4.1 Microsatellite marker analysis

Allele frequencies observed and expected heterozygosities and Hardy-Weinberg equilibrium tests were performed using Genepop 4.2. (Raymond and Rousset, 1995). The R package “PopGenReport” (Adamack and Gruber, 2014) was used to calculate number of alleles, fixation indices within samples (F_{IS}) and between samples (F_{ST}) by locus (Table 3), allelic richness, mean and total richness for each locus and sample location (Table 4). A Bayesian clustering algorithm implemented in STRUCTURE version 2.3.4 (Pritchard, Stephens and Donnelly, 2000) was used to determine the most probable number of genetic clusters (K) within the dataset. K was selected a priori ranging from 1 to 6 populations. After, the results from STRUCTURE were analysed following the Evanno method (Evanno, Regnaut and Goudet, 2005) and the K was determined in the STRUCTURE Harvester programme (Earl and VonHoldt, 2012). The principal component analysis (PCA) was applied aiming to correlate the relative differences among sampling locations (IRL, PT-CAM, PT-LIS, PT-VSA, MED and ADR) in relation to the allelic frequencies in each locus. The PRINCOMP procedure of R was applied after data standardization and PCAs were based on the correlation matrix. PCA data analyses, including the figures and the models presented, were performed on R 3.3.3 (R Core Team, 2017) and using packages “ggplot2” (Wickham, 2009) and “ggbiplot” (Vu, 2011).

6.3.4.2 Otolith shape analyses

Firstly, Principal Components Analysis (PCA) was applied to selected Elliptical Fourier Descriptors (EFDs) matrix (EFDs as columns and individual otolith as lines) of otolith contours (Rohlf and Archie, 1984). A subset of the resulting principal components was selected as otolith shape descriptors according to the broken stick model (Legendre and Legendre, 2012).

A cluster analysis was performed on the normalised Fourier harmonics to group individuals with similar otolith shapes. For this, Ward's hierarchical algorithm based on squared Euclidean distances was used. To visualise differences in otolith shape between groups of individual fishes, the average otolith shape of each group was formed by the outline reverse Fourier transform using the first 36 normalised Fourier harmonics.

To investigate potential explanations for otolith shape differences, a multivariate analysis of EFDs of otolith contours was carried out. To reduce the number of dimensions and to avoid collinearity between descriptors, Principal Components Analysis (PCA) was applied to the EFDs (for details see Mahe, Oudard, et al., 2016). Next, two Redundancy analyses (RDA) were carried out. RDA is an extension of multiple regressions to multivariate response data and an extension of principal component analysis (Legendre and Legendre 2012). The first RDA aimed at removing the variance explained by total fish length. In the second RDA, the residuals of the first RDA, referred to as RDA residual matrix, were related to explanatory variables of interest, i.e. sampling location (latitude, longitude), month, sex, fish total length on 118 individuals in (i); and sampling location (latitude, longitude), month, fish total length and depth on 305 individuals in (ii). A generalized linear model (GLM) was applied to analyse the magnitude of multicollinearity between variables. Residual plots were visually inspected for deviations from normality.

Statistical analyses were performed using R, packages “Vegan” (Oksanen et al., 2016) and “MASS” (Venables and Ripley, 2002).

6.4 Results

6.4.1 Microsatellite marker analysis

Deviations from the Hardy-Weinberg equilibrium, were observed for MpouBW09 locus in most of the sampled populations, similar to a previous study on this species (Ryan, Mattiangeli and Mork, 2005). The highest number of alleles was observed on this locus (MpouBW09) (n=35). The locus with the highest fixation index between samples was Mmer-UEAW01 (0.0491) and within samples was Tch06 (0.3724) (Table 6-3).

Table 6-3 The number of alleles, population wide Fit, fixation indices between samples (F_{ST}) and within samples (F_{IS}) for each locus (MpouBW13, MpouBW09, MpouBW08, Tch06, MpouBW07, Mmer-UEAW01) across all sample locations. The table is sorted in ascending order based on F_{ST}.

Locus	**Alleles (n)**	**Fit**	**F_{ST}**	**F_{IS}**
MpouBW13	16	0.0061	0.0023	0.0039
MpouBW09	35	0.2992	0.0040	0.2964
MpouBW08	17	0.0518	0.0055	0.0466
Tch06	28	0.3770	0.074	0.3724
MpouBW07	19	0.1518	0.0127	0.1409
Mmer-UEAW01	19	0.1647	0.0491	0.1216

The allelic richness on locus MpouBW09 for all the sample locations was between 17 and 21, and between 8 and 13 for the other loci (Table 6-4). PT-LIS was the area with higher mean and total allelic richness (Table 6-4).

Table 6-4 Allelic richness for each locus (MpouBW13, MpouBW09, MpouBW08, Tch06, MpouBW07, Mmer-UEAW01) and sample location (IRL, PT-CAM, PT-LIS, PT-VSA, MED and ADR).

Locus	**IRL**	**PT-CAM**	**PT-LIS**	**PT-VSA**	**MED**	**ADR**
MpouBW13	9.848	11.423	9.992	9.308	9.547	8.920
MpouBW09	17.048	21.807	21.833	19.984	17.628	21.782
MpouBW07	10.558	10.465	11.488	11.099	11.449	11.142
Tch06	12.261	9.756	12.568	12.011	10.923	13.730
Mmer-UEAW01	13.336	11.132	12.862	13.808	13.377	7.486
MpouBW08	10.778	11.232	10.992	9.832	9.440	10.781
Mean richness	12.305	12.636	13.289	12.674	12.062	12.307
Total richness	73.828	75.814	79.736	76.041	72.372	73.841

F_{ST} pairwise comparisons revealed a significant differentiation between blue whiting collected in the Adriatic Sea from other sampling locations (Table 6-5). Similarly, the Bayesian clustering method revealed two main clusters (Figure 6-2), one composed by the samples from the Northeast Atlantic and Mediterranean and the other by the Adriatic samples.

Table 6-5 P-values from the pair-wise F_{ST}-distance between sample locations (IRL, PT-CAM, PT-LIS, PT-VSA, MED and ADR) across all loci, determined using the Fisher's method from Genepop.

Sample location	IRL	PT-CAM	PT-LIS	PT-VSA	MED	ADR
IRL	-	0.1470	0.7345	0.800	0.0395	*
PT-CAM		-	0.4264	0.7651	0.0551	*
PT-LIS			-	0.1279	0.0001	*
PT-VSA				-	0.0678	*
MED					-	*
ADR						-

*Highly significant, P value < 0.0001

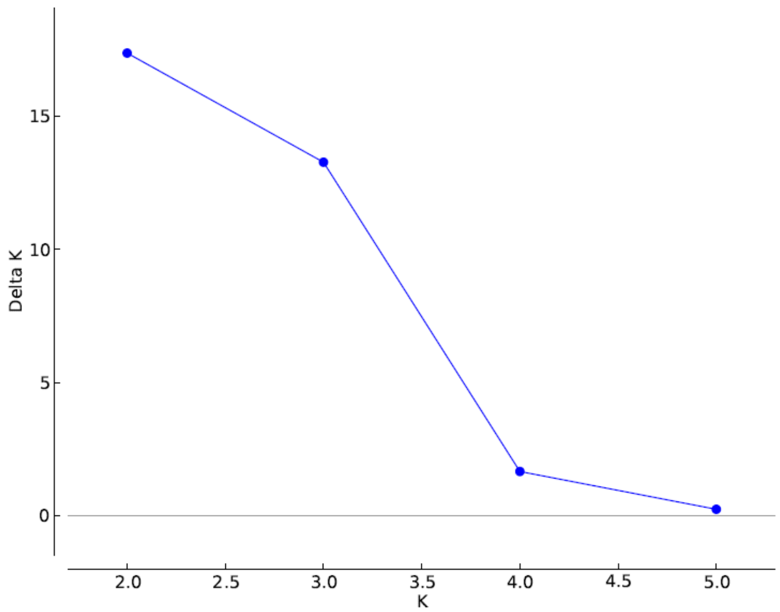

Figure 6-2 Magnitude of Delta (K) as a function of K (mean ± SD over 10000 replicates). Calculated for each model using the absolute values of the second order rate of change of the likelihood distribution (mean ± SD) according to the formula: $|L''(K)| = |L'(K+1) - L'(K)|$. Delta(K) ($\Delta K$) as $\Delta K = m|L''(K)|/s[L(K)]$. The modal value of this distribution is the true K(*) or the uppermost level of structure, here two clusters (according to the method by Evanno, Regnaut and Goudet, 2005).

A similar distribution was found on the PCA with the ADR samples detaching from the rest of the populations (Figure 6-3). Although, the Mediterranean samples do not present a highly significant differentiation from the remaining areas, but in some point a slightly differentiation still seems to exist, which could be observed in the results from the K-means estimation, between K=2 and K=3 (Figure 6-2), in the PCA (Figure 6-3) and on the results shown on Table 6-5.

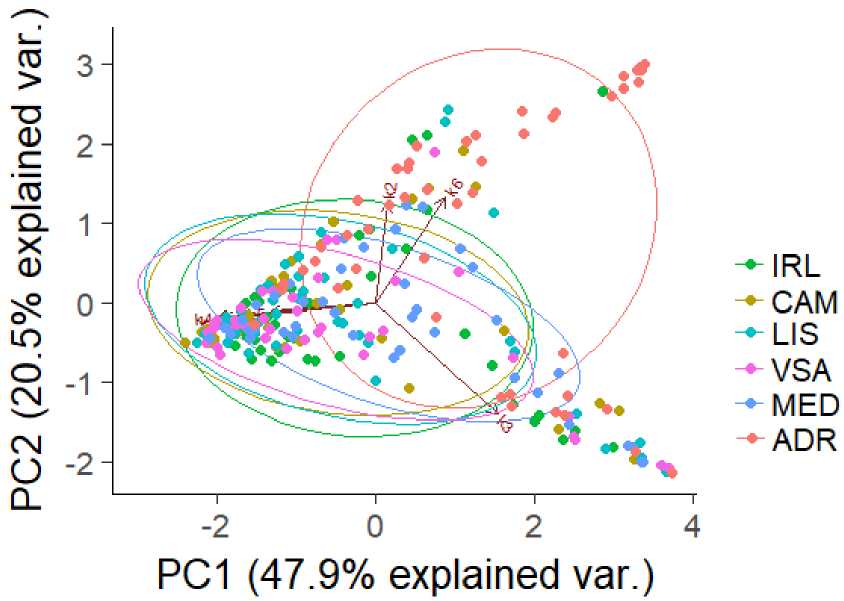

Figure 6-3 Relative relationships between the blue whiting collected in the different locations, in the Northeast Atlantic (IRL, PT-CAM, PT-LIS, PT-VSA), Mediterranean (MED) and Adriatic (ADR) Seas, according to Principal component analysis (PCA) based on correlation matrix of allele frequencies.

6.4.2 Otolith shape analysis

The multidimensional scaling plot of the first 36 Fourier harmonics from the blue whiting otoliths revealed a distinction in shape between three sample locations (IRL, PT-SW1 and MED) (i) (Figure 6-4a). The clustering method confirmed those 3 clusters and the outline from the reconstructed mean Fourier harmonics of each one of those clusters is shown in Figure 6-5. Similarly, in the results from the fitted generalized linear model significantly differences were shown in otolith shape between sample locations, months and fish total length (Table 6-6).

Table 6-6 Summary of the generalized model results applied to the RDA covariates of blue whiting otolith shape Fourier descriptors (PF=36) across the three areas (IRL, PT-SW1 and MED) (i).Interactions between factors are noted by a colon (:). df – degrees of freedom; Std. error – standard error. *< 0.001

	df	Estimate	Std. error	z-value	Probability	Probability(>|z|)
(Intercept)		31.370	5.673	5.530	5.02e-07	*
month	1	-2.521	0.433	-5.819	1.57e-07	*
sex	1	-0.024	0.019	-1.276	0.206	
latitude	3	-0.465	0.084	-5.523	5.15e-07	*
longitude	3	2.690	0.475	5.662	2.96e-07	*
fish total length	1	0.023	0.003	6.668	4.75e-09	*
latitude:longitude	3	-0.050	0.009	-5.620	3.51e-07	*

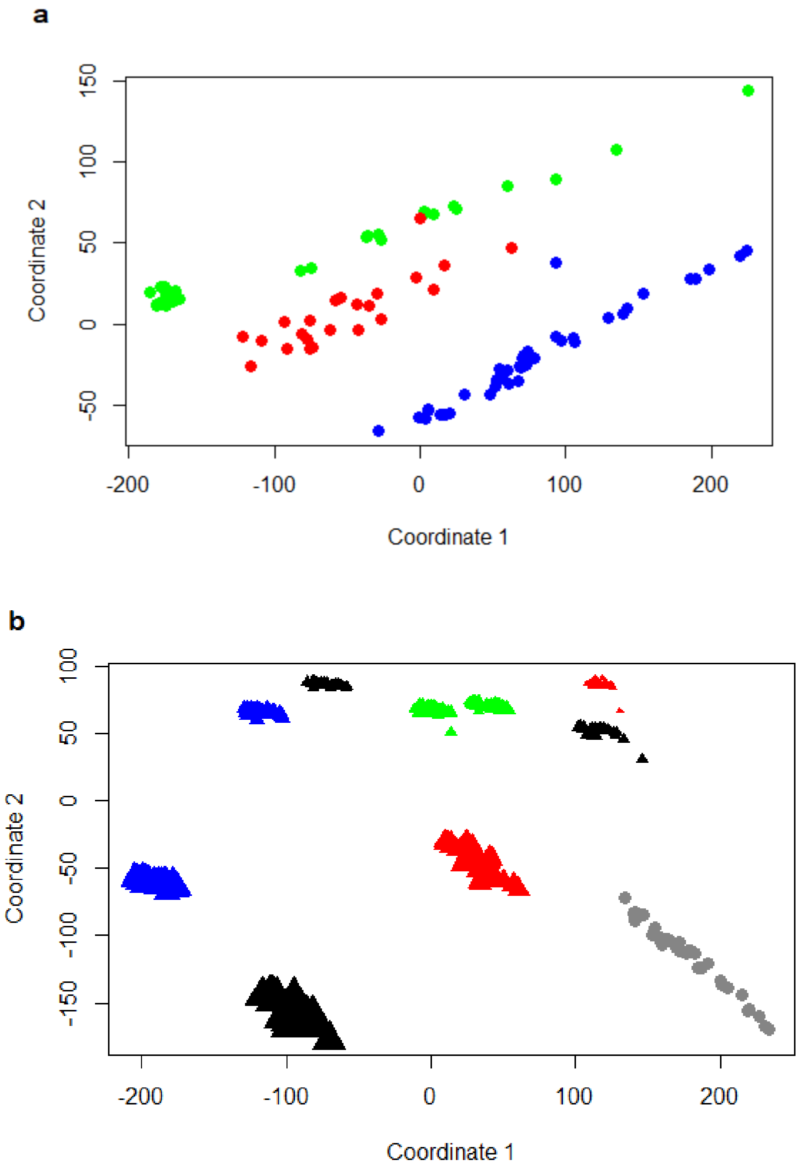

Figure 6-4 Multidimensional scaling plot of the otolith shape from the blue whiting samples: a) from IRL (red), PT-SW1 (blue) and MED (green) (i); b) only from the Portuguese coast, PT-CAM (green), PT-LIS (red), PT-SW1 (grey), PT-SW2 (black), and PT-VSA (blue) (ii). The symbols: ●- samples without depth information (commercial fishery); ▲ – samples in which the depth information was available (PT surveys). The size of ▲ is proportional to the increase in depth (m), the levels are: 100, 134, 147, 148, 180, 259, 324 and 385.

The position occupied by the blue whiting samples at PT-SW1 (commercial fishery) compared with the samples from the PT survey on the MDS plot, could give an indication that the depth of sampled was between 300m and 400m based on coordinate 2. The coordinate 1 could be related with a year effect on blue whiting otolith shape, since the only sample from 2016 is the one from the commercial fishery, or with the fish maturity (immature versus mature).

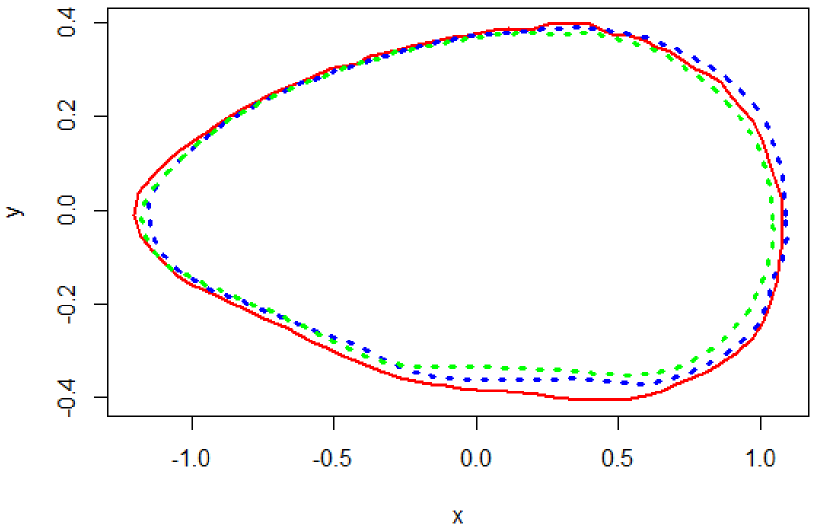

Figure 6-5 Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 36 Fourier harmonics showing the overlap and variations between three clusters (IRL (red), PT-SW1 (blue) and MED (green)).

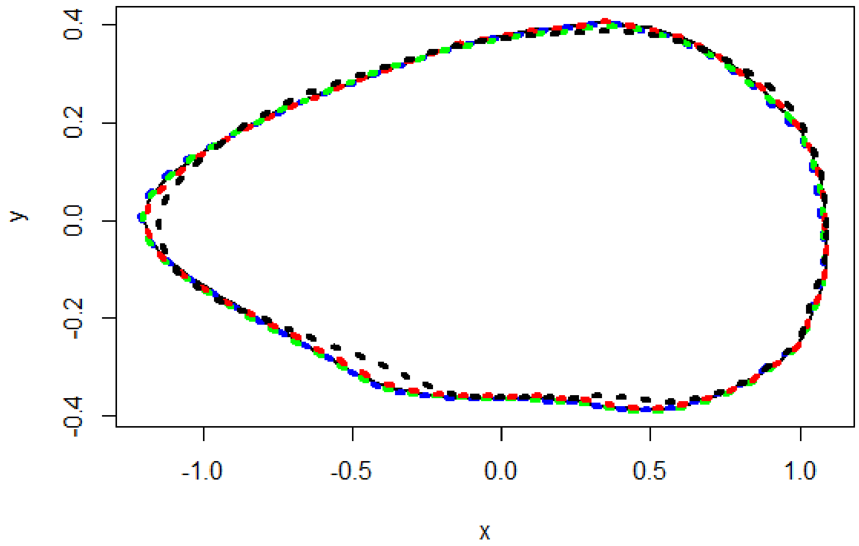

Figure 6-6 Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 36 Fourier harmonics showing the overlap between the Portuguese areas (PT-CAM (green dotted line), PT-LIS (red dotted line), PT-SW2 (black line), PT-VSA (blue dotted line) and PT-SW1 (black dotted line)).

The cluster analyses on blue whiting immature samples showed no significant differences in the mean outline shape between the Portuguese areas, but differences between immature and adults were identified (Figure 6-6).

Table 6-7 Summary of the generalized model results applied to the RDA covariates of immature blue whiting otolith shape Fourier descriptors (PF=36) from the Portuguese Coast (PT-CAM, PT-LIS, PT-SW2 and PT-VSA) (ii).Interactions between factors are noted by a colon (:).df – degrees of freedom; Std. error – standard error. *< 0.001

	df	Estimate	Std. error	z-value	Probability	Probability(>|z|)
(Intercept)		2.809e+01	2.115e+00	13.285	<2e-16	*
month	1	-1.086e-01	8.336e-03	-13.026	<2e-16	*
fish total length	1	-2.861e-04	1.616e-03	-0.177	0.860	
latitude	9	-7.311e-01	5.521e-02	-13.242	<2e-16	*
longitude	9	3.046e+00	2.282e-01	13.348	<2e-16	*
depth	1	-5.180e-05	3.667e-05	-1.413	0.159	
latitude:longitude	9	-8.242e-02	6.127e-03	-13.452	<2e-16	*

Although, the GLM results confirmed that the blue whiting otolith shape on immatures changed significantly along the Portuguese areas (latitude, longitude) and month of sampling (Table 6-7). The representation of the mean outline otolith shape with all the samples (Figure 6-7), confirmed the differentiation among the samples from the Portuguese coast, immature and mature fish, and all the others.

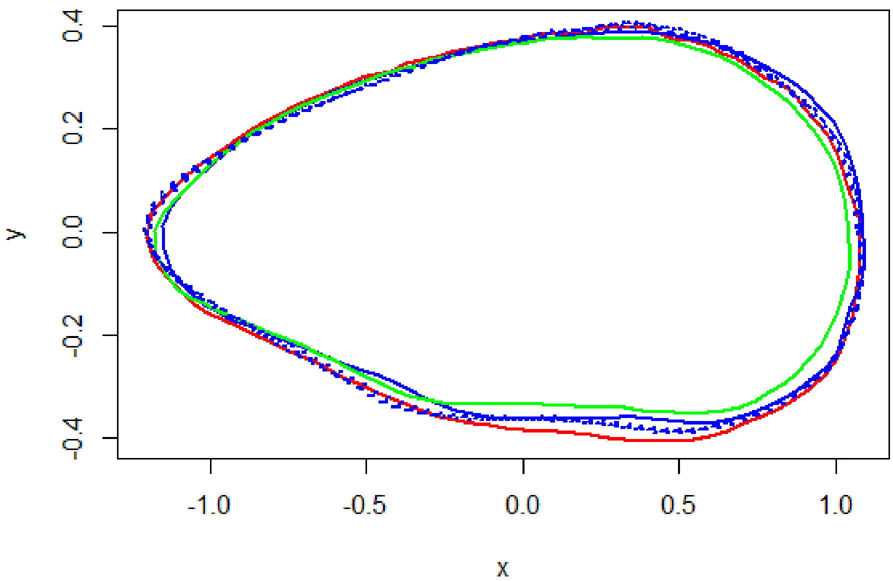

Figure 6-7 Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 36 Fourier harmonics showing the overlap between the Portuguese areas (PT-CAM, PT-LIS, PT-SW2, PT-VSA (blue dotted line) - immatures and PT-SW1 (blue) - matures), the IRL (red) and MED (green)).

6.5 Discussion

Microsatellites' based results show a lack of differentiation between samples from north to south of the NEA, and even at the Mediterranean coast of Spain showing what it seems to be a panmitic population. However, samples collected in the Adriatic show a clear differentiation from the rest of the samples. According to previous studies on the genetic variability of blue whiting populations, a genetic differentiation between the Mediterranean (East of Corsica), the Iberian coast (Bay of Biscay and Portugal) and the Porcupine Bank was observed also based on microsatellites (Ryan, Mattiangeli and Mork, 2005; Was, Gosling, Mccrann, *et al.*, 2008). In the present study, this differentiation was not evident, although the same molecular markers, loci, were used. These disparities suggest that either samples have not been randomly collected across studies or that some event must have occurred after 2005 (the year of sample collection in the study of Was, Gosling, Mccrann, *et al.*, 2008) to promote this homogeneity on gene pool. The hypothesis of temporal differentiation on genetic results was also been observed in two former studies on this species (Skogen, Monstad and Svendsen, 1999; Ryan, Mattiangeli and Mork, 2005).Those changes in their genetic structure can be associated with changes in population fitness (Carvalho, 1993), and are therefore relevant to fishery management.

Additionally, in marine fish populations, owing the continuous nature of their habitats and lack of evident physical barriers the adaptive significance of stock differentiation is more difficult to define, due to putative high gene flow (Waples, 1998). It is believed that a small number of migrants per generation are sufficient to homogenize the gene pool, between monospecific stocks (Carvalho and Hauser, 1994). A lack of genetically detectable stock separation may arise from different sources: sufficient gene flow to maintain panmixia and/or occasional 'sweepstake' events such as sporadic recruitment from distant, non-neighboring areas which could produce the appearance of panmixia (Carvalho and Hauser, 1994). In 2009-2010, the blue whiting stock abundance was the lowest of the time-series (ICES, 2016), since the surveys and the majority of catches were taken from what is believed to be the main spawning area (Porcupine Bank). The depletion in effective population sizes in this area might have caused a wider migration of individuals from/to other areas, and wherefore a higher genetic homogeneity of the population.

The otolith shape reflects its development under dual regulation: genetic conditions regulate the form of the otolith, while environmental conditions regulate the quantity of material deposited during the formation of the otolith (Lombarte and Lleonart, 1993; Aguirre and Lombarte, 1999; Gauldie and Crampton, 2002; Cardinale, Kastowsky and Mosegaard, 2004; Vignon, 2012). Considering the blue whiting otolith shape analysis results, three different otolith morphotypes were identified on the blue whiting based on different sample locations, i.e, from the Porcupine Bank, the Portuguese coast and the Mediterranean Sea. The existence and definition of these subpopulations is in agreement with the results from previous otolith shape analysis studies on this species (Keating *et al.*, 2014; Mahe, Oudard, *et al.*, 2016).

On the blue whiting off Portugal, the mean outline from the reverse Fourier transform, revealed only two distinct morphotypes, corresponding one to immature and the other to adult individuals. Some authors have highlighted that the most visible change in otolith shape occurred for a size corresponding to the onset of sexual maturity, a size at which the metabolism changes markedly, since sexual maturity influences fish growth and have an impact on otolith morphology (Tuset, Lozano, *et al.*, 2003; Cardinale *et al.*, 2004). Differences among individuals in the early life-stages seem to be governed by the genetically determined nature of otolith shape development and larvae size, while differences among adults may be the result of a combination of genetic and environmental effects, hence increasing the inter-individual disparity. In two species of coral reef fish species (*Amphiprion akindynos* and *Pomacentrus amboinensis*) ontogenetic trajectories showed that larger individuals were more efficiently differentiated as belonging to different locations than smaller sized ones (Gagliano and McCormick, 2004), which is in accordance with this study results.

Environmental factors affect somatic and therefore otolith growth are generally more influential determinants of otolith shape than genetic factors (Campana and Casselman, 1993). Otolith shape depends on the biological and ecological behavior of species (Lombarte and Lleonart, 1993; Cardinale *et al.*, 2004) and may be associated with differences in food, spatio-temporal niches and depth (Aguirre and Lombarte, 1999; DeVries, Grimes and Prager, 2002). Besides, feeding level has a direct effect on otolith shape, to the point that even recent feeding history creates discernable differences in otolith shape and also acts indirectly on otolith size through somatic growth (Gagliano and McCormick, 2004; Hüssy, 2008). Water temperature is also known to could influence the rate and pattern of material deposition during otolith growth producing changes in otolith's length and overall shape. This was been described in several studies on species of the genus *Merluccius* and *Coelorhynchus* (Lombarte and Lleonart, 1993) and sardines (*Sardine pilchardus*) (Jemaa *et al.*, 2015), although in cod (*Gadus morhua*) no influence was noted (Hüssy, 2008). The differences detected between the otolith shape of samples along the Northeast Atlantic, Portuguese coast and Mediterranean could result from differences in food availability and/or water temperature which can be reflected on different sampling depths, location and/or season. Longitude is clearly associated with depth in the western Portuguese coast and at southern is latitude. Differences in growth rates and otolith deposition patterns have previously been associated to seasonal and/or geographic variation in saury (*Scomberesox saurus saurus*), contributing to otolith shape variation between populations (western Mediterranean and the Northeast Atlantic) (Agüera and Brophy, 2011). Similar results, were found in species of the genus *Serranus*, where the differences in pattern were explained by depth distribution (Tuset, Lombarte, *et al.*, 2003) or a higher unpredictability of the environment, which resulted in a higher variability of larvae size, i.e. intrinsic metabolic rates (Garrido *et al.*, 2008). Consequently, since otolith shape is correlated with larvae growth rate and therefore with their probability of survival, this could be one of the causes of the encountered differences between samples of the NEA coast (Porcupine Bank and Portugal) and Mediterranean. The production of small larvae is advantageous for those species facing variable feeding conditions, where relatively slow-growing or small larvae would consume their yolk reserves more gradually, extending the endogenous period and increasing their chances to encounter adequate food patches (Garrido *et al.*, 2008).

This was the first study in which a larger number of immature blue whiting samples from the same area (Portuguese coast) were analyzed. The similar length range of those immature samples indicates that they should belong to the same cohort. The lower recruitments of recent years, observed regardless the Northeast Atlantic area considered, together with different growth rates on different locations of blue whiting distribution, stand along with the genetic population homogeneity. The oceanographic processes occurring off the Strait of Gibraltar may act as barriers to gene flow for fish (Bacha *et* *al.*, 2014 and references therein). Other studies suggested that on some species, individuals from the Mediterranean and Atlantic could be distinguished using otolith shape analysis, regardless belonging to genetically isolated populations or not, e.g. saury (*Scomberesox saurus saurus*) (Agüera and Brophy, 2011) and European anchovy (*Engraulis encrasicolus*) (Bacha *et al.*, 2014). The samples from the population components off Ireland, Portugal and the Mediterranean coast can be identified based on otolith shape analysis but on the microsatellites no significant differences were revealed. The samples from the Adriatic were the only ones presenting genetic heterogeneity amongst the other sample locations, which in part validate the application of the microsatellites technique and their ability into detect differences between populations in the current study.

This study once again confirmed (Begg and Waldman, 1999; Cadrin and Secor, 2009) that sock identification related questions can be addressed with otolith shape, but are not necessarily answerable like genetic studies. Therefore, genetic and otolith studies should complement rather than compete with each other. Thus otolith applications may help on the fish population identity (Campana and Thorrold, 2001), but is one more tool rather than the key for a definitive answer.

The genetic and otolith morphometry applied in this study suggest that blue whiting along the Portuguese coast belongs to a single component unit of the NEA stock. The major differences on otolith shape across the Portuguese coast were observed between immature and mature. The otolith shape within immature samples is related to larvae size and should constitute a species survival strategy. These sharp differences may be diluted as fish becomes larger due to an increasing environment impact on otolith growth. Based in the otolith shape analysis there are two blue whiting stock components along the NEA, one at south ending at the Portuguese coast and other at north off Ireland. Treating the blue whiting fishery as a single management unit can jeopardize the assessment framework, using unfit estimates of fishing effort, and arriving to inaccurate estimates of the main results, such as fishing mortality or spawning-stock biomass. In ultimate terms this mismanagement could lead to the collapse of some subpopulations within the stock (Carvalho and Hauser, 1994). There is a need to take into account the identification of phenotypic stocks as they may have unique demographic properties (e.g. growth rates, spawning sites) and responses to exploitation.

Therefore, the management of those subpopulations as separate stocks constitutes a more precautionary strategy, which will avoid overexploitation of smaller subpopulations and will allow larger components to be accordingly exploited.

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6.6 References

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Chapter 7

7. General Conclusions

General Conclusions

The present thesis aimed to study important aspects on the biology and structure of blue whiting in the southern part of stock distribution, the Portuguese coast. Furthermore, contributions to enlarge the knowledge of the input data used on the blue whiting assessment were made.

In chapter 2, the life history parameters (growth, population size-structure and maturity) and female spawning stock biomass, from the Portuguese bottom trawl surveys (PBTS) (1990 and 2011) were analysed in order to answer to the following questions: What does the data analysis reveal in terms of the blue whiting stock structure? Does this data show evidence of the existence of the two stock components?

The blue whiting in the Portuguese coast were mainly distributed between the 100m and 200m bathymetric line and up to a bottom depth below 500m. Large concentrations of immature females were found on shallow bottoms near the edge of the continental shelf, while concentrations of mature females are at depths greater than 100m. The spawning females were at deeper waters, extending down to 500m. The presence of immature and mature females, at all maturity stages (from immature, passing from mature to spawning), is an indicator of spawning across the Portuguese coast.

The length-weight relationships did not reveal differences between the two sexes, but significantly differences were found between semesters. Those differences in the length-weight amongst semesters were observed above the 20cm. This size corresponds to the length at first maturity (L50) for the Portuguese coast, according to the sex combined maturity ogive. The observed differences in the fish weight between semesters were due to the spawning occurrence at the beginning of the year.

Age of first maturity for the Portuguese coast is between 0 and 2 years, similar to the Mediterranean (Bas, 1959). On Faroese-Scottish-west Ireland waters, this species reached the first maturity also at 20cm, but at age between 2 and 4 years old (Raitt, 1966). On the North Sea, L50 was 25cm for an age of 2 or 3 years (Perry *et al.*, 2005), identical to the remaining northern areas (ICES, 2016). The growth rate decreases from southern to northern on blue whiting.

The female spawning stock biomass (FSSB) in the Portuguese coast, for the period from 2008 to 2011, presents the opposite trend observed on the combined stock (ICES, 2016). However, the recently decrease of FSSB in relation to the mean of FSSB, was higher in the combined stock. This biomass seems relatively stable around the mean since 2005 for the Portuguese coast, which was not the case for the whole stock, where a decline was observed from 2007 until 2010.

The blue whiting life-history parameters has shown differences in growth, length, age of first maturity, in the peak spawning and female spawning stock, between the Portuguese coast and the main spawning ground (west of Ireland Islands and Porcupine Bank). The results obtained are in line with the existence of two components on the blue whiting from the Northeast Atlantic.

The blue whiting female distribution off Portugal gave the indication of the possible existence of a different sex pattern along the areas (north, southwest and south) (chapter 2). In chapter 3, the blue whiting sex distribution, size structure and condition patterns, by area (north, southwest and south) and by season (autumn and winter) along the Portuguese coast, were further investigated (Gonçalves *et al.*, 2017).

Outside the spawning season blue whiting females occurred predominantly close to the sea bottom during the day. During spawning, males remained in great numbers close to the sea bottom surface. Differences in the fish condition's (K) were statistically significant between seasons and sexes. The lower condition in the winter was more pronounced in the north. The scarce number of spawners and the samples mostly composed by mature females (sex-ratio of 0.81) in a better condition in the south during winter survey, comparing with the other two areas, indicate that the main spawning is earlier in this area possible due to the warmer temperatures.

The modelling of blue whiting abundance relies on an age based model (SAM). Accurate and precise age estimates are critical for the effective management and understanding of this fish resource because dynamic rates of recruitment, growth and mortality depend on these data (Campana, 2001). The guidelines to count the rings and determine age are made to be objective (ICES, 2017), but the final classifications are always dependent on the reader's experience, which introduces an interpretation error in the process (Campana and Moksness, 1991). In chapter 4, an automatic system of age classification was developed (Gonçalves *et al.*, 2017). The demand behind was: Could ImageJ be used as an approach to improve the accuracy and precision of blue whiting ageing estimation?

In the first application, differences were found in the age estimation from readers and using the ImageJ plug-in (named as “OtoRing”). Taking into account that age by readers was a subjective process, even between readers differences were noted, the definitive test will be when otoliths in which the age is validated are used. A validation study on this species ageing (n=215), was conducted this year before the ICES workshop on the age reading of blue whiting (ICES, 2017) leading by IPMA (Gonçalves and Dores, 2017). The same approach will be repeated, but this time using a bigger otolith sample by area (n(area)=150). As soon as, a validate reference collection on blue whiting otolith ageing be available, a new test will be performed.

The OtoRing was developed and already made available to the scientific community as an open source (Gonçalves and Vaz da Silva, 2017). The principal advantages of OtoRing are: allowing the revision of age classifications in an easy way; performing calibrations between readers; and helping on the training of new readers. Apart, from all the potentialities of OtoRing, ImageJ by itself is a useful tool to obtain measurements and lead to image processing. The incorporation of both of these tools (OtoRing and ImageJ) makes the combination of otolith ageing and growth studies a more efficient and easy task.

The application of image analyses techniques on otoliths has been increasing, as shown in the chapter 4. The diagnosis on otoliths symmetry as a species characteristic is currently being done. Although, it is expected that the otolith surface (distal *versus* proximal) used to collect images should have no influence on the symmetry results, but this was never been proved before. Consequently the study presented in chapter 5 was conducted with the main goal to answer to the following question: Does the otolith surface position in the image matters, for fish otoliths pair’s symmetry studies?

On this study, the results enhanced the need to carefully evaluate the differences between the otoliths side, when obtaining images, before performing morphometry on those structures. The answer to the previous question was, that at least for blue whiting, the otolith images should always be obtained from the concave side (distal surface), because for symmetry studies the position real matter (Gonçalves, Mahé, *et al.*, 2017).

The results from chapter 2 support the hypothesis of two components in blue whiting population off the Northeast Atlantic. On their southern limit of blue whiting distribution, the Portuguese south coast is influenced from southeast Mediterranean waters while the colder North Atlantic waters went down the west coast. This oceanography mixed scenario could support the co-existence on Portuguese waters of blue whiting genetically and biologically closer to the Mediterranean at south and closer to the Celtic Sea at north. In chapter 6, the structure of blue whiting along the Portuguese coast was been studied by the integration of microsatellites and otolith shape methodologies. For that purpose, blue whiting muscle samples and otolith from different areas (Ireland coast, Portuguese coast, Mediterranean and Adriatic seas) and across the Portuguese coast (Caminha, Lisboa, Sines and Vila Real Sto. António) were used. Subsequently, also a larger number of immature blue whiting otolith samples from the Portuguese coast were analyzed based on shape analysis.

Microsatellites' based results show a lack of differentiation between samples from north to south of the NEA, and even at the Mediterranean coast of Spain showing what it seems to be a panmitic population. However, the samples collected in the Adriatic show a clear differentiation from the rest of the samples.

In the otolith shape of samples along the NEA, differences were detected between Ireland, Portuguese coast and Mediterranean. Small scale differences in the otolith shape of immature blue whiting were identified associated with different depth distributions along the Portuguese coast.

This study once again confirmed that sock identification related questions can be addressed with otolith shape, but are not necessarily answered like genetic studies (Begg and Waldman, 1999; Cadrin and Secor, 2009). Therefore, genetic and otolith studies should complement rather than compete with each other. The clear existence of two stock components is once again highlighted based in the otolith shape analysis. The main conclusion was that there are two blue whiting subpopulations along the Northeast Atlantic, one southwards ending at the Portuguese coast and other northwards off Ireland.

These phenotypic stocks, since they have unique demographic properties (e.g. growth rates, spawning sites) may as well have different responses to exploitation. Therefore, the management of blue whiting considering those stock components constitutes a more precautionary strategy, which will avoid overexploitation of smaller subpopulations and will allow larger components to be accordingly exploited.

Taking those findings into account, at this stage we are beginning to build the basis to assess the two components of the blue whiting stock. Firstly, it is necessary to try to apply the same effort on the age estimation and age criteria's to ensure that they were applied coherently mainly along the northern and the southern components. In the 2013 age reading workshop (ICES, 2013), the majority of otoliths used for the exchange exercise were from the main spawning ground (off Ireland) and the northern area of this species distribution, since the main catches of the single stock were taken from there. On the preparation of the 2016 exchange a lot of attention as taken to guarantee that otoliths samples from a wider distribution area were used to calibrate the ageing on blue whiting (ICES, 2017). Thus, otoliths from the following areas: ICES areas (27.2.a - 27.9.a and 27.14), Mediterranean and NAFO 1C (Western coast of Greenland at 64º15'N), were made available to readers to age classification. During the 2017 workshop on the age reading of blue whiting, the participants classified and discussed the main issues on the otoliths from each area. At this workshop a study aiming to identify the main causes of age misintrepretations by age readers and to propose a table with criterias' to ageing standing on age ring measurements was presented (Gonçalves and Dores, 2017). On the blue whiting from the Portuguese coast the otolith ring measurements disclosed a wider length of the first age ring, when compared with the values described on literature (Dores and Gonçalves, 2017). Both of those studies revealed important differences in otolith age ring growth between areas. It was pointed out that such differences must be consider when ageing, mostly the fact that the mean diameter of the first ring presents differences according to the area where the blue whiting was fished.

In conclusion, blue whiting presents a unique population structure along the Portuguese coast which is different from the populations at north (Ireland coast) and at the Mediterranean. In the course of this thesis new steps were established allowing in a nearly future a more sustainable and conservative management of this mesopelagic gadoid in the Northeast Atlantic.

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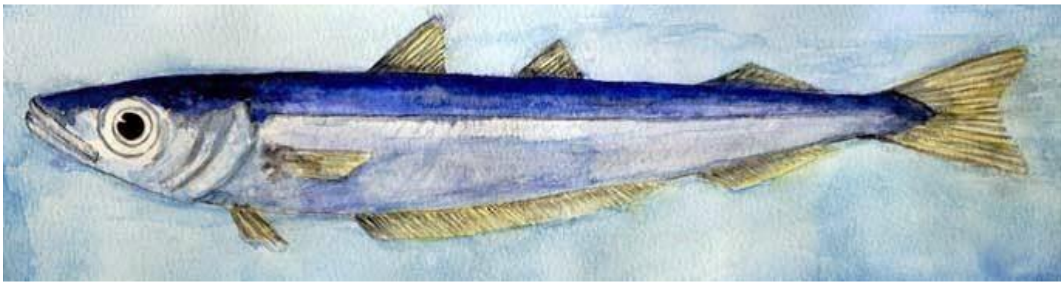
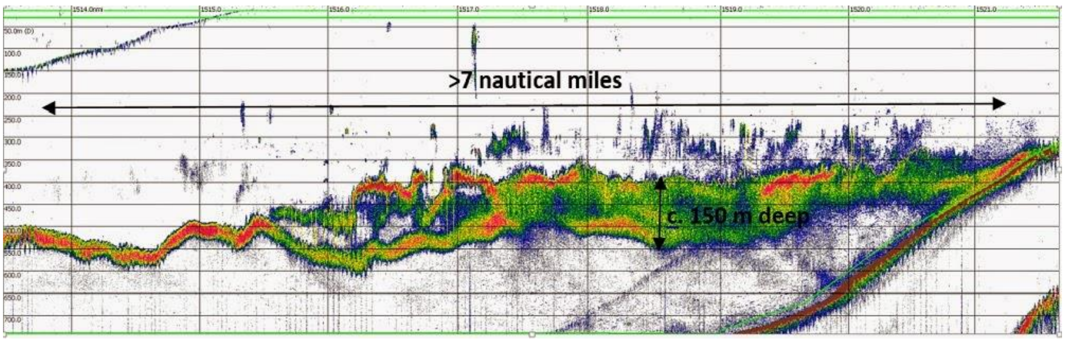
Chapter 8

8. Future Research





(Echogram of Blue Whiting School from IBWSS 2015)

Future Research

The present work contributes with advances aimed at enabling for the future assess of blue whiting in the Northeast Atlantic considering two different components. The next steps are enumerated on the following figure (Figure 8-1):

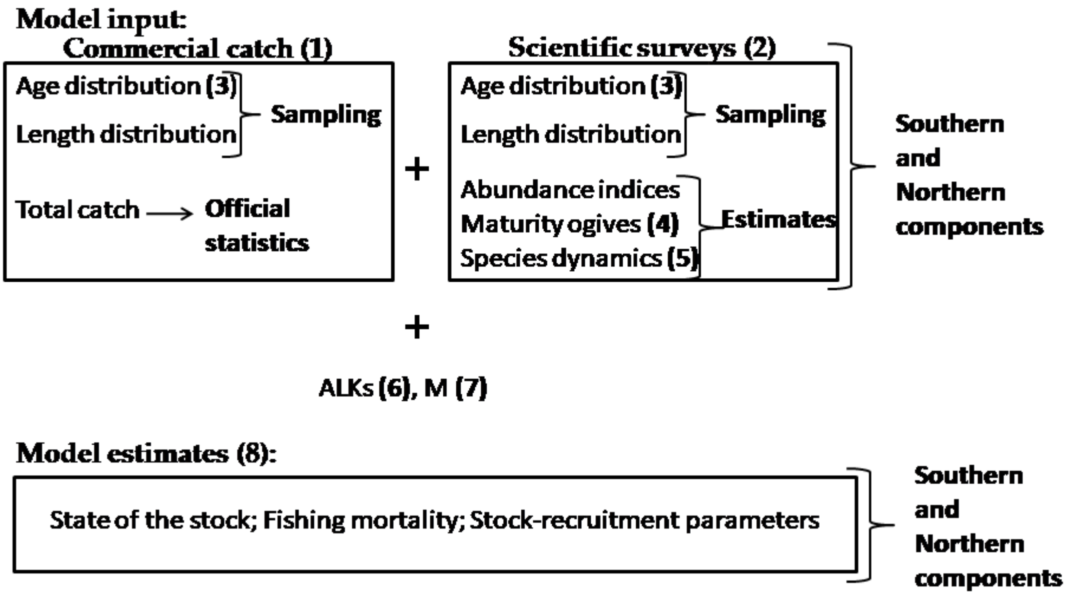


Figure 8-1 Future steps for the assessment of blue whiting considering the southern and the northern components. The numbers corresponds to the essential points in need to improve.

(1) The catch-at-age data from the mixing area (west coast of Ireland) will be splitted by component (southern and northern).

(2) The independent abundance estimates available from the surveys will be combined. The combination of data provided by the different surveys, even only for the area at south of Porcupine Bank, presents a bigger constraint. Demersal surveys are available apart from Portugal also from the other areas; their use could be one possibility for the southern stock assessment. The IBWSS the acoustic survey which provides independent data for the blue whiting assessment is carried out in March-April on the spawning grounds to the west of the British Isles and was established in its current form in 2004. In Spanish waters (Bay of Biscay and Galicia), besides regular demersal surveys, an acoustic survey during April have been adapted to produce abundance indices for this species, owed to their mesopelagic behaviour. From the Portuguese coast

only the PBTS data is available for blue whiting. But the combination of demersal and acoustic surveys was attempt in the past without any success. This constraint could be overtaken by one of this possibilities: or extend the area covered by the IBWSS to the southern areas, i.e. Iberian coast; or extend the area covered by the Portuguese acoustic survey design to sampling small pelagic fish during April, which currently only covers an area until the 200m bathymetry; or implement the acoustic echosounder records in the PBTS and also combining with an extend of the sampling stations to perform pelagic trawls. Any of these solutions, apart from helping to solve the lack of acoustic data from the blue whiting southern component, can contribute to improve the data collection for community and ecosystem studies. The implementation of acoustic as a routine can also help to understand the processes behind the vertical migrations on mesopelagic species off Portugal.

(3) The OtoRing will be improved. The ImageJ *plugin* developed under this thesis was presented during the last ICES workshop on the age reading of blue whiting (WKARBLUE2), the readers confirmed the potential of such a tool to be used as routine in this species ageing. Although, since the majority of readers made the ageing onboard the research vessels during scientific surveys and otolith images weren’t recorded, thus they can´t use it as a routine in such cases. The plugin will be modified to also allow the use of live images. This is still an ongoing project the next steps are: test it using validated data; introduce the age reading guidelines as an option, so the program can learn with the reader and that each of the reader’s decision into considering a specific ring as age ring can be recorded. Besides, independent age reading guidelines for the southern and northern will be implemented according to their specific otolith growth patterns.

(4) A new maturity ogive for each component based on microscopic validated data will be obtained.

(5) Otolith shape analysis must be applied as a routine to the data collected under the IBWSS, to allow the estimation of the proportion of abundance from each stock in the main spawning area. Furthermore, this will be used to correct the

abundance estimates from the survey and also to allocate the total catches (during quarter 1) to the respective stock.

(6) The Portuguese blue whiting sampling data collection for age-length distribution (ALKs) is sex stratified. The catch-at-age is subsequently estimated assuming a sex ratio of 1:1. The PBTS data analyses showed that the blue whiting sex ratio is significantly different by area and by season (inside and outside the spawning period). The sex composition of catches by season, area and fleet will also be determined. The use of separate age-length keys by season and area reflecting the sex composition in commercial catches will be evaluated in order to more accurately reflect the dynamic of blue whiting population off Portugal.

(7) The instantaneous rate of natural mortality will be estimated based on a food web model to determine the level of predation by age and applied to the data of each component, due to blue whiting being a forage species.

(8) Finally, the status of blue in the Northeast Atlantic will be assessed. If the decision was assess separately these two stocks, the application of a model like the Extended Survival Analysis (XSA) which will deal with different survivors at age estimates from more robust or more "noisy" surveys, could be applied to the southern component. Since the data quality used as input into the model is ensured, a simple and under parameterized model must be the best option. Another option could be also perform the assessment of the southern and the northern component separately, but by using the model currently applied to the single stock, the SAM. Furthermore, the application of a model like Stock Synthesis (SS), able to assess the two blue whiting stocks as a whole but considering their main differences, e.g. growth, maturity, must be further investigated. This type of model which could deal with genetic data, otolith shape data, two sets of maturity ogives, two sets of M by age, abundance indices from the two stocks and age composition of catches by stock, due to the migratory characteristic of blue whiting constitutes a valid and good option.

The mentioned points are the main topics, as a result of this thesis, that will be addressed in a near future. Notwithstanding, new and interesting challenges related to blue whiting biology and assessment will continue to lead our future research.