Characterization and influence of biotic and abiotic factors on the early life stages of European hake (*Merluccius merluccius* L. 1758) from the southern stock.

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Este es un logro alcanzado
gracias al esfuerzo realizado
y al gran apoyo de mi
esposa y mi familia.

This is an accomplishment
achieved by the effort
realized and the great
support given by my wife
and thewhole family.

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Ha sido un largo camino. Recuerdo llegar a un nuevo país (pensé que llegaba a España), cargado de ilusión y entusiasmo. Entusiasmo por conocer y aprender temas relacionados con la pesca y la acuicultura e ilusión por conocer una nueva cultura y unas nuevas personas. También me ilusionaba conocer la historia del viejo continente, su proyecto de vida y su progreso ya que el propósito también era poder aplicar de entre muchas cosas, el civismo, orden y desarrollo humano en un país como Colombia.

Entre mis planes solo estaba realizar el master y regresar cuanto antes a Colombia. Pero ooohh sorpresa me encontré con la adjudicación de una beca doctoral que no esperaba y con ello entonces mi radicación por otros 4 años en un pedazo de tierra que me supo acoger con paciencia como uno más de ellos.

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My plan was only to make the master and return to Colombia as soon as possible. But my surprise was to find the doctoral scholarship (without hope to obtain in that moment) and then, obtain the residence for another 4 years on a piece of land which acceptedto me patiently as one of them.

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CONTENTS

1. THESIS STRUCTURE

This PhD dissertation conform the following structure:

1: **Thesis structure.** The present section shows the chronological order and the structure of all thesis.

2: **Resumen**. En esta sección se presenta una completa recapitulación de los principales hitos alcanzados durante el desarrollo del presente estudio de investigación.

Summary.In this section the whole analysis text of the thesis is reduced. The main ideas that follow the logical development process of the entire document are written.

3: **Objectives of the study.** To give the basic tools that are the basis of planning of the thesis are the goal of this section.

4: General **introduction.** This **first chapter** includes information about European hake both economy and politic division and the eco-biology of this specie. Likewise, it is introduced the overall approach of the investigation project developed in this thesis. The general information (oceanographic and meteorology) about the study area and the basic material, methods and techniques used to develop this thesis is also given here.

5: **Chapter two**describes, from a biological point of view, the ecosystem where the hake habits (Galician area -NW, Iberian Peninsula-). In detail, the chapter is focused on providing information of the locations of spawning sites, timings of peak spawning and vertical and horizontal distribution of early life stages of European hake and the coexistence with other egg-fish species. Biological interactions analyzing the effect of different physical oceanographic features were analyzed.

6: **Chapter three**studies the effect of five different temperatures on the development, hatching and mortality of European hake eggs collected from adult stock (Southern stock) in captivity. The development time on 4 egg stages until hatching were analyzed.

7: Along the**Chapter four**is researched the effect of five temperatures on the yolk-sac larvae development until their death by starvation. Some indexes of larvae development were used to determine the morphological features changes.

8: **Conclusions.** This section presents the main conclusions of this PhD thesis and the Thesisas the answer to the working hypothesis set out in this research. Equally give the future analysis which could be revised.

9: **References.** Each revised reference which was analyzed and studied within of this study and that constitutes the body of literature of this thesisis included here.

2. RESUMEN

Conocer los factores que regulan el reclutamiento de las especies constituye uno de los objetivos clásicos en la biología pesquera. Pequeños cambios en la tasa de mortalidad durante los primeros estadios de vida de los peces (huevos y larvas), momento en el que la mortalidad es máxima, puede ocasionar cambios drásticos en la tasa de supervivencia y por tanto en la intensidad del reclutamiento de los juveniles y a mediano y/o largo plazo afectar la dinámica de la población adulta. La zona oeste del mar Cantábrico (zona de Galicia), es considerado como una zona de gran riqueza pesquera para las flotas que faenan en el área. En estas aguas desova una gran variedad de especies de gran interés económico para el sector pesquero; siendo la merluza una de las más importantes.

La merluza europea es una especie demersal distribuida en dos stock. Se encuentra en el Mediterráneo y en el Atlántico oriental, desde Islandia, Noruega, llegando ocasionalmente hasta Mauritania. Es una especie que alcanza tardíamente su madurez sexual, presentando un asincronismo en la época de desove por tanto es sensible a la explotación pesquera de ahí que surja la importante labor de estudiar sus estadios tempranos.

El trabajo de investigación se centró principalmente en el estudio de las fases tempranas de la merluza (HKE) dentro de su nicho de puesta y sus interacciones con el medio ambiente, el cual incluye tanto los factores abióticos, -temperatura, salinidad, hidrodinámica-, como bióticos -disponibilidad de alimento y de predadores-. Como parte del ecosistema también se incluye en el estudio los estadios tempranos de vida de otras siete especies(sardina –PIL-, anchoa –ANE-, caballa –MAC-, jurel –HOM-, lirio–

WHB-, gallo –MEG-, anchoa de fondo –MAM-)que cohabitan con la merluza y con las que comparte el nicho de puesta. El trabajo se llevó a cabo aguas de Galiciahacia el oeste del Océano Atlántico, Península Ibérica, dentro del stock sur de la merluza europea. Esta zona tiene una geomorfología particular, lo cual contribuye a mantener unas características oceanográficasy meteorológicas específicas. En consecuencia, estas características al parecer permiten la concentración de un alto porcentaje de especies en diferentes zonas. Se realizaron dos campañas oceanográficasdurante los dos picos principales de desove de esta especie tanto en invierno como en verano, durante el año 2012.

El estudio de los estadios tempranos de vida se desarrolló en dos fases. En primer lugar, se caracterizó la zona de desove mostrando la distribución espacial (horizontal y vertical) de huevos. Además, se analizóel efecto que diferentes factores ambientales bióticos –zoo e ictioplancton- y abióticos –variables físicas- pudieran tener sobre la abundancia y la distribución de las 8 especies de huevos y delas larvas de merluza. En segundo lugar se estudió, bajo condiciones de laboratorio; el efecto de cinco diferentes temperaturas (10, 13, 16, 19 y 22 °C) en el desarrollo y la mortalidad de huevos y larvas de merluza hasta la eclosión y la muerte por inanición, respectivamente.

Durante invierno se detectóun marcado frente de temperatura que separó las aguas más cálidas y salinas hacia el sur oeste de las aguas frías y de menor salinidad ubicadas hacia el noreste. Igualmente se observó que el frente separó las aguas frías localizadas hacia el interior de la plataforma de las aguas cálidas ubicadas fuera de ella. Según los registros del CTD, Las aguas frías provenientes de la Bahía de Vizcaya se extendieron hasta los 150 m de profundidad mostrando a lo largo del perfil las típicas condiciones de mezcla de aguas para esta época. En cuanto a los valores geostróficos estos fueron medianamente fuertes (velocidad geostrófica = < 10 cm/s en niveles superioresy < 5 cm/s por debajo de 100m; velocidad vertical entre 1-2m/día entre 50-80 m de profundidad) hacia el NE entre Coruña y Estaca de Bares.

En verano fueron notorias las aguas frías en el interior de la plataforma y cálidas hacia el exterior con valores de salinidad similares a los de invierno pero con temperaturas cercanas a los 20 ºC. Los valores geostróficos fueron mayores que los de invierno debido a los gradientes en la densidad (> 40 cm/s encima de la termoclina y entre 10-30 entre la termoclina y los 80 m de profundidad). En este periodo fue evidente la presencia de un meandro de corriente que contribuyó con la formación de estructuras mesoescalares (R < 20 km en ambos lados de la corriente). Se observó una estratificación vertical bien formada del agua (termoclina: 20-60 m de profundidad), con aguas ligeras que ocupan los niveles más superficiales de la columna de agua.

Los huevos y las larvas de merluza fueron más abundantes en invierno que en verano, mostrando una variabilidad estacional en su distribución vertical y horizontal. Se observó que ambos estadios tempranos estuvieron por debajo de 10 y 30 m en ambos periodos, respectivamente. En invierno, estuvieron entre 10-150 m de profundidad, mientras que, en verano, aparecieron a una profundidad de entre 30 a 150m. La mayor abundancia de huevos y larvas se presentó entre 40-60 m de profundidad en ambos periodos. Siendo la temperatura determinante en la distribución de huevos y larvas.

Los análisis estadísticos (la correlación de Spearman y el modelo general de aditivo –GAM-) mostraronque en invierno la distribución de huevos está determinada por la velocidad modular y la temperatura; mientras que para larvas el zooplancton pierde significancia, incluyendo en el modelo la salinidad. En verano, elreducido número de huevos y larvas recogidos no permitió obtener correlacionessignificativas con ninguna variable.

La mayoría de las especies de huevos capturados en invierno se concentraron hacia Estaca de Bares. Siete especies fueron capturadas en invierno (Excepto ANE), mientras que sólo cinco lo fueron en verano (excepto MAC, WHB y MEG).En invierno, PIL se distribuyó en mayor abundancia en la plataforma mientras que WHB y MAM fueron capturados hacia la zona oceánica. En contraste, los huevos de MAC HOM and HKE se distribuyeron balanceadamente entre ambas zonas: MAC y HOM con tendencia a ubicarse hacia el océano y HKE hacia el interior. En verano, PIL, HKE and MAM siguieron la misma distribución que en invierno. Los huevos de HOM se capturaron dentro de la plataforma. De otro lado la distribución de huevos de ANE fue similar en ambas zonas.

Especies como MAC, WHB, MEG y MAM se encontraron por debajo de 50 m de profundidad, mientras que HOM, ANE y PIL se distribuyeron por encima de 50 m. HKE, MAC, WHB and MAM mostraron los más altos índices de agregación en invierno y ANE y HOM en verano. Las primeras fases de desarrollo de huevo se recogieron a una mayor profundidad en la mayoría de las especies, para después subir hacia aguas menos profundas justo para la eclosión. Al parecer la profundidad de desove junto con el perfil hidrográfico de la temperatura y los vectores geostróficas representado por lavelocidad vertical fueron determinantes en la distribución de los huevos.

Respecto a los experimentos del efecto de la temperatura sobre el desarrollo de huevos de merluza realizado en cautiverio; las tasas de desarrollo y la mortalidad de estos mostraron diferencias significativas ($p < 0,05$) con la temperatura. Las tasas demortalidad fueronelevadas para todas las temperaturas, con valores más bajos a 12,7 °C (59%). En contraste, no toleraron temperatura por encima de 22 °C. El tiempo para completar las fases de desarrollo I, II, III y IV mostraron diferencias significativas ($P < 0{,}05$) entre este estudio y el reportado para los huevos de merluza del stock del Norte. Lacomparación en el tiempo de eclosiónen relación a la temperatura entre algunas especies de merluza mostró una gran homogeneidad.

Al igual que para los huevos, El efecto de las diferentes temperaturasinfluyó significativamente en los cinco indicadores morfológicos de desarrollo de las larvas recién nacidas (longitud estándar - SL, el volumen del saco vitelino - YSV, diámetro glóbulo de aceite - OD, altura del cuerpo - el peso húmedo BH y - BW). Las larvastuvieronmayor tamaño y peso a temperaturas bajas ($P < 0{,}05$). A lo largo del experimento, las tasas de cambios en SL, YSV y OD variaron con la temperatura ($P < 0{,}05$). Por el contrario, BH y BW se mantuvieron constantes y no mostraron relación significativa con el efecto de la temperatura ($P > 0{,}05$). Se identificaron dos fases de crecimiento SL: una fase de crecimiento rápido (desde 50 a 120 h a 19.5 y 10.5 °C, respectivamente), seguido de una lenta. Después del consumo total de YSV y OD, las larvas murieron entre los 8 y 14 días a 19,5 y 10,5 °C, respectivamente, alcanzando una longitud máxima de 4,3 mm a 10,5 °C. Además se estudiaron diferentes índices metabólicos para conocer el desarrollo de las larvas tales como: la temperatura umbral más baja (T_0); el desarrollo larvario efectivo ($Dº_{eff}$); las unidades térmicas acumuladas (TC) para completar el desarrollo larvario; el coeficiente de temperatura en la tasa de crecimiento en longitud (Q_{10}) y el factor de condición (K).

La temperatura, tanto en los análisis bajo condiciones controladas de laboratorio como en condiciones naturales, constituyó una variable determinante, si no la más, en la regulación de los procesos biológicos acelerando o desacelerando las tasas de desarrollo y mortalidad natural en huevos y larvas.

SUMMARY

To know the factors that regulate the recruitment of species is one of the classic objectives in fisheries biology. Small changes in the mortality rate during the early life stages of fish (eggs and larvae), the maximum mortality moment, can cause drastic changes in the survival rate and therefore in the recruitment intensity of the Juveniles and to medium and/or long term affect the dynamics of the adult population. The western part of the Cantabrian (Galicia area) sea, is considered as an area of great wealth for fishing fleets operating in the area. In this waters spawns a great variety of species with great economic interest for the fisheries sector; being the European hake one of the most important.

European hake is a demersal species distributed in two stocks. It is located in the Mediterranean Sea and in the eastern Atlantic Ocean from Iceland to Norway, occasionally reaching Mauritania. It is a species that reaches, belatedly, its sexual maturity, presenting an asynchronous in the spawning season therefore it is sensitive to the fishing exploitation. Hence, the important task of studying their early life stages.

The research work focused mainly on studying the early stages of hake (HKE) within its spawning niche and its interactions with the environment, which includes both abiotic and biotic factors such as temperature, salinity, hydrodynamics and food-predator availability, respectively. As part of the ecosystem, the early stages of life of seven species (sardine –PIL-, anchovy –ANE-, mackerel –MAC-, horse mackerel –HOM-, blue withing –WHB-, megrim –MEG- and silvery lightfish –MAM-), rooster and bottom anchovy) were also included in the study, which cohabit and share the spawning niche with the hake. The study was carried out in Galicia to the west of the Atlantic Ocean,

Iberian Peninsula, within the southernstock of European hake. This area has a particular geomorphology, which contributes to maintain specific oceanographic and meteorological characteristics. Consequently, these characteristics apparently allow the concentration of high percentage of species in different zones. Two oceanographic campaigns were carried out during the two main spawning peaks of this species in winter and summer in 2012.

The study of the early life history was developed in two phases. Firstly, the spawning area was characterized showing the spatial egg distribution (horizontal and vertical). Besides, the effect of different biotic and abiotic factors (Zoo and ichthyoplanktonand physical variables) on the abundance and distribution of 8 species of eggs and larvae were analyzed. Secondly it was studied under laboratory conditions; the effect of five different temperatures (10, 13, 16, 19 and 22 °C) on the development and mortality of hake eggs and larvae to analyze the hatching and starvation conditions, respectively.

During winter a marked temperature front which separated the warm and saline waters towards southwest from the cold waters with lower salinity located to the northeast was detected. It was also observed that this front separated the cold water located within the continental shelf from the warm waters located outside it. According to the CTD data, the cold waters from the Bay of Biscay extended to 150 m depth showing along the profile the typical mixed waters conditions for this time. The geostrophic values were moderately strong (geostrophic velocity = <10 cm/s at higher levels and <5 cm / s below 100m; vertical velocity between 1-2m/day to 50-80 m depht) towards NE between Coruña and Estaca de Bares.

In summer, the cold waters were notorious inside the continental shelf and warmer outward of it with similar salinities values to winter but with temperatures close to 20

°C. Geostrophic values were higher than in winter due to density gradients (> 40 cm / s above the thermocline and 10-30 between the thermocline and 80 m deep). Evident was the presence of meandering current that contributed with the formation of mesoscale structures (R <20 km on both current sides). Water stratification (20-60 m deep) was observed with an approximate thermocline to 60 m depth and slight waters occupying the surface levels of the water column.

The eggs and larvae of hake were most abundant in winter than summer, showing a seasonal variability in the vertical and horizontal distribution. It was observed that both early stages were placed under 10 and 30 m in both seasons, respectively. In winter, they were caught between 10-150 m of depth; while in summer appear between 30 to 150 m depth. The highest abundance of eggs and larvae was presented between 40 – 60 m of depth in both seasons. The temperature was determinant in the eggs and larvae distribution.

The statistical analysis (general additive model –GAM- and the Spearman correlation) showed in winter that the modular velocity and the temperature were determinant in the eggs distribution; while to larvae the model included the salinity. In summer, the little number of eggs and larvae collected did not permitted obtain significative correlations with any variable.

The highest amounts of species of eggs collected in winter were concentrated towards Estaca de Bares. Seven species were collected in winter (Except ANE) while only five in summer (Except MAC, WHB and MEG).In winter, PIL was distributed in highest abundance towards the inner shelf while WHB and MAM were captured to the outer shelf. By contrast, the eggs of MAC, HOM and HKE were distributed quite balanced by zones: for MAC and HOM slightly higher outer and for HKE inner. In summer, PIL, HKE and MAM followed the same distribution than winter. HOM eggs were preferably located on inner shelf. In the other hand, the eggs abundance from ANE was slightly similar in both zones. Species such as MAC, WHB, MEG and MAM were found under 50 m of depth while HOM, ANE and PIL were distributed above 50 m. HKE, MAC, WHB and MAM showed the highest aggregation indices in winter and ANE and HOM in summer. Early egg development stages were collected in higher depth in most of species; posteriorly they climbed to shallower waters just for hatching. Seem that spawning depth together hydrographic profile of temperature and the geostrophic vectors represented by the vertical velocity were determinant in their distribution.

Regarding the experiments of the effect of temperature on the development of hake eggs realized in captivity; the development rates and the mortality of hake eggsshowed significant differences ($p < 0.05$) with the temperature. Total egg mortality rates were high for all temperatures, with the lowest values observed at 12.7 °C (59%). In contrast, eggs did not tolerate temperature above 22 °C.The timeto complete the development stages I, II, III and IV showed significant differences ($P< 0.05$) betweenthis study and previous result reported for hake eggs from northern stock.An extensive review of some hake species from the Merluccius genera showed a big homogeneity in timeto hatch at each temperature.

The effects of the different temperatures also influenced the five morphological indicators of development of newly hatched larvae (standard length – SL, yolk-sacvolume – YSV, oil globule diameter – OD, bodyheight – BH and body wet weight – BW). The larvae had biggest size and weight at low temperatures ($P < 0.05$). Throughout the experiments, the rates of changes in SL, YSV and OD varied with temperature ($P < 0.05$). By contrast, BH and BW remained constant and did not showsignificant relationship with the effect of temperature ($P > 0.05$). Two growth phaseswere identified to SL: a rapid growth phase (from 50 to 120 h at 19.5 and 10.5°C, respectively) followed bya slow one. After total YSV and OD consumption, larvae died between 8and 14 days at 19.5 and 10.5°C respectively, reaching a maximum length of 4.3 mm at 10.5°C. Also, different metabolic indices to know the larvae development such as: lowest threshold temperature (T_0); effective larval development (D^{o}_{eff}); cumulative thermal units (TC) to complete larval development; the temperature coefficient on growth rate in length (Q_{10}) and the condition factor (K) were also studied.

The effect of temperature both under controlled laboratory conditions and natural conditions was a determinant variable, in the regulation of biological processes, accelerating or decelerating the development rates and natural mortality in eggs and larvae.

3. OBJECTIVES OF THE STUDY

The main objective of this thesis is to investigate the temporal spawning dynamic and the effect of different variables ondistribution patterns of early life phases (eggs and larvae) of European hake in the North West Atlantic waters (Galician zone) both in natural environment and under laboratory conditions. These processes have been studied in order to understand how the variables act during early life stages and how the mature spawn stock respond under these special features (Fig. 1).

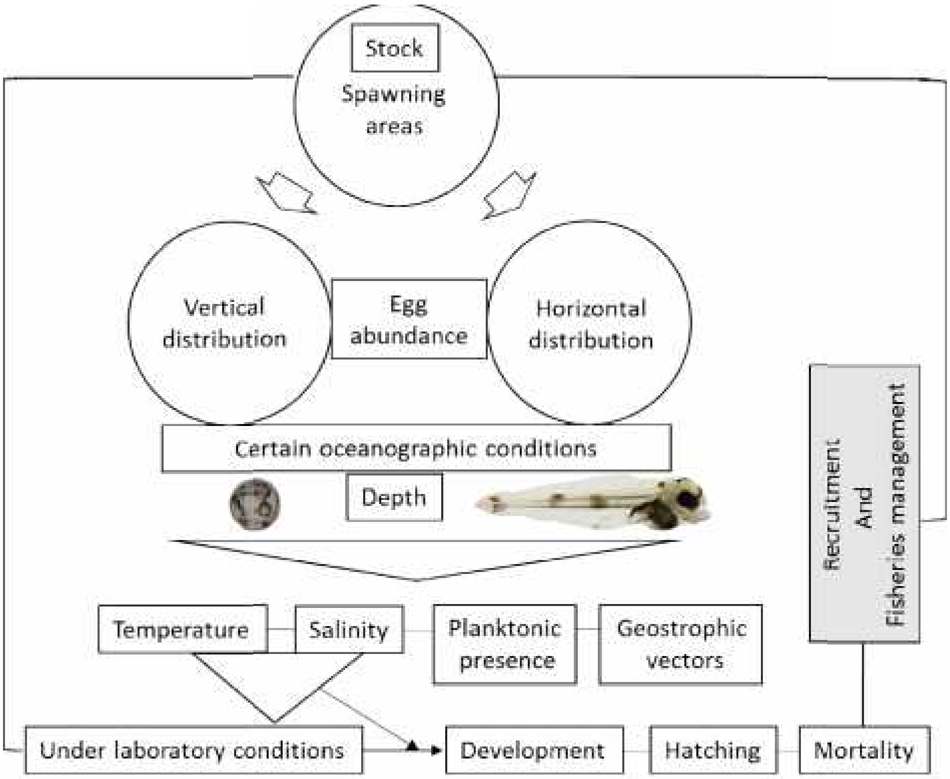


Fig. 1.Schematic diagram showing relationship that determines the survival of ELS of hake in Galician waters.

Therefore, itwere proposed the following specific objectives to achieve the main objective:

1.To study and describe the ecological environment of the spawning ground of European hake belonging to the southern stock and their coexistence with eight egg species from fishes with commercial and biological importance on the Galician area.

a. To describe the ecosystem where European hake (*M. merluccius*, L.) spawn.

b.To identify and staging classify the European hake eggs and their vertical distribution.

c. To know the spatial distribution (vertical and horizontal) of hake eggs and larvae in the Galician zone (North-west Atlantic Ocean).

d. To know the hatching time of *M. merluccius* on the Galician area.

e. To identify and staging classify seven species of egg fish such as: Mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*), Megrim (*Lepidorhombus whiffiagonis*), Blue whiting (*Micromesistius poutassou*), Sardine (*Sardina pilchardus*), Anchovy (*Engraulis encrasicolus*) and Silvery lighfish (*Maurolicus muelleri*).

f. To identify the vertical and horizontal distribution of those fish egg.

g. To know the effect of the environmental variables on the egg distribution.

h. To recognize and analyze the coexistence between eggs from *M. merluccius* with other egg-fish species.

2. To assess the effect of differenttemperatures on development and mortality of eggs under laboratory controlled conditions.

a. To know the optimal temperature range on hake eggs.

b. To estimate the hatching time to the different temperatures.

c. To compare the effect of temperatures on egg development and the hatching time with other studies.

d. To gauge the mortality egg rate at each temperature.

3. To assess the effect of five temperatures on larvae development until its death by starvation under laboratory controlled conditions.

a. To analyzethe five morphological indicators of development (yolk-sac volume, oil globule diameter, body height and body wet weight) until its death

b. To estimate different indexes to evaluate the larval conditions.

4. CHAPTER 1

INTRODUCTION

4.1. Description of study area.

4.1.1. Localization and geomorphology

The study area of this thesis is located along the Galician coast from Finisterre (42º52'59''N and 9º18'34''W) to Estaca de Bares (44º04'12''N and 7º37'06 ''W) (Fig. 1).

Figure 1.Localization of Galicia in Spain and the study area with the ICES divisions and subdivisions.

The Galician coastal geography has approximately 1600 kilometers, containing a lot of rivers (Rías). It has a top part with a flooded fluvial valley and intermediate areas with slopes without a fluvial origin. In consequence, the rivers are a main element of the Galician geographic area because of their particular features which make possible the presence of different organisms. In general, the rivers from the Cantabrian zoneare short and regular, while the rivers from the Atlantic side are longer and have gentle slopes (Álvarez, 2005).

Likewise the Galician coasts have a high amount of headlands, islands, mountainous areas and cliffs where is possible to find a variety of species owing to the variety of submerged habitats with various types of marine bottoms and coastal land (Prada et al., 2012).

Climate is basically wet Oceanic which tend to aridity in summer due to lower Rias. The prevailing winds come from the east and west in winter while in summer come from the north and northeast. The rainy seasons are irregular throughout the year with high values in November and February and evidently reduced in summer (Álvarez, 2005).

4.1.2. Water masses and general circulation patterns in the Galician area, North West from the Iberian Peninsula

The geomorphology features of Galicia make that this zone has an important contribution to the fishery in Spain and be one of the most important fishing areas in the world. Likewise, upwelling presence especially in the Rias Baixas to allow an increaseof phytoplankton evidently higher compared with similar latitudes (Two seasonal upwelling one in March and another in late summer) (Alvarez, 2005).

The upwelling on the Galician coast is part of a general system extending along the east coast of the North Atlantic from about 7.5 to 44º N (Wooster et al., 1976). Grossly, the Galician coast can be divided into three regions (Fig. 1). Cape Finisterre marks the abrupt change between the west coast and the rest of the coastal area and it is often an area of a stationary maximum upwelling (Blanton et al., 1984; Castro et al., 1994) and a recurring filament (Haynes et al., 1993). Along the west coast of Galicia, the upwelling phenomenon has been studied extensively both the platform and inside of some estuaries. Due to the orientation of the west coast of Galicia, the upwelling occurs under conditions of north wind (Alvarez, 2005). These winds arise mainly in spring and summer from April to October (McClain et al., 1986; Lopez-Jamar et al., 1992; Tilstone et al., 1994). In addition to this spring-summer upwellings, it has also found the existence of this phenomenon in winter (Alvarez et al., 2003). The upwelling in winter takes place under similar conditions to summer.

Hydrodynamic and hydrographic surveys during the time of upwellings are very important both inside estuaries and along the coast, because this phenomenon affects the distribution of temperature and salinity. Molina, (1972) found that this effect is produced from a depth of 500 m along the Galician coast. In the spring and summer 60% of the water entering in the estuaries corresponds with water from the North East Atlantic which is colder and saltier than the existing water in the estuaries, which influences their internal circulation (Fraga, 1981).

Besides hydrodynamics and hydrography descriptions, many biological studies have been conducted. The upwelling events have a significant biological implications because of the large amount of nutrients that entering into estuaries and are distributed along the coast(Prego & Bao, 1997; Prego et al., 1999). These nutrients, generate a primary productivity in the Galician area and thereby a high secondary productivity(Blanton et al., 1987).

A schematic diagram summarizing circulation in the mean downwelling and upwelling seasons is presented in Fig. 2 and 3. On the Galician coast there are two branches of Eastern North Atlantic Central Water (ENACW) from different sources. These branches are called ENACWp, when its origin is subpolar, and ENACWt when its origin is subtropical (Fiuza, 1984; Rios et al., 1992).

These two branches are directed towards the south and north respectively, forming a subsurface front when they meet (Fig. 2).

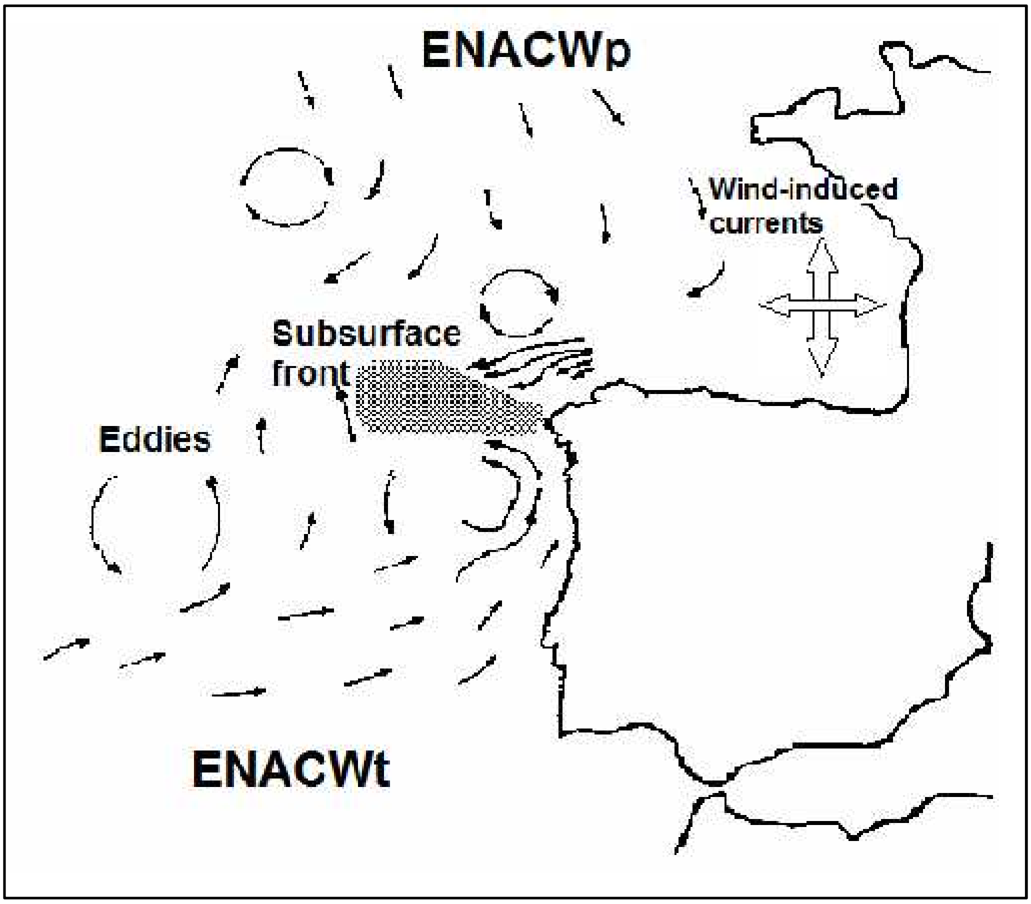


Figure 2.Scheme of ENACW distribution in the North Atlantic Ocean (Rios et al., 1992).

Rios et al (1992) defined in this area a subsurface area of convergence where the subpolar water source, as amended by the summer heat, can be mixed with water of tropical origin. This convergence is very marked in Finisterre, marking the abrupt change between the west coast and the rest of the coastal area and is usually an area of a maximum stationary upwelling (Castro et al., 1994) and a repeating upwelling filament (Haynes et al., 1993) (Fig. 3).

In this way, important differences between the waters of the coastal zone are established. On the west coast, the prevailing wind in summer (north direction) moves the surface water allowing ENACW which are laden with nutrient salts. The fertile characteristics of the Rias Baixas is caused by those nutrient supply, three times higher than normal, which penetrates through the bottom and transformed into organic matter that becomes part of the life cycle (Fraga & Margalef, 1979).

In the rest of the coastal zone, the upwelling occurs owing to easterly winds; the mixed winter water emerges from depths of 200 m and although is rich in nutrient salts, its concentration is less than ENACW. By contrast in spring the change in the wind triggers the upwelling of central water and provides the system with potential energy through elevation of isopycnals (Ruiz-Villarreal et al., 2006). This could be associated with the appearance of mesoscale structures in the west coast (Fiuza et al., 1998) and in the Cantabrian Sea, where a complex dynamics of fronts and eddies has been described in spring and summer (Gil et al., 2002). Mesoscale structures have implications in the distribution of nutrients and in the spring phytoplankton bloom (Bode et al., 1996, 2002; Gil et al., 2002; Lavin et al., 2006). In spring, the poleward current weakens on the slope (Fiuza et al., 1998; Lavin et al., 2006). Surface atmospheric warming originates a shallow thermal stratification in coastal waters (Lavin et al., 1998) that can be broken in upwelling pulses, especially in the west coast (Fig 3).

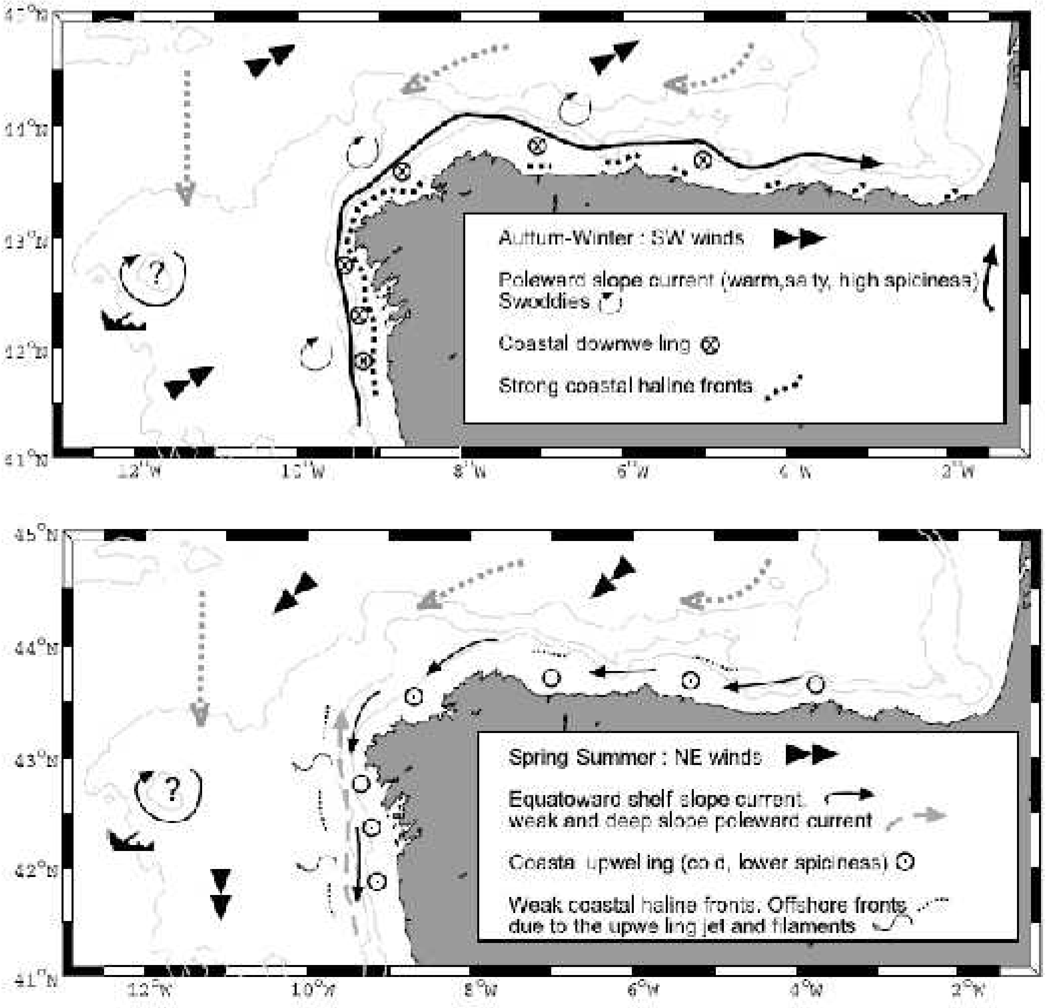


Figure 3.Schematic circulation in the area during typical upwelling (spring and summer) and typical downwelling (autumn–winter) season. Offshore current is dominated by the Portugal current, which is represented by a gray dotted arrow. Note that a typical season is a simplification and the system is subject to event variability that can dominate the response of the system (Ruiz-Villarreal et al., 2006).

4.1.3. Biodiversity of fish community

Cantabric Sea area belongs to subtropical/boreal transition zone of the Eastern Atlantic. Typical temperate water species from the south cohabit together with others of northern origin and, consequently, high biodiversity indices exist in relation to adjacent areas (Olaso, 1990; Sánchez, 1993). Moreover, the topographical complexity and the wide range of substrates on its narrow continental shelf give rise to many different types of habitats. This diversity is reflected in the biological richness of the region, which includes a wide range of species, many of which are of commercial interest(Sánchez & Serrano, 2003).

Within Cantabric Sea, the Galician area contains also, many of fish species with commercial importance from east Atlantic waters. In this sense, interaction, competition and depredation can occur between them (Sánchez 1993).

The studies dealing with the ecology of fish assemblageson the Galician coast are limited to the coastalembayments or rías (Iglesias, 1981, 1983; Iglesias & González-Gurriarán, 1984; González-Gurriarán et al., 1991), as opposed to the studies carried out in other areas of the Atlantic which focused on continental shelf and slope (Haedrich et al., 1975, 1980; Colvocoresses & Musick, 1984; Snelgrove & Haedrich, 1985; Overholtz & Tyler, 1985; Bergstad, 1990; Merrett et al., 1991; Gordon & Bergstad, 1992 cited by Fariña et al., 1997; Sánchez,1993). These studies have been traced to analyze the biology and ecology of different adult stocks and their interaction and to lesser degree, to explain the ELS behavior.

Competition for habitat suitability has not been widely analyzed for*M. merluccius*, but studies suggest a potential competition for food withother demersal and/or pelagic predators, playing an important role on theecosystems.

Population dynamics and productivity of these co-habiting species are interlinked and modulated by environmental conditions and trophic relationships. Predation is a very important source of mortality during embryonic and larval stages (Bailey & Houde, 1989; Bailey & Houde, 1989; Castro & Cowen, 1991; Gleason & Bengtson, 1996). The small size of eggs and larvae leads to numerous potential predators in the environment (Fortier & Villeneuve, 1996) and therefore can be of great importance for the future of the stock.

Apparently, adults of *M. Merluccius* compete for anchovy (*Engraulis encrasicolus*) with megrim(*Lepidorhombus boscii*), monkfish (*Lophius budegassa* and *L. piscatorius*), tuna and juveniles of hake in autumn, whereas in spring, anchovy might havealready achieved a size too large to be an important prey for *M. merluccius*(Goñi et al., 2011; López-López et al., 2012).

Starvation is the leading cause of larval mortality in some populations (Theilacker, 1986; Theilacker et al., 1996), therefore, the ability to locate, capture and ingest preys will be crucial to successful development. The starvation can be due not only by the low food availability but by competition. Among the hypotheses proposed to explain the failure of the larvae to reach external feeding, those that related to intra and interspecific food competition have paid considerable attention (McGurk et al., 1993).

Other studies carried out in Galician by Fariña et al., (1997) analyzed spatial structure associated with the variabilityof different environmental factors (mainly depth) and geographic location of this species as well as long-term temporalchanges due to environmental changes or to thefishery which directly effects a large part of thedominant species.

Similar situations occur with the interaction of early life stages of commercial species such as: mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*), sardine (*Sardine pilchardus*), anchovy (*Engraulis encrasicolus*), blue whiting (*Micromesistius pouttasou*); megrim (*Lepidorhombus whiffiagonis*) or not commercial such as *Maurolicus muelleri* Vs *M. Merluccius*. Unfortunately the spatial and temporal spawningdistributions of many fish species are out of the coveragecapability of most research cruises, which limitsthe understanding of the factors controlling spawning(Ibaibarriaga et al., 2007) or connection between them.

One exception to this limitation, in the area of NEA waters, the ICES triennialmackerel and horse mackerel egg survey. This ichthyoplankton survey has an exceptional spatial andtemporal coverage, from south of Portugal to north ofScotland and from January to July. Although the surveys are focused on mackerel and horse mackerel, the coverages and sampling periods facilitate the inclusion of an extensive number of other species which spawn in the area. An example of this applicability was the study published by Ibaibarriaga et al. (2007), where analysed the effect of different environmental variables on ELS of seven commercial fish species.

4.2. European hake.

4.2.1. Biology and fishery

The European hake (*Merluccius merluccius*) is widely distributed over the Northeast Atlantic shelf. Its distribution included the Mauritania coast to off the western coast of Norway and the south waters of Iceland (Casey & Pereiro, 1995), being more abundant from the British Isles to the south of Spain (ICES, 2008) (Fig. 4). It is also found in the Mediterranean and Black Sea (Casey & Pereiro, 1995) (Fig. 4). *M. merluccius* is a demersal and pelagic species where is one of major predator in the demersal ecosystem of the Cantabrian Sea (Velasco & Olaso, 1998). This species live between the depths of 70 and 370 m, although it is found in shallower and deeper waters, from coastal waters (30 m) down to the depths of 1000 m (Lloris et al., 2003). Adults live close to the bottom during day-time, but move off-bottom at night. Adults feed mainly on fish (small hakes, anchovies, pilchard, herrings, cod fishes, sardines and gadoid species), crustacean and squids. The young feed on crustaceans (especially euphausiids and amphipods). In this specie is possible to find a cannibal behavior (which increase with their growth) when the food is scarce (Pitcher & Alheit, 1995).

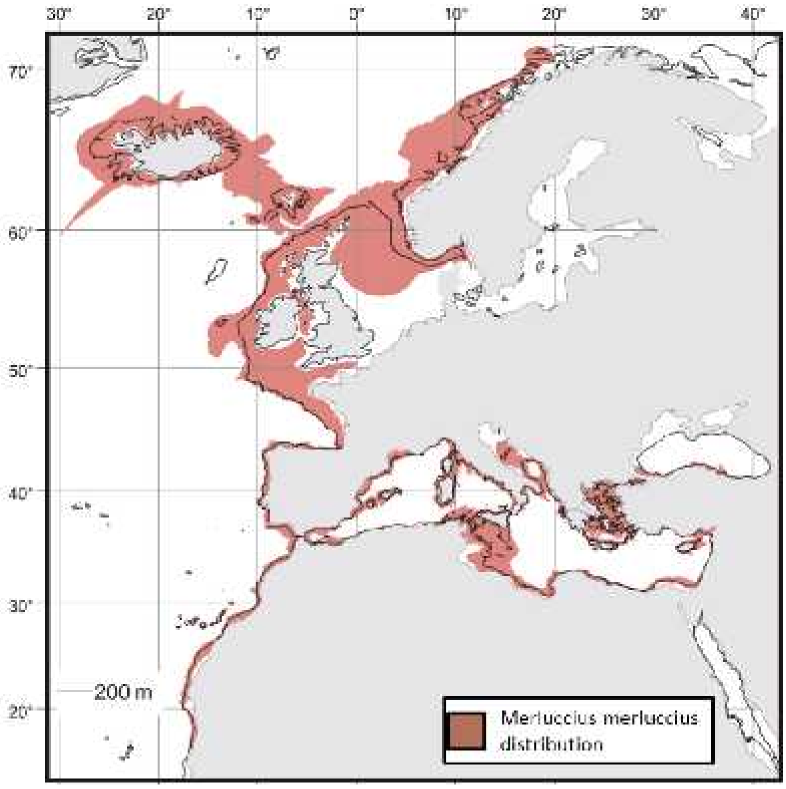


Figure.4. Distribution of *M. merluccius* (taken from Lloris et al., 2003).

European hake is one of the most heavily exploited fish species in Western European demersal fisheries and is taken as part of mixed-species fisheries in the Northeast Atlantic.Spain is responsible for most of the hake's landing following by France and Portugal. The highest catches occurred in 1948 (175 000 ton) and decreases until 80 000 ton at the end of 80's and beginning of 90's (Casey and Pereiro, 1995). In the last decade the catches lightly increase until close to 100 000 t in 2016 (ICES, 2016).

In the North east Atlantic waters hake population is divided in two stocks: southern stock which originates from the spawning areas to the north-west of Iberian peninsula (ICES divisions VIIIc and IXa) limited by the Gibraltar Strait (ICES, 2013), and northern stock which originates from Bay of Biscay, Celtic sea and other northern spawning areas (ICES Division IIIa, Sub-areas II, I, VI and VII and division VIIIa, b, d) (Fig. 5). The limit of both stocks is placed in Capbreton Canyon, close to the border between the French and Spanish coasts.

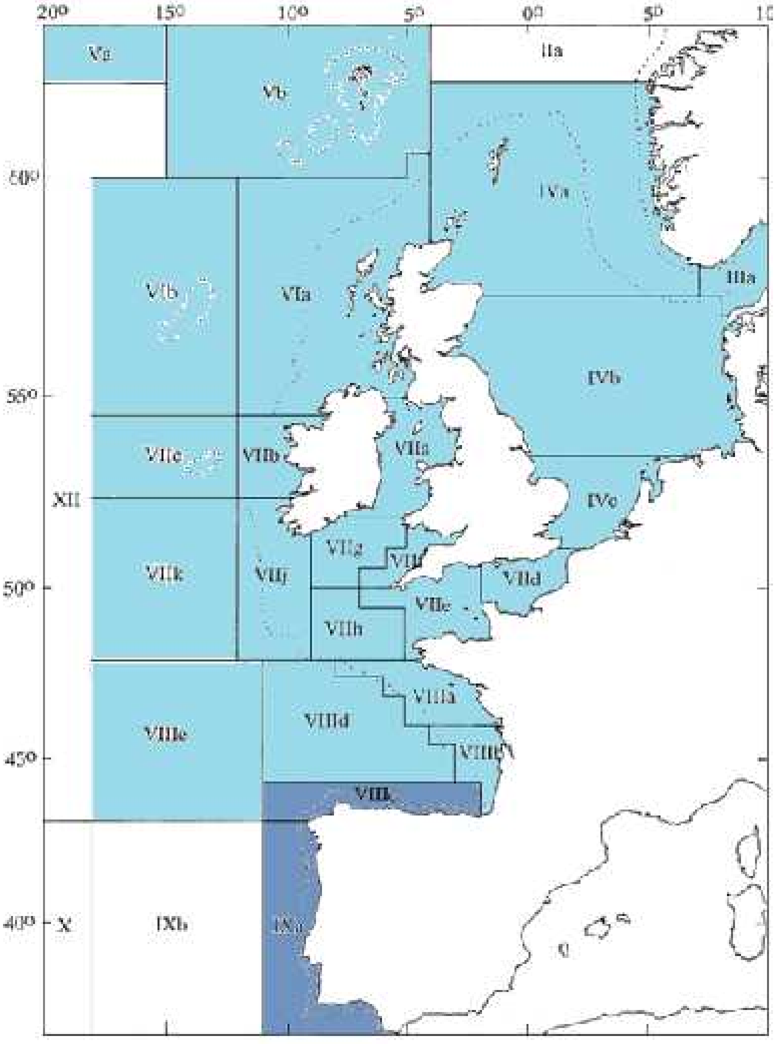


Figure 5. Statistical zones used for the collection of fishery statistics by the InternationalCouncil for the Exploration of the Sea (ICES, 2013).Southern stock in dark blue and northern stock in light Blue.

Nowadays, this geographical separation is still an controversial issue because of several genetic studies have showed that there are not differences between both stocks (Pla & Roldán, 1994; Roldán et al., 1998; Mattiucci et al., 2004; Castillo et al., 2005;Pita et al., 2010; 2014).

4.2.2. Reproduction and Spawning

The information available on the reproductive biology of European hake both in the South and North Atlantic areas is not extensive (Murua, 2010). Males mature at around 35 cm, whereas females mature between 45 and 50 cm total lengths (Domínguez-Petit et al., 2008;Lucio et al., 2000; Martin, 1991; Piñeiro & Sainza, 2003).However, in the Bay of Biscay and in particular in Galician waters,Domínguez-Petit et al.(2008) reported that since 1996the maturity size of hake varied as consequence of fishing pressure, changes in population biomass and the environmental conditions. The hakehave a sexual dimorphism and dioecism with males maturing earlier than females showing an external fertilization (Murua, 2010).The description of oocytes development cycle connotes that hake is regarded to be an indeterminate fecundity species (The recruitment of oocytes continues during all the spawning season until post-spawning period) (Hunter et al., 1985).The oocytes development is asynchronies with a large spawning period which varies between localities. Annual fecundity of females shouldbe estimated from the number of oocytes released per spawning (batch fecundity), the percentage of spawning females per day (spawning frequency) andthe extension of the spawning season (Hunter et al., 1985; Murua et al., 2010; Korta et al., 2015). Seems that the fecundity indeterminacy can be explained by the breeder strategy and the energy stores (Domínguez-Petit & Saborido-Rey, 2010).

As well as other hake species, *M merluccius* is a batch spawner, spawning several batches within the reproductive season (Ciechomski, 1967; Christiansen & Cousseau, 1971; Erkamov, 1974; Balbotin & Fischer, 1981; Alheit, 1986).

Two main areas ofspawning have been identified which coincide with the observed distributionof a-group recruits (fish in their first year of life). One area is located of the French coast in the Bay of Biscay and, to a lesser extent, in the Celtic Sea tothe west of the Isles of Scilly; the second spawning area occurs of the northwestcoast of the Iberian Peninsula(Pitcher & Alheit, 1995) (Figure 6).

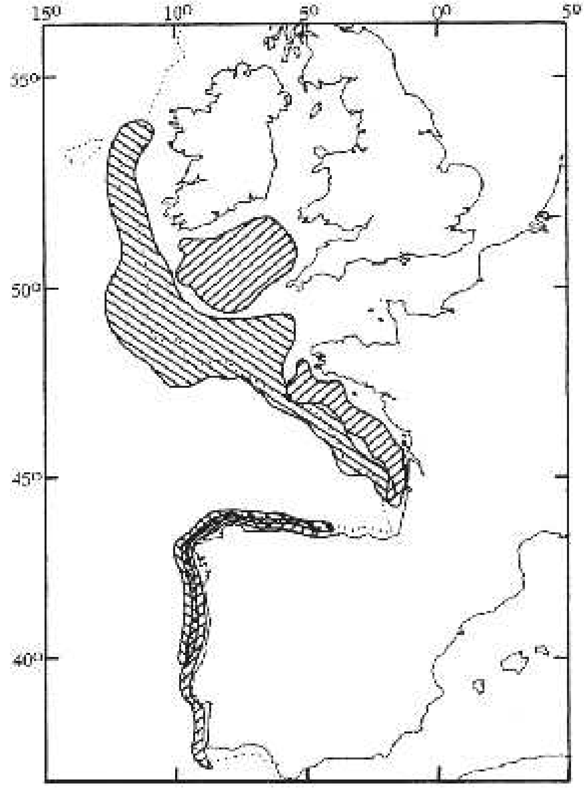


Figure 6. European hake: main spawning and nursery areas. Hatching sloping left to right spawning areas; hatching sloping right to left, main nursery areas (taken from Casey & Pereiro, 1995).

In general, the spawning season runs throughout the year with a clear seasonal peak, allowing to increase the survive opportunities owing to the decrease the natural mortality by exogenous factors. In the Gulf of Lions and the Catalan Sea, the main peak occur in autumn-early winter(Recasens et al., 1998). In the Northern stock the spawning seasons is observed from December to July from the Bay of Biscay to the south-west of Ireland with a spawning peak in March-June(Clark, 1920;Coombs & Mitchell, 1982; Horstman, 1988; Alvarez et al., 2001; Alvarez & Cotano, 2005).In western Ireland, spawning occurs from April to July and from May to August for sea areas west of Scotland (Hickling, 1930; O'Brien, 1986). Ripening hake hasbeen also recorded in the West Coast of Norway inAugust (Kvenseth et al., 1996). In the case of hake, this variability can be explained by a migration of spawners from south to north, or alternatively, by a spawning wave going from south to north as local population components reach maturity and spawn, or it can be a combination of both (Alvarez et al., 2000).

In the southern stock the main spawning areas are found towards the NW of Iberian Peninsula between January-Mayin the Cantabric Sea and on the Galician shelf with a secondary peak in June-July for the Galician area (Pérez & Pereiro, 1985; Domínguez-Petit, 2007; Korta et al., 2010; Murua & Motos, 2006;Murua, 2006). Distribution studies on hake eggs and larvae state than the reproductive stock spawned dispersedly eggs in open waters preferentially on the shelf break area where high concentration of eggs are commonly found(Alvarez et al., 2000; 2004).

4.3. The importance of early life stages studies

Nowadays, one of the major problems within fisheries science, is to understand the relationship between the biomass of the adult population, the development of early life stages and the amountof recruitsentering in the populations (Hjort, 1914).Gulland(1965), Hempel (1979) and Lasker (1987) considered that during the egg and larval stages the abundance of recruits entering into the fishery could be defined.This life stages represents an important period determining the year-class strength that in combination with ecological interactions can modulate each year class (Garvey et al., 2004). In this context, it is known that the study of eggs and larvae stages could resolve the questions about the problems with the recruitment, the low stock biomass and thereby the high variation in the hake catches from 1970 (Pitcher &Alheit, 1995).

Some studies deal directly with embryology and later ontogeny, other emphasize functional morphology or larval structure, other investigate the ecology of eggs and larvae, or use these stages to address fishery-related problems such as assessment of spawning stock size and recruitment success.

However, Information from egg and larval development from European hake, their critical features and their distribution are relatively scarce with is compared with other commercial fish species. Have been most studied the early stage of life from northern than the southern stock.The two main sources of information to obtain evidences on this issue are icthyoplankton surveys and rearing experiment. The first deal with aspects related to distribution, development of eggs and larvae which, linked to environmental variables try to dissentangle the factors causing that distribution/abundance or define preferential spawning or nursery habits. Rearing experiments represents a usefully source and information on biological development of egg and larvae under controlled conditions and facilitate the interpretation of process that occurs at sea.

4.3.1. Hake eggs and larvae distribution

The distribution of eggs and larvae is highly influenced by the localization of spawning and ocean circulation. Variations in circulation can transport eggs and larvae toenvironments of different characteristics and explain the changes in therecruitment of the stock (Campana, 1989; Bailey & Francis, 1985; Bailey et al., 1995, 1997).The retention or dispersion of ELSare relevant processes affecting the final fate of eggs and larvae.

Bailey et al. (1995) demonstrated that those larvae that were transportedoutside the continental shelf showed evidence of poor nutritional condition, so they could die of starvation or, ultimately, contribute little to recruitment in the area. In other systems, currents which occur near to the spawning zone, favor the transport ofeggs and larvae to high productivity areas (nursery areas) and if this transport fails, a very high mortality occurs(Shelton & Utchings, 1982).

Studies on European hake (Coombs and Mitchell, 1982; Alvarez et al 2000; 2004) reveal that hake eggs and early larvae are concentrated around the shelf break, although in the Celtic sea, this spawning area extends to shallower waters (Coombs and Mitchell, 1982; Horstman, 1988; Alvarez et al., 2000; Fives et al., 2001; Olivar et al., 2003). In this respect, Kacher & Amara (2005) found that the spatial distribution of

0-group hake is more onshore than the areas of concentration of larval stages in the Bay of Biscay (Alvarez et al., 2001) and Celtic Sea (Fives et al., 2001), suggesting an onshore migration during the first year of life.

The spatial distribution of hake larvae by size ranges showed important differences between years: 1983 and 1995 data indicated inshore transport of larvae, from the spawning areas (close to the 200 m depth isobath) to nursery areas (Alvarez et al., 2000 and 2004) while in 1998 it suggested offshore transport. These studies pointed out to wind, current and temperature as the environmental drivers responsible for the changes in the spatial and temporal abundance and distribution of ELS.

Regarding the vertical distribution of Atlantic hake eggs and larvae, it all boils down to the works carried out in the Northern stock by Coombs and Mitchell (1982) andMotos et al.(2000). The first, summarized eight years of sampling in an extensive area from the Bay of Biscay to west of Scotland from March to July, and the later derived from two surveys carried out in the Bay of Biscay in winter and early spring. Both works agreed that hake eggs and larvae occurred in the upper 150 m of the water column and may show monthly variability in their vertical distribution mostly depending on environmental conditions. In particular, Motos et al. (2000) observed important variations among winter and early spring which were interpreted on the basis of prevalent condition (upwelling or downwelling sceneries). The larvae were distributed throughout the water column down to depths exceeding 300 m. Although the majority of individuals were generally found between 50 and 100 m from the sea surface (Coombs & Mitchell, 1982; Motos et al., 2000) no relationship between vertical distribution and larval size or day/night was reported by these authors, coinciding totally with Rodriguez et al.(2015) for the southern stock. For *M. productus*, Bailey

(1982) shown that most of the small larvae (<8 mm) were caught in the 50–100 m depth interval, although he suggested that larvae >5 mm, located near the surface, were probably avoiding the net. This author stressed the close relationship between eggs and larvae vertical distribution and the vertical structure of the water column.

4.3.2. Early life development under controlled conditions

It is often a challenge to interpret the results observed during surveys carried out at sea because of the natural variables which affect ELS are many and no controlled. In this sense, laboratory procedures has provided the opportunity to extrapolate the ecological situations occurring at the sea with the purpose to know the best conditions for culture fish and thus supply the high global demand for fish in the future. The possibility of rearing eggs and larvae under controlled conditions to be compared with their wild counterparts has been long discussed (Ferron & Legget, 1994 for details). Today it is impossible to simulate in captivity the events occurring in the nature; however, it is accepted that can be a help to understand behaviors and mechanisms affecting the early life stages and the population dynamics of fish.

A non-exhaustive description of eggs and larvae of hake was given by Raffaele (1888) (in Coombs & Mitchel, 1982) based on artificial fertilization experiments using material from the Mediterranean Sea. This author detailed that eggs are transparent, smooth and spherical with a diameter between 0.9 - 1 mm and presented a yellow oil globule while larvae were elongated with presence of melanophores on the head and over the gut as wells as three vertical bands along the tail. All this features were confirmed posteriorly by several authors (Coombs & Mitchell, 1982; Bjelland & Skitesvick, 2006; Sanchez et al., 2011).

Other studies based on Raffaele results such as Schmidt (1907), Ehrenbaum (1905) and D´Ancona (1933) (in Coombs & Mitchell, 1982) made different description on eggs and larvae of hake. Posteriorly Russell(1976) illustrated the early life stages –ELS- of different fishes including to European hake. First studies to know the egg development was carried out by Coombs and Mitchell (1982) in the British Islands. Other studies such asMarrale et al.(1996) also studied the egg and larvae development in the Bay of Biscay. This work focused on morphological description along the development of eggs and yolk-sac larvae stages at no controlled temperature. Bjelland and Skitesvick (2006) carried out experiments with fertilized eggs from northern stock in captivity. Other works were made between IFREMER and Norwegian University (MerluNOR) which studied the captive broodstock obtaining a natural spawning. After that they reach the larval stage and achieve their maintained until their death being difficult to know breeding process (Geffen et al., 2008).From the southern stock Iglesias et al. (2010) published the process of capture, transport and maintained of adult brood stock in captivity. These aspects are highly relevant for this species because allowed to know more about their resistance to transportation owing to the pressure changes affect their demersal habits and with it, the swimming bladder. Sanchez et al. (2011) with those adults obtained the first spontaneous spawning of *M. Merluccius* from the southern stock and described the main characteristics of egg by stages and the hatching time. The authors provided valuable information on eggs and larvae development, including body Measurements from hatching to yolk-sac absorption and growth rates. The Instituto Español de Oceanografía (IEO) has earned the reputation as a reference point in the field of aquaculture, with special attention to hake, such as it was exhibited in different symposiums and congress (Iglesias et al., 2015).

5. CHAPTER 2

SPATIAL DISTRIBUTION OF EUROPEAN HAKE (*MERLUCCIUS MERLUCCIUS*, L.)EGG AND LARVAE FROM THE SOUTHERN STOCK (ICES VIIIC-WEST DIVISION) IN RELATION TO ENVIRONMENTAL CONDITIONS AND ITSCOEXISTENCE WITH OTHER EGG-FISH SPECIES.

Abstract

The distribution and abundance of southern European hake stock eggs and larvae and eggs of other seven species (*Engraulis encrasicolus* –ANE-, *Sardina pilchardus* –PIL, *Scomber scombrus* –MAC-, *Trachurus trachurus* –HM-, *Micromesistius poutassou* –WHB, *Lepidorhombus whiffiagonis*–MEG- and *Maurolicus muelleri* –MAM-) are investigated at the Galician coast (NW Iberian peninsula) during 2012. The effects of different environmental factors that may affect hake early life stages are also studied. For European hake, a total of 486 eggsand 421 larvae in winter and 138 eggs and 19 larvae in summer were captured. The vertical distribution of eggs and larvae showed seasonal variability being more abundant in winter than in summer. A preferential spawning and larval development was observed below 10 and 30 m isobaths in both seasons, respectively. In winter, hake spawned at 10-150 m depth while, in summer, it occurred at depth between 30-150m. Higher abundance of eggs and larvae between 40-60 m depth were found in both seasons. Sea temperature was shown to influence eggs and larvae distribution, observing higher concentration at temperatures between 11.5-12.5 ºC in winter and 11.5-14 ºC in summer. The statistical relationship between the abundance of eggs and larvae and the main environmental variables are further investigated using non-parametric analysis, such as General Additive Models (GAM) and the Spearman correlation. Seem that in winter, the temperature and module velocity were determinant to egg and larvae distribution; also the zooplankton abundance and salinity to eggs and larvae, respectively according by GAM and Spearman analysis. In summer the reduced number of eggs and larvae encountered did not permit to obtain significant correlations.Respect to other species studied, the most amount of all them were also captured in the first seasonstudied with a high distribution towards Estaca de Bares in both seasons.In this sense, the egg abundance along the three zones studied (Finisterre, Coruña and Estaca de Bares) showed significant differences between them in both seasons. All species of eggs were confined within the continental shelf. Six species were collected in winter (Except ANE) while only five in summer (Except WHB and MEG). Species such as MAC, WHB, MEG and MAM were found under 50 m of depth while HM, ANE and PIL were distributed above 50 m. MAC, HM, WHB showed the highest aggregation indices in winter and ANE and HM in summer. Early development stages were collected in higher depth in most of species. As development progressed, eggs went to the upper waters to hatching. Although no one variable showed a significative effect on the egg distribution of all species, seem that spawning depth, hydrographic profile of temperature and the geostrophic vectors such as vertical velocity were determinant in their distribution.

Key words:*M. merluccius***,** early life stages,interaction with other egg-fish species,spatial distribution, spawning, hydrographic variables, north-west Atlantic waters.

5.1. Introduction

Fisheries management depends largely on stock estimates and recruitment forecasts, some of which are based on studies of early life stages (ELS) development (Hempel, 1979; Chambers & Trippel, 1997; Murua, 2010).

ELS represent the most vulnerable phases of the life cycle of fishes (Hjort, 1914a). The female can spawn hundreds of thousands of eggs and only a few of them will be able to complete their maturation. Processes such as dispersion, influences in the egg mortality and consequently affect the larval abundance and recruitment (Laevastu & Hayes, 1981; Hollowed, 1992; Brodziak & O'Brien, 2005).

ELS survival depends of their adaptation to biotic and abiotic features of the local environment. Key factors affecting eggs and larvae development and survival include temperature, salinity, and food availability amongst others (Blaxter, 1973; Sánchez & Gil, 2000; Maynou, 2003).

Within most marine environments, biotic and abiotic factors often display horizontal and vertical gradients that structure the ecosystem and can affect the dynamics of ELS (Fortier& Harris, 1989; Lampert, 1989in Peck et al., 2012; Mintenbeck et al., 2012). In this sense studies on the vertical and horizontal distribution of fish eggs and larvae are required to know the transport and survival of ELS in relation to current systems, bio-ecological and environmental process in the water column (Coombs et al., 2001). Equally, they are a crucial pre-requisite for efficient sampling, e.g. for stock estimation base on plankton egg surveys (Coombs et al., 2001) in addition to be useful to define spawning ground and nursery areas in order to establish marine protected areas.

European hake (*Merluccius merluccius*) represents one of the most important stocks of demersal fisheries in Europe (Murua, 2010). This benthopelagic is widely distributed (Casey & Pereiro, 1995;Lloris et al. 2003; see chapter 1) and divided in two stocks (ICES Divisions VIIIc-IXa and ICES Division IIIa, Subareas IV, VI, and VII, and divisions VIIIa,b,d-northern stock) for fisheries management (see chapter 1) .

The females of European hake spawn pelagic eggs along the year in two principal laying peaks (Coombs & Mitchell, 1982; Motos 1995;Alvarez et al., 2001;Alvarez & Cotano, 2005; For more details see chapter 1). Within the southern stock, the Galician area (Gulf Artabro) has particular hydrographic characteristics which make it important to fishing of various species including the European hake (Vázquez-Seijas, 1998). Also, this area is one of the main spawning and nursery zone for this specie (Murua, 2010).

Throughout the time, the capture rate of this species has shown high fluctuations (Casey & Pereiro, 1995; Murua, 2010). This problem has been attributed to overfishing (Goikoetxea & Irigoien, 2013). However, it has been documented that the effect of overexploitation on the populations is not the only factor (Rothschild, 2011). Others factors such as adult age structure, the proportion of first time and repeating spawners, nutritional availability and finally, the survival, development and distribution of ELS as well as, the effect of different environmental conditions can determine also their viability (Brodziak & O'Brien, 2005; Murua, 2010).

Regarding the latest, the knowledge about the spatial and temporal distribution and abundance of European hake eggs and larvae in the southern stock especially within Galicia is almost nonexistent (Domínguez-Petit & Saborido-Rey, 2010).

The Galician area is under influence of the Atlantic Eastern Boundary Current (EBC) system which depicts a marked seasonal cycle, resulting from large-scale forcing (see chapter 1 for details). The induced prevailing geostrophic vectors define the first order picture of the variability of circulation in the EBC area (Wooster et al., 1976) with two mean seasons: upwelling season from April to September and downwelling season from October to March with winds predominant from NE and SW in spring-summer and autumn-winter, respectively (McLain et al., 1986; González-Pola Muñiz, 2006)(Fig. 1a). The Iberian Poleward Current, a seasonal slope current, is established in winter at the slope of the Iberian margin and reinforced by westerly winds, transporting subtropical, warm and salty waters toward the southern Bay of Biscay (see chapter 1). A high presence of eddies both cyclonic and anticyclonic central waters on time scales from a few days to weeks has been documented in Galicia in both seasons (Paillet, 1999; González-Pola Muñiz, 2006)(Fig. 1a).

Indeed, the effect of hydrographic conditions on the hake eggs distribution and development in this area is still unknown. Several previous studies on the relation between environment and the ecology of this species have focused in areas belonging to the northern stock and others such as Bay of Biscay, Celtic Sea and Mediterranean Sea (Alvarez et al., 2001, 2004; Coombs & Mitchell 1982; Maynou et al., 2003; Olivar et al., 2003). Since the circulation in the Galician oceanic area is well known to be complex and very variable in space and time, the characterization of the ELS distribution of European hake in relation to the local environment needed to be explored further.

However, in case of European hake there are other biological variables which could have an effect of their ELS development. Thus, in the western Atlanticarea *M. merluccius* share and interact with other fish species competing for food, protection and space between others necessities to live.

The Galician area (NW Galician, Spain) support greatly the fishery economy of Iberian peninsula(Vázquez-Seijas, 1998). Also, its importance as nursery area owing to their particular hydrographic features concentrate the ELS from pelagic and demersal species with fishery and bio-ecologic interest such as anchovy *Engraulis encrasicolus* (Linnaeus 1758), sardine *Sardina pilchardus* (Walbaun, 1792), mackerel *Scomberscombrus* (Linnaeus 1758), horse mackerel *Trachurus trachurus* (Linnaeus 1758), blue withing *Micromesistiuspottasou* (Riso, 1827),megrim *lepidorhombus whiffiagonis* (Walbaun, 1792) and silvery lightfish *Maurolicus muelleri* (Gmelin, 1789) between others (in Russell, 1976; Ibaibarriaga et al., 2007).

Physical and biological processes which occurring at the shelf break are thought to be important for the regulation of some fish populations, particularly because their recruitment is dependent on the dynamics during early life stages. In this context, spawning strategies of many species have been related to those processes and interpreted as adaptive responses that favor survival during early life stages (González-Quirós et al., 2003).

The reproductive strategies, spawning sites and develop and distributionof ELS of some species of fish such as anchovy, sardine, mackerel, horse mackerel, blue whiting, megrim and silvery lightfish have been studied. Extensive ichthyoplankton studies have been undertaken in the west Atlantic ocean (Arbault & Lacroix-Boutin, 1968, 1969, 1971, 1974;Dicenta et al., 1977, Villegas, 1979;Re 1979a, 1979b, 1981,1984;Re et al., 1982, 1983;Ferreiro & Labarta, 1988; Jhon & Kloppmann, 1993; Hillgruber et al., 1995;Valdes et al., 1996; Motos et al., 1996; Adlandsvik et al., 2001; Bernal et al., 2007; Allain et al., 2007; Ibaibarriaga et al., 2007). However, surveys attempting to cover the entire area of Galicia and the two main spawning seasons (winter and summer) of these species altogether have not previously been undertaken. In fact, the spatial and temporal spawning distributions of many fish species are out of the coverage capability of most research cruises, which limitsthe understanding of the factors controlling spawning (Ibaibarriaga et al., 2006).

In this context, the main aims of this study are focused in two aspects: 1.to determine the vertical and horizontal distribution both ELS of hake and the other egg-fish species during the main (winter) and secondary (summer) spawning peaks. 2. To establish how the environmental conditions could have influence on the ELS distribution and its development. Moreover, to characterize the spawning habitat of all cohabiting egg-species for understands the potential interactions among them.

5.2. Material and methods

5.2.1. Sample collection, laboratory procedure and data processing

This study is based on hydrographic data and plankton samples collected during two cruises (se details in chapter 1), carried out off the NW Iberian peninsula (NE Atlantic) from Finisterre to Estaca de Bares (Fig. 1a). The cruises took place in 2012 winter (from February 28 to March 13) and summer (from July 17 to 29) and covered 78 and 68 stations, respectively, arranged in 15 transects perpendicular to the coastline (Fig. 1 b). Transects were 8 nm apart and the distance between stations was 4 nm.

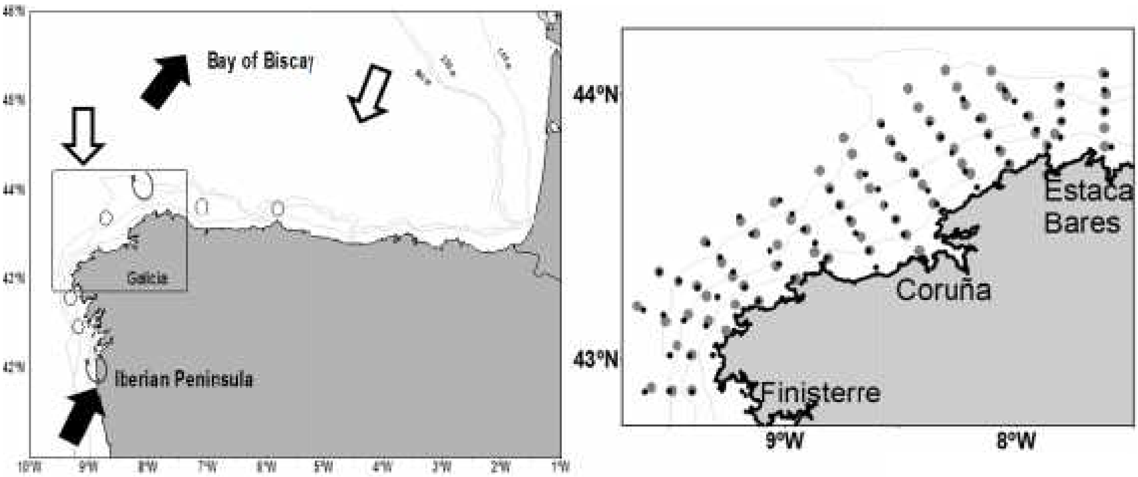


Figure 1a. General meteo-oceanographic characteristics of the northwestern coast of Spain (modified fromMcLain et al., 1986; Paillet, 1999; González-Pola Muñiz, 2006): SW winds in autumn-winter (   ), NE winds in spring-summer (⇩), river discharges (⇐), slope current (→), swoddies (↻), and presence of different upwellings and downwellings (○). b. The sampling stations for capturing eggs and larvae of *M. Merluccius* in the study area are also shown: winter (circle in grey) and summer (circle in black).

At every station, vertical profiles of temperature, salinity and pressure were recorded using a Sea-Bird SBE25 CTD. Temperature (Θ), Salinity (S) and density (Sigma.t) profiles were obtained from the surface to 10 m above the bottom.CTD data were processed using standard protocols for SeaBird instruments. Dynamic height (dh) was computed between adjacent stations using the following approach: dh at each level was calculated relative to the next (in the sense of the vertical spacing of the output grid); then, dh resulting profiles were interpolated onto the grid; and finally dh at each level was referred to the lowest one by adding the contributions of all the levels below. This method allows that profiles obtained at shallow stations take part in the recovery of the dynamic height field and has been previously tested over the continental shelf (e.g.Rubio et al., 2009). The horizontal interpolation of the hydrographic variables was performed using the Optimal Statistical Interpolation (OSI) scheme described in Gomis et al. (2001). The analysis was applied in a regular 34x50 grid, covering the study area with regular node distances of 5 x 3 km. For the OSI, a Gaussian function for the correlation model between observations (assuming 2D isotropy) was set up, with a correlation length scale of 11 km, chosen according to the observed correlation scales between individual profiles. Finally, all fields were spatially smoothed, with an additional low-pass filter with a cut-off length scale of 15 km, in order to avoid aliasing errors due to unresolved structures. To obtain 3D fields horizontal analyses were performed independently at 5 dbar intervals from 5 to 350 m (under this level, the information available is low and does not allow to obtain consistent horizontal fields). Geostrophic velocities (ug, vg), were obtained by the first-derivative of the 2D dh interpolated fields, and the module of the resulting vectors was calculated ([illegible]$_g$). Then, horizontal quasi-geostrophic relative vorticity (ξ_g) was obtained at a given z level as:

$$\xi_{gz} = \frac{\delta v_g}{\delta x} - \frac{\delta u_g}{\delta y} \qquad (1)$$

Quasi-geostrophic vertical velocities (ω_{qg}) was obtained using the Omega equation (Hoskins et al., 1978;Gomis & Pedder, 2005), in a similar approach to that used in Ruiz et al. (2014). In our case, the upper and lower boundaries have been set as $\omega_{qg} = 0$, while Neumann conditions are used for lateral boundaries. For a detailed study on the use of the Omega equation to diagnose vertical motions in the ocean, see Pinot et al. (1995). All the information registered by the CTD was confirmed using satellite images obtained by Instituto Español de Oceanografía –IEO- (ETREDAS-IEO-SANT 09/03/2015 and ETREDAS-IEO-SANT 24/07/2015).

Stratified plankton samples at 5 predefined and fixed depth strata (0-20, 20-40, 40-60, 60-100 and 100-200 m) were collected with a multiple opening/closing net

MultiNet MiDi, 50 x 50 cm aperture (MultiNet Hydro-Bios Apparatebau), equipped with 5 nets of 200 µm mesh size. The multinet, programmed to open/close at the predefined depths, was towed obliquely, sampling from 200 m depth or 10 m above the bottom at shallower station to the surface, at 2.5-3 knots and at a retrieval rate of 20 m per minute. The haul depth was monitored during every sampling by a Scanmar depth sensor mounted on the wire cable, 1 m above the net. The volume of water filtered in each stratum was measured by an electronic flowmeter located in the mouth of the net. Samples were immediately preserved in a 4% solution of buffered formalin and seawater.

At the laboratory, hake eggs and larvae were separated from the samples. Surface adhesion test method (SAT) and morphological features such as diameter of egg (1 to 1.1 mm), oil globule (0.25-0.28 mm) and egg pigmentation were used to identify the hake eggs (Porebsky, 1975; Coombs and Mitchell 1982; Alvarez et al., 2004). SAT method (Porebsky, 1975) is based on the fact that when the membrane of an egg of Merluccius spp. comes into contact with the air it tend to adhere to the liquid surface and does not descend to the bottom (For details see chapter 1, Fig. 15).

Development of hake eggs were classified in four stages (from I to IV) following Coombs & Mitchell (1982), Sanchez et al. (2011) and Jones (1972). Stage IA: 2-32 cellular division, IB: from cellular division to morula stage, II: from morula stage to primitive embryonic stage, III: from closure of blastopore to start of re-curvature of tail and IV: from start of re-curvature of tail to well-formed larvae around egg. Hake larvae identification was based on morphological characteristics and body pigmentation following the description given in the literature (Bigelow & Schroeder, 1953; Russell, 1976; Palomera et al., 2005).

The other fish eggs were sorted out, counted and stored in vials of 3.5 ml. If the number of eggs in the sample was more than 150 eggs a sub-sample of 1.5 ml were analyzed and identified. Total eggs (Y) in the plankton sample were estimated by equation:

$$Y = TotE\ (1/SubSa) \qquad (2)$$

Where TotE are the total egg identified in the subsampled and SubSa is the portion (%) of subsampled processed in every layer.

Other seven species of egg were identified and separated from the samples, namely: *Scomber scombrus* (MAC), *Trachurus trachurus* (HOM); *Engraulis encrasicolus* (ANE); *Sardina pilchardus* (PIL); *Maurolicus muelleri* (MAM); *Lepidorhombus whiffiagonis* (MEG) and *Micromesistius poutassou* (WHB). Different morphological features such as diameter of egg and oil globule as well as the presence of pigmentation and segmentation of yolk were used to identify all eggs (Russell, 1976; ICES, 2015). Subsequently, the morphological egg developments were classified in four stages (from I to IV) for HOM, ANE, PIL, MAM, MEG and WHB or five for MAC following the key identification given by ICES (2015) and Jones (1972): *a)* Stage IA: 2-32 cellular division, *b)* IB: from cellular division to morula stage, *c)* II: from morula stage to primitive embryonic stage, d) III: from closure of blastopore to start of re-curvature of tail, *d)* IV: from start of re-curvature of tail to well-formed larvae around egg and *e)* V: from well-formed larvae to the tail is crossing the head.

Concentrations of each egg species at each depth stratum on each station (A) were expressed as the number of individuals per 10 m^2 applying the following equation:

$$A = (C * D)/V \qquad (3)$$

Where, *C* is the number of eggs or larvae * 10, *D* is the depth (m) and *V* is the volume of sea water filtered (m^3)(flowmeter).

The volume of sea water filtered (m^3) by the sampler during the haul was calculated using the formula:

$$V=(r * a)/Cal * F \qquad (4)$$

Where *r* is the number of revolutions of the flow meter during tow, *a* is the area of the mouth opening of the sampler (m^2), *cal* is the number of flow meter revolutions per meter towed, obtained from the flume and *F* is the sampler efficiency from flume.

The egg and larvae concentration to *M. merluccius* and to other 7 eggs species at each depth stratum were calculated as the number of individuals per 1000 m^3 in order to represent their vertical distribution (Marrale et al., 1996; Sanchez et al., 2011).

Spawning time of hake eggs in a sample was determined by estimating the mean age of fish eggs <24 h old using embryo age prediction equations (see chapter 3;by Guevara-Fletcher et al., 2016a): $Y = 833.4\, X^{-1.85}$ which related the development time versus temperature.The determination of the spawning time was calculated subtracting egg age (hours) from the capture time.

Jaccard coefficient was analyzed to know the horizontal overlap between species(Legendre & Legendre, 1998):

$$I_1 = \frac{C_{11}}{C_{11}+C_{01}+C_{10}} \qquad (5)$$

Where *C01* is the number of samples in which only the second species is present, *C10* is the number of samples in which only the first species is present and *C11* is the number of samples in which both species are present. This index is the probability of two species being present given that at least one species is present. Thus, it takes values between 0 and 1. The value 0 corresponds to the case in which the species do not share any distribution areas and 1 to the case in which the distribution areas for both species are identical (Ibaibarriaga et al., 2007).

The distributions of eggs were mapped to show the spatio-temporal pattern of distribution. Moreover, this pattern was summarized by mapping the centroids of distribution of eggs and larvae densities. The centroids were estimated as the mean position (latitude and longitude) of positive stations for eggs weighted by the abundance of eggs at each station.

Sampling area was divided in three zones: Finisterre (F), Coruña (C) and Estaca de Bares (E) as well as the inner and outer shelf with the purpose to compare the egg distribution in these divisions.

Mesozooplankton individuals were semi-automatically counted using an image analysis technique (Bachiller & Fernandes, 2011). A subsample of 5 ml from each sample was stained for 24 hours with 0.1% eosin. The stained sample was scanned using the VueScan Professional Edition 8.5.02 software, generating an image of 256 (eight-bit) colors with a resolution of 2400 dpi. Images were processed using the ZooImage and the Image J 1.45s image analysis software. Each zooplankton individual was automatically counted (individuals number per m^3) and its length measured in a fraction ranging from 0.2 to 2 mm.

5.2.2. Data analysis and Statistical approach

For the statistical analysis the mean depth of each stratum (10, 30, 50, 80 and 150 m)was used as reference in the vertical distribution of ELS. Abundance of eggs and larvae in 10 m^2 were square roottransformed.

The effect of different environmental variables such as: temperature (Θ); salinity (Sal); zooplankton (Zoo) abundance between length of 0.2-2mm, geostrophic velocity module ($\bar{U}_g$) and latitude (Lat) on distribution of each species of eggsand larvae of European hake were analyzed by using different non-parametric statistical techniques.Variables such as density, geostrophic vorticity (ξg) and quasi-geostrophic vertical velocity (ωqg) were not included in the analysis because they are strongly correlated with temperature and salinity. Similarly, ξg and ωqg were also eliminate because they had approximates values than $\bar{U}_g$.GAM and Spearman index were used to analyze ELS of hake distribution while only GAM was applied for each 7 egg species. The use of two statistical analysisfor ELS of European hake permits to compare and/or corroborate the results between the effect of oceanographic variables on eggs and larvae.

Spearman coefficients are calculated from the order (ranks) of the data, rather than its same values. Consequently, they are less sensitive to outliers (non-normal distributions) than the Pearson correlation coefficients. This correlation test was used with the purpose to compare and corroborate the results obtained with GAMs analysis (Hays, 1981).

GAMs (Hastie & Tibshirani, 1990; Wood, 2006) were used to model occurrences for egg/larvae abundance as a function of environmental factors. The strength of GAMs is due to their capacity to deal with highly non-linear relationships between the response and the set of explanatory variables. This technique allows to obtain asymmetrical unimodal distributions, since interaction between species and extreme environmental gradients, may cause skewed responses (Oksanen & Minchin, 2002). The importance of variables was assessed firstly by removing variables that were not statistically significant and secondly, by adding and removing terms and noting the change in deviance or gain (>1%) in a forward stepwise procedure. For environmental variables, the degree of smoothness of model terms was restricted from 3-5 in order to assume a unimodal, ecologically meaningful niche model sensu (Hutchinson, 1957), but allowing asymmetry.

5.3. Results

5.3.1. Hydrographic conditions

Winter

The SST satellite image on March 9 and the 5 m temperature field from CTD data (Fig. 2), showed a marked temperature front separating the warm and saline waters at the SW of the study area from the colder (and less saline) waters at the NE of the domain. This colder water, entering to the study area from the Bay of Biscay, originated a cross-shelf front which is observed in CTD data to extend vertically until 150 m depth, with a clear signal in temperature, salinity and density (not shown). The winter TS diagrams (Fig. 6a), from the CTD profiles showed well mixed conditions (Fig. 6a) with temperature ranging from 11.7 to 12.8 ºC, salinities between 35.7 and 35.8 and water masses with low density variation following the 27- 27.2 isopycnals.

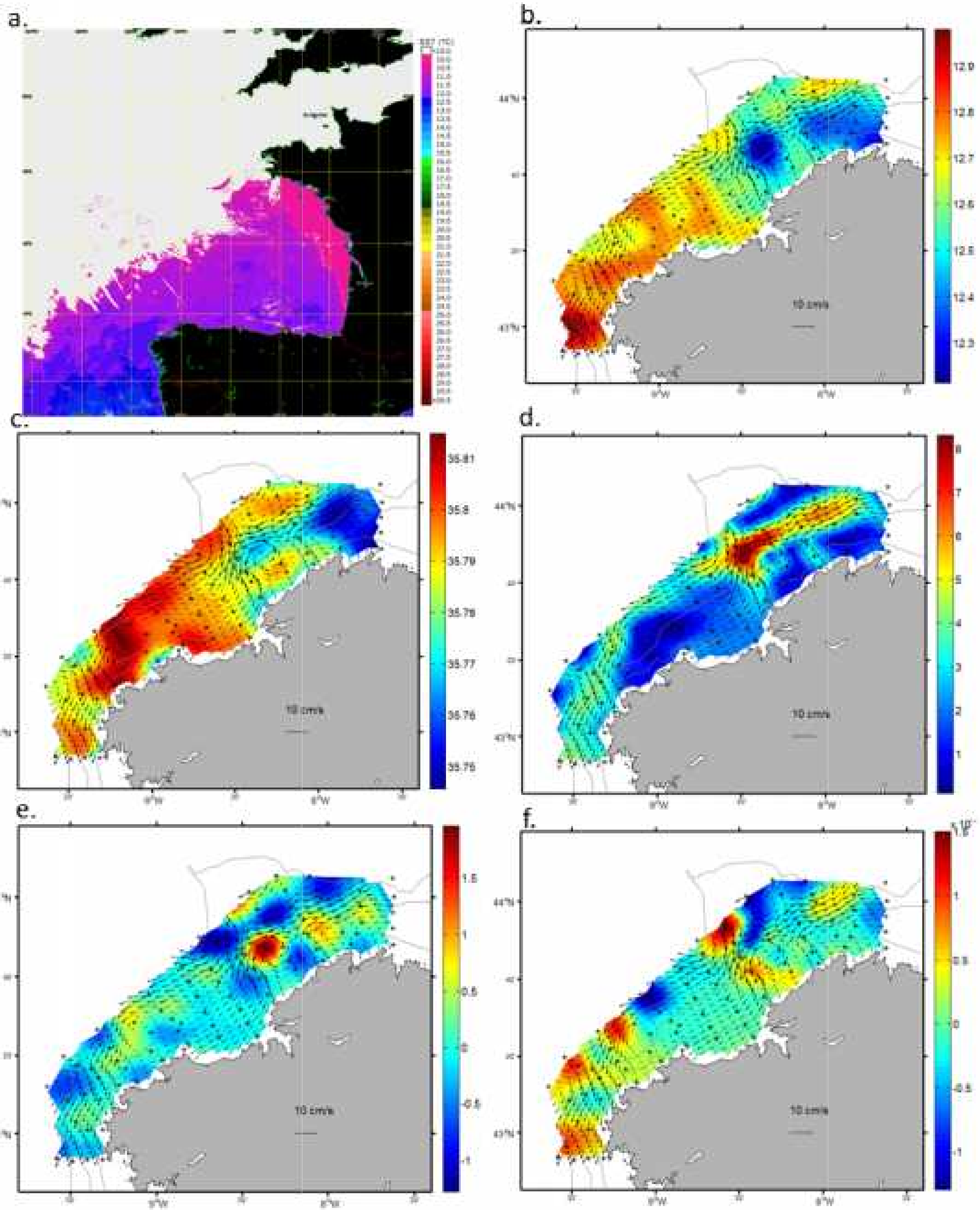


Figure 2. Hydrographic conditions during the winter campaign. a. Satellite images of sea surface temperature (ETREDAS-IEO-SANT 09/03/2015) and in-situ (CTD) fields at 10 m of b. Temperature (ºC), c. Salinity, d. Module geostrophic currents (cm/s), e. Geostrophic relative vorticity ($S^{-1} * 10^{-5}$) and f. Quasi-geostrophic vertical velocities ($m/day * 10^{-3}$). Geostrophic

currents vectors are superimposed to the fields in b-f. Gray lines are isobaths 100, 200 and 500 m.

The salinity distribution presented a coastal salinity minimum south of Coruña, but the lowest values were observed off, near to Estaca de Bares (associated to cold water masses) (Fig. 2). In addition to the aforementioned cross-shelf front, a less marked along-shelf front was also observed at all vertical levels until 150 m depth. This front separated the colder temperatures (and fresher waters) of the inner shelf from the warmer temperatures (and higher salinities) located offshore. This gradient resulted in an area of low dynamic heights at the inner shelf (over grounds < 100 m) and a south-westward current flowing along the 100 m isobath. Overall small variations in density and dynamic heights were observed and, hence, geostrophic velocities obtained were weak (< 10 cm/s at the upper levels, see Fig. 2, and < 5 cm/s under 100m). The geostrophic current was intensified at the NE part of the domain, between Coruña and Estaca de Bares, in the area where the temperature front was most intense. In this area were also observed the highest values of geostrophic relative vorticity (a well-defined cyclonic structure of Radio~ 25 km appears at 43.7ºN, 8.42ºW, associated to low temperature and low salinity, see Fig. 2) and the most intense vertical velocities (with maximum values between 1-2 m/day at 50-80 m depth).

Summer

The SST satellite image and temperature fields inferred from CTD data showed a clear along-shelf temperature front, with colder waters occupying the inner shelf and warmer waters offshore (Fig. 3). Salinity values were similar to those observed in winter but higher surface temperatures, up to 19.5ºC were recorded (Fig. 3).

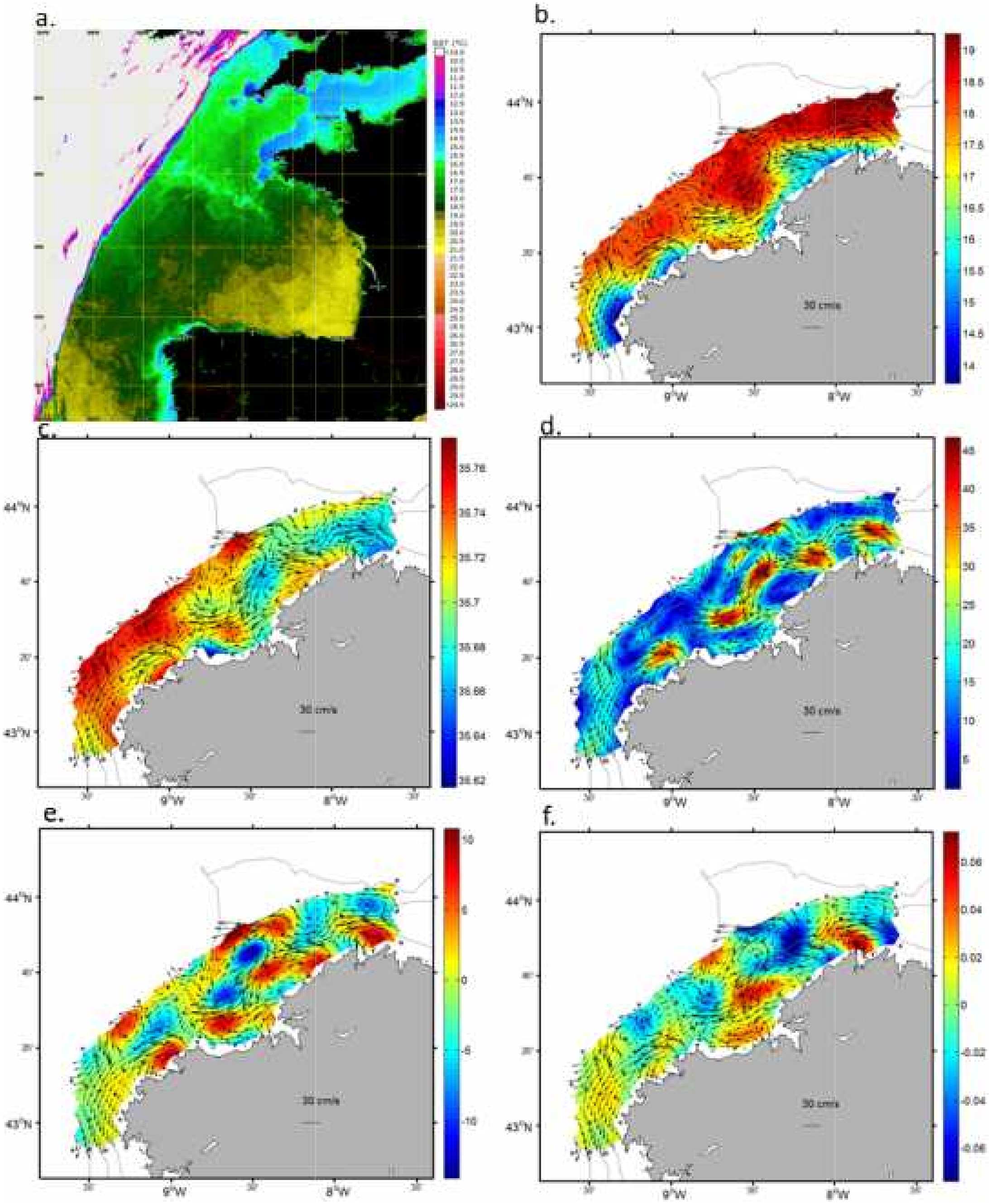


Figure 3. Hydrographic conditions during the summer campaign. a. Satellite images of sea surface temperature (ETREDAS-IEO-SANT 24/07/2015) and in-situ (CTD) fields at 10 m of b. Temperature (ºC), c. Salinity, d. Module geostrophic currents (cm/s), e. Geostrophic relative vorticity ($S^{-1} * 10^{-5}$) and f. Quasi-geostrophic vertical velocities ($m/day * 10^{-3}$). Geostrophic currents vectors are superimposed to the fields in b-f. Gray lines are isobaths 100, 200 and 500 m.

The highest temperatures were located at the NE of the study area and, as suggested by the SST image, surface temperature increases toward the E, approaching to the Cantabrian Sea (Fig. 3). The horizontal distribution of salinity also depicted an along-shelf front with fresher waters over the inner shelf, but a cross-shelf variation was also detected with the minimum salinities over the NE (linked to the warmest waters of the Cantabrian sea) (Fig. 3). Again, the resulting dynamic height, which responded mainly to the along-shelf temperature front, induced geostrophic current flowing south-westwards along the 100 m isobaths.

The density gradients were higher in summer than in winter, and thus more energetic geostrophic field was observed, with velocities over 40 cm/s over the thermocline and between 10 and 30 cm/s between the thermocline and at 80 m depth. The current had a meandering behavior and maximum (negative and positive) relative vorticity values were observed associated to this meandering along the shelf and mainly in the area in front of La Coruña (Fig. 3). Several small mesoscale structures (Radio<20 km) were observed at both sides of the current (Fig. 3). Also in the area in front of La Coruña, the highest vertical velocities, up to 5 m/day between 30 and 80 m depth were registered.

Stratified conditions were observed, with lighter waters occupying the shallower levels of the water column (Fig. 5b).

5.3.2. ELS abundance of European hake and diel periodicity of egg production

A total of 559 hake eggs and 440 hake larvae were collected during the two surveys, which were distributed as follow: 486 eggsand 421 larvae in winter and 138 eggs and 19 larvae in summer.

Horizontal and seasonal distribution of egg and larvae showed significant differences between winter and summer surveys. Table 1 shows the abundance of eggs collected in each sample period. Considering the egg stage, the most abundant corresponded to stage I (A + B), which comprised 63 and 65 % of total eggs captured in winter and summer, respectively; being the most abundant the eggs in stage IA.

Table 1.Egg abundance and mean depth (m) by development stage for European hake eggs in winter and summer.

Season	Stage		Egg abundance	Mean depth (m)
Winter	I	A	161	83
		B	143	89
	II		90	80
	III		35	77
	IV		57	80
Summer	I	A	58	73
		B	31	81
	II		36	78
	III		6	46
	IV		7	57

The daily abundance of the earliest eggs stage was used as reference to determine the hake daily spawning behavior. Table 2 shows the frequency distribution of hake egg in relation to hour of day.55% in winter and 62% in summer from total egg collected in stage I occurred during the darkness hour, being the time range 4:00-8:00 hours in winter and 0:00-04:00 in summer(Table 2).

Table 2.Frequency distribution of hake egg in stage I (Stage IA + IB) along the sampling time.

Hour	Winter Egg abundance	 Frequency (%)	Sumer Egg abundance	 Frequency %
0-4	28	9.2	33	37.1
4-8	98	32.2	1	1.1
8-12	54	17.8	24	27
12-16	39	12.8	27	30.3
16-20	51	16.8	4	4.5
20-24	34	11.2	0	0
Light time	136	45	55	62
Dark time	168	55	34	38

The spawning time was calculated 2:00 h before from the egg collected in stage IA according to Marrale et al. (1996).

Eggs in stage I transformed into age (hours) andassuming a mean temperature of 12.25ºC in winter and 13.25º C in summer showed that egg in stage I were 68.1 and 59.0 hours old respectively. The addition of 2 hours from fertilization to first division (Marrale et al., 1996) gives that those eggs in stage I were spawned 70.1 hours ago in winter and 61 hours in summer. The time sequence for the determination of the spawning time (calculated subtracting egg age (hours) from the capture time) is given in figure 4.

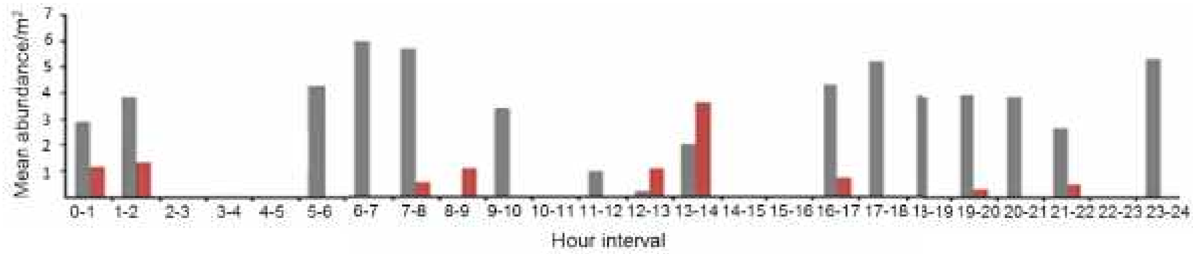


Figure 4. Temporal sequence of estimated hake spawning hour using the presence of hake eggs in stage 1.Winter (gray bar) and summer (red bar).

Spawning behavior cannot be clearly established from the hake egg time sequence, however it was evident the absence or low density of eggs in stage I from 10:00 to 16:00 hours. The low presence of hake eggs during the summer survey made it impossible to draw comparisons between them.

5.3.3. Spatio-temporal distribution of eggs and larvae of European hake and environment variables

Horizontal distribution

The horizontal distribution of hake eggs and larvae are given in Figure 5. In winter, eggs and larvae were distributed along the whole sampled area. However, important patches of E-L were observed around Finisterre and Estaca de Bares, respectively (Fig. 5a and Fig. 5b). The most of eggs appeared at temperatures between 11.7 to 12.5 ºC (Fig. 2 and Fig. 5). Higher eggs concentration was observed in areas with low zooplankton concentration, while the highest larvae concentration was observed in areas with highest zooplankton presence (Fig. 5). E-L were distributed within of 200 m isobaths with high egg abundance close to coast, over the 50 m isobath (Fig. 5a).

Higher egg densities were associated to higher temperature and salinity waters and low geostrophic currents, low vorticity and vertical velocities (Fig. 2 and Fig. 5a). Conversely, for the larvae, the highest concentrations were observed in an area (off Estaca de Bares) characterized by low temperature and salinity waters. Also in this area where observed an intense geostrophic currents and higher vorticity and vertical velocities associated to the presence of small (cyclonic mesoscale eddy) (Fig. 2 and Fig. 5b).

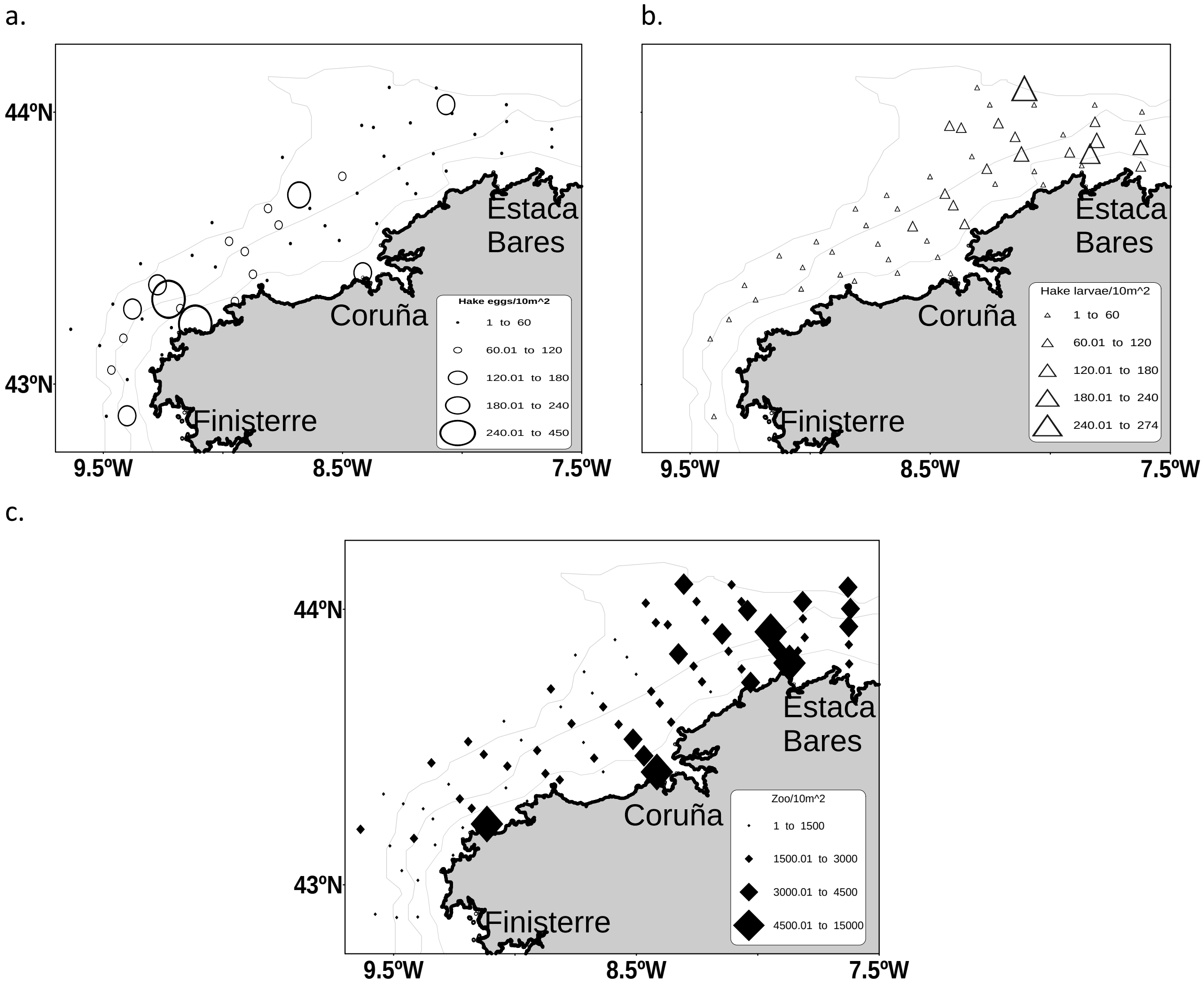


Figure 5.Horizontal distribution of eggs (a), larvae (b) and zooplankton (c) in winter.

In summer, the abundance of E-L reduced dramatically (Fig. 6). High eggs concentrations were localized from Coruña to Estaca de Bares within 200 m isobaths, in areas linked to low density of zooplankton (Fig. 6a and Fig 6c). The presence of larvae was not continuously distributed (limited at 12 positive stations) between Coruña and around Estaca de Bares and between the 200 m isobath and the coast (Fig. 6b).

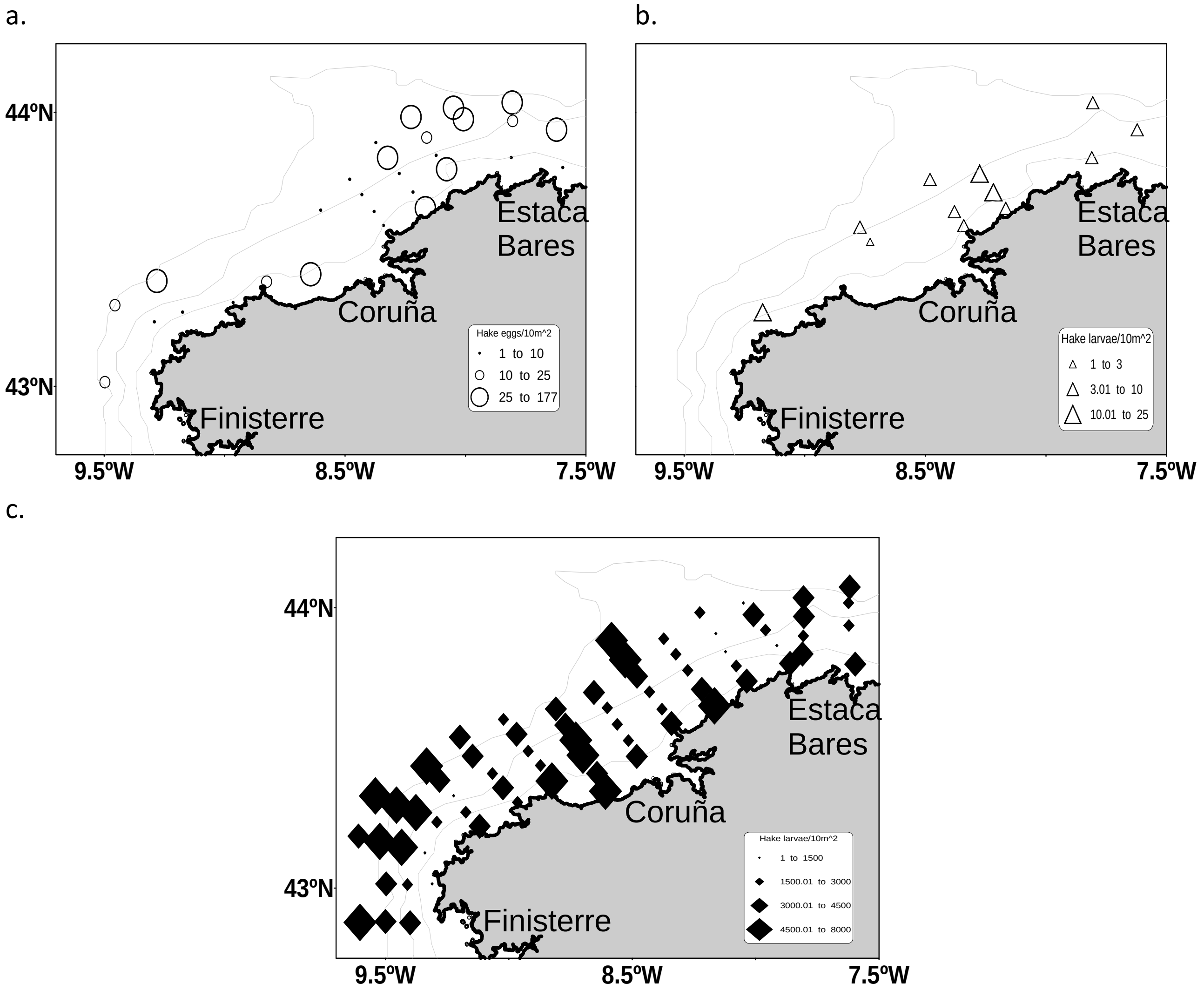


Figure 6.Horizontal distribution of eggs (a), larvae (b) and zooplankton (c) in summer.

It is worth noting the absence of larvae at stations located further 9 ºW. In relation to temperature, the early life stages were found preferentially at temperatures between 12.5 and 16.5 ºC (Fig. 3 and Fig. 6). Eggs were more abundant offshore in areas of weak geostrophic currents, high (positive and negative) vorticity values and high vertical velocities. Larvae were, as in winter, located at shallower waters than eggs, and concentrate in areas of low salinity, high (positive and negative) vorticity and high vertical velocity values, with less clear relationship with the intensity of the geostrophic currents (Fig. 3 and Fig. 6).

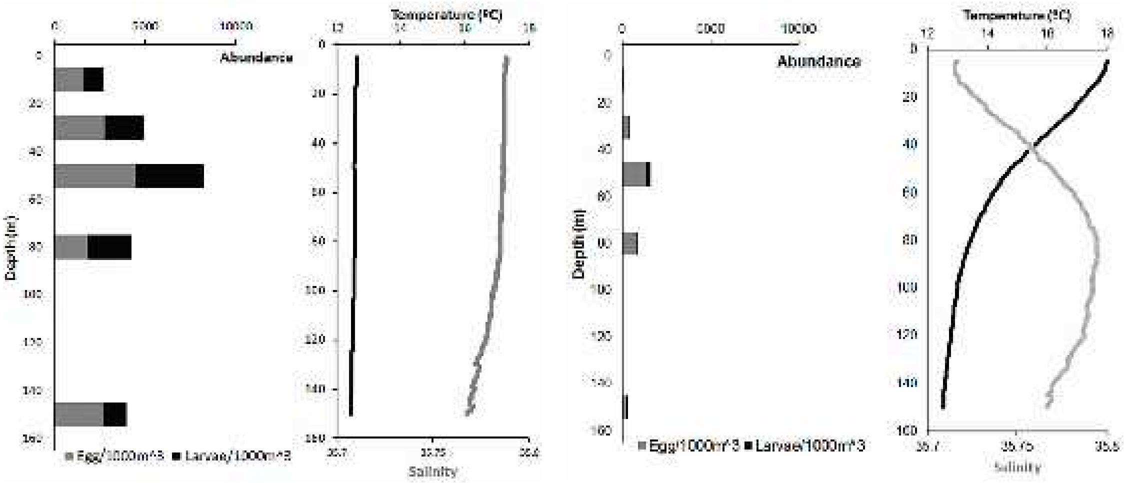
Vertical distribution

In this season, egg and larvae (E-L) were widely distributed thorough the water column from 10 to 150 m water depth (Fig. 7a). 66 and 71% of total eggs and larvae respectively, were collected below 40 m; being the range between 40 – 60 m which showed the highest concentration of them(Fig. 7a).It was observed that E-L occurred at temperatures, salinities and densities from 11.7 to 12.5 ºC; 35.7 to 35.9 and among 27-27.2, respectively, in winter (Fig. 6a).

Changes in the vertical distribution in each development stages were observed with early development stages found deeper than older ones (Table 2), although statistically the distribution was not different ($p > 0.05$). The vertical distribution showed that all stages were approx. distributed between 70 to 90 m depth (Table 1).

In summer, 84% of hake eggs and 87 % of larvae were distributed below 30 m (Fig. 7b). The E-L concentration occurred at all depth range in a broader range of temperature, between 11.7 to 17 ºC at the same salinity range than in winter while density showed values from 25.8 to 27.1 (Fig. 8b). However, the highest abundances were collected between 40-60 m at temperatures between 11.7 to 14 ºC (Fig. 8b).

During this season, all development stages were distributed between 40 to 80 m of depth (Table 1). Stage IA showed most abundance at high depths than the other development stages (Table 1). Statistical analysis not showed significant correlation between stage developments and depth (p > 0.05).



a.

b.

Figure 7.Vertical distribution of egg (grey) and larvae (Black) abundance of *M. Merluccius* and spatial mean of all data of temperature (black) and salinity (Grey) profiles registered in the sampled area in a. winter and b. summer, respectively.

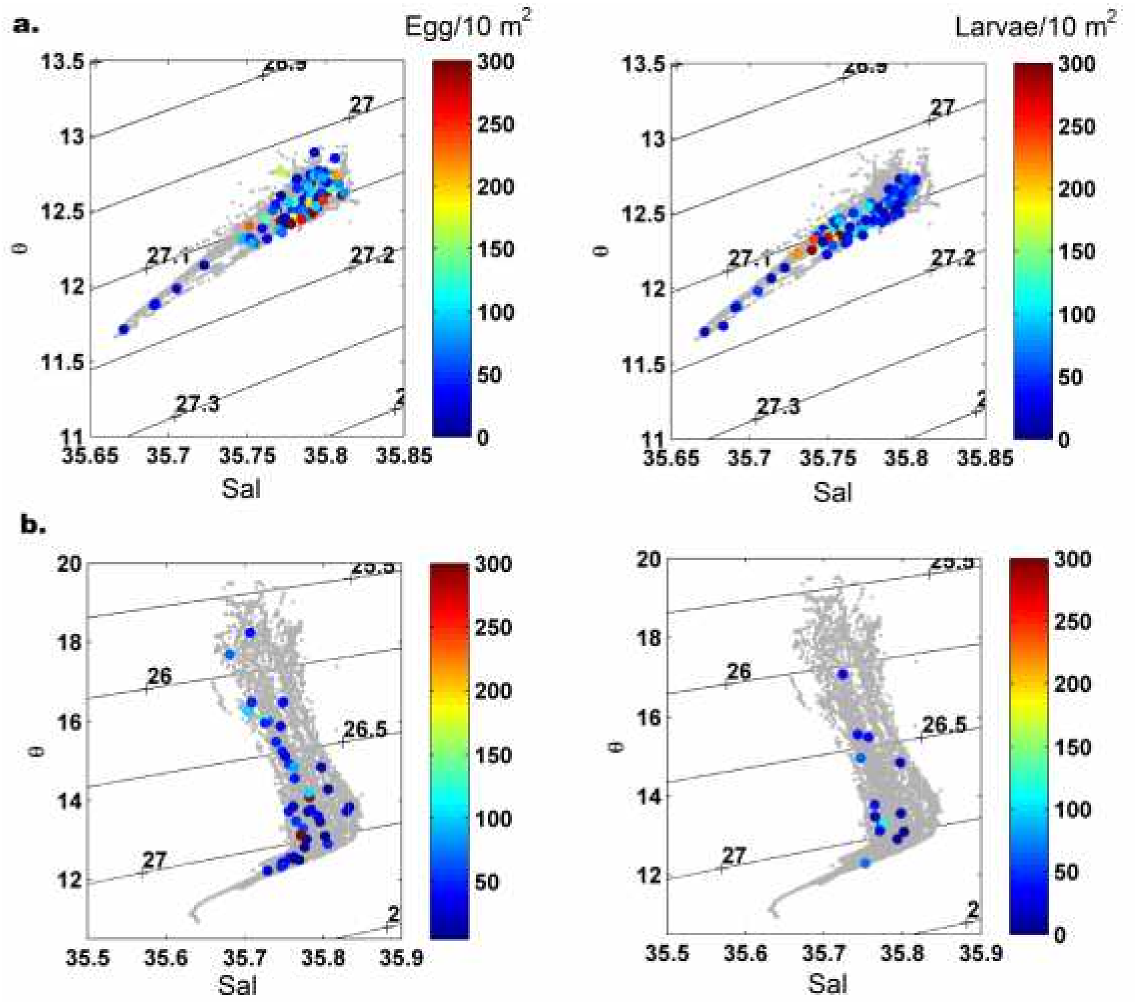


Figure8.Grey dots show the θ-S diagrams (sigma-ϴ values in diagonal lines) waterobtained using CTD profiles. Color dots show the joint distribution of ϴ -S values and egg and larvae abundance in number of individual per 10 m^{2}in (a) winter and (b) summer campaigns.

5.3.4. Statistical analysis to egg and larvae distribution of *M. merlucciusin* relation to bio-physical variables in winter.

Spearman correlation analysis showed that temperature and module velocity affected ($p < 0.05$) the eggs and larvae distribution; also, the zooplankton abundance to eggs and salinity to larvae in winter (Fig. 9). The values of correlation coefficients were, in general, low achieving maximums those obtained between temperature and salinity on larval distribution (Fig. 9). E-L abundance showed negative correlations with all variables (Fig. 9).

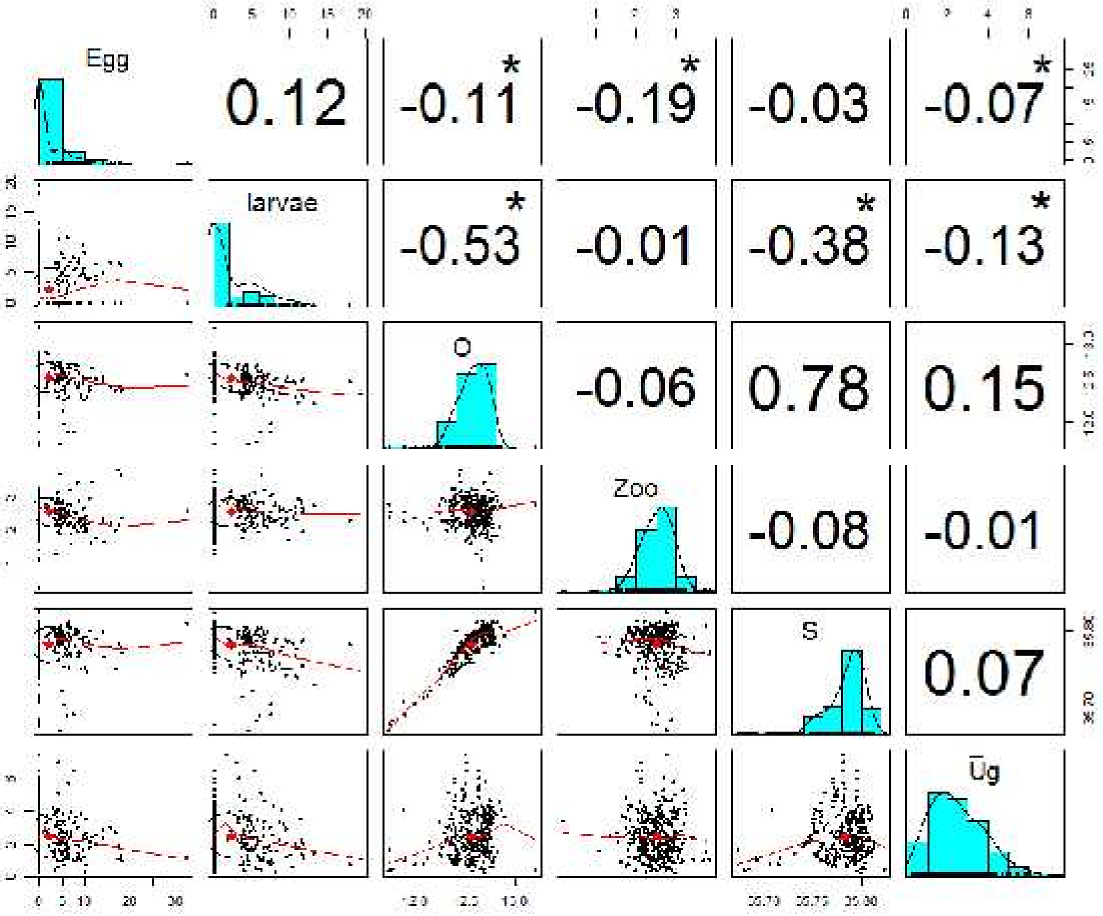


Figure 9.Spearman R correlation (*p* < 0.05) coefficients between egg (a) and larvae (b) abundance and environmental variables such as: temperature (Θ), salinity (S), zooplankton abundance (Zoo) and module velocity ($\bar{U}_g$) in winter.* means the correlation between variables ($p < 0.05$).

GAM corroboratedthe results obtained by Spearman analysis respect to variables affecting the eggs and larvae distribution ($P < 0.05$) (Table 3).However, in the case of eggs the deviance explained to each variable were low than 10 %. The random cross validation between variables showed that three variables were statistically significant ($p < 0.05$) with deviance explained of 11.2 % to all of them (Table 3).

Table 3. Generalized additive model (GAMs) to analyze the effect of each statistically significant variable and together ($p < 0.05$) on egg distribution in winter. Variables entered: temperature (ϴ), salinity (S), zooplankton (Zoo) and module velocity ($\bar{U}_g$). edf: estimated degrees of freedom. NA: not applicable in summer.

ELS	Season	Analysis of each variable	edf	Explained deviance to each variable (%)	Analysis of all variables	edf	Overall explained deviance to all variables (%)
Eggs	Winter	ϴ	1.9	2.4	ϴ	1.8	11.2
		Zoo	2.9	8.1	Zoo	2.9	
		$\bar{U}_g$	1	1.3	$\bar{U}_g$	1	
	Summer	NA			NA		
Larvae	Winter	ϴ	2.9	26.3	ϴ	2.9	30
		S	2.3	14.6	$\bar{U}_g$	1	
		$\bar{U}_g$	1	2.5			
	Summer	NA			NA		

Regarding larval abundance, its variability was highly explained by the temperature (Table 3). The random cross validation between variables showed that temperature and module velocity were statistically significant ($p < 0.05$) with deviance explained of 30 % (Table 3). High larvae abundance was related to low temperature and module velocity values (Fig. 10b).

The curve of response for each descriptor is shown in Figure 10. The model gave similar response for eggs and larvae versus temperature and velocity. For temperature, the optimal range of abundance occurred between 12.3 to 12.7 ºC. Concerning velocity module, the negative slope given in the analysis suggests than a higher velocity module producelower eggs and larval occurrence. Zooplankton abundance explained the 2.9% of eggs abundance. The curve of response indicated a negative slope for this variable with optima values from20 to 1000 abundance (take into account than the model presents an important bias for high values of zooplankton).

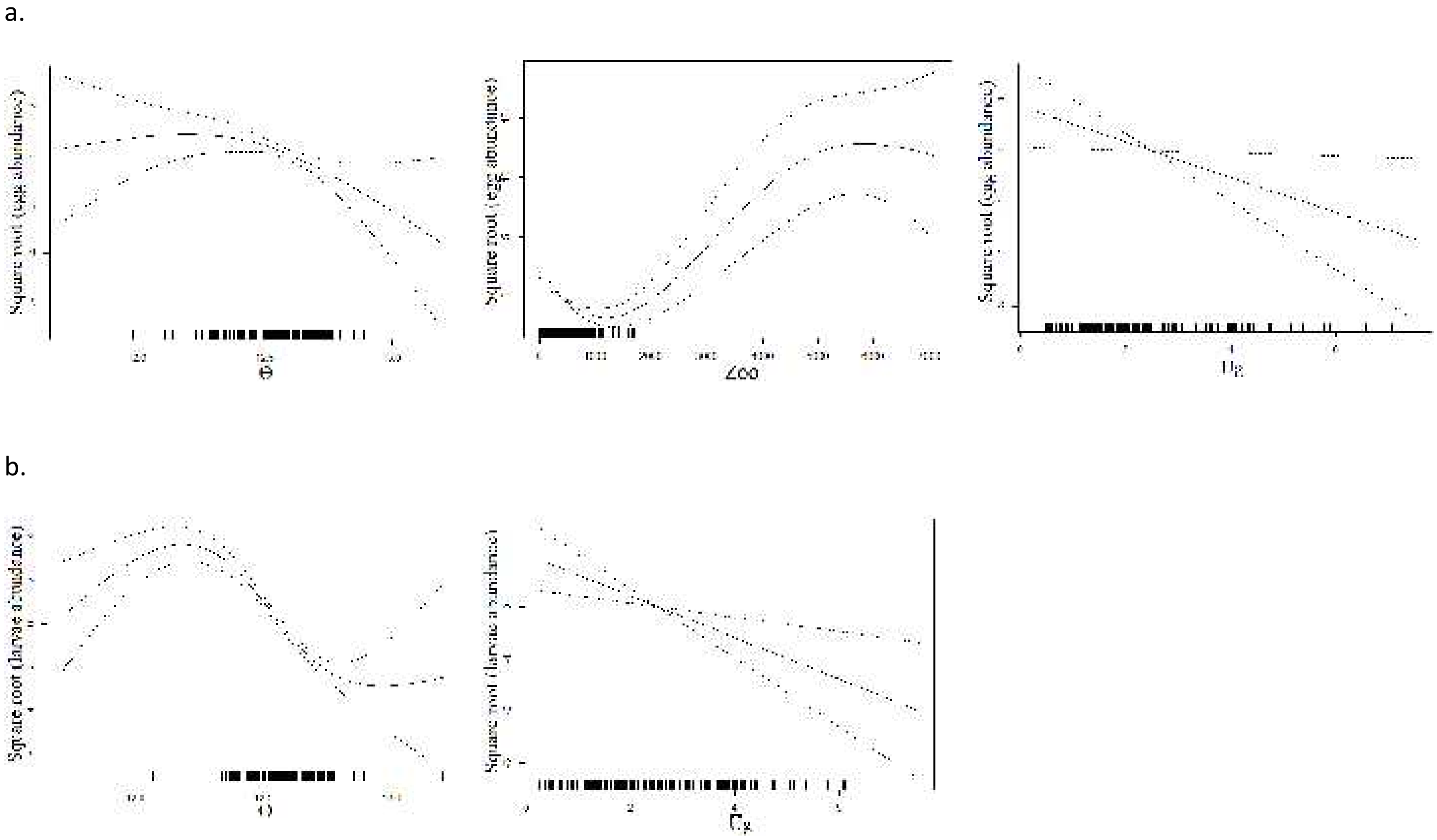


Figure 10. Generalized additive models images showing the effect of statistically significant variables (Analysis of all variables; $p < 0.05$) such as: temperature (Θ), zooplankton abundance (Zoo) and module velocity (U_g) on eggs (a) and larvae (b) distribution of *M. merluccius* in winter. Vertical lines Stacked on the “X” axis means high abundance of ELS Vs. the environmental variables studied.

5.3.5. Coexistence between European hake and other fish species.

Egg fish abundance and distribution of all egg-fish species

In total 97 797 eggs were collected and over 25213 were identified into species in both seasons. The abundance of eggs collected between winter and summer showed significant differences ($p < 0.05$) with highest amount of eggs captured in February (90 %) than July (10 %), respectively. In winter, MAC comprised the 55 % of total egg being, the dominant specie with high occurrence (see table 4). The second specie in importance was WHB, comprising 2.2 % of eggs collected. Three other species, HOM, PIL and MAM represented between 2 and 0.5 %, respectively (Table 4). ANE was absence in this period. In summer, 4 621 eggs were analyzed and identified from a total of 9 712 (18 %) collected. HOM was the dominant specie in this season comprising 16.3 % of total eggs. Species such as PIL and ANE occurred in moderate numbers making 2.2 and 2.7 %, respectively of total eggs (Table 4).

Table 4. Percentage representation of all eggs collected, identifiedand occurrence (Ocurr) during winter and summer (Occurrence mean the egg presence in each stations from the total sampling stations).

Specie	Winter (%)			Summer (%)		
	All eggs	Identified	Ocurr	All eggs	Identified	Ocurr
MAC	55.1	89.1	91.2	-	-	-
HOM	2.0	3.2	49.4	16.3	73.5	36.2
WHB	2.2	3.6	77.2	-	-	-
ANE	-	-	-	2.7	12.1	26.1
PIL	0.5	0.77	8.8	2.2	9.9	8.7
MEG	38.2	0.05	7.6	-	-	-
MAM	2.02	3.3	43.1	0.9	4.0	20.3
Other	38.2			77.8		

Horizontal distribution

Of the three areas evaluated, Estaca de Bares presented the highest egg abundance of all species studied both winter and summer while Finisterre area showed lower ones (Fig. 11). There was a significant difference ($p < 0.05$) in the egg abundance between the three zones in both seasons (Fig. 11).

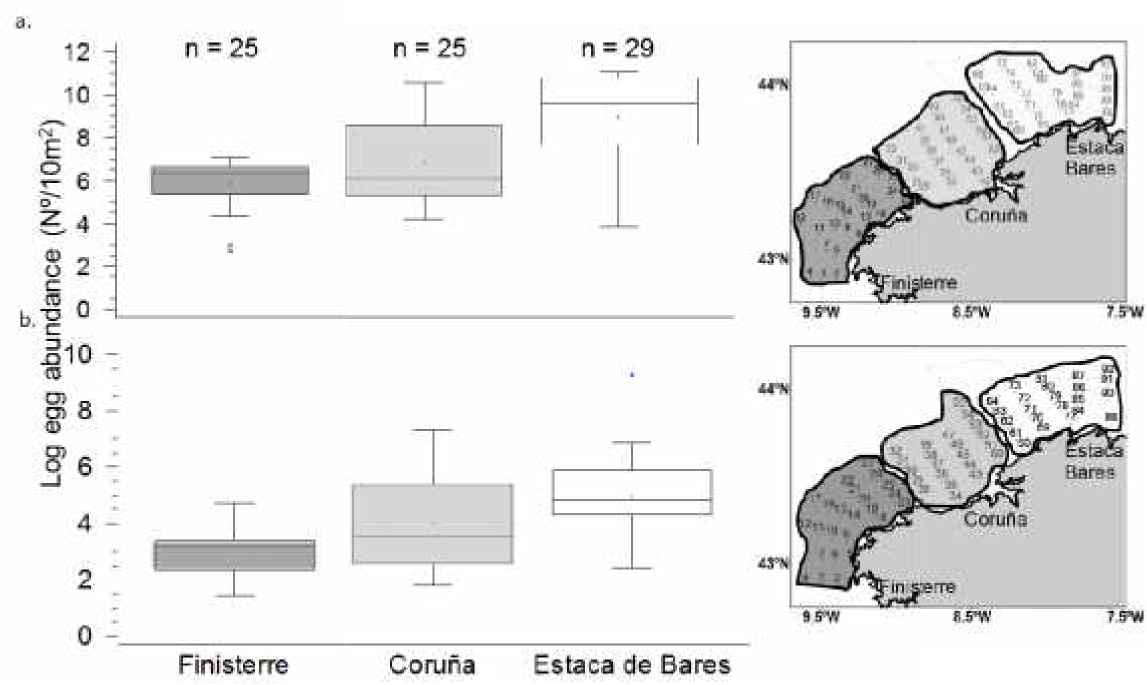


Figure.11. Log egg abundance (No/m^2) in three main zones (Finisterre, Coruña and estaca de Bares) within the sampling area from Galicia, NW Atlantic waters in winter (a) and summer (b). P < 0.05, significant differences.

Maps of the horizontal distribution of eggs from winter and summer are shown in Fig. 12. In winter, MAC eggs were the most abundant specie both in total number and frequency of occurrence. Eggs were found at 74 stations of the sampling grid with the highest densities in the NE (Estaca de Bares). Concentrations reached up to 7847 egg/m^2 and were consistently found within the 200 m contour line (Fig. 12a). HOM eggs were found at 39 stations of this region with numbers ranging between 0.7 and 194 eggs/m^2.Highest densities were encountered on the shelf north east of the area. Moderate numbers of eggs were found close to coastal line west of Coruña (Fig. 12b). WHB and PIL were distributed along the sampled area in 62 and 8, stations respectively; both species with highest abundances between Finisterre y Coruña within 50-200 m isobath (Fig. 12c and e).Eggs from WHB and PIL were concentrated between 610 egg/m^2 and 120 egg/m^2, respectively. MEG and MAM eggs were distributed in highest densities between Coruña-Estaca de Bares and Finisterre-Coruña, respectively (Fig. 12f y g). However, MAM was possible encounter along the sampled area (Fig. 12g). Both species were localized upper to 100 m isobath in 7 and 37 stations (Fig. 12f y g).

In summer, HOM showed the highest egg abundance respect to other species. HOM, ANE, PIL and MAM were distributed in highest abundance between Coruña and Estaca de Bares upper 50 m isobath in 25, 18, 6 and 14 stations, respectively (Fig. 12b, d, e and g).

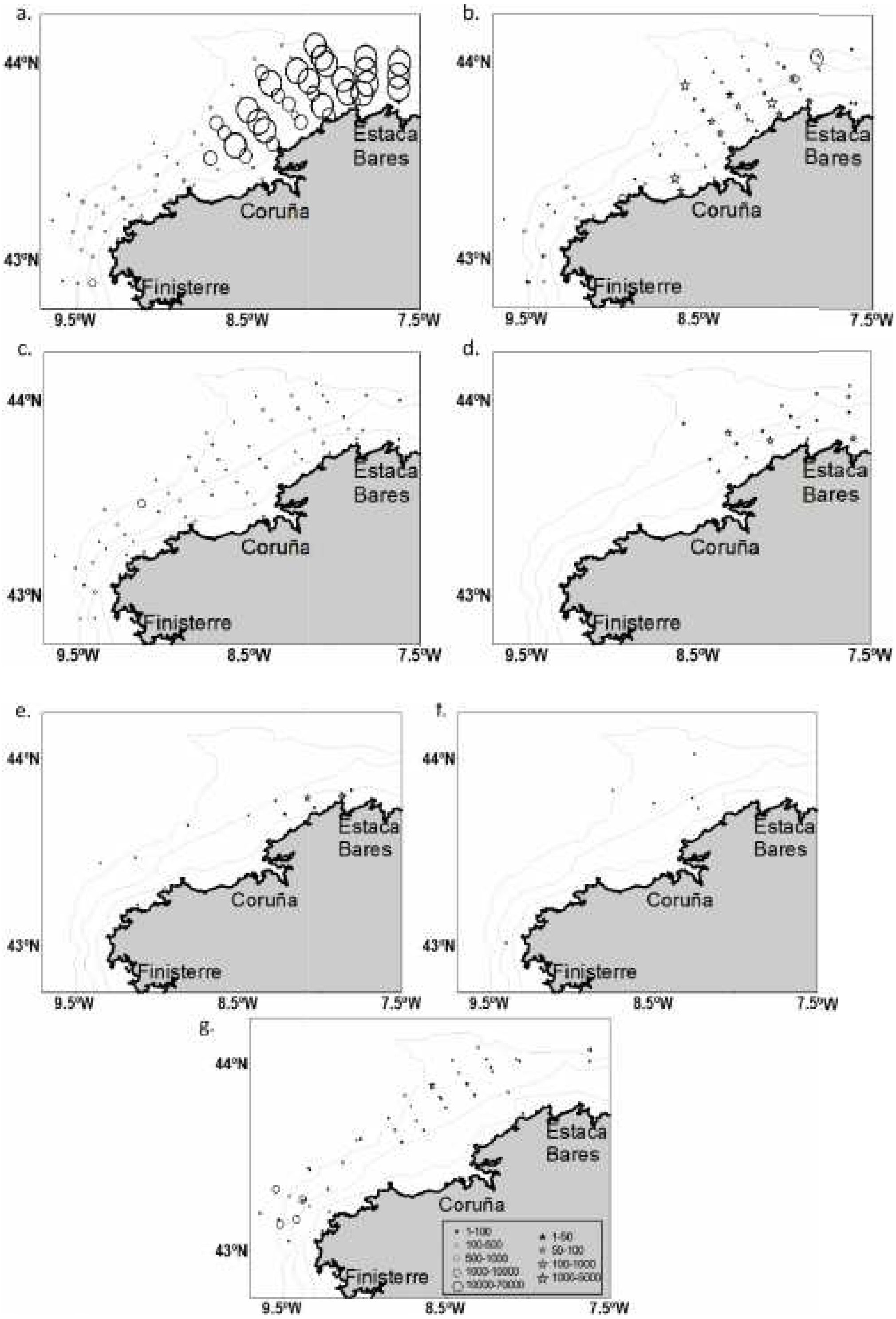


Figure.12. Horizontal distribution and abundance of egg such as: a. *Scomber scombrus* (MAC), b. *Trachurus trachurus* (HOM), c. *Macromesistius poutassou* (WHB), d. *Engraulis encrasicolus* (ANE), e. *Sardina pilchardus* (PIL), f. *Lepidorhombus whiffiagonis* (MEG), g. *Maurolicus muelleri* (MAM) in the sampling area from Galicia, NW Atlantic waters in winter (circles) and summer (stars).

The distribution of centroids of eggs (Figure 13) summaries the displacement of the spawning along the season. Comparing winter and summer the centroids for all species moved up and inwards. MAM and HKE centroids showed the longest displacement (51.4 and 40.5 km respectively) while the centroid of egg of HOM and PIL the shortest (10.3 and 11.8 km respectively). Only the centroid of density of zooplankton moved from the north to the south (17 km) between surveys.

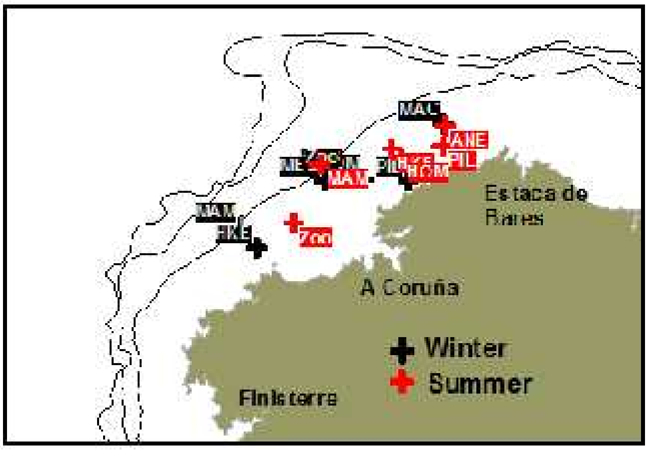


Figure 13: Maps of centroid distribution of density of eggs by species, zooplankton and periods, winter in black and summer in red.

In winter and summer 36 and 34 stations were localize on the inner shelf and 37 and 35 on the outer, respectively. The spatial distribution of eggs showed some preferences of species in relation to shelf or ocean waters (Table 5). In winter, PIL was distributed in highest abundance towards the inner shelf while WHB and MAM were captured to the outer shelf (Table 5). By contrast, the eggs of MAC, HOM and HKEwere distributed quite balanced by zones: for MAC and HOM slightly higher outer and for HKE inner (Table 5). In summer, PIL, HKEand MAM followed the same distribution than winter i.e. in the inner, both zones and outer, respectively. HOM eggs, however, were preferably located on inner shelf. In the other hand, the eggs abundance from ANE was slightly similar in both zones (Table 5).

Table 5.Distribution percentage of eggs abundance (Nº/10m^2) in relation to bottom depth.Shelf (Inner area) and Ocean (Outer area).

Season	Zone	MAC	HOM	WHB	ANE	PIL	MEG	MAM	HKE
Winter	Inner	42.4	41.8	36.4	-	96.9	38.8	7.7	51.8
	Outer	57.6	58.2	63.6	-	3.1	61.2	92.3	48.2
Summer	Inner	-	86.5	-	52.8	100	-	-	43.3
	Outer	-	13.5	-	47.2	-	-	100	56.7

For all the pairs of species the values of the Jaccard index for eggs were < 0.75 and 0.59 in winter and summer, respectively (Table 6). There was little overlap over the whole surveyed area in both seasons. In winter, the largest indices from highest to lesserwere presented between mackerel (MAC), blue withing (WHB), European hake (HKE), horse mackerel (HOM) and Silvery lightfish (MAM); all them mid-pelagic and demersal species and for anchovy (ANE) and sardine (PIL) in summer; both small pelagic species (Table 6).

Table 6. Jacquard index for eggs of each pair of species. Cells in grey (Below the main diagonal) correspond to eggs in winter and cells in white (above the main diagonal) to eggs in summer.

	MAC	HOM	WHB	ANE	PIL	MEG	MAM	HKE
MAC	-	NC	NC	NC	NC	NC	NC	NC
HOM	0.527	-	NC	0.593	0.222	NC	0.172	0.333
WHB	0.753	0.587	-	NC	NC	NC	NC	NC
ANE	NC	NC	NC	-	0.238	NC	0.208	0.393
PIL	0.080	0.097	0.130	NC	-	NC	0.063	0.129
MEG	0.067	0.071	0.081	NC	0	-	NC	NC
MAM	0.403	0.214	0.418	NC	0.107	0.081	-	0.105
HKE	0.701	0.412	0.662	NC	0.103	0.05	0.468	-

Vertical distribution

There were not differences in the mean depth of fish eggs between winter and summer. For winter spawner (MAC, WHB and MAM) the mean depth ranged from 73 to 90.2 m depth (Table 5). Eggs of MAC were distributed along all depth but 80 % of eggs abundance was found under 50 m depth. Eggs of MAM and WHB were encountered under30 m depth with highest abundance under 80 m. MEG eggs were found from 30 m to 150 m (Fig. 14). By contrast HOM and PIL were found in higher abundance in the upper 50 m depth (Fig 14).

In summer, ANE, HOM and PIL eggs were found in high abundance from 10 m to 80 m (Fig. 14). MAM eggs were localized from 50 to 150 m (Fig. 14). In this season the highest densities of egg species were located above 60 m just above where the mixed layer ends. The salinity range where they were distributed varied from 35.72 to 35.77; while temperature varied from13 to 16.3 ºC.

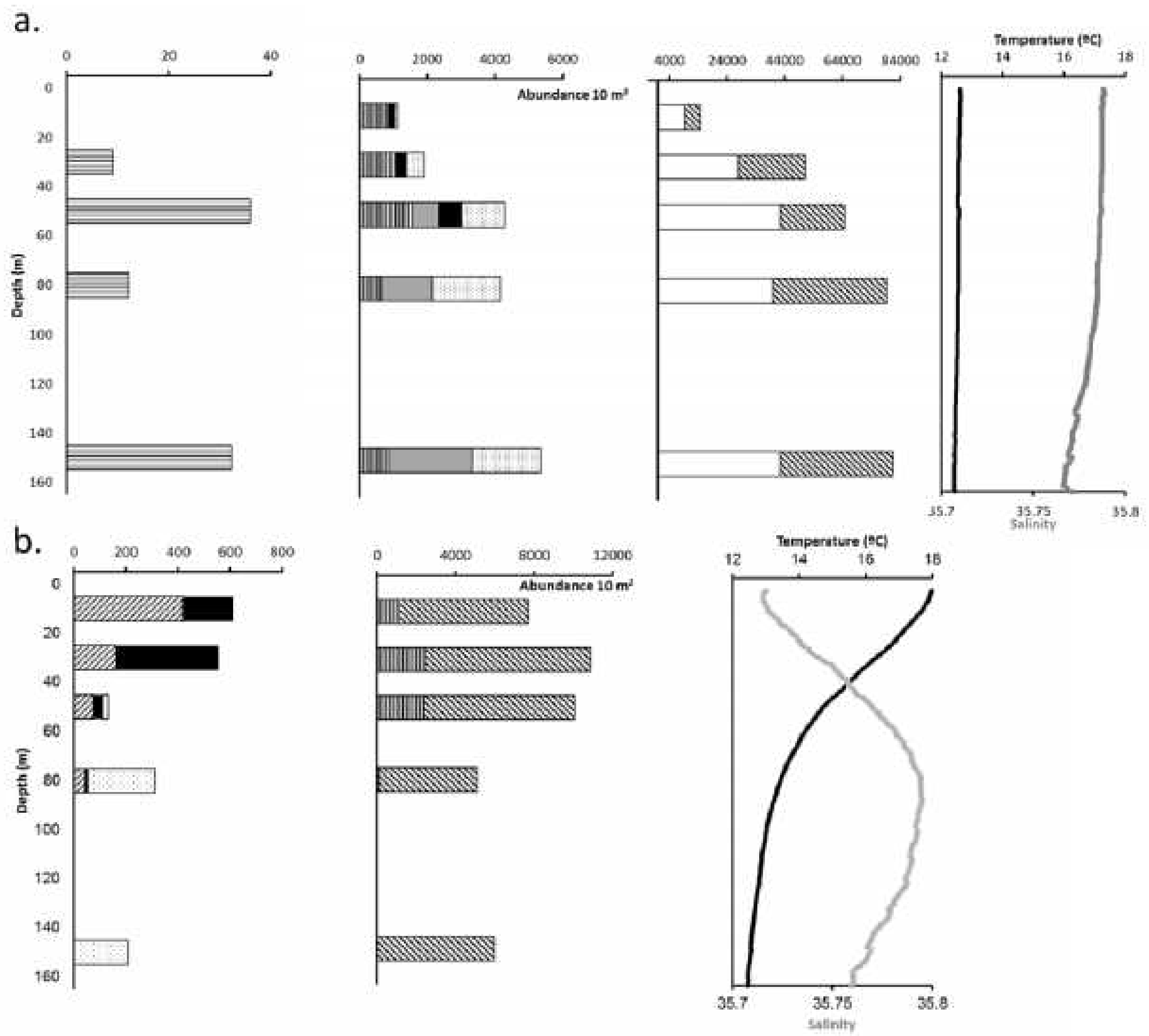


Figure 14. Vertical distribution of seven fish eggs abundance and spatial mean of all data of temperature (black)/salinity (grey) profiles registered in the sampled area in a. winter and b. summer, respectively. MAC (☐), HOM (▥), WHB (■), ANE (⧄), PIL (■), MEG (⊟), MAM (░), other (☐).

The depth at which the fish species spawn, can surmise knowing the depth at which the earliest egg stage is found. Table 7 shows the vertical distribution of eggs by development stages and season. The weighted mean depths of the different egg developmental stages were clearly different for either species. According to the mean depth of stage I, the species can be separated into three groups: G1- species spawning close to 100 m depth; G2- Species spawning at mid depth (50-80 m depth) and G3- species spawning at shallower depths (< 50 m depth). The group 1 corresponds to demersal species (MAM, MEG, WHB); the group 2 comprises two mid-pelagic species (MAC and HOM) and the group 3 the small pelagic species (ANE and PIL). In general, eggs at the latest stages of development tended to be shallower in the water column than earlier stages except sardine and MAM eggs. The largest differences in depth between the earliest and latest stages occurred for sardine (60 m) and the smaller for anchovy (7 m) in summer. By season, the vertical distribution of PIL eggs presented the most important differences (Table 7) when the mean depth of eggs in stage I moved from 29 m in winter to 79 m in summer.

Tables 7. Mean depth (m) by development stage for seven fish egg in winter (a) and summer (b). NC: Not captured.

a

Winter /Stage	**MAC/depth (m)**	**HOM/depth (m)**	**ANE/depth (m)**	**WHB/depth (m)**	**PIL/depth (m)**	**MEG/depth (m)**	**MAM/depth (m)**
IA	83.4	50.9	NC	99.3	29.4	94.2	96.1
IB	87.4	59	NC	87.7	49.5	149.6	88.8
II	74.5	49.2	NC	86	33.3	NC	95.7
III	56.9	60.2	NC	85.9	36.7	49.0	80.9
IV	42.8	26.8	NC	67.7	39.9	NC	76.7
V	44.9						
Mean depth (m)	73	57.7	NC	87.1	38.6	63.4	90.2

b.

Summer /Stage	MAC/depth (m)	HOM/depth (m)	WHB/depth (m)	ANE/depth (m)	PIL/depth (m)	MEG/depth (m)	MAM/depth (m)
IA	NC	53.7	NC	23	79.4	NC	95.2
IB	NC	28.4	NC	21.3	NC	NC	113.6
II	NC	14.7	NC	9.7	22.3	NC	124.2
III	NC	29.6	NC	23.3	22	NC	95.9
IV	NC	33.8	NC	15.7	18.6	NC	110.9
V	NC						
Mean depth (m)	NC	46.3	NC	14.8	9.5	NC	125.3

5.3.6. Relationship between seven egg fish distribution and environmental variables

GLM analysis to know the effect of each variable on egg distribution of each species is shown in table 8. Apparently, the analysis of each variable showed that presence of zooplankton (WHB, PIL and MAM) and localization on the latitude (MAC, WHB and MAM) followed by salinity (MAC and PIL) and temperature (MAC and WHB) were the most important variables ($p < 0.05$) which affect the egg distribution in winter (Table 8). These variables explain more than 5 % of deviance to each of them (Table 8). Other analysis of GLM on all variables together and for three variables together such as: temperature, salinity and vertical velocities showed that temperature (HM, PIL and MAM) and salinity (MAC, WHB and PIL) had an effect on the eggs distribution ($p < 0.05$; explained deviance upper than 10 %) (Table 8).

In summer, it was difficult to carry out GLM analysis to all species owing to the few amount of eggs collected (table 8).However, seems that variables such as temperature, salinity (PIL) and geostrophic vectors (HM) had significant effect on egg distribution (p

< 0.05; explained deviance upper than 35 %) on egg distribution from HM and PIL, respectively (Table 8). In ANE case, no variables affect the egg distribution.

Table 8. Generalized additive model (GAMs) to analyze the effect of each statistically significant variable and together ($p < 0.05$) on egg distribution in winter and summer. Variables entered: temperature (θ), salinity (S), zooplankton (Zoo), module velocity ($|u_g|$) and Latitude (Lat). edf: estimated degrees of freedom. Na: not applicable for not eggs captured or very little amount of eggs. * $P > 0.05$

Species	Season	Analysis of each variable	edf	Explained deviance to each variable (%)	Analysis of all variables	edf	Overall explained deviance to all variables (%)	Analysis of θ, S and $\|u_g\|$	edf	Overall explained deviance to three variables (%)
MAC	Winter	θ	2.7	41.3	S	1.9	43.2	S	1.9	43.3
		S	2.3	25.6						
		Lat								
	Summer	NA			NA			NA		
HM	Winter	*			θ	2	21.4	θ	2	14.2
	Summer	$\|u_g\|$	2.1	19.5						
WHB	Winter	θ	2.4	8	S	1	25.7	S	1	12.8
		Zoo	2.1	10.7	$\|u_g\|$	1				
		Lat	2.7	7.4						
	Summer	NA			NA			NA		
ANE	Winter	NA			NA			NA		
	Summer	*			*			*		
PIL	Winter	S	1	44.2	S	1	25.7	S	1.8	87.6
		Zoo	1.8	77.1	$\|u_g\|$	1		θ	1.9	
		$\|u_g\|$	1.7	55.9						
	Summer	θ	2.9	77.4	*			θ	1	59.9
		S	2.9	76.8						
MEG	Winter	NA			NA			NA		
	Summer	NA			NA			NA		
MAM	Winter	Zoo	1	14.2	θ	2	37	θ	1.8	10.3
		Lat	1	4.71	Zoo	1.4				
	Summer	*			*			*		

5.4. Discussion

The cruises carried out in Galicia during winter and summer provides useful information about the spawning distributions and ecology of eight species. The study of ELS of hake, as main target species in the project, underlines the important role of this area as a spawning and nursery habitat for hake and also as for other marine fishes studied here. Moreover, the study realized for seven species with commercial and biological importance, complement the work performed by different authors on the behavior of ELS in this zone (Jhon & Kloppmann, 1993; Hillgruber et al., 1995;Valdes et al., 1996; Motos et al., 1996; Adlandsvik et al., 2001; Bernal et al., 2007; Allain et al., 2007;(Ibaibarriaga et al., 2007) and report the first ichthyoplankton survey on European hake carried out in the Galician coast in the Southern stock. thus, our results represent the first reference of hake eggs distribution in this area.

5.4.1. Study of egg and larvae distribution of European hake (*M. merluccius*).

One of the reasons that explain the absence of previous studies on ELS of *M. merluccius* is related to the difficulties to collect and identify hake egg and to collect hake larvae from routine plankton surveys. The size and morphology of hake egg make difficult the identification, since they have similar characteristics to other eggs coexisting in the same area, mainly *Scomber sp*. Additionally, the Southern hake stock is relatively small compared to mackerel stock (ICES, 2015a and b) which implies that density of hake eggs at sea is consequently reduced (ICES, 2015a and b). Although SAT method has improved significantly the degree of reliability in the identification of hake eggs (Porebsky, 1975; Coombs & Mitchell, 1982; Alvarez et al., 2004); it requires highly trained and skilled personal well familiarized with this technique. For example eggs with the chorion damaged or deteriorated can float and be confused with hake eggs. Moreover, more than two repetitions of SAT method also can deteriorate the hake eggs affecting their identification. Porebsky (1975) affirmed that the eggs left for longer periods under air contact may undergo deformation. The limitations in the identification of hake eggs may be held liable for the unusual low number of eggs respect to larvae obtained in winter.In contrast the identification of larvae is relatively easy due to the punctual features such as the three bars along its body and pigmentation over its head.

Determining the distribution of eggs and larvae over both spatial and temporal scales, involves careful consideration of sampling strategy and the reproductive biology of the target species. The determination of spawning grounds requires, additionally, prior knowledge of likely spawning times and locations and the use of the earliest stages of eggs for minimizing the effects of drift. The reproductive strategy of hake consists in a protracted spawning period, with female spawning all year, with different level of spawning activity (Murua et al., 1998; Domínguez-Petit, 2007), being the main spawning period from January to March (Pérez & Pereiro, 1985; Alvarez et al., 2004). Hake eggs and larvae abundance were higher and wider distributed in winter than in summer. This result coincides with previous data on hake spawning periodicity given for others authors on the base of seasonal eggs density in the northern stock (Coombs & Mitchel, 1982; Motos et al., 2000; Alvarez et al., 2001) or gonad development in the southern and northern stock(Piñeiro & Sainza, 2003;

Domínguez-Petit, 2007; Murua, 2010) which indicated that spawning is especially intense during February and March.

Despite the fact that the sampling periods were designed in accordance with the expected peak of spawning for hake, the reduction of egg densities, in summer, was higher than expected. According to Mehault et al. (2010),the average monthly variation of the spawning fraction showed a main peak of spawning activity in February and secondary peak in May-June but lower than that presented in winter. Other results confirmed the decline in the presence of eggs and larvae in summer (Pérez & Pereiro, 1985; Alvarez et al., 2004). Small adjust in the timing of reproduction in relation to optimal parental (size and age of spawners) and environmental condition is a usual strategy for many fish species. It seems to be the case of hake, which is catalogued as an income breeder (Domínguez-Petit & Saborido-Rey, 2010), it means that adjust it food intake with breeding, with a minor relevance in energy store. The female size increases, the higher feeding activity and the better nutritional state conducive to greater production of high-quality eggs (Mehault et al., 2010). In these cases, the intensity of the spawning is going to be hardly link to actual condition state of the spawners. This characteristic constrains the ability of predict “a priori” spawning time of the specie. Other aspect to bear in mind is the coverage of the sampling. The study covered a small spawning area compared to the large area occupied by the European hake (Martin, 1991; Moser et al., 1997; Alvarez et al., 2004; Sanchez and Gil, 2000; Murua et al., 2010) although it corresponded to the most important spawning ground for the southern stock.It could be possible that in 2012 the spawning of hake moved in the space to other putative spawning grounds on in time, advancing or retarding the spawning (Alvarez et al, 2001, 2004).

One of the utilities of ichthyoplankton surveys is give information on the daily periodicity of spawning for species. The timing and site of egg release determine the environment first encountered by an egg and thus determine physical factors (e.g., salinity, temperature and currents), the presence of potential predators and the probability of egg fertilization(Lowerre-Barbieri et al., 2009). A number of methods are used to assess the time of spawning at the diel scale, including collection of newly fertilized eggs. Besides knowing the spawning strategy of specie, it can be applied if a daily egg production method is considered. Estimate the proportion of fish spawning daily is easier to calculate when the fish spawn at a regular and restricted time period each day (Walsh & Johnstone, 1992;Walters et al., 2009).The earliest development stage of egg identified in this study corresponded to stage IA. Independently from the season these eggs were practically collected during the whole day, but preferentially, during the darkness time between 00:00 to 08:00 which means that spawning can occur at any time of day and night (Figure 5), without identifying any preferential time for spawn.The no presence hake egg before dawn and around midday should be confirmed in further studies. Experiments in captivity carried out by Sanchez et al. (2011) andGuevara-Fletcher et al. (2016b) stated than the highest abundance of newly fertilized eggs were observed during the early morning. Nevertheless, the examination of female gonadal development demonstrated that hake is asynchronous species (Murua & Motos, 2006), which is considered as characteristic of an indeterminate reproductive species (Murua & Saborido-Rey, 2003). Our results partly match the observations described by those authors using different assessment methods, so it is a new evidence of asynchronic spawning for southern hake in Galician waters.

5.4.2. Horizontal distribution of hake eggs and larvae

Concerning the spatial distribution of spawning, the lack of information beyond the 500 m isoline should be taking in consideration in the interpretation of the results. However various aspects can be emphasized such as: the shelf appears as a preferential area for spawning in the Galician area and the shelf break limits the spawning area for hake as few positive stations were observed beyond 150 m isoline. This finding is similar with those reported in the literature for the Northern stock of hake (Motos et al., 2000; Alvarez et al., 2000 and 2004) and other hake studies from genus *Merluccius* (Nelson & Larkins, 1970; Bailey et al., 1982; Moser et al., 1997; Bustos et al., 2007;Landaeta & Castro, 2012). Those studies suggested that during the spawning, the ELS are generally distributed within 200 m of bottom depth at temperatures ranging from 10 to 13ºC and salinities around 35.2‰. The findings obtained here are consistent with those reported in the literature, which seemindicate that temperature is a key factor constraining the beginning and duration of the spawning season as well as the egg and larvae distribution.

Spatial movements of hake spawners along the spawning season have been reported for the northern stock (Alvarez et al. 2000 and 2004). These authors noted that the adult migration and/or temperature seemed to drive the spawning timing if this species. In the Galician area, we detected a sizeable displacement toward the area of Estaca de Bares of hake spawning from winter to summer. This area is coinciding with an aggregation of egg of other fish species as well.

Horizontal distribution of hake eggs and larvae seems to be different in winter but not in summer. Larvae were gathered to the NW from Coruña to Estaca de Bares while the eggs were concentrated to the SE from Coruña to Finisterre in winter. Evidently, the larvae concentration was related with the water temperature and geostrophic vectors (Cyclonic structures) presented in this area. Similar links between ELS distribution and hydrography have been confirmed by Landaeta et al. (2011) for *M. australis*.The slow flow currents and mesoscales structures corroborate the larvae retention in this area. Larval displacement due to the effect of the current (i.e. drifting) can result from the water flow exceeding the swimming capabilities of the larvae and/or if larvae decide not to swim despite being capable of doing so according toHogan & Mora(2005). In the case of eggs, they were distributed to southern, where temperature and the presence of predators of eggs were lower. In summer, the high egg and larvae concentrations to the NW coincide with strong mesoscale structures and high vertical velocity and energy (around Coruña and Estaca de Bares) which retain them in the area. Taking into account the currents, it is possible that the oldest eggs come from the east of the sampling area to hatch close to Coruña and Estaca de Bares. Also the spatial distribution of eggs increasing and/or changing depending to their development progresses according to Cowen & Sponaugle (2009).Pelagic eggs may be highly concentrated during first spawned but are quickly dispersed by ocean currents. These currents, which are usual in our study area(Sanchez & Gil, 2000) can contribute to supply the nursery located in this area. Additionally, the presence of a pronounced gradient of temperature within 200 m isobaths (colder temperatures in the inner shelf) also was determinant in their distribution.

5.4.3. Vertical distribution of hake eggs and larvae

The vertical distribution of hake eggs hardly changed from March to July. The mean depth of egg occurrence was located at 83 m in winter and raised at 74 in summer. The peak of abundance of early development egg stages (stage IA) in the water column occurred at 83 and 73 m depth, respectively, suggesting that hake spawning takes place near that depth. Little is known about the preferential depth at which European hake spawn beyond the data published by Coombs and Mitchell (1982) and Motos et al (1996). While Coombs and Mitchell (1982) indicated a spawning depth between 50-100 m (taking as reference 47 hake eggs from 4 positive stations), Motos et al (1996) extended the range to 120-50 m for hake egg in stage I. To make comparisons between different studies are not always simple because other factors can distort the results, e.g. the sampling design. Some authors affirmed than higher egg abundance both winter and summer are found out of shelf where exist high depth(Martin, 1991; Moser et al., 1997) while our sampling stations rarely extended beyond the continental shelf because there are lower depths. However,Alvarez et al. (2004), stated that between 68 to 97% of hake eggs were collected on the shelf.

The vertical distribution of eggs of a species may vary with local hydrography (Haug et al., 1986, Alvarez et al. 2004). In any case, our results confirm that the spawning depth of hake for the southern stock changed slightly from winter to summer and took place at lower depth than previously referred.

Changes in the vertical distribution in relation to development stage of eggs is common in fish egg species(Motos et al., 2000; Boyra et al., 2003; Ospina-Álvarez et al., 2012)because specific gravity of them are changing when the embryo development progress. Consequently, eggs modify their vertical distribution in the water column until reaching the neutral buoyancy(Sundby & Kristiansen, 2015). The difference observed between winter and summer in relation to egg development stages and their mean depth position seems to be presumably determined by the differences in the hydrography.

The depth at which the fish spawn has relevant ecological implications. Apparently, deeper spawning allows to eggs survive in zones where the light, important for plankton survival, could reduce the amount of predators. Moreover, the small ascent in the water column of eggs as their development progresses is related with the ontogenetic movement and the egg and water density which contributed with the migration to the upper euphotic zone (Moser et al., 1997;Hufnagl & Peck, 2011). Despite of the changes in depth were relatively small (around 13 and 20 m in winter and summer, respectively); it is noticeable that the latest egg stages moved to hatch at shallowerwaters. A similar behaviour showed the larvae which migrated to shallower depths to find food as their development advance (Grote et al., 2007). However, according to Rodriguez et al. (2015) there was little evidence of diel migration for hake larvae, arguing that it was due to the small size of range of the specimens captured.

According to Vázquez-Seijas(1998), dynamics and production of fish populations are strongly influenced by environmental and bio-ecological processes characteristics in area where inhabit. The results obtained here, shown that hake has a preferential depth to spawn and it kept invariable seasonally (bio-ecological behavior of this specie). Although the vertical distribution of eggs results from a combination of the depth of spawning and the egg buoyancy(Coombs et al., 2001), the earliest stages position is the most desirable approach to establish the spawning depth of this species.

The changes in vertical distribution as the egg development progresses are linked to different external factors:temperature, and geostrophic vectors, which seems to modulate the abundance and distribution of hake eggs and larvae in both seasons (Ibaibarriaga et al., 2007). In fact, the highest abundance at depths between 40-60 m and at temperatures from 11.7 to 12.5 and 11.7 to 14 ºC in winter and summer, respectively, corroborates this. The thermal niche for adult fish is usually, wider than the spawning thermal one. Therefore, eggs and larvae inhabit always within temperature ranges narrower than the specie may tolerate (Coombs & Mitchell, 1982; Bjelland & Skiftesvik, 2006; Persohn et al., 2009; Guevara-Fletcher et al., 2016a). Usually within this temperature range occurs the spawning, development and growth of early life stages of *M. merluccius* (Sánchez & Gil, 2000; Alvarez et al. 2001; Alvarez et al., 2004;Alvarez & Cotano, 2005; Motos et al., 2000; Guevara-Fletcher et al., 2016a; Guevara-Fletcher et al., 2016b). Equal temperature range was reported for other species of hake such as *M. productus* and *M. gayi* (Moser et al., 1997; Landaeta et al., 2011; Landaeta & Castro, 2012).

This study demonstrated that those variables which affect the egg distribution also had a significant effect on larval distribution. Highest abundances of larvae were found within the thermo-halocline between 40 – 60 m in summer and winter despite of mixed layer in this season. Similar results were obtained by Bailey et al.(1982) with Pacific hake larvae and Coombs and Mitchell (1982) and Rodriguez et al. (2015) with larvae of *M. merluccius*. According to these authors, hake larvae were most abundance towards the end of the mixed layer between February and March, usually about 50-100 m and above 100 m depths, respectively, at temperatures lower to 14 ºC. The results presented byNelson & Larkins(1970);Moser et al.(1997) also support that the vertical distribution of larvae seems be determined by stratification of the water column and the thermos-halocline barrier (Maynou et al., 2003; Rodriguez et al., 2015).However, the weak number of larvae collected during our mixed period did not permit to achieve a clear conclusion on this issue.

The mechanisms which concentrate the higher abundance of eggs and larvae on each zone; have been related with the effect of sea currents, circulation and hydrographic patterns (Sánchez & Gil, 2000). For example, in summer vertical velocities was considered as one of the main variable affecting the ELS distribution. Primary mechanisms responsible for transport of nutrients from lower to the most superficial levels of the ocean is due to vertical velocity concluded Ruiz (2000). Therefore, the relationship found between the module velocity to egg and larvae distribution seems to favour the retention, feeding behaviour and location of eggs and larvae in summer (Fig. 9 and Fig. 10). In fact, some studies affirmed that the hake spawn in upwelling areas within subsurface waters in the vicinity of the continental shelf break during late winter and early spring (Motos et al., 2000; Sundby et al., 2001; Vargas & Castro, 2001; Olivar et al., 2003). Several authors confirmed that upwelling conditions in the Galician waters from April to September (Casabella et al., 2012; Ruíz et al., 2014). However, it is notorious that between the end of February and March, the northeasterly winds permit the renovation of superficial waters for deeper waters, colder and richer in nutrients waters. Thus, the relationship between phytoplankton and the increase of zooplankton production could favor the selection of spawning area and the movement of larvae in the water column. The boundaries of the spawning population become confined to an area where the long-term probability of the larvae finding food is greater concluded Alvarez et al. (2004). Even though it was not possible to obtain data about phytoplankton (as an indirect indicator of zooplankton concentration); the close relationship between both of these parameters and the ELS distribution is widely justified (Blaxter, 1973; Lasker, 1975).

5.4.4. Fish eggs assemblages and environmental condition

Ichthyoplankton distribution of seven of the most important commercial species for the Galician waters reveals that, similar to hake, March is one of the main spawning period for most of them. In winter the dominant specie was mackerel and in summer horse mackerel. Horse mackerel, sardine, silvery lightfish and hake occurred in both season while eggs of mackerel, bluewhiting, megrim, and anchovy were constrained to one.The spatial and temporal distribution of mackerel and horse mackerel eggs in the north east Atlantic waters has been widely documented by the International ichthyoplankton program funding by European Union (TRIENNIAL surveys, ICES, 2015).

Based on this information, in our study area, ICES (2001 to 2016) recognized a peak of spawning for mackerel in March-April and an extended spawning season (Johnson, 1977; ICES, 2015c) for horse mackerel with not a clear peak. Our findings confirm that described for these species, suggesting that spawning activity of horse mackerel tends to be closer to summer than winter. As for later species, the distribution and abundance of eggs of sardine is extensive as well as this specie is triennially studied under the umbrella of an International program(ICES, 2015c). Sardine reproduces during autumn to springcoinciding with the months when temperatures of the sea surface were colder (Ferreiro & Labarta, 1984; Coombs et al., 2004; Ruiz et al. 2014). In Galicia sardine eggs were also presented in summer despite of increase of temperature

The spawning of anchovy extends from March to August when the sea surface temperature is warm (Coombs et al., 2004; Ruiz-Villarreal et al., 2006). In Galicia the spawning usually is rare, concentrating more to the east (Motos & Coombs, 2000), albeit the increases of its biomass in the last 5 years (Santos et al., 2016) could support an extension of the population westward, to colonize new spawning niches.

Bento-pelagic and mesopelagic egg species – bluewhiting, megrim and silvery lightfish- were well represented in winter. Bluewhiting demonstrated serial spawning with a pick in winter which differs from results obtained by Melo and Armstrong (1991)which reported a main peak in summer. Blue whiting and megrim spawning are associated to cold waters (Jhon & Kloppmann, 1993; Ibaibarriaga et al 2007). These authors did not reported presence of eggs of these species in our area of study, suggesting that the sampling depth was not suitable for collecting these species. However, our results did not support this statement, being highly likely that the distance among sampling stations- 30 nm (Ibaibarriaga et al 2007) vs 4nm (this study) - could in part be responsible of the lack of theses eggs.

The largest number of eggs of the seven investigated species was invariably found over the shelf, except for silvery lightfish in summer. The importance of the shelf area is due to in this zone, the primary production enhanced by fronts and internal waves (Pingree & Mardell, 1981; Joint et al., 2001, 2002). Also, the secondary production is more important than in the open ocean, which clearly enhance the presence of fishes spawning in that area.This positive effect has been proved for some species, so that zooplankton abundance explained, partially, the variability of abundance of eggs in GAM models. Other factors, such as geostrophic vectors (Cyclonic structures), mesoscale structure and the vertical velocities concentrate the egg in specific zones such as in the shelf of Estaca de Bares where a high abundance of eggs were found both in winter and summer (Fig. 8).The currents and geostrophic vectors (e.g. eddies, and vertical velocity)have been previously identified as variable determining the fate of ELS of different fish species (Hutchings, 1992;Fowler & Boyd, 1998; Alvarez et al., 2001; 2004;Grote et al., 2007; Landaeta et al., 2011). This oceanographic characteristics can retain ELS depending of their vertical and horizontal position (Govoni, 2005).

The results indicate a clear sequence of spawning of these species in relation to temperature and bathymetry. Other secondary variables such as presence and distribution of zooplankton and the vertical mixing determine the egg distribution and thereby the punctual locations along the sampled area in both seasons (Sundby & Kristiansen, 2015). The particular hydrographic conditions in the Galician area between Finisterre and Estaca de Bares seem to concentrate the eggs in this area (Vázquez-Seijas, 1998).

Their vertical distribution of eggs is determined by the relationship between physical properties of the eggs, sea water features and the degree of vertical mixing of the water column, according to Conway et al. (1997), Sundby (1983) and Adlandsvik et al. (2001). However, the egg abundance in one particular seasons may be due to three aspects: 1. properties of the eggs such as the buoyancy and the diameter which are key variables for modelling the vertical distribution according to Sundby (1991) and Sundby & Kristiansen (2015); 2. The different biological and hydrographic conditions presented in the two seasons which determine the amount of eggs collected and 3. The spawning season of each species with a particular natural history for each of them.

The vertical distribution of fish eggs provides highly relevant information for its application in different fields: i) to precise details in the ichthyoplankton surveys execution when a specific sampling depth needs to be selected and ii) for ecosystem models since the marine ecosystem is vertically structured. Nevertheless, the knowledge in this field is noticeably deficient in comparison with the information documented for the spatial distribution of eggs. The vertical distribution of earliest stages of eggs is defined, at the first time, by the depth at which de adults are spawning. Our findings showed that the depth at which the earliest stage (IA) was found varied between species (Section 6, table 2; and table 1). According to these features, we distinguish tree groups: i) Demersal and bento-pelagic species which occupy depths close to 100m, ii) mid-pelagic species which colonize the upper water column until 50 m depth, similar to that of hake, and iii) small pelagic species which, preferentially, spawn in the upper 50m depth.

The differences in the environmental conditions between winter and summer - winter mixing and summer stratified periods – affected to vertical distribution of eggs between seasons, except for silverylightfish. Mid-size pelagic fish (MAC and HOM) showed a wide depth range of distribution until 150 m depth, although mostly of the eggs (> 75%) were taken upper 80m depth. Both Walsh (1976) and Röpke (1989) indicate that before any significant thermocline is established, a great concentration of mackerel eggs may occur at depths beyond to 100 m which contradicts our results. Vertical distribution of eggs of sardine and horse mackerel moved upper during summer period, when the eggs where preferentially located upper the thermocline.

The vertical distribution of anchovy and sardine eggs confirms those described in the literature. It is generally restricted to the upper water column, concentrations peaking in the upper 10-30 m (Palomera, 1991;Motos et al., 1997;Conway et al., 1998;Olivar et al., 2001; Lopez-Jamar et al., 1992;Coombs et al., 2004; Baldó et al., 2006). However, subsurface peaks are often distinct, depending on local physical conditions(Motos & Coombs, 2000;Boyra et al., 2003) such as it was observed in winter in Galicia waters.

Silvery lightfisheggs occurred all around the water column. In summer, were collected in high abundance just below the thermocline similar to Shelton and Davies (1979)results for eggs localized off South Africa's Cape Peninsula. However, the eggs were spawned above 40 m in south Africa while in Galicia were collected below 80 m. Other distribution gave by Jhon and Kloppmann (1993), encountered that MAM eggs were distributed at depths beyond 80 m off Namibia. Worldwide shallow occurrences of Silvery lightfish eggs have been related to upwelling events directly or indirectly (Jhon & Kloppmann, 1993). However in Galician waters the vertical structures of water column did not affect to MAM egg distribution.

Vertical distribution of blue whiting (WHB) eggs was presentedunder 50 m with high abundance at 150 m within the inner shelf at temperatures mean of 12.5 ºC. These results differ with the eggs distribution given by Hillgruber et al. (1995) and Adlandsvik et al. (2001) in the west of Ireland. Those authors affirmed that blue whiting were distributed below 200 m close to the continental shelf at lower temperatures than this study. However, it is obvious that our vertical sampling area (never exceeding the 200 m of depth) can mask the interpretation and our finding could correspond to a small local population spawning occasionally on the shelf. Megrim (MEG) showed a limited and restricted presence in some stations in winter and null presence in summer similar to results obtained by Ibaibarriaga et al. (2007). Therefore, the low capture of WHB and MEG indicate their restricted spawning habitat as well as the necessity to widen the vertical and horizontal sampling.

The most significant environmental variable modelling the spawning of these fish species was the temperature such as it was shown in the GAM analysis.Temperature and velocity explained great part of variability in the abundance of egg for mackerel, sardine, and hake and secondarily for demersal species, megrim, blue whiting and silvery lightfish; the lack of relevance of temperature for these species could be related with the depth at which they spawn, where the temperature barely changes seasonally. However, zooplankton was not included in the most of models, being particularly significant for abundance of sardine eggs.

The horizontal and vertical distributions of fish eggs across the world oceans display a wide variety of distributions and adaptations by selection of spawning sites and development of egg buoyancies (Sundby & Kristiansen, 2015). Several variables such as spawning depth, hydrographic profile, egg specific features, are key information when investigating vertical egg distributions in field. This study gives information about the effect of those variables on the egg distribution and also the ecology these species in one of the most important nursery in southern Bay of Biscay. In addition, information about stage-dependent vertical egg distributions and evidence about spawning depths were given. However, bearing in mind of the offshore distribution of some our investigated species and their prolonged spawning season, it would be necessary to extend the surveys spatial and seasonally in order to cover as much as possible the whole spawning season and area.

The result obtained here, based on the two scientific cruises of a single year, was the first study which analyzes the effect of different factors on spatio-temporal distribution and abundance of eggs and larvae of M. merluccius from southern stock and complement new information about the ecological coexistence of hake eggs with other seven species of egg previously studied towards NE of the Bay of Biscay, Cantabric Sea and the Irish and Celtic Sea (Nichols et al., 1993; Velasco & Olaso, 1998;. A set of oceanographic variables such as depth, temperature, salinity, the module velocity and the zooplankton affect differentially to all eggs and hake larvae distribution. Consequently, extremes changes of these variablescould be a risk for the population dynamics of those species with a restricted spawning habitat, such anchovy or European hake(Peck et al., 2012).

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6. CHAPTER 3

EFFECT OF TEMPERATURE ON THE DEVELOPMENT AND MORTALITY OF EUROPEAN HAKE (*Merluccius merluccius* L.) EGGS FROM SOUTHERN STOCK UNDER LABORATORY CONDITIONS.

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Effect of temperature on the development and mortality of European hake (*Merluccius merluccius* L.) eggs from southern stock under laboratory conditions

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Abstract

The effects of five temperatures (10, 13, 16, 19 and 22 °C) on the survival, development and hatching time of fertilizedhake eggs from a spontaneous spawning were studied. Significant differences ($p < 0.05$) were found betweeneggs development at each temperature. The power law model provided good fits for the relationshipbetween temperature and development time, with r^2 values higher than 0.96. Total egg mortality rates werehigh for all temperatures, with the lowest values observed at 12.7 °C (59%). In contrast, eggs did not tolerate temperatureabove 22 °C. The timeto complete stages I, II, III and IV showed significant differences (p N 0.05) betweenthis study and previous result reported for hake eggs from northern stock. These differences could be related tothe different thermal history of the eggs, experimental conditions or differences in the grouping of developmentalstages. An extensive review of some hake species from the *Merluccius* genera showed a lowvariability in time to hatch at each temperature.

Keywords: European hake*, Merluccius merluccius*, temperature, egg development, hatching and mortality

6.1. Introduction

Temperature is one of the most important environmental factors affecting the Early Life Stages (ELS) such as incubation of fish embryos and their survival (Blaxter, 1969; Pepin, 1991; Martell et al., 2005; Laurel & Blood, 2011). In this sense, ELS tend to be more generally affected by exposition to geographic, seasonal or daily temperature variations (Ehrlich, 1988).

It is often difficult to estimate and quantify the specific effect of temperature on eggs in the field without considering the interaction of other environmental variables (Pepin & Myers, 1991; Murua, 2010).Consequently, several experimental studies such as Coombs and Mitchell (1982), Bjelland & Skiftesvik (2006) and Sanchez et al., 2011 with *Merluccius merluccius*, Morehead & Hart(2003) with *Latris lineata*, Gracia-López et al.(2004) with Mycteroperca rosacea, Mendiola et al., (2006)with *Scomber scombrus* and Bian et al. (2014)with *Gadus macrocephalus*between others have been conducted to know the specific effect of temperature on egg development and mortality under controlled laboratory conditions. The thermal sensitivity of the survival, development and hatching patterns of eggs from different fish species distributed in the eastern Atlantic Ocean have been examined across a wide range of temperatures (for a review see Peck et al.(2012). Many of these studies focused on assigning the age of eggs and spawning time and identifying different cohorts of eggs collected at sea where the water temperature has been determined (Ehrlich, 1998). Over the years, these studies have allowed to close the knowledge gap between the thermal physiology and ecology of eggs in captivity, and their behavior, development and hatching under natural conditions (Ehrlich, 1998).

The European hake (*M. merluccius*L. 1758) is an important case for study because along the time it has had a high market value and it has been widely consumed (Lloris et al., 2003). This demersal and benthopelagic fish is distributed in the north-east Atlantic Ocean from Norway and Iceland to the Gulf of Guinea in Africa, and throughout the Mediterranean and Black sea (Lloris et al., 2005), in coastal and oceanic waters at temperatures ranging from 10 to 14 °C (Coombs and Mitchell, 1982; Alvarez and Cotano, 2005), and at a depth of between 70 and 370 m (Cohen, Inada, Iwamoto, & Scialabba, 1990). Juveniles and adults fishes have been studied in captivity by some authors which have provided some basic parameters to rearing (De Pontual et al., 2003; Sanchez et al., 2011; Iglesias et al., 2014).

The first experiment concerning artificial fertilization in the European hake was carried out by Raffaele in 1888 (cited in Coombs and Mitchell, 1982). The author of that study identified the first main features of eggs and established the basis for future experiments. Since then, different studies, such as those by Ehrenbaun (1905), Lo Bianco (1909) and D'Ancona (1933), increased the understanding of this species using the methods developed by Raffaele in 1888 (Coombs and Mitchell, 1982).

The interest in advanced technical hake culture has increased recentlyto an industrial scale (Sanchez et al., 2011). In this regard, severalculture experiments have been performed in recent years up to the juvenile stage using eggs obtained from wild-caught specimens andfrom specimens that spawned in captivity (Bustos and Landaeta,2005; Bjelland and Skiftesvik, 2006; Sanchez et al., 2011). However, todate, there are very few studies dealingwith the effect of different temperatureson the development of European hake eggs (Coombs andMitchell, 1982; Marrale et al., 1996; Bjelland and Skiftesvik, 2006;Groison et al., 2010; Sanchez et al., 2011). Coombs and

Mitchell (1982) were the first authors to provide information concerning the rate of embryonicdevelopment of hake egg from northern stock over a specificrange of temperatures. As such, this study aims to compare the results reported by those authors by carrying out a similar experiment regardingthe effect of a range of temperatures on eggs from southern stock. This study aims to incubate hake eggs at five constant temperatures (10, 13, 16, 19 and 22 °C) in order to observe their development rateand survival. This information will provide a physiological basis for explaining the relationship between environmental factors and embryonic development under natural conditions.

6.2. Material and methods

6.2.1. Incubation conditions and egg collection

This work was carried out at the Vigo aquaculture station, which belongs to the Spanish Institute of Oceanography (IEO). European hake eggs were obtained by a spontaneous spawning, from an adult broodstock kept in captivity for the last seven years at 13–14 °C(Iglesias et al., 2010; Sanchez et al., 2011). Adult fish have been maintainedon a natural photoperiod and low light intensity. Also, theynever were sampled to avoid disturbing their behavior (Sanchez et al.,2011). A semi-moist feed of fish flour (35%), fish (30%), squid (17%), mussel (18%) and vitamin premix (6 mg kg−1), has been suppliedevery day ad libitum for the last 7 years (Sanchez et al., 2011).

A volume of approximately 200 mL of fertilized and floating eggs (n ~ 140000) in two mitotic divisions were collected in a plastic cylindrical collector (20 L) placed at the end of the broodstock tank on the morning of 19 November 2013 (Fig. 1). The mean of the number of eggs per milliliter was estimated by counting the eggs in five replicates of 1 mL each. The floating eggs were transferred to a darkened controlled room in which the temperature was maintained at 13 °C along the experiment. A quantity of 100 eggs was checked to observe the initial stage of development, and individual diameters were measuredusing a dissecting microscope. One plastic jar with a 500 µm meshwas outfitted and placed within five quadrate 60 L tanks measuring50 × 50 × 40 cm. These tankswere filledwith filtered (1 µm-mesh) seawater, which was allowed to run freely between the jars and the tanks.

Fig. 1.Fertilized eggs (in orange) collected from the broodstock tank. The eggs sampled to be transferred to each jar were taken in the middle of egg mass. (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article.)

The salinity within each tank was maintained at 34±0.9 psu. After theexperimental set-up, 3500 fertilized eggswere transferred to each jar toexamine egg development and mortality. In order to prevent dehydrationand desiccation of the eggs, a constant flow of seawater(0.678 L min−1) and air flowed into the jars through horizontal pipeswith a diameter of 1 cm (Bustos and Landaeta, 2005; Bjelland andSkiftesvik, 2006; Sanchez et al., 2011). The seawater was sprinkled overthe surface of the jars which caused a circular water movement. Thisflow moved the eggs gently down from the surface of the water (Sanchez et al., 2011). This is necessary because hake eggs exhibit astrongly hydrofugal nature (Porebsky, 1975; Coombs and Mitchell,1982) that causes them to adhere to the water surface where they eventuallydie. Eggs were incubated at five temperatures (10, 13, 16, 19 and22 °C) covering the whole temperature range for hake spawning (Alvarez and Cotano, 2005). Eggs were transferred immediately to eachjar at every tank after spawning. Temperatures were maintained usinga flow-through water cooler (Aqua-Medic, Titan 150) and the action ofthree automatic heaters (Jager100–150 L) except at 13 °C, whichcorresponded to room temperature. Every 36 h, 50% of the seawater inthe tanks was replacedwith freshly filtered seawater at 13–14 °C collectedfrom the Vigo estuary. Water was acclimatized before introduced ateach experimental temperature tank. The eggs used in this experimentwere not treated with any antibiotic solution throughout the experiment.

6.2.2. Sampling design, identification of developmental stages and hatchingtime

The eggs incubated in tankswere checked daily to determine the developmentrates with a sampling frequency that depended on the incubationtemperature. At temperatures ≤19 °C, five eggs were randomlyremoved to identify the developmental stages initially at intervals of4 h. However, at 22 °C, the experiment was suspended 2 h after starting due to total egg mortality. Sampling intervals were increased as the developmental stages progressed up to amaximum of 12 h over the hatchingperiod. The eggs taken for staging were immediately recorded, preserved in 4% formalin (neutralized with borax) and in some cases photographed. The egg developmental stageswere assigned using a stereoscopicbinocular microscope according to the morphological key reportedby Jones(1972): 2–32 cellular division (A), morula stage (B), blastula stage (C), gastrula stage (D), first embryonic vestige (DE) andembryonic stage (E), advanced embryonic stage (F), larval stage (G) and well-formed larval stage (H) until hatching. These nine categories were subsequently sequentially grouped into four morphological stages (stages I, II, III and IV), at the end of each embryonic stage, accordingto the egg classification proposed by Coombs and Mitchell (1982). Thereby, stages I, II, III and IV corresponded to development stages atD, E, F, and H, respectively.

6.2.3. Mortality

The hydrofugal nature of hake eggs makes it difficult to determinethe mortality over a specific time interval. As dead eggs do not shownegative buoyancy, and therefore do not sink immediately after death, inter-stage mortality. Consequently, mortality was only analyzedwhen eggs began to hatch by carefully removing, counting and preserving all dead eggs deposited at the bottom of the jar.

6.2.4. Statistical analysis

Comparisons between temperatures and their effect on egg development were analyzed by analysis of variance (ANOVA). Statistical significance was established at $p \leq 0.05$.

The influence of five temperatures on the process for all embryonic development classifications was studied using a power law model to fit data on time (h) from fertilization to hatching following Eq. 1 (1):$y=a \times T^b$, where y is the time (h) from fertilization, T is incubation temperatureand a and b are model parameters. The models were fittedusing ordinary least-squares for the log-transformed Eq. (2): $\ln I_i = \ln a_i + b_i \times T$. The temperature-dependent models were subsequentlycompared with those reported by Coombs and Mitchell (1982). AnANOVA analysis was performed to compare slopes and intercept at 95% confidence intervals.

The Statgraphics Centurion XV® (2007) statistical package for windows computers was used for all statistical analyses.

The number of eggs surviving (N_{sur}) was determined by subtracting total dead eggs fromthe initial number of eggs (N_{ini}). The totalmortality rate (MR) was estimated as follows:

$MR = (N_{ini} – N_{sur})/h$:

The percentage total mortality (%M) was determined according to the equation:

$\%M = (N_{ini} – N_{sur})/N_{ini}$

6.3. Results

6.3.1. Effect of temperature on egg development and hatching time

The eggs collected and incubated with two mitotic divisions had a diameter of 1.063 ± 0.021 mm (mean ± S.D. n = 100). They had a single golden oil globule with mean and standard deviation of 0.26 and 0.012 mm, respectively. According to Marrale et al. (1996) and Sanchez et al. (2011) this stage corresponds to eggs of 2 h old at 13–14 °C experimental temperature.

The duration of egg development differed for all temperatures. Comparison of each temperature and its effect on egg development showed significant differences between them ($p <0.05$). At 22 °C, all eggs were dead at the first sampling.

The time (h) fromfertilization to the end of the hatching period and the relationship between egg development and temperature are summarized in Table 1. The results reported in this table show that the embryonic period lasts up to 150.3 h at 10.7 °C and 51.4 h at 19.3 °C, at initial hatching (stage H) and 174.5 h at 10.7 °C and 72.9 h at 19.3 °C,when 100% egg hatched. Temperature has a pronounced effect on the duration of egg development. As expected, an inverse relationship was found between temperature and time to hatch (Fig. 2a). The equation and the values for parameters a andb are shown in Table 2. The incubation time variability (Y) ranged between 96% and 99% according to the temperature model.

Table 1. Estimated age (hours from fertilization) to the end of each development stage according to sampling time.

Stage	Grouped following the Coombs and Mitchell (1982) classification	10.7 °C (n = 147)	12.7 °C (n = 110)	16 °C (n = 80)	19.3 °C (n = 73)
Cellular division (A)		10.4	7.6	4.9	3.5
Morula (B)		27.9	21.2	14..7	10.9
Blastula (C)		48.2	34.8	22.4	15.6
Gastrula (D)		60.7	47.1	33.5	25.3
First embryonic vestige (DE)	I	66.3	50.3	34.7	25.6
Embryonic stage (E)	II	86.7	63.9	42.3	30.3
Advanced embryonic stage (F)	III	104.1	79.4	55.1	41.0
Larval stage (G)		126.3	94.9	64.5	47.2
Well-formed larval and hatching (H)	IV	150.3	110.1	72.3	51.4
Total Hatching		174.5	135.4	96.2	72.9

Parameters are presented in Table 2. Egg classifications by Coombs and Mitchell (1982): I. From fertilization to the first sign of primitive streak. II. From first sign of primitive streak to closure of blastopore. III. From closure of blastopore to star of re-curvature of tail. IV. From start of re-curvature of tail to 50% hatching.

Table 2.Values of the parameters for the relationship between incubation temperature and the time to the end of each stage of development given by the equation $y=a \times T^{b}$ represented at Fig. 1, where y is development time, T is temperature and a and b are constants.

Values of constants for the end of each stage	a	b	Correlation coefficient
A	833.35	-1.854	0.99
B	1208.5	-1.593	0.96
C	4460.6	-1.908	0.98
D	2025.4	-1.482	0.99
D-E	3011.3	-1.606	0.99
E	5889.9	-178	0.98
F	4403.7	-1.576	0.98
G	6613.4	-1.674	0.99
H	11235	-1.82	0.99
Total Hatching	5824.2	-1.488	0.97

Unsurprisingly, the duration of egg development was significantly shorter at high temperatures than at lower ones (Fig. 2a), with the duration time for each stage of egg development changing uniformly with temperature. The cumulative rate of development, from stage to stage, showed a transition period in the intermediate (DE) stage between the last mitotic division and initial embryonic development at all temperatures.The duration of the earliest developmental stages (from A to D) represented 35% of the total egg development time. These stages correspond to cellular division and appear to occur faster than later stages.The slight increase in the development rate during the embryonic phase (from E to Hatching) gave a higher slope (Fig. 2b). Embryo formation accounts for an average of 55% of total egg development time.

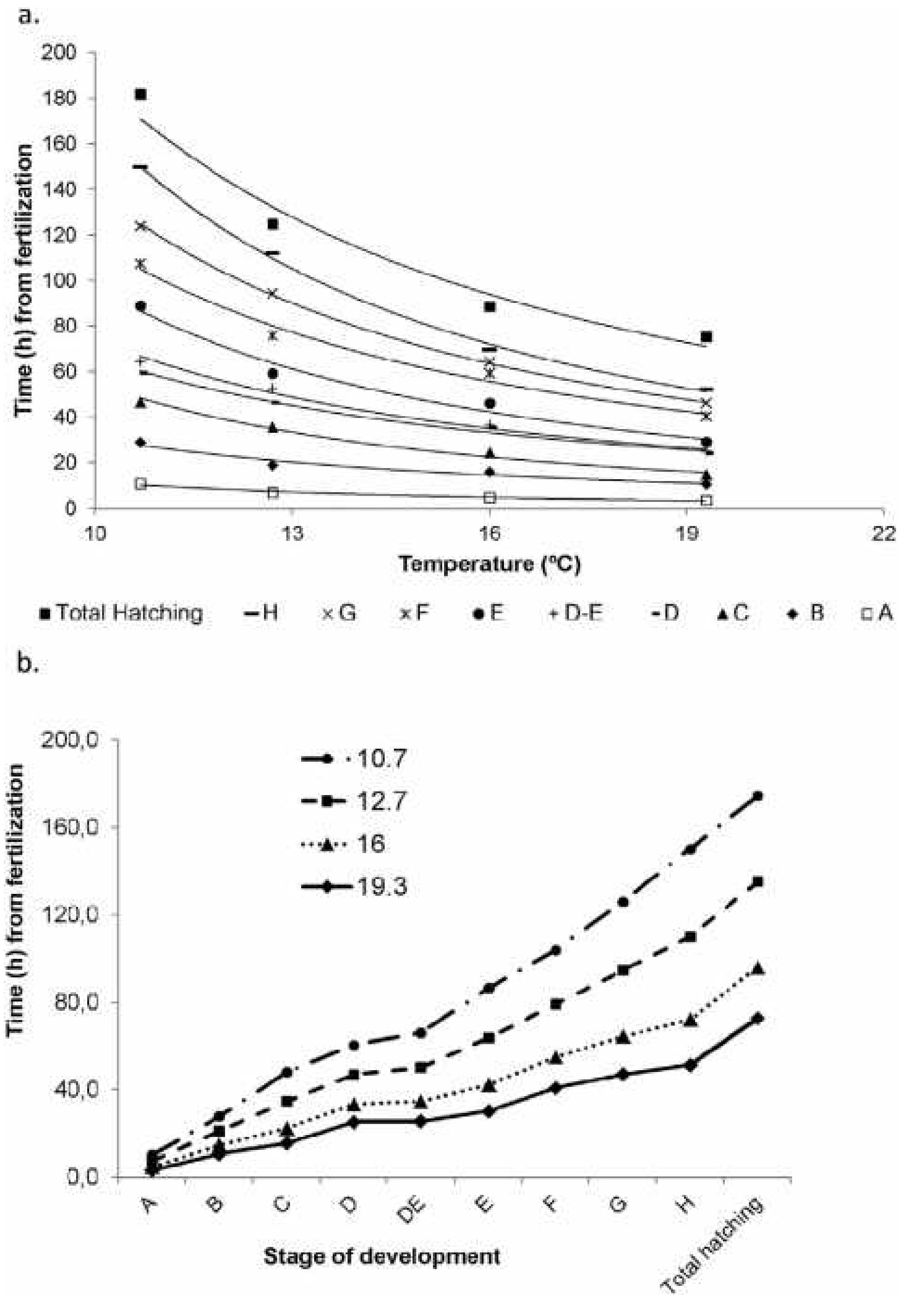

Fig. 2.a. Development observed of European hake eggs from fertilization to the age at the end of each stage at five experimental temperatures. Fitted lines are for regression model. b. Cumulative fitted age from fertilization to the end of each stage of development from stage A to total egg hatching.

A comparison of the inter-specific stage duration values for all developmental stages showed that stage DE was faster than the other eight.Moreover, the development time was lower for cell division (from stages A to D) than forembryonic growth (fromstages E to H) at all temperatures (Table 3). For most stages, the inter-specific stage duration ranged roughly between 30 min at high (19.3 °C) and 22.2 h at low (10.7 °C) temperatures.

Table 3.Total duration (specific stage duration) in hours of the stages of European hake egg development, for a range of four temperatures, derived from the fitted models.

Specific stage duration (egg development)	Temperature (ºC)			
	10.7	12.7	16	19.3
A	8.4	5.6	2.9	1.5
B	17.5	13.7	9.8	7.4
C	20.3	13.5	7.6	4.7
D	12.5	12.3	11.1	9.7
DE	5.6	3.2	1.2	0.3
E	20.4	13.6	7.7	4.7
F	17.4	15.5	12.8	10.7
G	22.2	15.5	9.4	6.2
H	24.1	15.2	7.8	4.2

The effect of temperature on egg stage duration between Coombs and Mitchell (1982) and this study showed that egg development times decreased significantlywith increasing temperature in both studies (Fig. 3). However, the timerequired to reach stage I in this study was longer (between 31 h and 6 h at 9 °C and 20 °C, respectively) than the values obtained by Coombs and Mitchell (1982) for all temperatures studied (Fig. 3a). In contrast, in this study the rates of development for stages II, III and IV were significantly slower at low temperature and faster at high ones, with a mid-point of coincidence at 15, 13 and 11 °C respectively in comparison to results given by Coombs and Mitchell (1982) (Fig. 3b, c and d). Significant differences were found between the slopes and intercept for the two studies ($p < 0.05$) for all egg developmental stages identified (Table 4). Eq. (2) and parameters “*a*” and “*b*” are shown in Table 4.

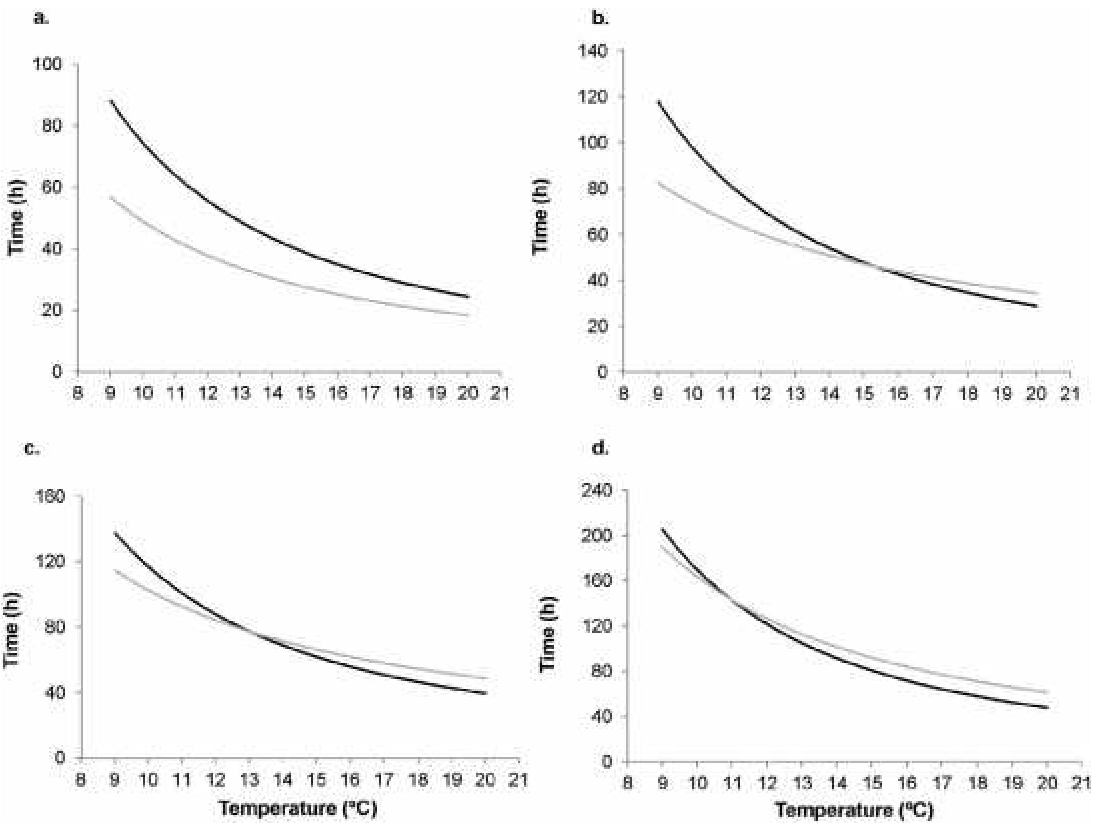

Fig. 3.Individual plots showing the time at the end of each egg developmental stage of European hake. a. Stage I, b. Stage II, c. Stage III and d. stage IV are in relation with temperature from9to 20 °C. The fitted curves using equation at those developmental stages compare the reported data by Coombs and Mitchell (1982) in gray and those from this study in black.

Table 4.Comparison of linear regression analysis (Eq. 2) between Coombs andMitchell (1982) (CM) and this study (TS) for each stage of development are shown. r^2: Correlation coefficient. $p<0.05$there are significant differences.

Stages		Coombs and Mitchell (1982)			This study 2013			Analysis of ANOVA				
CM	TS	a	b	r^2	a	b	r^2	d.f.	F intercept	p-value	F slope	p-value
I	DE	7.14	-1.41	0.99	8.01	-1.60	1	1	18,214,1	<0.01	335.8	<0.01
II	E	6.81	-1.09	0.99	8.65	-1.76	0.99	1	66.4	<0.01	539.6	<0.01
III	F	7.1	-1.07	0.99	8.37	-1.57	0.99	1	48.5	<0.01	520.9	<0.01
IV	H	8.34	-1.41	0.99	9.34	-1.83	0.99	1	539.2	<0.01	518.6	<0.01

6.3.2. Effect of temperature on egg mortality

The results of this study showed that total egg mortality was high atall incubation temperatures, varying from a minimum of 59% at 12.7 °C to a maximum of 100% at 22 °C (Table 5).

The lowest mortality rates recorded during the experiment occurredat the two lowest experimental temperatures, with 12.7 °C being themost suitable temperature for hake egg incubation. At 22 °C all eggsdied before the first sampling (Table 5).

Table 5. The effect of temperature on mortality of European hake eggs from fertilization to initial time to hatch. The number of eggs at the beginning of the experiment at each jar was 3500.

Temperature (ºC)	Nº total eggs dead	Experimental estimated duration (h)	Total mortality (%)
22	3500	2	100
19.3	2979	52.3	85
16	2772	69.8	79
12.7	2056	112	59
10.7	2210	149.8	63

The relationship between temperature and mortality rate wasexpressed by means of a linear regression, which gave the followingempirical equation: Mortality (%) = $0.035(\pm 0.0056) * T\ °C + 0.21(\pm 0.094)$; $r^2 = 0.92$. A significant relationship (ANOVA, $p < 0.05$) was observed between total egg mortality and temperature. According to this function, hake egg mortality increases at a rate of 3.5% per degree temperature increase.

The five temperatures showed a high presence of malformed eggs. Although the developmental stage atwhich all eggs diedwas not determined due to their degradation, it was observed that feweggs in acceptable conditions could have been developing during the morula stage.

6.4. Discussion

The mean egg diameter for European hake registered in this study isconsistent with the values obtained previously by Russell, (1976), Marrale et al. (1996), Bjelland and Skiftesvik (2006) and Sanchez et al.(2011). Similar egg diameters were found in other hake species (Bustos and Landaeta, 2005), thus meaning that the morphologicalcharacteristics of eggs belonging to Merlucciidae family are generallyvery similar (Ehrlich, 1998).

Egg development has been found to vary exponentially with temperaturefor most fish species (for a review see Peck et al., 2012). Thiss tudy is no exception, the relationship between temperature and timeelapsed from egg fertilization was observed. The rate of developmentof European hake eggs increased rapidly with temperature in a power law model in the temperature range 10–19 °C.

As expected, the egg development results showed that incubationtimes vary between stages and temperatures, with the intermediatestage developing more rapidly than the earlier and later stages. In the case of European hake eggs, development and successful hatching are primarily affected by the temperature at which they are incubated. Inmost species, the effect of temperature is fundamental as regards to fecundation, spawning dispersion and egg development (Ehrlich, 1998).

In fisheries, the development of individual-based models relatingegg development, incubation time and the effect of environmental factors, such as temperature, is essential in order to better understand recruitmentproblems (Murua, 2010). Indeed, a temperature-dependentregime best explains the variance in egg developmental stages (Pauly & Pullin, 1988). Moreover, temperature can help to detect long-termtrends in the distribution, composition, and abundance of fish populations(Hempel, 1979). The maintenance of stock size depends largelyon egg production and the rate of survival through egg and larval stages.In this regard, this study showed a clear effect of high temperatures onegg development and hatching, thereby affecting the population dynamicsof M. merluccius (Clark et al., 2003; Murua, 2010).

The incubation models for egg developmental stages obtained in the temperature range 10–19 °C were consistent with those reported by Coombs and Mitchell (1982) based on wild *M. merluccius* eggs belonging to northern stock. However, although both studies resulted in similar trend models, a comparison showed significant differences between stages I, II, III and IV. The differences in duration time between both studies are clear and might arise due to many different causes, including the experimental techniques used and the different egg classifications established. Thus, Coombs and Mitchell (1982) obtained wild eggs from stripped fish and examined them at intervals of 6 h. They assigned 25 egg developmental stages, whereas in this study the eggs were obtained from broodstock in captivity and only 10 stages were used which subsequently were grouped into four. Finally, it should be pointedout that stage IV was considered as the time fromstart of re-curvature oftail (initial stage IV) to start hatching and not at 50% such as these authors analyzed. It is difficult to determine the egg number at 50% without understating or overstating their amount owing to theirhydrophobic characteristics. It was evident that hake eggs continue floating for some time after they are dead. Although the separation ofegg development into a higher number of intervalsmeans that groupingthem into a smaller number is more flexible, the different taxonomicclassifications used make it difficult to accurately compare both studies(Blood et al., 1994).

Time to hatch differ significantly with temperature and also decreaseas temperature increased. Similar results have been reported in otherfish studies (Hart et al., 1996; Gracia-López et al., 2004; Yang & Chen, 2005; Mendiola et al., 2006; Peck et al., 2012). Peck et al.(2012) affirmed that for some species with thermal tolerances, eggs may drift for a month, a week, or only a few days prior to hatching.Also, they confirmed that the development rate versus temperatureamong 58 species showed that those using colder waters for spawningtended to have lower thermal sensitivities, indicated by lower values of *b* (constant of power law equation) and vice versa for warmer-water species. European hake tend to have relatively higher thermal sensitivities (b = 1.82, Table 2) regarding the incubation temperature range studied here. In fact, the variations in time to hatch differ between speciesand depend on cold or warm temperatures where they inhabit (Peck et al., 2012).

In spite of the *Merluccius*genera being extremely distributed allalong the ocean waters, time to hatch was reasonably uniform (Fig. 4a). A general relationship between hatching reported by differentsources and temperatures utilized during embryonic incubation for all species was described using a linear function. That dataset contained species within genera *Merluccius*, none of which appears to deviatefrom this general trend, except the hatching time to *M. merluccius*reportedby Breder & Rosen(1966)at 9.1 °C (Fig. 4b). Although thereis currently no general consensus regarding the origin of hake, this similarity could support the proposed hypothesis of only one ancestor for all genera (Murua, 2010). As such, it is possible to infer that *Merluccius* still maintains, adaptations to specific (local or regional) thermal conditionsin relation to egg development and time to hatch under specific environmental conditions as it can observed in Fig. 4 (Coombs andMitchell, 1982; Alvarez et al., 2004; Bustos et al., 2007; Sundby et al., 2001; Peck et al., 2012).

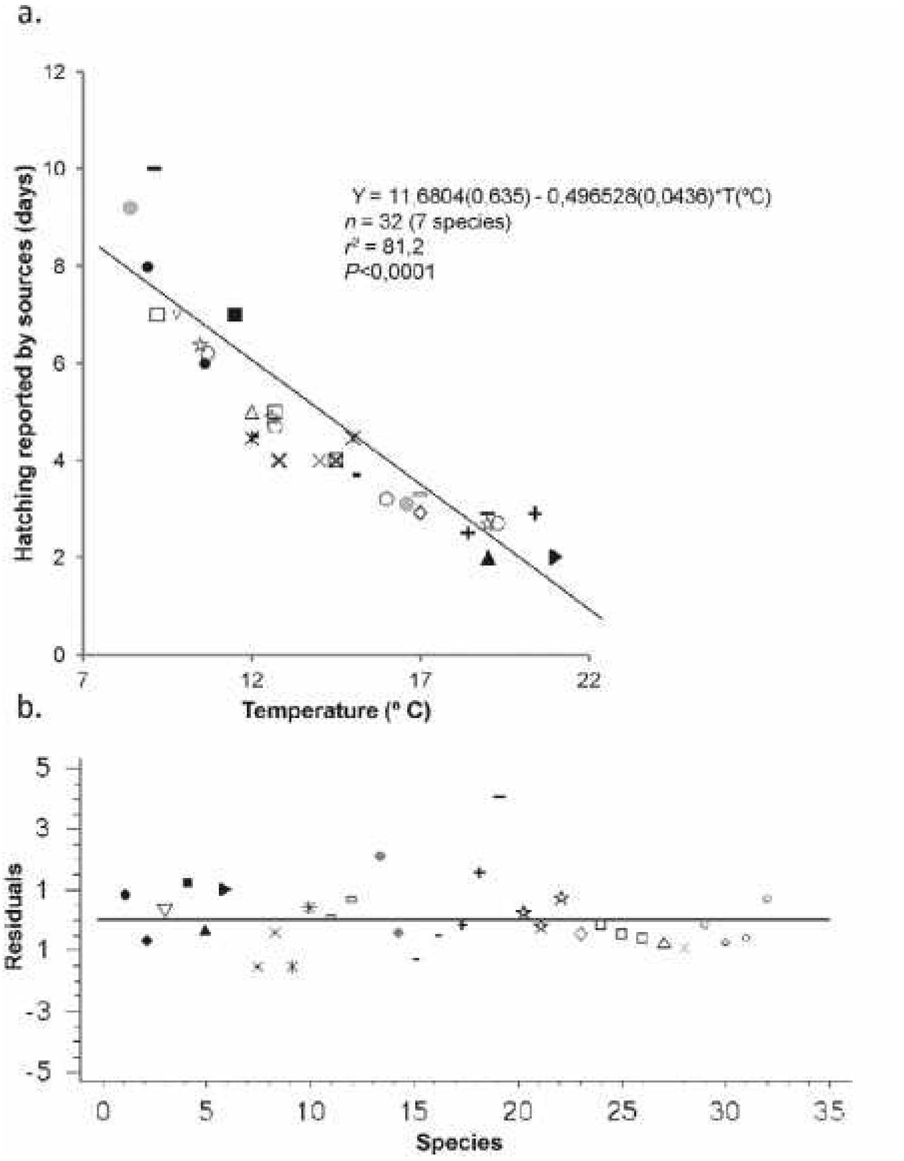

Fig. 4. Comparison of time to hatch for various hake species from genera Merluccius distributed in different places, including this study.● *M. albidus* (Marak, 1967);▽*M. albidus* (Hardy, 1978); ■*M. australis* (Bustos & Landaeta, 2005); ▲*M. bilienaris*(Bigelow & Schroeder, 1953); ▶*M. bilienaris* (Hardy, 1978); **X***M. gayi* (Fisher, 1959); **Ӿ***M. hubbsi*(Ehrlich, 1998); —*M. hubbsi*(Ciechomski & Weiss, 1974); ●*M. productus*(Nelson & Larkins, 1970); - *M. productus* (Bailey, 1982); +*M. merluccius* (Raffaele,1888 cited in Coombs & Mitchell, 1982); ▬*M. merluccius* (Breder & Rosen, 1966); ☆*M. merluccius* (Coombs & Mitchell,1982); ◇*M. merluccius* (Marrale et al., 1996); □ *M. merluccius* (Bjelland andSkitesvik, 2006); △*M. merluccius* (Groison et al., 2010); X*M. merluccius* (Sanchez etal., 2011); o This study.

Egg survival is relatively lowin nature and depends on different abioticand biotic variables, such as population increase, temperature, predation,cannibalism and egg dispersion, among others (Dahlberg, 1979;Murua, 2010). However, egg mortality patterns and their levels varywhen comparing the open sea work-field with experiments performed in captivity (Hempel, 1979). In this study, total egg mortality was above 59% at all temperatures, with high temperatures having a higher lethaleffect on this parameter. Similar findings were reported by Bjelland & Skiftesvik (2006), Coombs and Mitchell (1982) and Iglesias et al.(2014) with total egg mortality rates higher than 40%. Typically, thetemperature in each tank and specific hake egg features has an influence on mortality (Solemdal et al., 1995).

In this study, eggs died on the water surface despite the constantsupply of water on the jars (Bustos & Landaeta, 2005; Bjelland & Skiftesvik, 2006; Iglesias et al., 2010). Contact with air and circularwater movement caused the migration of eggs to the surface, wherethey eventually died from dehydration. Their adhesive and hydrofugal nature, which causes them to be trapped by surface tension, hasbeen described by different authors (Zaitsev, 1971; Porebski, 1976;Coombs & Mitchell, 1982; Marrale et al., 1996; Bustos & Landaeta,2005).This phenomenon is not a spontaneous adherence character, asthere has to be external forces (the air bubbles close to the eggs could make the eggs migrate faster to the water surface. However, if there isno air flow, equally, the eggs go to the top owing to their natural hydrophobiccondition) forcing the eggs up towards the surface (Bustos andLandaeta, 2005; Bjelland and Skiftesvik, 2006; Iglesias et al., 2010).This condition probably never occurs under natural conditions becausesouthern and northern hake spawn at depths of approximately 50–

200 m (Motos et al., 2000; Alvarez et al., 2001; Wheeler, 1969;Coombs & Mitchell, 1982).

Fish eggs are sensitive to temperature changes during the earlystages of development, particularly during mitotic division (Thompson & Riley, 1981; Rombough, 1996). Other studies carried out withinthe Gadidae group (*Gadus muruaa*nd *G. macrocephalus*) corroboratethis (Bonet, 1939; Bian et al., 2014). In this study, the majority of deadeggs collected had not reached the embryonic stages, thus suggesting that the critical period for high mortality during egg development occursfrom the morula to the gastrula stages (Riley, 1974; Dahlberg,1979; Bian et al., 2014). Eggs cannot tolerate high temperatures then they die in the early stages of development or tend to developabnormally (Ciechomski, 1967; Bunn et al., 2000). In contrast undernatural conditions, low water temperatures prolong egg developmentand therefore increase predation-related mortality (Dahlberg, 1979;Bachiller et al., 2013). These results show a similar behavior to otherfish species but adapted to each particular habitat (the location ofspawning and nursery areas, the bio-ecological history of the speciesand the oceanographic conditions of the area) (Hempel, 1979; Ehrlich,1998).

Short-term variations in temperature can result in significant changesin survival and egg development (Hempel, 1979). High mortality ofeggs at temperatures outside the optimal range from 10.7 and 12.7 °Cwas evident in comparison with other species studied (Mendiolaet al., 2006 with *S. scombrus*). Other factors such as egg manipulation and/or the egg and sperm quality; also have an effect on their mortality(Hempel, 1979; Abdoli et al., 2005). However, the specific hydrofugal nature of hake eggs represents an additional cause of mortality andcould therefore be chiefly responsible for the low survival valuesfound in this study. Under natural conditions, European hake fromsouthern and northern stocks normally spawn at temperatures anddepths ranging from 10 to 13 °C and 50–200 m, respectively (CoombsandMitchell, 1982; Motos et al., 2000; Alvarez et al., 2001). In addition, adult fish spawn within a preferred temperature range to produce themaximum number of viable eggs (Fry, 1971; Bunn et al., 2000).

The lowestmortality observed in this study occurred at 12.7 °C, closeto the water temperature at which the adult fish and egg spawning were maintained in captivity (Sanchez et al., 2011). Although, the temperaturetolerance of hake egg seems to be species-specific (a narrowtemperature tolerance range), some authors suggested that acclimatizationof adult broodstock might influence the thermal tolerance andsurvival of the embryos (Hempel, 1984; Abdoli et al., 2005). Bunnet al. (2000) have also suggested that the absolute temperature changethat most eggs can tolerate is about 6 °C and that susceptibility to mechanical pressure is dependent on the stage of development. In the presentstudy, fertilized eggs were immediately transferred to five temperatures, but only that which exceeded the range of tolerancecaused an extreme thermal shock and null survival (Iglesiaset al., 1995). Previous acclimatization of eggs at low temperatures could havebeen beneficial for the development rates at these temperatures (Mendiola et al., 2006). However, it is highly probable that the acclimatizationtime has an effect on egg developmental stages and incubation times.

The current study illustrated the strong temperature-dependence of hake egg development andmortality. Equally, temperature-development models provide biological information regarding the age of eggs in captivityand the probability to be compared with hake egg stages collected at sea. Such models could also be used to assess the extent to which climatechange and other thermal phenomena affect mortality rates andthe abundance and distribution of eggs; possibly leading in the futureto a better understanding of how to breed this species.

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7. CHAPTER 4

THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF YOLK-SAC LARVAE OF EUROPEAN HAKE (*Merluccius merluccius* L.) UNDER LABORATORY CONDITIONS.

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The effect of temperature on the development of yolk-sac larvae of European hake (*Merluccius merluccius* L.) under laboratory conditions

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Abstract

The effect of temperature (10, 13, 16, 19 and 22°C) on hatching, development and survival of yolk-sac larvae of European hake, *Merluccius merluccius*, was studied. At 22°C the experiment was suspended because all eggs died a few hours after incubation. Five morphological indicators of larval development (standard length – SL, yolk-sac volume – YSV, oil globule diameter – OD, body height – BH and body wet weight – BW) were analyzed. SL, YSV, OD and BW of newly hatched larvae were biggest in size and weight at low temperatures ($P < 0.05$). Throughout the experiments, the rates of changes in SL, YSV and OD varied with temperature ($P < 0.05$). By contrast, BH and BW remained constant and did not show significant relationship with the effect of temperature ($P > 0.05$). The potential model showed that the SL growth rate varied from 0.05 to 0.08 mm day 1 from 10.5 to 19.5°C respectively. However, at all temperatures two growth phases were identified: a rapid growth phase followed by a slow one. Rapid growth rate in length and depletion in yolk-sac and oil globule occurred at high temperatures. After total YSV and OD consumption (death by starvation), larvae died between 8 and 14 days at 19.5 and 10.5°C respectively, reaching a maximum length of 4.3 mm at 10.5°C. Metabolic indices such as: lowest threshold temperature (T_0); effective larval development ($D^{\text{o}}_{\text{eff}}$); cumulative thermal units (TC) to complete larval development; the temperature coefficient on growth rate in length (Q_{10}) and the condition factor (K) were also studied.

Keywords: *Merluccius merluccius*, temperature, larval development, threshold temperature, metabolic indices, starvation.

7.1. Introduction

All reactions that occur during the ontogeny ofearly life stages (structures formation, embryonicand larval development and first feeding, amongothers) are dependent on exogenous and endogenous factors (McGovern & Olney, 1996; Buun et al., 2000; Hurst et al., 2010). Between exogenous variables, the effect of temperature seems to be determinant on the developmentof fish larvae (Lasker, 1981; Bailey et al., 1982; Hempel,1979; Hurst et al., 2010; Ye et al., 2011)

Temperature has a different pressure throughoutlarval development (Lasker, 1981; Laurel & Blood,2011; Davidsen, 2012). Different studies have concludedthat some morphological structures fromnewly hatched larvae were malformed, or evenlacked most organs, owing to the previous effect oftemperature on egg development (Laurel & Blood,2011; Davidsen, 2012). In addition, the effect oftemperature on eggs shows that most larvae haveincomplete functional systems at hatching. Insome cases, larvae were smaller and had poorlydeveloped internal structures at hatching (Gracia-Lopez et al., 2004; Bjelland & Skiftesvik, 2006; Ahn. et al., 2012); or conversely, larvae were longer and hadbetter developed internal structures (Gracia-Lópezet al., 2004; Ahn et al., 2012). Similarly, sub- andsupra-optimal temperatures have been associatedwith increased levels of muscle, spinal and jawdeformities in larvae at hatching (Reitan et al., 1993;Davidsen, 2012). In fact, the tolerance of eggs tochanges in temperature has a posterior influenceon larval morphological development (Heming & Buddington, 1988). Under natural conditions, thenewly hatched larvae are almost static and vulnerableto any visual predator; if movement-relatedstructures are poorly developed, the probability ofbeing preyed increases (Wieser, 1995; Kjorsvik et al., 2011). In consequence, during the ontogeny, the development oforgans associated with movement is prioritised toincrease their chances of survival (Wieser, 1995;Kjørsvik et al., 2011).

During yolk-sac larvae development, depletionrates of internal structures such as yolk-sac andoil globule depend strongly on temperature (Morehead & Hart, 2003; Gracia-López et al., 2004; Bjelland & Skiftesvik, 2006; Davidsen, 2012). Theirslow or fast consumption can be determinant ontheir development and survival (Hewitt et al., 1985; Houde, 1987;Pepin, 1991;Trippel, 1998). For instance, the change from endogenousto exogenous feeding, first feeding phase, is criticalfor larval development (Blaxter & Hollyday, 1963). Thus it is considered as a high mortality periodrelated both to inadequate food availability (quantityand/or quality) and an inability to movethrough the water to search for food (Anderson, 1988).

The response of larval growth and developmentto changes in temperature has attracted considerableinterest due its applicability to different scientific fields. For example, the empirical modelshave been integrated into individual-based modelsto find out larval physiological responses vs.temperature (Miller, 2007). Thus, for successful culture of any species, it is appropriate to establishthe optimal conditions for maintaining larvaldevelopment (Yang & Chen, 2005).

Several studies have been carried out undercontrolled laboratory conditions to analyze theeffect of different abiotic parameters, such astemperature, on larval development (Coombs &Mitchell, 1982; Hempel, 1984; Hart et al., 1996; Weltzien et al., 1999; Morehead & Hart, 2003; Gracia-López et al., 2004;Yang & Chen, 2005; Bjelland & Skiftesvik, 2006;Mendiola et al., 2007; Peña et al., 2012; Yeet al., 2011; Bian et al., 2014). Nonetheless, the effect of awide range of temperatures on the larval developmentof European hake has been scarcely studied(Coombs & Mitchell, 1982; Marrale et al., 1996; Bjelland & Skiftesvik, 2006; Geffen et al., 2008; Iglesias et al., 2014).The considerable commercial importance of thisspecies in many places of the Northwest AtlanticOcean, as well as the need to know changes in itspopulation dynamics has led to it being reared incaptivity (Geffen et al., 2008; Murua, 2010; Sanchez et al., 2011). Itis evident that identifying how temperature actson larval development has important consequences, not only for the fishery industry, but alsofor the ecosystem (Murua, 2010).

Actually, knowledge of the development andbehaviour of larvae of *M. merluccius* to achievegood fish production on an experimental andindustrial scale continues to be a big challenge (Sanchez et al., 2011). Contributions to findinganswers about the mechanisms controlling larvaldevelopment to improve production of the hatcheriesand to understand the recruitment processesare aspects that need to be enhanced nowadays.

This paper aims to study the effect of five temperatureson the yolk-sac larvae development ofEuropean hake, *M. merluccius*, under laboratory conditions in terms of analyzing the effect of thosetemperatures on some morphological indicators oflarval development, from hatching to larvalstarvation.

7.2. Material and methods

7.2.1. Egg collection and incubation conditions

This work was carried out at the AquacultureStation at Vigo, which belongs to the Spanish Instituteof Oceanography (IEO). European hake eggswere obtained by spontaneous spawning from anadult broodstock kept in captivity for 7 years (Iglesias et al., 2014; Sanchez et al., 2011). Adult fishhave been maintained on a natural photoperiodand low light intensity within a 500 L isothermaltank covered with black curtains. Thirteen adultshake with reproductive capacity were within thetank. They never were sampled to avoid alter theirbehaviour, which may produce their death (Sanchez et al. 2011). A semi-moist feed offish flour (35%), fish (30%), squid (17%), mussel (18%) and vitamin premix (6 mg kg^{-1}), hasbeen supplied every day ad libitum during last7 years (Sanchez et al., 2011). A volume of approximately200 mL of fertilised and floating eggs(n = 140 000) was collected in a plastic cylindricalcollector (20 L) placed at the end of the broodstocktank on the morning of 19 November 2013,between 06:00 and 08:00 hours. The mean numberof egg per millimeter was estimated by counting the eggs in five replicates of 1 mL each. Thefloating eggs were transferred to a darkened controlledroom in which the temperature was maintainedat 12–13°C throughout the experiment.

The experimental assembly consisted of 1 Lplastic jars with a mesh of 500 lm which wereoutfitted and placed within five 60 L square tanksmeasuring 50 x 50 x 40 cm. These tanks were filled with filtered (1 µm-mesh) seawater from theVigo estuary, which was allowed to run freelybetween the jars and the tanks. The salinity within each tank was maintained at 34 ± 0.9 psu. 3500eggs were transferred and incubated into each of the jars arranged in the tanks at 10, 13, 16, 19and 22°C, until hatching. In each jar, at each experimental temperature was analysed the larvaedevelopment from egg hatching. A soft air flowwas supplied into the jars to maintain the wateroxygen. To prevent dehydration and desiccation ofthe eggs, a constant flow of seawater (0.678 L min^{-1}) was taken from the tanks to fallover the surface of the jars through horizontal pipe with a diameter of 1 cm (Bustos & Landaeta, 2005; Bjelland & Skiftesvik, 2006; Sanchez et al., 2011).This is necessary because hake eggs exhibit astrong hydrophobic nature that causes them toadhere to the water surface where they eventuallydie (Porebski, 1976; Coombs & Mitchell 1982). Thefive temperatures (10, 13, 16, 19 and 22°C) were maintained using a flow-through water cooler(Aqua-Medic Titan 150; AB Aqua Medic GMBH, Bissendorf, Germany) and three automatic heaters(Jager 100–150 L; EHEIM GmbH & Co KG, Stuttgart,Germany) in accordance with each temperature. This temperature range covered the wholetemperature range for hake spawning (Alvarez &Cotano, 2005). Every 36 h, 50% of the seawater ineach tank was replaced with freshly filtered seawater (1 µm-mesh) collected from the Vigo estuary. Seawater was acclimatized at each experimentaltemperature before be transferred to the tanks.

Immediately after egg hatching and every fourto twenty hours (depending on developmentadvancement), 1–3 individuals for each jar wereremoved randomly with a large-bore pipette todetermine the following morphological indicatorsof larval development: Standard length (SL), yolksacvolume (YSV), oil globule diameter (OD), bodyheight (BH) and body wet weight (BW). All theseparameters were measured using a dissectingmicroscope to the nearest 0.1 mm and a precisionscale to the nearest 0.01 mg. The SL of each larvawas measured from the tip of the snout to the endof the notochord. Yolk-sac volume (YSV) was obtained using the formula $mm^3 = p/6LH^2$, whereL is the yolk length and H is its height; OD wasdefined as the horizontal diameter of the oil globule; BH was measured vertically from the anus tothe maximum height of the larva body and to obtain BW, it was removed the formaldehyde withfreshwater and posteriorly, it was used a blotting paper to remove the water excess in each larvae. All larvae were photographed and preserved invials previously filled with formaldehyde 4% once all measurements were concluded.

7.2.2. Statistical analysis

All allometric data were gathered every 24 h usingmean values from larval hatching until the end ofthe experiments. The effects of incubation temperatureat hatching on each morphological indicatorof development were analysed using one-wayANOVA ($P < 0.05$). When the differences were significant, Newman–Keuls post-test was carried out todifferentiate statistically the specific differencesamong temperatures at 95% confidence level.

Three models were selected to fit the data ofeach morphological indicator versus time at eachtemperature: (1) a potential model was selected toanalyse the standard length growth (y):

$$Y = aT^{b} \tag{1}$$

where b represents the daily growth rate (GR,mm day^{-1}). (2) Yolk-sac consumption (Y) wasfitted to an exponential equation:

$$Y = ae^{bT} \tag{2}$$

and (3) the oil globule depletion, body height andbody weight increase (Y) were evaluated accordingto a lineal equation:

$$Y = a + b \times T \tag{3}$$

where$T$ is the experimental temperature and a and b represent the models constants.

The daily rates (constant, b) obtained for eachmorphological indicator of larval development ateach temperature given by the Eqns (1–3) werefitted with a lineal equation:

$$Y = a + b \times T \qquad (4)$$

Afterwards, the lowest temperature threshold (T0) was calculated for the daily growth rate (GR) in SL at which larvae could begin some physiologicalprocess (growth, feeding, spawning, etc.) usingthe equation:

$$T_0 = -a/b \qquad (5)$$

where a and b represent the parameters obtainedin the lineal Eqn (4) (Kamler, 1992; Weltzienet al. 1999; Chezik et al., 2014). The number of thermal units (degree-days) needed to complete total larval development ateach temperature was estimated using the equation:

$$TC = \Delta t \times T \qquad (6)$$

where Δt is total developmental time until larvaldeath in days at each temperature and T is eachexperimental temperature (Weltzien et al., 1999). In addition, the temperature-independent index oflarval development progress at which developmentis theoretically arrested at each temperature, called effective day-degrees (D^{o}_{eff}), was calculated over thelower threshold temperature (T_0) usingequation (Weltzien et al., 1999):

$$D^{o}_{eff} = \Delta t \times (T - T_0) \qquad (7)$$

where Δt and T were explained by Eqn (6).

The temperature coefficient (Q_{10}) to express theeffect of temperature on biological reactions suchas respiration, growth and so on was obtained for each experimental temperature range, using equation:

$$Q_{10} = (GR_2 = GR_1)^{(10/T2-T1)} \quad (8)$$

where R_1 and R_2 are the daily growth rates attemperature T_1 and T_2 (in °C), respectively and $T_1$and T_2 are the experimental water temperaturesduring the experiments (Weltzien et al., 1999; Yeet al., 2011).

Finally, to compare how 'healthy' a fish is, based on the hypothesis that the heaviest fish are in better condition (Andrade-lόpez, 2006) andassuming allometric growth, Fulton's conditionfactor (K) was used following the equation:

$$K = 100W = L^3 \quad (9)$$

where W = weight of larvae (mg) and L = Lengthof larvae (mm) (Ricker, 1975). Subsequently, apotential model was fitted between K and age (hours post-hatching) to find the behaviour *K* ateach temperature.

$$Y = a \times T^b \quad (10)$$

Where*T* is the experimental temperature and aand b represent the models constants. All statistics analyses were carried out usingStatgraphics® software.

7.3. Results

Newly hatched larvae had a non-functionalmouths, non-functional eyes and large yolk-sacand oil globule. The highest temperature (22°C) was suspended from the analysis because the eggmortality during their development was 100%.The newly hatched larvae showed that the standardlength, yolk-sac, oil globule and body weightwere inversely related to temperature ($P < 0.05$)(Fig. 1a–c,e). The SL, YSV, OD and BW of newlyhatched larvae showed the largest sizes andweights at low temperatures than at high ones (Fig. 1a–c,e). In contrast, body height did notshow any significant relationship with temperaturebecause it had a similar range size at alltemperatures ($P > 0.05$) (Fig. 1d). Newman–Keuls post-test revealed that SL did not show differencesneither between low (10.5–12.7°C) and norbetween high (16–19.5°C) temperatures at hatching($P > 0.05$) (Fig. 1a); Yolk-sac did not showsignificant differences at temperatures <19.5°C ($P > 0.05$) (Fig. 1b) while oil globule did not show significant differences at temperatures >12.7°C ($P > 0.05$) (Fig. 1c). Body weight did not show significant differences between 12.7 and 16°C ($P > 0.05$) (Fig. 1e).

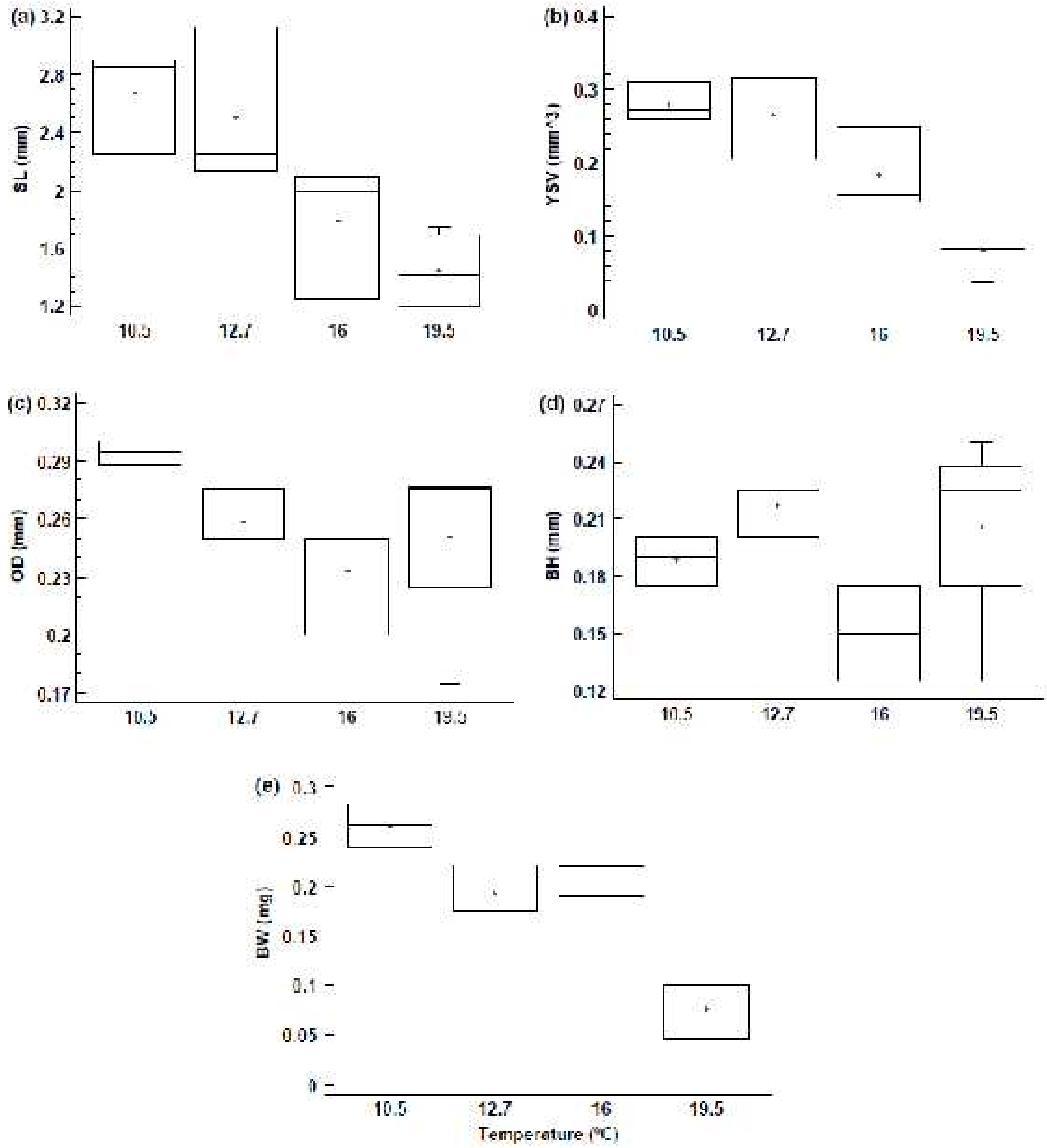

Figure 1.Box-Plots. Morphological indicators of larval development at hatching: (a) SL, standard length. (b) YSV,yolk-sac volume. (c) OD, oil globule diameter. (d) BH, body height and (e) BW, body weight, at each temperature.Black horizontal lines and grey crosses in box-plots are medians and average respectively.

During the experiments, the morphologicalindicators of larval development showed a highdependence on temperature ($P < 0.05$), except BWand BH which remained homogenous until the endof the study ($P > 0.05$). SL showed a potentialincrease throughout the experiment according toEqn (1) (Fig. 2a). In this sense, the growth of hakelarvae increased over time with an increase in temperature from 10.5 to 19.5°C ($P < 0.05$; Fig. 2a). Growth rate varied from 0.05 to 0.08 mm day^{-1}from 10.5 to 19.5°C respectively (Table 1). However, the shape of the standard length vs. age curve showed a phase with rapid growth from hatchingfrom 50 to 120 h at 19.5 and 10.5°C respectively,and after that, a phase of low growth until the endof each experiment (Fig. 2a). Consequently, larval growth followed two phases: high growth (Phase I) and low growth (Phase II).

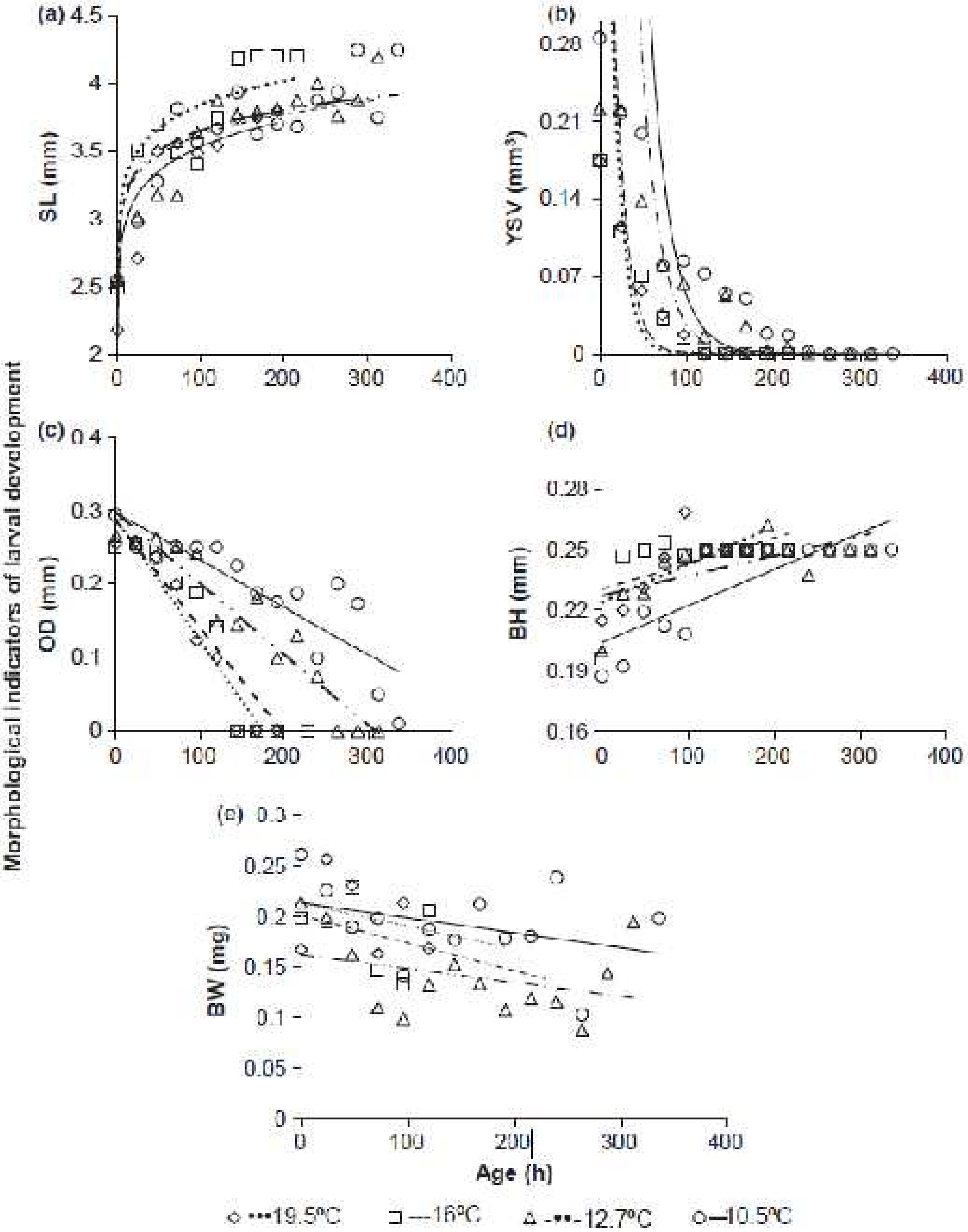

Figure 2.Temporal variation in morphological indicators of larval development throughout the experiment. (a) SL, standard length. (b) YSV, yolk-sac volume. (c) OD, oil globule diameter. (d) BH, body height and (e) BW, body weight. (a) Potential (SL), (b) exponential (YSV) and (c–e) linear (OD, BH and BW) equations were fitted for each temperature. Parameters are listed in Table 2.

Table 1 shows the parameters of linear regression lines fitted on each phase at each temperature. Maximum standard length of larvae ranged from 3.5 to 3.7 between 19.5 and 10.5°C in phase I, and from 3.8 to 4.3 in phase II at the same temperature interval. Growth rates decreased between 77% and 90% from phase I to phase II depending on temperature.

Table 1.Growth model: Parameter estimates (a and b) of the length at age (hours) potential equation for phase I+II given by Equation (1), and lineal equation for phase I and phase II (see details in the text) and mean SL at the end of each phase at each temperature.

Phase	Duration	Regression parameters for each phase				Mean SL at the end of phase (mm)
		T (ºC)	a	b	r^2	
I+II Potential model	From hatching to death	10.5	2.79	0.05	0.80	4.3
		12.7	2.76	0.06	0.81	4.2
		16	2.84	0.07	0.86	4.2
		19.5	2.50	0.08	0.89	3.8
I Lineal model	From hatching to beginning the growth slowdown (Larvae with big length growth)	10.5	2.7	0.009	0.78	3.7
		12.7	2.6	0.010	0.89	3.6
		16	2.8	0.013	0.57	3.5
		19.5	2.1	0.027	0.99	3.5
II Lineal model	From beginning growth slowdown to death by starvation (Larvae without big length growth).	10.5	3.4	0.002	0.34	4.3
		12.7	3.6	0.001	0.32	4.2
		16	3.5	0.003	0.47	4.2
		19.5	3.3	0.003	0.79	3.8

Yolk-sac and oil globule depletion were faster at high temperatures (4 and 6 days at 19.5°C) than at low ones (11 and 14 days at 10.5°C). The results showed significant differences between consuming rates at each temperature ($P < 0.05$). Moreover, yolk-sac was totally depleted before the oil globule (Fig 2b), which was completely absorbed 1–3 days after yolk-sac depletion (Fig. 2c). After total consumption of oil globule and yolk-sac (just the end of phase II) larvae diedbetween 8 and 14 days at 19.5 and 10.5°C respectively (Table 2). However, at 10.5°C larvae died before consuming the total oil globule (oil globule length was 0.01 mm at starvation death).The results showed that larval growth increaseswere related to yolk-sac and oil globule depletion (Table 2).

Table 2. Parameter values for the relationship among each morphological indicator of development and temperature (T) given by Equations (2) and (3). r^2: Determination coefficient and day of total larval death.

T (ºC)	YSV depletion rate (mm/hph) (2)			OD depletion rate (mm)/hph) (3)			BH growth rate (mm)/hph) (3)			BW Fattening rate (mg/hph) (3)			Death by starvation (day)
	a	*b*	r^2	*a*	*b*	r^2	*a*	*b*	r^2	*a*	*b*	r^2	consumption YSV-OD
10.5	3.46	-0.042	0.78	0.3	-0.0006	0.74	0.2	0.0002	0.67	0.21	-0.0001	0.13	14
12.7	2.44	-0.046	0.80	0.3	-0.001	0.92	0.2	0.0001	0.42	0.16	-0.0001	0.12	13
16	0.83	-0.07	0.87	0.3	-0.0014	0.88	0.2	0.0001	0.3	0.20	-0.0003	0.11	9.5
19.5	0.99	-0.08	0.82	0.3	-0.0016	0.94	0.2	0.0002	0.53	0.21	-0.0002	0.10	8

Conversely, body height and body weight remained almost constant with respect to incubation temperatures. For example, at 10.5°C thesetwo variables varied from 0.188 to 0.25 ± 0.025 mm and 0.26 to 0.1 ± 0.05 g, respectively,from hatching to starving (Fig. 2d,e). Accordingly, BH nor BW showed differences with temperatureduring the experiment ($P > 0.05$; Table 2).

Lineal relationships were fitted between thechange rates of SL, YSV, OD, BH and BW andexperimental temperatures (Fig. 3). Lineal regression defined the relationship between the rates obtained at constant b, using Equations (1–3) to each morphological indicator of larval development against every experimental temperature (Fig. 3).Larvae cultured at low temperatures showed the lowest growth rates and depletion of SL, YSV, OD, BH and BW than those at high ones (Fig. 3). However, there was a high ratio between SL, YSV and OD (r^2> 0.90) increase rates with respect totemperature increases (Fig. 3).

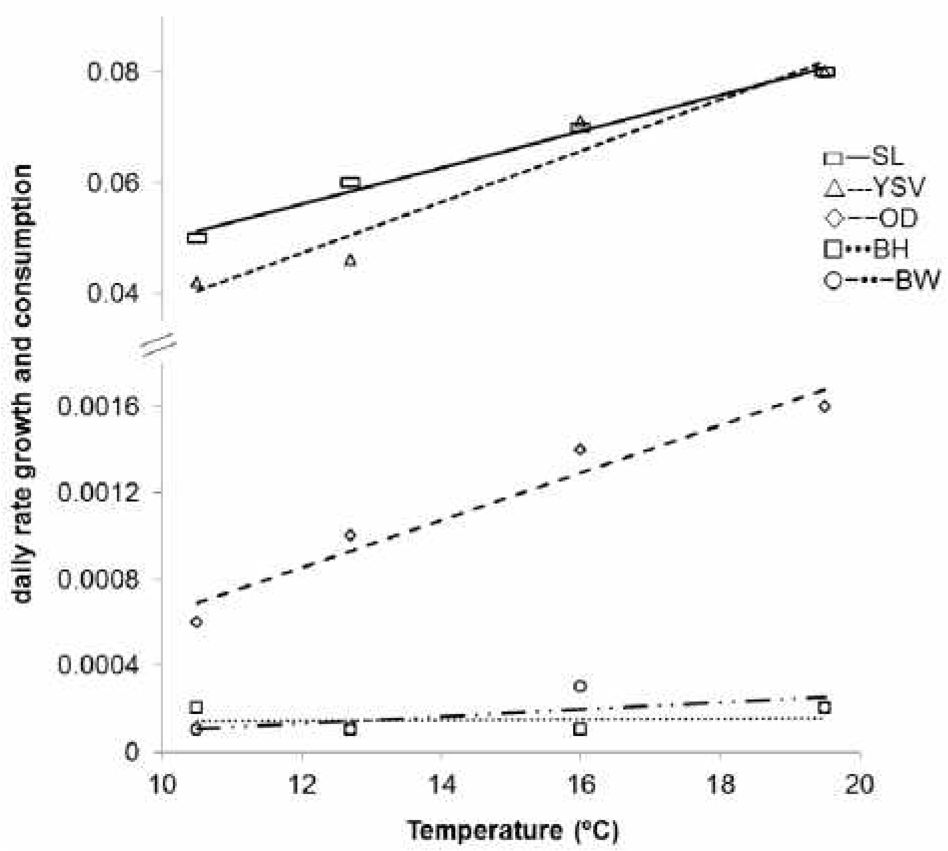

Figure 3.Changes in daily rates of SL (mm), YSV (mm^3), OD (mm), BH (mm) and BW (mg) of larvae of European hake, *M. merluccius*, for each temperature. Daily development rate from hatching to death by starvation at all temperatures allowed calculating the lower threshold temperature (T_0) according to linear Eqn (4): SL = 0.0033 (T) + 0.017, r^2 = 0.99.

Lower threshold temperature in which larvae begin a normal physiological process was calculatedat 5.2°C given by Eqn (5), according tolinear Eqn (4): $y = 0.0033 (T) + 0.017$; $r^2 = 0.99$, obtained from the daily growth rate ateach temperature (Fig. 3). Effective larval development in day-degrees (D^o_{eff}) showed a positive gradient from 10.5 to 19.5°Cranging from 74 to 114.4 (Table 3). The thermalconstant amount (*TC*) in degree-days to completetotal development time of *M. merluccius* larvae washigh at 12.7°C (165.1°C 9 day-degree) due tolarvae dying before consuming the oil globule at 10.5°C (147°C 9 days-degree) (Table 3).

Table 3. Calorific unit value given in cumulative thermal units (*TC*) and effective day-degrees (D^o_{eff}) index given by equations (6) and (7), respectively, of larvae of *M. merluccius* at each experimental temperature.

Temperature (ºC)	Effective day-degrees (Dºeff)	Cumulative of thermal units (day-degrees) (TC)
10.5*	74.2	147
12.7	97.5	165.1
16	102.6	152
19.5	114.4	156

* Larvae at 10.5 ºC died before total oil globule depletion.

The temperature effect (Q_{10}) on larval developmentalrates showed thermo-dependence at alltemperature ranges ($Q_{10} > 1.0$). However, larvalthermo-dependence was higher at low temperatures (Table 4).

Table 4. Temperature effect (Q_{10}) on growth rate given by equation (8) in larvae of *M. merluccius* at each experimental temperature.

Temperature range (ºC)	Q_{10} in developmental rate (GR)
10.5—12.7	2.3
10.5—16	1.8
10.5—19.5	1.7
12.7—16	1.6
12.7—19.5	1.5
16—19.5	1.5

Potential equations between condition indices (*K*) along the larval age (hours post-hatching) are shown in Table 5. According to the models, thebest and worst larval condition occurred at 19.5and 16°C respectively. Likewise, there was adecreasing trend in the condition factor fromhatching until the starvation condition at alltemperatures (Fig. 4), with greater loss of conditionat lower temperatures (12.7 and 10.5°Crespectively).

Table 5. Parameter values for the relationship among condition factor (K) vs. age (hours) at each experimental temperature given by equation (10). r^2: Determination coefficient.

Temperature(ºC)	Condition factor (*K*) parameters		
	a	*b*	r^2
10.5	1.09	-0.23	0.78
12.7	0.89	-0.25	0.78
16	0.86	-0.19	0.89
19.5	1.18	-0.19	0.67

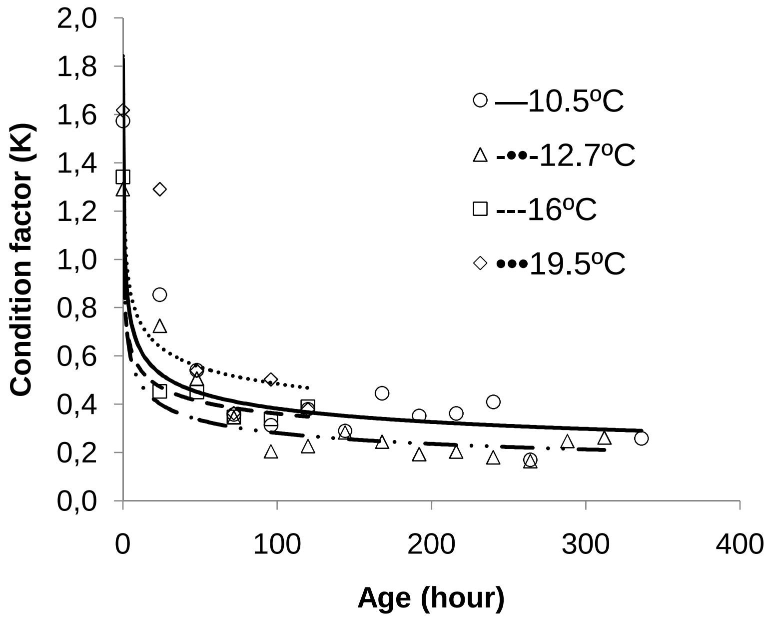

Figure. 4. Potential model (10) of condition factor at age (hour) for each experimental temperature.

7.4. Discussion

This study confirms the thermal effect on the physiological processes of *M. merluccius* such asthat different studies have reported with other fishlarvae such as *Notothenia rossi*, *Merluccius productus*,*Solea solea* and *Scomber scombrus* (Johnston et al., 1975; Bailey, 1982;Hempel, 1979; Blaxter, 1988; Kamler, 2002; Mendiolaet al., 2007).

In the results obtained here, it was evident thatthe morphological indicators of larval development such as SL, YSV, OD and BW were affected by theprevious thermal effect on eggs. Similar results ofthe temperature influence on the morphology andphysiology of larvae at hatching have been reportedby different authors (Blaxter, 1988; Morehead &Hart, 2003; Gracia-Lopez et al., 2004; Jordaan et al., 2006; Okamura et al., 2007; Ahn et al., 2012; Peña et al., 2012). For example, SL athatching was inversely related to increasingtemperature, being larger at lower temperaturesthan at higher ones (Blaxter, 1988; Jordaan et al., 2006; Peña et al., 2012).

After hatching, morphological indicators suchas SL, YSV and OD were affected by temperature during larval development, which is consistent with other fish larvae studies (Pepin et al., 1997;Mendiola et al., 2006; Ye et al., 2011; Bian et al., 2014). Infact, the yolk-sac and oil globule were consumed faster at higher temperatures than at lower ones, affecting larvae survival during their development. During the yolk-sac larvae phase, the food stored in the yolk and oil globule (endogenous food) isused for growth, with a species-specific nutritional composition that varies (it has a dependence) with respect to the mother's age, its weight and food availability in the spawning area (Kamler, 1976;Bustos et al., 2007). Also, the consumption of YSV and OD reserves presented a temporal sequence similar toother marine larvae (Gracia-Lopez et al., 2004;Bjelland & Skiftesvik, 2006; Mendiola et al., 2007; Peña et al., 2012). Yolk-sac consumption occurred earlier than for the oil globule at all experimental temperatures. Ciechomski et al.(1986)and Caldereg(2001) stated that larvae consumed proteins (YSV) faster to developspecific functions (vital structures for growth andfeeding, among others) and preserved their lipid(OD) reserves for other subsequent functions (energyreserves, hormonal regulator, among others) to find appropriate water mass for survival undernatural conditions.

Bustos and Landaeta (2005) and Bjelland andSkiftesvik (2006) reported to M. australis and *M.merluccius*, respectively, lesser days to complete YSV consumption (9 and 6 days at 11 and12.7°C, respectively) than this study (11 and 9days at 10.5 and 12.7°C respectively). There after,larvae continued searching for food for about 3 or4 days until starving to death on days 13 and 14 depending on temperature (12.7 and

10.5°Crespectively). However, Bjelland and Skiftesvik (2006) reported slightly similar results. These results showed differences in the experiments owing to the thermal effects applied to eggs, the supply of food to larvae, the bio-ecological life history of each species and the sampling methodsused (Bustos & Landaeta, 2005; Bjelland & Skiftesvik, 2006; Iglesias et al., 2014). It is known that all these factors could have an indirect effecton the survival and viability of fish larvae (Iglesiaset al., 2014). For instance, the supply of food somedays after larval hatching has influence on thepoint of no return (PNR, defined as the point atwhich larvae are still alive, but they are too weakto feed even if food becomes available) (Blaxter,1988). Also, the transition from the yolk-saclarvae stage to active first feeding is a criticalperiod in the life history of fish; therefore, successin food consumption determines their survival (Hjort, 1914).

Our results have shown that larvae developing between 10.5 and 19.5°C invested energy preferably in growing than in fattening. The total weight of the yolk-sac larvae remained almost constant throughout the experiment. This absence of increase in weight masked the conversion of yolk into new metabolically active biomass. Investing energy to generate organs of motion to find food or escape from predators seems to be a good strategy during this critical period. However, whenever non-food is supplied after YSV and ODare consumed, the ability of larvae to capture prey may be seriously jeopardised with serious consequencesfor survival (Ciechomski et al., 1986;Caldereg, 2001), this risk being even more severeat high temperatures (Fuiman & Werner, 2002).

The final growth-size reached by European hake larvae was bigger at low temperatures than at high ones and turned out to be similar to other species from the same genera (Fisher, 1959; Bailey,1982). Equally, in this study, the largest larvae were obtained at 10.5 and 12.7°C (4.3 and4.2 mm, respectively), coinciding with a maximum larval length reported by Marrale et al.(1996) and Iglesias et al. (2014), who argued that temperature had a significant influence on daily growth rate. The increase in growth rate withtemperature has been stated by other authors(Alvarez & Cotano, 2005; Bustos & Landaeta, 2005; Murua, 2010 and Sanchez et al., 2011).

Nonetheless, our growth estimates were slightly lower in comparison to theirs. As the larval growth rates were not constant during the experiment, an exponential model was selected to fit it.In fact, two growth phases occurred: a rapid growth phase during the first hours, followed by a slow-growth phase associated with the PNR, whenlarvae could not obtain exogenous food (Blaxter,1988). Marrale et al. (1996) for European hake and Mendiola et al. (2007) for mackerel larvae described similar patterns during yolk-sac larvae development. The rapid growth during phase Ishorten the larval period at which sensitivity to adverse conditions is higher. The notorious changes in this phase should be attributed to metabolic processes and the transition between internal differentiation and external morphogenesis which occur during this period (Conides & Glamuzina, 2001). In this regard, it seems thatwarmer temperatures, through higher grow thrates, imply a reduction in the time spent invulnerable life stages and therefore improves thelarvae survival. Nevertheless, an increase in temperature beyond the optimal temperature rangedid not result in a corresponding growth rateincrease because, outside of the optimal, energylosses would exceed the gains, negatively affectinglarval growth (Hart et al., 1996). Some morphologicalstructures, vital for larval survival, cannothave suitable development at warm temperatures(Blaxter, 1988; Gracia-Lopez et al., 2004; Jordaanet al., 2006; Okamura et al., 2007 and Ahn et al.,2012). In fact, it is known that the thermal rangeto which early life stages of *M. merluccius* were areable to develop properly under controlled conditionsvary from 10 to 13°C (Coombs & Mitchell,1982; Sanchez et al., 2011), coinciding with itsnatural spawning thermal niche at sea (Alvarez &Cotano, 2005).

T_0 seems to be species-specific (Weltzien et al.,1999) and in our study, it was estimated at 5.2°C.This value agrees with the results obtained by Bjelland and Skiftesvik (2006) which reported100% mortality in European hake larvae incubatedat 4°C and for other species which havesimilar spatial distribution (Weltzien et al., 1999).This close resemblance between hake larvae rearedboth in Norway and Spain may suggest that thisvalue could be, effectively, specific to North Atlantichake regardless of its origin.

For the first time for larvae of *M. merluccius*, the effective day-degrees (D^{o}_{eff}) as well as the amountof thermal units (TC) necessary to complete thelarval development at a specific temperature hasbeen reported. The early larvae development atlow temperatures and higher cumulative thermalunits at 12°C (low temperature) obtained herecoincide with the results obtained by Weltzienet al. (1999) for turbot larvae. They concluded that these are the best indices for describing developmentalprogress during the early life stages offish reared under different temperature conditionsand, in addition, they are practical tools for predicting larval phenological events.

The daily growth was thermo-dependent at alltemperature ranges, albeit the thermal sensitivity turned out to be higher at low temperatures, coinciding with the results reported for other different species (Otterlei et al., 1999; McGurk, 1984; and Ye et al.,2011). Lower thermal dependence of GR attemperatures beyond 13°C can be explained interms of faster increases of metabolic costs with temperature. Growth represents the difference between energy gained from food and the energylost via metabolic expenditure. Our results suggest that energy loss is more dependent on changes intemperature than on that gained, resulting in acontinuous reduction in Q_{10} at temperaturesoutside the optimum (Caldereg, 2001). In thissense, low temperatures studied here fall withinthe optimal temperature range in which larvae can develop. This may also explain why larvaereared at lower temperatures were of a larger sizeat metamorphosis (Ye et al., 2011).

Condition factor (*K*) reflects, through its variation,the physiological state of the fish in relationto its welfare (Abowei, 2010). Within this study, the condition of unfed hake yolk-sac larvae rapidly decreased over the few post-hatching days as theyolk-sac reabsorption progressed and the larvallength increased. At that point, larval survival may be compromised if external feeding does notcommence. The time at which the minimum Kvalue is observed (which is age-specific accordingto temperature) can be seen as the PNR in theindividual life. This decline in the condition factorhas been observed in other larval species (Blaxter & Hempel, 1963and Westernhagen & Rosenthal, 1981, in herring larvae cited by Ciechomski et al.,1986). Larvae, when still possessing yolk remainsduring the stage of mixed feeding, had a reduced condition factor since the supply food is useful tocounteract energy expenses. The minimum valueof *K* could be a proxy of the PNR, which is consideredas the ‘critical’ period of irreversible starvation (Ciechomski et al., 1986). The studies carriedout by Westernhagen and Rosenthal (1981) withPacific herring larvae (Ciechomski et al., 1986)related the decrease in the condition factor atpost-larval life with the learning process of how toobtain food and improve success in capturingprey.

In summary, this study confirms the effect oftemperature on larval growth from hatching untildeath by starvation. Similarly, the thermal effecton yolk-sac and oil globule depletion is evident aswell as the increase in standard length throughoutthe experiment. Our study showed that theoptimal temperature range for larval development, based on final larval size, total days until starvationcondition and the value of condition factor,was between 10.5 and 12.7°C. This range is inaccordance with sea surface temperatures during atural larval development (Alvarez & Cotano,2005). Further study of the interaction among ontogeny, behaviour and process, especially in relation to parental condition and genetics, would increase understanding of the development and mortality of early life stages and the role of early life-history traits for determining survival andrecruitment (Jordaan et al., 2006). Equally, adjusting the different laboratory processes with regard to the broodstock and eggs maintenance is important to obtain better results. This study gives the first approximation about rearing in captivity of European hake from southern stock, giving necessary tools to future experiments.

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8. CONCLUSIONS

In this thesis, changes inthe spatiotemporal distribution of eggs of European hake and other species with economic and bioecological relevance for fishery industry and the ecosystemhave been described and interpreted in relation to different environmental factors.The findings ofthese works have contributed significantly to a better understanding of thespawning of hake and the effect of temperature on the early life stages development, which will be beneficial to aquaculture, climate change effect andfuture stock management policies. It has been proved that:

On ELS distribution

1. From a methodological point of view the inclusion of SAT method in the routine process of egg identification facilitated the identification of hake eggs when other fish eggs species similar to hake are presented in the plankton samples.
2. The intensity of spawning'shake was higher in winter (March) than in summer (July), which matched with a period of well mixing water column and unusual down-welling conditions.
3. There is not a significant dial spawning pattern for hake, spawning occurs at any time of day or night.
4. The localization of spawning grounds of hake was, basically, defined by zooplankton, temperature and geostrophic currents which gathered and retained the ELS on the shelf.

5. Vertical distribution of eggs kept invariably between periods, despite of the difference in the vertical structure of water column. Most of the eggs occurred at the upper 100 m water column.
6. Concerning the species studied, the spawning activity was, especially important in winter. March was, undoubtedly, the peak of spawning of mackerel, while July was dominated by the spawning of *Trachurus trachurus* and *Sardine pilchardus* and, to a lesser extent, by *Engraulis encrasicolus*.
7. Main spawning grounds of the target species were highly determined by hydrography features and for some species, zooplankton abundance. Currents and eddies gathered eggs on the NW part of the domain. The center of eggs densities grouped the species into two clusters: costal spawners – *Trachurus trachurus*, *Sardine pilchardus* and *Engraulis encrasicolus* - and shelf spawners – *Scomber scombrus*, *Maurolicus muelleri, Lephidorhombus whiffiagonis and Micromesistius poutassou*.
8. According to spawning depth, three groups of species were differentiated: i) deep-water spawners, demersal species such as *Maurolicus muelleri, Lephidorhombus whiffiagonis and Micromesistius poutassou*; ii) mid-water spawners, fishes representing bento-pelagic and medium pelagic species; *Merluccius merluccius*, *Scomber scombrus* and *Trachurus trachurus*; and iii) shallow-water spawners, small-pelagic species*, Sardine pilchardus* and *Engraulis encrasicolus*. Vertical profiles of fish-eggs did not change seasonally.

On thermal effects on egg and larval development

9. Within the optimal thermal range, temperature affects the rates of eggs development, reducing hatching time and increasing egg mortality of European hake under controlled conditions.

10. The standard length (SL), yolk-sac volume (YSV), oil globule diameter (OD) and body weight (BW) of newly hatched larvae were also temperature dependent. Similarly, the larval development rates, the yolk-sac and oil globule absorption processes were positively affected by temperature, and in consequence the time to starvation as well.

11. Under starvation conditions, yolk-sac larvae survived between 8 and 14 days at 19.5 and 10.5 ºC respectively, reaching a maximum length of 4.3 mm at 10.5 ºC.

12. It has been demonstrated that the lowest tolerated temperature on hake larvae was at 5.2 ºC. Metabolic indices such as: effective larval development increases ($Dº_{eff}$), cumulative thermal units (*TC*) to complete larval development, the temperature coefficient on growth rate in length (Q_{10}) and the condition factor (*K*) presented different level of thermal dependency.

It is concluded that:

The spatial distribution of ELS of fish eggs in the Gulf Artabro, is, mainly explained by the hydrodynamics conditions and in second terms, by other biotic (zooplankton) and abiotic (salinity and temperature) factors (depending on the specie). For European hake the temperature highlighted as second factor determining its distribution. How the temperature can influence on the early life history of the hake has been experimentally *investigated under laboratory conditions. We showed that eggs and yolk-sac larval development and mortality are temperature-dependent. Based on the combination of both experimental biology and survey data, we were able to validate the hypothesis of this Thesis:*

"The distribution, development and survival of ELS of hake are affected by environmental conditions"

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