Universidade Vigo

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TESIS DOCTORAL 2011

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Diseño portada:
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Facultade de Ciencias do Mar
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Edad y crecimiento de la merluza europea *Merluccius merluccius* (Linnaeus, 1758) del Noroeste de la Península Ibérica: Evolución de un paradigma

Memoria presentada por Carmen G. Piñeiro Álvarez para optar al grado de
Doctora por la Universidad de Vigo

Dirigida por la Dra. Raquel Goñi Beltrán de Garizurieta, Científica Titular del Instituto Español de Oceanografía en el Centro Oceanográfico de Baleares y tutelada por el Dr. Bernardino González Castro, Profesor Titular en la Facultad de Ciencias del Mar de la Universidad de Vigo.

Fdo: Dra. Raquel Goñi Beltrán de Garizurieta

¡Triste época la nuestra! Es más fácil desintegrar un átomo que un prejuicio...
Albert Einstein

-Oes, fálame do mar, hou

-Que che fale. E que che hei falar…O mar…éche moi grande..

-O mar non. O mar non ten límites. Ti víchesllle algunha vez límite ao mar?

-mm ...

-Non ten, non ten límite. E non penses que é todo igual, porque é todo distinto, agora é así e o barco segue para adiante e logo o mar é doutra maneira, agora é liso e logo non, agora é manso e logo non. E onde era azul mañá é verde, e onde o mar facía ovelliñas coa espuma agora está chan. Nunca é igual, sempre cambia, sempre é o mesmo e sempre é diferente.

-Visto así…

-Non hai nada que ver, o mar non se deixa ver. Nunca ves como é, nada máis está aí. E non lle importa se ti vas a el ou non, se ti pasas por el ou non. Eu andei por todos os mares e o mar non me lembra, non sabe que existo sequera. Ai, este mal róeme as tripas.

-Chamo aos enfermeiros, doulle ao timbre?

-Non, non, deixa. Déixame. Non me fagas falar…

-En que pensas?

-En nada. No mar. Cando eu morra ha seguir alí...

No lombo do Atlántico, Suso de Toro, 2008

A Noa, Lucas e Holger…
…e demais familia…

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Agradecimientos

AGRADECIMIENTOS

Primeramente decir que lanzarse a realizar esta tarea después de superados los 25 años de vida profesional no ha sido nada fácil, sobre todo teniendo pluriempleo... Pero después de tantos años dedicados al estudio del crecimiento, también yo he crecido, no solo en dimensiones físicas y psíquicas, sino también como profesional y ahora estoy contenta de haber hecho este esfuerzo. Mi agradecimiento va dirigido a todos mis compañeras/os del IEO con los que he trabajado, en especial aquellos que valoran el esfuerzo de los demás y conciben el trabajo en equipo como el modelo que aporta siempre ventajas para mejorar no solo el conocimiento integral de los recursos marinos, sino también para enriquecernos nosotros mismos, como seres humanos.

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El contenido de esta tesis recoge, analiza y estudia una gran cantidad de datos y de resultados, fruto de varios proyectos financiados por la UE y programas de investigación del IEO (BIOSDEF, DEMASSESS, DEMERSALES, SAMFISH, DEMICES, EVICES, VEJMER y BIODEM, etc.) resultado del esfuerzo de muchas personas con las que he compartido muchas jornadas en el laboratorio y en el mar. Esto hace muy difícil mencionar a todas las personas que han colaborado en estos trabajos, por ello pido disculpas a aquellos de quienes me pueda olvidar: Pilar Pereda, Celso Fariña, Valentín Trujillo, Ignacio Olaso, Fran Velasco, Pepe Zabala, Jorge Fontenla, Roberto Morlán, Paco Sánchez, Roberto Gancedo, Baltasar Patiño, Jorge Landa, Begoña Villamor, Pablo Abaunza, Hortensia Araujo, Antonio Punzón, Tito Peleteiro, Urbano Autón y un largo etc. A los compañeros de AZTI, Paulino Lucio, Marina Santurtun, Iñaqui Quincoces, Susana Arego, etc. A las colegas del IPIMAR, Fátima Cardador, Cristina Morgado, Manuela Azevedo, Ivone Figueiredo, etc. He de destacar especialmente a mis compañeros del equipo de crecimiento del C.O. de Vigo, que en diferentes períodos de tiempo y situaciones laborales, han colaborado intensamente conmigo en los trabajos de investigación que aquí se recogen. En primer lugar a María Saínza, compañera de fatigas, que sigue a mi lado trabajando codo con codo y en ocasiones, en momentos difíciles. Ella, con su simpatía y paciencia, siempre está dispuesta a trabajar y a apoyarme, ¡Gracias María! A Antonio Gómez, Ana Leal, Lorena Fernández, Manolo Marín, Isabel Bruno, Cristina González, Isabel Loureiro, Fátima Saco, etc. He de agradecer especialmente la gran ayuda del tándem, María y Ana, en la fase final con la bibliografía y la edición de este trabajo. Resumiendo, ¡GRACIAS EQUIPO! porque de una u otra manera habéis sido fundamentales en este proyecto.

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RESUMEN

La finalidad de esta tesis es determinar el modelo de crecimiento de la merluza europea, *Merluccius merluccius* (Linnaeus, 1758), a través de una serie de estudios que muestran la evolución del paradigma de dicho modelo. Para ello se han planteado tres objetivos específicos: *i)* determinar la edad y el crecimiento a escala anual a partir de la macroestructura del otolito; *ii)* determinar la tasa de crecimiento diario a partir de la microestructura del otolito; y *iii)* determinar la tasa de crecimiento mediante técnicas de marcado-recaptura de ejemplares en su medio natural.

El primero de ellos se ha realizado mediante el análisis de la macroestructura del otolito y la elaboración de un protocolo de lectura de la edad, calibrado y aceptado internacionalmente. La aplicación de este protocolo indicaba que la merluza europea era una especie de crecimiento lento (modelo lento) cuya fase explotada estaba representada aproximadamente por 10 clases de edad. Sin embargo, esta metodología presentaba problemas, algunos de ellos claves para estimar la edad, como era la identificación del primer anillo anual, que hizo necesario abordar el estudio del crecimiento diario en juveniles.

El estudio de la microestructura de otolitos de los juveniles mostró que la tasa de crecimiento diario era superior a la obtenida a partir del análisis de la macroestructura del otolito. Sin embargo, la identificación del primer anillo anual, clave para la interpretación de la macroestructura, no fue posible debido a la gran variabilidad en el patrón de formación de anillos del otolito durante el primer año de vida.

Así, los resultados del estudio de la macro- y de la microestructura del otolito aportaron visiones contrapuestas sobre el crecimiento de la merluza europea —lento vs. rápido— poniendo de manifiesto la necesidad de llevar a cabo estudios de validación con métodos alternativos que, de forma independiente de la edad, permitieran conocer el crecimiento real de la especie.

La disponibilidad de nuevos métodos de pesca y marcado-liberación de merluza permitieron, por primera vez, realizar con éxito dos experiencias de marcado-recaptura en el Golfo de Vizcaya y en el noroeste peninsular. Los resultados concluyeron definitivamente que el crecimiento somático era el doble del estimado tradicionalmente por el modelo lento. Asimismo, el marcado simultáneo de los otolitos permitió conocer los anillos de crecimiento que se forman en el otolito entre el período de marcado y el de recaptura, lo que puso en evidencia que la tasa de crecimiento de la merluza había sido subestimada (por un factor de 2) debido al sobrestimación de edad. Así, tras siete décadas de investigación, el debate sobre el crecimiento de merluza pudo ser definitivamente resuelto, produciéndose un cambio del paradigma de «crecimiento lento» al de «crecimiento rápido».

Este descubrimiento puso de manifiesto la invalidez de las claves talla-edad utilizadas hasta 2009 por el Grupo de Trabajo del ICES para la evaluación del estado de los s*tocks* de merluza europea

y por lo tanto de las evaluaciones resultantes. Las implicaciones para la evaluación del estado de los *stocks* son importantes, ya que cambian sustancialmente la percepción del estado del recurso. Así, un crecimiento rápido, con una tasa de crecimiento el doble de la considerada tradicionalmente, indicaría que la mitad del número de clases de edad ha contribuido a la captura (5 en lugar de 10) y que la biomasa del *stock* reproductor es mucho menor y el reclutamiento muy superior a lo estimado hasta entonces. Esto significa que la especie presenta una dinámica más rápida que hace que la población responda a factores externos (ambientales o pesqueros) más rápidamente, de tal manera que su tamaño y producción serán más sensibles a alteraciones de esos factores. Esta mayor sensibilidad deberá condicionar las medidas de gestión para la regulación precautoria del recurso.

En conclusión, el compendio de estudios integrados en esta tesis muestra la evolución de un paradigma que ha cambiado la perspectiva del crecimiento de la merluza y la interpretación de su dinámica poblacional y de su capacidad de respuesta a factores ambientales.

Presentación de la Tesis: Objetivos y estructura

"Considero a los paradigmas como realizaciones científicas universalmente reconocidas que, durante cierto tiempo, proporcionan modelos de problemas y soluciones a una comunidad científica".

Thomas Kuhn, 1962

PRESENTACIÓNDE LA TESIS

OBJETIVOS

El objetivo general de esta tesis es determinar el modelo de crecimiento de la merluza europea *Merluccius merluccius* (Linnaeus, 1758) a través de una serie de estudios que muestran la evolución del paradigma de dicho modelo. Para ello se han planteado tres objetivos específicos:

1. Determinar la edad y el crecimiento a escala anual basándose en el análisis de la macroestructura del otolito, caracterizando para ello el patrón de formación de anillos y diseñando un método estándar de interpretación para estimar la edad.
2. Determinar la tasa de crecimiento diario e identificar el primer anillo anual mediante el análisis de la microestructura del otolito.
3. Determinar la tasa de crecimiento real mediante técnicas de marcado-recaptura en el medio natural.

ESTRUCTURA

La tesis consta de un compendio de publicaciones y está estructurada en seis capítulos:

Capítulo 1. Introducción general sobre el crecimiento de los peces, su estudio y los problemas asociados a la estimación de la edad. Además se incluye una breve presentación de la especie *Merluccius merluccius* (Linnaeus, 1758), de su importancia pesquera y de su biología y ecología, centrándose en el crecimiento y la evolución de su estudio y conocimiento, que constituye el eje vertebral de esta tesis. Esta información forma parte de la siguiente publicación (Anexo):

– Piñeiro C., Morgado C., Saínza M. and McCurdy W.J. (Eds), 2009. **Hake age estimation: state of the art and progress towards a solution.** *ICES Cooperative Research Report No. 294, 43 pp.*

Capítulo 2. Estudio del patrón de crecimiento anual mediante el análisis de la macroestructura del otolito, recogido en el artículo:

– Piñeiro C. and Saínza M., 2003. **Age estimation, growth and maturity of the European hake (*Merluccius merluccius*, Linnaeus, 1758) from Iberian Atlantic waters.** *ICES Journal of Marine Science, 60: 1086-1102.*

Capítulo 3. Estudio del crecimiento diario mediante el análisis de la microestructura del otolito, recogido en el artículo:

– Piñeiro C., Rey J., de Pontual H. and García A., 2008. **Growth of Northwest Iberian juvenile hake estimated by combining sagittal and transversal otolith microstructure analyses.** *Fisheries Research, 93: 173-178.*

Capítulo 4. Estudio del crecimiento real mediante marcado-recaptura en el medio natural en el Golfo de Vizcaya y el noroeste peninsular, recogido en las siguientes publicaciones:

– De Pontual H., Groison A.L., Piñeiro C. and Bertignac M., 2006. **Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation.** *ICES Journal of Marine Science 63: 1674-1681.*

– Piñeiro C., Rey J., de Pontual H. and Goñi R. 2007. **Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: First results support fast growth hypothesis**. *Fisheries Research 88: 150-154.*

Capítulo 5. Discusión global de los resultados de la tesis.

Capítulo 6. Conclusiones generales de la tesis.

Por último, el apartado de la Bibliografía incluye las referencias citadas en la tesis, a excepción de aquellas ya recogidas en las distintas publicaciones que la conforman.

COMENTARIOS DE LA AUTORA

Se sabe que a lo largo de la historia, la realidad a la que se refiere una palabra ha cambiado de forma o ha pasado a aplicarse a un nuevo referente. Teniendo en cuenta que en esta tesis se emplean palabras que tienen distintos significados y que pueden dar lugar a confusión en el contexto del trabajo que aquí se presenta, se explica a continuación el significado aplicado a las mismas.

Paradigma. Se emplea como una perspectiva científica o visión global de una época temporal atendiendo a la definición del filósofo y científico Thomas Kuhn (1962), que fue quien dio a paradigma su significado contemporáneo y lo adoptó para referirse a un marco, perspectiva o modelo bajo el cual se analizan y se tratan de resolver los problemas.

Modelo. Se refiere a un arquetipo o punto de referencia para imitarlo o reproducirlo y también a su conocida acepción en el ámbito de las ciencias puras como la representación matemática de fenómenos o procesos.

Estructura. Este término se emplea de forma generalizada en los estudios relacionados con el crecimiento y se refiere a la disposición y el orden de las partes dentro de un todo que en este caso es el otolito u otra pieza calcárea. La disposición de los anillos de crecimiento tanto a escala diaria como anual se denomina estructura y corresponde a la micro y macroestructura del otolito en función de la menor y mayor escala empleada para su observación.

Capítulo 1. Introducción general

Foto modificada de M.E.Garci

1. INTRODUCCIÓN GENERAL

La investigación aplicada a los recursos pesqueros tiene como misión aportar nuevos conocimientos sobre la biología y la dinámica de las poblaciones explotadas para determinar el estado en el que se encuentran estos recursos. Para ello es imprescindible conocer el ciclo vital y las características biológicas de las especies y poblaciones explotadas. El conocimiento del crecimiento es, por tanto, esencial para diagnosticar correctamente su potencial de explotación y el estado en el que se encuentran. Sin embargo, la merluza europea, *Merluccius merluccius* (Linnaeus, 1758), presenta serias dificultades en la estimación de la edad y del crecimiento y por ello es objeto de este estudio.

1.1. CRECIMIENTO EN LOS PECES

El crecimiento de un organismo es el incremento del tamaño de su cuerpo a lo largo del tiempo. En los peces, como en las plantas, parece ser indeterminado, siendo difícil conocer con certeza el límite máximo del tamaño de los individuos de una especie en particular (Weatherley & Gill, 1987). Una de sus principales características es su gran plasticidad, variable en función del patrón genético de la especie y, sobre todo, de factores ambientales, como la disponibilidad de alimento (cantidad y calidad), la densidad poblacional y la temperatura (Jobling, 2002).

La tasa de crecimiento de un individuo puede cambiar a lo largo de su ciclo vital y mostrar variaciones estacionales. En el caso de ecosistemas de latitudes medias y altas, las especies experimentan en general un crecimiento más rápido en verano que en invierno. Esta variabilidad se refleja tanto en el individuo como en la población, de modo que la tasa de crecimiento, estimada para una población y un período determinado, no puede aplicarse a otras poblaciones o a otros períodos. Por ello, los estudios sobre crecimiento han de extenderse en el tiempo lo suficiente para cubrir aquellos ciclos biológicos periódicos (estacionales y anuales) que llevan inherentes cambios en los patrones de crecimiento, como épocas de puesta o procesos de muda, entre otros (Panfili *et al.*, 2002). Dado que el crecimiento, al igual que la madurez y la mortalidad, son procesos dependientes del tiempo, la estimación de la edad es un elemento clave de su estudio.

1.1.2. ESTIMACIÓN DE LA EDAD DE LOS PECES

El estudio del crecimiento de una población de peces en general, se realiza en dos etapas:

***i)* Estimación de la edad individual**

El método de estimación de la edad individual más empleado en los peces es la observación del patrón de anillos en piezas duras o calcificadas (otolitos, escamas, vértebras, etc.), para lo que se utilizan diferentes técnicas de preparación y observación. En general, este método ha demostrado ser más informativo y preciso que otros métodos usados a escala poblacional (p. ej., métodos de progresión modal en distribuciones de frecuencias de tallas) o individual (p. ej., acumulación de pigmentos metabólicos) (Panfili *et al.*, 2002). Por tanto, la «esclerocronología», o estudio de las piezas calcificadas y su relación con el tiempo, ha surgido como una disciplina capaz de facilitar información muy valiosa sobre la edad de los organismos en diversos campos de la ciencia, particularmente en biología pesquera y ecología marina.

ii) Estimación de los parámetros de crecimiento

Los parámetros de crecimiento son valores numéricos de una ecuación que describe el tamaño corporal medio (talla o peso) de los individuos en función de la edad. En la mayoría de las especies el crecimiento suele ser exponencial asintótico, es decir, muy rápido en los primeros años y lento o casi nulo al ir aumentando la edad. Existen multitud de trabajos que abordan este tema (Beverton & Holt, 1957; Ricker, 1958; 1975), y todos coinciden en la dificultad de representar el crecimiento en las diferentes fases de la vida con una ecuación simple. El modelo más utilizado en estudios de biología pesquera es el de von Bertalanffy (1938), convirtiéndose en una de las piezas angulares de la biología pesquera, ya que generalmente se emplea en modelos complejos que describen la dinámica de poblaciones de peces (Sparre & Venema, 1997).

1.1.3. ESCLEROCRONOLOGÍA

La esclerocronología tiene como objetivo reconstruir la historia de los organismos mediante el estudio de sus piezas calcificadas. Sus métodos están basados en el examen de varios tipos de señales (estructurales, químicas y/o ópticas) que aportan referencias temporales. Los cambios metabólicos que tienen lugar por procesos endógenos pueden producir alteraciones en la proporción de materia orgánica e inorgánica depositada en algunos tejidos calcificados, como escamas, otolitos, vértebras, etc. Las fluctuaciones periódicas (ciclos día/noche, lunares, estacionales, etc.) de estos procesos se reflejan en la formación de anillos en dichas piezas duras (Wright *et al.*, 2002). En muchas especies de peces, el recuento de estos anillos periódicos permite estimar la edad con bastante exactitud. Sin embargo sucesos ocasionales, como enfermedades o condiciones ambientales u oceanográficas inusuales, pueden producir marcas no periódicas que dificultan la interpretación temporal del patrón de marcas en las piezas calcificadas. Las aplicaciones ecológicas y paleontológicas de tales datos incluyen el estudio de las respuestas adaptativas de las poblaciones a las presiones medioambientales (variaciones climáticas) o antropogénicas (p. ej., pesca, contaminación).

Por estas razones, la esclerocronología se ha convertido en parte integral del estudio de las poblaciones de peces explotadas, hasta el punto de que la mayoría de las instituciones responsables de la gestión de pesquerías recogen de forma rutinaria piezas calcificadas para este fin, principalmente otolitos.

Existe una clasificación de la esclerocronología según el tipo de pieza mineralizada que se estudie: esqueletocronología (estudia los huesos del esqueleto), escamimetría (estudia las escamas) y otolitometría (estudia los otolitos). El uso de una u otra pieza calcificada para estimar la edad dependerá, además, de la especie, de la facilidad para su obtención, conservación e interpretación.

Teniendo en cuenta el potencial informativo que tiene el análisis de estas piezas para conocer la edad individual y, por consiguiente, el crecimiento de los peces, existe una gran demanda de datos fiables procedentes de la aplicación de esta disciplina y por eso la esclerocronología de peces ha determinado el avance en el campo de la estimación de la edad durante décadas (Campana & Thorrold, 2001). Sin embargo, a pesar del largo camino recorrido, la esclerocronología está todavía lejos de ser una ciencia exacta y, en muchos casos, los protocolos de interpretación y descodificación del patrón de anillos de crecimiento de las piezas calcificadas continúan siendo motivo de debate, especialmente para algunas especies.

Entre las piezas calcificadas de los peces, los otolitos (Figura 1) están considerados como los mejores indicadores de la edad (Stevenson & Campana, 1992) y la interpretación de su patrón de anillos de crecimiento es el método más extendido para estimar la edad. A diferencia del resto de piezas calcificadas los otolitos, poseen tres propiedades que los hacen únicos como registros de la edad: *i)* son las primeras piezas duras que se forman, estando presentes desde el momento de la eclosión, y su crecimiento es continuo a lo largo de la vida del pez (Brothers, 1984); *ii)* no sufren resorción por lo que constituyen el registro más completo del crecimiento; y *iii)* son las únicas piezas que presentan marcas diarias, permitiendo así obtener información sobre el crecimiento en los primeros estadíos de vida.

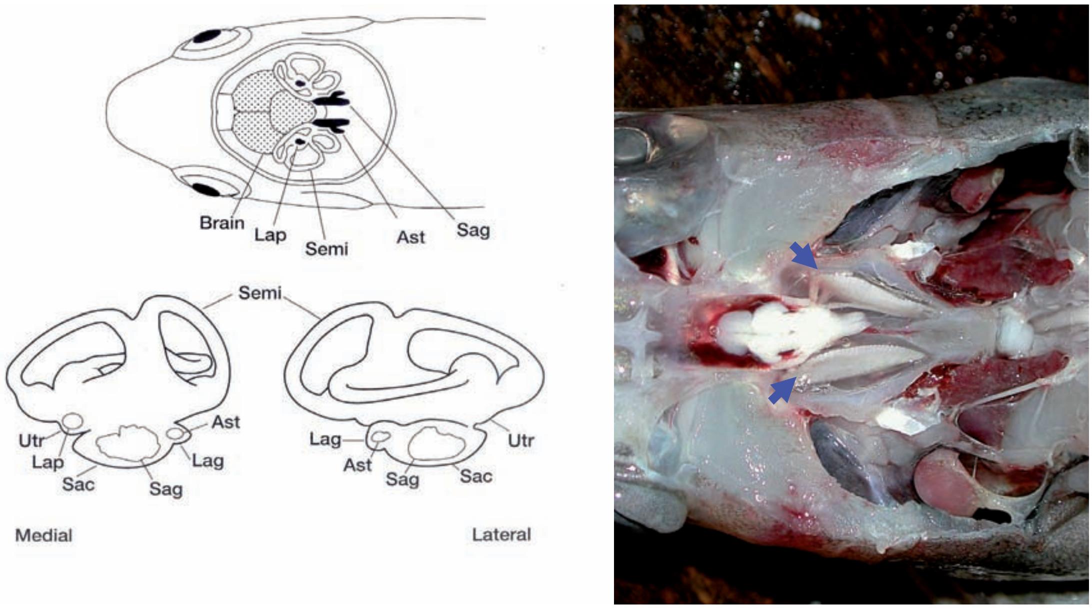

Figura 1. Esquema de la localización del oído interno en el cráneo de los peces teleósteos (modificado de Secor *et al.*, 1991). Vista dorsal de la cabeza (sección frontal) mostrando la posición de los otolitos en el oído interno de (izda. arriba); otolitos en el sistema laberíntico típico (izda. abajo): Ast, *asteriscus*; Lag, *lagena*; Lap *lapillu*s; Sac, *sacculus*; Sag, *sagitta*; Semi, canal semicircular del laberinto; Utr, *utriculus*. Disección de una cabeza de merluza mostrando la ubicación de los otolitos en el cráneo del pez, con flechas azules (foto dcha., C. Piñeiro).

Otolitos: estructura y aplicaciones

Los otolitos son concreciones de carbonato cálcico sometidas a un proceso de crecimiento continuo. Se trata de formaciones extracelulares constituidas por aragonito (una forma cristalizada de carbonato cálcico) fijado en una matriz orgánica compuesta por una proteína similar a la queratina que se llama otolina. Los otolitos están situados en el oído interno de los peces teleósteos (Figura 1) y además de sus funciones auditivas, también son responsables del equilibrio y de la orientación del pez (Popper & Lu, 2000). En la clase osteíctios existen tres sacos óticos a ambos lados de la cabeza (Figuras 1 y 2) (*sacculus*, *utriculus* y *lagena*) en los que se alojan los otolitos: *sagitta, lapillus y asteriscus* (Groison, 2003).

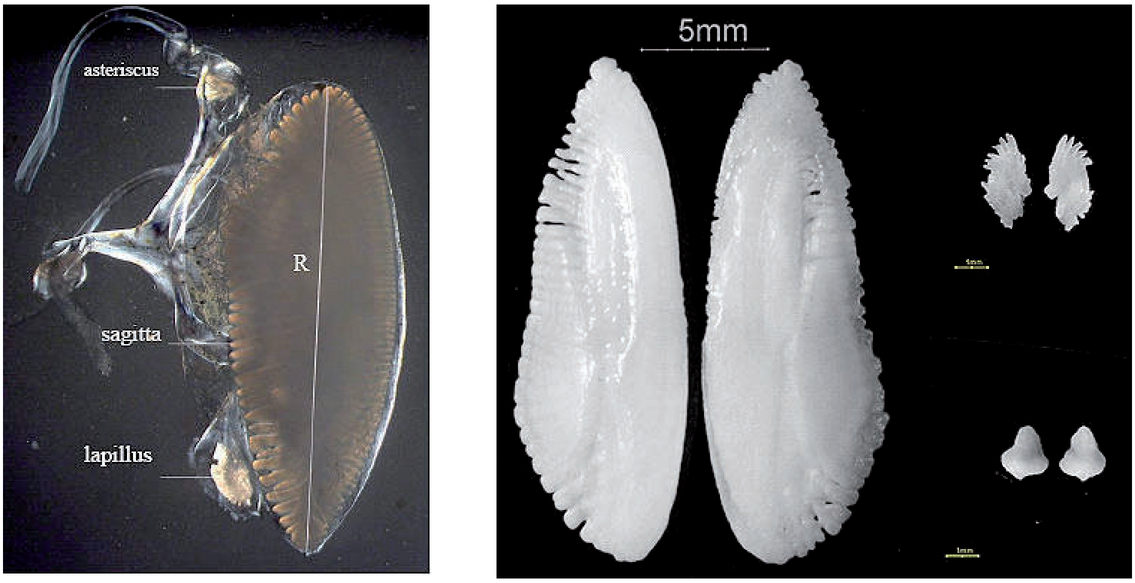

Figura 2. Sacos óticos del lado derecho del cráneo de *M. merluccius*, mostrando los tres otolitos: visión sagital de la superficie proximal de un *sagitta* (R, es el radio antero posterior de 16.7 mm de un ejemplar de 41 cm TL), un *lapillus* y *asteriscus* (izda., foto de Groison 2003, 10x). Forma y tamaño de los tres pares de otolitos (dcha., foto de A. Leal).

Generalmente, los otolitos están comprimidos lateralmente y presentan simetría bilateral, a excepción de los peces planos. Cada uno de ellos se diferencia en su localización, función, tamaño, forma y ultraestructura. De los tres, el *sagitta* es el más utilizado para determinar la edad por ser el de mayor tamaño y el más fácil de extraer en la mayoría de las especies. Así, de forma generalizada se utiliza el término otolito para referirse a el *sagitta.*

Las diferencias en la forma del otolito tienden a reflejar la filogenia del pez (Figura 3), aunque hay variaciones inter- e intraespecíficas asociadas a factores genéticos y ambientales (Lombarte & Lleonart, 1993; Torres *et al.*, 2000; Tuset *et al.*, 2008). Los otolitos también son muy útiles en los estudios de biología evolutiva y de relaciones tróficas, ya que su resistencia a la degradación permite identificar especies en sedimentos fosilizados (Nolf, 1985; 1995) y en contenidos estomacales (Hecht & Appelbaum, 1982; Olsson & North, 1997). Como su forma y composición química puede cambiar

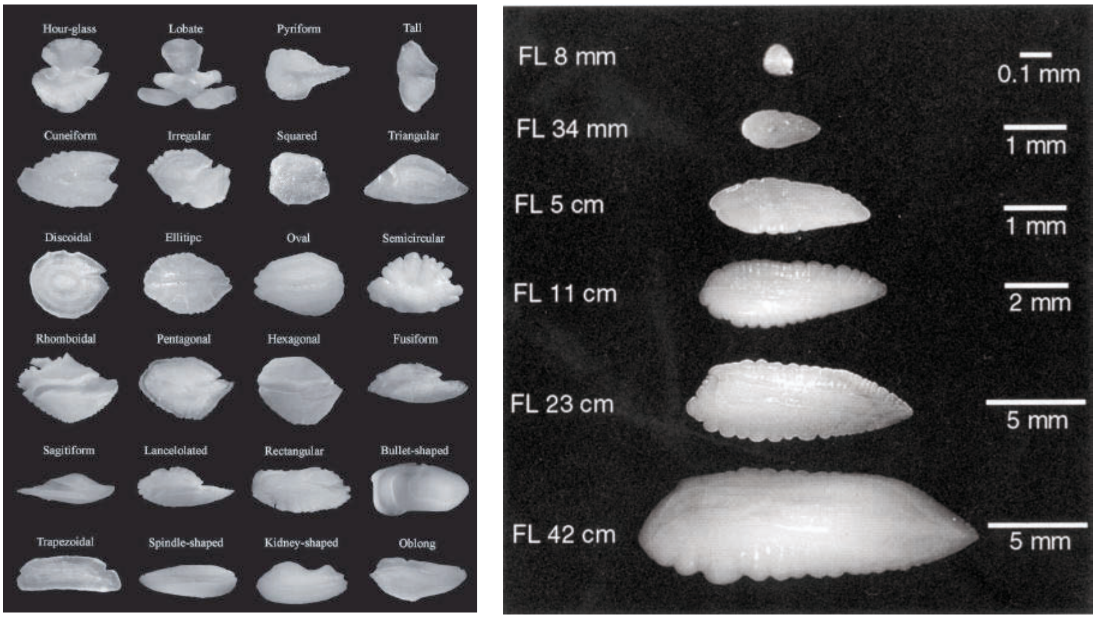

Figura 3. Variación de la forma del otolito (*sagitta*) y su denominación según las distintas especies (izda., foto extraída de Tuset *et al.,* 2008). Secuencia ontogénica del otolito *sagitta* de abadejo mostrando el cambio de tamaño desde el estado de larva al de un adulto de 6 años (dcha., foto extraída de Campana, 2004).

entre poblaciones, los otolitos han sido también empleados para caracterizar y diferenciar *stocks* de peces (Campana & Casselman, 1993; Stransky, 2005; Vignon & Morat, 2010).

La forma y la masa del otolito no son constantes a lo largo de la vida del pez (Figuras 3 y 4). Debido a sus funciones en el mantenimiento del equilibrio y orientación, los otolitos crecen a medida que lo hace el pez, por lo que hay una estrecha relación entre el tamaño del otolito y el tamaño del pez (Hunt, 1992). Así, conociendo la dimensión del otolito (longitud y/o peso) es posible estimar la talla del pez (Figura 4). Sin embargo, estas relaciones no deben aplicarse de forma generalizada, pues existen diferencias entre poblaciones o grupos de peces con distintas tasas de crecimiento (Campana, 1990).

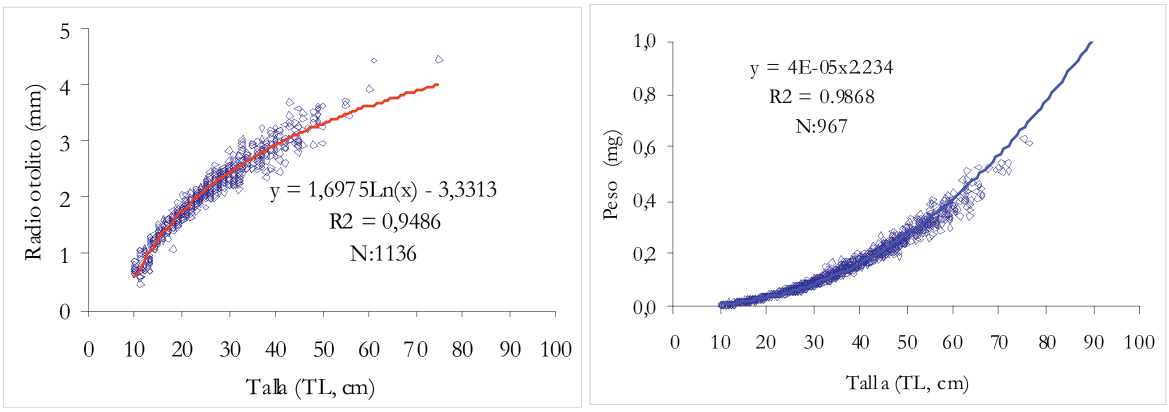

Figura 4. Relación entre la talla del pez y el tamaño del otolito *sagitta* (peso y radio ventral) de merluza del noroeste peninsular (datos del IEO elaborados por la autora).

El otolito crece por adición de capas concéntricas de proteínas y carbonato cálcico, que se depositan siguiendo los ritmos internos del metabolismo del calcio y de la síntesis de aminoácidos (Dannevig, 1956). Estos ciclos de deposición, o anillos de crecimiento, son de distinta densidad óptica y espesor (Figura 5), de manera que una vez confirmada su periodicidad (anual o diaria) permiten estimar la edad de los individuos (Pannella, 1980; Gauldie & Nelson, 1990).

Los primeros estudios sobre otolitos de peces datan de finales del siglo XIX. Reibisch (1899) fue el primero que observó al microscopio la presencia de anillos anuales en los otolitos, constituidos por bandas alternas, translúcidas y opacas. Desde entonces y hasta principios de los años 70, los otolitos se han utilizado fundamentalmente para la estimación de la edad mediante el recuento de los anillos anuales o estacionales en peces adultos (Figura 5). En 1971, Pannella documenta por primera vez la presencia de micro-incrementos de crecimiento de periodicidad diaria en los otolitos (Pannella, 1971; 1974; Brothers *et al.,* 1976). Este descubrimiento marca un hito en la esclerocronología, a partir del cual se abren nuevas vías para estudiar el crecimiento (Campana & Nelson, 1985), principalmente aplicables a los primeros estadíos del ciclo vital de peces y especies tropicales, donde los cambios estacionales son muy leves. La existencia de microincrementos diarios permite la estimación de la edad y el crecimiento diario con bastante exactitud en larvas, juveniles e incluso adultos (Campana, 2001; Wright *et al.*, 1991; 1998).

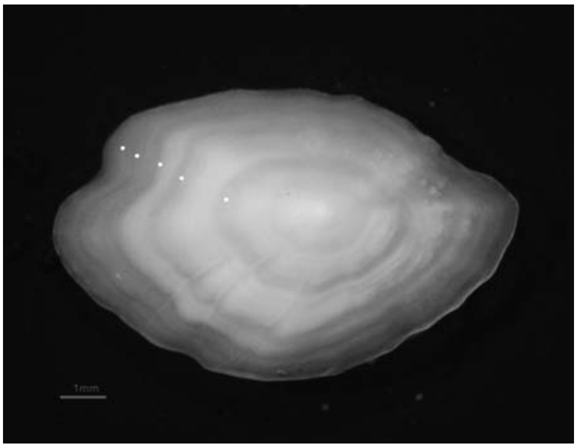

Figura 5. Otolito de gallo (*L. boscii)* de un individuo de 5 años, mostrando la secuencia de anillos traslúcidos y opacos (foto de C. Piñeiro).

El otolito presenta tres tipos de estructuras de crecimiento:

<u>Incrementos primarios</u>. Corresponden a procesos de ritmo circadiano endógeno sincronizados con cambios cíclicos del medio ambiente y son de periodicidad diaria. Un incremento diario es una microestructura compuesta por dos unidades de distinta composición química y apariencia óptica (Wright *et al.*, 2002). Estos incrementos solo son visibles al microscopio electrónico o de alta definición.

<u>Incrementos anuales</u>. Son macroestructuras que se forman con periodicidad anual (también llamadas *annuli*) y presentan una sucesión de varios incrementos estacionales, generalmente dos, de diferente opacidad y permiten estudiar el crecimiento de los adultos. Aunque se pueden observar a simple vista, se analizan con microscopio estereoscópico de baja definición.

<u>Discontinuidades</u>. Son estructuras no periódicas de naturaleza irregular y asociadas a períodos de estrés fisiológico y/o ambiental sufridos por el pez a lo largo de la vida. Las discontinuidades más evidentes denominadas *checks* corresponden a cambios vitales del individuo tales como la eclosión,

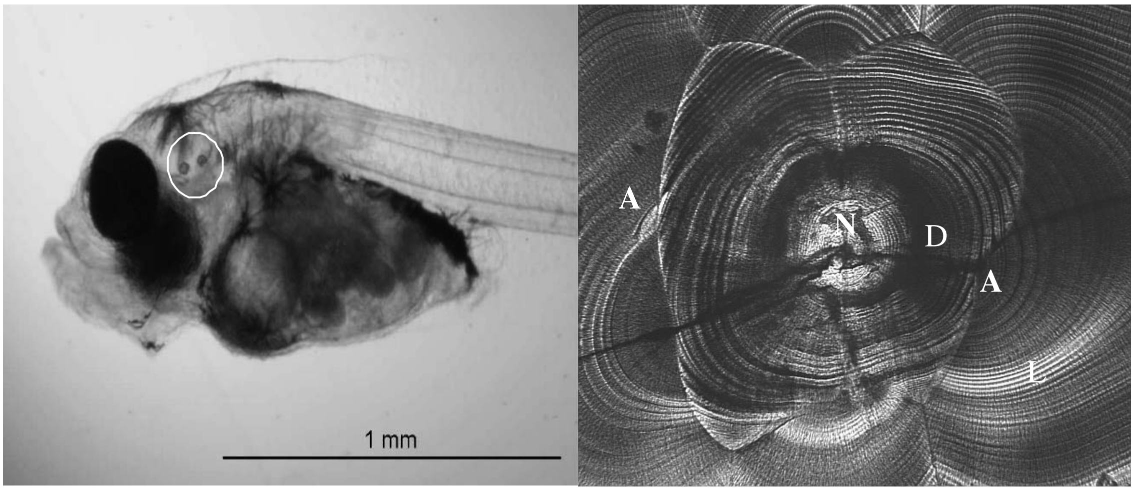

Figura 6. Cabeza de una larva de *M. merluccius* de 19 días de vida, mostrando con un círculo la posición del par de otolitos *sagitta* (izda., foto de Sánchez *et al.,* 2011). Forma característica del primordio del otolito una vez pulida la sección sagital del mismo: en la zona central se muestra el núcleo principal (N), los centros accesorios (A) y los primeros incrementos compuestos por una zona traslúcida (L) y una zona opaca (D) observada bajo luz transmitida (dcha., foto de J. Rey, 400x).

la primera alimentación u otros factores probablemente causados por ritmos de alimentación, madurez sexual, migraciones o cambios ontogénicos en las primeras fases del desarrollo (Brothers, 1978; Pannella, 1980; Campana & Neilson, 1982; Campana, 1983).

La formación del otolito se inicia en los estados más avanzados del desarrollo embrionario (4-6 días en el caso de la merluza) (Figura 6, izda.) con la formación de un núcleo central de materia orgánica, denominado *primordium,* sobre el que se depositan con periodicidad diaria sucesivas capas concéntricas de distinta densidad óptica (Campana & Neilson, 1985; Jones, 1986; Stevenson & Campana, 1992) (Figura 6, dcha.). La matriz orgánica proporciona un soporte que favorece la nucleación de los cristales de aragonito. El núcleo carece generalmente de anillos y los cristales crecen perpendicularmente desde él hacia la periferia del otolito en equilibrio isotópico con el agua del mar. En su etapa inicial, estas deposiciones no son muy obvias debido a su escaso grosor. Además, se pueden formar centros de crecimiento accesorios en relación con cambios fisiológicos, del hábitat o del comportamiento (A en Figura 6). Llegado el momento se hacen patentes las discontinuidades en forma de anillos opacos y traslúcidos. El primer anillo bien marcado se deposita en un período comprendido entre la eclosión del huevo y el comienzo de la alimentación activa de la larva, dependiendo de la especie. Existe una variada nomenclatura para los términos referidos a los anillos de crecimiento. En el 1er Simposio Internacional de Otolitos (*Fish Otoliths Research and Application*) celebrado en 1993, se propusieron los términos zona L (*light*) y zona D (*dark*) para las capas ricas en mineral y materia orgánica, respectivamente (Kalish *et al.,* 1995; Panfili *et al.*, 2002).

Muchos trabajos científicos asumen que los microincrementos se forman con una periodicidad diaria. Sin embargo, esto no es válido para todas las especies, ya que pueden registrarse incrementos sub-diarios, que junto a los *checks,* generan confusión en la interpretación de la microestructura del otolito (Volk *et al.*, 1995). Así, análisis comparativos entre lectores han demostrado que esta interpretación conlleva cierta subjetividad (Campana & Neilson, 1985; Campana & Moksness, 1991).

A escala macroscópica, los otolitos suelen mostrar una secuencia de anillos en la que se alternan opacos y traslúcidos de distinto grosor y que corresponden a períodos con diferentes tasas de crecimiento (Fablet *et al.,* 2009). Aunque existen múltiples hipótesis para explicar las causas de la formación de estos anillos y los patrones de deposición dependen de la especie (Wright *et al.,* 1990; Beckman & Wilson, 1995), se ha asociado la formación de anillos o zonas opacas a períodos de crecimiento rápido, alta actividad metabólica y la formación de zonas traslucidas a períodos de crecimiento ralentizado relacionados con períodos fríos o de menor producción (Wright *et al.,* 1991; 2002). Sin embargo, la relación no parece estar siempre clara y el amplio rango de interpretaciones existente refleja la influencia de una combinación de factores que pueden variar entre especies (Wright, 1990).

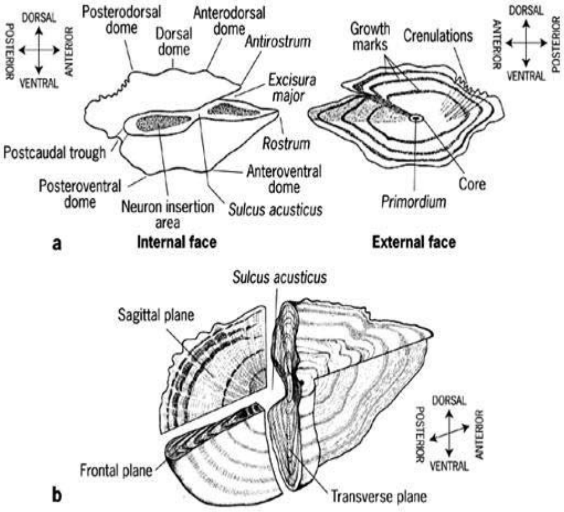

Figura 7. Anatomía del otolito *sagitta* mostrando los distintos planos de orientación que se usan habitualmente para la observación de los anillos de crecimiento: a) caras externa e interna del típico otolito; y b) tres planos de orientación del mismo: sagital, frontal y transversal.

En algunas ocasiones es posible observar estas macroestructuras sin necesidad de tratamiento previo, pero en general es necesario preparar de algún modo el otolito (Mc Curdy *et al.,* 2002).

Técnicas de preparación y observación de otolitos

Gran número de trabajos y manuales describen las técnicas para procesar y examinar otolitos con el fin de interpretar la edad a escala anual (Chilton & Beamish, 1982; Beamish & McFarlane, 1987; Pentilla & Dery, 1988; Panfili *et al.,* 2002) o diaria (Secor *et al.,* 1991; Stevenson & Campana, 1992). Las técnicas al uso son laboriosas y comprenden la extracción, limpieza, almacenamiento, obtención de la sección, pulido y un montaje adecuado para visualizar los incrementos (Stevenson & Campana, 1992). Las técnicas elegidas para procesar los otolitos dependen del objetivo del estudio, la morfología del otolito (Figura 7), preferencias del laboratorio y medios económicos disponibles. Sin embargo, en las dos últimas décadas se ha hecho patente la necesidad de estandarizar las metodologías empleadas para preparar y examinar los otolitos de cada especie con el fin de poder comparar las lecturas de edad aportadas por diferentes laboratorios, sobre todo cuando se trata de un mismo recurso explotado por distintos países (Eltink *et al.,* 2000; Mc Curdy *et al.,* 2000).

Para determinar la edad a escala anual, es necesario discernir los anillos de crecimiento anuales, descartando los falsos anillos o *checks*. En general, los anillos anuales se distinguen de los falsos por estar más marcados, por su formación regular en toda la pieza calcárea y por seguir el patrón de crecimiento del pez. Para ello es fundamental conocer la biología de la especie, su ciclo vital y las condiciones ambientales en las que vive.

La estimación de la edad a escala diaria se basa en la interpretación y recuento de los anillos diarios en el otolito (Figura 8). Para ello, existen dos aspectos que deben tenerse en cuenta: la edad en el momento del primer incremento y la certeza sobre la periodicidad diaria de los incrementos observados.

El tratamiento de los otolitos para estudios de crecimiento diario es más complicado que el anual, porque es necesario obtener una lámina fina que, al ser atravesada por la luz, muestre los microincrementos desde el núcleo del otolito hasta el borde (Figura 8). Para ello, se realiza un corte sagital o transversal del otolito. El núcleo indica el momento del nacimiento del pez y a partir de él se cuentan las marcas de crecimiento diario. La llegada de nuevos y sofisticados sistemas de análisis de imagen para identificar, contar y medir los microincrementos ha añadido rapidez y precisión a esta técnica. Además, cada vez se utiliza más la microscopía electrónica de barrido para contar correctamente, interpretar y validar la deposición diaria de anillos (Stevenson & Campana, 1992; Panfili *et al.* 2002).

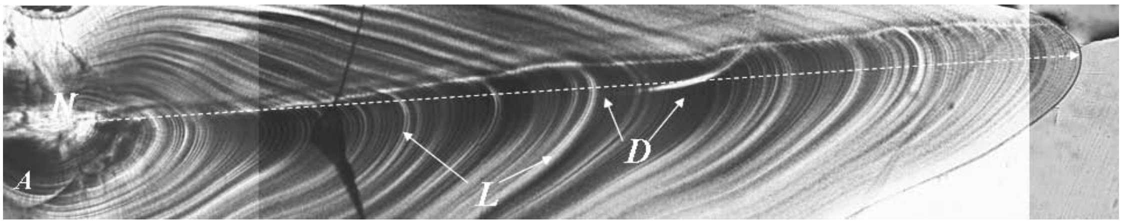

Figura 8. Sección transversal de un otolito *sagitta* de una ejemplar de *M. merluccius* de 244 mm (TL) capturado en septiembre del 2004, mostrando el núcleo (N), los centros accesorios (A), las zonas traslúcidas y opacas (L y D) bajo luz transmitida (200x) (Foto de C. Piñeiro).

1.1.4. VALIDACIÓN

Para estimar la edad, además de contar los incrementos o anillos de crecimiento visibles en los otolitos, es necesario confirmar con qué periodicidad se forman estos, es decir, se necesita la validación. Los problemas en la estimación de la edad a partir del recuento de anillos en los otolitos afectan tanto a la precisión como a la exactitud de las estimaciones. El primer problema se resuelve fácilmente, mientras que el segundo es muy difícil, debido a la falta de piezas calcáreas de edad conocida (Eltink *et al.* 2000; Campana, 2001).

En las últimas décadas ha surgido una gran preocupación en el ámbito científico por la validez de los métodos empleados en la estimación de la edad de los peces. Así, en la década de los 80, investigadores de reconocido prestigio cuestionaron las metodologías empleadas en las estimaciones de edad de muchas especies (Beamish & McFarlane, 1983; Casselman, 1983). Desde entonces, cada vez son más las instituciones involucradas en la determinación de la edad que han comprendido la importancia de los estudios de validación, lo que ha originado una revisión general de los procedimientos utilizados en la estimación de edades en peces y la proliferación de trabajos dedicados a la validación (Brothers *et al.*, 1976; Beamish & Mc Farlane, 1987; Panfili *et al.*, 2002; Kimura & Delsa, 2005).

En este contexto, la validación es un proceso que confirma la exactitud de un método para determinar la edad. La distinción entre validar la periodicidad con la que se forma un incremento y determinar la edad absoluta es importante. Beamish & Mc Farlane (1983) identificaron la validación de la periodicidad de un *annulus* con la validación de la edad. Pero esto implica que todos los grupos de edad deben ser validados para que se pueda confirmar la exactitud de la edad. Si esto se lleva a cabo de forma rigurosa, la validación de la formación de un *annulus* para cada uno de los grupos de edad equivaldría a la validación absoluta de la edad, sin embargo tanto rigor rara vez es posible (Campana 2001). En un glosario de Kalish *et al.* (1995) sobre la terminología al uso en otolitos se hace especial hincapié en que la validación se refiere más a validar el método que la edad, de modo que determinar la periodicidad del incremento solo sería una parte del método. La inmensa mayoría de los trabajos publicados equiparan la confirmación de la periodicidad anual de los incrementos con la validación de la edad. Desde que se publicó el trabajo de Beamish & McFarlane (1983), se han realizado muchos estudios sobre validación de la edad, de los cuales solo un 15% validan la edad absoluta de peces en su medio natural, mientras que más del 50% validaron la periodicidad del incremento para un solo grupo de edad. Lo que implica dejar sin examinar la mayoría de los grupos de edad problemáticos, como son los más jóvenes y los más viejos, que sin embargo tienen una gran influencia en la estimación del crecimiento, la longevidad y la mortalidad poblacional (Campana, 2001). Los estudios de validación son, por tanto, un requisito para estimar correctamente la edad a partir de piezas calcificadas. Panfili *et al.* (2002) agrupa los métodos disponibles para validar la frecuencia de formación de los incrementos o anillos de crecimiento en cuatro categorías, ordenadas de mayor a menor complejidad: validación indirecta, verificación, validación semidirecta y validación directa.

Validación indirecta

La validación indirecta implica la comparación de las edades individuales estimadas a partir de piezas calcificadas con edades estimadas estadísticamente, principalmente a partir de distribuciones de frecuencia de tallas. Es una técnica basada en la población y corrobora o no la interpretación de la edad. Sin embargo, no valida la periodicidad con que se forman los incrementos del otolito. Petersen (1891) fue el primero en identificar modas que correspondían a grupos de edad en las distribuciones de tallas y desde entonces se han desarrollado muchos métodos para identificar clases de edad a partir de la composición de tallas (Panfili *et al.,* 2002). Estos incluyen métodos gráficos (Cassie, 1954; Battacharya, 1967) y métodos computacionales como el de máxima verosimilitud de Hasselblad

(1966), combinado con información previa relacionada con las clases de edad presentes y su talla media (McDonald & Pitcher, 1979; Sparre & Venema, 1997) o incorporando información biológica (Schnutte & Fournier, 1980). Estimar la edad separando los componentes de edad de una distribución de frecuencias de tallas, es difícil cuando:

- la especie tiene un período de puesta relativamente largo en el que puede haber varios picos de freza (Hilborn & Walters, 1992);
- existe gran variabilidad en las tasas de crecimiento o en el caso de adultos de especies longevas, donde la variabilidad de sus tasas de crecimiento incrementa con la edad.

Ambas condiciones producen un gran solapamiento de tallas modales que impide la separación de grupos de edad. Por ello, éste método de validación sólo sirve para los primeros grupos de edad de especies de crecimiento rápido (Panfili *et al.,* 2002).

Otro método de validación indirecta es el análisis del crecimiento diario. Está demostrado que el análisis de los incrementos diarios puede ayudar a la interpretación de la macroestructura del otolito (Arneri *et al.,* 1998) y es utilizado normalmente para validar el primer *annulus*. Sin embargo, primero es necesario validar la formación diaria de los microincrementos (Campana, 2001).

Cuando el tamaño de la pieza calcificada crece linealmente con el tamaño del pez, se puede reconstruir la historia del crecimiento del pez (Figura 9). Esta técnica se denomina **retrocálculo** y permite estimar el crecimiento y las dimensiones corporales en edades o etapas previas de la vida del ejemplar estudiado. En concreto, se usan las dimensiones de los incrementos o anillos de crecimiento de la pieza calcificada, junto a la talla del pez en el momento de la captura, para estimar la talla en el tiempo de formación de cada anillo (Francis, 1990; 1995). Es necesario establecer primero una relación entre el tamaño de la pieza calcificada (ej. otolito) y el del pez. Así es posible, por ejemplo, calcular la talla teórica del pez en el momento de formación de cada anillo. Se distinguen dos aproximaciones: *i*) los métodos proporcionales; y *ii*) el método de regresión. Ambas utilizan los parámetros de la ecuación que relaciona la longitud del pez y el tamaño del otolito en el momento de la captura. El método de regresión no tiene en cuenta la variación en el tamaño del otolito entre los peces, mientras que los métodos proporcionales incorporan la información de cada pez, lo que tiene en cuenta la variación indivi-

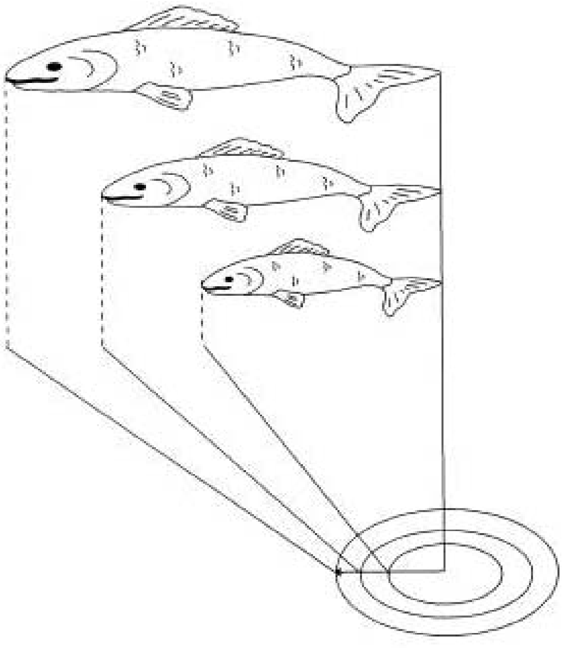

Figura 9. Esquema que muestra el retrocálculo del crecimiento en etapas previas de la vida del pez, mediante el uso de otolitos (Modificado de Bagenal & Tesch, 1978).

dual. Para aplicar esta técnica deben cumplirse las siguientes condiciones: *i)* no hay absorción ni degeneración de la pieza calcificada; *ii)* existe proporcionalidad entre el crecimiento somático y el crecimiento del otolito; y *iii)* conocer la edad de formación de los anillos de crecimiento (Folkvord & Mosegaard, 2002).

Esta técnica tiene además ciertas limitaciones y una de las más conocidas es el llamado fenómeno de Lee (1912) por el que la talla media retrocalculada para un grupo de edad es más pequeña cuando se recalcula a partir de peces más viejos que cuando se hace a partir de peces más jóvenes (Ricker, 1975). Este fenómeno puede deberse a la mortalidad selectiva, al muestreo sesgado, a problemas con la metodología aplicada al retrocálculo o a una combinación de todos ellos (Panfili *et al.,* 2002).

Verificación

En este contexto verificar la edad consiste en cuantificar el grado de acuerdo entre una o más lecturas de una o más piezas calcificadas. La verificación del criterio de interpretación de la edad para una determinada especie se realiza normalmente mediante la interpretación de una colección de piezas calcificadas, que se circula entre los lectores de varios laboratorios, se comparan los resultados y posteriormente se unifican los criterios de interpretación (Eltink *et al.,* 2000).

Para verificar la interpretación de la edad es importante estimar los errores que influyen en la precisión y en la exactitud de la misma. La precisión indica la proximidad de las medidas a un mismo valor y se describe mediante el coeficiente de variación, sin embargo, la exactitud es la proximidad de una estimación al valor real. Los índices de precisión se obtienen fácilmente y pueden dar información muy útil sobre las fuentes de error en los estudios de crecimiento (Campana, 2001; Morales-Nin & Panfili, 2002). Así, para evaluar el grado de dificultad que representa datar diferentes especies se suelen comparar métodos y/o los lectores distintos (Campana & Jones, 1992; Eltink *et al.,* 2000). Sin embargo, el acuerdo entre lecturas de diferentes piezas calcáreas no significa que los incrementos tengan un significado temporal.

Para determinar el grado de acuerdo y precisión entre lecturas y/o lectores se utilizan varios índices y pruebas estadísticas:

i) **Porcentaje de acuerdo** (PA, *percentage of agreement*). Es el más sencillo, basta calcular el porcentaje de lecturas coincidentes con respecto al total de lecturas realizadas.

ii) **Porcentaje medio de error** (APE, *average percentage error*) (Beamish & Fournier, 1981) que se calcula mediante la fórmula:

$$APE = 100\% \times \frac{1}{R}\sum_{i=1}^{R}\frac{\left|Xij - \overline{Xj}\right|}{\overline{Xj}}$$

Donde R = número de veces que es datado el pez

Xij =es la i-ésima estimación de la edad del j-ésimo pez

$\overline{X}j$ =edad media del j-ésimo pez

iii) **Coeficiente de variación** (CV, *coefficient of variation*)

$$CV = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(Xij - \overline{Xj}\right)^2}{R-1}}}{\overline{Xj}}$$

El CV es estadísticamente más robusto que el APE y más apropiado para comparar edades que el PA convencional, ya que tiene en cuenta la edad media del pez (Morales-Nin & Panfili, 2002).

Validación semidirecta

La validación semidirecta consiste en la observación de la evolución de la zona marginal de crecimiento de las piezas calcáreas de un gran número de individuos a lo largo del tiempo (Beckman & Wilson, 1995; Morales-Nin & Panfili, 2002). Para ello, se analiza el borde del otolito seleccionando una marca de referencia y siguiendo su formación en el tiempo (Figura 10). Esto se puede hacer de dos formas: cuantitativamente, midiendo las distancias del último anillo al borde del otolito, y cualitativamente, siguiendo el tipo de borde a lo largo del tiempo. El resultado es una media del porcentaje de observaciones en la población y se usa para validar la deposición estacional de incrementos o anillos.

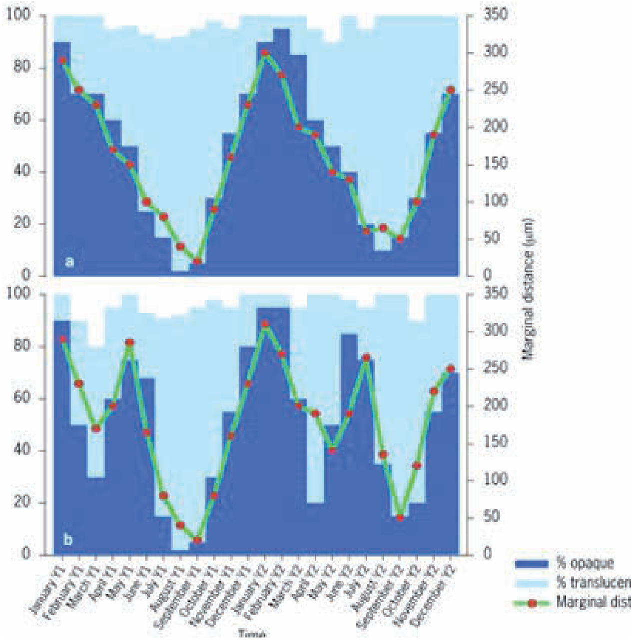

Figura 10. Evolución teórica del crecimiento marginal de una pieza calcificada mediante la observación microscópica de la anchura del borde y del tipo de borde en el tiempo. En azulón % borde opaco y en celeste % borde traslúcido (extraído de Panfili *et al.,* 2002).

Este método es muy popular, pues tiene pocos requerimientos de muestreo y es de bajo coste, aunque es uno de los más difíciles de validar por las dificultades técnicas asociadas tanto a la visualización del incremento marginal (muy dependiente de la refracción de la luz a través del borde del otolito, que se hace cada vez más fino cuanto más cerca del borde), como a la falta de comprensión de los mecanismos implicados en la formación de los incrementos (Beckman & Wilson, 1995). La aplicación de esta técnica puede, por lo tanto, ser muy subjetiva. Pese a ello, cuando no hay otro método de validación posible, es una técnica útil sobre todo para ejemplares jóvenes y de crecimiento rápido (Campana, 2001).

Validación directa

La validación directa se basa en la introducción o presencia de una marca de referencia temporal sobre la pieza calcárea. Esta marca puede ser química o intrínsecamente inducida. La inducción química se realiza mediante aplicación de una sustancia (ej. una terramicina como oxitiltetraciclina, OTC; alizarina, etc.) mediante inyección (Figura 11), inmersión o alimentación para que su rápida incorporación junto al calcio deje una marca permanente en las piezas calcificadas del pez marcado. Este tipo de marca se deposita en el incremento que se forma después del marcado y se hace visible con luz ultravioleta (Wright *et al.,* 2002).

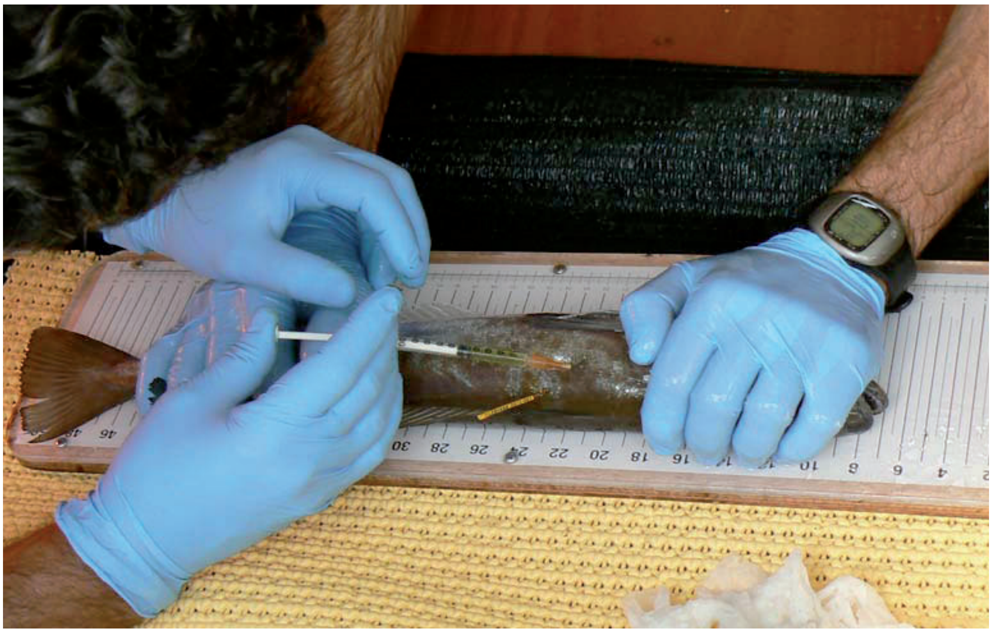

Figura 11. Proceso de marcado de merluza: marca externa intramuscular a la altura de la aleta dorsal (filamento de color amarillo, tipo «Floy Tag») e interna, inyección de oxitetraciclina (OTC). (Foto de C. Piñeiro).

Habitualmente, el marcado químico se combina con el marcado externo convencional (Figura 11), que consiste en la captura, marcado químico del otolito, marcado externo del pez, suelta y recaptura de ejemplares (Beamish & Chilton, 1982; Geffen, 1992; McFarlane & Beamish, 1995).

Es uno de los métodos más rigurosos para confirmar el significado temporal de las estructuras de crecimiento. La base de estos experimentos es el registro de la talla del pez, del tamaño del otolito y del número de anillos que se depositan en él en dos momentos conocidos: la primera captura y la recaptura, lo que permite calcular el incremento de talla del pez y del otolito en un período de tiempo conocido (Figura 12). Su ventaja es la alta fiabilidad de los datos individuales y su principal inconveniente, la gran cantidad de recapturas que son necesarias para poder estimar parámetros de crecimiento poblacionales. Este método se ha utilizado para validar la formación de los incrementos en una amplia variedad de piezas calcificadas de distintas especies (Casselman, 1974; Wild & Foreman, 1980; Beamish & Chilton, 1982; Brown & Gruber, 1988; Laurs *et al.*, 1985; Fowler, 1990; Geffen, 1992; Panfili *et al.,* 2002; Landa *et al.,* 2008).

Existen ciertos fenómenos o eventos medioambientales que pueden provocar la creación de marcas en las piezas calcificadas de todos los peces de una población (Eltink & Kuitier, 1989; Panfili *et al.,* 2002). Estas marcas de origen natural pueden emplearse para validar la formación de incrementos sobre una parte de la historia de la vida de los ejemplares de esa población. Así, se ha aplicado, por ejemplo, a la merluza del Pacífico (MacLellan & Saunders, 1995). Sin embargo, dichas marcas rara vez ocurren y es imprescindible que sean inequívocamente identificables (Casselman, 1987).

Desde que Beamish & McFarlane (1983) pusieron de relieve la importancia de la validación en las estimaciones de edad se ha avanzado mucho para asegurar un control de calidad de la estimaciones (Campana, 2001; Wright *et al.,* 2002). En Europa, el interés científico del tema ha motivado a la Unión Europa a apoyar proyectos de gran alcance como EFAN (*«European Fish Ageing Network»; EFAN; FAIR PL.96.1304: 1998-2000*) y TACADAR *(«Towards Accreditation and Certification of Age Determination of Aquatic Resources» 2002-2005*, Q5CA-2002-01891). Desde 1997 hasta 2006, estos proyectos han propiciado la colaboración en investigación destinada a asegurar el control de calidad de la estimación de la edad (ICES, 1994; Eltink, 1997; Eltink *et al.,* 2000, Appelberg *et al.,* 2005) para que se convierta en un proceso rutinario y fiable, desterrando así la concepción tradicional de que la estimación de la edad de los peces era más un «arte» que una ciencia (Panfili *et al.,* 2002; Kimura & Delsa, 2005).

Órganos consultivos internacionales como el ICES (*International Council for the Exploration of the Sea*), han reconocido también la importancia de la estimación de la edad y existen actualmente varias normas europeas que requieren datos de edades, como el Programa comunitario de recopilación de datos biológico-pesqueros de la UE (DCF), la Directiva Marco del Agua (DMA) y el Reglamento sobre la biodiversidad. Todo esto obligará a que en un futuro muy cercano las instituciones europeas tengan que obtener las certificaciones pertinentes que acrediten la calidad de la información que generan, como ya existe en instituciones de otras regiones del Pacífico y de América que trabajan en el mismo campo (ej. *International Pacific Tuna Commission, Pacific Halibut Commission*).

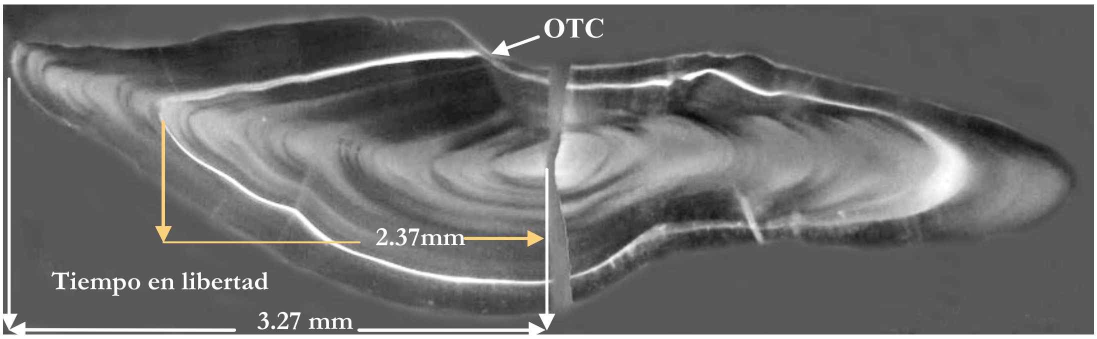

Figura 12. Sección transversal de un otolito *sagitta* de un ejemplar de *M. merluccius* marcado en octubre de 2004, con 29 cm de talla (TL) y recapturado después de 347 días en libertad. Crecimiento del otolito entre la captura (marca visible de OTC) y la recaptura (borde del otolito): 0.90 mm. (Foto de L. Rodríguez).

1.2. LA MERLUZA EUROPEA: *Merluccius merluccius* (Linnaeus, 1758)

La merluza europea (*Merluccius merluccius* L., 1758) es una de las especies demersales más importantes de los ecosistemas marinos de la plataforma y talud continental del Atlántico nororiental y Mediterráneo, tanto por su gran abundancia y amplia distribución, como por su papel en la cadena trófica. Es una especie comercialmente muy apreciada, empleándose casi exclusivamente para consumo humano (Casey & Pereiro, 1995; Oliver & Massutí, 1995; Papaconstantinou & Stergiou, 1995).

En la actualidad existen 13 especies descritas del género *Merluccius*, distribuidas principalmente en el Atlántico y Pacífico oriental (Lloris *et al.,* 2003). Aunque *M. merluccius* no es la más importante en términos de capturas, ostenta el mayor precio de mercado y es una de las más estudiadas (Alheit & Pitcher, 1995) (Figura 13).

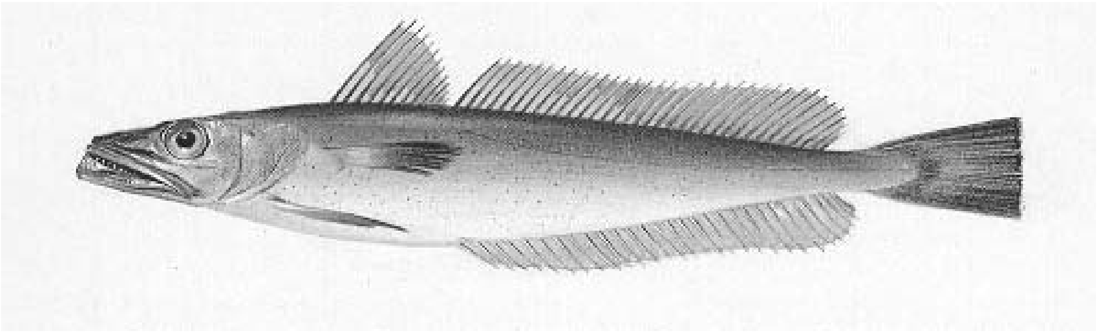

Figura 13. Merluza europea (*Merluccius merluccius*, Linnaeus, 1758) (imagen de Lloris *et al.,* 2003)

En cuanto a su posición taxonómica, la merluza *Merluccius merluccius* es un gadiforme de la clase *Actinopterygii*, cuya clasificación sistemática ha sido descrita por Inada (1989) y Cohen *et al.* (1990). Al igual que las otras especies del género, el cuerpo de la merluza es fusiforme simétrico y cubierto de escamas pequeñas (Figura 13). Su forma y sus huesos planos, junto a su cuerpo hidrodinámico, le dotan de una gran capacidad de aceleración (Alheit & Pitcher 1995). Descripciones más detalladas de esta especie pueden encontrarse en Svetovidov (1986), Inada (1989) y Lloris *et al.* (2003), entre otros.

1.2.1. IMPORTANCIA PESQUERA

La merluza europea (en adelante «la merluza») constituye un recurso pesquero de primera magnitud en el Atlántico nororiental, siendo España el país responsable de la mayor parte de los desembarcos, seguido de Francia y Portugal. Estos alcanzaron su nivel más alto en 1948, con 175 000 t, y descendieron hasta un valor medio de 80 000 t a finales de los años 80 y principios de los 90 (Casey & Pereiro, 1995). En la última década aumentaron ligeramente hasta superar las 88 000 t en 2010 (ICES, 2011) (Figura 14).

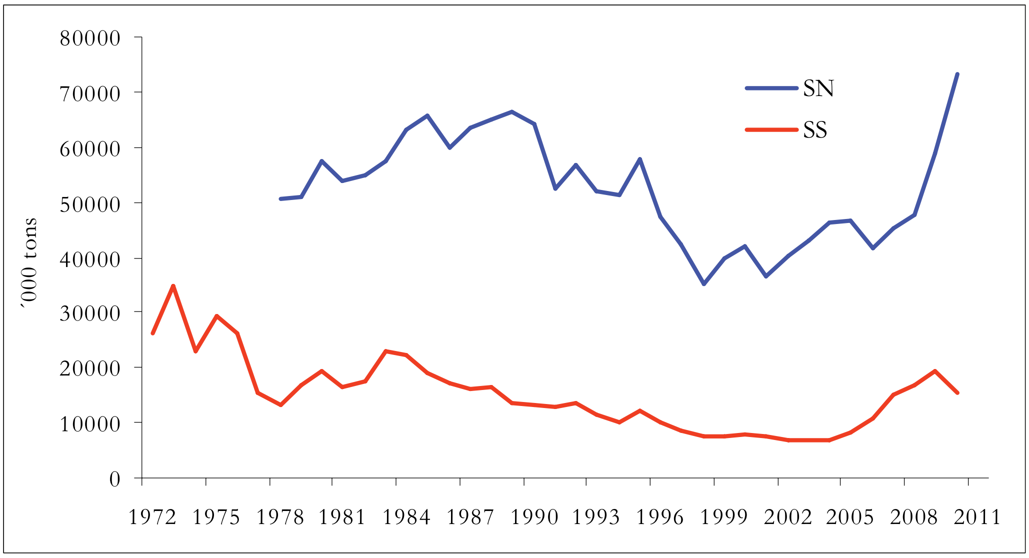

Figura 14. Evolución de los desembarcos de merluza en el Atlántico nororiental repartidos por *stocks*: norte (SN) y sur (SS) (WGHMM, 2011 ICES).

En el Atlántico y por motivos prácticos, esta especie se gestiona separadamente en dos unidades o *stocks*: norte y sur. Esta separación se basa principalmente en dos criterios: *i)* la existencia de Cap Bretón, un cañón submarino de 4 000 m de profundidad (Cassey & Pereiro 1995; ICES, 1979), tradicionalmente considerado una barrera geográfica que podría limitar cualquier intercambio entre las dos supuestas poblaciones; y *ii)* la observación de dos áreas de puesta, al norte en el Mar Céltico y Golfo de Vizcaya, y al sur a lo largo de la Península Ibérica. Se asume que la primera nutre a las pesquerías del *stock* norte, mientras que la segunda nutriría a las del *stock* sur. Así, desde 1978, el ICES evalúa anualmente el estado de explotación de ambos *stocks*. Sin embargo, la falta de evidencias concluyentes sobre la existencia de dos poblaciones de esta especie (ICES, 1979; Roldan *et al.,* 1998; Cassey & Pereiro, 1995; Pita *et al.,* 2011) y los resultados de investigaciones realizadas durante la última década, ponen en cuestión dicha separación. Así, estudios genéticos muestran una estructura más compleja que la simple existencia de dos *stocks* (Plá y Roldan, 1994; Roldán *et al.,* 1998; Lundy *et al.,* 1999), sugiriendo una posible conectividad entre poblaciones (Roldán *et al,* 1998; Castillo *et al.,* 2004; Pita *et al.,* 2010). Por otro lado, los estudios de Lundy *et al.* (2000) indican que Cape Bretón no es una barrera efectiva para la migración, al tiempo que estudios basados en parásitos han demostrado la existencia de una deriva genética entre el Atlántico norte y el Mediterráneo oriental (Mattiucci *et al.,* 2004; Cimmaruta *et al.,* 2005). A pesar de todo ello, hasta el momento estas evidencias no han sido consideradas suficientemente concluyentes como para reconsiderar las unidades de *stock* de esta especie (ICES 2007b).

En los últimos años, los *stocks* de merluza han estado sometidos a una alta presión pesquera, lo que ha provocado una gran preocupación en el ámbito científico (ICES, 2007a y b), que ha llevado a la Unión Europea a implementar planes de recuperación para ambos *stocks* (EC Nº 811/2004 y EC Nº2116/2005). Sin embargo, el conocimiento incompleto o erróneo de aspectos claves de su biología y ecología, principalmente del crecimiento y de la estructura espacial de sus poblaciones, introduce un alto grado de incertidumbre en la evaluación del estado de sus poblaciones en la que se basan tanto la gestión como los planes de recuperación.

1.2.2. DISTRIBUCIÓN Y HÁBITAT

Distribución geográfica

Merluccius merluccius (Linnaeus 1758) se distribuye en el Atlántico nororiental y centrooriental desde las costas de Noruega (aprox. 70º N) hasta Cabo Blanco, en Mauritania (aprox. 21º S) (Figura 15), donde cohabita con otras dos especies del género: *Merluccius senegalensis* y *Merluccius polli* (Cadenat, 1950). *Merluccius merluccius* es especialmente abundante entre las Islas Británicas y el sur de la Península Ibérica (Casey & Pereiro, 1995). Longitudinalmente se extiende desde Islandia hasta el Mar Negro (Lloris *et al.,* 2003) y también se ha observado ocasionalmente en las Azores (Casey & Pereiro, 1995; Inada, 1981, Lloris *et al.,* 2003 y Whitehead *et al.,* 1984). De las especies del género, *Merluccius merluccius* es la que ocupa el rango latitudinal y longitudinal más amplio, lo que refleja su gran capacidad de adaptación a las diferentes condiciones oceanográficas en su área de distribución. En general, se puede decir que es una especie que abunda en regiones de afloramiento con alta producción asociadas a corrientes oceánicas de alta variabilidad (Meiners, 2007).

Distribución batimétrica

La merluza se encuentra entre 25 y 1 075 m de profundidad (Stefanescu *et al.,* 1992; Lloris *et al.,* 2003; Domínguez-Petit, 2007), aunque ocupa preferentemente fondos de plataforma y talud continental entre 50 y 370 m de profundidad (Cohen *et al.,* 1990).

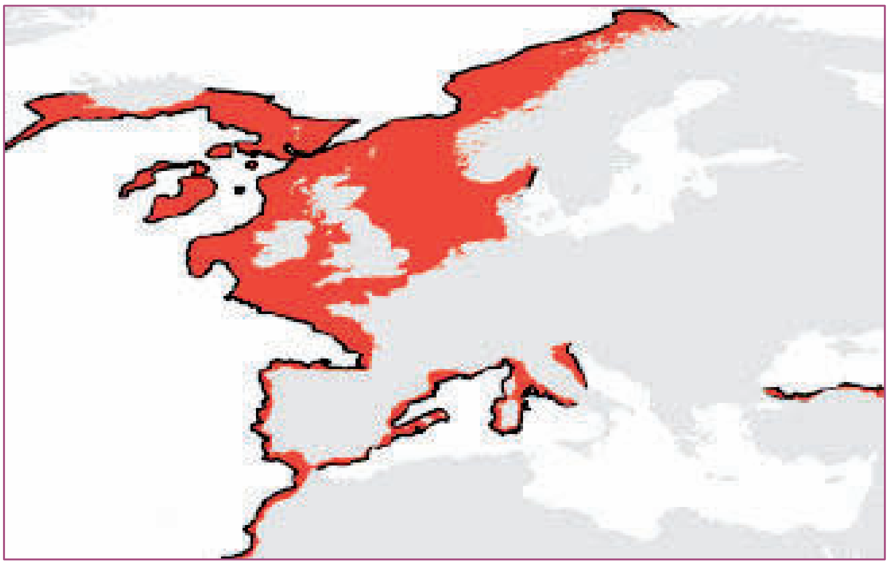

Figura 15. Distribución geográfica de la merluza europea en el Atlántico nororiental (mapa de la FAO disponible en *www.fao.org/figis/geoserver/factsheets/species.html#speciesSelectorPanel*).

Hábitat

Merluccius merluccius (Linnaeus 1758) es una especie de comportamiento demersal y bentopelágico, de aguas frías o templadas. Forma bancos laxos sobre fondos de arena, fango y más raramente rocas (Lloris *et al.,* 2003), en los que presenta una cierta segregación en función de la talla (Fariña *et al.,* 1991). Los juveniles ocupan fondos de lodo de la plataforma, mientras que los adultos se encuentran en el talud sobre fondos duros asociados a cañones y arrecifes (Sánchez & Gil, 2000; Velasco, 2007).

1.2.3. BIOLOGÍA REPRODUCTIVA

La merluza presenta una estrategia reproductiva combinada consistente en un amplio período de puesta, una alta fecundidad y la liberación de huevos en fases sucesivas, lo que permite la generación de varias cohortes anuales (Sarano, 1986; Murúa *et al.,* 1998; Domínguez-Petit, 2007).

El período de puesta se extiende prácticamente a lo largo de todo el año, lo que se interpreta como una estrategia adaptativa para maximizar la supervivencia de los estadíos tempranos, permitiendo a la merluza aprovechar las condiciones ambientales favorables (Domínguez-Petit, 2007). Como promedio una hembra de 60 cm LT produce entre 170 000 y 280 000 ovocitos por tanda o *batch* con una frecuencia de puesta de entre 5 y 12 días (Korta *et al.,* 2010).

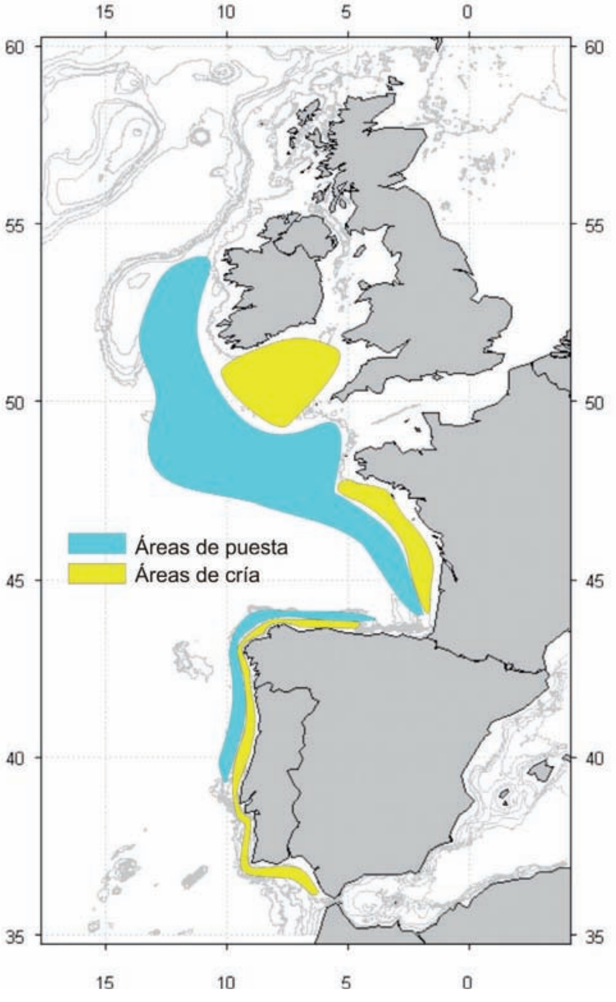

Figura 16. Principales áreas de puesta y cría de la merluza europea en el Atlántico nororiental.

Debido a su amplia distribución geográfica, existe un marcado gradiente latitudinal en los máximos de puesta (Cohen *et al.,* 1990), de manera que estos se retrasan a medida que aumenta la latitud (Casey & Pereiro, 1995; Murúa & Motos*,* 2006; Domínguez-Petit, 2007) (Tabla 3.2.1, Anexo).

La merluza realiza migraciones reproductivas, aunque no hay acuerdo sobre los hábitats específicos en los que se realizaría la puesta (Murúa, 2010). Las principales áreas de puesta (Figura 16) se concentran por un lado sobre el talud de la plataforma continental de la costa francesa en el Golfo de Vizcaya y en el Mar Céltico hacia el oeste de Irlanda (Álvarez *et al.,* 2001; 2004; Murúa, 2010) y por otro, sobre la plataforma del norte y oeste de la Península Ibérica (Pérez & Pereiro, 1985; Casey & Pereiro, 1995, Álvarez *et al,* 2004, Kacher & Amara, 2005).

En el Atlántico la talla de primera madurez (talla a la cual el 50% de los individuos son sexualmente maduros) se sitúa entre 39 y 47 cm (Lucio *et al,* 2000; Domínguez-Petit, 2007; Murúa, 2010), mientras que en el Mediterráneo la madurez se ha estimado en tallas entre 25 y 40 cm (Alheit & Pitcher, 1995; García-Rodríguez & Esteban, 1995; Ungaro *et al,* 2001). Existe una diferenciación sexual en la madurez. Así, los machos maduran antes y con una talla inferior que las hembras (Cohen *et al.,* 1990; Martin, 1991; Lucio *et al,* 2000a; Domínguez-Petit *et al.,* 2008). Finalmente, al igual que sucede en otras especies marinas, la talla de primera madurez se ve afectada tanto por factores dependientes de la densidad, como la abundancia o la estructura demográfica de la población, como por las condiciones ambientales (Domínguez *et al.,* 2008).

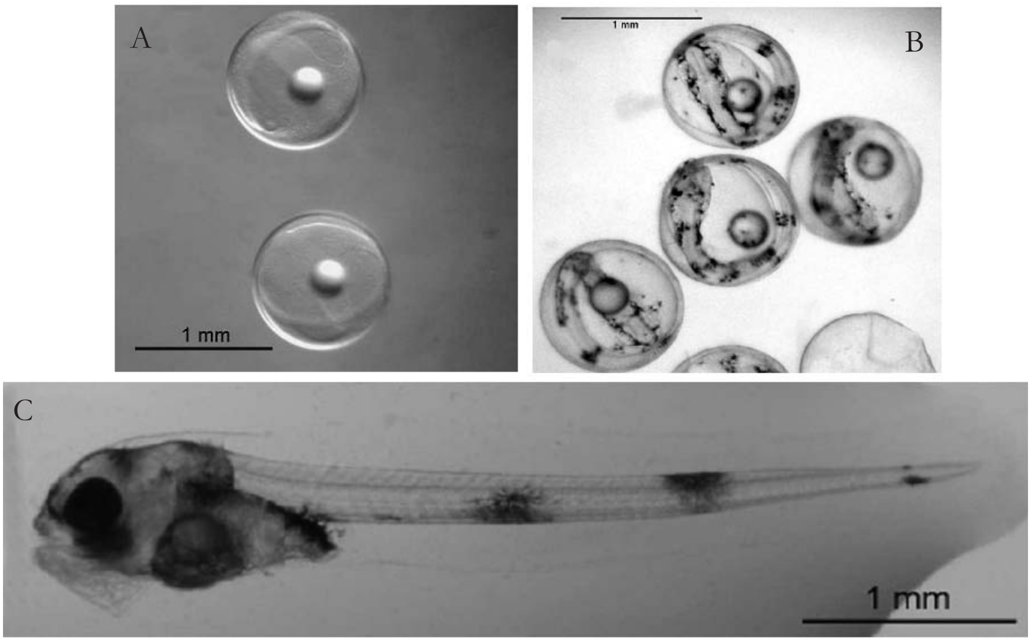

Figura 17. Primeros estadíos vitales de la merluza europea. Huevos en distinto estadío de desarrollo embrionario: (A) 26 horas, (B) 72 horas, (C) larva de 7 días (Sánchez *et al.,* 2011) (imágenes cedidas por el equipo de cultivos del C.O. de Vigo, IEO).

1.2.4. ECOLOGÍA LARVARIA

Los estadíos tempranos de la merluza son pelágicos. Huevos y larvas se concentran sobre la plataforma continental entre el final del invierno y principios de primavera (Olivar *et al.,* 2003), donde están sujetos a las corrientes del talud y a fenómenos hidrográficos de mesoescala y deriva. Las concentraciones máximas de huevos se encuentran entre 0 y 150 m (Álvarez *et al.,* 2001; Ibaibariaga *et al,* 2007) y las de larvas entre 50 y 150 m (Coombs & Mitchell, 1982; Motos *et al.,* 2000).

Los huevos eclosionan entre 4 y 6 días después de la puesta y las larvas de 4 mm de longitud (LT) tienen una mandíbula funcional que les permite alimentarse (Figura 17) (Sánchez *et al.,* 2011).

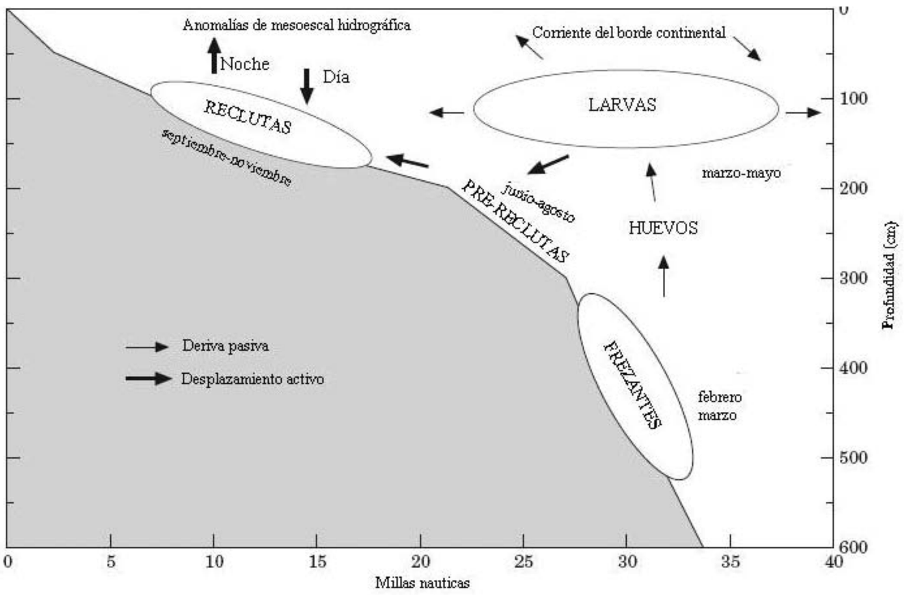

Figura 18. Esquema gráfico del ciclo vital de la merluza europea (modificado de Sánchez y Gil, 2000).

Durante el período larvario el crecimiento se limita a la cabeza y durante las primeras 3-4 semanas no hay aumento de talla. El desarrollo de las aletas, 30 días después de la eclosión, marca la transición entre la fase larvaria y la juvenil (Bjelland & Skiftesvik, 2006). Las larvas tienen una vida pelágica hasta que se asientan en el fondo 40-50 días después de la eclosión (Arneri & Morales-Nin, 2000; Álvarez & Cotano, 2005; Kacher & Amara, 2005). Las larvas de merluza (<8 mm LT) se encuentran distribuidas por todo el área de desove de la plataforma (Figura 18) (Álvarez *et al.,* 2001; 2004) y tras la metamorfosis a juveniles nadan activamente hacia áreas más profundas (>150 m), comenzando su reclutamiento a las zonas de cría a principios del verano (Sánchez & Gil, 2000).

En el noroeste de la Península Ibérica las mayores concentraciones de juveniles (tallas comprendidas entre 8-14 cm LT), se encuentran entre septiembre y noviembre (Sánchez & Gil, 1995; 2000) y más al sur entre otoño y primavera. Los procesos que afectan al reclutamiento de la merluza producen agregaciones de juveniles bien definidas y localizadas en determinadas zonas de la plataforma continental (Figura 18) (Sánchez & Gil, 2000). Estas concentraciones varían en densidad dependiendo de la fuerza del reclutamiento anual, aunque suelen ser más o menos estables en cuanto a su tamaño y localización (Sánchez & Gil, 2000). La constancia en el patrón de distribución de las agregaciones de juveniles ha llevado a asociarlas con ciertas condiciones oceanográficas particulares, como las estructuras de mesoescala de tipo *eddie*. Así, Sánchez *et al.* (2003) describen la relación entre los índices de reclutamiento de la merluza y una ventana ambiental óptima en el norte y noroeste de la Península Ibérica (Cury & Roy, 1989), de tal manera que se producirían reclutamientos altos en escenarios oceanográficos intermedios y bajos en situaciones extremas. Según estos autores, las condiciones oceanográficas que favorecerían reclutamientos altos serían: una corriente débil hacia el polo (conocida como *Poleward*), un afloramiento moderado y una fuerte actividad de mesoscala cerca de la plataforma (Sánchez & Gil, 1999).

Las áreas de cría o alevinaje de la especie se localizan próximas a las áreas de puesta (Figura 16), es decir, a lo largo de la plataforma de la costa francesa (Le Grand Vasiere) y mar Céltico, y en el norte y oeste de la Península Ibérica (Casey & Pereiro, 1995; Murúa, 2010).

1.2.5. ECOLOGÍA TRÓFICA

La merluza es una especie clave en la cadena trófica, donde ocupa un nivel alto entre los predadores de la comunidad demersal (Guichet, 1995; Velasco & Olaso, 1998; Cabral & Murta, 2002). Es un predador, activo, carnívoro y oportunista, cuyo carácter generalista puede cambiar a especialista dependiendo de la disponibilidad y diversidad de presas (Cassey & Pereiro, 1995; Bozzano *et al.,* 1997; Velasco & Olaso, 1998; Velasco, 2007). Realiza migraciones nictimerales asociadas a sus hábitos alimenticios. Así para alimentarse, los juveniles efectúan desplazamientos tanto verticales (Sánchez & Gil, 1995; Bozzano *et al.*, 2005) como oblicuos (Cartes *et al.,* 2004), mientras que los adultos se mantienen cerca del fondo durante las horas diurnas y se desplazan hacia aguas más someras al anochecer (Casey & Pereiro, 1995; Jolivet, *et al.,* 2009).

Existen básicamente tres fases tróficas en función de la composición de la dieta: *i)* basada en crustáceos durante la fase bentónica de larva y recluta; *ii)* basada en crustáceos y peces pelágicos en la fase juvenil (<20 cm TL); y *iii)* eminentemente ictiófaga basada en individuos de mayor talla. En esta fase, generalmente se alimenta de especies como bacaladilla (*Micromesistius poutassou* Risso, 1826), sardina (*Sardina pilchardus*; Walbaum, 1792), jurel (*Trachurus trachurus*, Linnaeus, 1758), caballa (*Scomber scombrus*, Linnaeus, 1758) y anchoa (*Engraulis encrasicholus*, Linnaeus, 1758), siendo la bacaladilla la presa principal sobre todo en el noroeste de la Península Ibérica (Velasco & Olaso, 1998; Velasco *et al.,* 2003; Velasco, 2007).

Por último, un componente importante de la alimentación de la merluza es el canibalismo, que es muy variable dependiendo del tamaño del depredador y de la abundancia de las presas alternativas (Hickling, 1927; González *et al,* 1985; Guichet, 1995; Oliver & Massutí, 1995; Bozzano *et al.,* 1997). La contribución del canibalismo a la dieta de la merluza puede alcanzar el 30% del volumen total de alimento ingerido, con valores medios del 5%, lo que contribuye a la alta mortalidad natural de los juveniles (Velasco & Olaso, 1998; Velasco, 2007).

1.2.6. CRECIMIENTO

Problemática de su estudio

El crecimiento es uno de los aspectos más problemáticos del estudio de la biología de la merluza en cualquiera de sus áreas de distribución y ha motivado discusión y debate durante décadas debido a la importancia que su conocimiento tiene para la evaluación y ordenación de las pesquerías.

Muchos años de investigación han apoyado la convicción de que los otolitos del genero *Merluccius* ofrecen registros de la edad interpretables y en consecuencia la posibilidad de conocer el patrón de crecimiento de las distintas especies: Hickling (1933) y Bagenal (1954) en *Merluccius merluccius* (Linneaus 1758); Dery (1988) y Hunt (1980) en *Merluccius bilinearis* (Mitchill, 1814); Fernández (1987) en *Merluccius gayi peruanus* (Guichenot, 1848); Renzi & Pérez (1992) en *Merluccius hubbsi* (Marini, 1933*)*; Ginsburg (1954) y Botha (1971) en *Merluccius paradoxus* (Franca, 1960) y Beamish (1979) en *Merluccius productus* (Ayres,1855). Sin embargo, y a pesar de los trabajos iniciales de Hickling (1933) y Bagenal (1954), los otolitos de *Merluccius merluccius* parecen ser la excepción. Así, los numerosos estudios del crecimiento basados en otolitos que han sido realizados desde entonces, no han permitido obtener estimaciones fiables de las tasas de crecimiento, abriendo así el debate de si *M. merluccius* tiene un patrón de crecimiento rápido o lento. Debate que ha permanecido abierto durante siete décadas.

Evolución de un paradigma: crecimiento lento – crecimiento rápido

Para comprender la errática evolución que ha seguido la investigación sobre el crecimiento de la merluza desde 1930, es útil mostrar su paralelismo con la dinámica del desarrollo científico descrita por Kuhn (1962) (Figura 19).

Kuhn (2006) explica que las ciencias progresan cíclicamente comenzando por una «fase precientífica» o de «ciencia inmadura» (Fase 1), caracterizada por la existencia de una serie de escuelas de pensamiento que aún no son ciencia, porque no tienen un paradigma común. En esta fase, no existe consenso y las teorías pueden ser, además de múltiples, incompletas e incompatibles. Le sigue una fase intermedia de «ciencia normal» (Fase 2), en la que existe un amplio consenso y un paradigma es aceptado por la comunidad científica e investigado durante un período de tiempo «normal» en la ciencia. En la fase siguiente de «ciencia revolucionaria» (Fase 3) existen discrepancias que si no son resueltas se convierten en anomalías y se acumulan produciendo una sensación creciente de que el paradigma existente ha dejado de funcionar. En la última fase se produce una «revolución científica» (Fase 4) en la que el antiguo paradigma es reemplazado por uno nuevo incompatible con el anterior.

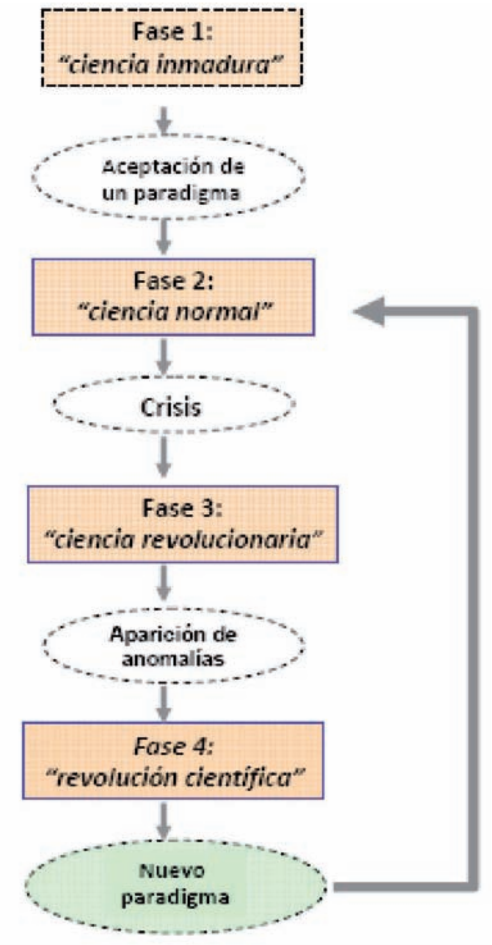

Figura 19. Esquema de las fases de la evolución de la ciencia según Thomas Kuhn (1962).

Fase 1. Ciencia inmadura: distintos estudios producen evidencias discordantes

El primero en obtener parámetros de crecimiento de *M. merluccius* a partir de otolitos (*sagitta*) fue Hickling

(1933), que concluyó que esta especie era de crecimiento lento, con una tasa de crecimiento media anual de 7 cm (TL). Poco después, Belloc (1935) realizó el primer experimento de marcado-recaptura y con solo un ejemplar recapturado concluyó que la merluza tenía un crecimiento rápido, cuya tasa anual podría alcanzar los 17 cm (TL). Sin embargo, el escaso éxito de esa experiencia de marcado debido a la baja supervivencia de los ejemplares tras la captura, motivó el abandono de esta técnica, quedando la interpretación de los otolitos como el único método viable para estimar la edad de la merluza y el modelo de crecimiento lento de Hickling (1933) como la referencia a seguir.

Desde los estudios promotores de los modelos de crecimiento lento y rápido (Hickling, 1933; Belloc, 1935) se ha producido una extensa literatura sobre el crecimiento de esta especie que ha aportado tasas de crecimiento muy dispares. Así, mientras estudios basados en la interpretación de los anillos de crecimiento del otolito generalmente producían resultados coherentes con el modelo de crecimiento lento (Meriel-Bussy, 1966; Guichet *et al.,* 1973; Robles *et al.,* 1975; Decamps & Labastie, 1978; Iglesias & Dery, 1981; Goñi, 1983; Goñi & Piñeiro, 1988; Piñeiro & Hunt, 1989; Morales-Nin & Aldebert, 1997), los resultados de estudios basados en el análisis de progresión modal en las distribuciones de tallas (Bagenal, 1954; Guichet, 1988; Piñeiro & Pereiro, 1993; Alemany & Oliver, 1995) apoyaban la hipótesis de crecimiento rápido de Belloc (1935). En esta fase se publicó también un trabajo que tenían en cuenta ambos modelos (Piñeiro*,* 1994) (Figura 3.1.1, Anexo).

Esta diversidad de resultados obtenidos en regiones distintas a lo largo de varias décadas (Tabla 3.4.1, Anexo) podía ser reflejo de una gran plasticidad en el crecimiento como resultado de factores endógenos o exógenos, del sesgo o inexactitud de los métodos empleados para estimar la edad, o bien de una combinación de ambos factores. Sin excepción, todos los autores encontraron dificultades para interpretar los otolitos debido a la complejidad de las estructuras de crecimiento. Los principales problemas observados fueron: *i)* la identificación del primer anillo anual; *ii)* la discriminación de los anillos anuales de los falsos; y *iii)* la clasificación del borde (Piñeiro *et al.,* 2009).

Por lo tanto, se puede decir que hasta bien entrada la década de los 90, trascurrió un período caracterizado por la falta de consenso y la escasa atención a la estandarización metodológica. Coexistían en aquella época distintas técnicas de preparación de los otolitos y diferentes métodos de interpretación. Los científicos involucrados en la determinación de la edad para la evaluación del recurso estimaban los parámetros de crecimiento basándose en la interpretación de la macroestructura de los otolitos y seguían sus propios criterios, sin realizar prácticamente ejercicios de calibración. Esta falta de consenso ralentizó el paso a la fase de «ciencia normal» en la que se podrían dar las condiciones para la creación del paradigma.

En este período surgieron iniciativas para impulsar la unificación de métodos de estimación de la edad a partir de otolitos. Se realizaron varios intercambios de otolitos y grupos de trabajo que obtuvieron los siguientes resultados: *i)* adopción de una técnica estándar para preparar y observar los otolitos (secciones transversales); *ii)* identificación de las principales fuentes de discrepancia entre lectores de edades basadas en secciones de otolitos; y *iii)* un mayor consenso sobre los criterios de lectura entre los lectores que aportaban edades al Grupo de trabajo de Evaluación del ICES (Francia y España). Sin embargo no fue suficiente para alcanzar una estandarización en el método de interpretación (FAO, 1982; ICES, 1983, 1984, 1986; Oliver *et al.,* 1989; Anónimo, 1994).

Este proceso permitió establecer el paradigma del crecimiento lento que dio paso a la fase de «ciencia normal» (Figura 19).

Fase 2. Creación del paradigma y ciencia normal

Entre 1997 y 2002 se produjo un fuerte impulso dirigido al estudio de la biología de esta especie con especial atención al crecimiento. Así, una serie de proyectos financiados por la UE (BIOSDEF, DEMASSES y SAMFISH) renovaron y ampliaron a nivel internacional los ejercicios de calibración y talleres de lectura (Piñeiro, 2000; Piñeiro *et al.,* 2000; Anon., 2002). Dado que el marcado-recaptura —único método que permitiría estudiar el crecimiento sin necesidad de determinar previamente la edad— no era viable por la alta mortalidad de las merluzas durante la captura (Lucio *et al.,* 2000b), se reforzó de nuevo la idea de que la solución estaba en mejorar la calidad de las estimaciones de edad. Simultáneamente se desarrollaban investigaciones destinadas a asegurar el control de calidad de la estimación de la edad para la evaluación de los recursos explotados y la merluza cobro especial atención (*EFAN, www.efan.no*). Todo este esfuerzo hizo de este período una etapa muy productiva.

En esta fase intermedia, según Kuhn (2006), existe un amplio consenso entre la comunidad científica, el paradigma es aceptado y es investigado durante un período de tiempo «normal» en la ciencia. De este modo, el paradigma del crecimiento lento fue aceptado de forma generalizada y se logró un marco de consenso para elaborar claves talla-edad que, durante años (1992 a 2009), permitieron evaluar el estado de las poblaciones de merluza y dar recomendaciones sobre las capturas máximas (WGHMM, ICES 2003).

Los resultados más relevantes de este período fueron los siguientes: *i)* se adoptó un método consensuado para interpretar el patrón de anillos de crecimiento en los otolitos hasta las primeras 5 edades, considerándose imposible proporcionar estimaciones de edad fiables para individuos de mayor edad; *ii)* se alcanzó el nivel más alto de precisión para edades de 0 a 5 años y se demostró que la experiencia del lector influye en el nivel de precisión de las estimaciones de edad; y *iii)* se reafirmó la necesidad de explorar técnicas alternativas de validación de las estimaciones de la edad (ej. marcado-recaptura, marcadores químicos o biológicos, etc.).

Fase 3. Crisis científica y ciencia revolucionaria

Según Kuhn (2006), la ciencia normal no está exenta de errores o contradicciones y pueden surgir anomalías que no se pueden resolver, es entonces cuando se produce una crisis científica. Pues bien, los problemas de la estimación de la edad de la merluza no afectaban solo a la precisión de las estimaciones, sino también, y lo que es más importante, a su exactitud, al no estar validadas (Piñeiro *et al.,* 2000). En este período aparecieron estudios discordantes con el modelo vigente, el paradigma del crecimiento lento de la merluza. Esta fase de «crisis» es la que Kuhn (1962) denomina como «ciencia revolucionaria» en la que se percibe que el paradigma existente ha dejado de funcionar (Figura 19).

En este período se realizó un intercambio internacional dirigido principalmente a los ejemplares de mayor edad que, a diferencia de todos los anteriores, partía de lecturas ciegas, es decir, se leían los otolitos sin conocimiento de la talla del pez (Piñeiro *et al.,* 2004). Los resultados revelaron una falta de consenso para interpretar los otolitos de los ejemplares más viejos y demostraron un importante sesgo en las estimaciones de edad debido a la subjetividad del método empleado, ya que conocer o no la talla del ejemplar influía en la edad asignada (Tabla 2.1.1, Anexo). Se alcanzó tal grado de incertidumbre que se recomendó la suspensión de la elaboración de claves talla-edad a partir de lecturas de otolitos (ICES CM 2005) mientras no se validara la metodología empleada para asignar edades (Piñeiro *et al.,* 2004).

En paralelo, diversos estudios aportaban resultados incongruentes con el modelo vigente. Así, el análisis del consumo alimenticio de la merluza mediante modelos bioenergéticos no era consistente con la baja tasa de crecimiento que indicaba el modelo lento (Riis-Vestergaard *et al.,* 2000). Al mismo tiempo, los escasos, pero reveladores resultados del segundo experimento de marcado-recaptura (Lucio *et al.,* 2000b) apuntalaban, 65 años después, la hipótesis de crecimiento rápido de Belloc (1935). Estos se sumaban a los obtenidos a partir del análisis de la progresión modal (Bagenal, 1954; Guichet, 1988; Piñeiro & Pereiro, 1993; Alemany & Oliver, 1995; García-Rodríguez & Esteban, 2002) que apoyaban también la hipótesis de crecimiento rápido. El empleo de estas técnicas, consideradas poco fiables para especies longevas (Panfili *et al.,* 2002), junto a sus resultados que no se ajustaban al paradigma establecido, fueron despreciadas y engrosaron así el conjunto de anomalías existentes en el modelo instaurado.

Por otro lado, en ausencia de técnicas de validación independientes de la edad, es sabido que el estudio del crecimiento diario puede ayudar a determinar la frecuencia de un *annuli* (Campana, 2001). Así, junto a los estudios volcados en la macroestructura del otolito, se investigó el crecimiento diario en la microestructura, a fin de conocer el crecimiento durante el primer año de vida e identificar el primer anillo anual (Panfili *et al.,* 2002). Los resultados demostraron la formación diaria de anillos de crecimiento (Morales-Nin & Aldebert, 1997; Arneri & Morales-Nin, 2000; De Pontual *et al.,* 2004; Morales-Nin & Moranta, 2004; Kacher & Amara, 2005; Belcari *et al.,* 2006), aunque se observaron variaciones en el crecimiento que afectan al desarrollo en el primer año de vida y que dificultan la identificación del primer anillo anual (Apartado 3.6, Anexo). Asimismo, la microestructura del otolito (Figura 3.6.1, Anexo) sirvió para analizar el significado biológico de los anillos formados durante el primer año de vida en el Atlántico (Alvarez & Cotano, 2005; Bjelland & Skiftesvik, 2006) y Mediterráneo (Arneri & Morales-Nin, 2000; Palomera *et al.,* 2005). En síntesis, los resultados indicaban una gran plasticidad individual y estacional en el crecimiento de larvas y juveniles, presentando un amplio rango de tasas de crecimiento en función de la fase de desarrollo y de la zona geográfica (Tabla 3.6.1, Anexo).

Fase 4. La revolución científica

La falta de coherencia de estos resultados con el modelo establecido, junto con los problemas que presenta la estimación de la edad basada en la macroestructura de los otolitos (Campana, 2001), puso de manifiesto, ya de manera incuestionable, la necesidad de un método que pudiera validar la periodicidad de las marcas de crecimiento de los otolitos (Piñeiro, 1994; Piñeiro & Saínza, 2002). A partir de ese momento se consideró que, sin dicha validación, sería imposible determinar el error de las edades y tasas de crecimiento estimadas (Piñeiro *et al.,* 2004).

Uno de los métodos más rigurosos para validar las tasas de crecimiento requiere de experimentos de marcado-recaptura de individuos en el medio natural (Campana, 2001). Desde que Belloc (1935) había descubierto el potencial del marcado de merluza tan solo Robles *et al.* (1975) y Lucio

Figura 20. Copo piscina llegando a la cubierta del barco en una campaña de marcado de merluza (Foto de C. Piñeiro).

et al. (2000b) habían intentado de nuevo el marcado siguiendo métodos convencionales de captura, pero con escaso éxito. La viabilidad del marcado de merluza vino más tarde gracias a adaptaciones especiales del sistema de captura, diseñadas por el laboratorio francés del IFREMER (De Puntual *et al.,* 2003). Con el desarrollo de un sobrecopo tipo piscina que sustituía al sobrecopo en un arte de arrastre tradicional, se logró aumentar la supervivencia de los ejemplares capturados, lo que hizo posible por primera vez el marcado de la merluza a gran escala (Figura 20).

Con una tasa de recaptura de 3.1%, el crecimiento somático observado fue superior al previsto por el modelo lento, mostrando una gran variabilidad individual (De Pontual *et al.,* 2003; 2004). Los resultados de esta primera campaña cerraron definitivamente el debate sobre el crecimiento de la merluza, dando el vuelco decisivo del viejo al nuevo paradigma: el crecimiento rápido de la merluza. Su éxito originó la inmediata puesta en marcha de estudios similares en diferentes zonas del Atlántico (Piñeiro, comunicación personal) y del Mediterráneo (Mellon-Duval *et al.,* 2010)

En resumen, esta tesis pretende ofrecer una visión global del estudio del crecimiento de la merluza europea. Su elaboración vino motivada por la necesidad de conocer el modelo de crecimiento de la merluza europea y comprender el largo proceso que condujo a él tras siete décadas de estudios. Para ello se ha utilizado un enfoque que se sustenta en la compleja evolución del conocimiento y en las técnicas de estudio aquí presentadas.

1.3. ÁREA DE ESTUDIO

El área de estudio de esta tesis es el Atlántico nororiental e incluye dos regiones de muestreo, el noroeste de la plataforma continental de la Península Ibérica y el Golfo de Vizcaya (Figura 21). Con fines de gestión, la merluza que habita esta regiones está separada por el ICES en dos unidades de stock, sur y norte, respectivamente. No obstante, la falta de evidencia directa de dicha separación, la existencia de indicios de conectividad entre ellas (Pita et al., 2011) y los resultados de las experiencias de marcado-recaptura realizados en ambas regiones, que indican tasas de crecimiento similares (Capítulo 4), apoyan que en esta tesis el crecimiento de la merluza europea y la problemática de la estimación de la edad sea tratada de manera unificada y con independencia del origen geográfico de las muestras empleadas en los distintos estudios presentados.

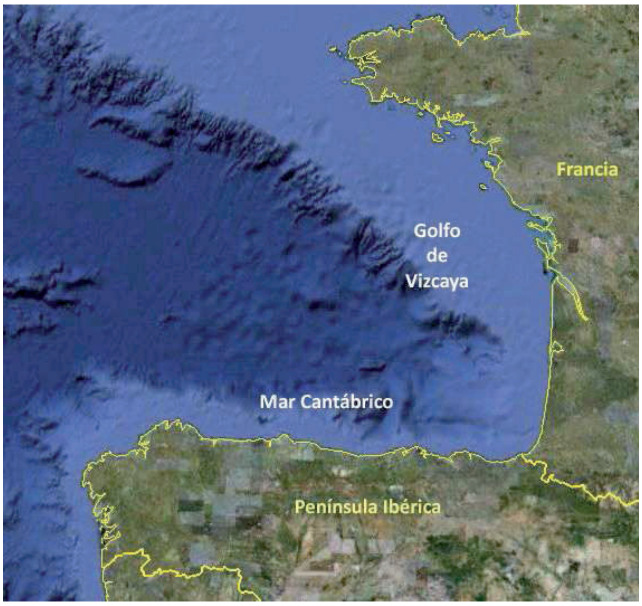

Figura 21. Mapa del área de estudio.

Capítulo 2. Estudio del patrón de crecimiento anual mediante el análisis de la macroestructura del otolito.

Age estimation, growth and maturity of the European hake (*Merluccius merluccius* Linnaeus, 1758) from Iberian Atlantic waters. **Piñeiro, C.**, and Saínza M., 2003. ICES Journal of Marine Science, 60:1086 1102.

Resumen

Las dificultades para estimar la edad de la merluza (*Merluccius merluccius*) han obstaculizado la evaluación de los *stocks* de esta especie. En este trabajo se describe por primera vez los criterios consensuados para interpretar el patrón de crecimiento del otolito y los métodos utilizados para la preparación de otolitos y asignación de la edad. Se presentan mejoras en las estimaciones de los parámetros de crecimiento según el modelo de von Bertalanffy, de la relación talla peso y de las ojivas de madurez para la merluza europea del las aguas del noroeste ibérico. Los resultados provienen de los estudios realizados a lo largo del periodo 1996-1997 y aportan los primeros datos sobre las características vitales de la merluza del *stock* sur. Los parámetros de crecimiento según Von Bertalanffy resultaron en L_∞= 70 cm, K= 0.18 año^{-1} , t_0 = -0.97 año para machos y L_∞= 89 cm, K= 0.13 año^{-1} , t_0= -1.15 año para hembras. El crecimiento fue diferente por sexos desde edad 3, siendo las hembras como promedio, más grandes y pesadas que los machos. La estimación de la relación: talla total (L, cm) - peso total (W, g) fue $W = 0.0132135L^{2.8134246}$ para machos y $W = 0.0086471L^{2.942563}$ para hembras. El periodo de puesta va desde diciembre a mayo presentando un pico en febrero. La talla y edad media de primera madurez es de 32.8 cm y 2.5 años para machos y 45 cm y 4.4 años para hembras. La aplicación del nuevo criterio de asignación de edad indica que las secciones de otolitos pueden ser empleadas para determinar la edad hasta los 5 años de una forma consistente. Estos resultados demuestran que la merluza del stock sur crece a una tasa superior y madura antes de lo que previamente se consideraba. Finalmente, se presenta un resumen de los parámetros que caracterizan su estrategia vital en las distintas áreas de su distribución incluyendo así información que no se encuentra fácilmente disponible por pertenecer a la literatura gris.

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Age estimation, growth and maturity of the European hake (*Merluccius merluccius* (Linnaeus, 1758)) from Iberian Atlantic waters

C. Piñeiro and M. Saínza

Piñeiro C., and Saínza M. 2003. Age estimation, growth and maturity of the European hake (*Merluccius merluccius* (Linnaeus, 1758)) from Iberian Atlantic waters. – ICES Journal of Marine Science, 60: 1086–1102.

Difficulties in age estimation for hake (*Merluccius merluccius*) have hampered the assessment of stocks. Here, we describe new, agreed ageing criteria based on the interpretation of the pattern of otolith growth. Improved estimates of von Bertalanffy growth parameters, and new estimates of maturity ogive parameters and length–weight relationships for European hake from Iberian Atlantic waters are presented. The results came from a study carried out during 1996–1997 and provide the first published account of the main life history traits of Southern stock hake. von Bertalanffy growth parameters of males were $L_\infty = 70$ cm, $K = 0.18$ year^{-1}, and $t_0 = -0.97$ year, and those of females were $L_\infty = 89$ cm, $K = 0.13$ year^{-1}, and $t_0 = -1.15$ year. Growth of sexes differed from age 3 onwards, with females being on average larger and heavier than males. The estimated total length (L, cm)–total weight (W, g) relationships were $W = 0.0132135L^{2.8134246}$ for males and $W = 0.0086471L^{2.942563}$ for females. Spawning took place from December to May with a peak in February. The mean length and age at first maturity were 32.8 cm at 2.5 years for males and 45 cm at 4.4 years for females.

Application of new ageing criteria showed that otolith sections may be used to determine ages up to 5 years in a consistent manner. These results indicate that hake of the Southern stock grow at higher rates and mature earlier than previously considered. Summaries of hake's life history parameters from other marine regions are also presented in order to make information that belongs largely to the grey literature available.

Keywords: age-at-maturity, growth, Iberian Atlantic waters, length-at-age, *Merluccius merluccius*, reproduction, southern stock, von Bertalanffy parameters.

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Introduction

The European hake (*Merluccius merluccius*) 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 (Casey and Pereiro, 1995). The assessment of hake in the area is undertaken annually by the Working Group of Southern Shelf Demersal Stocks (WGSSDS) of the International Council for the Exploration of the Sea (ICES). Despite the lack of a sound biological basis, since 1978 the WGSSDS distinguishes two hake stocks for assessment purposes: the Northern stock (ICES Division IIIa, Sub-areas IV, VI and VII and Divisions VIIIa-b) and the Southern stock (ICES Divisions VIIIc and IXa; Figure 1; ICES, 1979). A geographical barrier called Cap Breton Canyon separates these stocks.

European hake fisheries off the Iberian Peninsula (Southern stock) have been operating for many years. Hake is of major importance due to its high market value to both Portuguese and Spanish fisheries. It is caught as target or as by-catch by a variety of vessels and gears, the fishery is exclusively for human consumption and hake is consumed fresh.

Over the past few years, the WGSSDS has been concerned with the status of European hake stocks, which are considered outside safe biological limits (ICES, 1999). Knowledge of hake's biological traits (especially growth and maturity) is considered insufficient to improve the assessment of stocks. For the Southern stock in particular, catch-at-age

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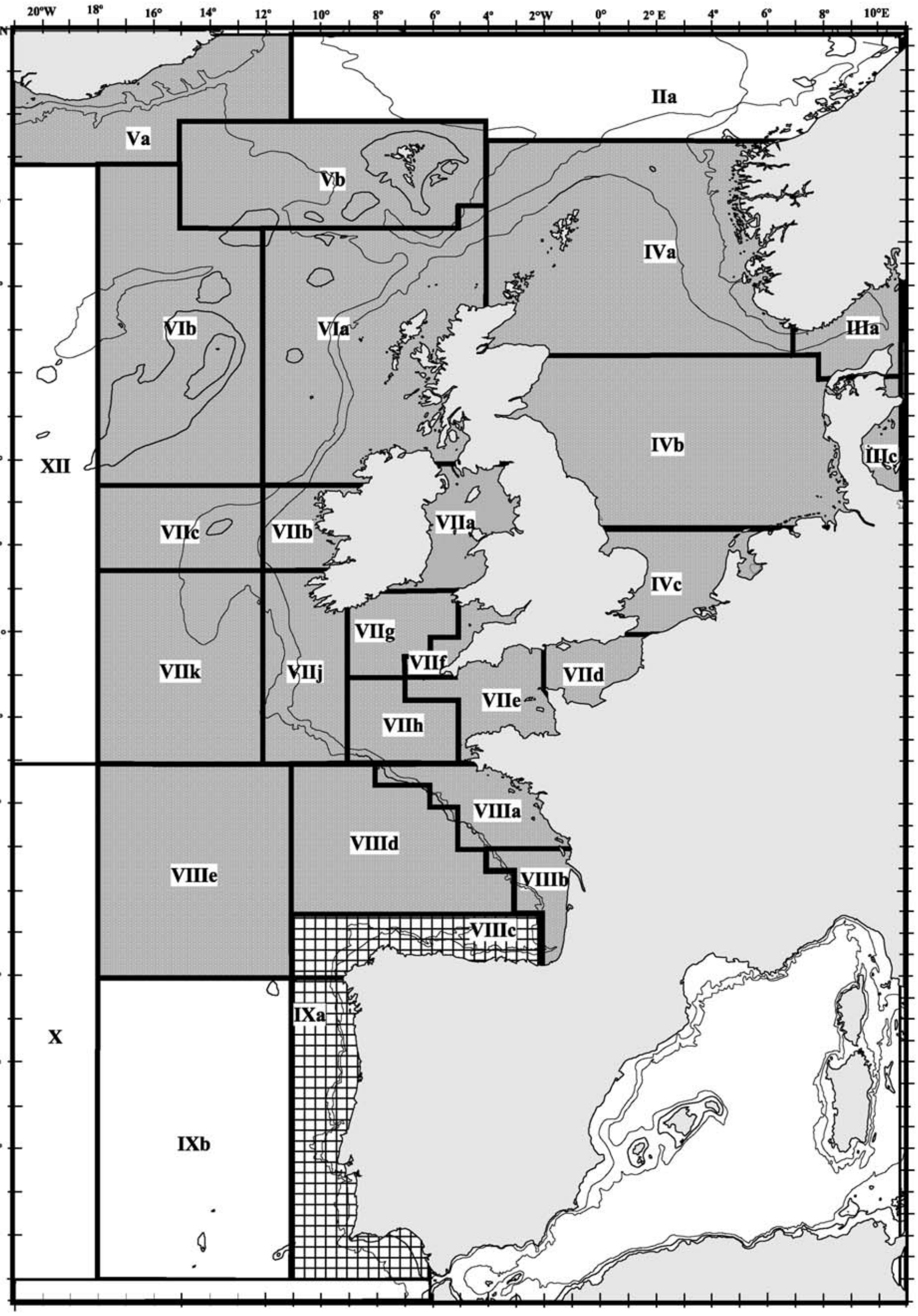


Figure 1. Map of ICES Divisions and location of study area (IXa and VIIIc) in squares design.

analyses have caused problems due to deficiencies in landing data and difficulties in age estimation (ICES, 1998). Since 1991, the latter problem has been overcome by converting length compositions of landings to age compositions using numerical methods (Kimura and Chikuni, 1987; ICES, 1994). Nevertheless, the annual assessment of Southern stock hake causes great scepticism, which emphasises the need to develop reliable ageing methods. In 1999, the WGSSDS made several changes supported by the results of two studies carried out under the auspices of the EU BIOSDEF (Anon., 1998) and DEMASSESS projects (Anon., 2000). These studies greatly enhanced knowledge of the biological parameters of hake, and improved age estimation procedures. One successful application of the latter was the use of empirical age-length keys (ALK) for the first time in the 1999 assessment of the Southern stock (ICES, 2000).

European hake age estimation relies on interpretation of the pattern of ring formation in otoliths. The unusual complexity of this task has been reported widely in the literature (Hickling, 1933; Bagenal, 1954; Meriel-Busy, 1966; Robles *et al.*, 1975; Descamps and Labastie, 1978; Iglesias and Dery, 1981; Goñi, 1983; Goñi and Piñeiro, 1988; Guichet, 1988; Piñeiro and Hunt, 1989; Piñeiro and Pereiro, 1993; Morales-Nin *et al.*, 1998), and several international workshops have been devoted to the development of a reliable ageing method for otoliths of this species (Anon., 1983, 1984, 1986). However, until recently these efforts had been fruitless. Two workshops on hake otolith interpretation conducted in 1997 and 1999, within the above-mentioned projects, developed standard ageing criteria to be adopted by age readers from institutions involved in hake stock assessment. The main achievement of these efforts was a set of internationally accepted interpretation criteria for hake otoliths up to age 5. Development of these ageing criteria was derived from previous research studies on the growth pattern of hake otoliths carried out by the authors of this work, who also led the above-mentioned workshops.

Despite the large number of available studies on the biology of European hake from the Northeast Atlantic, the majority of these have been published in national journals, collected in ICES contributions, or ICES working group and project reports, which are not widely distributed (Anon., 1998, 2002). Only in 2000, Lucio *et al.* published biological information of hake from the Bay of Biscay (Northern stock: ICES Divisions VIIIa,b,d). This paper presents improved estimates of the main biological characteristics of European hake from Iberian Atlantic waters (Southern stock) and for the first time includes growth information based on standardised otolith ageing criteria. It also constitutes the first published account of the life history parameters of hake from the Southern stock and includes a summary of the knowledge of hake's life history parameters obtained by different authors in the Northeast Atlantic, thus highlighting information that belongs largely to the grey literature.

Material and methods

Sampling

Hake specimens were collected from commercial landings during the BIOSDEF project (July 1996–June 1997) and from survey catches (September and October 1996) off the Northwest Iberian Peninsula. Sampling was done according to a random stratified design at quarterly intervals covering the length range of hake in the study area (ICES Divisions VIIIc and IXa, Figure 1). Total length (cm), total weight (g), sex and maturity state were recorded from all the specimens sampled. A summary of samples collected for this work is presented in Table 1.

Table 1. Summary of hake samples used in the tasks of this study (M, males; F, females; I, indeterminate).

Task		M	F	M + F + I
Growth	Number	315	469	1100
	Size range (cm)	17–60	15–78	6–78
	Age range (years)	0–9	0–11	0–11
Total length–total weight relationship	Number	441	716	1391
	Size range (cm)	17–60	16–78	6–78
	Weight range (g)	33–1297	7.6–2848	1.2–2848
Reproduction	Number	1141	1375	3639
	Size range (cm)	15–60	15–78	6–78

Age interpretation

The lack of agreement in the application of ageing criteria and procedures in hake otolith reading revealed the need to derive a set of internationally agreed ageing protocols, allowing otolith readers from different laboratories to age hake consistently. The otolith preparation and ageing methods used in this study were agreed in the two above-mentioned international workshops and rely heavily on our previous experience on hake ageing studies. The otolith terminology of Secor *et al.* (1995) has been used throughout this work.

Otoliths were sectioned on the dorso-ventral plane and thin sections mounted on glass slides using the technique described by Piñeiro *et al.* (1996). Two experienced readers aged the otoliths using a stereomicroscope under reflected light at ×20 magnification. Readers were calibrated with a reference collection available at the Instituto Español de Oceanografía (IEO) prior to viewing the study material. A microscope-mounted video camera and monitor was used for discussion. Only otoliths with which the age estimates of the two readers coincided were retained for the growth study.

Hake otolith sections have a concentric pattern of translucent and opaque bands around the nucleus. One annulus or annual ring consisted of an opaque and of a translucent ring or band. The appearance of bands or rings varies along the dorsal–ventral axis of the sections, showing subsidiary rings within the main opaque and translucent pattern. Counts of winter rings that appeared as dark narrow bands (translucent), preferably on the ventral region, were used to estimate ages. A winter ring consisted of a single translucent band or of a series of two or three clustered translucent rings (caused by intermittent growth during this period). To locate the first winter ring previous knowledge was employed, in particular the modes of the size distributions from the surveys carried out annually since 1983 by the IEO (Piñeiro *et al.*, 1992). These data show that the youngest fish enter the fishery during the third quarter with a modal size of 12–15 cm TL. This size corresponds to a radio distance of 1–1.5 mm from the otolith basis. Thus, in an average otolith the first winter ring may be found at around 1.3 mm from the nucleus. Additional information from discard surveys carried out in 1994 and in 1997–1999 indicates that the size of 0-age hake in December ranges between 17 and 20 cm (Pérez *et al.*, 1996).

Hake growth during the first year of life is characterised by the occurrence of three checks or "false rings" that appear around the nucleus of the otolith. The existence of these "false rings" has been reported by several authors and they have been associated with: (1) the larval phase, (2) the pelagic phase, and (3) the onset of the demersal phase (Descamps and Labastie, 1978; Iglesias and Dery, 1981; Goñi and Piñeiro, 1988; Piñeiro and Pereiro, 1993). The first annual ring (winter ring) appears either after the so-called "demersal" check or may be co-incident with it (Piñeiro and Hunt, 1989). The varying position of this annual ring relative to the nucleus appears to be related to the extended spawning period of the species.

Growth during the first and second years is large by comparison with later increments. A well-marked check is frequently found on the otolith during this period (at around 1.5 mm from the nucleus). This check is not an annual ring but it may be co-incident with the first or with the second annual rings (Goñi and Piñeiro, 1988). It appears independently of the season, is linked to some still unknown biological or behavioural event, and is considered a reference mark when otolith reading is undertaken. The second annual ring (winter ring) appears after this check at around 2 mm from the nucleus although its position varies. The third annual ring appears afterwards and is usually preceded by another translucent ring.

Classification of the otolith edge type (translucent or opaque) tends to be complicated by the high incidence of false rings. Nevertheless, in this study we assumed that otolith edges followed the predominant pattern of translucent in winter and opaque in summer, and by convention an otolith with a translucent edge is not considered to be 1 year older until the 1st of January. Edge interpretation of the otolith sections in the study area was especially difficult for samples caught between late spring (May) and summer (July) and in otoliths from young fish. Very often sections presented faint rings at the edge and the mounting procedure did not permit checking the edge type on both faces, therefore whole otoliths were preferred for this matter. A length-stratified sub-sample of 350 whole otoliths of fish 8–40 cm long were selected to analyse the evolution of the marginal growth of otoliths through an annual cycle. Larger fish were not selected because the thickness of whole otoliths from older individuals hides the translucent bands. A selection of otolith sections ($n = 526$) from hake of the same size range and collected in the same area of study, was used to compare the evolution of the otolith edge with both procedures.

Precision of readers was assessed by comparing the readings in a random sample of 100 otolith sections viewed independently by two readers. The average percentage of error (APE) was calculated following the method of Beamish and Fournier (1981).

Growth parameters

Age length keys (ALK) were produced for males, females and for the two sexes combined, and their mean lengths at age and standard deviations calculated. von Bertalanffy growth curves (von Bertalanffy, 1938) were fitted to the data applying the least-squares approach of the ® Microsoft Excel Solver routine using the Newton algorithm. This growth curve describes fish length as a function of age and its equation is as follows:

$$L_t = L_\infty(1 - e(-K(t - t_0)))$$

where L_t is the mean length at age t, L_∞ is the asymptotic mean length, K is a rate constant that determines how fast L_t approaches L_∞ and t_0 is the age at which the mean length is zero. Initially, the three parameters were estimated for both sexes and the two sexes combined without any restriction. Because the estimated value of L_∞ for the sexes combined was lower than the largest hake found in the study area, in a second trial we decided to adjust it to the largest size observed in the commercial landings with a 5% of increase to approximate the asymptotic mean length (Taylor, 1960, 1962; Pauly, 1985). Thus, to estimate K and t_0, for the sexes combined, L_∞ was fixed at 120.5 cm.

Given that sets of growth parameters for European hake estimated by different authors were available, we decided to evaluate their reliability using the wide application phi-prime test (Φ') (Pauly and Munro, 1984). This test provides an indication of the reliability of age estimates since it has been suggested that phi-prime test values are similar for the same species and genera. The test is based on

$$\Phi' = \log K + 2 \log L_\infty$$

Comparisons between all growth parameters estimated for both sexes were carried out using the Kruskal–Wallis non-parametric analysis. Data analysis was carried out using SPSS © (Statistical Package for the Social Sciences, 1999).

Length–weight relationships

Total length (L) and total weight (W) relationships were estimated for males, females and both sexes combined by fitting non-linear regressions to the data. The model fitted was $W = aL^b$ and the resulting parameters (a,b) were obtained using the Newton algorithm from the Microsoft ® Excel Solver routine.

Reproduction

Sex determination of small fish was difficult and hake smaller than 15 cm long were considered indeterminate. The sex ratio was therefore obtained from this length onwards. The spawning season was determined by macroscopic examination of the gonads for maturity stage according to a four-point scale validated histologically by Lucio *et al.* (2000).

Size at maturity was defined as the size at which 50% of males and females become mature (L_{50}) and was estimated using specimens over 15 cm long collected during the months of maximum reproductive activity (December–May). The percentage mature by length class and sex were fitted to a logistic function using the Newton algorithm from ® Microsoft Excel solver routine

$$P(l) = 1/1 + e^{-(a+bl)}$$

where P(l) is the proportion of mature fish at length l, and a and b the parameters of the logistic equation. The same procedure was used to obtain the maturity ogive at age.

Results

Age estimation

The pattern of annual otolith increments could be clearly seen by experienced readers along the dorsal–ventral axis of otolith sections. Interpretation of the otolith pattern of growth presented two main difficulties: the central area close to the nucleus, where the growth pattern of the first year of life is laid down, and the area beyond the sixth annual ring. As mentioned in the previous section, the central area possessed a number of "false rings". Taking into account the age interpretation methods described earlier, the first annual ring (1) was identified despite the presence of checks (−3, larval; −2, pelagic; −1, demersal) around the nucleus (Figure 2). Although the position of the first annual ring varied, its recognition was aided by the frequent presence of a well-marked translucent ring along the dorsal–ventral axis of the section, between two first annual bands.

Growth increments between the first, second and third annual rings had two translucent checks per year (winter and summer rings). These false rings were not clearly discernible in subsequent years. In older fish, the increments were narrower and more visible on the ventral apex, with a progressive shift in growth towards the internal face. The area beyond the sixth annual ring was complex, the frequent appearance of faint lines between annual rings combined with the gradual reduction in the spacing between them made defining a consistent reading method difficult.

Due to the high incidence of checks, the edge of hake otoliths did not strictly follow the expected pattern of translucent bands in winter and opaque bands in summer. Translucent edges appeared all year round. On average, more than 60% of the whole otoliths had translucent edges, indicating a high incidence of checks particularly in summer samples (Figure 3). Overall, two peaks of translucent edges per year were observed in whole otoliths. The most important occurred in winter (November) and the secondary one in spring–summer (April–June). These two peaks appeared later in otolith sections, the first in February and the second in July. However, the optical characteristics of the thin sections under variable light could have affected these results.

Of the total number of otoliths read, 12.4% were rejected for being unreadable. Precision estimates gave an average percent of error between readers of 7.7% and a mean coefficient of variation of 12%.

Growth parameters

The mean length at age for males, females and sexes combined are presented in Table 2. The maximum age recorded was 9 years for males and 11 years for females. Males larger than 60 cm total length were not caught and all fish older than 9 years were females. The sample was dominated by specimens belonging to age groups 1–5. Kruskal–Wallis tests comparing length distributions for each age of males and females showed highly significant differences from age 3 onwards ($p < 0.001$). Comparison of mean lengths at age estimated by other authors with this study (Table 3) showed that in the study area, hake of any given age are larger than was estimated before, reaching mean sizes close to those estimated for hake of the Northern stock.

The estimated growth parameters of males were $L_\infty = 70.0$ cm; $K = 0.184$ and $t_0 = -0.973$ and of females $L_\infty = 88.7$ cm; $K = 0.127$ and $t_0 = -1.157$ (Table 4). For the sexes combined, the resulting parameters were $L_\infty = 88.0$ cm; $K = 0.128$ and $t_0 = -1.174$. For the option where L_∞ was fitted to the observed data (120.5 cm), the results were $K = 0.075$ and $t_0 = -1.715$. The estimated growth curves were plotted together with those estimated by other authors (Figure 4).

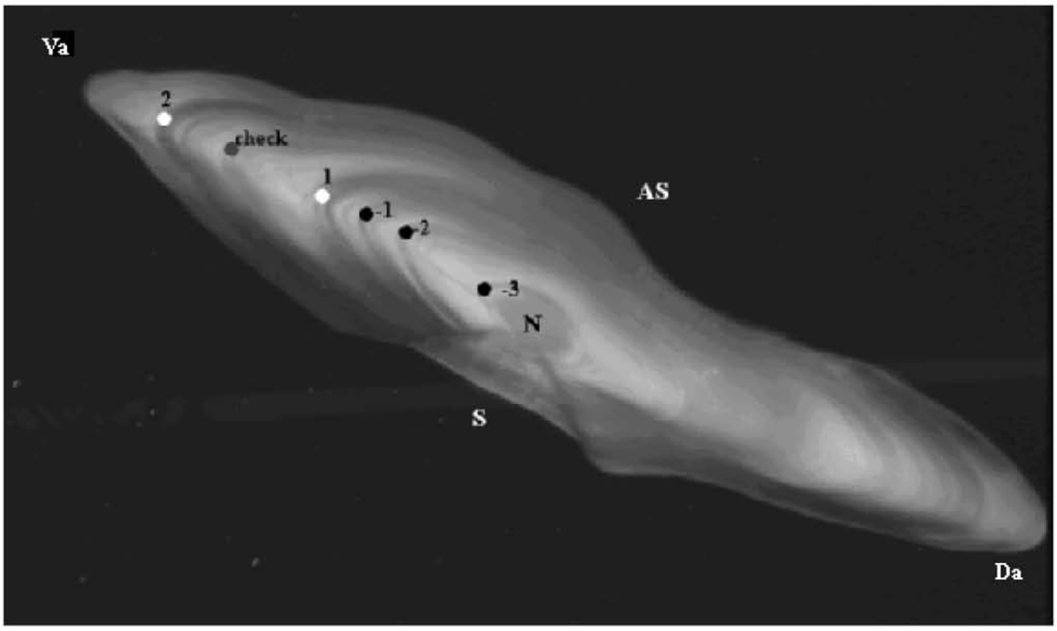
The phi-prime test (Φ') was used to compare the growth parameters L_∞ and K estimated for the sexes combined with those obtained in other studies (Table 4). Within the same geographic area, Φ' values obtained from our growth parameters and those of Robles *et al.* (1975) were the same, while those of Iglesias and Dery (1981) and ICES (1991) gave lower Φ' values. For hake from the Bay of Biscay (Northern Stock) similar (ICES, 1993) and higher Φ' values were reported (Lucio *et al.*, 2000). Finally, for waters off Morocco Goñi (1983) also calculated lower Φ' values.

Length–weight relationship

The total length–total weight relationships were: $W = 0.013L^{2.813}$ for males, $W = 0.008L^{2.942}$ for females (Figure 5), and $W = 0.00733L^{2.981}$ for the sexes combined. Females were heavier than males. Comparison of weights at age of males and females showed highly significant differences ($p < 0.001$) from age 3 onwards. Estimates of parameters of the length–weight relationships for combined sexes are presented together with those of other authors in Table 5.

Sex ratio

The sex ratio was close to 1 : 1 in hake smaller than 45 cm (Figure 6). Males outnumbered females in the size range of 25 to 45 cm, after which females predominated and rapidly increased in relative abundance to reach 100% in fish larger than 60 cm.



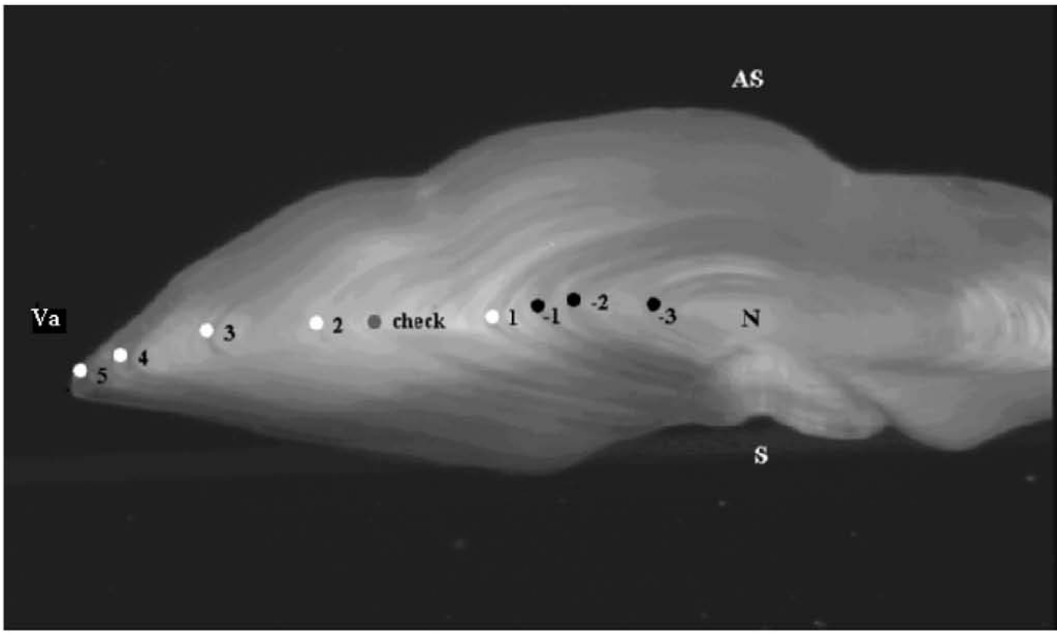


Figure 2. Transverse sections of *sagitta* otoliths of two hake specimens collected in Iberian Atlantic waters in 1997. Above: a male of 30 cm total length caught in October and aged 2+ years old. Below: a female of 47 cm total length caught in April and aged 5 years old. Photographs (reflected light) show central area around the nucleus (N), where three false rings (−1, −2, −3) are visible before the first annulus and one (check) between the first and the second annulus. Visible as translucent zones (white dots) are the annual rings. Da, dorsal apex; Va, ventral apex; S, sulcus; AS, antisulcus.

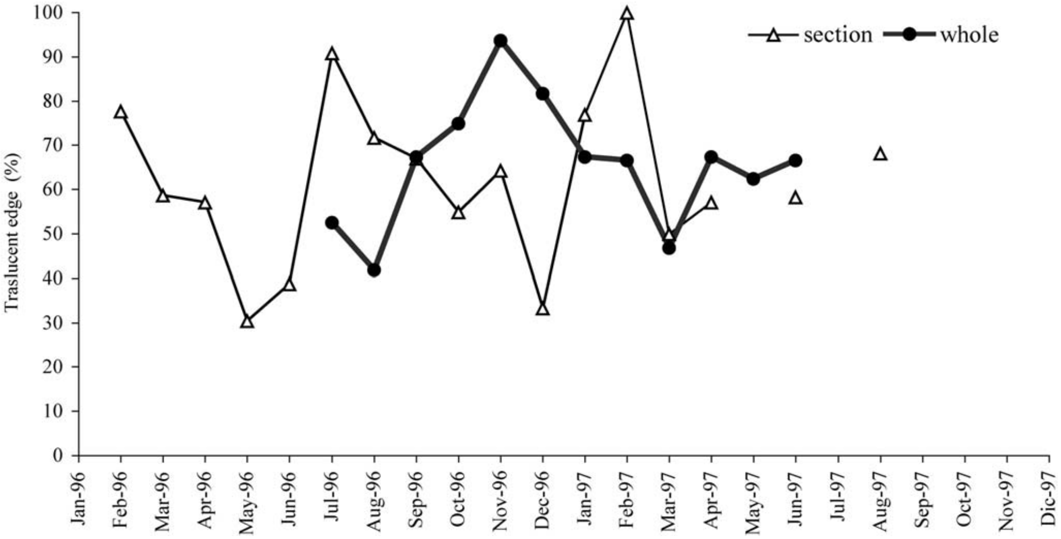


Figure 3. Plots of percentage of individuals with a translucent otolith edge by month throughout the period February 1996 to August 1997. Number of whole otoliths = 350; number of sectioned otoliths = 526.

Spawning season and size at maturity

Resting stage females appeared in April and disappeared in September, with mature females increasing from December to May, with a peak in February. Mature males were present almost throughout the year, although the highest proportion occurred from February to April, and a small amount of resting males were present from June to July and from September to October. Immature individuals were frequent throughout the year.

The estimated mean size at first maturity of males was 32.8 cm and the range of maturation (L_{75}–L_{25}) was 4.2 cm. The females' mean size at first maturity was 45.4 cm and the range of maturation (L_{75}–L_{25}) was 6.2 cm. The mean size at maturity for the sexes combined was 37.9 cm and the range of maturation (L_{75}–L_{25}) was 11.5 cm (Figure 7). The estimated age at which 50% of individuals become mature was 2.5 years for males, 4.4 years for females and 3.2 years for the sexes combined. The reproductive parameters of hake obtained by different authors and in different areas are summarised in Table 6.

Discussion

Age interpretation

The wide range of length at age estimates of European hake obtained by different authors reveals the lack of agreement in

Table 2. Mean lengths at age (ML, cm) and standard deviations (SD) (M, males; F, females; I, indeterminate).

	M			F			M + F + I		
Age	Otoliths read	ML	SD	Otoliths read	ML	SD	Otoliths read	ML	SD
0	2	17.0	0.0	5	16.8	1.3	118	11.9	4.2
1	58	21.0	2.1	60	20.9	2.2	137	20.6	2.3
2	95	29.5	3.1	96	28.4	3.5	193	29.0	3.3
3	68	36.4	2.7	70	37.0	3.2	139	36.7	2.9
4	60	42.7	2.4	58	44.5	3.1	135	43.8	2.7
5	18	45.7	2.3	56	48.7	3.3	91	50.0	3.2
6	7	49.7	2.7	52	53.7	3.4	68	55.4	3.5
7	6	54.2	3.5	39	56.4	3.4	49	58.3	4.9
8	–	–	–	24	62.3	4.1	25	63.1	5.0
9	1	60.0	–	7	68.7	4.1	8	67.1	6.8
10	–	–	–	1	75	–	1	75	–
11	–	–	–	1	78	–	1	74	–
Total	315			469			965		

Table 3. Mean lengths (cm) at age of European hake from different areas by sex (1 = Males, 2 = Females, 3 = Sexes combined), obtained by different authors. The ageing method used is also specified.

			Age											
Author	Age	Sex	0	1	2	3	4	5	6	7	8	9	10	Ageing method
Hickling (1933)	Ireland	3			19.6	25.4	35.1	43.2	51.4	63.4	68.0	72.9		Otoliths and scales
Hickling (1933)	Scotland	3			20.9	25.6	34.6	42.1	50.9	59.8	67.9	74.1	81.2	Otoliths
Bagenal (1954)	Scotland	3		22.3	43.2	58.7	70.0							Otoliths-Petersen
Guichet *et al.* (1973)	Ireland	1			22.5	20.1	32.5	40.1	47.3	53.0	59.8	63.9	66.0	Otoliths
		2			19.1	27.9	33.8	42.0	49.6	56.5	62.2	69.9	76.9	Otoliths
Meriel-Busy (1966)	Bay of Biscay	3		11.0	19.6	28.0	36.6	43.5	51.2	58.7	65.1	69.6	80.1	Otoliths
Descamps and Labastie (1978)	Bay of Biscay	1		15.7	24.9	32.9	39.8	45.7	50.9	55.3	59.1	62.4	65.2	Otoliths
		2		15.9	25.2	33.7	41.4	48.4	54.7	60.4	65.6	70.3	74.5	Otoliths
Guichet (1988)	Bay of Biscay	3	16.3	24.1	32.3	39.1								NORMSEP Modal Progression
ICES (2000)	Bay of Biscay	3	11.2	20.7	26.9	34.3	41.8	50.4	59.3	63.7	90.3			Otoliths (ALK from 1999)
Lucio *et al.* (2000)	Bay of Biscay	3	13.1	17.8	24.7	33.1	42.6	51.6	60.9	67.7	72.7	85.1		Otoliths
Iglesias and Dery (1981)	N and NW Iberian waters	1		17.6	23.8	29.5	34.4	38.6	43.1	45.2	47.8	50.1		Otoliths
		2		19.6	24.7	29.4	33.8	38.0	41.9	45.5	49.0	52.2		Otoliths
		3		20.2	24.9	29.3	33.4	37.3	41.0	44.4	47.7	50.7		Otoliths
Robles *et al.* (1975)	NW Iberian waters	3		10.6	19.7	27.8	35.2	42.7						Otoliths
Goñi (1983)	Morocco	1		16.0	21.4	26.5	31.3	35.7	39.9	43.8	47.3	50.9		Backcalculated/Otoliths
		2		15.4	21.4	26.9	32.2	37.1	41.7	46.0	50.0	53.8		Backcalculated/Otoliths
		3		14.8	20.7	26.3	31.4	36.3	40.9	45.2	49.2	53.0		Backcalculated/Otoliths
Goñi and Piñeiro (1988)	N and NW Iberian waters	3		15.0	23.0	28.0	32.0	36.0	40.5					Backcalculated/Otoliths
ICES (1991)	N and NW Iberian waters	3	12.0	18.0	25.0	30.0	36.0	41.0	45.0	49.0	53.0	57.0	60.0	Kimura and Chikuni (1987)
ICES (1999)	N and NW Iberian waters	3	14.9	21.5	29.4	36.8	43.0	48.6	54.4	58.0	63.6	67.0	77.0	Otoliths (ALK from 1998)
ICES (2000)	N and NW Iberian waters	3	12.6	21.4	28.7	33.8	41.8	48.7	53.4	58.1	63.7	69.9	79.0	Otoliths (ALK from 1999)
This study	N and NW Iberian waters	1	17.0	21.0	29.5	36.4	42.7	45.7	49.7	54.2	60.0			Otoliths
		2	16.8	20.9	28.4	37.0	44.5	48.7	53.7	56.4	62.3	68.7	75.0	Otoliths
		3	11.9	20.6	29.0	36.7	43.8	50.0	55.4	58.3	63.1	67.1	75.0	Otoliths

Table 4. Parameters of the von Bertalanffy growth equation (K, L_∞ and t_0) obtained by different authors, by sex, area, and method of age determination; phi-prime test (Φ') for sexes combined.

Author	Sex	Method	Stock	Area	K	L_∞	t_0	Φ'
Hickling (1933)	Combined	Otoliths	Ns	SW Ireland	0.087	128.6	–	3.16
Bagenal (1954)	Combined	Petersen	Ns	Scotland	0.710	126.4	–	4.05
Bagenal (1954)	Combined	Otoliths	Ns	Scotland	0.204	125.9	–	3.51
Guichet *et al.* (1973)	Males	Otoliths	Ns	NE Ireland	0.069	124.0	–	
	Females	Otoliths	Ns	NE Ireland	0.070	124.0	–	
Guichet *et al.* (1974)	Males	Otoliths	Ns	NE Ireland	0.024	268.2	–	
	Females	Otoliths	Ns	NE Ireland	0.087	123.7	–	
Meriel-Busy (1966)	Combined	Otoliths	Ns	Bay of Biscay	0.059	171.8	–	3.24
Descamps and Labastie (1978)	Males	Otoliths	Ns	Bay of Biscay	0.148	83.0	−0.420	
	Females	Otoliths	Ns	Bay of Biscay	0.098	116.0	−0.510	
ICES (1993)	Combined	Otoliths	Ns	Bay of Biscay	0.073	127.5	−1.130	3.07
Lucio *et al.* (2000)	Combined	Otoliths	Ns	Bay of Biscay	0.124	110.0	−0.452	3.18
	Males	Otoliths	Ns	Bay of Biscay	0.181	80.0	−0.724	
	Females	Otoliths	Ns	Bay of Biscay	0.122	110.0	−0.619	
Robles *et al.* (1975)	Combined	Otoliths	Ss	NW Iberian waters	0.070	125.4	–	3.04
Iglesias and Dery (1981)	Combined	Otoliths	Ss	NW Iberian waters	0.060	99.9	−2.740	2.78
	Males	Otoliths	Ss	NW Iberian waters	0.150	63.4	−1.030	
	Females	Otoliths	Ss	NW Iberian waters	0.060	99.8	−2.360	
Goñi (1983)	Combined	Otoliths/Back-calculated	Ss	Morocco	0.064	110.0	−0.760	2.89
	Males	Otoliths/Back-calculated	Ss	Morocco	0.067	100.0	−1.090	
	Females	Otoliths/Back-calculated	Ss	Morocco	0.065	110.0	−0.820	
ICES (1991)	Combined	Otoliths	Ss	N and NW Iberian waters	0.080	100.0	−1.420	2.90
This study	Combined	Otoliths	Ss	N and NW Iberian waters	0.075	120.5[a]	−1.715	3.04
	Combined	Otoliths	Ss	N and NW Iberian waters	0.128	88.0	−1.174	
	Males	Otoliths	Ss	N and NW Iberian waters	0.184	70.0	−0.973	
	Females	Otoliths	Ss	N and NW Iberian waters	0.127	88.7	−1.157	

[a] L_∞ fixed.

the interpretation of ring patterns and the difficulties inherent to ageing European hake otoliths. The ageing criteria used here have been elaborated on the basis of research studies carried out by our team and have been universally accepted among hake otolith readers from European Institutions involved in hake ageing (Anon., 2000).

Every ageing method requires validation (Beamish and McFarlane, 1983) but due to the difficulties in accomplishing this in European hake, only age corroboration methods (Campana, 2001) have been used to support the age estimates presented here. Thus, Piñeiro and Hunt (1989) and Piñeiro and Pereiro (1993) provided evidence in support of the length range of the age one group by means of length frequency analysis and back-calculation of otolith ring measurements. Additionally, a recent study (I. Meneses, pers. comm.) on daily growth of juvenile hake from Portuguese waters (Southern stock) found that otoliths of fish 13–20 cm long caught between June and October presented three checks and 279–413 daily increments (Anon., 2000). Furthermore, in the Mediterranean Sea, studies of the otolith microstructure and length frequency analysis have found a mean size of 16 cm at the end of the first year of life of juvenile hake (Morales-Nin and Aldebert, 1997). The ageing criteria used here were also supported by the progression of the 1993 to 1999 year-classes in the Southern stock (Anon., 2000) from which the age estimations were obtained independently from two different laboratories (Figure 8).

The characteristic checks or "false rings" observed in hake otoliths from different geographic areas are related to biological or seasonal environmental changes (spring–summer rings) affecting a large proportion of the population. Some of these checks have been identified in this study. In particular, the check between the first and second annual rings may be related to the feeding behaviour of this species. In the study area, Olaso (1994) and Velasco and Olaso (1998) described a drastic change in the diet of hake 15–20 cm long from 88% invertebrates (crustaceans) to 97% fish. This event could explain the presence of this check since it appears independently of the season when fish attain about 19 cm (Goñi and Piñeiro, 1988). In the past, this ring may have been interpreted as the second annual ring leading to underestimation of hake growth (Robles *et al.*, 1975; Iglesias and Dery, 1981; Goñi, 1983; ICES, 1991).

The second check, observed between the second and third annual rings, is formed mainly in summer and at present there is no clear explanation of its significance. However, differences in the interpretation and counting of these translucent rings appear to be the main source of discrepancies in estimates of size at age. The shift in pattern

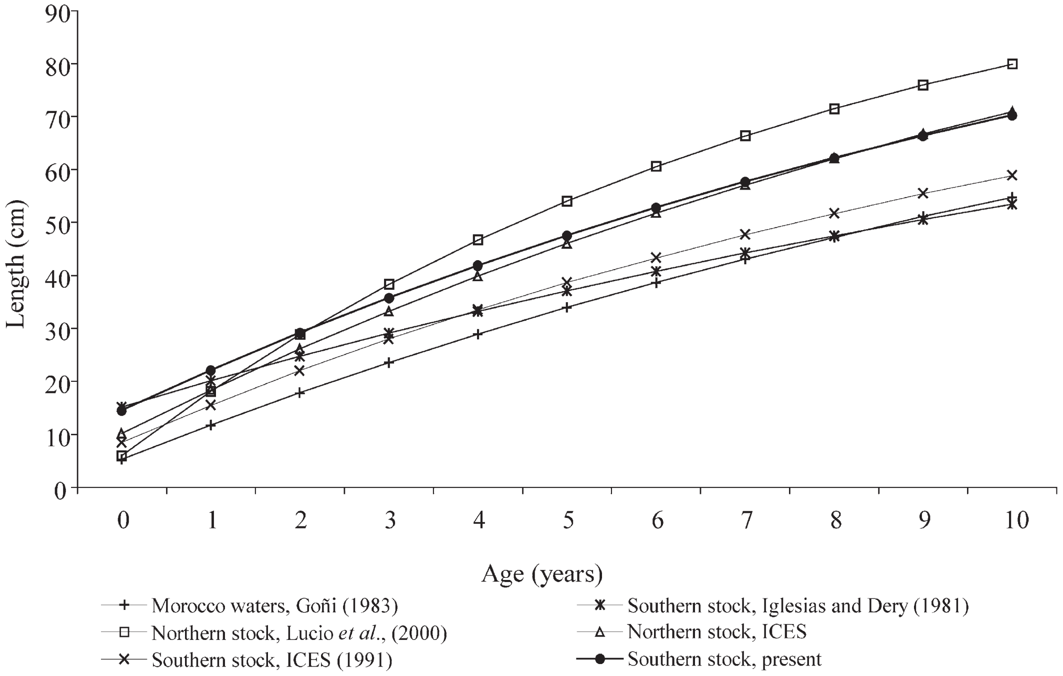


Figure 4. von Bertalanffy growth curves of European hake from ICES areas estimated by different authors.

of otolith growth from forming mainly two translucent rings per year during the first three years of life, to forming only one thereafter, has been associated with the process of sexual maturation (Hunt, 1980).

The seasonal pattern of translucent and opaque edges of hake otoliths found in our study, with a high incidence of translucent edges all year round, has also been reported by other authors (Iglesias and Dery 1981; Goñi, 1983; Piñeiro and Hunt, 1989). The edges of otolith sections were difficult to interpret, and we therefore recommend using whole otoliths when interpreting marginal growth. Nevertheless, further studies of fish larger than 40 cm are required to clarify this matter.

The complexity of the pattern of ring formation in otoliths indicates that European hake grow in an intermittent manner, undergoing a series of growth interruptions during the first years of life. In addition to the diet changes mentioned earlier, some of these interruptions could be related to the depth stratification of hake in relation to size and age (Pereiro and Fernández, 1983; Fariña and Abaunza, 1991). The predatory and reproductive behaviour of hake results in seasonal movements through habitats with

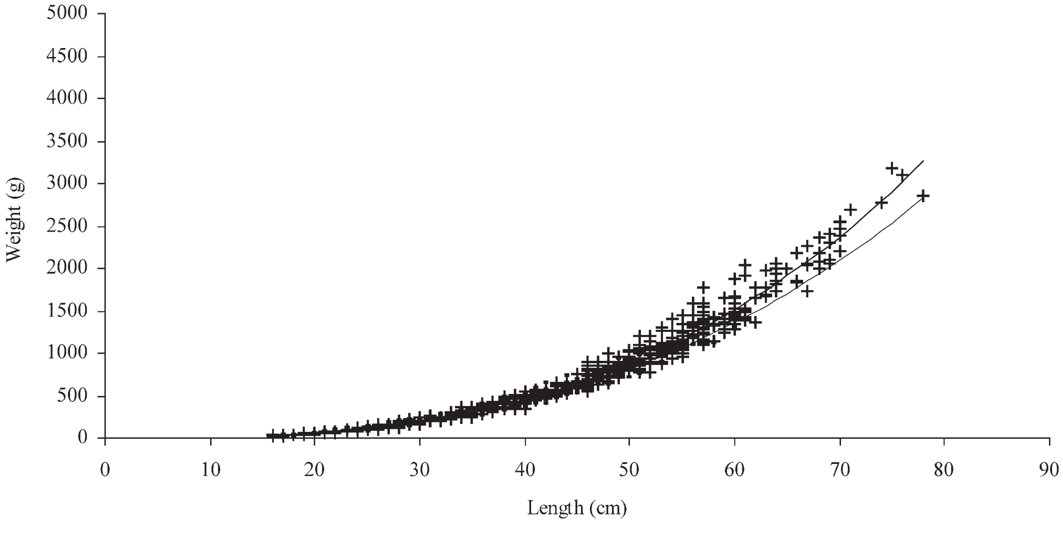


Figure 5. Length (cm)/weight (g) relationship of European hake in the study area by sex. Males: observed (–) vs. fitted (broken line); Females: observed (+) vs. fitted (solid line).

Table 5. Total length (L, cm)/total weight (W, g) relationships of hake from the Northern (Ns) and Southern (Ss) stocks (sexes combined) obtained by different authors.

Authors	Stock	Area	$W = a \times L^b$	Number	r^2	Weight range	Length range	Period
ICES (1991)	Ns	Bay of Biscay and Grand Sole	$W = 0.00513 \times L^{3.074}$	–	–	–	–	–
Lucio *et al*. (2000)	Ns	Bay of Biscay	$W = 0.00632 \times L^{3.019}$	4536	0.99	3–7300	8–103	1996–1997
Lucio *et al*. (unpublished)	Ss	N and NW Iberian waters	$W = 0.00659 \times L^{3.017}$	79 159	0.99	1–4378	5–86	1980–1998
Fontenla and Trujillo (unpublished)	Ss	N and NW Iberian waters	$W = 0.00473 \times L^{3.100}$	7429	0.99	9–2802	11–79	1984–1992
Piñeiro *et al*. (unpublished)	Ss	N and NW Iberian waters	$W = 0.00558 \times L^{3.060}$	14 339	0.99	1–4377	6–86	1984–1997
This study	Ss	N and NW Iberian waters	$W = 0.00733 \times L^{2.981}$	1391	0.99	1–2848	6–78	1996–1997

different environmental conditions. The presence of false rings has also been reported in European hake from other Atlantic waters (Hickling, 1933; Descamps and Labastie, 1978; Goñi, 1983) and from the Mediterranean (Morales-Nin *et al*., 1998). A similar pattern of false rings has been observed in otoliths of other species of the genus *Merluccius*, such as *M. bilinearis* (Hunt, 1980) and *M. hubbsi* (Renzi and Pérez, 1992).

Growth pattern

The mean lengths-at-age obtained in this study indicate that hake from the Southern stock grow faster than it has been traditionally believed. For any given size, hake is now about 1 year younger than previously estimated. This would seem realistic and coincides with studies on food consumption using bioenergetics models that indicate that hake in the study area exhibit rapid growth rates (Riis-Vestergaard *et al*., 2000). This new length-at-age estimate makes the number of year classes that contribute to the catch smaller and the age at first maturity lower (ICES, 2000). It has important implications for the estimation of essential hake population parameters, such as recruitment, fishing mortality and spawning stock biomass.

Males grow slightly faster than females up to age 2 and from age 3 onwards female growth rates surpass those of males. Females also reach larger sizes and grow older than males. The difference in growth rate coincided with the onset of sexual maturity. Lucio *et al*. (2000) are the only authors who have described the growth and reproduction of hake simultaneously. They noted a similar pattern of differential growth between males and females, with males growing faster than females before reaching sexual maturity and the reverse afterwards. Greater and faster growth in females than in males is a common phenomenon in many species of demersal fishes (Landa and Piñeiro, 2000) and may be related to differences in metabolism between sexes, such as differences in oxygen consumption

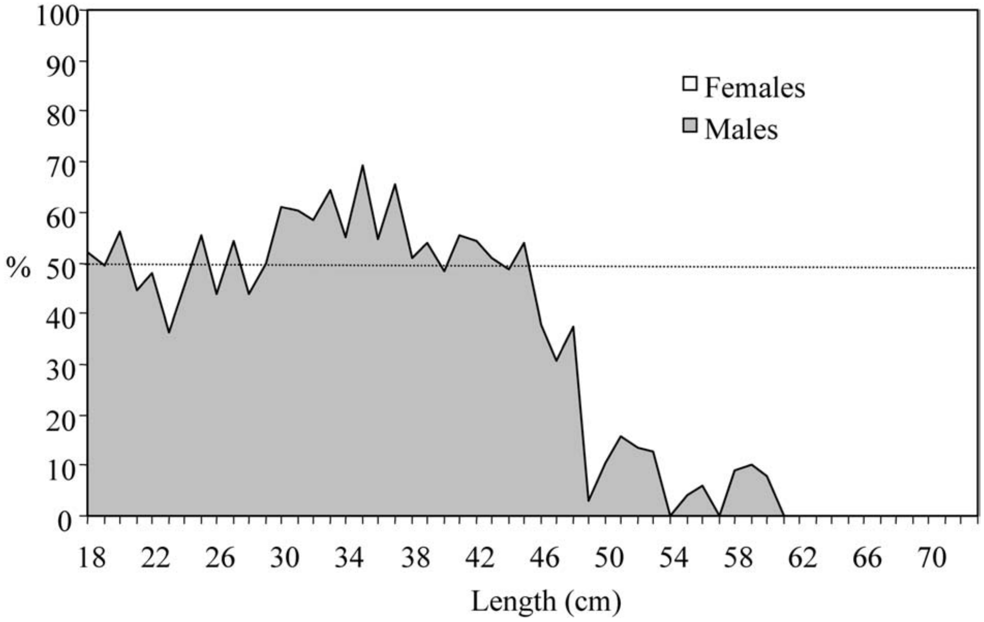


Figure 6. Sex ratio by total length of European hake from the study area. Number of males = 1382; number of females = 1156.

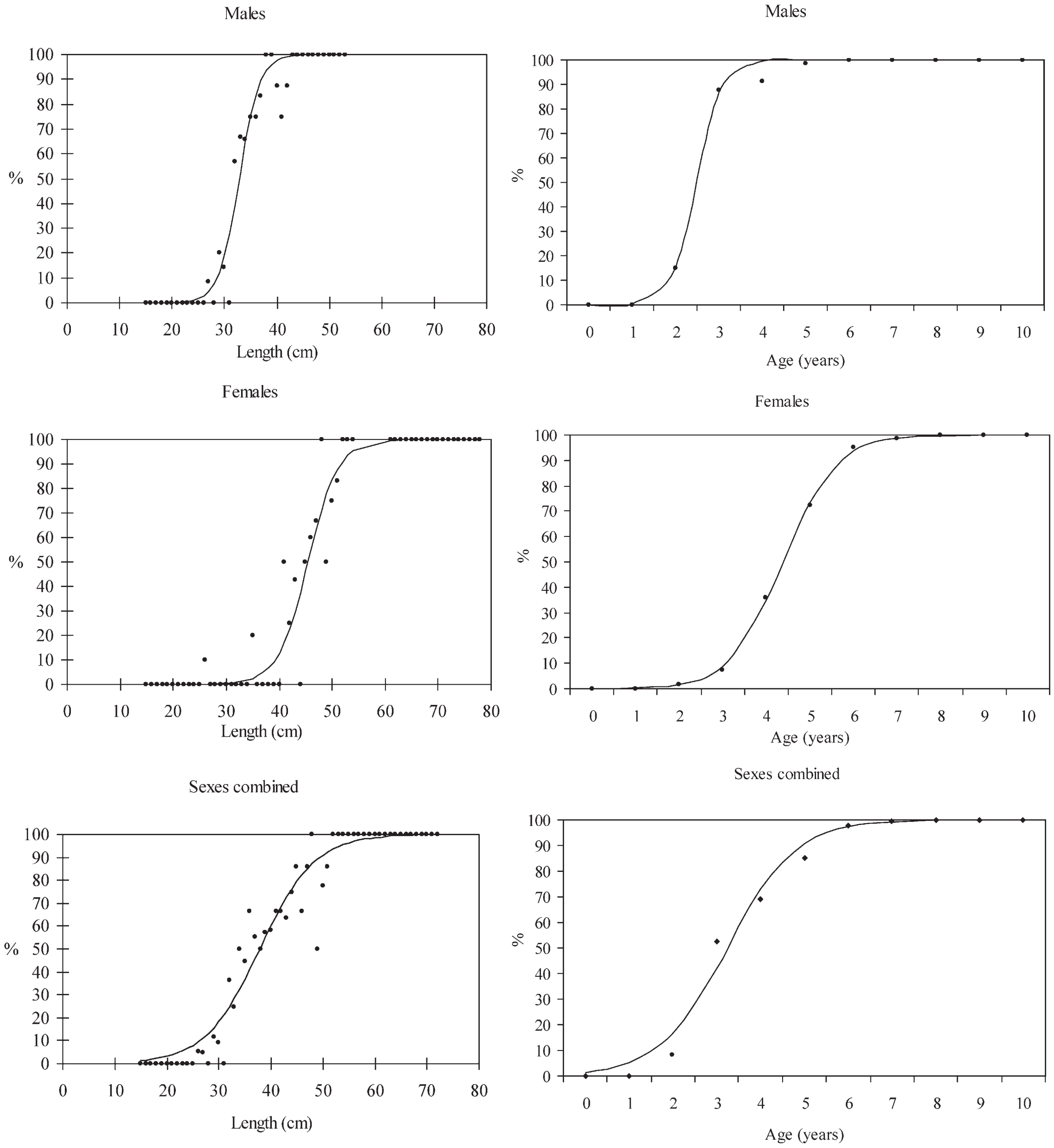


Figure 7. Maturity ogives at length and maturity ogives at age of males, females and both sexes combined of European hake from the study area.

(Pauly, 1994) and/or to differences in the level of surplus energy between reproduction and somatic growth (Rijnsdorp and Ibelings, 1989).

Bearing in mind that comparison with other growth studies can be difficult due to differences in age estimation procedures, a visual comparison suggests that hake growth rates from this study are higher than previous estimates by Iglesias and Dery (1981) and ICES (1991) in the same study area, and much higher than growth rates obtained by Goñi (1983) in hake off Morocco. However, growth rates obtained here were lower than those estimated by Lucio *et al.* (2000) in the Bay of Biscay (Northern Stock), although similar rates have been reported for hake elsewhere in the Northern stock (ICES, 1993). We contend that these discrepancies are primarily due to differences in the otolith interpretation criteria used by the various authors, although factors such as sampling strategy used could also play a role.

The main features of the sex ratio observed in this study were a ratio close to 1 : 1 up to 45 cm and a predominance of females thereafter, reaching 100% after 60 cm. There is a moderate increase in the proportion of males in the interval of 25–45 cm TL which is due to the slowdown of the males' growth rate after the onset of maturity and the

Table 6. Size at first maturity (cm) of European hake from different geographic areas. Range of maturation ($L_{75}-L_{25}$), number of individuals sampled and size range of the sample are also specified when provided by the authors.

Author	Area	Month	Year	L_{50}	L_{75}–L_{25}	Number	Range
Males							
Meriel-Busy (1966)	Bay of Biscay	April, May	1965, 1966	36.0–37.0[a]	–	–	–
Martin (1991)	Bay of Biscay	January–May	1987–1990	37.7	4.8	1129	20–62
Lucio *et al.* (2000)	Bay of Biscay	January–May	1996–1997	37.8	3.1	2865	–
ICES (1986)	Grande Sole and Bay of Biscay	–	1983–1986	38.4	11.2	854	12–85
ICES (1990)	Grande Sole and Bay of Biscay	March, December	1987–1989	33.1	6.3	–	–
ICES (1982)	NW Iberian waters	March–June	1980, 1981	33.0	–	–	–
ICES (1983)	NW Iberian waters	April–March	1980–1983	32.0	–	–	–
ICES (1986)	NW Iberian waters	June	1983	31.6	–	2252	–
ICES (1986)	NW Iberian waters	June	1984	28.6	–	199	–
ICES (1986)	NW Iberian waters	January–September	1981	37.0	–	428	–
ICES (1986)	NW Iberian waters	January–June	1982	36.5		207	
ICES (1988)	NW Iberian waters	Febuary–March	1987	27.5	–	1254	–
ICES (1986)	NW Iberian waters	January–June	1984	37.0	–	176	–
ICES (1986)	NW Iberian waters	January–June	1985	32.0	–	176	–
ICES (1982)	N and NW Iberian waters	January–December	1980–1981	39.0	–	–	–
Pérez and Pereiro (1985)	N and NW Iberian waters	January–December	1980–1982	36.5–39.5	–	1896	25–65
ICES (1986)	N and NW Iberian waters	April–December	1980	39.5	–	1230	
Cardador *et al.* (unpublished)	N and NW Iberian waters	December–July	1980–1998	31.9	5.4	9588	14–67
This study	N and NW Iberian waters	December–May	1996–1997	32.8	4.5	247	15–60
Females							
Meriel-Busy (1966)	Bay of Biscay	April, May	1965, 1966	54.0–57.0[a]	–	–	–
Martin (1991)	Bay of Biscay	January–May	1988–1990	50.5	7.0	1013	20–75
Lucio *et al.* (2000)	Bay of Biscay	January–May	1996–1997	48.8	3.8	–	–
Anon. (1986)	Great Sole and Bay of Biscay	–	1983–1986	50.4	8.9	781	12–85
ICES (1990)	Great Sole and Bay of Biscay	March, December	1987–1989	59.2	5.0	–	–
Monteiro and Lima Dias (1965)	NW Iberian waters	April–September	1959	57.7	8.4	2023	40–86
ICES (1982)	NW Iberian waters	March, June	1980, 1981	49.0	–	–	–
ICES (1983)	NW Iberian waters	April–May	1980–1983	49.0	–	–	–
ICES (1986)	NW Iberian waters	January–September	1981	49.5	–	285	–
ICES (1986)	NW Iberian waters	January–June	1982	47.0	–	190	–
ICES (1986)	NW Iberian waters	January–June	1983	46.0	–	141	–
ICES (1986)	NW Iberian waters	June	1983	55.5	–	453	–
ICES (1986)	NW Iberian waters	January–June	1984	45.0	–	320	–
ICES (1986)	NW Iberian waters	January–June	1985	47.0	–	263	–
ICES (1986)	NW Iberian waters	June	1984	58.0	–	271	–
ICES (1988)	NW Iberian waters	June February–March	1986 1987	5.10	–	874	–
ICES (1982)	N and NW Iberian waters	January–December	1980–1981	56.0	–	–	–
Pérez and Pereiro (1985)	N and NW Iberian waters	January–December	1980–1982	47.0–58.0	–	1232	25–75
ICES (1986)	N and NW Iberian waters	April–December	1980	58.0	–	832	–
Fontenla and Trujillo (unpublished)	N and NW Iberian waters	November–April	1984–1993	40.9	9.2	3298	11–74
Cardador *et al.* (unpublished)	N and NW Iberian waters	December–July	1980–1998	45.3	7.7	8234	14–86
This study	N and NW Iberian waters	December–May	1996–1997	45.4	6.2	312	15–78
Sexes combined							
Martin (1991)	Bay of Biscay	January–May	1988–1990	41.4	8.1	2142	20–75
Lucio *et al.* (2000)	Bay of Biscay	January–May	1996–1997	42.2	5.9	–	–
Saínza and Pérez (unpublished)	Grand Sole	February–May	1994	44.9	10.5	6497	31–108
ICES (1986)	Grand Sole and Bay of Biscay		1983–1986	43.3	12.5	1635	12–85
ICES (1990)	Grand Sole and Bay of Biscay	March–December	1987–1989	40.0	13.6	–	–
ICES (1987)	NW Iberian waters		1982–1984	39.0	–	–	–
Cardador *et al.* (unpublished)	N and NW Iberian waters	December–July	1980–1998	36.0	10.0	17 882	14–86
This study	N and NW Iberian waters	December–May	1996–1997	37.9	11.5	559	6–78

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[a] Derived from the figure provided by the author.

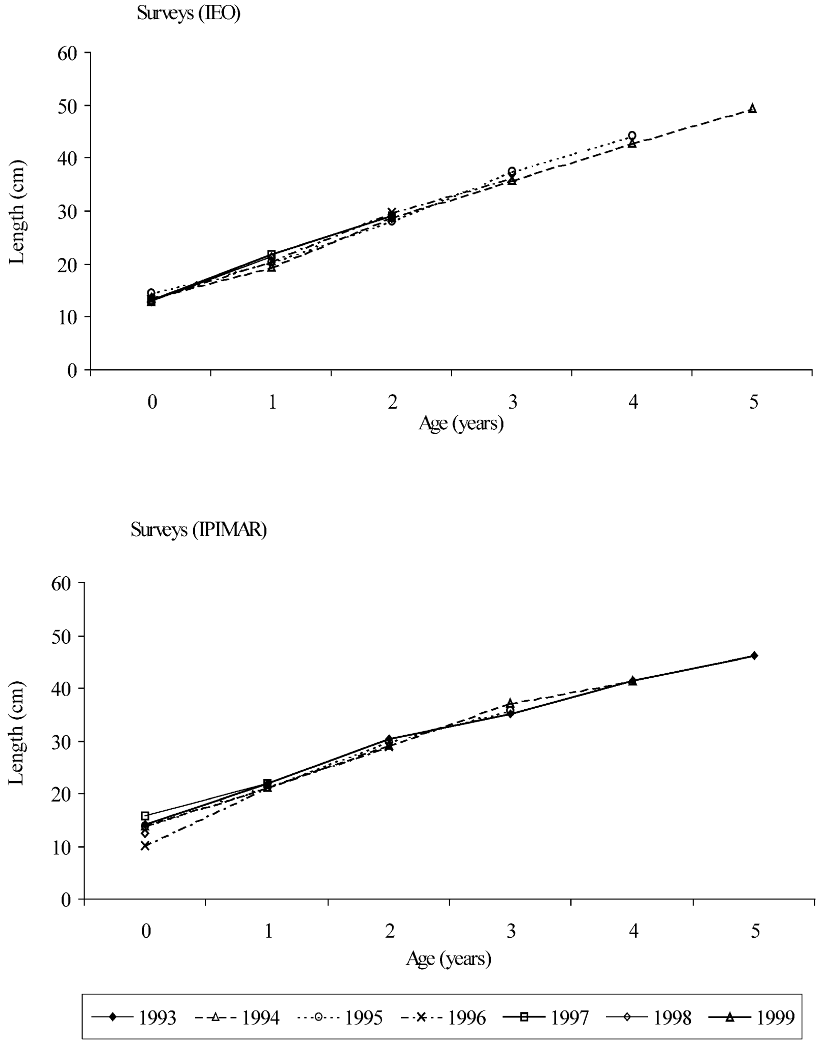

Figure 8. Mean lengths at age of different cohorts (1993–1999) obtained from ALK constructed with survey data of the Southern Stock by the IEO (Spain) and IPIMAR (Portugal) Institutes. Adapted from the Final Report of DEMASSESS (EU Project S.C. 97/015).

subsequent accumulation of individuals in that size range. A similar pattern of sex ratio by size has been described by Pérez and Pereiro (1985) in the study area, by Lucio *et al.* (2000) in the Bay of Biscay and Fariña and Fernández (1986) in western Ireland.

These results suggest that the natural mortality rate of old males may be much higher than that of females as males older than 9 years disappear from the fishery. However, the reason may be related to the different growth rates of males and females and/or to their different behaviour and consequently different accessibility to the fishing gear. Roff (1982) suggested that having reached the size at which the fish can successfully reproduce, the growth of males in some species of fish declines in part as a response to the divergence of energy into reproduction, and in part, as a response to decreased foraging activity. If male hake grow at a lower rate, particularly after the onset of maturity, the combined effect of growth and size-specific mortality would lead to a higher proportion of females in the larger size classes.

Length–weight relationships

In general, the estimates of length–weight relationships obtained here are close to those from previous studies. Differences in the numbers of samples at length distribution margins, especially in larger sizes, can explain the small differences with values reported in other studies.

Spawning season and size at maturity

The spawning season of hake in this study area extends from December to May and peaks in February. However, because hake is a partial spawner (Sarano, 1986; Murua *et al.*, 1996) mature, immature and resting females occurred simultaneously during this period. These results agree with those of Martin (1991) and Lucio *et al.* (2000).

Estimates of the size at maturity of hake obtained in this study fall within the range of estimates obtained for hake from other areas. However, our results suggest that the size-at-maturity in the Iberian waters has decreased over the last years. Sustained high fishing mortalities in this stock may be responsible for this change (ICES, 1996; Goñi, 1998; Rochet, 1998).

Conclusions

The successful application of ageing criteria presented here demonstrate that, with experience, otolith sections may be used to estimate hake age reliably up to age 5. Despite these advances, our ability to age European hake is still not good enough. Hake is a long-lived species reaching ages over 10 years and older fish are difficult to age. Thus, further research on alternative methods for ageing, and on age validation, are still required for hake. However, overall it is recognised that a significant improvement has been attained with the establishment of ageing criteria for the first 5 years of life. These criteria are already in use by the readers of the countries involved in hake stock assessment (ICES, 2000). This is particularly important because fish from ages 0 to 5 years make up 95% of the catch (mean of 1991–2000). Thus, this represents an essential step towards an accurate assessment of the status of the Southern stock.

Many efforts have been made to improve the knowledge of biological parameters of European hake in the Northeast Atlantic, but so far most of them have not produced sufficient validated information on which to base stock assessment. Our findings indicate that for European hake the age estimation of the first 5 years of life is reliable and that it will allow the successful application of empirical ALKs for the assessment of the Southern stock. Our estimates of size at maturity of hake from Iberian waters indicate there has been a decrease over the past few years, a worrying trend that will require further investigation as it may indicate over-fishing. Our results further indicate that hake from the Southern stock grow at higher rates than previously believed, approaching the values of the Northern stock. This result supports the hypothesis of Sanchez *et al.* (ICES, 1999) that there is no biological basis for European hake from ICES areas being separated by Cap Breton canyon into two different stocks (Anon., 1999).

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References

Anon. 1983. Confrontation technique sur les methodologies utilisées pour la lecture des otolithes de merlu. Report. 24 pp.

Anon. 1984. Report of the ad hoc hake otolith working group. La Rochelle, 28th May–1st June 1984. ICES CM 1984/G: 74. 15 pp.

Anon. 1986. Report of the ad hoc study group on hake. Lowestoft, 3–6 June 1986. ICES CM 1986/G: 93. 19 pp.

Anon. 1998. Biological Studies of Demersal Fish to European Commission. Annex III of the Final Report to the Commission of European Communities (BIOSDEF Study contract 95/038). 522 pp.

Anon. 1999. Evaluation of Demersal Resources of South-western Europe from standardised ground fish surveys. Final Report to the Commission of European Communities (SESITS, 2000; Study contract 96/029). 195 pp.

Anon. 2000. Report of New Assessment and Biology of the Main Commercial Fish Species: Hake and Anglerfish of the Southern Shelf Demersal Stocks in the South Western Europe. Appendix I of Final Report to the Commission of European Communities (DEMASSESS Study Contract 97/015). 497 pp.

Anon. 2002. Report of the Improving Sampling of Western and Southern European Atlantic Fisheries. Appendix 19 in the Final report to the Commission of European Communities (SAMFISH, Contract No. 99/009). 184 pp.

Bagenal, T. B. 1954. Growth rate of the hake (*Merluccius merluccius* L.) in the Clyde and other Scottish areas. Journal of the Marine Biology Association of the United Kingdom, 33: 69–95.

Beamish, R. J., and Fournier, D. A. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences, 38: 982–983.

Beamish, R. J., and McFarlane, G. A. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society, 112: 735–743.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

Casey, J., and Pereiro, F. J. 1995. European hake (*M. merluccius*) in the North-east Atlantic. *In* Hake: Fisheries, Ecology and Markets. Fish and Fisheries Series 15, pp. 125–148. Ed. by J. Alheit, and T. J. Pitcher. Chapman and Hall, London. 478 pp.

Descamps, P., and Labastie, J. 1978. Note sur la lecture et l'interpretation des otolithes du merlu. ICES CM 1978/G: 41. 21 pp.

Fariña, A. C., and Abaunza, P. 1991. Contribución al estudio de los juveniles de merluza entre Cabo Villano y Cabo Prior (NW Galicia) mediante prospecciones pesqueras. Boletín Instituto Español Oceanografía, 7(2): 155–163.

Fariña, A. C., and Fernández, A. 1986. Datos biológicos de la merluza del oeste de Irlanda. Informes Técnicos Instituto Español Oceanografía, 47: 1–13.

Goñi, R. 1983. Growth studies of European hake (*Merluccius merluccius* L.) from the Northwest African shelf. ICES CM 1983/G: 10. 3 pp.

Goñi, R., and Piñeiro, C. 1988. Study of the growth pattern of European hake (*Merluccius merluccius* L.) from the Southern stock: ICES Divisions VIIIc and IXa. ICES CM 1988/G: 18. 13 pp.

Goñi, R. 1998. Ecosystem effects of marine fisheries: an overview. Ocean and Coastal Management, 40: 37–64.

Guichet, R., Quero, J. C., and Labastie, J. 1973. Estimation du stock de merlu au nord et a l'ouest de l'Irlande. ICES CM/G: 5. 9 pp.

Guichet, R., Dardignac, J., and Guégen, J. 1974. Distribution des jeunes merlus des grupes I, II, III, dans le golfe de Gascogne. ICES CM 1973/G: 7. 3 pp.

Guichet, R. 1988. Étude de la croissance du merlu europeen (*Merluccius merluccius* L.) au cours de ses premières années. ICES CM 1988/G: 53. 27 pp.

Hickling, C. F. 1933. The natural history of the hake. Part 3. Seasonal changes in the condition of hake. U.K. Fishery Investigations Series II, 12(1): 120 pp.

Hunt, J. 1980. Guidelines for age determination of silver hake (*Merluccius bilinearis*) using otoliths. Journal Northwest Atlantic Fishery Science, 1: 65–80.

ICES. 1979. Report of the Working Group on Assessment of Hake Stocks. ICES CM 1979/G: 27. 65 pp.

ICES. 1982. Report of the Working Group on Assessment of Hake Stocks. ICES CM 1982/Assess: 14. 63 pp.

ICES. 1983. Report of the Working Group on Assessment of Hake Stocks. ICES CM 1983/Assess: 20. 55 pp.

ICES. 1986. Report of the Working Group on Assessment of Hake Stocks. ICES CM 1986/Assess: 1. 48 pp.

ICES. 1987. Report of the Working Group on the Assessment of the Stocks of Hake. ICES CM 1987/Assess: 1. 57 pp.

ICES. 1988. Report of the Working Group on the Assessment of the Stocks of Hake. ICES CM 1988/Assess: 24. 51 pp.

ICES. 1990. Report of the Working Group on Fisheries Units in Sub-areas VII and VIII. ICES CM 1990/Assess: 23. 129 pp.

ICES. 1991. Report of the Working Group on the Assessment of the Stocks of Hake. ICES CM 1991/Assess: 20. 181 pp.

ICES. 1993. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. ICES CM 1994/Assess: 3. 92 pp.

ICES. 1994. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. ICES CM 1995/Assess: 3. 598 pp.

ICES. 1996. Report of the Working Group on Ecosystem Effects of Fishing Activities. ICES CM 1996/Assess/ENV: 1. 131 pp.

ICES. 1998. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. ICES CM 1998/Assess: 4. 664 pp.

ICES. 1999. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. ICES CM 1999/ACFM: 4. 655 pp.

ICES. 2000. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. ICES CM 2000/ACFM: 4. 744 pp.

Iglesias, S., and Dery, L. 1981. Age and growth studies of hake (*Merluccius merluccius* L.) from ICES Divisions VIIIc and IXa. ICES CM 1981/G: 38. 6 pp.

Kimura, D. K., and Chikuni, S. 1987. Mixtures of the empirical distributions: an iterative application of the age-length key. Biometrics, 13: 23–35.

Landa, J., and Piñeiro, C. 2000. Megrim (*Lepidorhombus whiffiagonis*) growth in the north-eastern Atlantic based on back-calculation of otolith rings. ICES Journal of Marine Science, 57: 1077–1090.

Lucio, P., Murúa, H., and Santurtún, M. 2000. Growth and reproduction of hake *(Merluccius merluccius)* in the Bay of Biscay during the period 1996–1997. Ozeanografika, 3: 325–354.

Martin, I. 1991. A preliminary analysis of some biological aspects of hake (*Merluccius merluccius* L.) in the Bay of Biscay. ICES CM 1991/G: 54. 30 pp.

Meriel-Busy, M. 1966. La croissance du merlu dans le Golfe de Gascogne. ICES CM 1966/G: 17. 5 pp.

Monteiro, R., and Lima Dias, M. 1965. On some aspects of the ovary development in the hake (*Merluccius merluccius,* L.) of the Portuguese coast. ICES, Doc. C.M. 1965/G: 37.

Morales-Nin, B., and Aldebert, Y. 1997. Growth of juvenile *Merluccius merluccius* in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length frequency analysis. Fisheries Research, 30: 77–85.

Morales-Nin, B., Torres, G. J., Lombarte, A., and Recasens, L. 1998. Otolith growth and age estimation in the European hake. Journal of Fish Biology, 53(6): 1155–1168.

Murua, H., Motos, L., and Marrale, D. 1996. Reproductive modality and batch fecundity of the European hake (*Merluccius merluccius*). ICES CM 1996/G: 40. 27 pp.

Olaso, I. 1994. Posición trófica de la merluza en la plataforma cantábrica. *In* El estado actual de los conocimientos de las poblaciones de merluza que habitan la plataforma continental atlántica y mediterránea de la Unión Europea con especial atención a la península ibérica, pp. 217–223. Ed. by A. González-Garcés, and F. J. Pereiro. Publicación privada, Vigo. 327 pp.

Pauly, D., and Munro, J. L. 1984. Once more the comparison of growth in fish and invertebrates. ICLARM Fishbyte 2. 21 pp.

Pauly, D. 1994. On the sex of fish and the gender of scientists: essays in fisheries science. Fish and Fisheries series. Chapman and Hall, London. 264 pp.

Pauly, D. 1985. Population dynamics of short lived species with emphasis on squids. NAFO Science Council Studies, 9: 143–154.

Pereiro, F. J., and Fernández, A. 1983. Relación entre las edades y la profundidad, e índices y áreas de reclutamiento de la merluza, en Galicia y aguas adyacentes. Boletín Instituto Español Oceanografía, 1(1): 45–54.

Pérez, N., and Pereiro, F. J. 1985. Aspectos de la reproducción de la merluza *Merluccius merluccius* de la plataforma gallega y cantábrica. Boletín Instituto Español Oceanografía, 2(3): 39–47.

Pérez, N., Pereda, P., Uriarte, A., Trujillo, V., Olaso, I., and Lens, S. 1996. Descartes de la flota española en el área del ICES. Datos y Resúmenes Instituto Español Oceanografía, 2: 142 pp.

Piñeiro, C., and Hunt, J. J. 1989. Comparative study on growth of European hake (*Merluccius merluccius* L.) from southern stock using whole and sectioned otoliths, and length frequency distributions. ICES CM 1989/G: 37. 16 pp.

Piñeiro, C. G., Fariña, A. C., and Abaunza, P. 1992. Resultados de las campañas realizadas en las zonas de veda al arrastre en la plataforma continental Gallega (NW España), 1989–1990. Informes Técnicos Instituto Español Oceanografía, 130. 39 pp.

Piñeiro, C. G., and Pereiro, J. A. 1993. Study on juvenile growth pattern of European hake (*Merluccius merluccius* L.) using whole otoliths and length frequency distributions from commercial catches and fish surveys. ICES CM 1993/G: 12. 14 pp.

Piñeiro, C., Padín, A., and Loureiro, I. 1996. Metodología y técnicas en determinación de edad. Informes Técnicos Instituto Español Oceanografía, 163. 29 pp.

Renzi, M., and Pérez, M. A. 1992. Un criterio para la determinación de la edad en juveniles de merluza (*Merluccius hubbsi*) mediante la lectura de otolitos. Frente Marítimo, 11(A): 15–31.

Rijnsdorp, A. D., and Ibelings, B. 1989. Sexual dimorphism in the energetic of reproduction and growth of North Sea plaice, *Pleuronectes platessa* L. Journal of Fish Biology, 35: 401–415.

Riis-Vestergaard, J., Velasco, F., Hill, L., and Olaso, I. 2000. Food consumption of European hake (*Merluccius merluccius*) estimated by application of bioenergetics model: is the growth of hake underestimated? ICES CM 2000/Q: 12. 12 pp.

Robles, R., Pereiro, J. A., Iglesias, S., and Pereiro, F. J. 1975. Étude sur la croissance du merlu europeen, *Merluccius merluccius* L. du NW de L'Espagne. ICES CM 1975/G: 8. 17 pp.

Rochet, M. J. 1998. Short-term effect of fishing of life history traits of fishes. ICES Journal of Marine Science, 55: 371–391.

Roff, D. A. 1982. Reproductive strategies in flatfish: a first synthesis. Canadian Journal of Fisheries and Aquatic Sciences, 39: 1686–1698.

Sarano, F. 1986. Cycle ovarien du Merlu. *M. merluccius*, poisson à ponte fractionée. Revue des Travaux de L'Institut des Péches Maritimes, 48: 65–76.

Secor, D. H., Dean, J. M., and Campana, S. E. (eds.). 1995. Recent developments in fish otolith research. University of South Carolina Press, South of Carolina, Columbia. 735 pp.

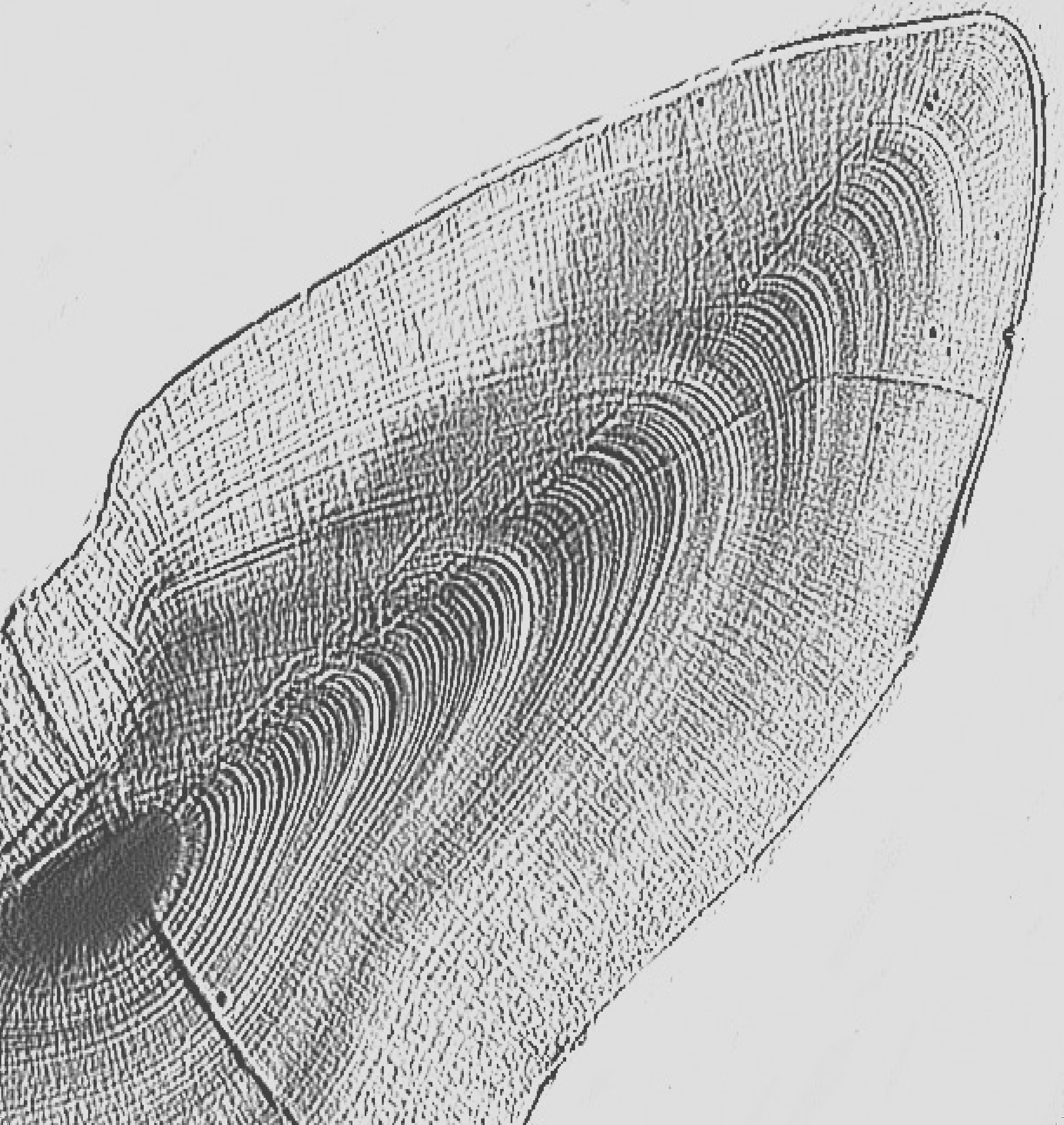
Taylor, C. C. 1960. Temperature, growth and mortality—the Pacific cockle. ICES Journal Conseil, 26: 117–124.

Taylor, C. C. 1962. Growth equation with metabolic parameters. ICES Journal Conseil, 23: 366–370.

Velasco, F., and Olaso, I. 1998. European hake (*Merluccius merluccius* L. 1758) feeding in the Cantabrian Sea: seasonal, bathymetric and length variations. Fisheries Research, 38: 33–44.

von Bertalanffy, L. 1938. A quantitative theory of organic growth. Human Biology, 10: 181–213.

Capítulo 3. Estudio del crecimiento diario mediante el análisis de la microestructura del otolito



Growth of Northwest Iberian juvenile hake estimated by combining sagittal and transversal otolith microstructure analyses. **Piñeiro, C.,** Rey, J., de Pontual, H., García, A., 2008. Fisheries Research, 93: 173-178.

Resumen

Se ha estimado el crecimiento diario de los juveniles de merluza del noroeste de la Península Ibérica empleando un nuevo enfoque que consiste en la combinación de dos análisis realizados sobre las secciones transversales y sagitales del otolito (*sagitta*) a lo largo del radio ventral. Se ha estimado la edad de juveniles de merluza para un rango de tallas comprendido entre 3 cm y 25 cm de longitud (TL) recogidos durante la primavera de 2002. El crecimiento somático estimado se ajusta a un modelo potencial tipo: Talla del pez, $TL = 3.3254 \cdot t^{0.7336} (r^2 = 0.87, p < 0.001, n = 76)$ del que resulta una tasa media de crecimiento individual de 0.66±0.06 mm.día^{-1}. El modelo de crecimiento indica que los juveniles de merluza pueden alcanzar los 25 cm de talla al final del primer año de vida. El radio ventral del otolito presenta un tamaño comprendido entre 401 y 1842 µm y el número de incrementos diarios observado está entre 104 y 387. El crecimiento del pez y el del otolito están estrechamente relacionados $(r^2 = 0.92, p < 0.001, n = 76)$. Estos son los primeros resultados de tasas diarias de crecimiento para la merluza del *stock* sur y corroboran la hipótesis de crecimiento rápido de esta especie. Asimismo, la evolución de la anchura de los incrementos en el otolito desde sus comienzos, fecha de eclosión en adelante, revela un importante componente estacional, con picos de crecimiento durante julio-agosto y octubre-noviembre. Asimismo, se comparan y discuten los resultados aquí obtenidos con los de otros autores, teniendo en cuenta además los resultados de los trabajos recientes de marcado y recaptura de esta especie.

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Growth of Northwest Iberian juvenile hake estimated by combining sagittal and transversal otolith microstructure analyses

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ABSTRACT

Daily growth of Atlantic juvenile hake from Northwest Iberia has been estimated employing a new approach combining analyses of transversal and sagittal sections of the otoliths along the ventral radius. Age of juvenile hake ranging from 3 to 25 cm collected during a spring 2002 survey was estimated. Somatic growth followed a power fit: Fish size (TL) = 3.3254*age$^{0.7336}$ ($r^2 = 0.87$, $p < 0.001$, $n = 76$), yielding an average individual growth rate of 0.66 mm/day (±0.06). The growth model indicates that after a year's life a juvenile can reach 25 cm. Otolith ventral radius ranged from 401 to 1842 μm and daily increments were between 104 and 387. Fish growth and otolith growth were closely related ($r^2 = 0.92$ $p < 0.001$, $n = 76$). These first results of daily growth rates for the Southern stock corroborate the fast-growth hypothesis of this species. The evolution of increment widths from hatch dates onwards reveals important seasonal growth peaks during July–August and October–November. A comparison with prior data and discussion is also presented in the light of recent work on hake juveniles and tagging-recapture experiences.

1. Introduction

European hake (*Merluccius merluccius*) is a demersal finfish inhabiting Atlantic and Mediterranean waters. It is widely distributed in the Northeast Atlantic from Norway to Mauritania, being more abundant from the British Isles to the south of Spain (Casey and Pereiro, 1995). It is mainly found between 50 and 370 m depth though its distributional bathymetric range is from 30 to 1000 m (Domínguez-Petit, 2007). In the Western European fisheries hake is one of the most valuable and heavily exploited demersal species. In recent years the status of European hake stocks (Northern and Southern) has been cause for concern, particularly the Southern stock that is considered to be outside safe biological limits (ICES, 2007). However, the biology of hake is insufficiently known to make accurate predictions, and in particular little is known about growth, although such knowledge is critical for accurate stock assessment.

European hake is a batch spawner (Murúa and Motos, 2006) with an indeterminate fecundity. In Iberian waters it has a protracted spawning season that covers the whole year, though most spawning activity occurs from December to July, depending on the year, with a main peak between January and March and a secondary peak in June–July (Domínguez-Petit, 2007). On the Galician and Cantabrian continental shelves there are important nursery areas located where juveniles measuring 8–14 cm long have been found from September to November (Sánchez and Gil, 1995, 2000).

The importance of growth prediction is reflected in the fast versus slow hake growth debate that has been ongoing since the 1930s (Hickling, 1933; Belloc, 1935). However, recent tagging experiments (de Pontual et al., 2003, 2006; Piñeiro et al., 2007) have provided direct evidence supporting the fast-growth hypothesis, as anticipated by Belloc (1935) and Bagenal (1954), which has implications on the management of the resource (Bertignac and de Pontual, 2007).

Tagging small juvenile fish is difficult and has not been successful yet because of the high natural mortality rate that occurs during early life stages. Therefore, an alternative approach based on otolith microstructure analysis was developed to analyze growth during the first year of life. Otolith incremental patterns have been validated for European hake at daily level, directly in the Bay of Biscay (de Pontual et al. unpublished data); indirectly in the Adriatic Sea (Arneri and Morales-Nin, 2000) and from a larval rearing experiment in Norway (Morales-Nin et al., 2005).

Growth studies on juvenile hake have been carried out in different areas of the Mediterranean Sea (Morales-Nin and Aldebert, 1997; Morales-Nin et al., 1998; Arneri and Morales-Nin, 2000; Morales-Nin and Moranta, 2004; Belcari et al., 2006) and on the

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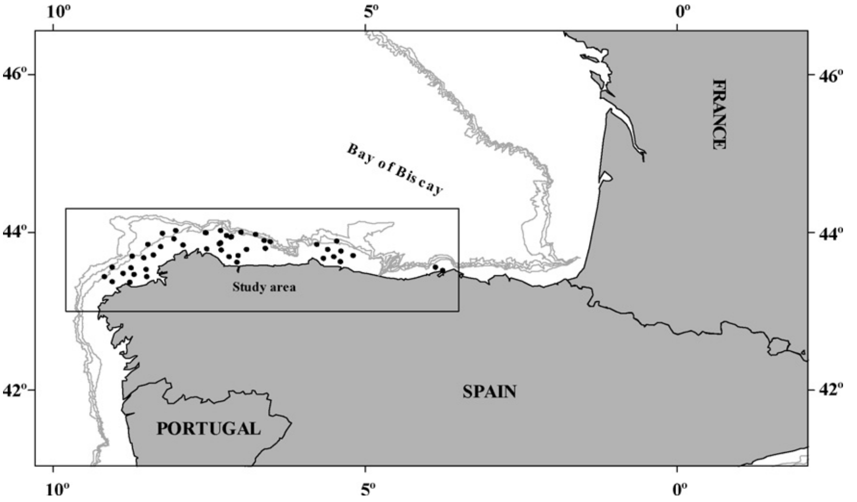

Fig. 1. Map showing the survey area and the locations from which otoliths samples were obtained.

northern stock in Atlantic waters (Kacher and Amara, 2005). Nevertheless, growth estimations are still missing for hake of the so-called Southern stock.

The purpose of this study is to estimate, for the first time, the growth rate of juvenile hake from Northwestern Iberian waters which are home to an important nursery ground (Southern stock, ICES Divisions VIIIc and IXa). The study is based on age estimates from microincrement analyses of saggital and transversal otolith sections. Seasonal growth is analyzed to improve the understanding of growth variability over time at the juvenile life stage. The results obtained are compared with growth models reported by other authors and discussed in the light of recent work carried out on juvenile of hake and tag-recapture experiments.

2. Material and methods

Juvenile hake were collected during a bottom trawl survey carried out in Spanish Atlantic waters (Fig. 1) from the 22nd to 30th of April 2002 on board the R/V “Cornide de Saavedra”. The sampling scheme was randomly stratified according to depth and geographical criteria (Sánchez, 1994; Sánchez and Gil, 2000). Hauls were performed during daylight hours at a depth range of 40–325 m. Sex, catch date and total length (±1 mm) of fish were recorded on board, while otoliths (sagittae) were carefully removed from five specimens by length class and stored dry in vials for subsequent analysis in the laboratory. The terminology used for otolith sections is based on the glossary edited by Panfili et al. (2002).

A total of 157 pairs of otoliths were prepared from individuals between 3 and 25 cm (TL) long. Otoliths were embedded in polyester resin. Thin transversal sections (TS) were obtained from the right otoliths ($N = 108$) using an ISOMET saw machine. Sections were ground with sandpaper between 400 and 1200 μm and polished with a graded series of aluminium oxide of 3, 1 and 0.3 μm until thin slides with clear microincrement sequence along the ventral axis were obtained. The left otoliths ($N = 49$) were ground equally on both sides along sagittal plane (SS) until the central zone

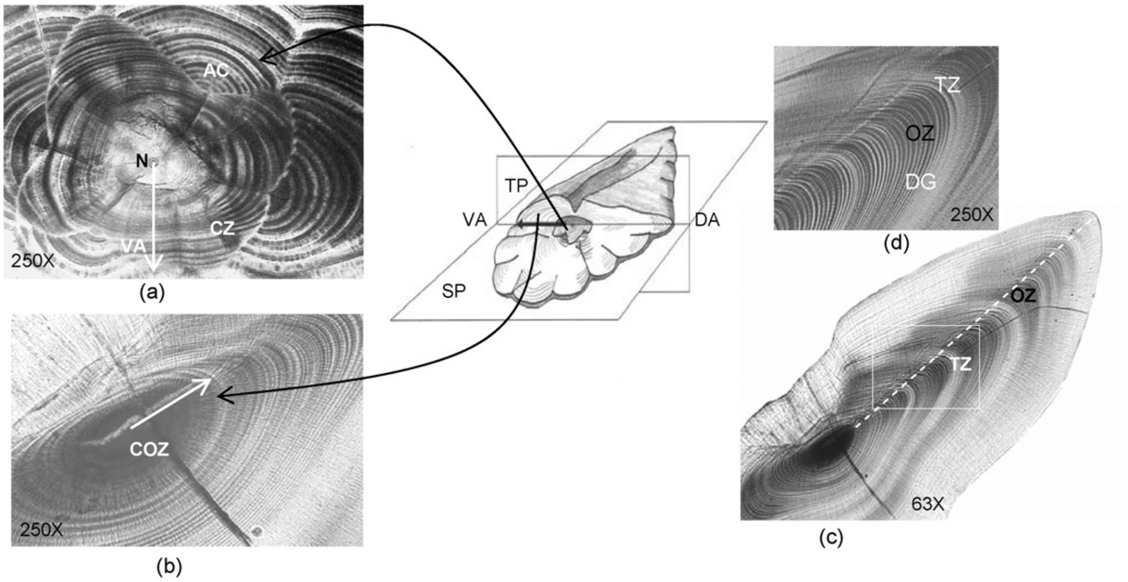

Fig. 2. Diagram showing the two sectioned planes of a hake otolith: saggital (SP) and transversal planes (TP) and the dorso–ventral axis (DA, VA). Photographs show: (a) saggital section showing the CZ, with an arrow indicating the ventral axis used for counting (VA), the nucleus (N), and accessory centers (AC); (b) transversal section showing the central opaque zone (COZ) where increments are indistinguishable; (c) view of the ventral axis chosen for radius measurement and growth increments along with changes in increment width; (d) detail of a sequence of growth increments (DG) showing translucent (TZ) and opaque bands (OZ).

(CZ) increments were totally readable from the nucleus to the edge of accessory growth centers.

Age interpretation was carried out using a light microscope connected to a video camera that projects a live image of the otolith using the Image Analysis System OTO v3 software designed by Andersen and Moksness (1988). Increments were counted and increment widths measured at magnifications from ×250 to ×1000 where the greatest magnification corresponded to the core region (SS).

On TS, counts and measures were made along the ventral axis between the edge of the central opaque zone (COZ) and the otolith edge (Fig. 2). This otolith axis has been used for decades for macrostructural interpretation (Piñeiro and Saínza, 2003) and has been therefore chosen as the reference axis to undertake daily increment counts. To ensure that the same axis was used in both sections, SS counting was done throughout the ventral axis from the first discernible increment to the boundary with the accessory contiguous growth center. A linear relationship between the number of increments and the radius of CZ was obtained from the SS. This relationship was applied to estimate the number of increments within COZ on TS. Thus, the age estimation was a 3-step process: (1) counting increments on TS from the edge of the central opaque zone to the otolith margin (Fig. 2c), (2) estimating the number of microincrements deposited in the CZ from the above relationship and (3) adding both estimates to obtain the total age. To assess age estimation errors, the average percentage of error (APE) (Beamish and Fournier, 1981) and CV were calculated for the two readings made on TS.

Assuming that no age differences occur between right and left otoliths, the growth through the life cycle of juvenile hake was fitted to a power function. Individual growth increments of each larva were estimated from the derivative of the power function of growth and corrected using residuals of estimated to observed sizes as described in Ramirez et al. (2001).

Changes in increment width under transmission light microscopy of transversal sections were examined in relation to the presence of translucent and opaque zones in order to understand the seasonal growth variation pattern.

3. Results

A total of 76 transversal and 27 sagittal otolith sections could be read, while the rest were discarded due to over-polishing or an imprecise increment pattern (Table 1). The fish and otolith size, number of increments and the total radius recorded are summarized in Table 2. The saggital and transversal sections have a different appearance. The central region of SS is characterized by a core surrounded by accessory growth centers, with individual increments being almost contiguous across the growth zones and corresponding to different points of nucleation (Fig. 2a). This complex structure appears as an opaque zone (COZ) on TS with poorly defined increments difficult to interpret (Fig. 2b). From the COZ edge, increments tend to be wider and regularly spaced on the ventral axis (Fig. 2b–d).

Table 1
Number of sagittal (SS) and transversal sections (TS) otolith sections analyzed by fish length class

TL (cm)	SS	TS
<5	1	–
5–10	1	3
11–15	8	27
16–20	14	26
21–25	3	20
Total	27	76

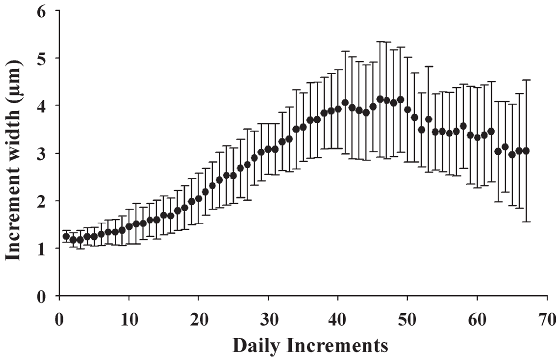

Fig. 3. Increments width (mean ± S.D.) within the CZ area of sagittal sections.

3.1. Sagittal section

The core and accessory growth centers were completely formed in individuals at a minimum size of 3 cm TL, (Fig. 2a). Radius measurements along the ventral axis to the accessory growth centers were highly variable due to its irregularly shaped border. Thus, radius measurements to the accessory growth centers were independent of fish size.

The number of increments in the CZ ranged from 38 to 69, with a mean value of 51 (±8.0) and a radius range of 89–212 µm with a mean value of 141 µm (±31.6) (Table 2). Increment counts started from a first check observed at 24 µm (±3.9), presumably corresponding to a "first feeding check" (FFC). From this check onwards visible increments appeared with a progressively wider pattern (1–2 µm) from the FFC to the edge of accessory growth centers, with a mean value of 3 µm (±0.9) and reaching a maximum value of 4 µm at 40 increments (Fig. 3). From this point increment widths decrease gradually to 3 µm.

The ventral radius of CZ versus increment counts showed strong relationships both by linear and power functions fixing an intercept at 16.3 µm as hatch check reported by Palomera et al. (2005)

Table 2
Summary statistics of data measurements obtained from the analysis of sagittal section (SS) and transversal section (TS) otoliths: fish length, first ring observed, radius of CZ (µm), increment width of CZ (µm), daily increments of CZ, ventral radius (VR, µm) of COZ, total ventral radius, daily increments without COZ and total daily increments

SS	Fish length (TL; mm)	First ring (µm)	Radius CZ (µm)	Increment width CZ (µm)	Daily increments CZ
Mean	163	24	141	3	51
Range	32–229	15–31	89–212	1–4	38–69
S.D.	41.7	3.9	31.6	0.9	8.0

TS	Fish length (TL; mm)	Total VR (µm)	VR of COZ (µm)	VR without COZ (µm)	Daily increm. without COZ	Total daily increm.
Mean	175	1211	142	1069	173	224
Range	93–256	400–1842	76–208	230–1707	46–331	104–387
S.D.	39.7	367.8	31.9	371.2	66.9	65.9

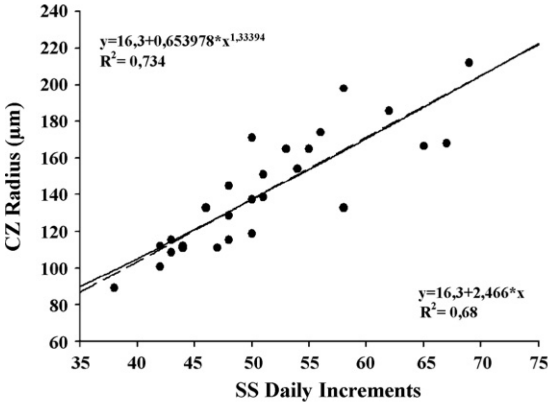

Fig. 4. Relationships (Power and Linear) between CZ radius and CZ daily increments of the sagittal section.

(Fig. 4). Nevertheless, the power function has been considered more adequate to explain the early life otolith growth of this species (Radius = 16.3 + 0.653978*age$^{1.33394}$; R^2 = 0.734).

3.2. Transversal section

The radius of ventral axis in TS ranged from 400 to 1842 µm with a mean value of 1211 µm (±367.8) (Table 2). From the COZ edge, TS showed on the ventral axis, clear increment sequence with an average width of 6 µm (±1.6). Sequences of wide and narrow increment widths viewed under transmitted light at small magnification appear as translucent and opaque bands (see Fig. 2c). The number of increments observed from the COZ to the otolith edge ranged from 46 to 331. The APE and CV obtained from the readings were 4.5 and 1.3, respectively.

Fish growth and otolith growth are linearly correlated as indicated by the relationship between otolith ventral radius in the TS and fish total length ($y = 0.1035x + 50.019$; $r^2 = 0.92$, $p < 0.001$; $n = 76$). This allows fish size to be inferred from otolith size.

3.3. Growth model

The sum of estimates of SS and TS sections provide age estimates of the fish considered in this study. The estimated age–length relationship was best explained by a power fit whose origin intercepts at 0, which would lead to a juvenile size of 25.2 cm at one year (Fig. 5). No significant difference was observed when introducing a biological intercept at 2.5 mm, size at hatch according to Palomera et al. (2005). Individual growth increments show a significant decreasing trend with age (Fig. 6), which overall averaged is 0.66 mm/day (±0.06).

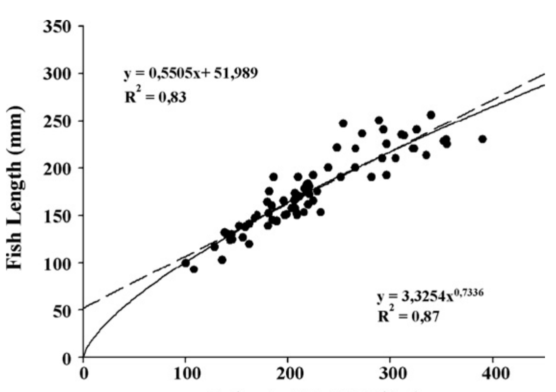

Fig. 5. Growth models (Power and Linear) of hake juveniles using estimated daily increments counts from TS and SS otoliths.

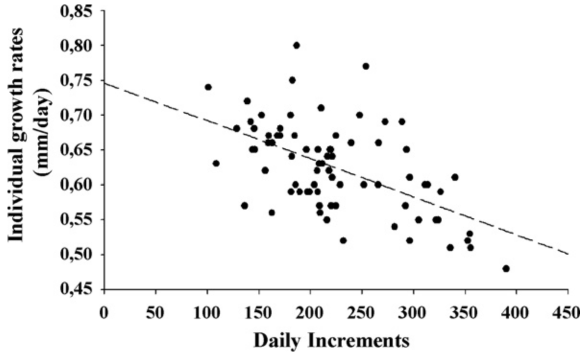

Fig. 6. Individual growth rates of hake juveniles by age.

3.4. Seasonal growth

Dating increments backwards from the most recent that coincide with capture date to the COZ area, increment width ostensibly varied with time (Fig. 7), corresponding to seasonal growth pattern. Two seasonal peaks of high growth are observed corresponding to July–August and October–November, when increment widths are greater than 6 µm. During the months of March–April and September, increment widths decrease substantially to widths measuring around 4 µm. Therefore, juvenile growth shows a seasonal trend where growth slows down in spring and early autumn (width <6 µm) and increases in summer and winter.

4. Discussion

To understand the process of growth through the juvenile life cycle it was considered important to gain insight of increment formation and deposition in early larval stages. Since larval growth studies on European hake are at present rather scarce due to their usually low catch of larvae by plankton gear (Palomera et al., 2005; Álvarez and Cotano, 2005) on European hake from Mediterranean and Atlantic waters, this study allows us to infer larval growth pattern from the interpretation of juvenile otoliths.

Larval otoliths (sagittae) are disc shaped and, as they evolve over time, become increasingly asymmetrical and gain complexity with the formation of accessory growth centers until they reach a point at which age estimation of juveniles is difficult. The approach used in this study attempts to overcome this issue by combining age estimation from saggital and transversal planes to estimate the age of an individual.

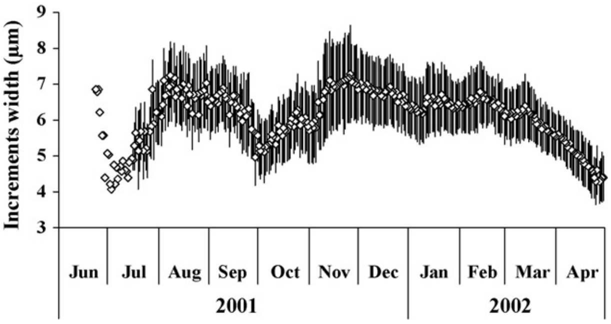

Fig. 7. Increments (mean widths) from individual capture date (most recent increment) to the COZ area of the transversal section.

Table 3
Summary table of daily growth data obtained by different authors in different areas: growth rate (GR), central zone (CZ) and its daily growth increments (DGI), mean length at the first year (ML), otoliths sampled and fish length (FL) range and geographical area

Author	GR	CZ size ø = diameter ® = radius (μm)	*N* (mean ± S.D.) or range number of DGI into the CZ	ML at first year of life (TL, cm)	Otoliths sampled and FL range	Area
Morales-Nin and Aldebert, 1997	1.15 cm/month	162 ± 43 ø	(43.9 ± 19.7)	16	81 10.5–20.7 cm (TL)	Gulf of Lions
Arneri and Morales-Nin, 2000	1.1–1.6 cm/month	–	–	15	145 1.6–16.4 cm (TL)	Central Adriatic
Morales-Nin and Moranta, 2004	1.2–2.5 cm/month	–	63	–	153 2.5–25 cm (TL)	Mediterranean Sea
Kacher and Amara, 2005	0.72–0.74 mm/day	–	(39 ± 7, *n* = 13)	23.8	107 6–22 cm (TL)	Bay of Biscay and Celtic Sea
Palomera et al., 2005	0.15–0.19 mm/day	(18.9–221.1) ø	6–26	–	71 2.5–9.1 mm (SL)	Mediterranean Sea
Álvarez and Cotano, 2005	0.15–0.17 mm/day	–	3–40	–	40 2–12 mm (SL)	Bay of Biscay
Morales-Nin et al., 2005	1.8 cm/month	461.3 ø	64	–	1 13.5 cm (TL)	Rearing conditions
Belcari et al., 2006	1.3–1.7 cm/month	–	(52 ± 2)	18.3	579 4–20 cm (TL)	Tyrrhenian Sea
This study	0.66 ± 0.06 mm/day (mean ± S.D.).	(89–212)®	(51.2 ± 8, *n* = 27)	25.2	103 3–25 cm (TL)	NW Spanish Atlantic

Generally, it is assumed that increment formation starts at hatching (Wright et al., 2002a). The studies of Palomera et al. (2005) and Álvarez and Cotano (2005) describe a dark spot in the center of the sagitta forming a clear check around it that they attribute to a hatch check at a distance of 16.3 and 15.7 μm, respectively. These authors observe a number of intermediate increments before the next check formation (4–6: Álvarez and Cotano, 2005; 4–5: Palomera et al., 2005), which presumably corresponds to the FFC, practically coincident with Bjelland and Skiftesvik (2006) who found that hake larvae start first feeding at 6 days after hatch from a larval rearing experiment. The first discernible increment observed on our SS was found at a mean distance of 24 μm (±3.9) from the core. From the relationship of CZ radius and SS increment counts in which 16.3 μm is assumed as hatch check, after 6 days we would find the FFC at 23.5 μm, in agreement with our assumed FFC.

Formation of the CZ and accessory growth centers are attributed to the pelagic life of the larval stage of hake and recruitment to the bottom (Morales-Nin and Aldebert, 1997; Arneri and Morales-Nin, 2000). The pelagic phase estimates from our observations (51 ± 8 days) are within the temporal range found by other studies (see Table 3), such as the work of Belcari et al. (2006), but slightly lower than that reported by Morales-Nin and Moranta (2004) on Mediterranean hake, and greater than that reported for Atlantic hake by Kacher and Amara (2005). These differences may be due to either the different methodological approaches and/or to site dependent growth characteristics of hake living in different environmental conditions.

Larval otolith analyses also show differences in maximum age estimates of hake larvae between the Atlantic and Mediterranean (Álvarez and Cotano, 2005; Palomera et al., 2005), but these studies did not consider the whole early life history corresponding to CZ formation.

The TS used to account for the juvenile phase presented particularly clear sequence of microincrements (Fig. 2d) which at a macroscopic scale constituted successive wide opaque zones (OZ) and narrower translucent zones (TZ). The latter corresponds to either seasonal growth structures or fish specific responses to endogenous or environmental factors (Courbin et al., 2007).

This plane of sectioning was chosen in this study because sagittal and frontal sections did not allow accounting for the entire fish life (from the core to the edge) due to curvilinear growth of hake otoliths in respectively the distal–proximal and antero–posterior axis. The choice of a common interpretation axis between SS and TS (Fig. 2) allowed a complete reconstruction of the life history on the assumption of left–right symmetry generally fulfilled except in flatfish and catfish (Wright et al., 2002b).

The measurement trajectory allows measuring the overall otolith ventral radius and estimating the number of increments, while the relationship between the ventral radius of the TS is strongly correlated with fish length ($R^2 = 0.92$, $p < 0.001$) and therefore allows a reliable estimation of fish size from otolith size.

Juvenile hake size showed clear exponential growth with the approach undertaken in this study. The relationship is supported by a strong relationship between otolith size, measured by the ventral radius, and daily increment counts which were highly correlated with linear and power fits. However, we consider that the power model provides a better description of otolith growth ($r^2 = 0.92$; $p < 0.001$).

Averaged individual growth rates (0.66 mm/day ± 0.06) were high and the growth model indicates that at one year juvenile hake may attain 25.2 cm. These values are higher than most of the reported ones (Table 3), although they are in line with recent estimations by Morales-Nin et al. (2005), and Kacher and Amara (2005) which defend the fast-growth hypothesis. Furthermore, recent tag-recapture experiments provided direct evidence for the fast-growth hypothesis (de Pontual et al., 2006; Piñeiro et al., 2007), showing that NE Atlantic hake (*M. merluccius*) may reach 25 cm TL at the end of the first year, instead of the 20 cm TL estimated from an internationally agreed age estimation method (Piñeiro and Saínza, 2003).

Growth studies based on otolith microstructure analysis have produced great discrepancies in the estimated growth rates in the juvenile phase and consequently on the size attained at the end of the first year of life. The highest daily growth rates (0.71–0.74 mm/day) were found in Atlantic hake (Kacher and Amara, 2005) which would yield a juvenile TL of 23.8 cm at the end of a first year, while lower estimates have been obtained in the Mediterranean (see Table 3). These differences may be due to various factors such as geographical locations, period of sampling and methodological approaches.

The method employed in this study allows a unidirectional linear measurement path for daily age interpretation, as recommended by Campana (1992). The main difficulty in age estimation is found in the transition area between the CZ and the prisms of accessory growth centres.

Analysis of otolith microstructure of the ventral axis indicated a variation of increment widths showing seasonal growth variations. There is a decrease in growth in spring and to a lesser extent in early autumn (width <6 μm), while there are more stable growth rates in winter and summer. Atlantic hake therefore follows a series of pulses of varying growth intensity throughout the year as also reported for Mediterranean hake (Morales-Nin and Moranta, 2004) and even undergoing a series of growth decreases during their first year of life in the Atlantic and Mediterranean hake (Piñeiro and Pereiro, 1993; Morales-Nin and Aldebert, 1997). The microstructural examination of increment width variations with time of this study confirms hake growth variability.

In conclusion, we applied a novel approach involving TS and SS microstructural analysis to estimate the growth of juvenile hake. The proposed approach will be useful for the comparison of structural patterns at micro and macroscopic scales. Such analyses are required for a better understanding of the typology of hake otolith macrostructure in relation to the biological meaning and mechanisms (endogenous and environmental) that control increment deposition. This approach also provides a new framework for the development of an alternative method of age estimation of this species as the current one has been shown to be inaccurate (de Pontual et al., 2006).

Our study indicates that, during their first year of life, hake grow at a faster rate than commonly accepted. Estimation of hake size at one year is in agreement with the hake growth model derived from tag-recapture data in both hake stocks (de Pontual et al., 2006; Piñeiro et al., 2007).

Such results are of real interest as underestimation of growth has implications stock assessment and management. As has been demonstrated for hake northern stock (Bertignac and de Pontual, 2007), bias in estimating age affects the absolute levels of fishing mortality and stock biomass estimates, and also impacts on the predicted trend in SSB indicating that the stock may be more reactive to any change such as for instance fishing level, which affects medium and long term forecasts.

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References

Andersen, T., Moksness, E., 1988. Manual for reading daily increments by the use of a computer program. Flødevigen Meldingen 4. Directorate of Fisheries. Flødevigen Biological Station. Arendal.

Álvarez, P., Cotano, U., 2005. Growth, mortality and hatch-date distributions of European hake larvae, *Merluccius merluccius* (L.), in the Bay of Biscay. Fish. Res. 76, 379–391.

Arneri, E., Morales-Nin, B., 2000. Aspects of the early life history of European hake from the central Adriatic. J. Fish Biol. 56 (6), 1368–1380.

Bagenal, T.B., 1954. Growth rate of the hake (*Merluccius merluccius* L.) in the Clyde and other Scottish areas. J. Mar. Biol. Assoc. U.K. 33, 69–95.

Belcari, P., Ligas, A., Viva, C., 2006. Age determination and growth of juveniles of the European hake, (*Merluccius merluccius* L., 1758). Fish. Res. 78, 211–217.

Belloc, G., 1935. Etude monograhique du merlu *Merluccius merluccius* L. 3ème partie. Rev. Trav. Off. Pêches marit. 8, 145–202.

Bertignac, M., de Pontual, H., 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES J. Mar. Sci. 64, 981–988.

Beamish, R.J., Fournier, D.A., 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38, 982–983.

Bjelland, R.M., Skiftesvik, A.B., 2006. Larval development in European hake (*Merluccius merluccius* L.) reared in a semi-intensive culture system. Aquacult. Res. 37, 1117–1129.

Campana, S.E., 1992. Measurement and interpretation of microstructure of fish otoliths. In: Stevenson, D.K., Campana, S.E. (Eds.), Otolith Microstructure Examination and Analysis, 117. Can. Spec. Publ. Fish. Aquat. Sci., pp. 59–71.

Casey, J., Pereiro, F.J., 1995. European hake (*M. merluccius*) in the North-east Atlantic. In: Alheit, J., Pitcher, T.J. (Eds.), Hake, Biology, Fisheries, and Markets. Chapman & Hall, London, pp. 125–147.

Courbin, N., Fablet, R., Mellon, C., de Pontual, H., 2007. Are hake otolith macrostructures randomly deposited? Insights from an unsupervised statistical and quantitative approach applied to Mediterranean hake otoliths. ICES J. Mar. Sci. 64, 1191–1201.

de Pontual, H., Bertignac, M., Battaglia, A., Bavouzet, G., Moguedet, P., Groison, A.L., 2003. A pilot tagging experiment on European hake (*Merluccius merluccius*): methodology and preliminary results. ICES J. Mar. Sci. 60, 1318–1327.

de Pontual, H., Groison, A.L., Piñeiro, C., Bertignac, M., 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES J. Mar. Sci. 63, 1674–1681.

Domínguez-Petit R., 2007. Study of Reproductive Potential of *Merluccius merluccius* in the Galician Shelf. Doctoral Thesis. University of Vigo (Spain), 253 pp. + xxii.

Hickling, C.F., 1933. The natural history of hake. 4. Age determination and growth rate. UK Ministry of Agriculture, Fisheries and Food. Invest. Ser. 2 13 (2), 120.

ICES, Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk and Megrim. ICES CM2007/ACFM:21, 700 pp.

Kacher, M., Amara, R., 2005. Distribution and growth of 0-group European hake in the Bay of Biscay and Celtic Sea: a spatial and inter-annual analyses. Fish. Res. 71, 373–378.

Morales-Nin, B., Aldebert, Y., 1997. Growth of juvenile *Merluccius merluccius* in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length frequency analysis. Fish. Res. 30, 77–85.

Morales-Nin, B., Torres, G.J., Lombarte, A., Recasens, L., 1998. Otolith growth and age estimation in the European hake. J. Fish Biol. 53, 115–1168.

Morales-Nin, B., Moranta, J., 2004. Recruitment and post-settlement growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. Sci. Mar. 68 (3), 339–409.

Morales-Nin, B., Bjelland, R.M., Moksness, E., 2005. Otolith microstructure of a hatchery reared European hake (*Merluccius merluccius*). Fish. Res. 74, 300–305.

Murúa, H., Motos, L., 2006. Reproductive strategy and spawning activity of the European hake (*Merluccius merluccius* L.) in the Bay of Biscay. J. Fish Biol. 69, 1288–1303.

Palomera, I., Olivar, M.P., Morales-Nin, B., 2005. Larval development and growth of the European hake *Merluccius merluccius* in the northwestern Mediterranean. Sci. Mar. 69 (2), 251–258.

Panfili, J., Meunier, F.J., Mosegaard, H., Troadec, H., Wright, P.J., Geffen, A.J., 2002. Glossary. In: Panfili, J., Pontual, H. (de), Troadec, H., Wright, P.J. (Eds.), Manual of Fish Sclerochronology. Ifremer-IRD Co-edition, Brest, France, pp. 373–384.

Piñeiro, C.G., Pereiro, J.A., 1993. Study on juvenile growth pattern of European hake (*Merluccius merluccius* L.) using whole otoliths and length frequency distributions from commercial catches and fish surveys. ICES C.M. 1993/G 12, 14.

Piñeiro, C., Saínza, M., 2003. Age estimation, growth and maturity of the European hake, (*Merluccius merluccius* Linnaeus, 1758) from Iberian Atlantic waters. ICES J. Mar. Sci. 60, 1086–1102.

Piñeiro, C., Rey, J., de Pontual, H., Goñi, R., 2007. Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: first results support fast growth hypothesis. Fish. Res. 88, 150–154.

Ramirez, T., Cortés, D., García, A., 2001. Growth of Alborán Sea sardine larvae estimated by otolith microstructure, nucleic acids and protein content. J. Fish Biol. 59, 403–415.

Sánchez, F., 1994. Campaña de evaluación de recursos pesqueros “Demersales0991”. Inf. Téc. Inst. Esp. Oceanog. 155, 51.

Sánchez, F., Gil, J., 1995. Influencia de anomalías térmicas de mesoescala sobre la distribucion de peces demersales. In: Cendrero, O, Olaso, I. (Eds.), Actas del IV Coloquio Internacional de Oceanografía del Golfo de Vizcaya. Inst. Esp. de Oceanografía, Santander, pp. 49–54.

Sánchez, F., Gil, J., 2000. Hydrographic mesoscale structures and Poleward Current as a determinant of hake (*Merluccius merluccius*) recruitment in southern Bay of Biscay. ICES J. Mar. Sci. 57, 152–170.

Wright, P.J., Panfili, J., Folkvord, A., Mosegaard, H., Meunier, F.J., 2002a. Direct validation. In: Panfili, J., de Pontual, H., Troadec, H., Wright, P.J. (Eds.), Manual of Fish Sclerochronology. Ifremer-IRD Co-edition, Brest, France, pp. 114–127.

Wright, P.J., Panfili, J., Morales-Nin, B., Geffen, F.J., 2002b. Otoliths. In: Panfili, J., de Pontual, H., Troadec, H., Wright, P.J. (Eds.), Manual of Fish Sclerochronoloy. Ifremer-IRD Co-edition, Brest, France, pp. 31–57.

Capítulo 4. Estudio del crecimiento real mediante el marcado y la recaptura de la especie en el medio natural

Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. De Pontual, H., Groison, A.L., **Piñeiro, C.,** Bertignac, M., 2006. ICES Journal of Marine Science. 63: 1674-1681

Resumen

En 2002 se realizó una experiencia piloto de marcado de merluza usando una metodología específicamente desarrollada para capturar y manipular peces vivos. A finales del 2005, 36 merluzas y cinco marcas han sido recuperadas (3.1% de tasa de recaptura) con un tiempo máximo de libertad de 1 066 días. El crecimiento somático de los ejemplares recapturados fue dos veces más rápido de lo esperado, según el modelo de crecimiento de von Bertalanffy publicado para esta especie en el Golfo de Vizcaya. La subestimación del crecimiento se relaciona con la sobreestimación de la edad como se demuestra en los dos análisis independientes realizados. El primero esta basado en la interpretación «a ciegas» de los otolitos marcados que ha sido realizada de forma independiente por dos expertos europeos que están involucrados en la estimación de la edad de la merluza europea. Los resultados muestran que las estimaciones de edad obtenidas no son ni exactas (inconsistentes con la posición de la marca de OTC en el otolito), ni tampoco precisas. El segundo análisis compara el crecimiento esperado del otolito con el crecimiento observado y el resultado obtenido presenta una gran discrepancia entre ambos tipos de datos. Estos dos análisis indican que el método de estimación de la edad acordado a nivel internacional no es válido demostrándose la necesidad de investigar más. Este estudio, aunque basado en datos limitados, hace hincapié en la necesidad de mejorar el conocimiento biológico de la especie para un mejor asesoramiento y gestión del recurso y refuerza las razones para llevar a cabo estudios de validación.

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Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation

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de Pontual, H., Groison, A. L., Piñeiro, C., and Bertignac, M. 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. – ICES Journal of Marine Science, 63: 1674–1681.

In 2002, a pilot experiment on hake tagging was carried out using methodology specifically developed to catch and handle fish in good condition. By the end of 2005, 36 hake and five tags had been returned to the laboratory (a 3.1% return rate) with a maximum time at liberty of 1066 days. The somatic growth of the recoveries proved to be twofold higher than that expected from published von Bertalanffy growth functions for the species in the Bay of Biscay. The growth underestimation was related to age overestimation, as demonstrated by two independent analyses. The first was based on a blind interpretation of marked otoliths conducted independently by two European experts involved in the routine age estimation of hake. The result shows that the age estimates were neither accurate (inconsistent with oxytetracycline mark positions) nor precise. The second approach compared the predicted otolith growth with the observed growth, and the discrepancy between the two data sets was large. Both types of analyses invalidate the internationally agreed age estimation method and demonstrate a need for further research. Although based on limited data, the study highlights the need to improve biological knowledge of the species in order to improve assessment and management advice. It also strengthens the argument for age validation.

Keywords: age, age estimation method, age validation, European hake, growth, *Merluccius merluccius*, otolith, tagging.

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Introduction

European hake (*Merluccius merluccius*) are distributed widely over the Northeast Atlantic shelf, from Norway to Mauritania, with greatest density between the British Isles and south of the Iberian Peninsula (Casey and Pereiro, 1995). For several decades, they have been targeted by demersal fisheries, being landed either as a target or as incidental catch using a variety of gear, including bottom trawl, net, and longline.

Two stocks of European hake are considered by the International Council for the Exploration of the Sea (ICES) for assessment purposes. The northern stock is distributed north of the Cape Breton Canyon, which separates the waters of France from those of Spain in the southeastern Bay of Biscay, and the southern stock is south of this physical barrier. Total annual landings of European hake declined from about 120 000 t in the early 1960s to a current level of some 50 000 t (ICES, 2005). In recent years, assessments have raised concern about the state of the stocks, and in 2004 a Recovery Plan was implemented by the EU Commission (EC Regulation No. 811/2004). Spawning-stock biomass declined from the mid-1980s to the beginning of the 1990s, and has since remained low, although there has been sign of a slight recovery since 2000. Recruitment declined during the 1990s.

The stock assessment model currently used by ICES is an age-based sequential population model (Extended Survivors Analysis, XSA; Darby and Flatman, 1994), a model that has proved useful in establishing a diagnosis on stock

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status. However, the current assessment has several limitations and shortcomings, including scientific misgiving about growth rate, which obviously impacts the age distributions of the catch and abundance indices used in the age-structured model.

The debate about whether hake is a fast- or a slow-growing species has been going on since the 1930s (Hickling, 1933; Belloc, 1935). Studies since those days in various areas have reported very different growth estimates for hake in both the Northeast Atlantic (Bagenal, 1954; Guichet *et al.*, 1973; Robles *et al.*, 1975; Decamps and Labastie, 1978; Iglesias and Dery, 1981; Lucio *et al.*, 2000a; Piñeiro and Sainza, 2003) and the Mediterranean (Aldebert and Recasens, 1996; Morales-Nin and Aldebert, 1997; Garcia-Rodriguez and Esteban, 2002). It is important to assess whether such diversity is biologically meaningful or whether it reflects bias in the estimation methods.

In most studies, growth functions are provided, using otoliths to estimate age. Errors in age estimation can be caused by accuracy and/or precision issues (Campana, 2001). Accuracy refers to the closeness between measurements (here, the age estimates) and their actual (true) values. Precision (also referred to as repeatability or reproducibility) pertains to the closeness of a set of repeated observations to each other (here, several readings of the same otolith). Hake otoliths are difficult to interpret and the age estimation method has not been validated, although progress has been made recently regarding precision (Piñeiro and Sainza, 2003). To address this, a pilot experiment was carried out in 2002 in the northern Bay of Biscay, and this resulted in the development of a suitable tagging method for this reputedly very fragile species (de Pontual *et al.*, 2003). Here we report on the somatic and otolith growth of the hake recovered from that tagging work, and compare both with what would have been expected according to current knowledge of the species in the Bay of Biscay. We also examine the accuracy and precision of the internationally agreed age estimation criteria (here called the agreed age estimation method). Questions raised by this analysis are then discussed.

Methods

For a detailed description of the tagging method, the reader is referred to de Pontual *et al.* (2003). Basically, the innovative aspects of that method concern both the capture method (trawling with a codend specially designed to minimize mortality) and the handling method. The tagging process consists of measuring hake total length (TL) to the centimetre below, inserting a T-bar tag (Floy Tag® FD-94 Anchor tag) at the base and in front of the second dorsal fin, and injecting the fish with Terramycine®, a veterinary solution of oxytetracycline (OTC), at a dose of 60 mg kg^{-1}. The individual OTC dose was adjusted using the weight–length relationship $W = 5.13 \times 10^{-6} L^{3.0744}$ (Dorel, 1986). As this antibiotic deposits a fluorescent mark on growing calcified structures, it is conventionally used for age validation (see Wright *et al.*, 2002).

Analysis of somatic growth

Recaptured tagged fish were stored frozen until subsequent processing. Prior to otolith extraction, they were measured and weighed, and sex was recorded if possible, i.e. for fish returned ungutted.

To our knowledge, all hake growth models are based on the von Bertalanffy growth function (VBGF), as rearranged by Beverton (1954) and Beverton and Holt (1957):

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

where L_t is the predicted length at time t, L_∞ the asymptotic maximum length reached when t tends to infinity, K the Brody growth coefficient, and t_0 is the hypothetical time at which length is assumed to be zero.

The growth expected for a given period, for instance, the time elapsed between tagging and recapture, can be derived from Equation (1) as follows:

$$L_2 - L_1 = \left(L_\infty - L_1\right)\left(1 - e^{-K(t_2-t_1)}\right), \tag{2}$$

where L_2 and L_1 are the lengths at recapture and tagging, respectively, and t_2 and t_1 are the corresponding dates of catch and tagging (i.e. t_2-t_1 is the time interval between catch and tagging). Equation (2) was therefore used to compute the growth predicted by the VBGF (L_∞ and K) as previously published, so allowing comparison of observed and predicted growth.

We then fitted the VBGF to the tagging data in order to obtain relationships of length at age, which were used for various comparisons.

Otolith growth analysis and age estimation

Otolith extraction was performed under clean conditions, as required for chemical analysis. The left and right sagittae were stored in clean polypropylene vials and stored in a dessicator awaiting further treatment. For this study, we systematically used the left sagittae (an arbitrary choice for standardization). The otoliths were weighed to the nearest µg, then embedded in epoxy resin. Transverse sections (TS) through the core were made with a precision saw, embedded on a glass slide with epoxy resin, and finally ground and polished using a polishing machine. Impurities were removed by rinsing the TS in an ultrasonic bath with Milli Q water. The remaining anterior and posterior otolith parts were kept for subsequent use.

The TS were observed under a compound microscope equipped with three light sources (transmitted, reflected, and UV). We used TNPC software (Fablet and Ogor, 2005) to acquire calibrated numerical images and to make appropriate otolith measurements. For each otolith, images were digitized under all three light modalities.

Otolith analysis was carried out in two different ways: first, we used an interpretation exercise using the agreed age estimation method, i.e. following the specific interpretation criteria described in Piñeiro and Sainza (2003); second, we compared observed and expected otolith growth. The blind interpretation of marked otoliths was carried out independently by two European experts involved in the routine age estimation of hake. No information was provided to them except for the date of fish recapture. The experts were asked to store interpreted images with positions of the reading axis, false rings (FR), and winter rings (WR), for subsequent comparisons.

Otolith growth analysis was carried out by first fitting an otolith growth model to data (size of ventral radius by age group) acquired during the 2002 international exchange of European hake otoliths (Anon., 2002). It is worth noting that this data set was obtained using the agreed age estimation method. The otolith growth model was fitted using monthly data. The conversion of age groups to age data was performed using catch dates and considering 1 January as the birth date. In this study, we considered the observed otolith growth of tagged fish to be the ventral radius at recapture (Rv2 = nucleus to otolith edge) minus the ventral radius at tagging (Rv1 = nucleus to OTC mark). This observed otolith growth was compared with the expected otolith growth using the same approach as for somatic growth analysis. The otolith analysis took place in June 2003, and subsequent recoveries were used for somatic growth analysis only.

Results

With respect to previously published data (de Pontual *et al.*, 2003), the recovery rate has increased slightly. We now have 41 recoveries (36 fish returned to the laboratory plus five tags sent without the fish to which it was attached), corresponding to a recapture rate of 3.1%. Time at liberty varies from 1 to 1066 days.

Somatic growth

Fish that had spent fewer than 20 days at liberty had a nil or even negative somatic growth rate. Therefore, growth analysis was performed excluding recoveries for which $L_2 - L_1 \leq 0$. For combined sexes, the growth rate was estimated at 0.038 ± 0.004 cm d^{-1} (mean ± 1 s.d.; $n = 20$). The growth rates of 0.029 ± 0.006 cm d^{-1} ($n = 7$) and 0.039 ± 0.005 cm d^{-1} ($n = 8$) for males and females, respectively, did not differ significantly (t-test, $p = 0.269$). The growth rate, when restricted to recoveries that had at least one summer and one winter at liberty, was estimated to be 19.82 ± 1.49 cm y^{-1} ($n = 6$).

Figure 1 illustrates the results of a more thorough analysis that compared the observed growth with that predicted by current growth models. Observed and expected growth coincide for fish that had been at liberty less than ca. 50 days. Thereafter, growth is about twice as high as expected according to Equation (2) and using the values of L_∞ and K parameters published by ICES (1993) and Lucio *et al.* (2000a) for European hake of the northern stock in the Bay of Biscay (see Table 1). Although few returns had a long time at liberty, they do provide a strikingly consistent data set with relatively small individual variability, and faster growth of fish tagged at relatively smaller length (see, e.g., fish tagged at 21 cm and 23 cm on Figure 1).

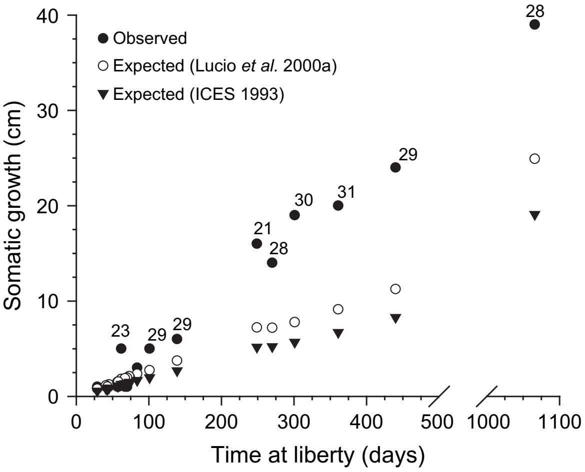

Figure 1. Comparative analysis of observed somatic growth and growth predicted by ICES (1993) and Lucio *et al.* (2000a) von Bertalanffy growth functions for hake in the Bay of Biscay. Numbers above the points indicate fish lengths (in cm) at tagging.

Fitting VBGF parameters on sex-combined data ($L_2 - L_1 > 0$, $n = 20$) with a non-linear regression model provided the estimates for model 1 of $L_\infty = 89.9$ cm (66.8−112.9 cm asymptotic 95%CI, $r^2 = 0.959$) and $K = 0.362$ (0.176−0.549 asymptotic 95%CI). The correlation between K and L_∞ estimates was −0.977. This good correlation and the scarcity of data are the main reasons for the large confidence intervals. In order to obtain a better estimate of K, we set L_∞ to 110 cm, a value that is included in the model 1 CI and that corresponds to the estimate of Lucio *et al.* (2000a). For model 2, K was estimated at

Table 1. Von Bertalanffy growth parameters fitted from recapture data with respect to reference models for hake from the northern stock of the Bay of Biscay. See Equation (1) for the significance of the parameters.

Author	Sex	K	L_∞ (cm)	t_0 (years)
ICES (1993)	Combined	0.073	127.5	−1.130
Lucio *et al.* (2000a)	Combined	0.124	110.0	−0.452
	Males	0.181	80.0	−0.724
	Females	0.122	110.0	−0.619
This study	Combined	0.362	89.9	—
This study (L_∞ fixed)	Combined	0.250	110	—
	Males	0.436	80	—
	Females	0.261	110	—

0.250 (0.225–0.276 95%CI, $r^2 = 0.955$). This value is twofold higher than the value of K estimated by Lucio *et al.* (2000a; Table 1). Figure 2 provides a graphic comparison between our estimated growth models and the available reference models. Models 1 and 2 are very close in the size range of recoveries (maximum length 67 cm). However, we consider model 2 (referred to as the "new VBGF" hereafter) to be more reliable than model 1, because L_∞ is probably larger than 90 cm, and because of the reduced CI on K.

Fitting the von Bertalanffy growth function onto data separated by sex was not feasible if both L_∞ and K were estimated. A very large CI was obtained for males ($n = 7$), and no convergence at all for females ($n = 8$). We therefore fixed the L_∞ values as those of Lucio *et al.* (2000a), respectively, 80 cm and 110 cm for males and females, which led to an estimated K of 0.436 (0.336–0.536 95%CI, $r^2 = 0.976$) for males and 0.261 (0.204–0.319 95%CI, $r^2 = 0.902$) for females. A graphic presentation of the results compared with reference material is given in Figure 3.

According to these estimates, the ages at first maturity could well be 1+ and 2+ for males and females, respectively, on the basis of known sizes at first maturity in the Bay of Biscay ($L_{50} = 37.8$ cm and 48.8 cm for males and females, respectively; Lucio *et al.*, 2000a).

Age estimation and otolith growth

The first approach to otolith analysis consisted of the blind interpretation exercise based on the agreed age estimation method. The precision of the age estimates ($n = 33$) provided independently by two European expert readers was quite low, agreement being just 48.5% (Figure 4a). This poor precision was highlighted by comparing the annotated images, which revealed inconsistencies between numbers and positions of WRs and FRs identified by the two experts. Figure 4a shows a cross-comparison of estimates provided by the two. The recoveries aged between 1 and 4 years by expert A were aged between 2 and 5 years by expert B. The discrepancies between estimates did not exceed 1 year except in the case of 9% of the fish (two fish aged 4 years by expert A were aged 2 years by expert B, and one fish aged 2 years by expert A was aged 5 years by expert B; Figure 4a).

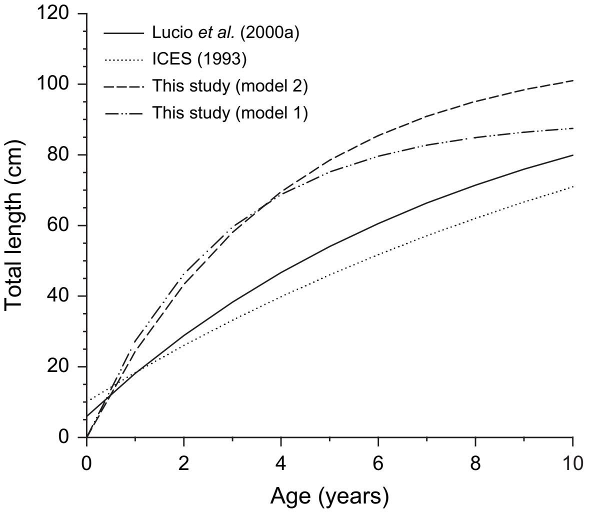

Figure 2. Comparison of a sexes-combined von Bertalanffy growth function of hake in the Bay of Biscay (ICES, 1993; Lucio *et al.*, 2000a), and the same function fitted from recapture data (in model 1, both K and L_∞ were estimated, whereas in model 2, L_∞ was set to 110 cm).

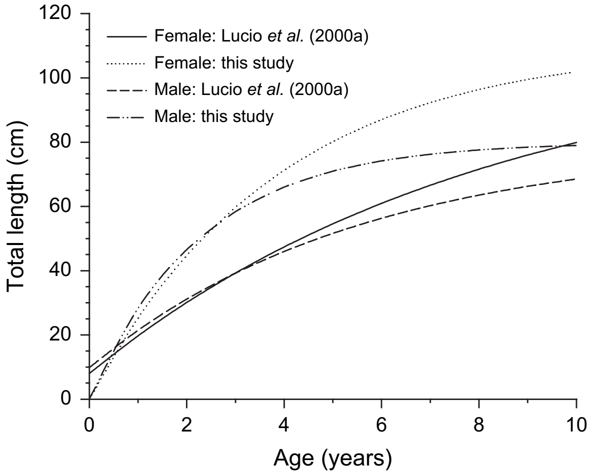

Figure 3. The von Bertalanffy growth function fitted from recapture data for male and female hake and the corresponding function given by Lucio *et al.* (2000a). Fitting was performed fixing L_∞ values to those given by Lucio *et al.* (2000a): 80 cm and 110 cm for males and females, respectively.

The important outcome of this analysis is evidence that the agreed age estimation method leads to inaccurate estimates of age. Figure 5 shows an otolith of a hake that had been at liberty for less than 1 year (301 days) after tagging. Considering the position of the OTC mark, it is clear that macrostructures interpreted as WRs were actually FRs. The fish first aged 4+ years was then aged 2+ years. The inconsistency between otolith interpretation by experts and the OTC mark position was observed in all fish displaying significant otolith growth (i.e. sufficient time at liberty). A prospective interpretation exercise guided by the OTC mark position was undertaken. The age range provided by the new interpretation (referred to as new age in Figure 4b) was 1–2 years, whereas the range of the mean ages provided by the blind reading was 2–4 years. In all cases but one, the new interpretation provided younger ages than the rounded mean ages estimated from the blind interpretation exercise (Figure 4b). Estimates also shifted towards younger ages (Figure 4c) when they were derived from the new VBGF (model 2).

The second approach to analysing marked otoliths consisted of comparing observed with predicted otolith growth. In order to obtain an otolith growth model based on the agreed age estimation method, we tested three methods of

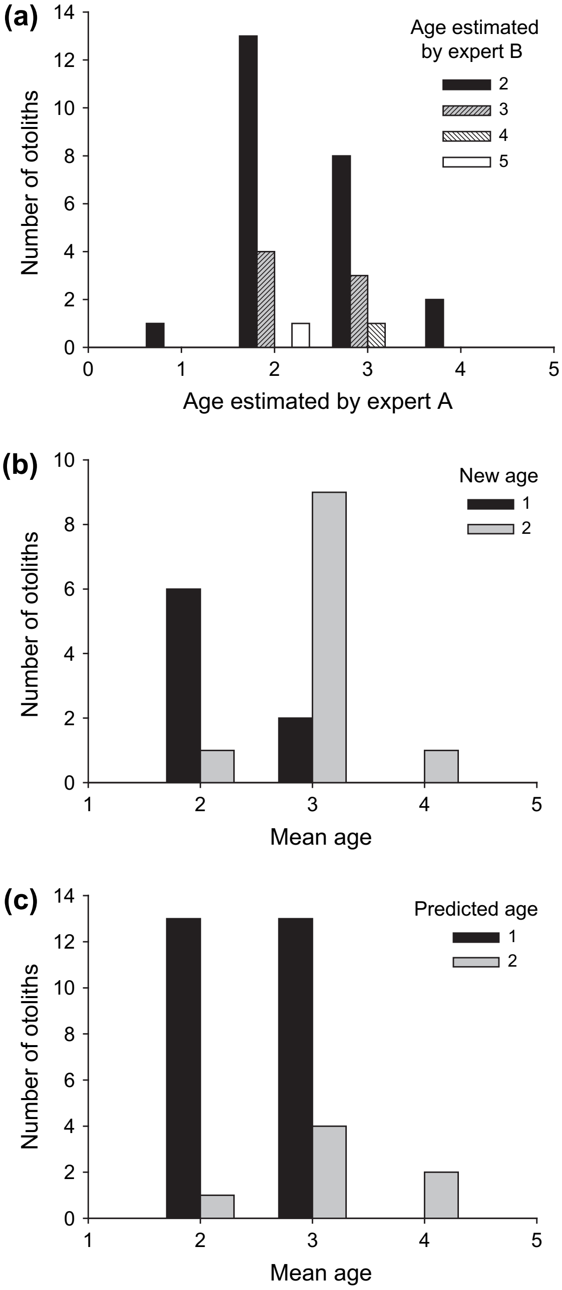

Figure 4. Results of marked otolith interpretation analyses. (a) Blind reading: a cross-comparison of age estimates (in years) provided by experts A and B. (b) Comparison of the rounded mean ages given by the experts and ages subsequently assigned using the oxytetracycline mark position to guide interpretation. (c) Comparison of the rounded mean ages given by experts and the ages predicted using the new von Bertalanffy growth function ($L_\infty = 110$ cm, $K = 0.250$).

fitting otolith growth data (size of ventral radius vs. fish age) acquired during the 2002 international otolith exchange for European hake. The von Bertalanffy otolith growth model turned out to be better than the linear and power models ($r^2 = 0.924$, $r^2 = 0.879$, $r^2 = 0.907$, respectively; $n = 99$). Otolith growth was estimated as follows:

$$\mathrm{Rv} = 5.018\left(1 - \mathrm{e}^{(-0.168(t+0.80))}\right), \tag{3}$$

where Rv is the otolith ventral radius (in mm) and t the age (in years).

This model was used to compute expected otolith growth following the argument used to analyse somatic growth (see Equation (2)). Figure 6 is a comparison of expected and observed otolith growth; there is great discrepancy between the two data sets. Otolith growth was measurable on fish that had a very short time at liberty, and the discrepancy between expected and observed growth occurred even for early recoveries. Although individual variability is quite high, the observed otolith growth is, overall, more than twice as high as expected from the otolith growth model of Equation (3). This is further evidence that the agreed age estimation method gives biased age estimates for hake.

Discussion

Somatic growth

For the first time since research on hake started in the 1920s, observed growth data are available for comparison with estimations. Two hake tagging experiments were reported before our pilot study. Lucio *et al.* (2000b) reported three recaptures from 151 released tagged fish, but only one fish was retrieved after a very short time at liberty. An interesting pioneer experiment also succeeded with the recovery of just one tagged fish after 255 days at liberty (Belloc, 1935). The fish lengths at tagging and recapture were, respectively, 28.9 cm and 40.6 cm, corresponding to a growth rate of 16.7 cm y^{-1}. From his single observation, Belloc (1935) said that hake was a fast-growing species, contradicting the slow-growth hypothesis of Hickling (1933), who estimated the species growth rate at 8.9 cm y^{-1}. The disagreement between these authors could not be explained by geographic area because both studies took place off the south coast of Ireland. Numerous studies have since reported very different hake growth patterns for areas between Ireland, the British Isles (Bagenal, 1954; Guichet *et al.*, 1973), and Morocco (Goñi, 1983), including the Bay of Biscay (Decamps and Labastie, 1978; ICES, 1993; Lucio *et al.*, 2000a) and off the Iberian Peninsula (Robles *et al.*, 1975; Iglesias and Dery, 1981; Goñi and Piñeiro, 1988; Piñeiro and Sainza, 2003). We focused our comparative analysis on data published by ICES (1993) and Lucio *et al.* (2000a), in order to restrict comparisons to the Bay of Biscay.

Negative growth rates observed in some fish that spent a very short time at liberty may be the result of shrinkage following freezing, as has been observed for other species (Armstrong and Stewart, 1997; Al Hassan *et al.*, 1999), and/or the consequence of poor precision of measurement at sea (always to the centimetre below). Stress induced by capture and tagging is likely to lower the somatic growth

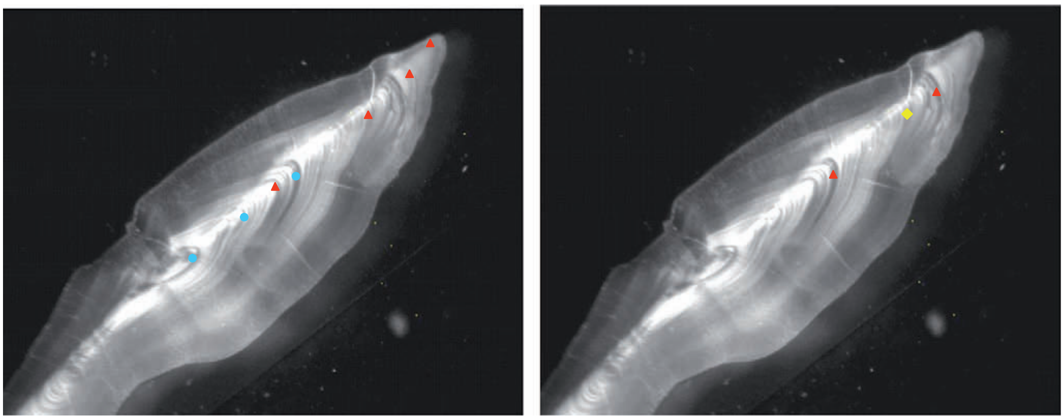

Figure 5. Transverse section of a marked otolith (#1356) observed under reflected light. (a) Blind interpretation (age 4+ years); (b) new interpretation (age 2+ years). Blue indicates the false rings (FR) described by Piñeiro and Sainza (2003), red the winter rings (WR), and yellow the oxytetracycline (OTC) mark. The fish was recaptured 301 days after tagging (TLcap: 30 cm, TLrecap: 49 cm).

rate for some time, as observed here for fish that had been at liberty for less than ca. 50−80 days (see Figure 1). However, our results clearly show that the growth rate of hake that spent sufficient time at liberty diverges by a factor of 2 from previous estimates. They provide direct evidence for the fast-growth hypothesis defended by Bagenal (1954), who used *in toto* otolith readings, and Piñeiro and Pereiro (1993), who examined the modal progression of size frequency. Interestingly, they also confirm the recently published study on 0-group hake from the Bay of Biscay and the Celtic Sea, which states that the mean length of juvenile hake at the end the first year of life was 23.8 cm (Kacher and Amara, 2005). Those authors based their estimate on counts of otolith micro-increments, assuming a daily rhythm of deposition. Marked otoliths have allowed us to validate this hypothesis (work in progress). According to the VBGF fitted to our tag-recapture data, the length at age 1 would be 24.3 cm (sexes combined, model 2), very close to the estimate of Kacher and Amara (2005). Different growth rates in males and females have been extensively reported (Casey and Pereiro, 1995; Piñeiro and Sainza, 2003). The non-significant *t*-test comparing the growth rates of males and females may be questionable because it considered fish that were tagged at different sizes and that had spent different times at liberty. The VBGF fitted on sex-based data (Figure 3) may change our perception, assuming that the L_∞ values given by Lucio *et al.* (2000a) are correct. However, these models remain tentative because of the maximum sizes of the recovered hake (67 cm and 49 cm for males and females, respectively), and the insufficiency of the data. Nevertheless, hake probably mature much younger than previously believed, whatever the sex, a finding that will undoubtedly have a substantial impact on the understanding of the population dynamics of the species.

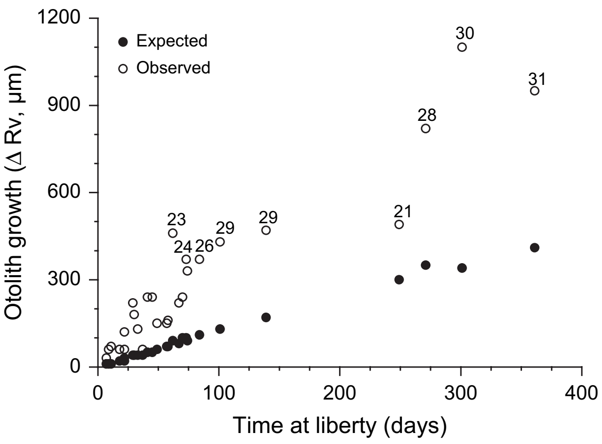

Figure 6. Comparative analysis of the expected otolith growth and the observed otolith growth with respect to time at liberty. Otolith growth (ΔRv) is the distance (in μm) from the oxytetracycline mark to the edge, measured on the ventral radius. Numbers above the points indicate fish lengths (in cm) at tagging.

The VBGFs derived from the tagging data (sexes combined or separate) need to be refined, and interannual variability linked to environmental conditions and/or density-dependence has to be investigated. We might have other alternative growth models from our data, for instance deciding on L_∞ from historical data or from the maximum reported length of 140 cm (Cohen *et al.*, 1990). However, our data do not support the hypothesis of a very high L_∞ (see the CIs of model 1), but they do support the L_∞ value given by Lucio *et al.* (2000a). Moreover, recent findings on fisheries-induced rapid evolution of key life-history traits in harvested populations (Olsen *et al.*, 2004) incline one to be cautious when using historical data. More data are obviously needed. Some should be provided in the near future, perhaps from large-scale tagging surveys carried out in 2004 and 2005 in the northern Bay of Biscay. Recent mark-recapture studies from several research institutes should also produce valuable information on the geographic variability in hake growth rates. Waters off the Iberian Peninsula, the southern Bay of Biscay, and the Mediterranean (Gulf of Lions and the Balearic Isles) have been under consideration until now. For the first area, the preliminary results of a pilot tagging experiment carried out in 2004 indicate that the fast-growth hypothesis of hake could also be valid for the southern stock.

Age estimation and otolith growth

The analyses performed on marked otoliths clearly show that age overestimation is the reason for growth underestimation. This is an important issue, because the annual assessment of hake stocks by ICES requires that reliable age-length keys (ALK) be provided annually by different countries. Regarding hake, considerable effort has been made to improve the precision of age data through successive international reading exercises and workshops. This goal has been partially achieved, and experts have recently agreed on criteria that provide acceptable precision for ages up to 5 years (Piñeiro and Sainza, 2003), i.e. ages of up to 2–3 years in the context of faster growth shown here. However, those authors emphasized the difficulty in interpreting ring patterns, which are particularly complex in hake otoliths, and the need to validate the agreed interpretation criteria. Our results clearly show the unsuitability of these criteria. Both precision, which was assessed by a cross-comparison of estimates provided by two independent experts, and accuracy, which was assessed by analysing the consistency of the ages provided by the experts with the oxytetracycline mark positions, have turned up questionable results. Poor precision (Figure 4a) is inconsistent with the progress previously achieved in international workshops and exchanges. It is worth pointing out too that the experts involved in our marked otolith interpretation were considered as the most experienced in the international comparison. The percentage agreement they achieved during the last international exchange (Anon., 2002) was 68%, when computed on the size range of the recoveries. Poorer precision may at least partly result from the fact that fish length was not provided, although one expert usually exploits this extra information during his routine annual hake age estimation. Access to this information may well have improved precision. In fact the use of auxiliary data can reduce errors as well as introduce bias (Morison *et al.*, 2005). Specific validation studies are necessary to assess accuracy (see review by Campana, 2001). For European hake, all previous attempts have proved unsuccessful, so our results point to progress on this topic. Results reported in Figures 4b, c, and 5 lead to a conclusion that the agreed age estimation method provides overestimated age and underestimated growth. From a more general perspective, such results highlight the fact that precision management in the absence of accuracy cannot, under any account, guarantee data quality.

Otolith growth analysis (Figure 6) provides further evidence of biased age estimates for hake. Interestingly, results show that otolith growth is measurable on very quick recoveries, even on the dorso-ventral axis where growth is relatively compressed. Therefore, the hake otolith continues to grow even though somatic growth has seemingly ceased, perhaps because of stress. This observation may illustrate the possible uncoupling of otolith and somatic growth under particular conditions (Mosegaard *et al.*, 1988; Folkvord *et al.*, 2000). The relatively high individual variability may be explained by several factors, including length at capture, season, and environmental conditions, such as the temperature that fish actually experience.

Validated data are currently insufficient to develop an alternative robust age estimation method for European hake. Data expected from recent tagging experiments should help to achieve this goal, but attention must be focused on drawing up a typology of macrostructures and on understanding their biological meaning, as well as on understanding the mechanisms (environmental and endogenous) that control their deposition.

The results of the present study raise concern over the production of potentially inaccurate ALKs for stock assessment and management advice. The impact of biased age estimates on the European hake stock assessment conducted in ICES is currently under investigation, and it may well be considered as critical by managers, the consequence of both the recent concern about the state of Atlantic stocks and in the context of an international commitment to exploit stocks to their maximum sustainable yields by 2015 (UN, 2002).

Besides being a contribution to knowledge of Atlantic hake, our results have a more generic interest, because they strengthen the argument for age validation. This point needs to be stressed, because uncertainty in age estimation in a number of ICES stocks is well recognized.

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References

Al Hassan, L. A. J., Bujawari, J. A., and El Siline, O. A. 1999. Additional report on the effect of preservatives and freezing on morphological characters of four sparid fish species collected from Benghazi, Libya. Journal of Animal Morphology and Physiology, 46: 57–62.

Aldebert, Y., and Recasens, L. 1996. Comparison of methods for stock assessment of European hake *Merluccius merluccius* in the Gulf of Lions (Northwestern Mediterranean). Aquatic Living Resources, 9: 13–22.

Anon. 2002. Report on improving sampling of western and southern European Atlantic Fisheries. Final report to the Commission of European Communities, Appendix 19, SAMFISH, Contract No. 99/009. 184 pp.

Armstrong, J. D., and Stewart, D. C. 1997. The effects of initial length and body curvature on shrinkage of juvenile Atlantic salmon during freezing. Journal of Fish Biology, 50: 903–905.

Bagenal, T. B. 1954. Growth rate of the hake (*Merluccius merluccius* L.) in the Clyde and other Scottish areas. Journal of the Marine Biological Association of the UK, 33: 69–95.

Belloc, G. 1935. Etude monographique du merlu *Merluccius merluccius* L., 3ème partie. Revue des Travaux de l'Office des Pêches maritimes, 8: 145–202.

Beverton, R. J. H. 1954. Notes on the use of theoretical models in the study of the dynamics of exploited fish populations. Miscellaneous Contribution of the US Fisheries Laboratory, Beaufort, North Carolina, 2. 159 pp.

Beverton, R. J. H., and Holt, S. J. 1957. On the dynamics of exploited fish populations. Fishery Investigations London, Series 2, 19. 533 pp.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–202.

Casey, J., and Pereiro, J. A. 1995. European hake (*M. merluccius*) in the North-east Atlantic. *In* Hake: Biology, Fisheries and Markets, pp. 125–147. Ed. by J. Alheit, and T. J. Pitcher. Chapman and Hall, London. 478 pp.

Cohen, D. M., Inada, T., Iwamoto, T., and Scialabba, N. 1990. FAO species catalogue, 10. Gadiform fishes of the world (order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date. FAO Fisheries Synopsis, 10(125): 442.

Darby, C. D., and Flatman, S. 1994. Virtual Population Analysis: Version 3.1 Windows/Dos User Guide. MAFF Information Technology Series, 1. Directorate of Fisheries, Lowestoft.

Decamps, P., and Labastie, J. 1978. Note sur la lecture et l'interprétation des otolithes du merlu. ICES Document CM 1978/G: 41.

de Pontual, H., Bertignac, M., Battaglia, A., Bavouzet, G., Moguedet, P., and Groison, A. L. 2003. A pilot tagging experiment on European hake (*Merluccius merluccius*): methodology and preliminary results. ICES Journal of Marine Science, 60: 1318–1327.

Dorel, D. 1986. Poissons de l'Atlantique Nord-Est: relations taille-poids. Ifremer Report DRV.86.001/RH/NANTES. 165 pp.

Fablet, R., and Ogor, A. 2005. TNPC: digital processing for calcified structures. [online] http://www.ifremer.fr/lasaa/TNPC/manuel_tnpc4.pdf.

Folkvord, A., Blom, G., Johannessen, A., and Moksness, E. 2000. Growth-dependent age estimation in herring (*Clupea harengus* L.) larvae. Fisheries Research, 46: 91–103.

Garcia-Rodriguez, M., and Esteban, A. 2002. How fast does hake grow? A study on the Mediterranean hake (*Merluccius merluccius* L.) comparing whole otoliths readings and length frequency distributions data. Scientia Marina, 66: 145–156.

Goñi, R. 1983. Growth studies of European hake (*Merluccius merluccius* L.) from the northwest African shelf. ICES Document CM 1983/G10.

Goñi, R., and Piñeiro, C. 1988. Study of the growth pattern of European hake (*Merluccius merluccius* L.) from the southern stock: ICES Divisions VIIIc and IXa. ICES Document CM 1988/G: 18.

Guichet, R., Quero, J. C., and Labastie, J. 1973. Estimation de la composition du stock du merlu au nord et à l'ouest de l'Irlande. ICES Document CM 1973/G: 5.

Hickling, C. F. 1933. The natural history of hake. 4. Age determination and growth rate. UK Ministry of Agriculture, Fisheries and Food, Investigation Series 2, 13(2). 120 pp.

ICES. 1993. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. ICES Document CM 1994/Assess: 3. 92 pp.

ICES. 2005. Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk and Megrim. ICES Document CM 2005/ACFM: 02.

Iglesias, S., and Dery, L. 1981. Age and growth of hake (*Merluccius merluccius* L.) from ICES Divisions VIIIc and IXa. ICES Document CM 1981/G: 38.

Kacher, M., and Amara, R. 2005. Distribution and growth of 0-group European hake in the Bay of Biscay and Celtic Sea: a spatial and inter-annual analysis. Fisheries Research, 71: 373–378.

Lucio, P., Murúa, H., and Santurtún, M. 2000a. Growth and reproduction of hake (*Merluccius merluccius*) in the Bay of Biscay during the period 1996–1997. Ozeanografika, 3: 325–354.

Lucio, P., Santurtun, M., and Quincoces, I. 2000b. Tagging experiments on hake, anglerfish and other species in the Bay of Biscay. ICES Document CM 2000/Z: 09.

Morales-Nin, B., and Aldebert, Y. 1997. Growth of juvenile *Merluccius merluccius* in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length-frequency analysis. Fisheries Research, 30: 77–85.

Morison, A. K., Burnett, J., McCurdy, W. J., and Moksness, E. 2005. Quality issues in the use of otoliths for fish age estimation. Marine and Freshwater Research, 56: 773–782.

Mosegaard, H., Svedäng, H., and Taberman, K. 1988. Uncoupling of somatic and otolith growth rates in Arctic charr (*Salvelinus alpinus*) as an effect of differences in temperature response. Canadian Journal of Fisheries and Aquatic Sciences, 45: 1514–1524.

Olsen, E. M., Heino, M., Lilly, G. R., Morgan, M. J., Brattey, J., Ernande, B., and Dieckmann, U. 2004. Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature, 428: 932–935.

Piñeiro, C., and Sainza, M. 2003. Age estimation, growth and maturity of the European hake, *Merluccius merluccius* (Linnaeus, 1758) from Iberian Atlantic waters. ICES Journal of Marine Science, 60: 1086–1102.

Piñeiro, C. G., and Pereiro, J. A. 1993. Study on juvenile growth pattern of European hake (*Merluccius merluccius* L.) using whole otoliths and length frequency distributions from commercial catches and fish surveys. ICES Document CM 1993/G: 12.

Robles, R., Periero, J. A., Fernandez, A. M., and Garcia, J. M. 1975. Estudios de cartografia, selectividad y marcado de merluza europea, *Merluccius merluccius* L., frente al litoral Gallego. Boletin del Instituto Español de Oceanografia, 190. 39 pp.

UN. 2002. Report of the World Summit on Sustainable Development. Johannesburg, South Africa, 26 August–4 September 2002, A/CONF.199/20*, New York. [online] http://www.johannesburgsummit.org/html/documents/documents.html.

Wright, P. J., Panfili, J., Folkvord, A., Mosegaard, H., and Meunier, F. J. 2002. Direct validation. *In* Manual of Fish Sclerochronoloy, pp. 114–127. Ed. by J. Panfili, H. de Pontual, H. Troadec, and P. J. Wright. Ifremer–IRD Co-edition, Brest, France.

Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: First results support fast growth hypothesis. **Piñeiro C**, J. Rey, H. de Pontual and R. Goñi, 2007. Fisheries Research 88: 150-154.

Resumen

En el primer experimento de marcado de merluza europea (*Merluccius merluccius L.*) realizado en aguas del noroeste de la Península Ibérica con el fin de estudiar el crecimiento de la especie en libertad, se soltaron al mar 527 ejemplares marcados. La supervivencia tras la captura y marcado fue del 58%. La mortalidad en el proceso de captura está positivamente correlacionada con la profundidad y negativamente correlacionada con el tamaño de los ejemplares. Quince meses después del marcado, siete ejemplares (1.3%) han sido recapturados después de transcurrir un periodo de tiempo en libertad que va desde 29 a 446 días. En este trabajo se aportan los primeros datos obtenidos sobre tasas reales de crecimiento en libertad de la merluza europea del *stock* sur y se comparan con tasas obtenidas en experiencias de marcado realizados en otras regiones así como con tasas de crecimiento derivadas del método convencional de lectura de otolitos. La tasa media de crecimiento somático del conjunto de los ejemplares recapturados fue de 0.032 ± 0.016 cm.día^{-1} (n=7, sexos combinados), mientras que la tasa media de los dos ejemplares de merluza con más de 340 días en libertad fue de 0.052 ± 0.003 cm.día^{-1} (sexos combinados). Estos resultados indican que el método convencional de lectura de otolitos sobreestima la edad y subestima el crecimiento.

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Short communication

Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: First results support fast growth hypothesis

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Abstract

In the first tagging experiment of European hake (*Merluccius merluccius* L.) conducted off the NW Iberian Peninsula to study hake growth in the wild we released 527 live tagged individuals. The survival rate after capture and tagging was 58%. Mortality during capture was positively correlated with depth of capture and negatively correlated with hake size. Fifteen months after tagging, seven individuals (1.3%) had been recaptured with times at liberty ranging from 29 to 466 days. We provide the first direct measurements of growth rates of Southern stock European hake in the wild and compare them with rates obtained from tagging experiments in other regions and with rates derived from conventional otolith age reading. The mean somatic growth rate of all recaptured hake was $0.032 \pm 0.016\,\text{cm day}^{-1}$ (sexes combined), while the mean growth rate of the two hake with over 340 days at liberty was $0.052 \pm 0.003\,\text{cm day}^{-1}$ (sexes unknown). These results indicate that conventional otolith age reading methods overestimate age and underestimate growth.

Keywords: European hake; Growth rates; Tag-recapture; Age estimation; Northeast Atlantic

1. Introduction

The European hake *Merluccius merluccius* L. is widely distributed throughout the Northeast Atlantic and Mediterranean and reaches greatest density between the British Isles and the Southern Iberian Peninsula (Casey and Pereiro, 1995). It is one of the most valuable and heavily exploited demersal species in Western European fisheries. The status of *M. merluccius* populations in the NE Atlantic is assessed annually by the International Council for the Exploration of the Sea (ICES) on the basis of two separate stocks, the Northern stock and the Southern stock, to the north and south of Cape Breton Canyon (Bay of Biscay), respectively (ICES, 1979). Recent assessments of the status of the Southern stock (Spanish and Portuguese waters) reveal a dramatic decline of the spawning stock biomass that has been attributed to overfishing (ICES, 2006). Despite numerous studies focusing on the biology of *M. merluccius* (see Piñeiro and Saínza, 2003 for a review), many gaps remain in our knowledge on the species. In particular, there is a lack of knowledge on the growth rate, which is vital for population assessment using age-based models. Problems with hake otolith interpretation and age estimation have to date hindered efforts to produce reliable age length keys for age-based models (Piñeiro and Saínza, 2003).

One of the most reliable methods of validating the growth of fishes is tagging and recapturing individuals in nature (Campana, 2001). Until recently few tagging experiments of hake had been conducted due to the poor survival of individuals captured by conventional methods (Belloc, 1935; Robles et al., 1975; Lucio et al., 2000). In 2002 a new trawl gear specially designed for the capture of live individuals was successfully tested by de Pontual et al. (2003). The survival rate after capture and tagging was 68% and the rate of recapture after 3 years was 3.1%. The results of that experiment indicated that the rate of somatic growth of Northern stock hake was two times higher than predicted by current growth models derived from internationally agreed otolith ageing criteria (de Pontual et al., 2006).

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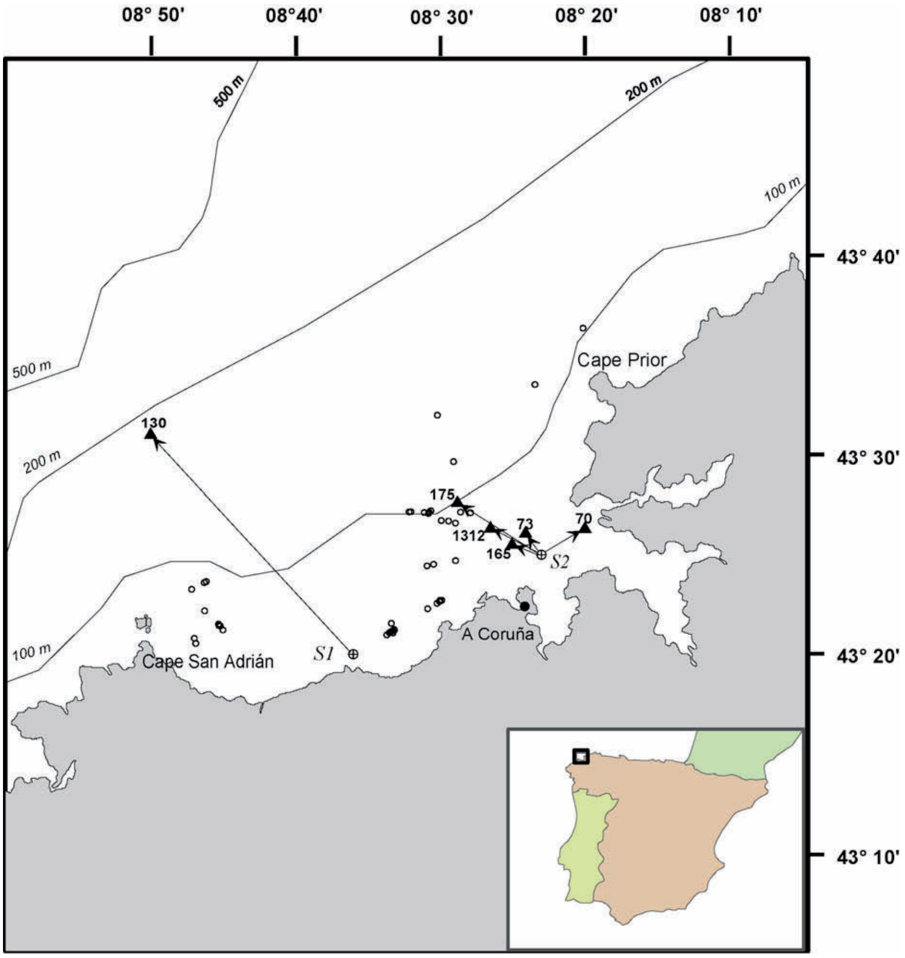

Fig. 1. Study area indicating capture (circles), release (cross circles S1, S2) and recapture (triangles) locations with fish codes.

Internationally agreed criteria for hake otolith interpretation and age estimation, summarized by Piñeiro and Saínza (2003), have never been validated and have been questioned by the first results of Northern stock hake tag-recapture experiments (de Pontual et al., 2003, 2006) as well as by ICES (2005). Here we present estimates of somatic growth rates obtained from the first successful tag-recapture experiment of Southern stock hake and compare them with growth rates obtained by conventional ageing criteria, as well as with those derived from tag-recapture experiments of Northern stock hake (Belloc, 1935; Lucio et al., 2000; de Pontual et al., 2003, 2006).

2. Materials and methods

Tagging was carried out in September–October 2004 off the NW Iberian Peninsula at depths from 40 to 130 m. Locations of capture, release and recapture are shown in Fig. 1. The fishing gear, a trawl (GOC-73) modified with a specially designed cod-end, and the handling method were as described in de Pontual et al. (2003). All hake were measured (total length, TL) and individuals < 40 cm TL were tagged with FF-94 Floy® anchor tags while individuals ≥ 40 cm TL were tagged with the larger FD-94 Floy® anchor tag. All hake were also tagged internally by injection with Terramicine® (OTC) at a dosage of 60 mg kg^{-1}, using the length–weight relationship $W_t = 0.00733L^{2.981}$ (Piñeiro and Saínza, 2003). Tagged hake were released in two locations selected to keep the animals temporarily safe from commercial fishing operations (Fig. 1). The release was done through a smooth PVC pipe while seabirds were scared away to minimize predation. Recaptures came from commercial tangle-net and bottom trawl hake fisheries operating in the region. Whenever possible, recaptured individuals were measured and sexed, while the otoliths were removed for growth analysis based on the Terramicine® mark laid down at the time of tagging.

Somatic growth from release to recapture was computed for each recaptured individual. Mean growth rates were estimated for all the recaptures combined and separately for two recaptures with times at liberty > 340 days. Survival rates after capture and after tagging were calculated and their relationship with fishing depth was investigated by logistic regression analysis. Mortality due to seabird predation was estimated by eye.

3. Results

A total of 1131 hake were caught in 45 fishing sets (Fig. 1). The proportion of hake alive in the catch was 58%, of which 80% survived the tagging process. In total 527 hake (46.6%

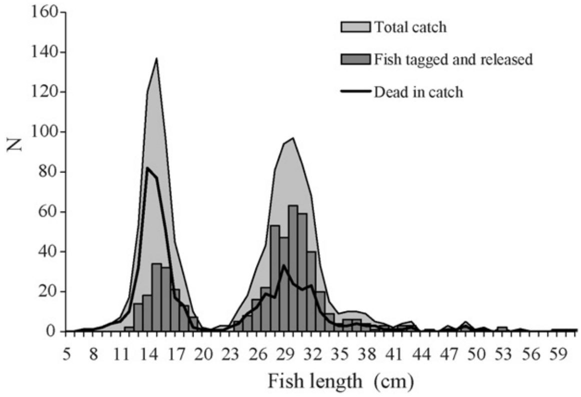

Fig. 2. Size frequency distributions (*N*) of the total catch, dead after captured fish and tagged-released fish.

of the catch) were returned tagged to the sea. We estimated that about 10% of the released fish were affected by seabird predation.

The size structure of hake in the catch ranged from 7 to 61 cm TL and had two distinct groups with modal sizes of 15 and 30 cm TL (Fig. 2). The proportion of hake dead in the first group was greater than in the second, suggesting a negative relationship with size (Fig. 2). Mortality rates during capture and tagging were positively correlated with depth of capture, which explained 72% and 98% of their variation, respectively ($r^2 = 0.72$, $p = 0.07$; $r^2 = 0.99$, $p < 0.01$). However, the correlation between fishing depth and hake size, the smaller hake being more abundant at greater depth, precluded contrast across the two effects.

One year after tagging, six tagged hake had been returned to the laboratory and one tag was recovered without the fish, resulting in a recapture rate of 1.3% (Table 1). The time between release and recapture ranged from 29 to 466 days and the distance moved from release to recapture ranged from 1 to 14.7 nautical miles (Table 1, Fig. 1).

The mean growth rate (±S.D.) of recaptured fish was 0.032 ± 0.016 cm TL day^{-1} ($n = 6$, sexes combined), while that of the individuals recaptured within 3 months of release was 0.021 ± 0.003 cm TL day^{-1} ($n = 4$, sexes combined). The mean growth rate of the two specimens that had spent over 340 days at sea was 0.052 ± 0.003 cm TL day^{-1} (Table 2).

4. Discussion

The first tagging trial of Southern stock hake was carried out off the NW Iberian Peninsula by Robles et al. in 1975. This experiment confronted high mortality and yielded no recaptures. Almost 30 years later, results of the second tag-recapture experiment in the region confirm the suitability of the method developed and successfully tested in the northern Bay of Biscay by de Pontual et al. (2003). In our experiment the survival rate after capture (58%) was lower than in de Pontual et al. (2003) (68%), but survival rates can be improved in future surveys by targeting hake in shallow waters, thus reducing barometric stress, and by using a device to limit the size of the catch in the cod-end. The relatively low recapture rate of 1.3%, in comparison with that obtained by de Pontual et al. (2006) of 3.1%, may be partially attributed to the limited geographic scope of our publicity campaign to fishermen and to the lack of an economic reward for the return of tagged individuals.

In spite of the many efforts devoted to age estimation of *M. merluccius* over the years (see Piñeiro and Saínza, 2003 for a review), interpretation of otolith ring patterns remains controversial and uncertainty about the reliability of age estimates has not declined. Direct measures of growth derived from mark-recapture experiments provide invaluable data for the resolution of this problem. Until now, these data were only available for Northern stock hake. Here for the first time we provide direct measures of the growth rate of Southern stock hake. Our results support the fast growth model proposed by de Pontual et al. (2006) for Northern stock hake by which *M. merluccius* reach about 25, 45 and 60 cm TL at the end of the first, second and third year of life, instead of 20, 29 and 37 cm TL as estimated from otoliths using the internationally agreed age interpretation method (Piñeiro and Saínza, 2003). The striking consistency between our results and somatic growth rates from tag recoveries of Northern stock hake (Fig. 3) is of prime interest because concurs with genetic studies which do not find significative differences between both stocks (Cimmaruta et al., 2005). Although earlier tagging studies had very low tag return rates (Belloc, 1935; Lucio et al., 2000), their findings also indicate a much faster growth rate of this species (Table 2).

The mean daily growth of individuals recaptured within 3 months (fall season) was less than half that of the hake recaptured after nearly 1 year at sea. This discrepancy may be due to

Table 1
Tag-recapture information of hake tagged during the experiment

Tagging data				Recapture data					
Fish code	Date	Depth (m)	TL (cm)	Date	Time since release (days)	Distance (nautical miles)	TL (cm)	Sex	TL increment (cm)
165	4 October 2004	61	30.7	3 November 2004	29	1.04	31.2	U	0.5
1312	2 October 2004	63	30.7	4 November 2004	32	4.48	31.5	F	0.8
70	3 October 2004	61	31.1	22 November 2004	49	2.45	32.1	F	1
149	4 October 2004	61	36.2	29 November 2004	55	–	No fish	–	No fish
73	3 October 2004	61	32.2	26 December 2004	83	1.09	34.1	F	1.9
130	4 October 2004	61	29	21 September 2005	347	14.7	46.5	U	17.5
175	4 October 2004	61	30.7	20 January 2006	466	2.45	56	U	25.3

F: female; U: unknown (gutted).

Table 2
Summary of results of available tag-recapture studies of European hake

Tagging experiment			Recapture results							
Author	Location	Number of individuals released	Number of tagged fish	Number of tags[a]	TL at release (cm)	Time since release (days)	Distance nautical miles (nm)	TL at recapture (cm)	Growth rate (cm day^{-1} mean ± S.D.)	Recapture rate (%)
Belloc (1935)	NS: SW Ireland	78	1	–	28.9	255	130	40.6	0.046	1.3
Lucio et al. (2000)	NS: South Bay of Biscay	152	1	2	56	24	20	60	0.166	1.9
de Pontual et al. (2006)	NS: Bay of Biscay	1307	36	5	21–40 mode = 29	1–1066	0–112	24–67	0.038 ± 0.004	3.1
de Pontual et al. (2006)	NS: Bay of Biscay	–	10[b]	–	21–33	101–1066	11–112	34–67	0.052 ± 0.009	–
This study	SS: NW Iberian Peninsula	527	6	1	(29–36) mode = 29	29–466	1–15	31–56	0.032 ± 0.016	1.3
This study	SS: NW Iberian Peninsula	–	2[b]	–	29–30	347–466	2–15	46.5–56	0.052 ± 0.003	–

NS: Northern stock; SS: Southern stock.

[a] Only tag (no fish) recaptured.

[b] Information of fish that were >100 days at sea.

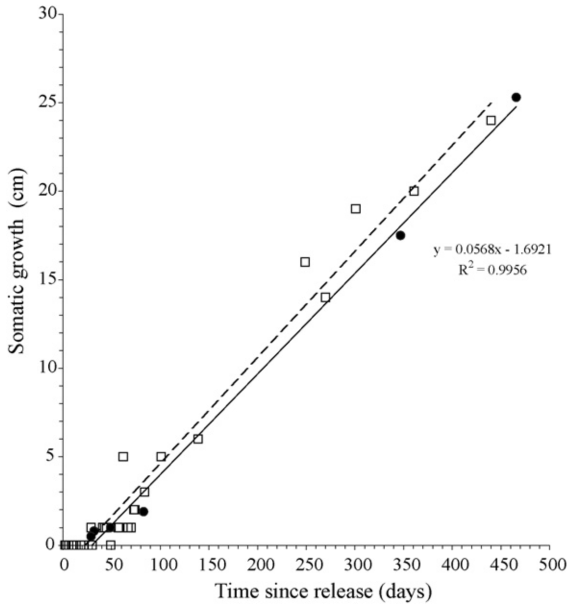

Fig. 3. Somatic growth increment (TL, cm) as a function of time since release. Black dots and solid line: this study; squares and dashed line: data of Northern stock hake (de Pontual et al., 2006) restricted to comparable time period (up to 500 days since release).

seasonal variations in growth (Norbis et al., 1999; Morales-Nin and Moranta, 2004), although short-term growth slow-down as a result of capture and tagging stress cannot be discounted.

Interestingly, the fast growth of this species had been repeatedly hypothesized, first by Bagenal (1954) on the basis of otolith readings and size frequency data and later by Piñeiro and Pereiro (1993) and Alemany and Oliver (1995) on the basis of size modal progression analysis, by Riis-Vestergaard et al. (2000) based on consumption rates and bioenergetic requirements, and by Kacher and Amara (2005) based on daily growth. A further indication of the failure of current otolith age estimation procedures is the lack of coherent evolution of cohorts in age structured models used by ICES (2005).

In sum, our results coincide with all available tag-recapture data to indicate that the growth rate of European hake in the size range studied is about double that derived by the conventionally accepted and routinely applied otolith age interpretation method. We expect that these results will contribute to the discussion on European hake population dynamics as recent work by Bertignac and de Pontual (2007) has shown how bias in age estimations affect assessments of Northern stock hake. Our results thus emphasize the need to carry out large-scale tagging experiments covering the main geographic range of the species in order to develop new, validated ageing criteria for routine age estimation.

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References

Alemany, F., Oliver, P., 1995. Growth of female hake in the Balearic Sea: a proposal of new growth model with higher growth rates. Rapp. Comm. Int. Mer. Médit., 34.

Bagenal, T.B., 1954. Growth rate of the hake (*Merluccius merluccius* L.) in the Clyde and other Scottish areas. J. Mar. Biol. Assoc. U.K. 33, 69–95.

Belloc, G., 1935. Etude monograhique du merlu *Merluccius merluccius* L. 3ème partie. Rev. Trav. Off. Pêches marit 8, 145–202.

Bertignac, M., de Pontual, H., 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES J. Mar. Sci. 64, 981–988.

Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish. Biol. 59, 197–202.

Casey, J., Pereiro, F.J., 1995. European hake (*M. merluccius*) in the North-east Atlantic. In: Alheit, J., Pitcher, T.J. (Eds.), Hake: Biology, Fisheries, and Markets. Chapman & Hall, London, pp. 125–147.

Cimmaruta, R., Bondanelli, P., Nascetti, G., 2005. Genetic structure and environmental heterogeneity in the European hake (*Merluccius merluccius*). Mol. Ecol. 14, 2577–2591.

de Pontual, H., Bertignac, M., Battaglia, A., Bavouzet, G., Moguedet, P., Groison, A.L., 2003. A pilot tagging experiment on European hake (*Merluccius merluccius*): methodology and preliminary results. ICES J. Mar. Sci. 60, 1318–1327.

de Pontual, H., Groison, A.L., Piñeiro, C., Bertignac, M., 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES J. Mar. Sci. 63, 1674–1681.

ICES, 1979. Report of the Working Group on Assessment of Hake Stocks. ICES C.M. 1979/G:27.

ICES, 2005. Report of the Working Group on the Assessment of Southern Stocks of Hake, Monk, and Megrim. ICES C.M. 2005/ACFM: 02.

ICES, 2006. Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk and Megrim. ICES C.M. 2006/ACFM: 29.

Kacher, M., Amara, R., 2005. Distribution and growth of 0-group European hake in the Bay of Biscay and Celtic Sea: a spatial and inter-annual analyses. Fish. Res. 71, 373–378.

Lucio, P., Santurtun, M., Quincoces, I., 2000. Tagging experiments on hake, anglerfish and other species in the Bay of Biscay. ICES C.M. 2000/Z:09.

Morales-Nin, B., Moranta, J., 2004. Recruitment and post-settlement growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. Scientia Marina 68 (3), 399–409.

Norbis, W., Lorenzo, M.I., Torres, G.J., 1999. Intra-annual growth variations of young of the year hake (*Merluccius hubbsi*) of the Uruguayan continental shelf based on otolith analysis. Fish. Res. 44, 129–137.

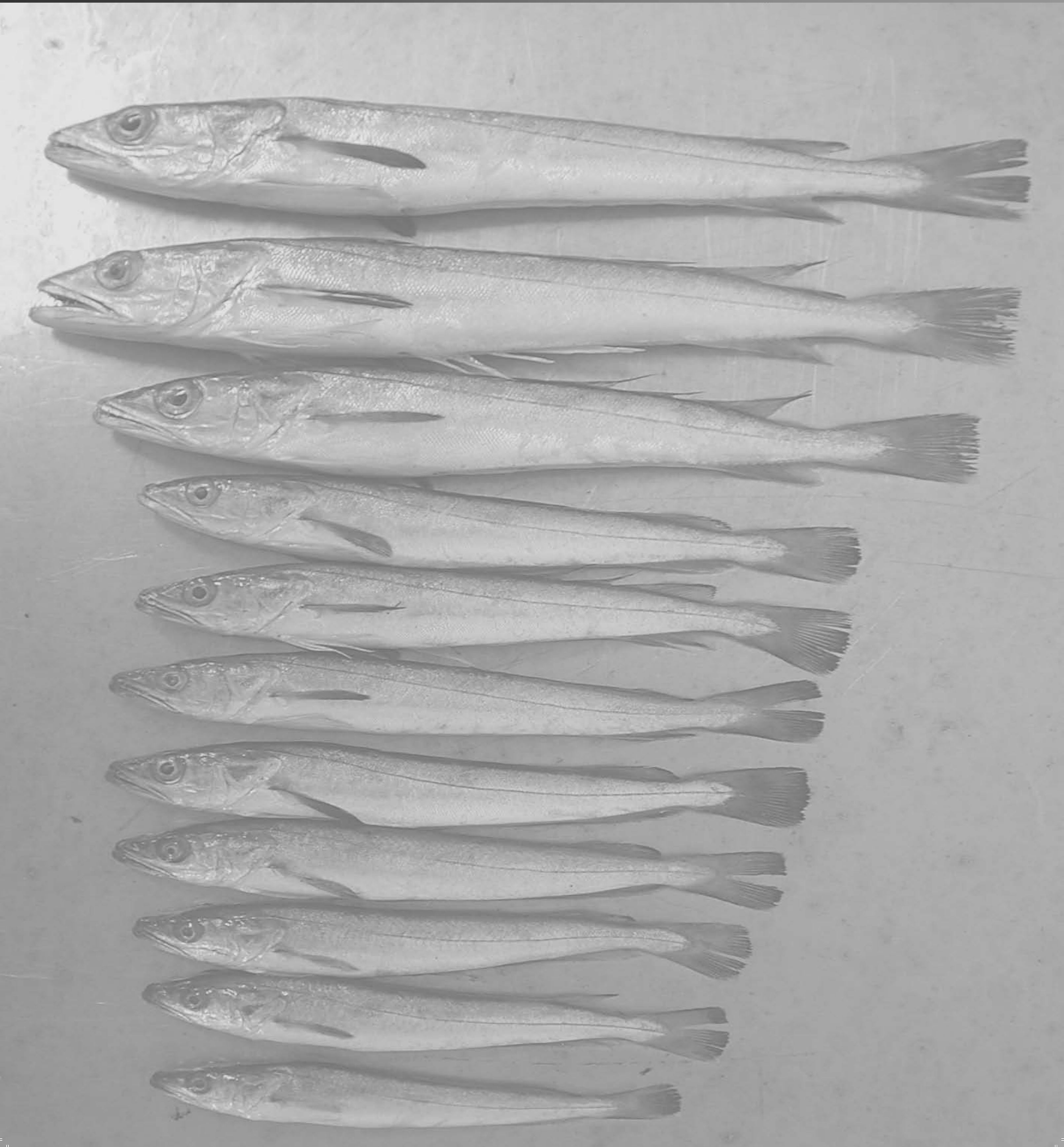
Piñeiro, C.G., Pereiro, J.A., 1993. Study on juvenile growth pattern of European hake (*Merluccius merluccius* L.) using whole otoliths and length frequency distributions from commercial catches and fish surveys. ICES C.M. 1993/G: 12, 14 pp.

Piñeiro, C., Saínza, M., 2003. Age estimation, growth and maturity of the European hake (*Merluccius merluccius* Linnaeus, 1758) from Iberian Atlantic waters. ICES J. Mar. Sci. 60, 1086–1102.

Riis-Vestergaard, J., Velasco, F., Hill, L., Olaso, I., 2000. Food consumption of European hake (*Merluccius Merluccius* L.) estimated by application of a bioenergetics model: is the growth of hake underestimated? 9ICES C.M. 2000/Q:12, Ref. R.

Robles, R., Pereiro, J.A., Fernandez, A.M., García, J.M., 1975. Estudios de cartografía, selectividad y marcado de merluza europea (*Merluccius merluccius* L.), frente al litoral Gallego. Bol. Ins. Esp. Oceanog. 190, 1–39.

Capítulo 5. Discusión general



DISCUSIÓN GENERAL

A pesar de que la merluza *Merluccius merluccius* (Linnaeus, 1758) constituye un importante recurso pesquero en el Atlántico nororiental y el Mediterráneo, el conocimiento de su biología y ecología es todavía limitado. En particular, el estudio del crecimiento presenta serias dificultades debido a la complejidad del patrón de anillos de los otolitos en el que tradicionalmente se ha basado. Los trabajos de investigación recogidos en esta tesis ilustran el proceso de construcción, discusión y cambio de paradigma del modelo de crecimiento de la merluza. Este, establecido inicialmente hace siete décadas y reiteradamente cuestionado, se mantuvo vigente hasta que, con la llegada de nuevos métodos de estudio y las evidencias que estos aportaron, se pudo descartar. Durante este tiempo, la incertidumbre sobre el modelo de crecimiento de la merluza ha tenido especial relevancia por sus implicaciones en los resultados de los métodos de evaluación al uso, basados en la captura por edad (WGHMM, Working Group of Hake Megrim and Anglerfish; ICES 2007) y sus consecuencias para la correcta gestión de sus pesquerías.

Uno de los objetivos de la ciencia es encontrar modelos que expliquen la mayor cantidad posible de observaciones dentro de un marco coherente. La ciencia normal según Kuhn (1962), intenta adecuar la teoría a la práctica, pero pueden existir ciertas discrepancias que, si no son resueltas, se convierten en anomalías. Si estas se acumulan se produce una crisis que conlleva la caída del antiguo paradigma y una revolución científica en la que es reemplazado por uno nuevo incompatible con el anterior (Kuhn, 2006).

El debate acerca de si *M. merluccius* es una especie de crecimiento lento (Hickling, 1933) o rápido (Belloc, 1935) se inició en los años 30. El escaso éxito obtenido por Belloc (1935) en su experimento pionero de marcado, que con solo un ejemplar recapturado postuló un modelo de crecimiento rápido (tasa anual ~17 cm/año), propició que durante décadas la investigación del crecimiento de *M. merluccius* se basara casi enteramente en edades obtenidas mediante la lectura de otolitos y en el modelo de crecimiento lento (tasa anual ~7 cm/año) propuesto por Hickling (1933).

Con el fin de permitir comparaciones y reducir la gran variabilidad que acompañó sistemáticamente a las estimaciones de la edad (Piñeiro *et al.,* 2009, Anexo), a partir de los años 80 se avanzó en el desarrollo y el establecimiento de un protocolo estándar de lectura y estimación de la edad basado en la macroestructura del otolito y en la biología de la especie (Goñi, 1983; Goñi & Piñeiro, 1988; Piñeiro & Hunt, 1989; Piñeiro *et al.,* 1992; Lucio *et al.,* 2000a; Godiho *et al.*, 2001).

El protocolo de lectura de la edad mediante el análisis de la macro-estructura del otolito que se presenta en esta tesis (Capítulo 2, Piñeiro & Saínza, 2003) resultó en sus inicios muy prometedor. Por primera vez se hacía explícito el criterio de interpretación, permitiendo obtener estimaciones de edad de distintos lectores de forma estandarizada (Anon., 1998; Anon, 2002; Piñeiro & Saínza, 2002; ICES, 2003; Piñeiro *et al.,* 2009). Así se mejoró el nivel de precisión de las estimaciones para un rango de edades (0 a 5 años) que comprendía el 90% de las desembarcos comerciales de la especie (ICES 2004). Su aplicación tuvo como resultado una tasa de crecimiento algo superior a la estimada por medio de otolitos hasta entonces (Capítulo 2, Tablas 2 y 3).

Este aumento con respecto a los estudios anteriores, que sugería un crecimiento más lento (Piñeiro *et al.,* 2009), se debía a que el nuevo método de interpretación asumía explícitamente la deposición de dos pares de bandas o anillos traslúcidos y opacos por año durante los tres primeros años de vida y solo un par anual a partir de entonces (Capítulo 2, Figura 3). Este cambio en la frecuencia de formación de anillos se consideraba relacionado con el proceso de maduración sexual (Recasens *et al.,* 1998; Morales-Nin *et al.,* 1998; Lucio *et al.,* 2000a; Piñeiro *et al.,* 2009).

Sin embargo, este protocolo de lectura presentaba limitaciones. La primera y más importante, era la dificultad de identificar el primer anillo anual. Esto se debía a que el otolito suele presentar anillos no anuales (o *checks*) bien marcados antes, simultáneamente y/o después de la deposición del primer anillo anual (Iglesias & Dery, 1981; Goñi, 1983; Piñeiro & Pereiro, 1993; Piñeiro *et al.,* 2009). La localización de un anillo característico asociado a un cambio en la pauta alimenticia de la especie (Goñi & Piñeiro, 1988; Velasco & Olaso, 1998; Velasco, 2007) sirvió como marca de referencia para su identificación (Goñi & Piñeiro, 1988; Piñeiro & Hunt, 1989), reduciendo así la variabilidad en la interpretación (Capítulo 2, Figura 2). Este *check* posiblemente fue contabilizado en el pasado como anual, dando como resultado una tasa de crecimiento menor (Capítulo 2, Piñeiro & Saínza, 2003).

La segunda de las limitaciones se encontraba en el análisis del crecimiento marginal (borde) del otolito (Capítulo 2, Figura 3). La presencia frecuente de *checks* enfrentaba sistemáticamente a los lectores y el protocolo de lectura no ofrecía soluciones (Piñeiro *et al.,* 2009). Estos *checks* han sido relacionados por distintos autores con el desplazamiento estacional entre hábitats como consecuencia del comportamiento predatorio y reproductivo de la merluza europea (Fariña & Abaunza, 1991; Beckman & Wilson, 1995; Morales-Nin *et al.,* 1998; Velasco & Olaso, 1998) y han sido descritos también en otras especies del género (Beamish, 1979; Hunt, 1980; ICSEAF, 1983; Morales-Nin, 1986; Renzi & Perez, 1992; Norbis *et al.,* 1999; Torres *et al.,* 1996).

Se probaron métodos automatizados para analizar la tipología macroestructural del otolito y evitar la subjetividad del lector, que arrojaron resultados contrapuestos. Así, Morales-Nin *et al.* (1998) concluyó que los anillos marcados en los otolitos de la especie no pueden considerarse anuales (*annuli*) porque responden a factores ontogénicos. Sin embargo, Courbin *et al.* (2007) observaron ciertas secuencias estructurales que podrían estar relacionadas con una respuesta a escala poblacional, frente a las variaciones estacionales o acontecimientos ontogénicos y que según estos autores, servirían para asignar edades. Sus resultados fueron consistentes con los obtenidos en los estudios de la composición microquímica del otolito de merluza (ej. variaciones Sr/Ca) que muestran variaciones endógenas (ontogenia) y exógenas (Tomas *et al.,* 2006).

En definitiva, este protocolo de lectura, calibrado y aceptado por la comunidad científica internacional, permitió estimar con niveles de precisión relativamente aceptables la edad de ejemplares de merluza europea hasta los 5 años (Anon., 2000; ICES, 2000; Piñeiro, 2000, Piñeiro *et al.,* 2004, ICES, 2010a; Piñeiro *et al.,* 2009). Sin embargo para ejemplares más longevos no existía un marco de interpretación, debido a las dificultades encontradas en la unificación de criterios entre lectores. No obstante, este problema, aunque importante, no parecía tener consecuencias negativas debido a la escasez de merluzas de mayor edad en las poblaciones explotadas (ICES 2003). Así que la necesidad imperiosa de disponer de un método de lectura estandarizado de aplicación general para elaborar claves talla-edad anuales resultó en la aceptación del protocolo de lectura sin debatirlo en profundidad ni ponerlo a prueba con nuevos análisis. Esto no era un caso insólito, ya que la evaluación de los *stocks* de otras especies del género, como *M. productus* y *M. hubbsi*, se basan en estimaciones de edad a partir de otolitos que también tienen limitaciones en los métodos de validación (MacFarlane & Beamish, 1985; Torres, 1992; Renzi & Perez, 1992; MacLellan & Sanders*,* 1995; Torres *et al.,* 1996; Renzi *et al.,* 2009). Por otro lado, en esta fase también eran evidentes las dificultades para alcanzar un consenso sobre el método de lectura frente a resultados no consistentes o contrapuestos (Piñeiro *et al.,* 2009, Anexo).

Pese a que el protocolo de lectura resultaba útil, la identificación del primer anillo anual seguía sin resolverse, por lo que se dirigió la atención al estudio de la microestructura del otolito de juveniles, método habitual de validación indirecta del primer anillo anual (Campana, 2001; Panfili *et al.,* 2002). Estos estudios avalaron la formación de anillos de crecimiento diario en el otolito de esta especie (Morales & Aldebert, 1997; Arneri & Morales-Nin, 2000; Groison, 2003; Morales-Nin *et al.,* 2005). Parte de ellos indicaban que la merluza podía alcanzar los 24-25 cm TL al final del primer año de vida (Piñeiro *et al.,* 2004; Katcher & Amara, 2005; Capítulo 3, Piñeiro *et al.,* 2008), lo que significaba una tasa diaria de crecimiento superior a la obtenida mediante el análisis de la macroestructura, y similar a la derivada del análisis de progresión modal en las distribuciones de tallas (Guichet, 1988; Piñeiro & Pereiro, 1993, Anexo, Tabla 3.3.1). Los resultados del análisis microestructural indicaban también que el crecimiento diario sufría pulsos de distinta intensidad a lo largo del año, siendo las tasas más estables en invierno y verano (Capítulo 3, Figura 7). Esto confirmaría la influencia de las condiciones ambientales en el crecimiento y su relación con la época de nacimiento, como ha sido observado por varios autores (Morales & Moranta, 2004; Álvarez & Cotano, 2005; Katcher & Amara, 2005; Otxotorena *et al.,* 2010).

A pesar de estos avances, no se pudo identificar de manera sistemática el primer anillo anual debido a la complejidad de las estructuras de crecimiento, que muchos autores han asociado a la dilatada época de puesta de esta especie (Martin, 1991; Casey & Pereiro, 1995; Anon., 1998; Lucio *et al.,* 2000a; Domínguez-Petit, 2007), la más extensa entre todas las especies del género *Merluccius* estudiadas (Murúa, 2010). Su estrategia reproductiva, que combina la liberación de huevos en fases sucesivas y un amplio período de puesta, tiene como consecuencia la aparición de reclutas de tamaños diferentes en el mismo año del calendario, lo que contribuye a la gran variabilidad del patrón de crecimiento del otolito (Domínguez-Petit *et al.*, 2008; Piñeiro *et al.*, 2009; Korta *et al.*, 2010; Murúa, 2010). En el caso de *M. productus,* y *M. hubbsi,* sin embargo se ha identificado el primer anillo anual (Mc Farlane & Beamish, 1985; Torres, 1992; Renzi & Pérez, 1992) y se ha observado que su tamaño está relacionado con la época y el área de puesta, así como con las condiciones medioambientales, fundamentalmente la temperatura (Torres *et al.*, 1996; Norbis *et al.*, 1999).

De manera que, simplificando, por un lado el análisis de la macro- y la microestructura del otolito aportaba visiones discordantes sobre el crecimiento de *M. merluccius* —lento vs. rápido—, mientras que, por otro, el surgimiento gradual de las anomalías y la incapacidad de explicarlas con el paradigma vigente, preparó el camino al cambio de paradigma, no sin ciertas dificultades que se manifestaron en la resistencia a este cambio (Kuhn, 2006). Un claro ejemplo de esta resistencia fueron los cuatro años que el WGHMM utilizó estimaciones de edad de los otolitos cuando ya estaba demostrada la invalidez de las mismas (De Pontual *et al.,* 2006). Una de las anomalías fue la imposibilidad de seguir la progresión de las cohortes en la matriz de capturas por edad y en los índices de abundancia de las campañas, lo cual cuestionó la credibilidad del método de estimación de edades a partir de la macroestructura del otolito y, por tanto, del modelo de crecimiento de la especie y de los resultados de las evaluaciones del estado de los *stocks* (ICES, 2005).

Todos los indicadores apuntaban hacia un modelo de crecimiento rápido (Piñeiro & Pereiro, 1993; Alemany *et al.,* 1993; Riis-Vertegaard *et al.,* 2000; Garcia-Rodríguez & Esteban, 2002; Courbin *et al.,* 2007), sin embargo, la corriente dominante del crecimiento lento y la inercia del uso de la metodología empleada para la evaluación de poblaciones que de este modelo se derivaba, hacían muy difícil implantar cambios sin la disponibilidad de pruebas fehacientes. Solo las aportadas por la técnica de marcado-recaptura, método independiente de la edad, tuvieron absoluta credibilidad e invalidaron de forma incuestionable el protocolo internacional de lectura de edades en otolitos, abriendo el paso al cambio de paradigma de crecimiento lento a crecimiento rápido de la merluza (ICES 2010b).

Hasta el desarrollo de una metodología viable de marcado no se disponía de otro método alternativo para estudiar el crecimiento de la merluza que el de estimar la edad empleando otolitos, pese a que la comunidad científica lo consideraba inválido. Afortunadamente, en 2006 salieron a la luz los primeros resultados procedentes de una experiencia de marcado-recaptura a gran escala, en la que, no con una, sino con 36 recapturas, se pudo determinar el crecimiento real de merluza en el medio natural (Capítulo 4, De Puntual *et al.,* 2006). La tasa de crecimiento obtenida para aquellos ejemplares que pasaron al menos un invierno y un verano en libertad fue 19.8±1.5 cm/año (n=6) en el Golfo de Vizcaya, lo que indicaba que el crecimiento somático era aproximadamente el doble del estimado tradicionalmente. Aunque con menos muestras, se obtuvieron resultados similares (19.1±0.9 cm/año, n=2) en el noroeste de la Península Ibérica, (Capítulo 4, Piñeiro *et al.,* 2007). Además, el marcado simultáneo de los otolitos con OTC (Campana, 2001; Wright *et al.,* 2002), permitió conocer los anillos de crecimiento que se forman en el otolito entre el período de marcado y el de recaptura (De Puntual *et al.,* 2006). Así, las lecturas ciegas de los otolitos marcados con OTC pusieron en evidencia que la tasa de crecimiento de la merluza estaba siendo subestimada (por un factor de 2) debido a la sobrestimación de edad (Capítulo 4, De Pontual *et al.,* 2006).

Los resultados de estos trabajos produjeron una conmoción, así como la constatación palpable de que la estimación de la edad de la merluza europea era incorrecta, avanzándose ya de manera irreversible hacia el cambio de paradigma (Kuhn, 2006). Así, se alertó sobre la escasa fiabilidad de las claves talla edad aportadas rutinariamente a los grupos de trabajo para la evaluación (Piñeiro *et al.*, 2004) y autores como Bertignac & De Pontual (2007) advirtieron sobre los errores que podían producirse en las estimaciones de la mortalidad pesquera y de la biomasa del *stock* reproductor de la especie (ICES 2007a). La coherencia observada entre las anomalías pasadas por alto hasta entonces y la evidencia incuestionable que aportaban los datos de marcado-recaptura provocaron el cambio de paradigma. Las experiencias recientes de marcado realizadas en el Mediterráneo han demostrado que la subestimación del crecimiento no es una cuestión regional (Mellon-Duval *et al.,* 2010) y han proporcionado nuevos datos de gran relevancia que confirman la generalidad del nuevo modelo de crecimiento rápido (Piñeiro *et al.,* 2009; ICES 2010a).

Existen, sin embargo, dificultades y problemas metodológicos en esta técnica, como son la elevada mortalidad de individuos de talla inferior a 25 cm TL (Piñeiro *et al.,* 2007) y la baja tasa de recaptura de individuos marcados, que oscila según el estudio entre el 3% y el 7%, para un período de tiempo de hasta 1 066 días (De Puntual *et al.,* WD3 ICES 2010a; Mellon-Duval *et al.,* 2010; Piñeiro & Saínza, 2011; ICES 2010a). Además, las condiciones vitales del ejemplar y su tamaño condicionan la supervivencia de los individuos marcados (De Puntual *et al.,* WD3, ICES 2010a) y producen bajas que influyen en la tasa de recaptura (Jovilet *et al.,* 2009). Por otro lado, el escaso número de individuos recapturados con más de un año en libertad (ICES 2010), unido a su limitado rango de tallas (35-45 cm TL), no ha permitido hasta el momento elaborar un método robusto y fiable de estimación de la edad que pueda reemplazar al anterior (ICES 2010a y c). A estas dificultades se une la escasa colaboración del sector pesquero en la notificación de las recapturas, debido posiblemente a una información y sensibilización insuficientes (Piñeiro & Saínza, 2011).

Desde los años 90, se han estado aportando datos de edad para la evaluación de los *stocks* de merluza asumiendo la hipótesis de crecimiento lento, inherente a los criterios de interpretación de otolitos consensuados internacionalmente. De este modo, hasta 2009, los *stocks* se han estado gestionando con arreglo a los resultados de evaluaciones con modelos analíticos estructurados por edad (XSA) que se consideraban fiables para diagnosticar el estado del recurso. A partir del 2010, las evaluaciones se realizan con modelos basados en tallas (ICES, 2010b), fijando ciertos parámetros de crecimiento según von Bertalanffy (t_0=0, L_∞=130 cm) y estimando el parámetro K con la información de las distribuciones de tallas de la captura comercial y de campañas (*K*=0.165).

El nuevo paradigma sobre el crecimiento tiene notables implicaciones para la evaluación del estado de los *stocks* cuya finalidad es dictar recomendaciones sobre medidas de gestión. En el caso concreto de la evaluación de la merluza del *stock* sur, el modelo de crecimiento lento (tasa de crecimiento de la ecuación de von Bertalanffy, k=0.075) usado en el pasado, indicaba que la captura comercial estaba representada por aproximadamente 10 clases de edad, la biomasa del *stock* reproductor era 17 219 t y el reclutamiento 45.7 millones de individuos (media del período 2006-2008, ICES, 2010b y c). En la actualidad, con el modelo de crecimiento rápido (k=0.165) ha cambiado notablemente la percepción del estado del recurso, siendo la mitad el numero de clases de edad que contribuyen a la captura, la biomasa del *stock* reproductor mucho menor (11 589 t) y el reclutamiento muy superior (61.2 millones de individuos) (ICES, 2010b y c).

En cuanto a las implicaciones futuras, un crecimiento más rápido conlleva una dinámica más rápida, que permite una renovación de las distintas clases de edad que componen la población en menos tiempo. De esta forma la población responderá más rápidamente a factores externos (ambientales o pesqueros), con lo que su tamaño y producción será más sensible a alteraciones de esos factores (Haddon, 2001). Esta mayor sensibilidad del *stock* a factores externos debería condicionar las medidas de gestión que deben aplicarse para la regulación precautoria del recurso (FAO, 1995).

Por otro lado es importante resaltar que, al igual que la merluza europea, existen otras muchas especies explotadas con problemas semejantes en la determinación de la edad, como es el rape blanco, *Lophius budegassa* (Spinola,1807), cuyo crecimiento estaba también subestimado (Landa *et al.,* 2008), y el jurel, *Trachurus trachurus* (Linnaeus,1758). Así, el jurel, por ejemplo, fue considerada inicialmente una especie de crecimiento rápido y después de años pudo validarse el método de estimación de la edad gracias al fuerte reclutamiento de una clase anual, confirmándose el crecimiento lento de esta especie que puede llegar a alcanzar los 40 años de edad (Abaunza *et al.,* 2003).

En conclusión, el compendio de estudios integrados en esta tesis ilustra la evolución de un paradigma que ha cambiado la perspectiva del crecimiento de la merluza y la interpretación de su dinámica poblacional y de su capacidad de respuesta a factores ambientales. El cambio de modelo o paradigma tiende a ser dramático en las ciencias, ya que estas aparecen como estables y maduras. Sin embargo, como afirmaba Kuhn (1962, 2006) «*las sucesivas transiciones de un paradigma a otro, vía alguna revolución, es el patrón de desarrollo habitual de la ciencia madura*». El cambio de paradigma no es una mera revisión o transformación de un modelo a otro, sino que también cambia la manera en que los científicos abordan su objeto de estudio y, acaso más importante aún, el tipo de preguntas y respuestas que se consideran válidas.

A la vista de los resultados presentados y discutidos en la tesis, la posibilidad de que un día se pueda estimar la edad de la merluza de manera rutinaria depende de que lleguemos a comprender el significado biológico de las señales que aparecen en las piezas calcificadas (ICES 2010a). El estudio combinado de la micro- y la macroestructura de los otolitos, junto con los datos de marcado-recaptura a gran escala, son en la actualidad la única vía posible para elaborar métodos alternativos fiables para conocer el crecimiento a nivel poblacional.

Para terminar, los resultados de esta tesis indican que, a pesar de la importancia de la merluza europea en el Atlántico nororiental y de haber sido ampliamente estudiada, el conocimiento de su biología y ecología presenta todavía importantes lagunas. La biología de la especie parece mostrar características contrapuestas a la de otros gádidos: tasa de crecimiento alta, fecundidad indeterminada y temporada de desove prolongada. Este patrón parece indicar que la merluza europea ha adoptado una estrategia de vida oportunista, lo que posiblemente se deba a su gran capacidad adaptativa frente condiciones muy heterogéneas que demuestran una gran plasticidad biológica.

Capítulo 6. Conclusiones

CONCLUSIONES

De los estudios realizados en esta tesis sobre la estimación de la edad y el crecimiento de la merluza europea se desprenden las siguientes conclusiones:

1. El estudio de la macroestructura del otolito proporcionó estimaciones de la edad que indicaban que la merluza presentaba un crecimiento lento, de manera que la fase explotada estaba representada aproximadamente por 10 clases de edad, lo que se estableció como modelo de crecimiento convencional (denominado «*modelo lento*»).
2. El protocolo estándar para interpretar la macroestructura del otolito acordado a nivel internacional no despejó las dudas sobre la estimación de la edad de la merluza. La identificación del primer anillo anual en la macroestructura del otolito, pieza clave de la estimación de la edad, no fue posible debido a la prolongada puesta de la merluza, que hace que coexistan reclutas de tamaños muy diferentes y que, por consiguiente, se registre una gran variabilidad en el patrón de crecimiento del otolito durante el primer año de vida.
3. A partir de esta constatación se concluyó que era necesario contrastar el modelo de crecimiento lento con métodos alternativos y estudios de validación, como el estudio del crecimiento diario y experimentos de marcado-recaptura.
4. Los resultados del estudio del crecimiento diario a partir de la microestructura del otolito mostraron una tasa diaria de crecimiento superior a la obtenida a partir del análisis de la macroestructura del otolito, situándose en la franja superior del rango de valores observados en otras áreas.
5. Los experimentos de marcado-recaptura resolvieron finalmente la cuestión, aportando datos concluyentes sobre el crecimiento de la especie en el medio natural, con valores similares a los obtenidos en el estudio de la microestructura del otolito.
6. La lectura de secciones de otolitos marcados procedentes de los ejemplares recapturados confirmaron que el protocolo de interpretación de la edad mediante el análisis de la macroestructura del otolito sobrestimaba la edad y subestimaba el crecimiento, demostrándose así irreversiblemente que esta metodología no era válida.
7. Finalmente, siete décadas después de comenzado el debate sobre el crecimiento de la merluza europea, se produjo un cambio del paradigma de crecimiento lento al de crecimiento rápido. Esto fue posible al demostrarse, de forma independiente de las estimaciones de edad, que la merluza crece a una tasa dos veces superior a la considerada tradicionalmente.
8. Este descubrimiento puso de manifiesto que las claves talla-edad utilizadas en el Grupo de Trabajo del ICES para la evaluación del estado de los s*tocks* de merluza

europea, desde su inicio en 1992 hasta 2009, no eran válidas, como tampoco lo eran las evaluaciones resultantes. Este reconocimiento no eludía la necesidad de disponer de estimaciones de edad de merluza para la evaluación anual del estado de sus poblaciones.

9. Cualquier progreso en esta dirección requiere la puesta en marcha de estudios, en condiciones controladas, para entender las señales que aparecen en los otolitos en relación a los factores que gobiernan la formación de anillos: fotoperíodo, temperatura, disponibilidad de alimento, crecimiento, actividad reproductiva y sus efectos. El empleo combinado de la macro y microestructura de la sección del otolito facilitará el análisis comparativo del crecimiento a escala anual y diaria, permitiendo comprender las relaciones existentes entre los anillos depositados y los sucesos medioambientales y fisiológicos.

10. Además, el análisis de los otolitos marcados puede proporcionar el conocimiento necesario sobre el significado biológico de los anillos de crecimiento. Para ello es necesario disponer de un mayor número de recapturas, incluyendo también ejemplares más grandes y longevos. Sin ellos, la incógnita sobre la periodicidad de los anillos de crecimiento del otolito de la merluza seguirá abierta y no será posible disponer de un método fiable para estimar la edad individualmente y de forma rutinaria.

11. El nuevo paradigma del crecimiento tiene claras implicaciones para la evaluación del estado de los *stocks* y ha cambiando sustancialmente la percepción del estado del recurso. Así, basándose en el modelo de crecimiento rápido, con una tasa de crecimiento (K=0.165) el doble de la considerada tradicionalmente (K=0.075), se estima que en el año 2010 el número de clases de edad que ha contribuido a la captura es 5, en lugar de 10, la biomasa del stock reproductor es mucho menor y el reclutamiento muy superior.

12. En cuanto a las implicaciones futuras, un crecimiento más rápido, conlleva una dinámica más rápida, que permite una renovación de las distintas clases de edad que componen la población en menos tiempo, con lo que ésta responde más rápidamente a factores externos (ambientales o pesqueros). Esta mayor sensibilidad del stock a factores externos deberá condicionar las medidas de gestión para la regulación precautoria del recurso.

Anexo

Foto cedida por I. Bruno

Hake age estimation: state of the art and progress towards a solution. **Piñeiro, C. G.,** Morgado, C.; Saínza, M.; Mc Curdy, W. J. (Eds). 2009. ICES Cooperative Research Report No. 294. 43 pp.

Resumen

Desde 1992, la evaluación de las poblaciones de merluza del norte y del sur (*Merluccius merluccius*) han utilizado los datos de edad basándose en el análisis del otolito. Estos datos son proporcionados por diferentes instituciones, lo que implica un nivel de precisión necesario en las lecturas de edad aportadas para poder estimar indicadores de calidad de la evaluación. Durante el período 1997-2004, se hizo un esfuerzo considerable para mejorar la precisión de los datos de edad por medio de sucesivos ejercicios de calibración de edades, intercambios y talleres internacionales. Este objetivo se logró en parte y los expertos acordaron un método estándar (Piñeiro y Saínza, 2003) que permitió obtener una precisión aceptable hasta los 3 años (Piñeiro *et al.,* 2004). Sin embargo, estos criterios no han sido validados y los resultados recientes obtenidos por las experiencias de marcado y recaptura no están en consonancia con los resultados de las estimaciones de edad basadas en el método estándar de lectura de otolitos.

El impacto producido por el sesgo en la estimación de la edad sobre los resultados de la evaluación y en consecuencia sobre el asesoramiento en la gestión, junto a la preocupación sobre el estado de los stocks de merluza (ICES, 2007a, 2007b) hizo necesario la elaboración de un informe científico sobre el estado actual del crecimiento de esta especie. El objetivo principal de este informe es aportar una síntesis de la labor realizada durante años por los investigadores que han trabajado en la estimación del crecimiento y edad de la especie, revisando principalmente los resultados obtenidos en los consecutivos ejercicios de calibración y talleres de lectura de edad realizados. Asimismo se presenta un resumen del conocimiento actual y los últimos avances sobre el estudio del crecimiento de la merluza europea. Este informe también incluye recomendaciones dirigidas a lograr la validación de los métodos de lectura de otolitos para estimar la edad.

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Hake age estimation: state of the art and progress towards a solution

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Contents

Executive summary

Since 1992, northern and southern hake (*Merluccius merluccius*) stock assessments have used age data based on otolith analysis. Age data for stock assessment is provided by different institutions, which implies a quantification of age-reading precision to estimate assessment quality indicators. During this period, considerable effort has been made to improve the precision of age data by means of successive age-reading calibration exercises, exchanges, and workshops in 1997, 1999, 2001, and 2004. This goal was partly achieved, and experts recently agreed on standard criteria (Piñeiro and Saínza, 2003) that allowed an acceptable precision to be reached for ages up to 3 years (Piñeiro *et al.*, 2004). However, these criteria have never been validated, and recent mark–recapture experiments are not in line with ageing results based on the standard criteria.

Given the impact of bias in age estimation on stock assessment results, consequent management advice, and concern about the state of the hake stocks (ICES, 2007a, 2007b), a report on the current state of the art is needed. The main goal of this report is to present a synthesis of the work carried out over the years by researchers involved in providing age data for stock assessment, mainly on age-reading calibration exercises, and current knowledge regarding the growth and ageing of this species. This report also includes recommendations for future work aimed at achieving validated age-reading criteria.

1 Introduction

The European hake (*Merluccius merluccius* Linnaeus, 1758) is one of the most valuable and heavily exploited demersal species in the western European fisheries. It is widely distributed in the Northeast Atlantic, from Norway to Mauritania, being more abundant from the British Isles to south of Spain (Casey and Pereiro, 1995) and in the Mediterranean and Black seas. It is found mainly at depths between 50 and 370 m, although its distributional bathymetric range is 30–1000 m (Domínguez-Petit, 2007). Hake is a batch-spawner (Murúa *et al.*, 1996; Domínguez-Petit, 2007) with a protracted spawning season extending throughout the entire year, but mainly from December to July along the continental shelf edge.

The assessment of hake is undertaken annually by the ICES Working Group on the Assessment of Southern Shelf Stocks of Hake, Megrim, and Monkfish (WGHMM). Since 1978, despite the lack of a sound biological basis, ICES has distinguished two separate hake stocks for management purposes: (i) the northern stock (ICES Division IIIa, Subareas IV, VI, and VII, and Divisions VIIIa–b); and (ii) the southern stock (ICES Divisions VIIIc and IXa), split at Cape Breton Canyon (ICES, 1979). Total annual landings of European hake declined from ca. 125 000 t in the early 1960s to the current level of ca. 44 000 t for the northern stock and 15 000 t for the southern stock. In recent years, the relatively depleted levels of both stocks have been a cause for concern to ICES (ICES, 2007a, 2007b).

The assessments for hake include several areas of uncertainty, such as growth, population structure, and stock definition, with growth being one of the most limiting factors for stock assessment. Currently, both stock assessments use age-structured models. Age data are provided by different countries and follow agreed international ageing criteria (Piñeiro and Saínza, 2003) based on the results of successive international age-reading exercises and workshops (held in 1997, 1999, 2001, and 2004). A working document presented at the 2002 WGHMM meeting demonstrated that the northern hake assessment had a better fit to the assessment model if the plus group were set at age 10 instead of age 8 (ICES, 2003). However, the confident ages from the previous otolith age estimation workshop ranged from 0 to 5 years and, because the scientists involved in hake ageing and growth drew the attention of WGHMM to the low reliability of age 10, the working group decided to continue to use age 8 as the plus group.

The ages recorded for both stocks of hake usually range from 0 to 12 years, although the landing and survey data are dominated by specimens belonging to age groups up to three years.

The assessment of the southern hake stock has been based on age-reading data since the 1999 assessment, with age–length keys (ALKs) from 1992 onwards (ICES, 2000). The northern stock assessment used ALKs based on otolith readings from 1992 (ICES, 1994a). The ALKs that were applied to catch data prior to 1992 are described in the WGHMM report (ICES, 2007a). For both stocks, when the assessment was performed without ALKs, the age composition of the catches was estimated using a numerical method developed by Kimura and Chikuni (1987).

In hake, the *sagitta* otoliths are routinely used for ageing. The annual rings (*annuli*) have proven difficult to interpret because of the complexity of the otolith macrostructure, and the age estimation method currently in use has not been validated. Recently, however, progress has been made regarding the precision of age data. More than five exchanges and four workshops have taken place in the past two decades. The lack of high precision, despite these efforts, is a strong indication of the difficulty of hake age interpretation.

At the end of 1990s, within the framework of EU Study Contract 95/038 "Biological Studies of Demersal Fish" (BIOSDEF; see Anon., 1998), standard criteria for the ageing of hake otoliths were developed. These criteria were adopted by age readers from all the institutions involved in hake stock assessment.

In recent years, new relevant knowledge has been obtained concerning the growth of this species, based on the mark–recapture method (de Pontual *et al.*, 2003; Piñeiro *et al.*, 2007). Results based on a blind interpretation of marked hake otoliths concluded that age estimates were neither accurate nor precise and invalidated the age estimation method (de Pontual *et al.*, 2006). The results from mark–recaptures are not compatible with the growth rate obtained by mean otolith age estimation, which raises concerns about the use of potentially inaccurate age data for stock assessment and resulting management advice. The impact of biased age estimates on European hake stock assessment is currently under investigation (Bertignac and de Pontual, 2007).

The main goal of this report is to summarize the current status of age estimation for European hake and the work carried out over the years by researchers involved in providing age data for stock assessment purposes. The current knowledge of the age and growth of this species will be described, together with the results of recent international exchanges and workshops held before and after the availability of hake growth information from mark–recapture programmes. In addition, guidelines are provided with a view to furthering progress in ageing studies of this species. Considering the long history of efforts devoted to resolving this problem and the need to have reliable age-structure data for stock assessment, this review is long overdue.

2 Review of otolith age estimation calibration exercises: exchanges and workshops

2.1 Overview of otolith age-reading exchanges and workshops

Although several hake ageing problems have yet to be addressed, several advances have taken place in the past two decades that have involved many scientists from different countries. Table 2.1.1 summarizes the main exchanges and workshops carried out to date. It should be mentioned that several researchers and otolith readers have devoted special attention to the improvement of age-reading precision and the establishment of an internationally agreed ageing protocol for this species. The revision of the previous calibration exercises is important because the outcomes and reports from these exercises have not been published and are collected in contributions to ICES working groups and project reports. The ICES Planning Group on Commercial Catch, Discards, and Biological Sampling (PGCCDBS) provides access to reports on age calibration (age reading) exchanges and workshops via its document repository at http://www.ices.dk/reports/acfm/pgccdbs/PGCCDBSdocrepository.asp.

In the 1980s, the scientists involved in hake age reading for stock assessment purposes also used the data to determine growth parameters, whose wide range suggests that different age-reading methods were employed. To detect these differences, several international *ad hoc* working groups and exchanges were conducted (FAO, 1982; ICES, 1983, 1984, 1986; Oliver *et al.*, 1989). The analysis performed was based mainly on percentage of agreement; the low precision of the results indicated that age interpretation of hake otoliths was very complex and further work was needed on standardization of protocols (Table 2.1.1).

A workshop preceded by an exchange was conducted in 1994 between IFREMER (French Research Institute for the Exploitation of the Sea) and IEO (Spanish Institute of Oceanography), based on the recommendations of the ICES Workshop on Sampling Strategies for Age and Maturity (ICES, 1994b). The main objective of this exchange was to assess age-reading precision, using the recommended protocol (Piñeiro and Meixide, 1995). At the workshop, the general criteria adopted were based on the number of annual rings (consisting of one opaque and one translucent, or hyaline, zone) and used 1 January as the birthday. Readers were not made aware of the length of the fish. The readings were carried out using a stereomicroscope (magnification × 20) under reflected light, but transmitted light was also used to help discriminate between translucent and opaque growth rings. A video camera connected to a large screen was used to facilitate discussion of the interpretation criteria. The results indicated a low level of agreement between the two readers. Since that time, the guidelines for organizing exchanges and workshops, the methods used, and the analysis of the data have remained unchanged.

Within the framework of BIOSDEF (Anon., 1998), a workshop was organized in 1997 (Piñeiro, 2000) to improve the method of ageing hake in countries that share the same stocks. Prior to the workshop, an exchange was carried out during 1995/1996. The otolith readers applied the protocol used at the 1994 workshop, and the analysis of the age-reading results was carried out using the methodology proposed by Eltink (1997). Following the recommendation given by ICES (1994), several statistical indices and tests were used for analysing the results:

- Wilcoxon's rank test, to assess bias between a pair of readers
- bias plots of the average age, ±2 s.d. (standard deviation), obtained by each age reader; all age reader results were plotted against modal age, considered as the referential age
- average percentage error (APE), from Beamish and Fournier (1981), to assess a measure of the precision of a series of readings
- mean coefficient of variation (CV)
- index of precision (Chang, 1982)
- box-whisker plots for visual presentation of the observations

These analyses provided information on the precision of age readings by age group for both individual readers and all readers combined. An interpretation criterion for the first three ages was agreed, but precision levels were low for the age-reading exercises performed. However, this workshop was considered to be a further step towards reaching a consensus for age-reading criteria (Table 2.1.1). It was agreed that more training was necessary to increase precision levels, and a further exchange was recommended.

Another workshop was carried out in 1999 to analyse results from the exchange circulated in the previous year (Piñeiro *et al.*, 2000). This workshop was planned within the framework of EU Study Contract No. 97/015 "New Assessment and Biology of the Main Commercial Fish Species: Hake and Anglerfish of Southern Shelf Demersal Stocks in the South Western Europe" (DEMASSESS; see Anon., 2000). New readers started to be involved in hake otolith ageing, and their participation at the workshop was considered worthwhile in facilitating their understanding of the ageing criteria. Two otolith samples prepared by the IFREMER and IEO laboratories were read during the exchange, and a copy of the protocol used at the last workshop (1997) was provided to all participants. Fish length information from IEO otolith collections was available and digital images of sectioned otoliths were used and circulated for the first time. For the age-reading analysis, a more extensive study was performed to compare the age interpretations of the readers. However, the Wilcoxon's rank test was not used because it was considered inappropriate to perform multiple paired comparisons when more than two readers were involved in ageing the same collection (Zar, 1996). In order to determine which groups of readers showed the higher levels of agreement, cluster analysis and multiscaling dimensions were used.

For reproducibility measures (APE and CV), the values for all readers in general had decreased since the last exchange, particularly for the IFREMER sample. The CV of the expert readers was reduced, but the APE index remained the same (Figure 2.1.1). It should be emphasized that these measures of agreement should be interpreted with caution because of the influence of sample size and, in particular, of younger ages in CV calculations. At the 1999 workshop, it was recognized that the level of reader experience is an important aspect of the achievement of higher precision for hake age reading, particularly in the identification of false (non-annual) rings. Results indicated that it is possible to use the annual ALK instead of numerical methods to estimate age composition of catches for the southern hake stock assessment, taking into account the observed agreement between the readers involved.

Until this time, the age-structure composition of the northern hake stock catches had been based solely on IFREMER ALKs. However, in light of the agreement on age reading that was reached, the possibility of applying ALKs from AZTI-Tecnalia (the Basque Technological Centre for Marine and Food Investigation) in addition to those from IFREMER was recommended. The agreement between readers for ages 0–4 was improved compared with the previous workshop (1997), mainly in the identification of the first annual ring. Despite improvements in precision for all readers, the results continued to highlight the difficulties in interpreting the age of hake, and it was recommended to hold another exchange and workshop in two years. Furthermore, it was recommended that alternative techniques (tagging, microchemistry, etc.) for validation of the age estimation of this species be explored.

Under EU Contract No. 99/009 "Improving Sampling of Western and Southern European Atlantic Fisheries" (SAMFISH), an otolith exchange on hake was undertaken in 2001 (Piñeiro and Saínza, 2002) from both northern and southern ICES stocks in order to calibrate the age readings and to estimate the precision level. The terminology, guidelines, and tools used for age-reading comparison were carried out according to the recommendations of Eltink *et al.* (2000). Readers had access to the collection of otoliths used in the exchange and to the digitized images from the otolith sections. Information on catch date, sex, and total length were available to the readers. The same procedures as for the previous exchange were followed (1998), and the exploratory and statistical analyses revealed the highest level of agreement in the location of the first three annual rings as a result of the adoption of the ageing criteria. The results indicated that, although an improvement in precision was evident for almost all readers, the estimation of older fish (> 5 years) appeared to be the major difficulty. The latter result justified the need for another exchange among readers that provided stock assessment age data, with particular focus on higher ages.

In 2002, the ICES WGHMM demonstrated the difficulties in the assessment of hake that result from uncertainty in age estimation for older fish, which led the working group to use a plus group at age 8. To deal with these problems, an otolith exchange focusing mainly on older fish was conducted in 2003 between readers involved in the assessment (Piñeiro *et al.*, 2004). In contrast to the previous exchange (2001), no information on fish length was available to the readers. The exchange results indicated that the precision of age readings had decreased. The comparison of the results obtained from this exchange (2003) and the previous exchange (2001; Figure 2.1.1) highlights the difficulty of hake age estimation, and it was recommended that an international workshop specifically aimed at solving these problems should be held.

The 2003 exchange demonstrated a strong bias in age estimations of older fish. The precision (CV) of age estimation had decreased from ca. 17 to 32% for the ALK readers between the previous two exchanges (2001, 2003) for otolith collections within the same fish length range (Figure 2.1.1). The age range regarded by age readers as confident also dropped: from 0–5 years to 0–3 years. This negative result was a consequence of the difficulty of hake otolith reading using non-validated age determination criteria. The lack of knowledge of fish length in this exchange, in contrast to the availability of fish length information during the 2001 exchange, could also contribute to the reduction in precision and the "confident" age range. This mainly affected less experienced readers, who were not accustomed to measuring ring radius when they performed routine hake age readings.

An international age-reading workshop was conducted in 2004 to tackle the problem of providing age estimates of fish older than 5 years (see Section 2.2).

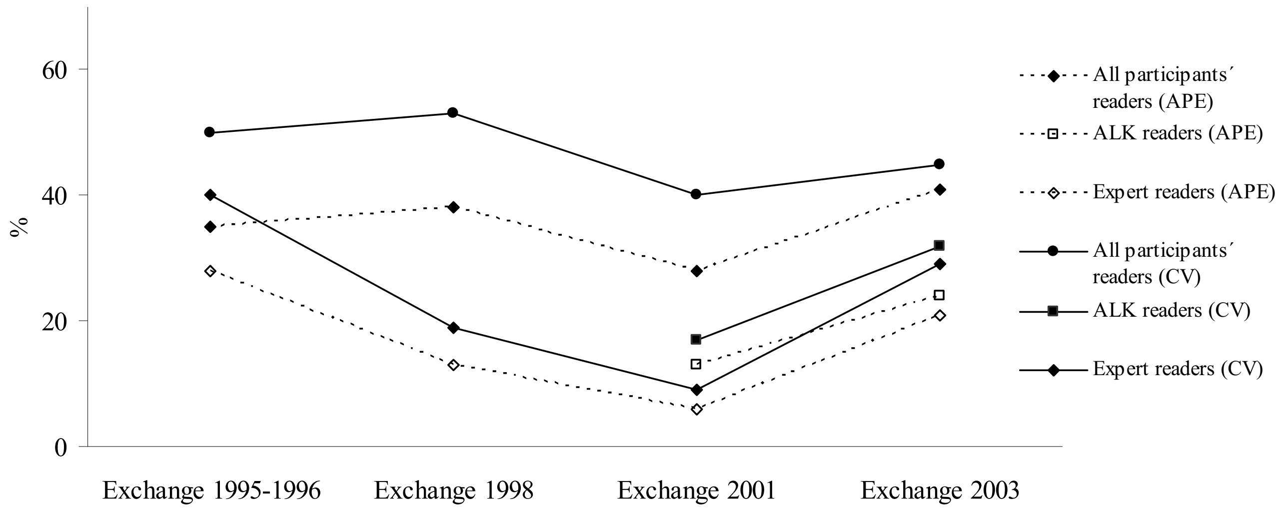

Figure 2.1.1. Average percentage error (APE) from Beamish and Fournier (1981) and coefficient of variation (CV) for exchanges between 1995/1996 and 2003 for all participants, readers that provided age data for stock assessment purposes (ALK readers), and expert readers.

Table 2.1.1. Overview of hake age estimation events conducted during the past three decades, with information on sample sets read, objectives, and achievements.

<table>
<tr><th>Year</th><th>Event</th><th>Participating countries</th><th>Area</th><th>No. of readers</th><th>Sample: no. otoliths & length range (TL cm)</th><th>Objectives</th><th>Main results</th></tr>
<tr><td>1981</td><td>Workshop methodologies used for fish age reading</td><td>France, Italy, Morocco, Senegal, and Spain</td><td>Mediterranean
NE Atlantic</td><td>14</td><td></td><td>Compare preparation techniques and reading methods</td><td>No standard method agreed</td></tr>
<tr><td>1983</td><td>Workshop</td><td>France, Spain</td><td>NE Atlantic</td><td>4</td><td rowspan="4"></td><td rowspan="4">Preparation techniques selection and agreed age-reading criteria</td><td rowspan="4">

- Standard preparation technique adopted using transversal sections of otoliths
- Improvement in agreement on otolith interpretation, but no standardization
- Identification of three sources of different interpretation:
  - difficulty in locating first *annulus*
  - discrimination of true rings from checks
  - interpretation of the edge

</td></tr>
<tr><td>1984</td><td>*Ad hoc* WG</td><td>Canada, France, and Spain</td><td>NE Atlantic</td><td>5</td></tr>
<tr><td>1986</td><td>*Ad hoc* WG</td><td>Canada, France, Portugal, Spain, and UK</td><td>NE Atlantic</td><td>14</td></tr>
<tr><td>1989</td><td>Workshop</td><td>France, Greece, Spain, and Italy</td><td>Mediterranean</td><td>9</td></tr>
<tr><td>1994</td><td>Exchange and workshop</td><td>France and Spain</td><td>NE Atlantic</td><td>2</td><td>95
(15 – 67)</td><td>Assess age-reading precision</td><td>

- Main difficulties related to correct identification of first *annulus*
- No difference in growth pattern between otoliths from northern and southern stocks

<table>
<tr><th>Readers</th><th>Agreement between first and second reading</th><th>CV (%) (intra-reader)</th></tr>
<tr><td>Spanish</td><td rowspan="2">43 – 98</td><td>75</td></tr>
<tr><td>French</td><td>50</td></tr>
</table>
</td></tr>
<tr><td>1995 – 1996</td><td>Exchange</td><td>France, Portugal, Spain, and UK</td><td>NE Atlantic</td><td>7</td><td>192
(16 – 55)</td><td>Assess age-reading precision and agreed age-reading criteria</td><td>
<table>
<tr><th></th><th>APE (%)</th><th>CV (%)</th></tr>
<tr><td>All readers</td><td>35</td><td>50</td></tr>
<tr><td>ALK readers</td><td>28</td><td>39</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Year</th><th>Event</th><th>Participating countries</th><th>Area</th><th>No. of readers</th><th>Sample: no. otoliths & length range (TL cm)</th><th>Objectives</th><th>Main results</th></tr>
<tr><td>1997</td><td>Workshop</td><td>France, Portugal, Spain, and UK</td><td>NE Atlantic</td><td>9</td><td>95
(16 – 55)</td><td>Assess age-reading precision and agreed age-reading criteria</td><td>

Otolith interpretation criteria agreed for first three ages
No difference in growth pattern between otoliths from both stocks
Reference factor adopted to locate first *annulus*
Two or three checks before first *annulus*
Difficulty interpreting otolith edge

<table>
<tr><th></th><th>APE (%)</th><th>CV (%)</th></tr>
<tr><td>All readers</td><td>16</td><td>21</td></tr>
<tr><td>ALK readers</td><td>11</td><td>16</td></tr>
</table>
</td></tr>
<tr><td>1998</td><td>Exchange</td><td>France, Portugal, Spain, and UK</td><td>NE Atlantic</td><td>11</td><td>100 (IEO)
(12 – 69)
107 (IFREMER)
(19 – 102)</td><td>Assess age-reading precision and agreed age-reading criteria</td><td>
<table>
<tr><th></th><th colspan="2">APE (%)</th><th colspan="2">CV (%)</th></tr>
<tr><th>Sample sets</th><th>IEO</th><th>IFREMER</th><th>IEO</th><th>IFREMER</th></tr>
<tr><td>All readers</td><td>37</td><td>33</td><td>53</td><td>44</td></tr>
<tr><td>ALK readers</td><td>13</td><td>5</td><td>19</td><td>7</td></tr>
</table>
</td></tr>
<tr><td>1999</td><td>Workshop</td><td>UK, France, Ireland, Portugal, and Spain</td><td>NE Atlantic</td><td>11</td><td>54 (IEO)
(12 – 69)
41 (IFREMER)
(20 – 76)</td><td>Assess age-reading precision and agreed age-reading criteria</td><td>

Adoption of ageing criteria for ages 0 – 4
High level of variability between readers for ages greater than 5
High agreement in locating first *annulus*
Precision level highly related to ageing experience
Difficulty interpreting age patterns on hake otoliths justifies need for periodic exchange exercises

<table>
<tr><th></th><th colspan="2">APE (%)</th><th colspan="2">CV (%)</th></tr>
<tr><th>Sample sets</th><th>IEO</th><th>IFREMER</th><th>IEO</th><th>IFREMER</th></tr>
<tr><td>All readers</td><td>29</td><td>21</td><td>42</td><td>31</td></tr>
<tr><td>ALK readers</td><td>13</td><td>10</td><td>13</td><td>14</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>YEAR</th><th>EVENT</th><th>PARTICIPATING COUNTRIES</th><th>AREA</th><th>NO. OF READERS</th><th>SAMPLE: NO. OTOLITHS & LENGTH RANGE (TL CM)</th><th>OBJECTIVES</th><th>MAIN RESULTS</th></tr>
<tr><td>2001</td><td>Exchange</td><td>UK, France, Ireland, Portugal, and Spain</td><td>NE Atlantic</td><td>12</td><td>199
(11 – 83)</td><td>Assess age-reading precision and improvement in agreed age-reading criteria; new readers started to be in-volved in otolith ageing</td><td>
• Highest level of agreement obtained so far in location of first three *annuli* as result of using established ageing criteria
• Highest level of agreement (%) achieved for first 5 age groups
• APE and CV indices for all readers are best from all exchanges
• No significant differences between otoliths from both stocks (p > 0.05)
<table>
<tr><th>READERS</th><th>AGREEMENT (%)</th><th>APE (%)</th><th>CV (%)</th></tr>
<tr><td>All</td><td>58</td><td>28</td><td>40</td></tr>
<tr><td>ALK</td><td>75</td><td>13</td><td>17</td></tr>
<tr><td>Expert</td><td>84</td><td>6</td><td>9</td></tr>
</table></td></tr>
<tr><td>2003</td><td>Exchange</td><td>UK, France, Ireland, Portugal, and Spain</td><td>NE Atlantic</td><td>13</td><td>200
(11 – 84)</td><td>Assess age-reading precision and improvement in age-reading precision of ages >5 years</td><td>
• Decrease in level of agreement for ALK readers
• Strong bias found in age estimates of older fish
• High level of agreement in locating first three *annuli*
• Consensus ageing methods for ages >5 years to be established at next workshop
<table>
<tr><th>READERS</th><th>AGREEMENT (%)</th><th>APE (%)</th><th>CV (%)</th></tr>
<tr><td>All</td><td>47</td><td>41</td><td>45</td></tr>
<tr><td>ALK</td><td>57</td><td>24</td><td>32</td></tr>
<tr><td>Expert</td><td>57</td><td>21</td><td>29</td></tr>
</table></td></tr>
<tr><td>2004</td><td>Workshop</td><td>Belgium, Denmark, UK, France, Ireland, Portugal, and Spain</td><td>NE Atlantic</td><td>14
(70)</td><td>S1: 127
(11 – 59)
S2: 187
(10 – 58)
S3: 70
(14 – 87)</td><td>Try to establish ageing criteria for older fish; checking precision and relative bias of ALK readers; incorporation of new readers</td><td>
• No agreed criteria for older fish
• Difficult to maintain precision for fish older than 3 years
• Confident age range dropped from 5 to 3 years from 2001 to 2003 exchanges, respectively
• Studies on hake growth presented at workshop indicate that actual ageing criteria not accurate
<table>
<tr><th>READERS</th><th>SAMPLE</th><th>AGREEMENT (%)</th><th>APE (%)</th><th>CV (%)</th></tr>
<tr><td>All</td><td>S3</td><td>-</td><td>24 – 24</td><td>32 – 31</td></tr>
<tr><td>ALK</td><td>S1–S2</td><td>72 – 60</td><td>19 – 35</td><td>25 – 48</td></tr>
<tr><td>ALK</td><td>S3</td><td>-</td><td>20 – 15</td><td>25 – 20</td></tr>
<tr><td>Expert</td><td>S3</td><td>-</td><td>22 – 15</td><td>31 – 20</td></tr>
</table></td></tr>
</table>

2.2 Summary of the most recent (2004) hake otolith workshop report

Although the 2004 workshop report is available via the PGCCDBS document repository on the ICES website, we summarize it here because of the extent of the conclusions compared with previous workshop reports. See http://www.ices.dk/reports/acfm/pgccdbs/PGCCDBSdocrepository.asp

2.2.1 Introduction

In 2002, the WGHMM (ICES, 2003) recommended addressing hake ageing problems by means of (i) a hake otolith exchange programme (scheduled for 2003) focusing mainly on older fish and (ii) a subsequent workshop to discuss the results (scheduled for 2004). The results of the 2003 otolith exchange programme indicated a decrease in the precision of age readings compared with the precision of the 2001 exchange and a strong bias in the estimates for older fish.

The main goals of the 2004 workshop were to increase the precision of European hake otolith age estimation reached in previous exchanges (in 1997, 1999, 2001, and 2003) for ages up to 5 years and to improve the confidence in age estimation for older age groups (> 6 years).

The initial terms of reference (ToRs) for the 2004 workshop were changed as a result of recent advances reported in age and growth estimation from tagging experiments. The revised ToRs were:

- discussion of the results of the fourth otolith exchange (2003)
- discussion of new information regarding the estimation of age and growth and alternative methods of obtaining age–length keys (ALKs) for assessment purposes (e.g. elaboration of synthetic ALKs)
- checking the precision and bias of age readers involved in stock assessment
- discussion of age-reading criteria and problems identified for young and/or old fish
- incorporation of new readers in hake age estimation

2.2.2 Material and methods

The workshop was held from 18 to 20 October 2004 in Vigo (Table 2.1.1). A subset of 70 otoliths (S3) from an exchange of the 2003 collection was read in this workshop in the absence of information on fish length. This subset was selected to include otoliths that represented the full range of agreement obtained in the previous exchange (2003).

At the 2003 exchange, an ageing protocol was circulated together with a CD-ROM containing digitized images from otolith sections and the adopted ageing criteria (Piñeiro and Saínza, 2003). All readers were asked to measure the distances between the nucleus and (i) the first three translucent rings considered to be *annuli*, (ii) the check ring, and (iii) the edge. These measurements were marked on the images. This was to determine whether readers were counting the same translucent rings. In order to make analytical comparisons, the readers were split into three groups based on their level of experience: ALK readers, expert readers, and all readers.

Digital images, projected on a screen using a computer running TNPC Image Analysis Software, were used to facilitate individual interpretations and discussions between the readers.

Two analyses were undertaken:

1) comparison of readings from the 2001 exchange (S1) and the 2003 exchange (S2) for ALK readers, excluding otoliths from fish larger than 60 cm, in order to assess reader bias

2) comparison of the age-reading results from a subset of 70 otoliths (S3) with the 2003 exchange collection (S2; Table 2.1.1, last row)

The workshop analyses of the age-reading results were performed using the "Guidelines and Tools for Age Reading Comparisons" (Eltink *et al.*, 2000). Several indices and tests were used to assess the age-reading consistency of several readers from the same calcified structures, as recommended by Campana (2001):

1) **The percentage of agreement** is the ratio (as a percentage) between the number of coincident readings and the total number of readings and is one of the simplest methods available. However, Beamish and Fournier (1981) recognized that this percentage did not take into account the absolute age of the fish. Coefficient of variation (CV) and average percentage error (APE) are now preferred for the study of age-reading precision.

2) **The average percentage error** (APE; Beamish and Fournier, 1981) is an index of reading precision that is very useful for comparing series of observations. It is defined as

$$APE = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{1}{r}\sum_{j=1}^{r}\frac{\left|x_{ij}-\overline{x}_i\right|}{\overline{x}_i}\right)$$

where n is the number of otoliths, r is the number of readings for each otolith, x_{ij} is the j value of age estimation for otolith i, and $\overline{x}_i$ is the mean age of otolith i. When averaged across many fish, it becomes an index of mean APE.

3) **The coefficient of variation** (CV) describes the precision errors in age reading by age group. It is statistically more robust and flexible than APE. It should be remembered that CV is very sensitive to low age values.

$$CV = \frac{100}{n}\left[\sum_{i=1}^{n}\left(\frac{\sigma}{\overline{x}_i}\right)\right]$$

where n is the number of otoliths, σ is the standard deviation for the otolith i, and $\overline{x}_i$ is the mean age of otolith i.

Because no validated ages are available, the reference age assumed is the modal age from readers who provide ALKs for stock assessment. In the case of bimodality, the estimate of modal age was based on data from the most expert readers.

The first three annual rings and check ring measurements were analysed using box-whisker plots by age and reader. Age-bias plots demonstrate both types of age-reading errors (precision and accuracy); however, because known-age material was not available, the bias in age reading was only assessed by precision.

Moreover, evidence from mark–recapture experiments, daily growth, and elaboration of synthetic ALKs (Annexes 6, 7, and 8 respectively of the 2004 workshop report; see Piñeiro *et al.*, 2004) were available and were presented during the workshop. This evidence gives insight into differential growth; thus, relevant results on growth contribute to the main conclusions that are crucial to assessment and management of the hake stocks.

2.2.3 Results and discussion

2.2.3.1 Comparison of results from the last two exchanges: 2001 (S1) and 2003 (S2)

The results revealed that, between the 2001 (S1) and 2003 (S2) exchanges, agreement dropped from 72 to 60%, whereas the APE increased from 19 to 35% and the CV increased considerably from 25 to 48%. The values obtained from the APE and CV indicated that precision of age estimation has decreased between both exchanges (Figure 2.1.1).

2.2.3.2 Comparison of results from the 2003 exchange (S2) and 2004 workshop (S3)

In general, the majority of readers tended to give lower ages for S3 than for S2. The ages assigned by readers to fish older than 3 years demonstrated a higher level of variability. The results indicated that it is difficult to maintain precision for fish older than 3 years (reference – or modal – age, not true age). In the S3 readings, the amplitude of confidence intervals generally decreased for all readers, and they tended to underestimate ages above 3 years (Figure 2.2.1).

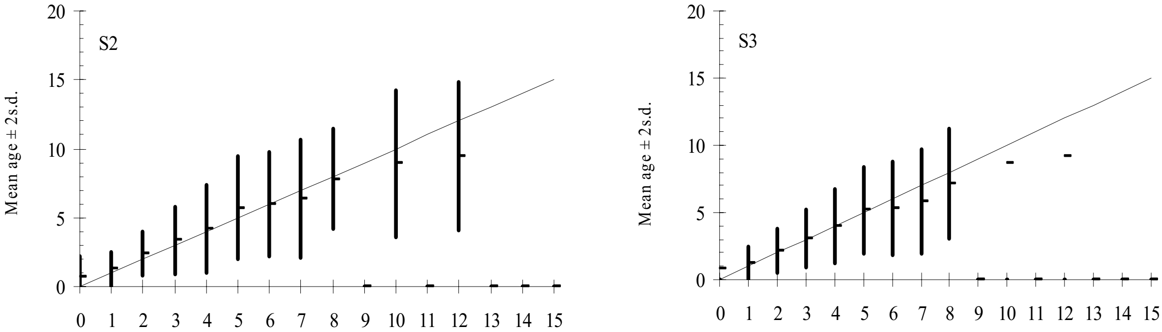

Figure 2.2.1. Age-bias plots for the same readers involved in the 2003 exchange (S2) and the 2004 workshop (S3). Mean age recorded ± 2 s.d. (standard deviation) are plotted against the modal age.

2.2.3.3 Analysis of fish less than 60 cm total length (S4)

The values obtained for APE and CV demonstrated hardly any improvement for the three groups of readers.

The radius measurement for the first three annual rings and the check ring indicated that all readers can clearly distinguish between these rings because similar median distances for these rings were obtained (Figure 2.2.2). This indicates the consistent nature of the ageing criteria for these first rings.

The classification of the otolith edge (opaque or translucent) was also discussed. Most of the confusion was caused by the frequent occurrence of a translucent edge through the whole year in young fish.

Considering the incorporation of new readers in hake age estimation, the results of this workshop demonstrate the difficulties in applying the established ageing criteria and reflect the importance of training in otolith age reading.

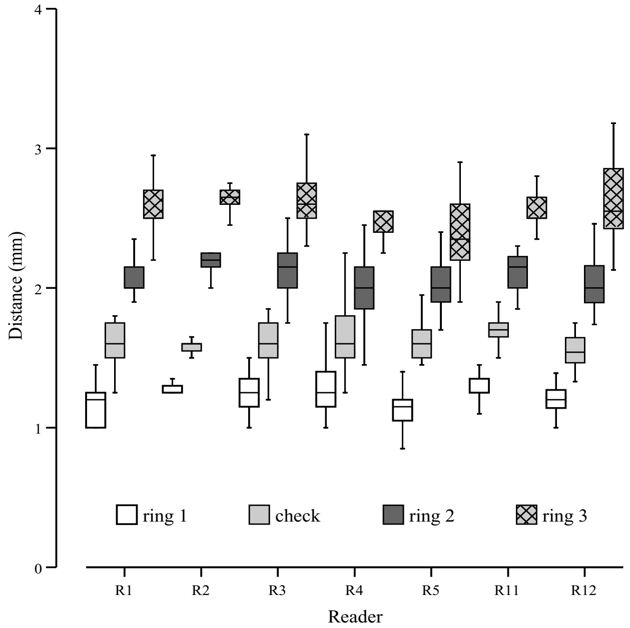

Figure 2.2.2. Ring measurement (mm) from the nucleus to the end of the translucent band for the following rings by all readers who provided stock-assessment age data: first annual ring (ring 1); check; second annual ring (ring 2); third annual ring (ring 3).

2.2.4 Conclusions

The ages assigned by readers to fish older than 3 years demonstrated a higher level of variability compared with those for younger fish. Taking into account the low precision obtained for those lengths, no agreed criteria were established for older fish.

The results of studies on hake growth presented at the 2004 workshop indicate that the ageing criteria are inaccurate.

The results from mark–recapture experiments provide evidence that ages are overestimated when using the internationally agreed criteria (de Pontual *et al.*, 2003).

2.2.5 Recommendations

Taking into account the loss of precision in the last exchange (2003) and workshop (2004), and the inaccuracy of ageing criteria evidenced by tagging experiments, workshop participants recommended that the supply of age-reading data for the elaboration of ALKs for stock assessment purposes should be interrupted in order to reallocate effort to the establishment of validated ageing criteria.

Validation studies are essential to the progress of hake ageing studies. Taking into account the recapture rate obtained in recent studies (de Pontual *et al.*, 2003), tagging is a very promising method for validating hake ageing.

In the meantime, the effort (for both time and people) that, until now, has been expended on the reading of otoliths should be allocated to other tasks, such as:

- tagging surveys, financed by national data collection and management programmes, in order to provide the reference material essential to the development of accurate age criteria that will yield reliable ALKs
- creation of a database of otolith images, weight, and other complementary biological information
- otolith microstructure studies (daily growth, etc.)

- length distribution analysis of surveys and available commercial catches
- research studies of the macrostructure pattern of otoliths in order to establish an understanding of the typology of the rings (annual rings and checks) and the mechanisms that control their deposition

Workshop participants recognized that these recommendations represent a turning point in the stock assessment of this species. In light of the results obtained in this workshop, as well as the recent advances in hake age validation (mark–recapture experiments, daily growth), it is important to avoid expending time in obtaining age data without assurance of its precision.

3 Summary of current knowledge

Over the years, many initiatives have been launched to address the problems of age determination of European hake. These have been reported in the scientific literature and very frequently in "grey literature", available in the public domain but not widely distributed. This section describes important recent studies that have contributed directly or indirectly to a better understanding of hake growth.

Initial attempts at studying the growth of European hake were made using mark–recapture methods (Belloc, 1935) and reading of scales (Birtwistle and Lewis, 1925; Belloc, 1929). Subsequently, most researchers have relied on the interpretation of rings in bony structures (otoliths) and on analyses of length–frequency distributions, such as modal progression analysis and the separation of modal components (Gulland and Rosenberg, 1992). Daily growth of this species has also been studied and has yielded important information about first-year growth (Morales-Nin and Aldebert, 1997; Arneri and Morales-Nin, 2000; Morales-Nin and Moranta, 2004; Kacher and Amara, 2005; Belcari *et al.*, 2006; Piñeiro *et al.*, 2008). Recent mark–recapture experiments are an important tool in improving the current status of knowledge of European hake growth. Although independent studies may indicate growth rates different from the ones inherent in standard age-reading criteria, mark–recapture results have been the driving force behind the general discussion on the hake fast-growth hypothesis.

Over the years, several techniques for making hake otoliths easier to examine have been employed (Christensen, 1964; Albrechtsen, 1968; Nichy, 1969) but even so, all of the researchers have encountered difficulties in interpreting the pattern of growth ring formation on otoliths of this species consistently. Therefore, studies on annual growth have reported very different growth estimates for hake in both the Northeast Atlantic and the Mediterranean Sea (Hickling, 1933; Bagenal, 1954; Meriel-Bussy, 1966; Robles *et al.*, 1975; Iglesias and Dery, 1981; Goñi, 1983; Goñi and Piñeiro, 1988; Guichet, 1988; Lucio *et al.*, 2000; Godinho *et al.*, 2001; Piñeiro and Saínza, 2003; ICES 2000, 2008a). Figure 3.1.1 shows the wide range in total length by age class and sexes combined. To minimize bias caused by different sample size and range, the estimated sample mean lengths-at-age are presented as the only comparable growth information available throughout the studies reviewed. It is important to assess whether such a variety of results is a consequence of diverse biological growth, an outcome of bias and inaccuracy in the age estimation methods, or a combination of both these factors.

3.1 Age-reading criteria

In order to standardize age-reading procedures, otolith preparation and ageing methods were agreed at two workshops conducted in 1997 and 1999 within the framework of the EU projects BIOSDEF (Anon., 1998) and DEMASSESS (Anon., 2000). The main achievement of these workshops was a set of internationally accepted interpretation criteria for hake otoliths up to the age of 5 years, allowing otolith readers from the various institutions involved in stock assessment to age hake consistently. The elaboration of these ageing criteria was a direct consequence of previous research studies on the growth pattern of hake otoliths, which were carried out by a group of experts on hake otolith patterns and biology. The agreed criteria rely on the experience obtained from previous hake ageing studies (Goñi and Piñeiro, 1988; Piñeiro and Hunt, 1989; Piñeiro *et al.*, 1992; Piñeiro and Pereiro, 1993). Additional information was also employed, namely length–frequency distributions from research and discard surveys (Piñeiro *et al.*, 1992; Pérez *et al.*, 1996). However, these criteria were never validated (Piñeiro and Saínza, 2003).

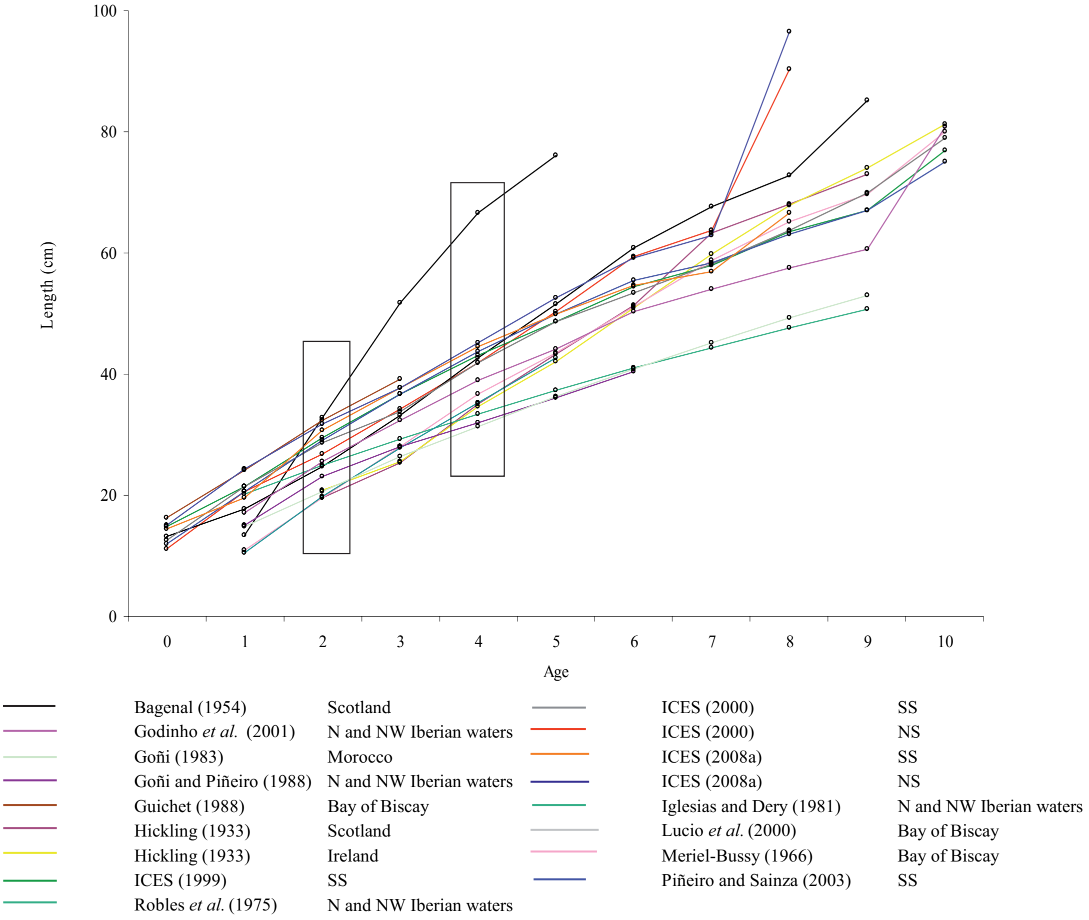

Figure 3.1.1. Mean length-at-age (sex combined) obtained by mean otolith age readings from different authors in Northeast Atlantic waters (NS, northern stock; SS, southern stock).

The standard age estimation criteria are derived from hake otolith sections, which have a concentric pattern of translucent and opaque bands around the nucleus when viewed under reflected light. One annual growth zone, or *annulus*, consists of one opaque and one translucent ring or band. Counts of annual translucent rings, preferably on the ventral region, are used to estimate ages. The first annual ring (1) is identified despite the presence of checks (−3, "larval"; −2, "pelagic"; −1, "demersal") around the nucleus (Figure 3.1.2). Growth during the first and second years is great compared with subsequent annual increments. Although the position of the first annual ring varies, its recognition is aided by the frequent presence of a well-marked translucent ring along the dorso-ventral axis of the section between the two first annual bands. This check is frequently found on the section otolith at ca. 1.57 mm (mean value ± 0.13, $n = 608$) from the nucleus. The pattern of otolith growth presents two translucent rings per year during the first 3 years of life and thereafter only one, which has been associated with the process of sexual maturation.

Classification of the otolith edge type (translucent or opaque) tends to be complicated by the high incidence of false rings. Translucent edges appear year-round. On average, more than 60% of the otoliths presented translucent edges, indicating a high incidence of checks, particularly in summer (Piñeiro and Saínza, 2003). In general, two peaks of translucent edges per year were observed. The most important peak was in winter (November) and the secondary peak was in spring–summer (April–June). Nevertheless, it is assumed that otolith edges follow the predominant pattern of translucent-in-winter and opaque-in-summer; by convention, an otolith with a translucent edge is not considered to be one year older until 1 January of every year.

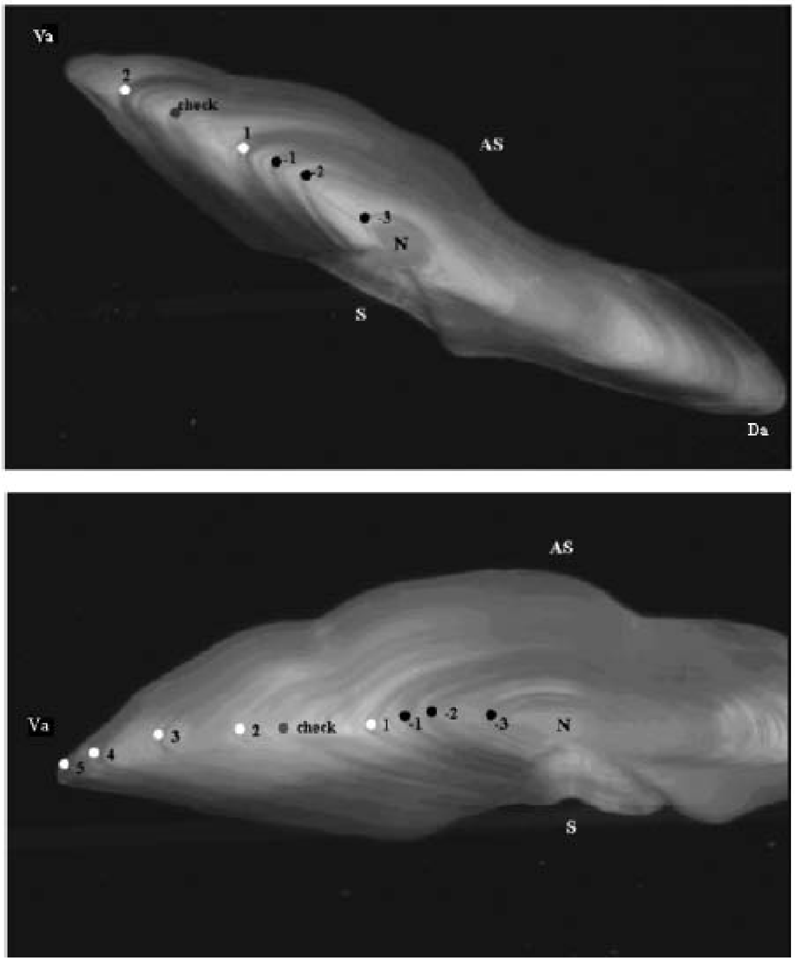

Figure 3.1.2. Transverse sections of *sagitta* otoliths of two hake specimens collected in Iberian Atlantic waters in 1997. Top: a male of 30 cm total length caught in October and aged 2 years. Bottom: a female of 47 cm total length caught in April and aged 5 years. Photographs (× 20, reflected light) show central area around the nucleus (N) where three false rings (−1, −2, and −3) are visible before the first *annulus* and one (check) between the first and the second *annulus* (1 and 2). Annual rings are visible as translucent zones (white dots). (Da = dorsal apex; Va = ventral apex; S = sulcus; AS = antisulcus; from Piñeiro and Saínza, 2003).

3.2 Reproductive behaviour

One of the key issues in relating length and age for hake is the extended spawning period. Spawning of hake in the Northeast Atlantic has been studied by several authors (Lucio *et al.*, 2000; Piñeiro and Saínza, 2003; Lannin, 2006; Murúa, 2006; Murúa *et al.*, 2006; Domínguez-Petit, 2007). Results from these studies indicate that spawning takes place over an extended period and occurs earlier in more southerly latitudes and progressively later towards the northern latitudes. It is possible to find mature specimens throughout the year, but prolonged spawning is more evident in females (Murúa and Motos, 2006; Table 3.2.1). Spawning is one of the more important biological aspects that will be reflected in the otolith growth patterns if this species registers the energetic effort of spawning as rings in the otoliths (Morales-Nin and

Moranta, 2004). In the Mediterranean Sea, Oliver (1991) also reported a long spawning season for this species, with peaks in spring and autumn demonstrating a marked interannual variability related to individual growth variability. Morales-Nin *et al.* (1998) also reported that the ring pattern depends on sex and sexual activity.

The European hake is a batch-spawner with indeterminate fecundity. Reproductive modality of this species is highly asynchronous, both at the individual level, namely oocyte development, and at the population level, namely spawning pattern (Murúa, 2006; Domínguez-Petit, 2007). These fluctuations may increase the chances of survival of the offspring and decrease the natural mortality of the cohorts (Murúa *et al.*, 1996). The extended spawning season also indicates the occurrence of individuals of very different sizes belonging to the same year class (born in the same calendar year). These size differences will be greater with faster growth rates. This factor will always be an obstacle to accurate age estimation for hake.

A study of whole and sectioned hake otoliths from Galician waters and the Cantabrian Sea, based on ring measurement analysis, found that otolith ring pattern was a function of hatch date (Piñeiro and Hunt, 1989). So, for average total length at age 1 (which can vary from 10 to 20 cm), two extreme otolith types were evident, corresponding to early and late hatching.

In relation to the spawning areas, available studies demonstrate that hake migrate to spawn, although spawning behaviour appears to vary with latitude. Belloc (1935) and Hickling (1933) observed that mature adults concentrate in deep waters but spawn in shallower waters. Males reach first maturity at a shorter length and younger age than females. Therefore, in the Northeast Atlantic, the northern stock has a mean length and age at first maturity of ca. 39 cm and 3.4 years for males and 47 cm and 4.2 years for females (Lucio *et al.*, 2000; ICES, 2007a). For the southern stock, Piñeiro and Saínza (2003) reported ca. 33 cm and 2.5 years for males and 45 cm and 4.4 years for females. Males grow slightly faster than females up to age 2 years, but from age 3 years onward, female growth rates surpass those of males. Females also grow to larger sizes and live longer than males. The difference in growth rate coincided with the onset of sexual maturity.

Table 3.2.1. Hake spawning season and their main peaks found by study (dark grey = main peak of the spawning season; light grey = peak of the spawning season).

		Month											
Area	**Study**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Bay of Biscay	Lucio *et al.* (2000)	░	■	■	░	░							
Bay of Biscay	Álvarez *et al.* (2004)	░	■	■	░	░							
Bay of Biscay	Murúa and Motos (2006)	■	■	■	░	░	░	░	░	░	░	░	░
Celtic Sea	Fives *et al.* (2001)				■	■	■						
Celtic Sea	Lannin (2006)	░	░	░	░	░	░	░	░	░			
Galician waters and Cantabrian Sea	Alcázar *et al.* (1983); Pérez and Pereiro (1985)	░	■	■	░								░
Galician waters and Cantabrian Sea	Piñeiro and Saínza (2003)	░	■	░	░	░							░
Galician waters	Domínguez-Petit (2007)	■	■	■	░	░	■	■					
Gulf of Cadiz	Silva *et al.* (2007)	░	░			░	░	░	░	░			
Mediterranean Sea	Oliver (1991)			■	■	■				■	■	■	░
Adriatic Sea	Arneri and Morales-Nin (2000)						■	■	■	░	░	░	░
Mediterranean Sea	Morales-Nin and Moranta (2004)						░	░	░	■	■	■	░
Greek waters	Mytilineou and Vassilopoulou (1988)	░	░	░	░	░	░	░	░	░	░	░	░

3.3 Length–frequency analysis

The analysis of length–frequency distributions can provide useful estimates of relative age and growth of fish. Several studies have been carried out using length–frequency distributions of hake in the Atlantic (Hickling, 1933; Bagenal, 1954; Guichet, 1988; Piñeiro and Pereiro, 1993; Godinho *et al*., 2001) and the Mediterranean (Orsi-Relini *et al*., 1989; Recasens, 1992; Alemany and Oliver, 1995; Morales-Nin and Aldebert, 1997; García-Rodriguez and Esteban, 2002). The most commonly used length-based method is the relationship between otolith radius and fish size (Goñi, 1983; Goñi and Piñeiro, 1988; Piñeiro and Hunt, 1989; Recasens, 1992; Table 3.3.1). However, the extended spawning period and the influx of new recruits throughout the year make the length-progression analysis difficult to interpret because modal age groups may not be distinguished and/or more than one mode may belong to the same year class (Goñi, 1983; Piñeiro and Hunt, 1989; Godinho *et al*., 2001; Domínguez-Petit, 2007).

Table 3.3.1. Overview of European hake length–frequency analysis studies: growth rate in first year and total length (TL) for age 1 by author, area, and method.

STUDY	AREA	GROWTH RATE, FIRST YEAR, (CM MONTH^{-1})	TL AT AGE 1 (CM)	METHOD
Hickling (1933)	West of Ireland		10.5	Petersen (1891)
Bagenal (1954)	Scotland		22.3	Petersen (1891)
Goñi (1983)	NW African Shelf		13 – 16.4	Backcalculation
Goñi and Piñeiro (1988)	Galician waters and Cantabrian Sea		15	Backcalculation
Guichet (1988)	Gulf of Biscay		24.1	NORMSEP
Piñeiro and Hunt (1989)	Galician waters and Cantabrian Sea		10 – 20	Backcalculation
Orsi-Relini *et al.* (1989)	Ligurian Sea	0.8 – 1.2 (spring) 0.7 – 0.9 (autumn)		Modal progression analysis
Recasens (1992)	Catalan Sea	1.1		Modal progression analysis
Piñeiro and Pereiro (1993)	Galician waters and Cantabrian Sea		20	Petersen (1891)
Alemany and Oliver (1995)	Balearic Sea	1.8 (only females)		Bhattacharya (1967) FISHPARM software package
Morales-Nin and Aldebert (1997)	Gulf of Lion	1.15	14.7	MIX (MacDonald and Pitcher, 1979)
Godinho *et al.* (2001)	Portuguese continental waters		14.4 – 21.8	Bhattacharya (1967) FiSAT software package

3.4 Growth parameters

The literature on European hake growth abounds with estimates of mean lengths-at-age and growth parameters obtained for different geographic regions using a variety of ageing methodologies. Almost all studies on hake growth deal with fitting von Bertalanffy (1938) or other growth functions to length-at-age data, where the age data are inferred either from calcified structures or by length–frequency distributions. They are difficult to assess and compare because their estimation depends greatly on the size and age ranges covered in the study. Very few of these studies use validated ages. A summary of growth parameters estimated by sex, both separately and combined, is presented in Table 3.4.1. It is difficult to make consistent comparisons because of differences in the sampling design, age estimation method used (e.g. otolith age estimation, length–frequency analysis), calcified structure (e.g. otoliths, scales) and parameter estimation procedures (e.g. assumed asymptotic length). The wide range of results may be a consequence of the methods used for age estimation (bias/inaccuracy) or attributable to biological factors, such as the complexity of otolith pattern.

Table 3.4.1. Growth parameters obtained by von Bertalanffy growth function (K, L_∞, and t_0) in different studies by sex (m = males; f = females; c = combined sexes), age estimation method, stock (M = Mediterranean Sea; N = Atlantic northern stock; S = Atlantic southern stock), and geographic area.

STUDY	SEX	METHOD	STOCK	AREA	K (CM YEAR $^{-1}$)	L_∞ (CM)	T_0 (YEAR)
Belloc (1929)	c	Scales	–	Africa	0.078	56.6	−0.586
Hickling (1933)	c	Otoliths	N	S Ireland	0.087	128.6	–
Bagenal (1954)	c	Petersen	N	Scotland	0.710	126.4	–
	c	Otoliths			0.204	125.9	–
Meriel-Bussy (1966)	c	Otoliths	N	Bay of Biscay	0.059	171.8	–
Guichet *et al.* (1973)	m	Otoliths	N	NE Ireland	0.069	124.0	–
	f				0.070	124.0	–
Guichet *et al.* (1974)	m	Otoliths	N	NE Ireland	0.024	268.2	–
	f				0.087	123.7	–
Robles *et al.* (1975)	c	Otoliths	S	Galician waters and Cantabrian Sea	0.070	125.4	–
Descamps and Labastie (1978)	m	Otoliths	N	Bay of Biscay	0.148	83.0	−0.420
	f				0.098	116.0	−0.510
Iglesias and Dery (1981)	c	Otoliths	S	Galician waters and Cantabrian Sea	0.060	99.9	−2.740
	m				0.150	63.4	−1.030
	f				0.060	99.8	−2.360
Goñi (1983)	c	Otoliths/ Back-calculated	S	NW African Shelf	0.064	110.0	−0.760
	m				0.067	100.0	−1.090
	f				0.065	110.0	−0.820
ICES (1991)	c	Otoliths	S	Galician waters and Cantabrian Sea	0.080	100.0	−1.420
Alemany *et al.* (1993)	f	FISHPARM/ Bhattacharya	M	Balearic Sea	0.214	113.2	0.311
ICES (1993)	c	Otoliths	N	Bay of Biscay	0.073	127.5	−1.130
Aldebert and Recasens (1995)	m	FISHPARM/ Bhattacharya	M	Gulf of Lion	0.149	72.8	−0.383
	f				0.124	100.7	−0.350
Alemany and Oliver (1995)	c	FISHPARM/ Bhattacharya	M	Balearic Sea	0.184	126.9	0.035
García-Rodriguez and Esteban (1995)	c	Petersen	M	Mediterranean Sea	0.123	113.2	0.137
Lucio *et al.* (2000)	c(1)	Otoliths	N	Bay of Biscay	0.124	110.0	−0.452
	m(1)				0.181	80.0	−0.724
	f(1)				0.122	110.0	−0.619
Godinho *et al.* (2001)	c	Otoliths	S	Portuguese continental waters	0.08	110	−0.97
García-Rodriguez and Esteban (2002)	m	FiSAT (subp. ELEFAN)	M	Mediterranean Sea	0.20	93.0	−0.091
	f				0.21	108.0	0.115
	c				0.21	108	0.115
Piñeiro and Saínza (2003)	c(1)	Otoliths	S	Galician waters and Cantabrian Sea	0.07	120.5	−1.72
	c				0.13	88.01	−1.17
	m				0.18	70.06	−0.97
	f				0.13	89.04	−1.15
de Pontual *et al.* (2006)	c	Mark – recapture	N	Bay of Biscay	0.362	89.9	–
	c(1)				0.250	110	–
	m(1)				0.436	80	–
	f(1)				0.261	110	–

(1) L_∞ fixed.

3.5 Bioenergetics studies

In the Northeast Atlantic, the hake is an ichthyophagous species. Adults feed on fish, mainly blue whiting, other gadoids, and pelagic fish, whereas juveniles prey mainly upon planktonic crustaceans (mainly euphausids, copepods, and amphipods). Cannibalism of juveniles by adults has also been reported (Velasco and Olaso, 1998).

In Galician waters and the Cantabrian Sea, the hake is one of the top predators of the demersal community and occupies one of the highest trophic levels (Velasco *et al.*, 2003). The variability in diet depends mainly on two factors: length and depth (Velasco, 2007). Velasco (2007) indicated an important change, which occurs at a fish length of ca. 18 cm, from a benthic diet based on crustaceans to a pelagic and mesopelagic diet based on small fish. This change may be responsible for the characteristic check found in the ring pattern of hake otoliths described by several authors (Goñi and Piñeiro, 1988; Piñeiro and Hunt, 1989). The presence of this check (ca. 1.5 mm; see Section 3.1), which appears to be well marked in the otolith (Piñeiro and Saínza, 2003), may be linked to some biological or behavioural event, such as a dramatic change in diet. Velasco and Olaso (1998) noted that, in the Calabrian Sea, the diet of hake between 15 and 20 cm in length changed from 88% invertebrates to 97% fish. This might explain the presence of the check because it appears to be independent of season and occurs when the fish attain ca. 19 cm in length (Goñi and Piñeiro, 1988).

A study conducted by Riis-Vestergaard *et al.* (2000) on the rate of food consumption by hake, with estimates based on a bioenergetic model and gastric evacuation methods, revealed that the growth rate may be underestimated. Although bioenergetics studies do not permit accurate age estimation, they can be used as an external support for age estimation.

3.6 Daily growth

The study of daily growth increments is a useful tool for the examination of growth and length-at-age of young fish (Pannela, 1971; Morales-Nin and Aldebert, 1997). This methodology has been indirectly validated in larvae and juvenile European hake in the Adriatic Sea (Arneri and Morales-Nin, 2000) and in experimentally reared conditions in Norway (Morales-Nin *et al.*, 2005). Otolith microstructure analysis has been used to estimate juvenile growth rates and to determine the length corresponding to the first year of life of hake in different areas of the Mediterranean Sea (Morales-Nin and Aldebert, 1997; Arneri and Morales-Nin, 2000; Morales-Nin and Moranta, 2004; Belcari *et al.*, 2006) and in Atlantic waters (Kacher and Amara, 2005; Piñeiro *et al.*, 2008). This method was also used to study larval growth and development, and mortality rates, in both the Atlantic (Álvarez and Cotano, 2005; Bjelland and Skiftesvik, 2006) and the Mediterranean (Palomera *et al.*, 2005).

Hake growth during the first year of life is characterized by the occurrence of different check rings in the otolith. These are probably related to physiological and environmental events and make it difficult to locate the first annual translucent ring (Morales-Nin *et al.*, 1998; Piñeiro and Saínza, 2003). Erroneous interpretation of these rings could be the source of the differences in the length estimated in the first year in several studies (Morales-Nin *et al.*, 1998; Piñeiro and Saínza, 2003). Morales-Nin and Aldebert (1997) noted that, in the Gulf of Lion, hatching occurred throughout the year, indicating that irregular growth during the first year of life may be related to seasonal variations in the times of hatching and recruitment to the bottom producing different growth rates. Morales-Nin and Moranta (2004) found hake recruits year-round in the Mediterranean Sea, with modes between 7 and 11 cm total length (TL), depending on the season. Otolith analysis indicated monthly variations in growth rates (1.2–2.5 cm m^{-1}), with a maximum in summer corresponding to fish hatched the previous autumn. Arneri and Morales-Nin (2000) studied the early development of hake otoliths and obtained a mean total length of 15 cm at the end of the first year, with monthly variable growth rates (1.1–1.6 cm month^{-1}). Kacher and Amara (2005) note that, in age 0 hake (age < 365 d) in the Bay of Biscay and Celtic Sea (northern stock area), two hyaline rings were observed and, in one individual of 22.5 cm, a third ring corresponding to 329 d from the accessory growth centre was detected. They found that juvenile hake reach daily growth rates ranging from 0.71 mm d^{-1} to 0.74 mm d^{-1}. These results suggest that juvenile hake growth rates are faster than previously thought for this species (Table 3.6.1) and are consistent with the growth rates from tagging studies. The size at first year estimated by Kacher and Amara (2005) is close to the size when hake lay down their second-year ring (age group 1), according to the age data obtained by Descamps and Labastie (1978), Goñi and Piñeiro (1988), and Piñeiro and Saínza (2003).

A daily growth study using hake otoliths from the northwest Mediterranean obtained a mean total length of 18.3 cm at the end of the first year of life and a mean growth rate of ca. 1.5 cm month^{-1} (Belcari *et al.*, 2006). A new approach to the estimation of daily growth in Atlantic juvenile hake, based on both sagittal and transversal otolith sections (Piñeiro *et al.*, 2008), found that, by the end of the first year, hake from northwest Iberian waters may attain a length of 25.3 cm, demonstrating an average individual growth rate of 0.66 mm d^{-1} ± 0.06 (Table 3.6.1). These authors observed that the transversal section used to study the juvenile phase presented a particularly clear sequence of microincrements which, at macroscopic scale, consisted of successive wide opaque zones (OZ) and thinner translucent zones (TZ), as depicted in Figure 3.6.1. The latter zones correspond either to seasonal growth structures or to fish-specific responses to endogenous or environmental factors (Courbin *et al.*, 2007).

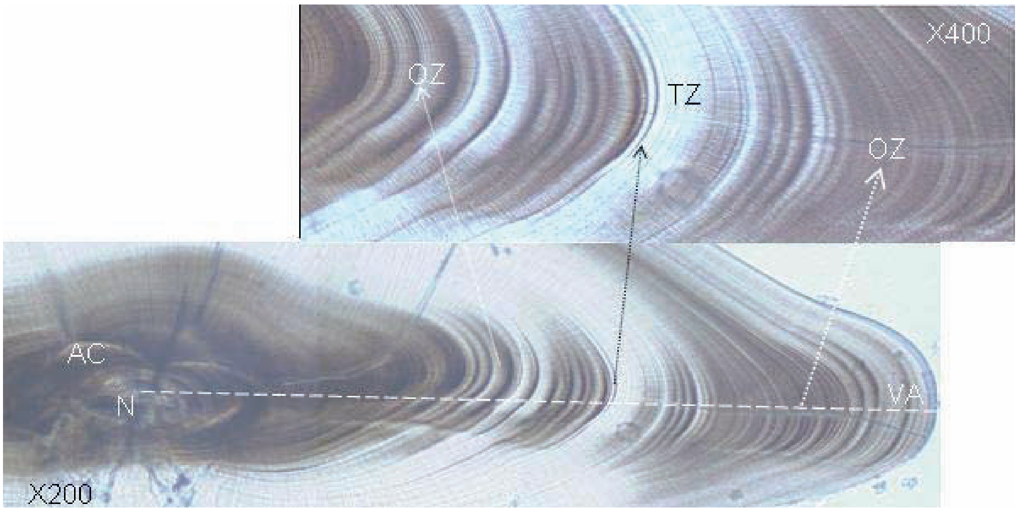

Figure 3.6.1. Bottom: hake otolith, sectioned on the transversal plane (under transmitted light, × 200 magnification), demonstrating a central zone with the nucleus (N) and accessory-growth centres (AC). The daily growth increments visible on the ventral axis (VA) are used for counting. Top: detail of a sequence (× 400 magnification) of growth increments grouping in the translucent (TZ) and opaque zones (OZ) and demonstrating the variation of increment widths that indicates seasonal growth variations.

Growth studies based on otolith daily age estimates have produced great discrepancies in the estimated growth rates throughout the juvenile phase and, consequently, in the size attained at the end of the first year of life. The highest daily growth rates (2.16–2.22 cm month^{-1}) were found in Atlantic hake (Kacher and Amara, 2005), which would result in a juvenile total length of 23.8 cm at the end of the first year. Lowest growth rates were observed in Mediterranean hake (Table 3.6.1). Nonetheless, it must be stated that these differences may be the consequence of different methodological approaches, geographical location, and seasonal origin of the samples.

Table 3.6.1. Overview of hake daily growth data obtained by various studies: growth rate (GR), mean length (ML) at first year of life, number of otoliths sampled, total length (TL) range, and study area.

Study	GR (cm month^{-1})	ML at first year of life (cm)	No. otoliths	TL (cm)	Area
Morales-Nin and Aldebert (1997)	1.15	16	81	10.5 – 20.7	Gulf of Lion
Arneri and Morales-Nin (2000)	1.1 – 1.6	15	145	1.6 – 16.4	Central Adriatic
Morales-Nin and Moranta (2004)	1.2 – 2.5	–	153	2.5 – 25	Mediterranean Sea
Kacher and Amara (2005)	2.16 – 2.22 * (0.72 – 0.74 mm d^{-1})	23.8	107	6 – 22	Bay of Biscay and Celtic Sea
Palomera *et al.* (2005)	0.45 – 0.57 * (0.15 – 0.19 mm d^{-1})	–	71	0.25 – 0.91	Mediterranean Sea
Álvarez and Cotano (2005)	0.45 – 0.51 * (0.15 – 0.17 mm d^{-1})	–	–	0.2 – 1.2	Bay of Biscay
Morales-Nin *et al.* (2005)	1.8	–	1	13.5	Rearing conditions
Belcari *et al.* (2006)	1.3 – 1.7	18.3	579	4 – 20	Tyrrhenian Sea
Bjelland and Skiftesvik (2006)	1.8	–	–	–	Rearing conditions
Piñeiro *et al.* (2008)	1.98 ± 0.18 * (0.66 ± 0.06 mm d^{-1})	25.2	103	3 – 25	Galician waters and Cantabrian Sea

*** Data calculated from original study.**

3.7 Mark and recapture

Age determination of hake is accompanied by two main sources of error: accuracy and/or precision (Campana, 2001). Marking (tagging) and recapturing fish is one of the best methods available for validating the periodicity of growth increment formation. Validation studies on age estimation for hake based on tagging were only accomplished very recently because of the fragility of the species and its bathymetric distribution (Lucio *et al.*, 2000; de Pontual *et al.*, 2003; Piñeiro *et al.*, 2007). An early experiment carried out by Belloc (1935) recovered only one individual, with a growth rate of 16.7 cm year^{-1} after eight months at sea. Lucio *et al.* (2000) reported three recaptures from 151 released tagged fish, but only one fish was retrieved after a very short time at liberty (23 d).

Results from tagging experiments (de Pontual *et al.*, 2006) conducted in the Bay of Biscay demonstrated that the somatic growth of the recoveries was double that expected from published von Bertalanffy growth functions (VBGFs) for the species (Figure 3.7.1). The growth rate, when restricted to recoveries that had spent at least one summer and one winter at liberty, was estimated to be 19.82 ± 1.49 cm year^{-1} ($n = 6$). Growth underestimation was related to age overestimation, which was demonstrated by two independent analyses: (i) results based on a blind interpretation of marked otoliths by two experts involved in routine hake age estimation demonstrated that the age estimates were neither accurate nor precise (Figure 3.7.2); (ii) the predicted otolith growth was inconsistent with the observed otolith growth (Figure 3.7.3). Both types of otolith analyses invalidated the international agreed age-estimation method and demonstrate the need for further research (de Pontual *et al.*, 2006).

Results from a tagging experiment conducted off the northwest Iberian Peninsula indicated that hake growth rate, within the total length range studied, is about double that derived by the standard agreed criteria of otolith age estimation (Piñeiro and Saínza, 2003). Results from this work support the fast-growth hypothesis of this species (Figure 3.7.4). The results indicate that European hake would reach a total length of ca. 25, 45, and 60 cm at the end of the first, second, and third years of life, respectively, instead of 20, 29, and 37 cm, as estimated using the standard agreed criteria described by Piñeiro and Saínza (2003).

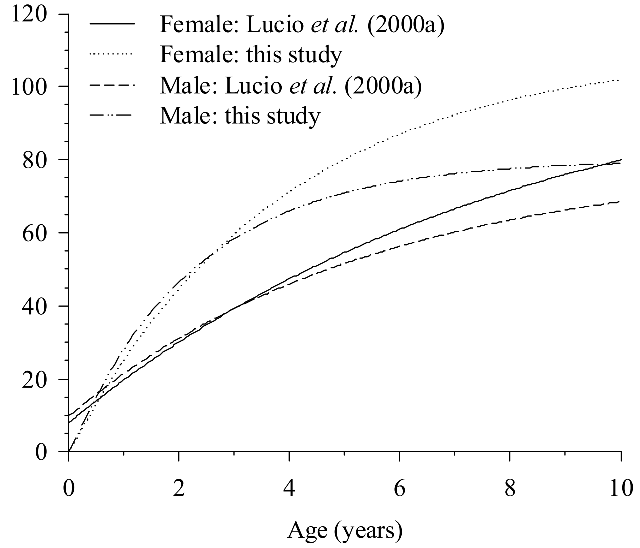

Figure 3.7.1. The von Bertalanffy growth function (VBGF) fitted from recapture data for male (dashed line) and female (solid line) hake and the corresponding VBGF given by Lucio *et al.* (2000); male (dotted and dashed lines), female (dotted line). Fitting was performed using fixed L_∞ values given by Lucio *et al.* (2000): 80 and 110 cm for males and females, respectively (from de Pontual *et al.*, 2006).

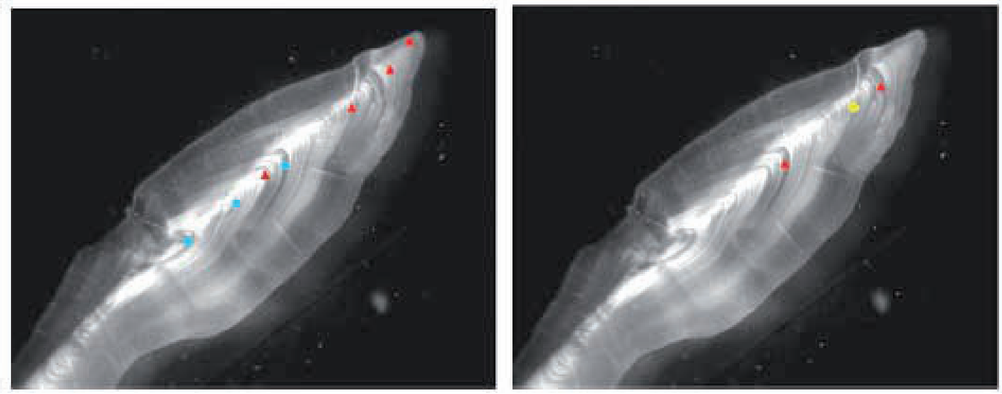

Figure 3.7.2. Transverse section of an oxytetracycline (OTC) marked otolith observed under reflected light. Left: blind interpretation (age 4 + years); right: new interpretation (age 2 + years). Blue indicates the false rings (FR) described by Piñeiro and Saínza (2003); red indicates the winter rings (WR); and yellow indicates the OTC mark. The fish was recaptured 301 d after tagging (TL at capture, 30 cm; TL at recapture, 49 cm; from de Pontual *et al.*, 2006).

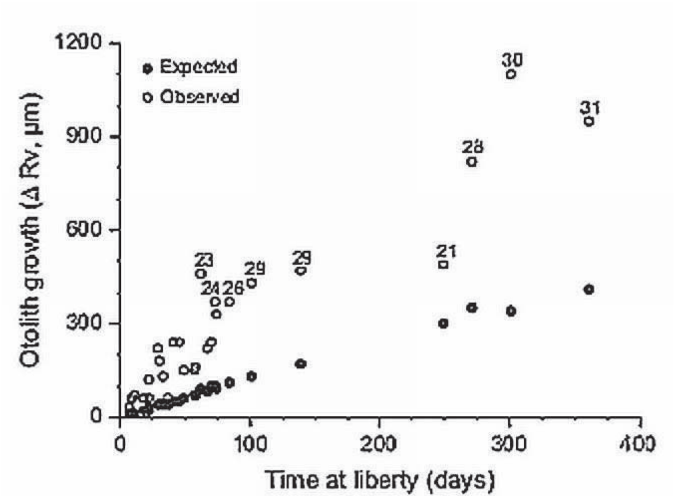

Figure 3.7.3. Comparative analysis of expected and observed otolith growth with respect to time at liberty. Otolith growth (ΔRv) is the distance (in mm) from the oxytetracycline mark to the edge, measured on the ventral radius. Numbers above the points indicate fish lengths (in cm) at tagging (from de Pontual *et al.*, 2006).

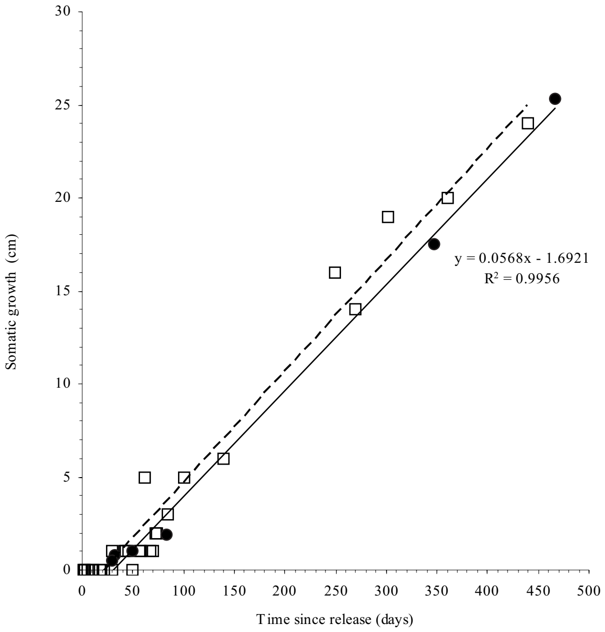

Figure 3.7.4. Somatic growth increment (total length, cm) as a function of time since release. Black dots and solid line, data from southern hake stock tagging experiment (Piñeiro *et al.*, 2007); squares and dashed line, data from northern hake stock tagging experiment (de Pontual *et al.*, 2006) restricted to comparable period (up to 500 d since release; from Piñeiro *et al.*, 2007).

Although earlier tagging studies had very low tag return rates (Belloc, 1935; Lucio *et al.*, 2000), their findings also indicated a much higher growth rate for this species (Table 3.7.1). The striking consistency between the results and somatic growth rates from tag recoveries of both northern and southern hake stocks (Piñeiro *et al.*, 2007) is in accordance with genetic studies, which do not find significant differences between the two stocks (Cimmaruta *et al.*, 2005).

Unfortunately, there are currently insufficient validated data to develop an alternative robust age estimation method for European hake. Direct measures of growth derived from mark–recapture experiments provide invaluable data for the resolution of the ageing problem.

Table 3.7.1. Summary of results of available mark–recapture studies of European hake (NS = northern stock; SS = southern stock; TL = total length; GR = growth rate).

Tagging experiment			Recapture results					
Study	Location Stock	No. released	No. tagged	TL release (cm) (mode)	Days since release	TL recapture (cm)	GR (cm d^{-1} mean ± s.d.)	Recapture rate %
Belloc (1935)	SW Ireland NS	78	1	28.9	255	40.6	0.046	1.3
Lucio *et al.* (2000)	South Bay of Biscay NS	152	1	56	24	60	0.166	1.9
de Pontual *et al.* (2006)	Bay of Biscay NS	1307	36	21–40 (29)	1–1066	24–67	0.038 ± 0.004	3.1
de Pontual *et al.* (2006)	Bay of Biscay NS	–	10 *	21–33	101–1066	34–67	0.052 ± 0.009	–
Piñeiro *et al.* (2007)	NW Iberian Peninsula SS	527	6	29–36 (29)	29–466	31–56	0.032 ± 0.016	1.3
Piñeiro *et al.* (2007)	NW Iberian Peninsula SS	527	2 *	29 – 30	347–466	46.5–56	0.052 ± 0.003	–

* Fish that were > 100 days at sea.

4 Key issues

The key issues concerning hake are representative of the main problems encountered in the assessment of many European stocks. A large number of otolith exchanges and workshops have been held, aimed at improving the consistency and precision of age reading for hake. Usually, the reports of these studies have not been published in refereed journals and have been available only in grey literature. However, much of the work provides useful insights into the problems of age determination in hake and, as a result, some of the key findings and issues raised from these reports are listed below. The report from the most recent workshop (in 2004, summarized in Section 2.2) provides detailed information on the problems encountered.

4.1 Complexity of the otolith growth pattern

The interpretation of otolith growth marks is often a difficult task in which subjectivity increases with the complexity of the structural pattern of the otolith, as is the case for hake (Courbin *et al*., 2007). Improved understanding of the biological meaning of growth marks and the development of a quantitative framework aimed at defining an objective procedure for otolith interpretation are complementary solutions to reducing interpretation subjectivity, and are especially needed for complex species (Courbin *et al*., 2007).

Macrostructures visible on otoliths may have different origins. The identification of these macrostructures and the establishment of the link between the macrostructure and an event in the life cycle represent a fundamental step in accurate age estimation. Hake otolith interpretation is complex because of the presence of many macrostructures. There are many thin translucent zones (TTZs) that probably correspond to short environmental and/or physiological events, and the difficulty in interpreting such otoliths often increases with the size of the fish (Courbin *et al*., 2007).

Goñi and Piñeiro (1988) found an average number of 11 translucent rings laid down in hake otolith sections of individuals within the length range 32–38 cm. If it is assumed that fish of that size were 3–5 years old (according to the standard age-reading criteria described by Piñeiro and Saínza, 2003), this would mean six to eight false rings in addition to the true annual rings.

The complexity of hake otolith macrostructure and growth variability is believed to be related to the long spawning season (Casey and Pereiro, 1995; Anon., 1998; Domínguez-Petit, 2007). The almost continuous recruitment throughout the year, as a result of multiple spawning, causes variable ring patterns. The sexual dimorphism in growth rates and different ring patterns, depending on the hatching time and geographical area, render otolith interpretation more difficult and contribute to the discrepancies that exist between different otolith readers. Piñeiro (2000) noted that the major difficulties in otolith age estimation were (i) the location of the first annual ring (first *annulus*), (ii) the classification of the rings as annual or false, and (iii) the interpretation of the edge. The identification of the first annual ring is problematic because of the presence of several checks associated with juvenile growth (Table 4.1.1).

Piñeiro and Saínza (2003) observed that these difficulties could be related to some generally well-marked false rings that very frequently occur before, during, or after the first *annulus* deposition. This makes it very difficult to identify the first annual ring and constitutes the most important source of uncertainty in hake otolith age reading. The wide range of length-at-age estimates given by different authors for European hake in 2003 was also an issue.

Casey and Pereiro (1995) held similar views and concluded that there is a large number of different descriptions of European hake growth, depending on the area of capture and on the interpretation of the rings on the otoliths. However, it was impossible at that time to determine whether the reported differences were real or the result of difficulties in age determination.

Fariña and Abaunza (1991) considered that the growth interruptions marked on hake otoliths could be related to depth stratification in relation to size and age. The predatory and reproductive behaviours of hake result in seasonal movements through habitats with different environmental conditions (Fariña and Abaunza, 1991).

Table 4.1.1. False ring distances (backcalculated) identified in hake otoliths before the first annual ring in different studies.

STUDY	GEOGRAPHICAL AREA	FALSE RING DISTANCES (CM)
Descamps and Labastie (1978)	Bay of Biscay	7 and 12
Iglesias and Dery (1981)	Galician waters and Cantabrian Sea	Three rings (<12)
Goñi (1983)	NW African Shelf	2, 4, 7, and 10
Goñi and Piñeiro (1988)	Galician waters and Cantabrian Sea	4, 7, 9, and 12
Piñeiro and Pereiro (1993)	Galician waters and Cantabrian Sea	Hyaline band (7–10)

Courbin *et al.*, (2007) investigated the macrostructure pattern of hake otoliths from the Mediterranean Sea. Unsupervised statistical analysis of the distribution of the characteristics of these macrostructures led to the definition of three main macrostructure types: (i) a wide translucent zone close to the nucleus (WTZC), (ii) a wide translucent zone distant from the nucleus (WTZD), and (iii) a thin translucent zone or check (TTZ), associated with a large variation in distance to the nucleus. The TZZ type is therefore probably associated mainly with specific fish responses to environmental or endogenous factors. In contrast, the spatial extent of the WTZC and WTZD types are more clearly defined, suggesting that they are relevant at a population level, for instance in response to seasonal variations or major ontogenetic events.

The interpretation of the otolith growth pattern will be a fundamental step towards the establishment of validated age-reading criteria.

4.2 Lack of validated ageing criteria

Age validation is the confirmation of the temporal meaning of the growth structure used for age determination. However, it is not easy because the ageing has to be validated for the entire age range of the population and for spawning stocks in different geographic areas. Different techniques can be used for validation (Morales-Nin, 1997). Attempts were initially made using mark–recapture methods (Belloc, 1935; Fritz, 1959) for the study of European hake growth, but most researchers have relied on the interpretation of rings in bony structures (otoliths) and on analyses of length–frequency distributions, such as modal progression analysis and the separation of modal components (Gulland and Rosenberg, 1992). Although several techniques for making hake otoliths easier to examine have been employed (Christensen, 1964; Albrechtsen, 1968; Nichy, 1969), all researchers have encountered difficulties reaching a consistent interpretation of the pattern of growth ring (annual growth zone) formation on otoliths of this species.

The 1999 workshop permitted the establishment of age-reading criteria (Piñeiro and Saínza, 2003). The decrease in age-reading agreement that occurred at the last workshop, although detrimental, is obviously related to the use of hake estimation criteria that have not been validated. The use of validated criteria will encourage better results because it will reduce the subjectivity inherent in age reading, increase the confidence of readers in assigning a given age, and facilitate the identification of macrostructures in the otoliths that could easily be related to life-history events. The lack of knowledge of hake otolith growth patterns (see Section 4.1) makes it impossible to establish reliable ageing criteria.

4.3 Alternative methods

A variety of methods or approaches has been used in growth studies. The analyses of hake catch length distributions have been applied to hake age distribution (see Section 3.3). However, results of this methodology are difficult to interpret owing to the difficulty in splitting overlapping normal distributions or establishing the correspondence between normal distribution and year classes. Even where these analyses are possible, they are limited to the first age class or to the few age classes that make up the bulk of the landings.

Prior to 1997, the ICES Working Group for the Assessment of Southern Shelf Demersal Stocks (WGSSDS) – previously in charge of hake stock assessments – applied numerical methods (Kimura and Chikuni, 1987) to the annual length composition of catches in order to obtain a catch-at-age matrix for the assessment of the southern hake stock (ICES, 1993, 1994, 1995). However, the WGSSDS considered the use of ALKs to be more reliable for obtaining catch-at-age data (Piñeiro *et al.*, 2000). The application of length–frequency analysis is problematic because of the hake's extended spawning period. More than one mode may relate to the same year class, and the correct identification of length mode is subjective. Additional information can be achieved by age validation methods, including both direct and indirect approaches, in order to reduce growth and age uncertainties.

The mark–recapture of wild fish is one of the best methodologies to facilitate age-reading validation if the otoliths are chemically marked (with OTC) when the tagged fish are released. This approach, used successfully with hake, is very expensive because a large number of fish must be marked in order to obtain sufficient returns. Success is highly dependent on the recapture rate, which is often very low. The major difficulty is related to the tagging of small hake because of the high mortality rate that occurs during early life stages (Piñeiro *et al.*, 2007). For this reason, alternative approaches, such as otolith microstructure analysis, can be very useful in confirming growth in the first year of life.

Daily growth increment analysis is also a very important tool for the establishment of a criterion for the identification of the first annual ring. Furthermore, this analysis provides a better understanding of the typology of hake otolith macrostructure in relation to the biological meaning and mechanisms (endogenous and environmental) that control incremental deposition. The approach of comparing the structural pattern at micro- and macroscopic scales provides a new framework for the development of an alternative method of age estimation for this species.

Chemical-composition analysis of otolith growth marks may provide information about fundamental differences between opaque and translucent bands that will help to explain the processes governing their formation and, therefore, aid in their interpretation (Morales-Nin *et al.*, 2005; Tomás *et al.*, 2006). An electron probe study, based on calcium, strontium, and sodium, of some European hake populations has revealed relative changes in composition associated with opaque and translucent bands in their otoliths (Tomás *et al.*, 2006).

A combination of all methodologies, the description of otolith patterns, length–frequency analysis, daily growth increment analysis, and mark–recapture results is necessary to establish validated criteria for reliable age reading of hake otoliths. The application of each individual methodology in isolation, which has been the case until now, has not permitted the necessary advances in hake age reading.

4.4 Fast growth vs. slow growth

The debate about whether hake is a fast- or slow-growing species has continued since the 1930s (Hickling, 1933; Belloc, 1935). Results of recent mark–recapture studies demonstrate that growth is underestimated as a consequence of age overestimation (de Pontual *et al.*, 2006). Underestimation of growth during the first year of life has also been recently demonstrated, based on daily growth increments (Arneri and Morales-Nin, 2000; Morales-Nin and Moranta, 2004; Kacher and Amara, 2005; Belcari *et al.*, 2006; Piñeiro *et al.*, 2007). Bioenergetics studies also supported an underestimation of growth rate (Riis-Vestergaard *et al.*, 2000; see Section 3). The number of wide translucent zones (WTZ) detected by Courbin *et al.* (2007) were more in line with a fast-growth rather than a slow-growth hypothesis.

Scientists who provided age data for stock assessment purposes assumed a slow-growth hypothesis that is inherent to the standard criteria, which were agreed following the results of several workshops. However, complementary and recent studies indicate that the slow-growth hypothesis is less likely to be valid. Bearing in mind that the age criteria used are not validated and that the fast-growth hypothesis is consistent with mark–recapture results, a reliable and non-subjective methodology that allows accurate results is necessary. It is clear that conventional otolith age estimation needs to change in order to be consistent with the fast-growth hypothesis. According to the fast-growth hypothesis, hake at the end of the first, second, and third years of life would reach a TL of 25, 45, and 60 cm respectively, instead of 20, 29, and 37 cm (Piñeiro *et al.*, 2007), as estimated from otoliths using the internationally agreed ageing method (Piñeiro and Saínza, 2003).

5 Future work

5.1 Exchange programme

The objective of otolith exchanges is to estimate precision and relative/absolute bias in the age readings made by readers from different age-reading laboratories in order to determine whether or not these parameters are still within acceptable levels (ICES, 2008b). In this case, a new otolith exchange has been scheduled for 2009 to identify the nature of the translucent rings that contributed to the decrease in the precision of age estimations between the last two exchanges (2001 and 2003, where the CV increased from 25 to 48%) for fish of the same length range. Taking into account the known discrepancy between the standard ageing criteria and the results of mark–recapture experiments, the next exchange needs not only to assess the precision of age estimation but also to start developing new validated age-reading criteria. This can be achieved by using otoliths collected from both tagging material and previous workshop collections; it is essential to compile a collection of currently recognized known-age material that can be used for validation. The position of known annual translucent rings on otoliths from marked and recaptured fish can be compared with translucent rings identified by individual readers as annual rings on raster layers of images of the otoliths.

5.2 Otolith biometrics

The purpose of inferring age from the correlation between the age of a fish and otolith biometrics, such as otolith weight or two-dimensional otolith shape, is to estimate the proportions-at-age within a fish population rather than to assign ages to individual fish (FAbOSa, 2002; Francis and Campana, 2004). The development of an otolith growth model is hampered by the lack of validated material (mark–recapture or other data on known age); this prevents not only validation, but also the development of statistical methods to update historical data (ICES, 2006).

Recent advances in image acquisition and the development of image analysis techniques for fish otoliths offer new possibilities for the automation of aspects of image processing that can improve the objectivity of reader interpretation within a quality-control framework.

5.3 Otolith macrostructure typology

The low levels of precision in the age interpretation of older hake otoliths are partly the result of the complexity of the otolith macrostructure. Problems in age interpretation can only be resolved by establishing descriptors for the key features within the otolith macrostructure and developing a better understanding of their biological significance and the mechanisms that control the deposition of the growth rings. Courbin *et al.* (2007) have developed a methodological framework that, when applied to a set of Mediterranean hake otoliths, discriminates between two types of macrostructure that are clearly not random and are meaningful at a population or group level. However, the authors note that, although this proposed measure of similarity of otolith patterns can be applied to the patterns interpreted by experts with a view to quantifying interpretation differences, it would rely on a predetermined macrostructure typology agreed among experts.

5.4 Experiments in a controlled environment

Laboratory experiments to confirm the increment formation of known-age or chemically marked fish are common (Geffen, 1992). However, it is seldom possible to mimic in the laboratory natural conditions, such as photoperiodicity, temperature and feeding cycles, and vertical migrations. Nevertheless, it can be helpful to know when important events, such as growth and behaviour, occur at different stages in otolith growth pattern formation. This information can only be obtained from rearing experiments. Semi-intensive culture of the eggs of European hake has allowed descriptions to be made of larval development, growth during early life stages, and the influence of temperature on development and survival (Morales-Nin *et al.*, 2005; Bjelland and Skiftesvik, 2006). Studies on the correlation between otolith micro- and macrostructure and feeding, temperature, and fish growth require empirical observations in laboratory experiments. Recently, Martínez de Murguía *et al.* (2005) demonstrated that, with regular feeding, it is possible to keep adult hake in captivity for more than a year. This experiment opens the possibility of future research for studying the optical signal of otolith structures and other issues concerning their biology under controlled conditions.

5.5 Developing a new protocol for hake age estimation

The otolith exchange in 2009 will be followed by a Workshop on Age Estimation of European Hake (WKAEH), which will take place at IEO in Vigo, Spain, in October/November of the same year. Identification of the problematic translucent zones and a better understanding of the features that make them difficult to interpret are essential steps in the development of an improved protocol that can increase the precision of hake age estimation for stock assessments. A comparison of the position of known annual rings on otoliths from marked and recaptured fish, and the identification of these rings by experienced otolith readers during the exchange, will be a key topic of the workshop. Otolith microstructure analysis and observed growth rates from tagging experiments will be used to inform discussions on the improvement of the criteria required to distinguish annual growth rings from other rings, as will the results of recent research. The presence of stock assessment experts at this workshop, and their interaction with age readers, will help lead to the provision of age data that can be used reliably for stock assessments.

5.6 Summary of recommendations for future work

It is clear from the difficulties previously described that a great deal of work still needs to be done to develop stable and acceptable levels of precision for European hake age estimation. Recommendations include:

- an exchange programme (2009), using a set of otoliths (images) collected from both tagging material and previous workshop collections, for the purpose of intercalibration between age readers involved in stock assessment
- a workshop in 2009 to analyse and discuss the results of the exchange programme between age-reading laboratories
- a meeting between international experts on growth and age estimation and scientists involved in stock assessments, aimed at producing age data that can be reliably used for stock assessments

- provision of supplementary data in order to perform statistical approaches to estimating age distributions, including:
    - otolith biometrics and weight
    - historical data from length–frequency distribution from surveys and commercial catches
- continued collection of data and otolith for future work
- a large-scale tagging experiment covering both northern and southern stocks
- continuation of daily growth studies in order to establish seasonal growth variations and to locate the first annual ring by area and stock
- establishment of a typology of otolith macrostructure and an understanding of its biological meaning, as well as an understanding of the mechanisms (environmental and endogenous) that control otolith ring (growth zone) deposition
- development of a refined model of otolith formation, including growth patterns and otolith shape
- production of new validated standard criteria for age interpretation that take all the above items into account

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6 References

Albrechtsen, K. 1968. A dyeing technique for otolith age reading. Journal du Conseil International pour l'Exploration de la Mer, 32: 278–280.

Alcázar, J., Carrasco, F., Llera, E., Menéndez, M., Ortea, J., and Vizcaíno, A. 1983. Recursos Pesqueros de Asturias. 3: Biología, Dinámica y Pesca de la Merluza en Asturias. Consejería de Agricultura y Pesca del Principado de Asturias, Gijón, Spain. 135 pp.

Aldebert, Y., and Recasens, L. 1995. Estimation de la croissance de merlu dans le Golfe de Lion par l´analyse des frequences de tailles. CIHEAM, Cahiers Options Méditerranéennes, Dynamique des Populations Marines, 10: 49–50.

Alemany, F., and Oliver, P. 1995. Growth of female hake in the Balearic Sea: a proposal of a new growth model with higher growth rates. Rapports et Procès-Verbaux des Réunions – Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée, 34: 236.

Alemany, F., Oliver, P., and Reñones, O. 1993. Growth of hake of Balearic Islands stock. *In* Etùde pour l'aménagement et la gestion des pêches en Méditerranée occidentale. Rapport final. CEE FAR. 474 pp.

Álvarez, P., and Cotano, U. 2005. Growth, mortality and hatch-date distributions of European hake larvae, *Merluccius merluccius* (L.), in the Bay of Biscay. Fisheries Research, 76: 379–391.

Álvarez, P., Fives, J., Motos, L., and Santos, M. 2004. Distribution and abundance of European hake *Merluccius merluccius* (L.), eggs and larvae in the North East Atlantic waters in 1995 and 1998 in relation to hydrographic conditions. Journal of Plankton Research, 26: 811–816.

Anon. 1998. Biological Studies of Demersal Fish to European Commission. Annex III of the Final Report to the Commission of European Communities (BIOSDEF, EU Study Contract 95/038). 522 pp.

Anon. 2000. Report of New Assessment and Biology of the Main Commercial Fish Species: Hake and Anglerfish of the Southern Shelf Demersal Stocks in the South Western Europe. Appendix I of the Final Report to the Commission of European Communities (DEMASSESS, EU Study Contract 97/015). 497 pp.

Arneri, E., and Morales-Nin, B. 2000. Aspects of the early life history of European hake from the central Adriatic. Journal of Fish Biology, 56: 1368–1380.

Bagenal, T. B. 1954. Growth rate of the hake (*Merluccius merluccius* L.) in the Clyde and other Scottish areas. Journal of the Marine Biology Association of the UK, 33: 69–95.

Beamish, R. J., and Fournier, D. A. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences, 38: 982–983.

Belcari, P., Ligas, A., and Viva, C. 2006. Age determination and growth of juveniles of the European hake (*Merluccius merluccius* L., 1758). Fisheries Research, 78: 211–217.

Belloc, G. 1929. Étude monographique du merlu. Revue des Travaux de l'Office des Pêches Maritimes, Revue Trimestrielle, Tome II, Fasc. II (Paris). 67 pp.

Belloc, G. 1935. Etude monographique du merlu *Merluccius merluccius,* 3ème partie. Revue des Travaux de l'Office des Pêches Maritimes, 8: 145–202.

Bertignac, M., and de Pontual, H. 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES Journal of Marine Science, 64: 981–988.

Bhattacharya, C. G. 1967. A simple method of resolution of a distribution into Gaussian components. Biometrics, 23: 115–135.

Birtwistle, W., and Lewis, H. M. 1925. Hake investigations. Reports of the Lancashire Sea Fisheries Laboratory, 1925: 36–56.

Bjelland, R. M., and Skiftesvik, A. B. 2006. Larval development in European hake (*Merluccius merluccius* L.) reared in a semi-intensive culture system. Aquaculture Research, 37: 1117–1129.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

Casey, J., and Pereiro, F. J. 1995. European hake (*M. merluccius*) in the North-East Atlantic. *In* Hake: Fisheries, Ecology and Markets. Fish and Fisheries Series 15, pp. 125–147. Ed. by J. Alheit and T. J. Pitcher. Chapman and Hall, London. 508 pp.

Chang, W. Y. B. 1982. A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries and Aquatic Sciences, 39: 1208–1210.

Christensen, J. M. 1964. Burning of otoliths, a technique for age determination of soles and other fish. Journal du Conseil Permanent International pour l'Exploration de la Mer, 29: 73–81.

Cimmaruta, R., Bondanelli, P., and Nascetti, G. 2005. Genetic structure and environmental heterogeneity in the European hake (*Merluccius merluccius*). Molecular Ecology, 14: 2577–2591.

Courbin, N., Fablet, R., Mellon, C., and de Pontual, H. 2007. Are hake otolith macrostructures randomly deposited? Insights from an unsupervised statistical and quantitative approach applied to Mediterranean hake otoliths. ICES Journal of Marine Science, 64: 1191–1201.

de Pontual, H., Bertignac, M., Battaglia, A., Bavouxet, G., Moguedet, P., and Groison, A. L. 2003. A pilot tagging experiment on European hake (*Merluccius merluccius*): methodology and preliminary results. ICES Journal of Marine Science, 60: 1318–1327.

de Pontual, H., Groison, A. L., Piñeiro, C., and Bertignac, M. 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES Journal of Marine Science, 63: 1674–1681.

Descamps, P., and Labastie, J. 1978. Note sur la lecture et l'interpretation des otolithes du merlu. ICES Document CM 1978/G:41. 21 pp.

Domínguez-Petit, R. 2007. Study of reproductive potential of *Merluccius merluccius* in the Galician Shelf. PhD thesis, University of Vigo. 253 pp.

Eltink, A. T. G. W. 1997. Horse mackerel otolith exchange in 1996. ICES Document CM 1997/H:24. 30 pp.

Eltink, A. T. G. W., Newton, A. W., Morgado, C., Santamaria, M. T. G., and Modin, J. 2000. Guidelines and tools for age reading comparisons, European Fish Ageing Network (EFAN), EFAN Report 3/2000. Available online at http://www.efan.no/NewsAndReports/EFANReport32000.htm.

FAbOSa. 2002. Final Report to the European Commission. EU Contract no. FAIR CT97 3402 for the FAbOSa (Fish Ageing by Otolith Shape Analysis) Project.

FAO. 1982. Report of the technical consultation on methodologies used for fish age-reading. FAO Fisheries Report 257. 109 pp.

Fariña, A. C., and Abaunza, P. 1991. Contribucion al estudio de los juveniles de merluza entre Cabo Villano y Cabo Prior (NW Galicia) mediante prospecciones pesqueras. Boletín del Instituto Español Oceanografía, 7: 155–163.

Fives, J. M., Acevedo, S., Lloves, M., Whitaker, A., Robinson, M., and King, P. A. 2001. The distribution and abundance of larval mackerel, *Scomber scombrus* L., horse mackerel, *Trachurus trachurus* L., hake *Merluccius merluccius* L., blue whiting *Micromesistus poutassou* (Risso,

1826) in the Celtic Sea and west of Ireland during the years 1986, 1989 and 1992. Fisheries Research, 50: 17–26.

Francis, R. I. C. C., and Campana, S. E. 2004. Inferring age from otolith measurements: a review and a new approach. Canadian Journal of Fisheries and Aquatic Sciences, 61: 1269–1284.

Fritz, R. L. 1959. Hake tagging in Europe and the United States, 1931–1958. Journal du Conseil Permanent International pour l'Exploration de la Mer, 24: 480–485.

García-Rodríguez, M., and Esteban, A. 1995. Algunos aspectos sobre la bilogía y pesca de la merluza mediterranea (*Merluccius merluccius* L.) en la Bahia de Santa Pola (Sureste de la Peninsula Iberica). Boletín. Instituto Español de Oceanografía, 11: 3–25.

García-Rodríguez, M., and Esteban, A. 2002. How fast does hake grow? A study on the Mediterranean hake (*Merluccius merluccius L.*) comparing whole otoliths readings and length frequency distributions data. Scientia Marina, 66: 145–156.

Geffen, A. J. 1992. Validation of otolith increment deposition rate. *In* Otolith Microstructure Examination and Analysis, pp. 101–113. Ed. by D. K. Stevenson, and S. E. Campana. Canadian Special Publication of Fisheries and Aquatic Sciences, 117. 126 pp.

Godinho, M. L., Afonso, M. H., and Morgado, C. 2001. Age and growth of hake *Merluccius merluccius* Linnaeus, 1758 from the Northeast Atlantic (ICES Division IXa). Boletín Instituto Español de Oceanografía, 17: 255–262.

Goñi, R. 1983. Growth studies of European hake (*Merluccius merluccius* L.) from the northwest African shelf. ICES Document CM 1983/G:10. 3 pp.

Goñi, R., and Piñeiro, C. 1988. Study of the growth pattern of European hake (*Merluccius merluccius* L.) from the southern stock: ICES Divisions VIIIc and IXa. ICES Document CM 1988/G:18. 13 pp.

Guichet, R. 1988. Étude de la croissance du merlu Européen (*Merluccius merluccius* L.) au cours de ses premières années. ICES Document CM 1988/G:53. 27 pp.

Guichet, R., Quero, J. C., and Labastie, J. 1973. Estimation de la composition du stock du merlu au nord et à l'ouest de l'Irlande. ICES Document CM 1973/G:5. 9 pp.

Guichet, R., Quero, J. C., and Labastie, J. 1974. Composition du stock du merlu au nord et a l'ouest de l'Irlande (deuxiéme note). ICES Document CM 1974/G:8. 5 pp.

Gulland, J., and Rosenberg, A. 1992. A review of length-based approaches to assessing fish stocks. FAO Technical Report 323. 100 pp.

Hickling, C. F. 1933. The natural history of the hake. Part 3. Seasonal changes in the condition of hake. UK Fishery Investigations Series II, 12(1). 120 pp.

ICES. 1979. Report of the Working Group on Assessment of the Hake Stocks. 28–31 May 1979, Charlottenlund. ICES Document CM 1979/G:27. 65 pp.

ICES. 1983. Confrontation Technique sur les Methodologies Utilisées pour la Lecture des Otolithes de Merlu. *In* Report of the Working Group on Assessment of Hake Stocks, pp. 49-55. 29 June–6 July 1983, Copenhagen. ICES Document CM 1983/Assess:20. 55 pp.

ICES. 1984. Report of the *ad hoc* Hake Otolith Working Group, 28 May–1 June 1984, Lowestoft. ICES Document CM 1984/G:74. 19 pp.

ICES. 1986. Report of the *ad hoc* Study Group on Hake, 3–6 June 1986, Lowestoft. ICES Document CM 1986/G:93. 19 pp.

ICES. 1991. Report of the Working Group on the Assessment of the Stocks of Hake. 29 April–8 May 1991. ICES Document CM 1991/Assess:20. 25 pp.

ICES. 1993. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks, Copenhagen, 8–17 September 1992. ICES Document CM 1993/Assess:3. 181 pp.

ICES. 1994a. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks, Copenhagen, 6–15 September 1993. ICES Document CM 1994/Assess:3. 92 pp.

ICES. 1994b. Report of the Workshop on Sampling Strategies for Age and Maturity, Copenhagen, 3–9 February 1994. ICES Document CM 1994/D:1. 67 pp.

ICES. 1995. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks, Copenhagen, 31 August–6 September 1994. ICES Document CM 1995/Assess:6. 598 pp.

ICES. 2000. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks, Copenhagen, 14–23 September 1999. ICES Document CM 2000/ACFM:4. 744 pp.

ICES. 2003. Report of the Working Group on the Assessment of Southern Stock of Hake, Monk, and Megrim (WGHMM), Lisbon, Portugal, 21–30 May 2002. ICES Document CM 2003/ACFM:01. 499 pp.

ICES. 2006. Report of the Study Group on Ageing Issues of Baltic Cod (SGABC), 16–19 May 2006, Gdynia, Poland. ICES Document CM 2006/BCC:08. 45 pp.

ICES. 2007a. Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk, and Megrim (WGHMM), 8–17 May 2007, Vigo, Spain. ICES Document CM 2007/ACFM:21. 700 pp.

ICES. 2007b. Report of the ICES Advisory Committee on Fisheries Management, Advisory Committee on the Marine Environmental and Advisory Committee on Ecosystems, 2007. Book 7: Bay of Biscay and Western Iberia. ICES Advice. 94 pp.

ICES. 2008a. Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk, and Megrim (WGHMM), 30 April– 6 May, Copenhagen. ICES Document CM 2008/ACOM:07. 622 pp.

ICES. 2008b. Report of the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS), 3–7 March 2008, Cyprus, pp. 70–75. ICES Document CM 2008/ACOM:29. 91 pp.

Iglesias, S., and Dery, L. 1981. Age and growth studies of hake (*Merluccius merluccius,* L.) from ICES Divisions VIIIc and IXa. ICES Document CM 1981/G:38. 6 pp.

Kacher, M., and Amara, R. 2005. Distribution and growth of 0-group European hake in the Bay of Biscay and Celtic Sea: a spatial and interannual analysis. Fisheries Research, 71: 373–378.

Kimura, D. K., and Chikuni, S. 1987. Mixtures of the empirical distributions: an iterative application of the age–length key. Biometrics, 13: 23–35.

Lannin, A. 2006. The biology, dynamics and fisheries for hake (*Merluccius merluccius*) in the waters around Ireland. PhD thesis, University College Cork. 182 pp.

Lucio, P., Murúa, H., and Santurtún, M. 2000. Growth and reproduction of hake (*Merluccius merluccius*) in the Bay of Biscay during the period 1996–1997. Ozeanografika, 3: 325–354.

MacDonald, P., and Pitcher, T. 1979. Age groups from size frequency data: a versatile and efficient method of analysing distribution mixtures. Journal of the Fisheries Research Board of Canada, 36: 987–1001.

Martínez de Murguía, A., Leé Herrero, D., Gilisagasti, J., Sanchez, S., Casares, P., and Lucio, P. 2005. First attempt at keeping *Merluccius merluccius* European hake in closed tanks. *In* Proceedings of the 21st Meeting of the European Union of Aquarium Curators (EUAC), Vienna, Austria. Bulletin de l'Institut Océanographique, Monaco, 77: 133–139.

Meriel-Bussy, M. 1966. La croissance du merlu dans le golfe de Gascogne. ICES Document CM 1966/G:17. 5 pp.

Morales-Nin, B. 1997. Review on the European hake age determination and validation problems. European Fish Ageing Network, EFAN Report, 1/1997.

Morales-Nin, B., and Aldebert, Y. 1997. Growth of juvenile *Merluccius merluccius* in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length–frequency analysis. Fisheries Research, 30: 77–85.

Morales-Nin, B., and Moranta, J. 2004. Recruitment and post-settlement growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. Scientia Marina, 63: 399–409.

Morales-Nin, B., Torres, G. J., Lombarte, A., and Recasens, L. 1998. Otolith growth and age estimation in the European hake. Journal of Fish Biology, 53: 1155–1168.

Morales-Nin, B., Bjelland, R. M., and Moksness, E. 2005. Otolith microstructure of a hatchery reared European hake (*Merluccius merluccius*). Fisheries Research, 74, 300–305.

Murúa, H. 2006. Reproductive fundamentals for the estimation of egg production of the European hake, *Merluccius Merluccius*, in the Bay of Biscay. PhD thesis, University of Basque Country (Spain). 158 pp.

Murúa, H., and Motos, L. 2006. Reproductive strategy and spawning activity of the European hake (*Merluccius merluccius* L.) in the Bay of Biscay. Journal of Fish Biology, 69: 1288–1303.

Murúa, H., Motos, L., and Marrale, D. 1996. Reproductive modality and batch fecundity of the European hake, *Merluccius merluccius*. ICES Document CM 1996/G: 40. 28 pp.

Murúa, H., Lucio, P., Santurtún, M., and Motos, L. 2006. Seasonal variation in egg production and batch fecundity of European hake *Merluccius merluccius* (L.) in the Bay of Biscay. Journal of Fish Biology, 69: 1304–1316.

Mytilineou, Ch., and Vassilopoulou, V. 1988. The reproductive cycle and sex ratio of hake, *Meluccius merluccius*, in Patraikos and Korinthiakos gulfs and the Ionian Sea. Proceedings of the 4th Hellenic Congress of Ichthyologists, 4: 164–177.

Nichy, F. E. 1969. Growth patterns on otoliths from young silver hake, *Merluccius bilinearis* (Mitch). International Commission for the Northwest Atlantic Fisheries Research Bulletin, 6: 107–117.

Oliver, P. 1991. Dinámica de la población de Merluza (*Merluccius merluccius*, L.) de Mallorca. Reclutamiento, crecimiento y mortalidad. PhD thesis, Universitat de les Illes Baleares. 392 pp.

Oliver, P., Alvarez, F., and Morales-Nin, B. 1989. Report of the age-reading workshops on Mediterranean European hake and sardine, 10–15 April 1989, Palma de Mallorca, Spain. FAO Rapport sur les Pêches, 447, 79–86.

Orsi-Relini, L., Cappanera, M., and Fiorentino, F. 1989. Spatial-temporal distribution and growth of *Merluccius merluccius* recruits in the Ligurian Sea: observations on the 0 group. Cybium, 13: 263–270.

Palomera, I., Olivar, M. P., and Morales-Nin, B. 2005. Larval development and growth of the European hake *Merluccius merluccius* in the northwestern Mediterranean. Scientia Marina, 69: 251–258.

Pannela, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science, 173: 1124–1127.

Pérez, N., and Pereiro, F. J. 1985. Aspectos de la reproducción de la merluza (*Merluccius merluccius*) de la plataforma gallega y cantábrica. Boletín del Instituto Español Oceanografía, 2: 39–47.

Pérez, N., Pereda, P., Uriarte, A., Trujillo, V., Olaso, S., and Lens, S. 1996. Descartes de la flota española en el área del ICES. Datos y Resúmenes Instituto Español de Oceanografía, 2. 142 pp.

Petersen, C. G. 1891. Eine Methode zur Bestimmung des Alters und Wuchses der Fische. Mitteilungen. Deutscher Seefisherei-Verein, 11: 226–235.

Piñeiro, C. 2000. Report on workshop on hake otolith age reading, June 1997, Vigo. European Fish Ageing Network, EFAN Report, 6/2000.

Piñeiro, C., and Hunt, J. J. 1989. Comparative study on growth of European hake (*Merluccius merluccius* L.) from southern stock using whole and sectioned otoliths, and length frequency distributions. ICES Document CM 1989/G:37. 16 pp.

Piñeiro, C., and Meixide, M. 1995. Analysis of results obtained in the Spanish-French meeting about otolith age reading of European hake. *In* Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks, Copenhagen, 6–15 September 1994. ICES Document CM 1995/Assess:6. 598 pp.

Piñeiro, C. G., and Pereiro, J. A. 1993. Study of the growth pattern of juvenile European hake (*Merluccius merluccius* L.) using the whole otoliths and length frequency from commercial catches and groundfish surveys. ICES Document CM 1993/G:12. 14 pp.

Piñeiro, C., and Saínza, M. 2002. Report of 3rd exchange on European hake age readings. Annex of the Final Report of EU Contract 99/009, Improving Sampling of Western and Southern European Atlantic Fisheries – SAMFISH.

Piñeiro, C., and Saínza, M. 2003. Age estimation, growth and maturity of the European hake, *Merluccius merluccius* (Linnaeus, 1758) from Iberian Atlantic waters. ICES Journal of Marine Science, 60: 1086–1102.

Piñeiro, C. G., Fariña, A. C., and Abaunza, P. 1992. Resultados de las campañas realizadas en las zonas de veda al arrastre en la plataforma continental Gallega (NW España), 1989–1990. Informes Técnicos Instituto Español Oceanografía, 130. 39 pp.

Piñeiro, C., Afonso, M. H., Arego, S., Bellail, R., Labastie, J., Loureiro, I., Lucio, P., *et al.* 2000. Report of 2nd international workshop on hake otolith age reading, February 1999, Vigo, Spain. European Fish Ageing Network, EFAN Report, 7/2000.

Piñeiro, C., Saínza, M., Morgado, C., de Pontual, H., Hoey, S., Beattie, S., Lucio, P., *et al.* 2004. Report of 4th Exchange on European Hake Age Readings, 16–20 October 2004, Vigo, Spain. ICES Document CM 2005/ACFM:02. 18 pp.

Piñeiro, C., Rey, J., de Pontual, H., and Goñi, R. 2007. Tag and recapture of European hake (*Merluccius merluccius* L.) off the northwest Iberian Peninsula: first results support fast-growth hypothesis. Fisheries Research, 88: 150–154.

Piñeiro, C., Rey, J., de Pontual, H., and García, A. 2008. Growth of Northwest Iberian juvenile hake estimated by combining sagittal and transversal otolith microstructure analyses. Fisheries Research, 93: 173–178.

Recasens, L. 1992. Dinámica de poblaciones i pesqueria del lluc (*Merluccius merluccius*) al Golfo de Lleon i la mar Catalana. PhD thesis, Universitat de Barcelona. 398 pp.

Riis-Vestergaard, J., Velasco, F., Hill, L., and Olaso, I. 2000. Food consumption of European hake (*Merluccius merluccius*) estimated by application of a bioenergetics model: is the growth of hake underestimated? ICES Document CM 2000/Q:12. 12 pp.

Robles, R., Pereiro, J. A., Iglesias, S., and Pereiro, F. J. 1975. Étude sur la croissance du merlu Européen, *Merluccius merluccius* L. du NW de L'Espagne. ICES Document CM 1975/G:8. 17 pp.

Silva, L., Vila, Y., Acosta, J. J., and Tornero, J. 2007. Size at first maturity in European hake: estimates based on different maturity criteria. Working Document (WD10) presented to the Workshop on Sexual Maturity Staging of Hake and Monk (WKMSHM), 21–24 November 2007, Lisbon, Portugal. ICES Document CM 2007/ACFM:34. 82 pp.

Tomás, J., Geffen, A. J., Millner, R. S., Piñeiro, C., and Tserpes, G. 2006. Elemental composition of otolith growth marks in three geographically separated populations of European hake (*Merluccius merluccius*). Marine Biology, 148: 1399–1413.

Velasco, F. 2007. Alimentación de la merluza europea (*Merluccius merluccius* L.) en el mar Cantábrico. PhD thesis, Complutense University of Madrid. 257 pp.

Velasco, F., and Olaso, I. 1998. European hake (*Merluccius merluccius* L. 1758) feeding in the Cantabrian Sea: seasonal, bathymetric and length variations. Fisheries Research, 38: 33–44.

Velasco, F., Olaso, I., and Sánchez, F. 2003. Annual variations in the prey of demersal fish in the Cantabrian Sea and their implications for foodweb dynamics. ICES Marine Science Symposia, 219: 408–410.

von Bertalanffy, L. 1938. A quantitative theory of organic growth. Human Biology, 10: 181–213.

Zar, J. H. 1996. Biostatistical Analysis, 3rd edn. Prentice-Hall, Englewood Cliffs, NJ. 662 pp.

Acronyms

ALK	age−length key
APE	average percentage error
AZTI−Tecnalia	Instituto Tecnológico, Pesquero y Alimentario (Technological Center, Fishing and Food Research)
BIOSDEF	Biological Studies of Demersal Fish to the European Commission (EU Study Contract 95/038)
CV	coefficient of variation
DEMASSESS	New Assessment and Biology of the Main Commercial Fish Species: Hake and Anglerfish of the Southern Shelf Demersal Stocks in the South Western Europe (EU Study Contract 97/015)
IEO	Instituto Español de Oceanografía (Spanish Institute of Oceanography)
IFREMER	Institut Français de Recherche pour l'Exploitation de la Mer (French Research Institute for the Exploitation of the Sea)
WGHMM	Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk, and Megrim

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Referencias bibliográficas

BIBLIOGRAFÍA

Abaunza, P., Gordo, L., Karlou-Riga, C., Murta, A., Eltink, A.T.G.W., García Santamaría, M.T., Zimmermann, C., Hammer, C., Lucio, P., Iversen, S.A., Molloy, J., Gallo, E. 2003. Growth and reproduction of horse mackerel, *Trachurus trachurus* (Carangidae).Reviews in Fish Biology and Fisheries, 13: 27-61.

Alemany, F., Oliver, P., Reñones, O. 1993. Growth of Hake of Balearic Islands Stock. Final Report of the Study for the assessment and management of fisheries in western Mediterranean Sea . Part 2. 14 pp.

Alemany, F. and Oliver, P. 1995. Growth of female European hake in the Balearic Sea: a proposal for new growth model with higher growth rates. Rapports et procès verbaux des réunions - Commission internationale pour l'exploration scientifique de la mer Méditerranée., 34: 236.

Alheit, J., Pitcher, T.J. 1995. Hake Fisheries, Ecology and Markets. Chapman and Hall, London. 478 pp.

Álvarez, P., Motos, L., Uriarte, A., and Egaña, J. 2001. Spatial and temporal distribution of European hake, *Merluccius merluccius* (L.), eggs and larvae in relation to hydrographical conditions in the Bay of Biscay. Fisheries Research, 50: 111–128.

Álvarez, P., Fives, J., Motos, L., Santos, M. 2004. Distribution and abundance of European hake *Merluccius merluccius* (L.), eggs and larvae in the North East Atlantic Waters in 1995 and 1998 in relation to hydrographic conditions. Journal of Plankton Research, 26:1-16

Álvarez, P. and Cotano, U. 2005. Growth, mortality and hatch-date distributions of European hake larvae, *Merluccius merluccius* (L.), in the Bay of Biscay. Fisheries Research, 76: 379–391.

Anónimo, 1994. Report of the Workshop on sampling strategies for age and maturity. ICES CM 1994/D:1.

Anónimo, 1998. Biological Studies of Demersal Fish to European Commission. Annex III of the Final Report to the Commission of European Communities (EU BIOSDEF Study contract 95/038). 522 pp.

Anónimo, 2000. Report of New Assessment and Biology of the Main Commercial Fish Species: Hake and Anglerfish of the Southern Shelf Demersal Stocks in the South Western Europe. Appendix I of Final Report to the Commission of European Communities (DEMASSESS, Study Contract 97/015). 497 pp.

Anónimo, 2002. Report of the Improving Sampling of Western and Southern European Atlantic Fisheries. Appendix 19 in the Final report to the Commission of European Communities (SAMFISH, Contract No. 99/009). 184 pp.

Appelberg, M., Formigo, N., Geffen, A.J., Hammer, C., McCurdy, W., Modin, J., Mokness, E., Mosegaard, H., Morales-Nin, B., Troadec, H. and Wrigth, P. 2005. A cooperative effort to exchange age reading experience and protocols between European fish institutes. Fisheries Research, 76: 167-173.

Arneri, E., Mosegaard, H., Wright, P. J., and Morales-Nin, B. 1998.Microstructural validation of annual increments. In The PresentStatus of Otolith Research and Applications, pp. 54–59. Ed. by P. J.Wright. EFAN report 1/98.

Arneri, E. and Morales-Nin, B. 2000. Aspects of the early life history of European hake from the central Adriatic. Journal of Fish Biology, 56: 1368–1380.

Bagenal, T.B. 1954. Growth rate of the hake (*Merluccius merluccius* L.) in the Clyde and other Scottish areas. Journal of the Marine Biology Association of the UK, 33: 69–95.

Bagenal, T. B. and Tesch, F.W. 1978. Age and growth. In T. Bagenal (ed) Methods for assessment of fish production in fresh waters. IBP Handbook No.3, 3rd Edition. Balckwell Scientific Publicatons, London.

Beamish, R.J. 1979. Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. Journal of the Fisheries Research Board of Canada, 36: 141-151.

Beamish, R.J. and Fournier, D.A. 1981. A method for comparing the precision of a set of age determination. Canadian Journal of Fisheries and Aquatic Sciences 38, 982-983.

Beamish, R.J. and Chilton, D.E. 1982. Preliminary evaluation of a method to determine the age of sable fish (*Anoploma fimbria*). Canadian Journal of Fisheries and Aquatic Sciences, 39: 277-287.

Beamish, R.J. and McFarlane, G.A. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society, 112:735-743.

Beamish R.J. and McFarlane G.A. 1987. Current trends in age determination methodology pp. 1-21. Department of Fisheries and Oceans. Fisheries Research Branch. Pacific Biological Station. Nanaimo.

Beckman, D.W. and Wilson, C.A. 1995. Seasonal timing of opaque zone formation in fishes otoliths. In recent developments, in Fish Otolith Research (Secor, D.H., Dean, J.M. and Campana S.E. eds.) pp. 27 – 34. Columbia SC: University of shout Carolina Press.

Belcari, P., Ligas, A., and Viva, C. 2006. Age determination and growth of juveniles of the European hake (*Merluccius merluccius* L., 1758). Fisheries Research, 78: 211–217.

Belloc, G. 1935. Etude monograhique du merlu (*Merluccius merluccius*) troisième partie. Revue des Travaux de l'Office des Peches Maritimes, 8: 145–202.

Bertignac, M. and de Pontual, H. 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES Journal of Marine Science, 64: 981–988.

Beverton, R.J.H. and Holt, S.J. 1957. On the dynamics of exploited fish populations. Fisheries Investigation London, Ser. II 19, 533.

Bhattacharya, C.G. 1967. A simple method of resolution of a distribution into Gaussian Component Biometrics, 23: 115–135.

Bjelland, R.M. and Skiftesvik, A.B. 2006. Larval development in European hake (*Merluccius merluccius* L.) reared in a semi-intensive culture system. Aquaculture Research, 37: 1117–1129.

Botha, L. 1971. Growth and otolith morphology of the cape hakes *Merluccius capensis* Cast. and *M. paradoxus* Franca. Investigational Report, Division of Sea Fisheries, South Africa, 97: 1-32.

Bozzano A., Recasens L., Sartor P. 1997. Diet of de European hake *Merluccius merluccius* (Pisces: Merluccidae) in the western Mediterranean (Gulf of Lions). Science Marine, 61: 1-8.

Bozzano, A., Sarda, F., Rios, J. 2005. Vertical distribution and feeding patterns of the juvenile European hake, *Merluccius merluccius* in the NW Mediterranean. Fisheries Research 73: 29-36.

Brothers E.B, Mathews, C.P. and Lasker, R. 1976. Daily growth increments in otoliths from larval and adults fishes. Fishery Bulletin 74: 1-8.

Brothers, E.B. 1978. Exogenus factors and the formation of daily and subdaily increments in fish otoliths. American Zoologist 18: 631–632.

Brothers, E.B. 1984. Otolith studies. American Society of Ichthyologists and Herpetologists Special Publication, 1: 50–57.

Brown, C.A. and Gruber, S.H. 1988. Age assessment of the lemon shark, *Negabrion brevirostris*, using tetracycline validated vertebral centra. Copeia 3: 747-753.

Cabral, H.N. and Murta, A.G., 2002. The diet of blue whiting, hake, horse mackerel and mackerel off Portugal. Journal of Applied Ichthyology, 18, 14-23.

Cadenat, J. 1950. Note sur le merlus de la côte occidentale d'Afrique. Pp. 128-130. In: Congrés de Pêches et Pêcheries dans l'Union Français d'Outre-Mer, Oct.1950. Marseille (Institut Colonias de Marseille).

Campana, S.E. 1983. Feeding periodicity and the production of daily growth increments in otoliths of steelhead trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Canadian Journal of Zoology, 61: 1591-1597

Campana, S.E. 1990. How reliable are growth back-calculation based on otoliths?. Canadial Journal of Fisheries and Aquatic Sciences 47, 2219-227.

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

Campana, S.E. 2004. Photographic atlas of fish otoliths of the Northwest Atlantic Ocean. NRC Research Press, Ottawa, Ontario. 284pp.

Campana, S.E. and Neilson, J.D. 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influence of some environmental variables in their production. Canadian Journal of Fisheries and Aquatic Sciences, 39:937-942.

Campana, S.E. and Neilson, J.D. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences, 42:1014-1032.

Campana, S.E. and Moksness, E. 1991. Accuracy and precision or age and hatch date estimates from otolith microstructure examination. ICES Journal of Marine Science, 48: 303-316.

Campana, S.E. and Jones, C. M. 1992. Analysis of otolith microstructure data. In: Stevenson D K, Campana S E (eds), Otolith microstructure examination and analysis. Canadian Special Publication of Fisheries and Aquatic Sciences. 117. pp. 73-100.

Campana, S.E. and Casselman, J.M. 1993. Stock discrimination using otolith shape analysis. Canadian Journal of Fisheries and Aquatic Sciences, 50: 1062-1083

Campana, S. E. and Thorrold, S. R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding or fish populations? Canadian Journal of Fisheries and Aquatic Sciences, 58:30-38.

Cartes, J. E., Rey, J., Loris, D., Gil de Sola, L. 2004. Influence or environmental variables on de feeding an diet or European hake (*Merluccius merluccius*) on de Mediterranean Iberian coasts. Marine Biological Association of the U.K., 84: 831-835.

Casey, J. and Pereiro, F.J. 1995. European Hake (*M. merluccius*) in the North-East Atlantic. In Hake: Fisheries, Ecology and Markets. Fish and Fisheries Series 15, pp. 125–147. Ed. by J. Alheit, and T. J. Pitcher. Chapman and Hall, London. 508 pp.

Casselman, J.M. 1974. Analysis of hard tissue of pike *Esox lucius l.* with special reference to age and growth. In The ageing of fish (Bagenal, T:B. ed.) pp. 13-27. Olsl Woking, UK: Unwin Brother's Limited.

Casselman, J.M. 1983. Age and growth assessment of fish from their calcified structures: techniques and tools. In Proceedings of the international workshop on age determination of oceanic pelagic fishes: Tunas Billfishes and Sharks (Prince, E:D.and Gill, H.S., ed.)Pp.1-17, NOAA Technical Report NMFS 8. .

Casselman, J.M. 1987. Determination of age and growth. In A. H. Weatherley and H.S. Gill (eds.). The Biology of Fish Growth. Academic Press, London. P. 209-242.

Cassie, R.M. 1954. Some use of probability paper in the analysis of size frequency distributions. Australian journal of Marine and Freshwater Research, 5: 513-522.

Castillo, A.G.F., Martinez, J.L. and Garcia-Vazquez, E. 2004. Fine spatial structure of Atlantic hake (*Merluccius merluccius*) stocks revealed by variation at microsatellite loci. Marine Biotechnology, 6: 299–306.

Chilton, D.E. and Beamish, R.J. 1982. Age determination methods for fishes studied by the Groundfish Program at the Pacific Biological. Station. Canadian Special Publication of Fisheries and Aquatic Sciences, 60.

Cimmaruta, R., Bondanelli, P. and Nascetti, G. 2005. Genetic structure and environmental heterogeneity in the European hake (*Merluccius merluccius*). Molecular Ecology, 14: 2577–2591.

Cohen, D.M., Inada, T., Iwamoto, T. and Scialabba, N. 1990. Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes know to date. FAO species catalogue.Vol. 10.

Coombs, S. H. and Mitchell, C.E. 1982. The development rate of eggs and larvae or the hake, *Merluccius merluccius* (L.) and their distribution to the west of the British-Isles. ICES Journal of Marine Science, 40: 119-126.

Courbin, N., Fablet, R., Mellon, C. and de Pontual, H. 2007. Are hake otolith macrostructures randomly deposited? Insights from an unsupervised statistical and quantitative approach applied to Mediterranean hake otoliths. ICES Journal of Marine Science, 64: 1191–1201.

Cury, P. and Roy, C. 1989. Optimal environmental window and pelagic fish recruitment succes in upwelling areas. Canadian Journal of Fisheries and Aquatic Sciences, 46: 670-680.

Dannevig, E.H. 1956. Chemical composition of the zones in cod otoliths. Journal du conseil permanent international pour l'exploration de la mer, 21:156–159.

De Pontual, H., Bertignac, M., Battaglia, A., Bavouxet, G., Moguedet, P., and Groison, A. L. 2003. A pilot tagging experiment on European Hake (*Merluccius merluccius*):

methodology and preliminary results. ICES Journal of Marine Science, 60: 1318–1327.

De Pontual, H., Groison, A.L. and Piñeiro, C. 2004. Do we need to revise growth estimation of European hake (*Merluccius merluccius*)?. ICES CM 2004/K: 66.

De Pontual, H., Groison, A.L., Piñeiro, C. and Bertignac, M. 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES Journal of Marine Science, 63: 1674–1681.

Dery, L.M. 1988. Silver hake *Merluccius bilinearis*. In: Age determination methods for Northwest Atlantic species. J. Penttila and L. M. Dery (eds.) 8: 41-47. NOAA [National Oceanic and Atmospheric Administration] Technical Report, National Marine Fisheries Service (Woods Hole), USA.

Descamps, P. and Labastie, J. 1978. Note sur la lecture et l'interpretation des otolithes du merlu. ICES CM 1978/G: 41, 21 pp.

Domínguez-Petit, R. 2007. Study of Reproductive Potential of *Merluccius merluccius* in the Galician Shelf. Doctoral Thesis. University of Vigo. 253 pp.

Domínguez-Petit, R., Korta, M., Saborido-Rey, F., Murua, H., Sainza, M., and Pineiro, C. 2008. Analysis of the influence of stock structure and environmental changes in size at maturity for both European hake Atlantic populations. Journal of Marine Systems. 71, 260–278.

Eltink, A.T.G.W. 1997. Horse mackerel otolith exchange in 1996. ICES Document CM 1997/H: 24. 30 pp.

Eltink, A.T.G.W. and Kuiter, C.J. 1989. Validation of ageing techniques on otoliths of horse mackerel (*Trachurus trachurus* L.). ICES CM 1989/H:43, 15 p.

Eltink, A.T.G.W., Newton, A.W., Morgado, C., Santamaria, M.T.G. and Modin, J. 2000. Guidelines and tools for age reading, European Fish Ageing Network (EFAN), EFAN Report3–2000, *ww.efan.no*/News and Reports/EFAN Report 32000.htm.

Fablet, R., Chessel, A., Carbini, S., Benzinou, A. and de Pontual, H. 2009. Reconstructing individual shape histories of fish otoliths: a new image-based tool for otolith growth analysis and modeling. Fisheries Research, 96(2-3):148-159.

FAO. 1982. Report of the Technical Consultation on Methodologies used for Fish Age-Reading. FAO Fisheries Report, no. 257. 109pp.

FAO. 1995. Code of Conduct for responsible fisheries. Roma, FAO. 41 pp

Fariña, A. C., and Abaunza, P. 1991. Contribución al estudio de los juveniles de merluza entre Cabo Villano y Cabo Prior (NW Galicia) mediante prospecciones pesqueras. Boletín del Instituto Español Oceanografía, 7: 155–163.

Fernández, F. 1987. Edad y crecimiento de la merluza peruana (*Merluccius gayi peruanus*). Boletín Instituto del Mar del Peru 11 (6): 191-220.

Folkvord, A. and Mosegaard, H. 2002. Some uses of age data. In Manual of Fish sclerochronology (Panfili J., Pontual H (de), Troadec H., Wright P.J., eds), pp 143-165. Brest, France: Ifremer-IRD coedition.

Fowler, A.J. 1990. Validation of annual growth increments in the otoliths of a small, topical coral reef fish. Marine Ecology Progress Series 64:25-38.

Francis, R.I.C.C.1990. Back-calculation of fish length: a critical review. Journal of Fish Biology 36, 883-902.

Francis, R.I.C.C. 1995. The analysis of otolith data: a mathematician's perspective (What, precisely, is your model?). In Recent developments in fish otolith research (Secor, D.H., Dean, J.M. and Campana, S.E., eds.), pp. 81-95. Columbia, SC, USA: University of South Carolina Press.

García-Rodríguez, M. and Esteban, A. 1995. Algunos aspectos sobre la bilogía y pesca de la merluza mediterranea (*Merluccius merluccius* L.) en la Bahia de Santa Pola (Sureste de la Península Ibérica). Boletín del Instituto Español de Oceanografía, 11: 3–25

García-Rodríguez, M. and Esteban, A. 2002. How fast does hake grow? A study on the Mediterranean hake *(Merluccius merluccius* L.) comparing whole otoliths readings and length frequency distributions data. Scientia Marina, 66: 145–156.

Gauldie, R.W. and Nelson, D.G.A. 1990. Otolith Growth in Fishes. Comparative Biochemistry and Physiology. Vol. 97A, nº 2, pp. 119-135.

Geffen, A.J. 1992. Validation of otolith increment deposition rate. *In* Otolith Microstructure Examination and Analysis, pp. 101–113. Ed. by D. K. Stevenson, and S. E. Campana. Canadian Special Publication of Fisheries and Aquatic Sciences, 117, 126 pp.

Ginsburg, I. 1954. Whitings on the coast of the American continents. Fishery Bulletin U.S., 56: 187-208.

Godinho, M.L., Afonso, M.H. and Morgado, C. 2001. Age and growth of hake *Merluccius merluccius* Linnaeus, 1758 from the Northeast Atlantic (ICES Division IXa). Boletín Instituto Español de Oceanografía, 17: 255–262.

González, R. Olaso, I. and Pereda, P. 1985. Contribución al conocimiento de la alimentación de la merluza (*Merluccius merluccius* L.) en la plataforma continental de Galicia y del Cantábrico. Boletín del Instituto Español de Oceanografía, 2: 49–60.

Goñi, R. 1983. Growth studies of European hake (*Merluccius merluccius* L.) from the Northwest African shelf. ICES Document CM 1983/G: 10, 3 pp.

Goñi, R. and Piñeiro, C. 1988. Study of the growth pattern of European hake (*Merluccius merluccius* L.) from the southern stock: ICES Divisions VIIIc and IXa. ICES Document CM 1988/G:18. 13 pp.

Groison, A.L. 2003. Validation des estimations de l'âge et de la croissance du merlu ((Merluccius merluccius L.,1758) dans le golfe de Gascogne: résultats préliminaires. Memoire de DEA.

Guichet, R. 1988. Étude de la croissance du merlu Européen (*Merluccius merluccius* L.) au cours de ses premières années. ICES Document CM 1988/G:53. 27 pp.

Guichet, R. 1995. The diet of European hake (*Merluccius merluccius*) in the northern part of the Bay of Biscay. ICES Journal of Marine Science 52, 21–31.

Guichet, R., Quero, J.C. and Labastie, J. 1973. Estimation de la composition du stock du merlu au nord et à l'ouest de l'Irlande. ICES Document CM 1973/G: 5. 9 pp.

Haddon, M. 2001. Modelling and Quantitative Methods in Fisheries. CRC/Chapman and Hall. 406pp.

Hasselblad, V. 1966. Estimation of parameters for a mixture of normal distributions. Technometrics, 8: 431-444.

Hecht, T. and Appelbaum, S. 1982. Morphology and taxonomic significance of the otoliths of some bathypelagic Anguilloidei and Saccopharyngoidei from the Sargasso Sea. Helgol. Meeresunters, 35: 301-308.

Hickling, C.F. 1927. The natural history of the Hake—Parts I and II. Fish. Invest. Ser. II X (2), 79pp.

Hickling, C.F. 1933. The natural history of the hake. Part 3. Seasonal changes in the condition of hake. U.K. Fishery Investigations Series II, 12 (1). 120 pp.

Hilborn, R. and Walters, C. J. 1992. Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. New York, USA: Chapman and Hall, Inc.

Hunt, J.J. 1980. Guidelines for age determination of silver hake, *Merluccius bilinearis*, using otoliths. Journal of Northwest Atlantic Fishery Science, Vol. l: p. 65–80

Hunt, J.J. 1992. Morphological characteristics of otoliths for selected fish in the Northwest Atlantic. Journal of Northwest Atlantic Fishery Science, Vol. 13.

Ibaibarriaga, L., Irigoien, X., Santos, M., Motos, L., Fives, J. M., Franco, C., Lago de Lanzos, A., Acebedo, S., Bernal, M., Bez, N., Eltink, G. and Farinha, A. 2007. Egg and larvae distribution of seven fish species in the North East Atlantic waters. Fisheries Oceanography, 16(3): 284–293.

ICES. 1979. Report of the Working Group on Assessment of the Hake Stocks. 28– 31 May 1979, Charlottenlund. ICES Document CM 1979/G:27. 65 pp.

ICES. 1983. Confrontation Technique sur les Methodologies Utilisées pour la Lecture des Otolithes de Merlu. Report of the Working Group on Assessment of Hake Stocks. ICES CM 1983/Assess: 2.

ICES. 1984. Report of the ad hoc Hake Otolith Working Group. ICES CM 1984/G: 74.

ICES. 1986. Report of the ad hoc Study Group on Hake. ICES CM 1986/G: 93.

ICES. 1994. Report of the Workshop on Sampling Strategies for Age and Maturity. ICES CM 1994/D: 1. 67 pp.

ICES. 2000. Report of the Working Group on the Assessment of Southern Shelf Demersal Stocks. ICES CM 2000/ACFM: 4. 744 pp.

ICES. 2003. Report of the Working Group on the Assessment of Southern Stock of Hake, Monk, and Megrim, ICES Document CM 2003/ACFM: 01. 499 pp.

ICES. 2005. Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk and Megrim. ICES C.M. 2005/ACFM: 02.

ICES. 2007a. Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk, and Megrim. ICES Document CM 2007/ACFM: 21. 700 pp.

ICES. 2007b. Report of the ICES Advisory Committee on Fisheries Management, Advisory Committee on the Marine Environmental and Advisory Committee on Ecosystems, 2007. ICES Advice. Book 7: 94pp

ICES. 2010a. Report of the Workshop on Age estimation of European hake (WKAEH), 9-13 November 2009, Vigo, Spain . ICES CM 2009/ACOM: 42. 68 pp.

ICES. 2010b. Report of the Benchmark Workshop on Roundfish (WKROUND), 9–16. February 2010, Copenhagen, Denmark. ICES CM 2010/ACOM:36. 183 pp.

ICES. 2011. Report of the Working Group on the Assessment of Southern Shelf stocks of Hake, Monk and Megrim (WGHMM), 5 - 11 May 2011, ICES Headquarters, Copenhagen. ICES CM 2011/ACOM:11. 625 pp.

ICSEAF. 1983. Otolith interpretation guide of hake. International Commission For Southeastern Atlantic Fisheries,1: 1 - 70.

Iglesias, S. and Dery, L. 1981. Age and growth studies of hake (*Merluccius merluccius* L.) from ICES divisions VIIIc and IXa. ICES, C.M. 1981/G:38.

Inada, T. 1981. Studies on the Merlucciid fish. Bulletin Far Seas Fisheires Research Laboratory Shimizu, Japan, 18: 1–172.

Inada, T. 1989. Current status of the systematics of Merlucciidae. In: Cohen E D (ed), Papers on the systematics of Gadiformes. Natural History Museum of Los Angeles County. Science Series, 32: 197-207.

Jobling, M. 2002. Enviromental factors and Rates of Development and Growth . in Paul J.B. Hart and John D. Reynolds (eds) Handbook of Fish Biology and Fisheries. Blackwell Science Ltd.

Jones, C. 1986. Determining age of larval fish with the otolith increment technique. Fisheries Bulletin, 84: 91-104.

Jovilet A., de Pontual, H., Garren, F. and Bégout, M. L. 2009. Effects of T-Bar and DST Tagging on Survival and Growth of European hake. In: Nielsen *et al.* (eds), Tagging and tracking of Marine Animals with Electronic Devices. Dordrecht: Springer: 181-192.

Kacher, M. and Amara, R. 2005. Distribution and growth of 0-group European hake in the Bay of Biscay and Celtic Sea: a spatial and interannual analyses. Fisheries Research, 71: 373–378.

Kalish, J.M., Beamish, R.J., Brothers, E.D., Casselman, J.M., Francis, R.I.C.C., Mosegaard, H., Panfili, J., Prince, E.R., Thresher, R.E., Wilson, C.A. and Wright, P.J. 1995. Glossary for otolith studies. In: Secor, D.H., Dean, J.M., Campana, S.E. (Eds.) Recent developments in Fish Otolith Research. University of South Carolina Press, Columbia, SC pp. 723-729.

Kimura, D.K and Delsa, M.A. 2005. Quality control of age data at the Alaska Fisheries Science Center. Marine and Freshwater Research, 56: 783-789.

Korta, M., Domínguez-Petit, R., Murua, H. and Saborido-Rey, F. 2010. Regional variability in reproductive traits of European hake *Merluccius merluccius L.* populations. Fisheries Research, 104: 64–72.

Kuhn Thomas, S. 1962. The Structure of Scientific Revolutions. Edicíon autorizada por «The University of Chicago Press, Chicago, Ill., EUE. 1962, 1970, The Univesity of Chicago Press. La estructura de las revoluciones científicas/ Thomas S, Kuhn; traducción de Carlos Solis Santos. 3ª Edición. Mexico FCE, 2006. 361pp.

Kuhn Thomas, S. 2006. La estructura de las revoluciones científicas. Traducción de Carlos Solis Santos. 3ªedicion. México: FCE, 2006

Landa, J., Duarte, R. and Quincoces, I. 2008. Growth of white anglerfish (*Lophius piscatorius*) tagged in the Northeast Atlantic, and a review of age studies on anglerfish. ICES Journal of Marine Science, 65: 72–80.

Laurs, R.M., Nishimoto, R. and Wetherall, J.A. 1985. Frecuency of increment formation on sagittae of North Pacific albacore (*Thunnus alalunga*). Canadian Journal of Fisheries and Aquatic Sciences, 42: 1552-1555.

Lee, R.M. 1912. An investigation into the methods of growth determination in fishes. Cons. Perm. Int. Explor Mer., Publ. Circonstance 63

Linnaeus, C. 1758. Systema Naturae. X edn. vol. 1, 824 p. Nantes y Pisces, 230–338.

Lloris, D., Matallanas, J. and Oliver, P. 2003. Merluzas del mundo (Familia Merlucciidae). Catálogo comentado e ilustrado de las merluzas conocidas. FAO Catálogo de especies para los Fines de la Pesca. No. 2. 57 pp.

Lombarte, A. and Lleonart, J. 1993. Otolith size changes related with body growth, habitat depth and temperature. Environmental Biology of Fishes 37:297-306.

Lucio, P., Murúa, H. and Santurtún, M. 2000a. Growth and reproduction of hake (*Merluccius merluccius*) in the Bay of Biscay during the period 1996–1997. Ozeanografika, 3: 325–354.

Lucio, P., Santurtun, M. and Quincoces, I. 2000b. Tagging experiments on hake, anglerfish and other species in the Bay of Biscay. ICES C.M. 2000/Z:09.

Lundy, C.J., Moran, P., Rico, C.,Millner, R S. and Hewitt, G. M. 1999. Macrogeographical population differentiation in oceanic environments: A case study of European hake (*Merluccius merluccius*), a commercially important fish. Mol. Ecol., 8: 1889–1898.

Lundy, C. J., Rico, C. and Hewitt, G.M. 2000. Temporal and spatial genetic variation in spawning grounds or European hake (*Merluccius merluccius*) in the Bay. Mol Ecol 9: 2067-2079.

MacDonald, P. and Pitcher, T.1979. Age groups from size frequency data: a versatile and efficient method of analyzing distribution mixtures. Journal of the Fisheries Research Board of Canada, 36: 987 – 1001.

MacLellan, S.E. and Saunders, M.W. 1995. A natural Tag on the Otolith of Aacific Hake (*Merluccius productus*) with Implications for Age Validation and Migration. In Recent developments in fish otolith research (Secor, D.H. Dean, J.M.and Campana, S.E., ed.), pp. 567-580. Columbia, SC. USA: University of South Caroline Press.

Martin, I. 1991. A preliminary analisis of some biological aspects of Hake (*M. merluccius* L. 1758) in the Bay of Biscay. ICES CM 1991/G: 54 (Demersal Fish Commitee).

Mattiucci, S., Abaunza, P., Ramadori, L. and Nascetti, G. 2004. Genetic identification of Anisakis larvae in European hake from Atlantic and Mediterranean waters for stock recognition. J. Fish. Biol., 65: 495–510.

McCurdy, W.J., Appelberg, M., Ayers, R., Belcari, P., Bolle, L., Cendrero, O., Easey, M.W., Fossum, P., Hammer, C., Hansen, F.I. and Raitaniemi, J. 2000. Guidelines for quality assurance of fish Age determination, EFAN Report 5/2000, 9 p.

McCurdy, W.J., Panfili, J., Meunier, F.J., Geffen, A.J. and de Pontual, H. 2002. Preparation of calcified structures. In: J. Panfili, H. de Pontual, H. Troadec and P.J. Wright (eds.), Manual of sclerochonology, pp. 129-134. Ifremer-IRD edition, Brest.

McFarleane, G.A and Beamish, K.M 1985. Biology and fishery of the Pacific whiting, *Merlucius productus*, resource and industry: An Overview. Marine Fishers revies 47:23-34.

McFarlane, G.A. and Beamish, R.J. 1995. Validation of the otolith cross-section method of age determination for sablefish (*Anoplopoma fimbria*) using oxytetracycline. In: Secor, D.H. et al. (Eds.) Recent Developments in Fish Otolith Research. University of South Carolina Press, pp. 319-329.

Mellon Duval, C., de Pontual, H., Metral, L. and Quemener, L. 2010. Growth of European hake (*Merluccius merluccius*) in the Gulf of Lions based on conventional tagging. ICES Journal of Marine Science, 67: 62–70.

Meriel-Bussy, M. 1966. La croissance du merlu dans le golfe de Gascogne. ICES C.M.1966/ G:17 Comite Poissons Gadoides, 5 pp.

Meiners, C.G. 2007. «Importancia de la variabilidad climática en las pesquerías y biología de la merluza europea *Merluccius merluccius* (Linnaeus, 1758) de la costa Noroccidental africana», PhD Tesis. Universidad Politécnica de Cataluña

Morales-Nin, B. 1986. Structure and composition of *Merlucius capensis* otoliths. South African Journal Marine Science, 4: 3 -10.

Morales-Nin, B. and Aldebert, Y. 1997. Growth of juvenile *Merluccius merluccius*in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length frequency analysis. Fisheries Research, 30:77–85.

Morales-Nin, B., Torres, G.J., Lombarte, A. and Recasens, L. 1998. Otolith growth and age estimation in the European hake. J. Fish Biol., 53: 1155-1168.

Morales-Nin, B. and Panfili, J. 2002. Verification. In Manual of Fish Sclerochronoloy, pp. 139-142. Ed. by J. Panfili, H. de Pontual, H. Troadec, and P. J. Wright. Ifremer-IRD Co-edition, Brest, France.

Morales-Nin, B. and Moranta, J. 2004. Recruitment and post-settletment growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. Scientia Marina, 63: 399-409

Morales-Nin, B., Bjelland, R.M. and Moksness, E.2005. Otolith microstructure of a hatchery reared European hake (*Merluccius merluccius*). Fisheries Research, 74: 300 – 305.

Motos, L., Alvarez, P. and Uriarte, A. 2000. Distribution pattern of hake (*Merluccius merluccius,* L) eggs and larvae in relation to environmental conditions in the Bay of Biscay in winter and early spring 1995. Ozeanografika, 3: 273-292.

Murua, H. 2010. The Biology and Fisheries of European Hake, *Merluccius merluccius*, in the North-East Atlantic. In Michael Lesser editor: Advances in Marine Biology - 58, Burlington: Academic Press, 2010, pp. 97-154. ISBN: 978-0-12-381015-1 © Copyright 2010 Elsevier Inc. Academic Press.

Murua, H., Motos, L. and Lucio, P.1998. Reproductive modality and batch fecundity of the European hake (Merluccius merluccius L.) in the Bay of Biscay. California Cooperative Oceanic Fisheries Investigations Reports, 39:196–203.

Murua, H. and Motos, L. 2006. Reproductive strategy and spawning activity of the European Hake *Merluccius merluccius* (L.) in the Bay of Biscay. Journal of Fish Biology., 69: 1288–1303.

Nolf, D. 1985. Otolithi Piscium. Handbook or Paleoichthyology, Vol. 10A. Paul Durey, New York.

Nolf, D. 1995. Studies on fossil otoliths:the state of the art. In Recent developments in fish otolith research (Secor, D.H. Dean, J.M.and Campana, S.E., ed.), pp. 513-544. Columbia, SC. USA: University of South Caroline Press.

Norbis W., Lorenczo, M.I.and Torres, G. 1999. Intra-annual growth variations of the young of the year hake (*Merluccius hubbsi*) of the Uruguayan continental shelf, based on otolith analysis. Fisheries Research, 44:129-137.

Olivar, M.P., Quilezz, G. and Emelianov, M. 2003. Spatial and temporal distribution and abundante of European hake, *Merluccius merluccius*, eggs an larvae in the Catalan coast (NW Mediterranean). Fisheries Research, 60: 321-331.

Oliver, P., Álvarez, F. and Morales-Nin, B.1989.Report of the age-reading workshops on Mediterranean European hake and sardine, Palma de Mallorca, Spain, 10-15 April 1989. FAO Rapport Pêches, pp:447.

Oliver, P. and Massutí, E. 1995. Biology and ficheries of western Mediterranean hake (*M. merluccius*). In: Alheit J, Pitcher T J (eds), Hake Fisheries, Ecology and Markets. Champman and Hall, London, pp: 181-202.

Olsson, O. and North, A.W. 1997. Diet of the king Penguin Aptenodytes patagonicus during three summers at South Georgia. Ibis, 139: 504-512.

Otxotorena, U., Díez, G., López de Abechuco, E., Santurtún, M. and Lucio, P. 2010. Estimation of age and growth of juvenile hakes (Merluccius merluccius Linneaus; 1758) of the Bay of Biscay and Great Sole by means of the analysis of macro and microstructure of the otoliths. Fisheries Research, 106: 337-343.

Palomera, I., Olivar M.P. and Morales-Nin, B. 2005. Larval development and growth of the European hake *Merluccius merluccius* in the north-western Mediterranean. Scientia Marina, 69(2): 251-258.

Pannela, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science, 173: 1124–1127.

Panella, G. 1974. Otoliths growth patterns: an aid in age determination in temperate and tropical fishes. In the aging of fish (Bagenal, T.B., ed), pp: 28-39. London, UK:Unwin Borthers Ltd.

Panella, G. 1980. Growth pattern in fish sagittae. In Skeletal growth of aquatic organisms. Biological records of environmental change. (Rhoads, D.C.andLutz, R.A., ed.), pp: 519-560. New York, USA and London, UK:Plenun Press.

Panfili, J., Meunier, F.J., Mosegaard, H., Troadec, H., Wright, P.J. and Geffen A.J., 2002. Glossary. In: Manual of Fish Sclerochronology, pp. 373-384. Panfili, J., Pontual, H. (de)., Troadec H., Wright P.J. (eds), 2002. Brest, France: Ifremer-IRD Co-edition, 464 pp.

Papaconstantinou, C. and Stergiuou, K I. 1995. Biology and fisheries of eastern Mediterranean hake (M. merluccius). In: Alheit J, Pitcher T J (eds), Hake: ficheries, ecology and Markets. Chapman and Hall, London, p 149-180.

Penttila J. and Dery L.M. 1988. Age Determination Methods for Northwest Atlantic Species. In: National Marine Fisheries Center, NOAA.

Pérez, N., Pereiro, F.J. 1985. Aspectos de la reproducción de la merluza *Merluccius merluccius* de la plataforma gallega y cantábrica. Boletín Instituto Español Oceanografía, 2(3): 39-47.

Petersen, C.G. 1891. Eine Methode zur Bestimmung des Alters und Wuchses der Fishe. Mitteilungen. Deutscher Seefisherei-Verein, 11: 226–235.

Piñeiro, C. 1994. Estado actual del estudio del crecimiento de la merluza europea del Atlántico Norte. Estado actual de los conocimientos de las poblaciones de merluza que habitan la plataforma continental atlántica y mediterránea de la Unión Europea con especial atención a la Península Ibérica. Ed. por A. González-Garcés y F.J. Pereiro. Publicación privada, pp 217.

Piñeiro, C. 2000. Report on workshop on hake otolith age reading, Vigo, June 1997. European Fish Ageing Network, EFAN Report, 6/2000, pp 32.

Piñeiro, C. and Hunt, J.J. 1989. Comparative study on growth of European hake (*Merluccius merluccius* L.) from Southern stock using whole and sectioned otoliths, and length frequency distributions. ICES CM 1989/G: 37. 16 pp.

Piñeiro, C.G., Fariña, A.C. and Abaunza, P. 1992. Resultados de las campañas realizadas en las zonas de veda al arrastre en la plataforma continental Gallega (NW España), 1989-1990. Informes Técnicos Instituto Español Oceanografía, 130: 39 pp.

Piñeiro, C.G. and Pereiro, J.A. 1993. Study of the growth pattern of juvenile European hake (*Merluccius merluccius* L.) using the whole otoliths and length frequency from commercial catches and groundfish surveys. ICES CM/G: 12. 14 pp.

Piñeiro, C., Afonso, M.H., Arego, S., Bellail, R., Labastie, J., Loureiro, I., Lucio, P., Marecos, L., Mc Cormick, H., Moguedet, P., Morgado, C., Sainza, M., Santurtún, M., Trujillo, V.,. Watson, T. and Woods, F. 2000. Report on 2nd International Workshop on Hake Otolith Age Reading. Instituto Español de Oceanografía IEO, Vigo, 1999. Spain, EFAN Report 8/2000. pp 70.

Piñeiro, C. and Sainza., M. 2002. Age estimations of European hake for assessment purposes. Instituto Español de Oceanografía IEO, Vigo, Spain . WD7 presented to the WGHMM, Lisbon, 20-30 of May, 2002, ICES CM 2003/ACFM: 01

Piñeiro, C. and Sainza M. 2003. Age estimation, growth and maturity of the European hake (Merluccius merluccius (Linnaeus, 1758) from Iberian Atlantic waters. ICES Journal of Marine Science, 60: 1086-1102.

Piñeiro, C., Sainza, M., Morgado, C., de Pontual, H., Hoey, S.,Beattie, S.and Lucio, P. 2004. Report of 4th Exchange on European Hake Age Readings. ICES Document CM 2005/ACFM: 02. 18 pp.

Piñeiro, C., Rey, J., de Pontual, H. and Goñi, R. 2007. Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: First results support fast growth hypothesis. Fisheries Research, 88: 150–154.

Piñeiro, C., Rey, J., de Pontual, H. and García, A. 2008. Growth of Northwest Iberian juvenile hake estimated by combining saggital and transversal otolith microstructure analyses. Fisheries Research, 93: 173–178.

Piñeiro, C.G., Morgado, C., Sainza, M. and McCurdy, W.J. (Eds). 2009. Hake age estimation: state of the art and progress towards a solution. ICES Cooperative Research Report No. 294. 43 pp.

Piñeiro, C.G. and Sainza, M. 2011. Crecimiento de la merluza europea: ¿lento o rápido? Foro dos Recursos Mariños e da Acuicultura das Rías Galegas,13: 129-148. Santiago de Compostela, A Coruña, España.

Pla, C. and Roldán, M. I. 1994. Estructura genética de la merluza europea (*Merluccius merluccius*) y su relación con la gestión pesquera. In «Estado actual de los conocimientos de las poblaciones de Merluza que habitan la plataforma continental atlántica y mediterránea de la Unión Europea con especial atención a la Península Ibérica». (Vigo, 13 a 17 de Diciembre de 1993)» (A. González-Garcés and F. J. Pereiro, eds.), pp: 327. Publicación Privada, Vigo.

Pita. A., Presa, P. and Pérez, M. 2010. Gene Flow, Individual Assignment and Genetic Structuring of European Hake (*Merluccius Merluccius*) Stocks. Thalassas, 25 (3) Special issue: 129-133.

Pita. A., Perez, M., Presa, P. and Perez, M. 2011.What can gene flow and recruitment dynamics tell us about connectivity between European Hake stocks in the Eastern North Atlantic? Continental Shelf Research, 31: 376-387.

Popper, A.N. and Lu, Z.M. 2000. Structure funtion relationship in fish otolith organs. Fisheries Research, 46: 15-25.

Recasens, L., Lombarte, A., Morales-Nin, B., and Torres, G.J. 1998. Spatiotemporal variation in the population structure of the European hake in the NW Mediterranean. Journal of Fish Biology, 53: 387–401.

Reibisch, J. 1899. Ueber di Einzahl bei *Pleuronectes plateassa* und die Altersbestimmung dieser Form aus den Otolithen. Wissenschaftliche Meeresuntersuchungen (Kiel) 4, 233-248.

Renzi, M. and Pérez, M. 1992. Un criterio para la determinación de la edad en juveniles de merluza (*Merluccius hubbsi*) mediante la lectura de otolitos. Frente Marit., 11(A):15-31.

Renzi, M., Santos, B. and Abachian, V. 2009. Crecimiento de la merluza (*Merluccius hubbsi*) del Atlántico sudoccidental entre 1993-2003 al Norte y Sur de 41° S*. INIDEP Informe Técnico 76. 33 pp.

Ricker, W.E. 1958. Handbook of computation for biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 119.

Ricker, W.E. 1975 Computation and Interpretation of Biological Statistics of Fish Populations. Fisheries Research Board of Canada, 191: 1-382.

Riis-Vertergaard, J., Velasco, F., Hill, L. and Olaso, I. 2000. Food consumption of Europeas hake (*Merluccius merluccius)* estimated by application of bioenergetics model: is the growth of hale underestimated? ICES CM 2000/Q: 12. 12pp.

Robles, R., Pereiro, J.A., Iglesias, S. and Pereiro, F.J. 1975. Étude sur la croissance du merlu europeen, *Merluccius merluccius* L. du NW de L'Espagne. ICES CM 1975/ G: 8, 17 pp.

Roldán, M.I., García-Marín, J.L., Utter, F.M. and Pla, C. 1998. Population genetic structure of European hake, *Merluccius merluccius*. Heredity, 81: 327–334.

Sánchez, F. and Gil, J. 1995. Influencia de anomalías térmicas de mesoescala sobre la distribución de peces demersales. In Actas del IV Coloquio Internacional de Oceanografía del Golfo de Vizcaya, pp. 49–54. Ed. by O. Cendrero, and I. Olaso. Inst. Esp. de Oceanografia, Santander.

Sánchez, F., and Gil, J. 2000. Hydrographic mesoscale structures and Poleward Current as a determinant of hake (*Merluccius merluccius*) recruitment in southern Bay of Biscay. ICES J. Mar. Sci. 57, 152–170.

Sánchez, F.J., Cal, R.; Otero, J.J., Lago, M.J., Gómez, C. and Iglesias, J. 2011. The first spontaneous spawning of European hake (*Merluccius merluccius* L.): characteristics of eggs and early larval stages. Aquaculture Research, doi:10.1111/j.1365-2109.2011.02966.x.

Sánchez, R., Sánchez, F. and Gil, J. 2003. The optimal environmental window tht controls hake (*Merluccius merluccius*) recruitment in the Cantabrian Sea. ICES Marine Science Symposia, 219:415-417.

Sarano, F. 1986. Cycle ovarien du Merlu, *M. merluccius*, poisson a‘ ponte Fractione´e. Revue des Travaux de l'Institut des Peches Maritimes 48(1et 2), 65–76.

Schnutte, J. and Fournier, D.1980. a new approach to length-frequency analysis: growth structure, Canadian Journal of fisheries and Aquatic Sciences 37, 1337-1351.

Secor, D.H., White, M.G. and Dean, J.M. 1991. Immersion marking of larval and juvenile hatchery-produced striped bass with oxytetracycline. Transactions of the American Fisheries Society, 120:261-266.

Sparre, P. y Venema, S.C. 1997. Introducción a la evaluación de recursos pesqueros tropicales. Parte I. Manual FAO. Documento Técnico de Pesca nº 306. 1 Rev. 2: 420pp.

Stefanescu, C., Lloris, D. and Rucabado, J. 1992. Deep-living demersal fishes in the Catalan sea (Western Mediterranean) below a depth of 1000 m. Journal of Natural History, 26: 197-213.

Stevenson, D.K. and Campana, S.E. 1992 Otolith microstructure examination and analysis. Canadian Special Publication of Fish Aquatic Science, 117: 126 pp.

Stransky, C. 2005. Geographic variation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the North Atlantic based on otolith shape analysis. ICES Journal of Marine Science, 62:1691-1698.

Svetovidov, A.N. 1986. Merlucciidae. In: Whitehead P J P, Bauchot M L, Hureau J C, Nielsen J, Tortonese E (eds) FNAM, Unesco, Paris, 2: 677-679.

Tomás, J., Geffen, A. J., Millner, R. S., Piñeiro, C. and Tserpes, G. 2006. Elemental composition of otolith growth marks in three geographically separated populations of European hade (*Merluccius merlucius*). Marine Biology, 148: 1399 – 1413.

Torres, G. 1992. Estudio sobre crecimiento de la merluza (*Merluccius hubbsi*) del Atlántico Sudoccidental. Universidad de la República. Facultad de Ciencias. Tesis para optar Licenciatura en Oceanografía Biológica. 105 pp.

Torres, G., Norbis, W. and Lorenzo, M.I. 1996. Variation in the measures of hake (*Merluccius hubbsi*) rings otoliths during their first year: evidence for stock separation?. Scientia Marina, 60: 331-338.

Torres, G.J., Lombarte, A. and Morales-Nin, B. 2000. Sagittal otolith size and shape variability to identify geographical intraspecific diffrences in three species of the genus *Merluccius*. Journal of the Marine Biological Association of the United-Kingdom, Plymouth, 80: 333-342.

Tuset, V.M., Lombarte, A. and Assis, C.A. 2008. Otolith atlas for the western Mediterranean, north and central eastern Atlantic Scientia Marina, 72: 7-198.

Ungaro, N., Vrgoc, N. and Manzini, P. 2001. The biology of the stock assessment of the *Merluccius merluccius* (L.) in the Adriatic Sea: a historical review by geographical management units. FAO Scientific Cooperation to the Support Responsible fisheries in the Adriatic Sea (AdriaMed). Italia, 12 pp.

Velasco, F. 2007. Alimentación de la merluza europea (*Merluccius merluccius*) en el mar Cantábrico. Tesis doctoral. Universidad de Santander. 256 pp.

Velasco, F. and Olaso, I. 1998. European hake (*Merluccius merluccius* L. 1758) feeding in the Cantabrian Sea: seasonal, bathymetric and length variations. Fisheries Research, 38: 33 – 44.

Velasco, F., Olaso, I. and Sánchez, F. 2003. Annual variations in the prey of demersal fish in the Cantabrian Sea and their implications for food web dynamics. ICES Marine Science Symposia, 219: 408-410.

Vignon, M. and Morat, F. 2010. Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. Marine Ecology Progress Series 411: 231-241.

Volk, E.C., Mortensen, D.G. and Wetheimer, A.C. 1995. Non-daily otolith increments and seasonal changes in growth of a pink salmon (*Oncorhynchus gorbuscha*) population in Auke Bay, Alaska. In Recent developments in fish otolith research (Secor, D.H. Dean, J.M.and Campana, S.E., eds.), pp. 211-225. Columbia, SC. USA: University of South Caroline Press.

Von Bertalanffy, L. 1938. A quantitative theory of organic growth. Human Biology, 10: 181-213.

Weatherley, A.H. and Gill, H.S. 1987. The biology of Fish Growth. Academic press, London, 433 pp.

Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J.and Tortonese, E. 1984. Fishes of the north-Eastern Atlantic and the Mediterranean, Unesco, I: 510pp.

Wild, A. and Foreman, T.J. 1980. The relationship between otolith increments and time for yellowfin and skipjack tuna marked with tetracycline. Inter-American Tropical Tuna Commission, Bulletin 17, 509-560. 9.

Wright, P.J. 1990. The periodicity and formation of otolith increments in *Salmo salar* and *Gasterosteus aculeatus*. PhD Thesis, University of Glasgow, 268 pp.

Wright, P.J., Metcalfe, N.B. and Thorpe, J.E. 1990. Otolith and somatic growth rates in Atlantic salmon parr, Salmo salar L.: evidence against coupling. Journal of Fish Biology, 36: 341-249.

Wright, P.J., Rowe, D. and Thorpe, J.E. 1991. Daily growth increments in the otoliths of Atlantic salmon parr, Salmo salar L., and the influence of environmental factors on their periodicity. Journal of Fish Biology, 39: 103-113.

Wright, P.J., Geffen, A.J., Gordon, J., Morales-Nim, B., Palomeres, J.I. and Rebelo, J. 1998. Interpreting otolith microstructure. In Otolith preparation and analysis (Wright, P.J., ed.), EFAN Report 2/1998.

Wright, P. J., Panfili, J., Morales-Nin, B. and Geffen, A.J. 2002. Type of calcified structures. In Manual of Fish Sclerochronoloy, pp. 31-87. Ed. by J. Panfili, H. de Pontual, H. Troadec, and P. J. Wright. Ifremer IRD Co-edition, Brest, France.

ACRÓNIMOS

BIOSDEF: «Biological Studies of Demersal Fish» (EU Study Contract 95/038), proyecto financiado por la UE.

DEMASSESS: «New Assessment and Biology of the Main Commercial Fish Species: Hake and Anglerfish of Southern Shelf Demersal Stocks in the South Western Europe»; (EU Study Contract 97/015) proyecto financiado por la UE.

CO: Centro Oceanográfico.

CV: Coeficiente de Variación.

DCF: Programa comunitario de recopilación de datos biológico-pesqueros y económicos, así conocido por sus siglas en inglés «Data Collection Regulation Framework» de la UE. Esta basado en la utilización de actividades pesqueras como estratos de muestreo (Decisión de la Comisión 2008/949/EC).

DMA: Directiva Marco del Agua.

EFAN: «European Fish Ageing Network»; proyecto financiado por la UE (FAIR PL.96.1304: 1998-2000).

ICES: International Council for the Exploration of the Sea (CIEM en español).

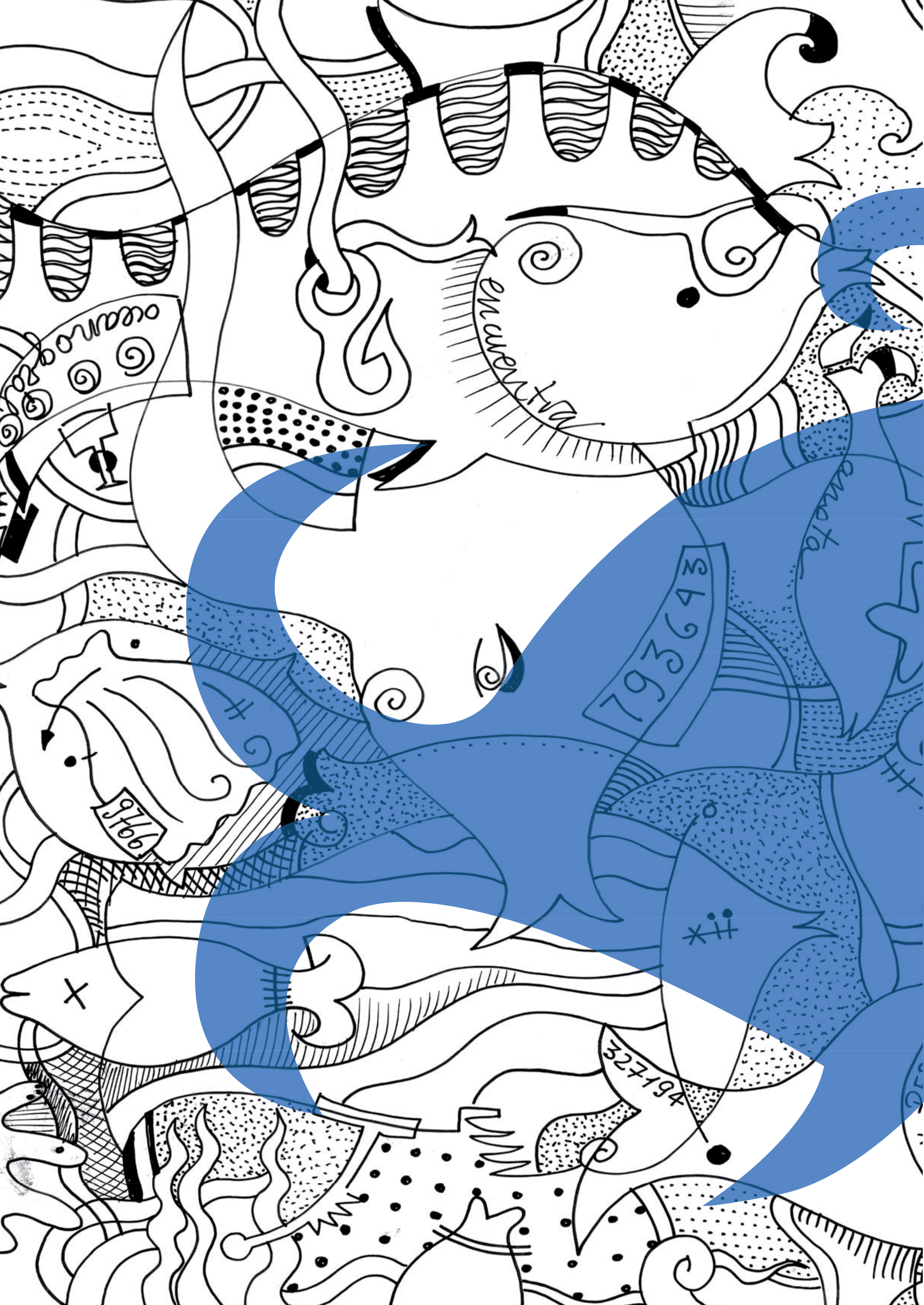
IFREMER: Institut Français de Recherche pour L'exploitation de la Mer.

IEO: Instituto Español de Oceanografía.

SAMFISH: «Improving Sampling of Western and Southern European Atlantic Fisheries»; (EU Contract No. 99/009) proyecto financiado por la UE.

TACADAR: «Towards Accreditation and Certification of Age Determination of Aquatic Resources», proyecto financiado por la UE (2002-2005, Q5CA-2002-01891).

UE: Unión Europea.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