Departamento de Ingeniería hidráulica, Marítima y Ambiental
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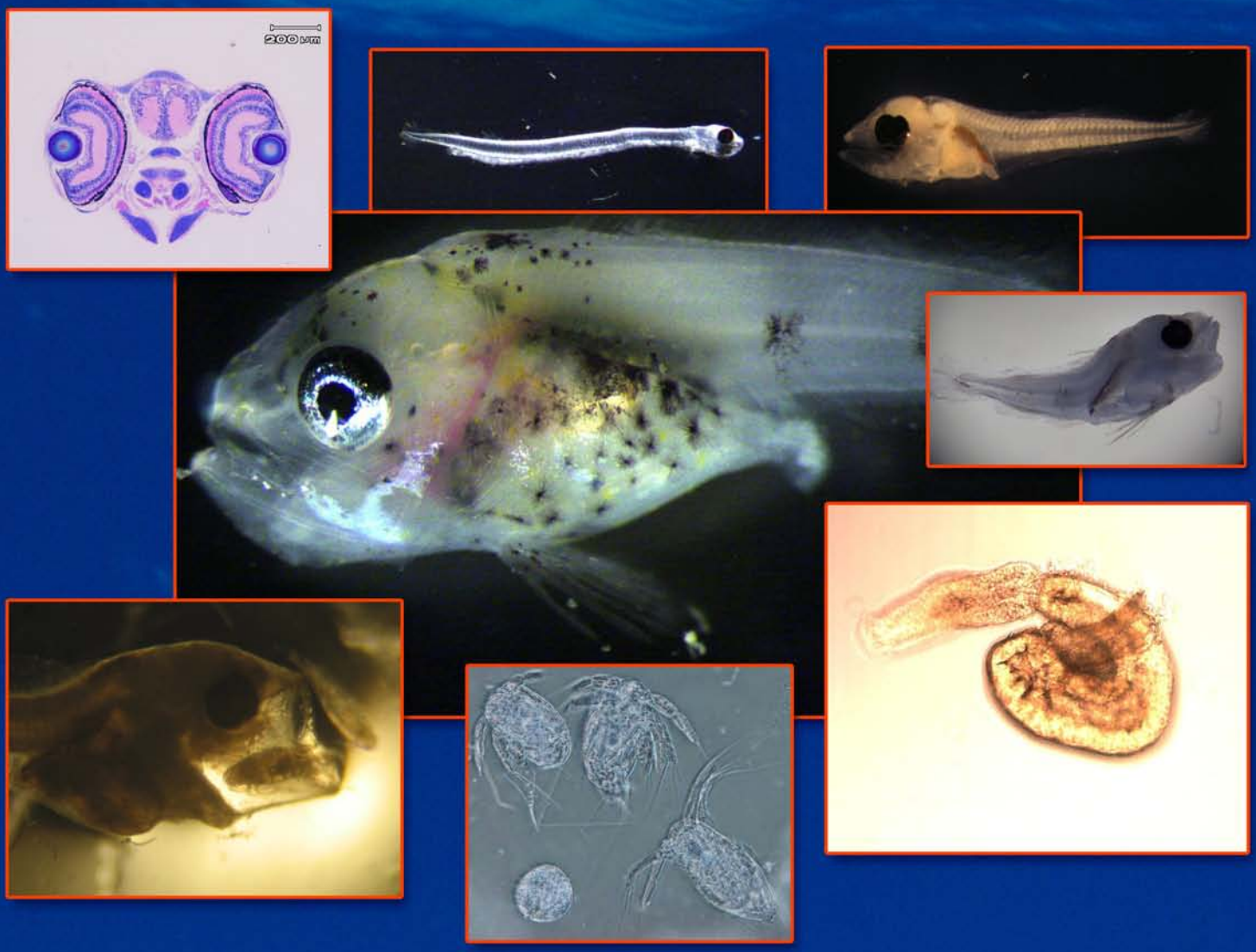
Trophic ecology of hake, anchovy, sardine, round sardinella and bullet tuna larvae of NW Mediterranean

Influence of trophic environment and ontogeny



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TROPHIC ECOLOGY OF HAKE, ANCHOVY, SARDINE, ROUND SARDINELLA AND BULLET TUNA LARVAE OF NW MEDITERRANEAN

Influence of trophic environment and ontogeny

TESIS DOCTORAL

ELVIRA MOROTE CÓRDOBA

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A mis padres Antonio y Elvira; a mis hermanas Cristina y Alicia...Porque de ellos he aprendido la lección más importante de la vida

ORGANIZACION DE LA TESIS

Esta Tesis Doctoral ha sido realizada gracias a una beca/contrato predoctoral del Ministerio de Ciencia e Innovación (España) durante 4 años que se encuadra dentro del Programa de Formación de Personal Investigador (FPI) y ha comprendido un periodo de tiempo desde el 1 de Agosto de 2005 hasta el 30 de julio de 2009.

Esta tesis esta compuesta por tres pilares que son los tres primeros capítulos:

Los capítulos 1 y 3, ambos escritos en castellano, corresponden a la Introducción y a la Discusión general respectivamente, mientras que el capítulo 2 de Resultados (en inglés) contiene los estudios de ecología trófica por especies y publicados en forma de artículos científicos en revistas internacionales (subcapítulo 2.2 los clupeiformes; subcapítulo 2.3, la melva y la merluza).

Las otras partes del trabajo son las conclusiones (en inglés) enunciadas en el capitulo 4, las referencias de la introducción y la discusión en el capítulo 5, y al final de la tesis aparece un resumen global (en inglés y en castellano). La utilización de las lenguas española e inglesa según capítulos es una opción personal elegida por el hecho de que esta tesis opta a la mención de doctorado europeo.

Los artículos publicados que forman el capítulo 2 son los resultados de los estudios de distribución y alimentación de las larvas de las especies objetivo de la tesis:

Olivar MP, Emelianov M, Villate F, Uriarte I, Maynou F, Alvarez I, **Morote E** (2010) The role of oceanographic conditions and plankton availability in larval fish assemblages off the Catalan coast (NW Mediterranean). Fish Oceanogr 19 (3): 209-229

Morote E, Olivar MP, Villate F, Uriarte I (2010) A comparison of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) larvae feeding in the Northwest Mediterranean: influence of prey availability and ontogeny. ICES J Mar Sci 67: 897-908

Morote E, Olivar MP, Villate F, Uriarte I (2008b) Diet of round sardinella, *Sardinella aurita*, larvae in relation to plankton availability in the NW Mediterranean. J Plankton Res 30: 807–816

Morote E, Olivar MP, Pankhurst PM, Villate F, Uriarte I (2008a) Trophic ecology of bullet tuna *Auxis rochei* larvae and ontogeny of feeding-related organs. Mar Ecol Prog Ser 353: 243–254

Morote E, Olivar MP, Bozzano A, Villate F, Uriarte I. (2011). Feeding selectivity of European hake (*Merluccius Merluccius*) larvae in relation to ontogeny and visual capabilities. Mar Biol 158 (6): 1349-1361

Además, existen otros trabajos publicados en el que la doctoranda ha participado durante su periodo de doctorado y que complementan la temática tratada en esta tesis doctoral pese a que no se han incluido en esta memoria:

Olivar, M. P., Emelianov, M., Uriarte, I., Villate, F., Álvarez, I., **Morote, E.**, Fuerstenau, B., and Molí, B. 2007. Demographic structure of early stages of *Engraulis encrasicolus* and *Sardinella aurita* and water mass circulation in the southern Catalan sea. Rapp. Comm. int. Mer Médit, 38: 559.

M. P. Olivar, **E. Morote** and M. Emelianov. 2010. Autumn overlapping of *Sardina pilchardus* and *Merluccius merluccius* early stages in the NW Mediterranean: distribution and diet. Rapp. Comm. int. Mer Médit., 39: 607.

M. Faria; T. Muha; **E. Morote** and M. A. Chícharo. 2011. Influence of starvation on the critical swimming behaviour of the Senegalese sole (*Solea senegalensis*) and its relationship with RNA/DNA ratios during ontogeny. 2011. Sci Mar, 75 (1): 87-94

Los resultados a lo largo de estos años de investigación, además de reflejarse en la publicación de estos artículos, han ido contribuyendo a la ciencia mediante su exposición en congresos, conferencias y simposiums: SIEBM (Barcelona 2005), GLOBEC (Valencia 2006), ALFC (New Foundland 2007, Kiel 2008, Portland 2009), CIESM (Turquía 2007, Venecia 2010).

Esta tesis confirma datos de la ecología trófica de las larvas de anchoa, sardina, merluza, alacha y melva, que son esenciales para la supervivencia y subsiguiente reclutamiento de estas especies. Las investigaciones llevadas a cabo en la zona de estudio se realizaron en periodos con condiciones ambientales opuestas para un estudio comparativo no solo de las características de cada especie sino también de su entorno (oceanografía y composición del zooplancton).

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INTRODUCTION

INTRODUCCIÓN

1.1 CARACTERIZACIÓN DEL AREA DE ESTUDIO: EL MAR CATALÁN (MEDITERRÁNEO NOROCCIDENTAL). RELACIÓN CON LA HIDROGRAFÍA Y EL PLANCTON

El mar Catalán, donde este estudio se ha llevado a cabo, está ubicado en el Noroeste del Mediterráneo (Fig. 1.1). Es un mar templado y relativamente productivo si se compara con el carácter oligotrófico del mar Mediterráneo (Estrada y Margalef 1988). La circulación superficial está dominada por dos frentes salinos: el frente de la plataforma-talud Catalán localizado sobre el talud de la península ibérica, y el frente Balear localizado sobre el talud insular. El primer frente separa aguas menos salinas (<37.5) influenciadas por el agua Atlántica y las descargas de los ríos, de las aguas de mar abierto (> 38.1) (Font et al. 1988). Está asociado con el flujo de la corriente Catalana (CC), cuya intensidad tiene una importante componente estacional, menor en verano y con una reintensificación en otoño (Pinot y Ganachaud 1999). Además de las características de la corriente hay otros cambios estacionales en la hidrografía que juegan un papel importante en los procesos de fertilización de la región. La mezcla invernal de la columna de agua y las intrusiones de agua en el borde de la plataforma-talud durante el invierno, causan un máximo de la biomasa del fitoplancton y valores de producción distribuidos uniformemente en la capa fótica. Por el contrario, la fuerte estratificación estival con una marcada termoclina, conlleva que conforme el verano avanza se produzca un agotamiento de los nutrientes en las capas superficiales. Como consecuencia, el máximo de producción primaria durante esta parte del año ocurre en la profundidad donde la intensidad de luz y la concentración de nutrientes lo permite (DCM), seguido por el pico de biomasa del zooplancton. En este periodo, además, hay un incremento de la productividad gracias a la variabilidad a corto plazo de la meteorología e hidrografía (Estrada 1996; Salat et al. 2002; Alcaraz et al. 2007).

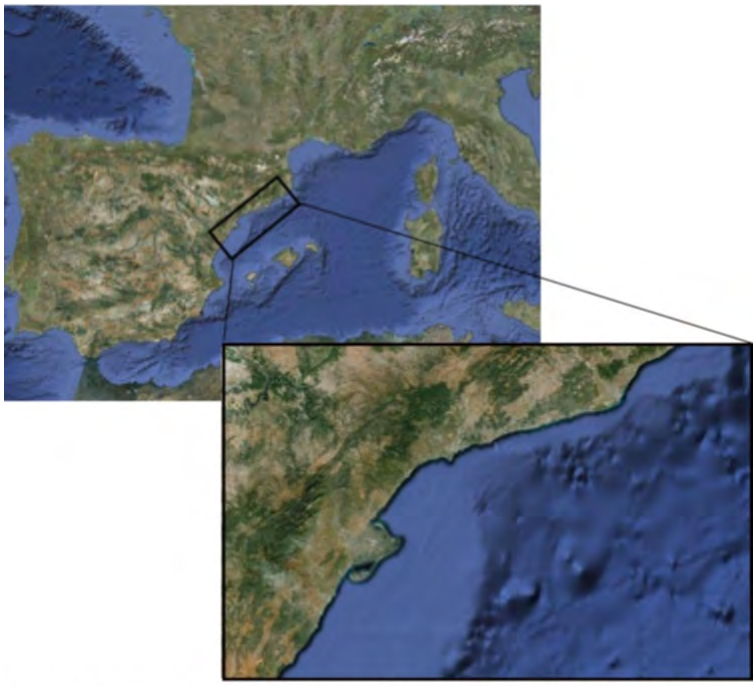


Figura 1.1. Noroeste del Mediterráneo con la zona de muestreo a lo largo de la costa Catalana; NW Mediterranean with study area along the Catalan coast

El zooplancton juega un importante papel en la estructura y funcionamiento de las redes tróficas marinas, constituyendo el enlace principal entre los productores primarios y los peces pelágicos. En la zona de estudio se da una estacionalidad prominente en la composición y abundancia del

zooplancton. Algunas especies están presentes solo durante algunos meses (como los cladóceros en verano o los poliquetos y larvas de cirrípedos en invierno), mientras que otras especies (como ciertos copépodos) se observan durante todo el año (Calbet et al. 2001). El papel que juega el espectro de tamaños de los organismos en la transferencia de materia y energía a través de la cadena trófica es de gran importancia para las larvas de peces. Si durante su desarrollo larvario tienen disponible un amplio espectro de tallas pueden adaptar su alimentación a las particulares capacidades depredadoras de cada estadio, centrándose en presas pequeñas, abundantes y poco móviles (dinoflagelados, microzooplancton) en los primeros estadios, y cambiar progresivamente a presas más grandes y nutritivas (mesoplanctónicas), que contribuyen al crecimiento de una forma más importante (Hunter 1981). Los copépodos son la presa más común de las larvas de peces en todos los océanos, especialmente en sus primeros estadios (nauplios) para las larvas más jóvenes. A medida que crecen, las larvas cambian, y depredan copepoditos y copépodos adultos (Last 1980; Voss et al. 2003; Sassa 2010). Algunas especies de copépodos se encuentran en el mar Catalán tanto en verano como en invierno (como *Clausocalanus* spp. o *Oithona* spp.), mientras que otras especies solo se dan en ciertos meses (*Temora* sp. o *Centropages* sp.) (Calbet et al. 2001; Fernández de Puelles et al. 2003). A lo largo del año los diferentes estadios de desarrollo de los copépodos proveen un stock continuo de recursos alimentarios para las larvas de peces (Mazzocchi y Ribera d'Alcala 1995). Los nauplios de copépodos, uno de los elementos más importantes de la comunidad zooplancton, pueden llegar a representar el 59% del total de zooplancton en áreas del noroeste del Mar Mediterráneo (Calbet et al. 2001).

El "Ictioplancton" es el contingente zooplanctónico constituido por las etapas tempranas de los peces que se desarrollan en el entorno planctónico durante sus fases de huevo y larva (Ahlstrom y Moser 1976). En el mar Catalán también es posible observar una variabilidad estacional de la comunidad ictioplanctónica en relación tanto con el número de especies como en su abundancia a lo largo del año (Sabatés et al. 2007), más evidente en los taxones de la plataforma que en los oceánicos (Olivar et al. 1998). La mayoría de las especies que se reproducen en el noroeste del Mar Mediterráneo lo hacen durante el verano, mientras que en otoño el número de especies es inferior (Palomera y Olivar 1996), a pesar de que la biomasa de plancton es mayor en otoño. El ensamblaje de larvas de peces en el Mediterráneo noroccidental está dominado por especies de clupeiformes que se alternan durante el año como la anchoa (*Engraulis encrasicolus*) y la alacha (*Sardinella aurita*) durante la primavera y verano y la sardina (*Sardina pilchardus*) en otoño. Las especies que solo aparecen en verano junto con los clupeiformes mencionados son *Coris julis*, *Mullus barbatus* y diversas especies de la familia de los escómbridos. En otoño-invierno el número de especies que coinciden en su distribución temporal con la sardina es reducido, entre ellas el *Pagellus bogaraveo*, algunos gádidos y ammodítidos. Las especies que aparecen durante los dos periodos son más abundantes en verano que en otoño, excepto *Merluccius merluccius* que tiene su pico de reproducción en otoño. Las especies oceánicas como los mictófidos no muestran grandes diferencias en diversidad y abundancia entre los dos periodos, y su distribución está más relacionada con corrientes que con variables tróficas (Olivar et al. 2010).

El ictioplancton está considerado por definición dependiente de las corrientes horizontales para su desplazamiento, aunque puede darse migración activa (especialmente movimientos verticales) en las larvas incluso en los primeros estadios de alimentación. La distribución del ictioplancton será resultado del lugar de puesta de los adultos reproductores, la hidrodinámica y la movilidad vertical de las larvas, pero la supervivencia de las larvas de peces estará determinada de manera crucial por la falta de alimento y la depredación que sufran. La alimentación de las larvas de peces además puede llegar a tener un impacto sobre la comunidad de zooplancton en la que habitan. Por un lado, el impacto directo sobre el nivel trófico del que se alimentan (grazing impact) cuando la densidad de larvas de peces es tan alta que puede llegar a hacer mermar la población de determinadas especies (Peterson y Ausubel 1984), y por otro el efecto sobre otros niveles tróficos superiores en el ecosistema. Por ejemplo, cuando la presión de depredación de las larvas de peces modifica el tamaño medio de las presas requeridas también por peces adultos, pudiendo llegar a afectar al crecimiento de éstos.

1.2 LA IMPORTANCIA DEL ESTADIO LARVARIO PLANCTÓNICO EN LOS PECES

Según la clasificación de Balon (1981) el desarrollo de los peces puede categorizarse en base a la anatomía y funcionalidad como precocial y altricial. En el desarrollo precocial, típico de peces de baja fecundidad que ponen huevos grandes demersales o adheridos al fondo, la forma anatómica básica y los órganos internos aparecen temprano dentro del embrión o de las larvas lecitotróficas. En cambio, en el desarrollo altricial, típico de peces de elevada fecundidad que ponen huevos pequeños, los órganos internos y las capacidades funcionales se desarrollan de modo más tardío, durante el periodo larvario o en la metamorfosis a juveniles. Es este tipo de desarrollo el que caracteriza a las especies estudiadas en esta investigación, y por tanto al que nos vamos a referir a lo largo de toda la tesis.

El periodo larvario se extiende desde el momento de la eclosión y el inicio de la metamorfosis al estadio juvenil (Blaxter 1969). De forma general la larva eclosiona teniendo reservas alimentarias en forma de saco vitelino rico en energía (larva lecitotrófica), luego se reabsorbe y la larva depende de la captura de presas de su entorno para poder sobrevivir. Durante este periodo, podemos clasificar a las larvas según la flexión de la última vértebra de la notocorda y el estadio de desarrollo de la aleta caudal: larva en estadio de pre-flexión, flexión y post-flexión (Kendall et al. 1984). El desarrollo de esta característica va asociado también al desarrollo de otras estructuras que marcan el progreso de la capacidad locomotora (radios de las aletas, forma corporal). La larva pasa al estadio juvenil cuando adquiere la apariencia de un pequeño adulto: la respiración cutánea deja paso a la respiración branquial, la boca y mandíbulas son totalmente funcionales (algunas formas de alimentación como la succión pueden empezar a llevarse a cabo), el tracto digestivo se diferencia en compartimentos especializados (como el estómago), el riñón pasa de pronefrítico a mesonefrítico, etc. (Gerking 1994).

1.2.1 FACTORES QUE AFECTAN A LA SUPERVIVENCIA LARVARIA

Durante el periodo larvario ocurre la mayor mortalidad en la vida de una especie. En la mayoría de las especies de peces con desarrollo altricial, el número de larvas que alcanzan el final del estadio larvario suele estar por debajo del 0.1% (Houde 1989). Las causas de esta elevada mortalidad larvaria están relacionadas con la falta de alimento y con la depredación. Hjort (1914) definió el momento de la primera alimentación exógena como periodo crítico de supervivencia puesto que las larvas han agotado sus reservas vitelínicas y son más vulnerables a la inanición que en ningún otro momento, y por esto el alimento planctónico disponible en el medio se convierte según este autor en uno de los posibles determinantes de la fuerza de la clase anual. Hjort (1914) también estableció que pequeñas fluctuaciones en la mortalidad durante este periodo van a determinar la variabilidad en el reclutamiento de la especie.

Esta relación entre el éxito alimentario, supervivencia y reclutamiento en el momento en el que la larva pasa a alimentación exógena ha sido puesta en entredicho por otros autores que, de forma unánime, afirman que hay relación entre abundancia de comida en el momento que la larva empieza a alimentarse del entorno y la supervivencia, pero que esta relación no es siempre la determinante para el reclutamiento de las especies ya que también afecta en los otros estadios larvarios y juveniles (May 1974; Bradford 1992). La hipótesis de Cushing (1972, 1990) del acoplamiento-desacoplamiento (Match-mismatch Hypothesis) relaciona también la inanición y mortalidad larvaria en una línea similar pero menos restrictiva que la de Hjort. Cushing propuso que la supervivencia de una cohorte larvaria está ligada a la abundancia de comida entre el lugar de la puesta y el área donde se desarrollan las larvas hasta la metamorfosis a juveniles, de forma que la producción larvaria tiene que estar acoplada en el tiempo y espacio a la producción del plancton (Fig. 1.2). En esta teoría se defiende que la limitación por comida durante todo el periodo larvario (y no solo en el periodo crítico de Hjort) es el mayor determinante de la supervivencia larvaria y por tanto del reclutamiento.

La depredación es también una fuente importante de mortalidad de las larvas como han documentado muchos estudios (Purcell 1984; Bailey y Houde 1989; Pedersen et al. 2009). Existe una gran variedad de organismos invertebrados como eufasiáceos, medusas, ctenóforos, anfípodos y algunos copépodos carnívoros que son capaces de alimentarse de huevos y larvas de peces, pero las larvas de mayor tamaño no son tan vulnerables a los depredadores como las larvas lecitotróficas (hipótesis “más grande es mejor”, -Bigger is Better-) (Bailey y Houde 1989; Leggett y Deblois 1994). La depredación y la inanición están estrechamente relacionados entre sí porque a medida que las larvas crecen, pasan menos tiempo en el plancton, y tienen mas capacidad de escape frente a depredadores. La hipótesis “crecimiento-mortalidad” sugiere que las larvas de crecimiento más rápido pueden ser capaces de ganar ventajas en la supervivencia al acortar una fase de desarrollo (mecanismo de estadio y duración: Houde 1987) y reducir la depredación de modo potencial y/o el riesgo de muerte por inanición al adquirir un tamaño mayor a una edad determinada (y esto se

relaciona también con el mecanismo "más grande es mejor" -bigger-is-better-, Miller et al. 1988).

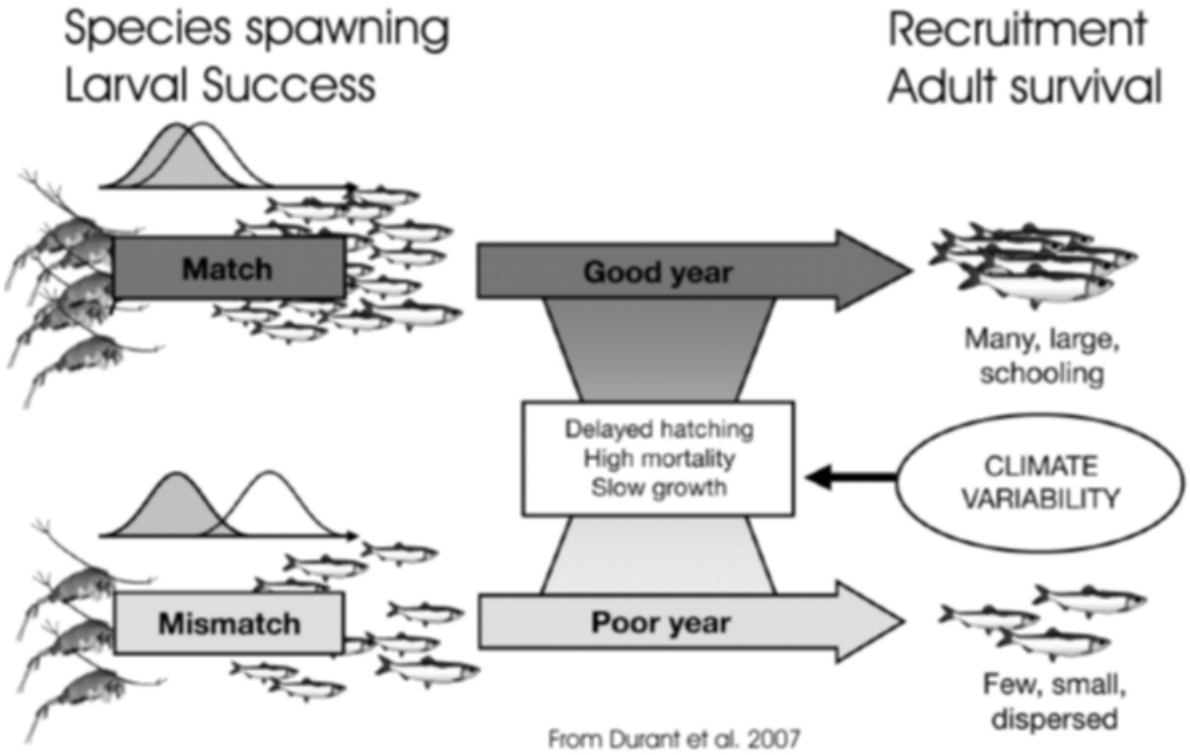


Figura 1.2. Hipótesis de acoplamiento-desacoplamiento entre producción del plancton y larvas de peces para la supervivencia larvaria y éxito en el reclutamiento;

Además de la depredación y la inanición existen otros factores de naturaleza abiótica, principalmente la hidrografía, que pueden tener un impacto positivo o negativo sobre las especies afectando también a la supervivencia larvaria y que interaccionan con los factores anteriormente descritos de diversas formas. Las condiciones oceanográficas pueden afectar a la tasa de alimentación de las larvas o al éxito de captura de sus presas. La temperatura del agua y la intensidad de luz pueden afectar a la tasa de desarrollo o a las estrategias alimentarias en las larvas (Frank y Legget 1981; Johnston et al. 2001). Fenómenos de turbulencia a microescala pueden facilitar el encuentro predador-presa y traducirse en un aumento de la tasa de alimentación en las larvas (Sundby y Fossum 1990). Las corrientes marinas pueden transportar a las larvas a zonas con mayor o menor densidad de presas que en las zonas anteriores, favoreciendo o perjudicando su éxito alimentario, así zonas de frentes como los que ocurren en el Noroeste del Mediterráneo pueden concentrar las larvas y sus presas presentando un panorama favorable (a priori) para que las larvas puedan sobrevivir.

Así pues, teniendo en consideración la influencia de la alimentación en la supervivencia larvaria y por consiguiente en el reclutamiento de la especie, esta tesis aborda el estudio de la ecología trófica de especies comercialmente importantes de las pesquerías mediterráneas: merluza, anchoa, sardina, alacha y melva. El estudio de la alimentación de las larvas de dichas especies se ha abordado en relación al desarrollo ontogénico de sus larvas y a la variación en la disponibilidad de presas en su entorno con objeto de esclarecer, por un lado, qué estrategias conducen a un mayor

éxito alimentario durante los estadios tempranos y por otro, el papel trófico de estas especies en el ecosistema.

1.2.2 ÉXITO ALIMENTARIO

La abundancia de presas (densidad y distribución) y otros factores involucrados en el encuentro depredador-presa como el comportamiento nadador de las presas y la respuesta de escape, la visibilidad de la presa y la experiencia previa de la larva (Govoni et al. 1986; Buskey et al. 1993) pueden marcar el éxito alimentario de las larvas. El éxito de las larvas de peces a la hora de alimentarse depende también de otros factores entre los cuales las condiciones de luz ambiental juegan un papel fundamental puesto que la mayoría de las larvas son depredadores visuales (Blaxter 1986). Las larvas de algunas especies pueden ser comedores oportunistas, ingiriendo presas en la misma proporción que su abundancia en el ambiente, mientras que otras larvas pueden mostrar preferencia por presas de un cierto tamaño (Scharf et al. 2000). Generalmente las larvas de peces son muy flexibles y modifican su comportamiento alimentario (Munk 1992, 1995), y las restricciones que las larvas tienen al eclosionar son progresivamente superadas gracias al crecimiento temprano de las estructuras para las necesidades básicas, y posteriormente las prioridades van cambiando durante la ontogenia (Osse et al. 1997).

La zooplanctivoria inicial es necesaria en las larvas de teleósteos para alcanzar sus requerimientos metabólicos y poder crecer puesto que no están preparadas para ser herbívoras, incluso si una vez convertidas en adultos éste es su tipo de alimentación. El 50% del peso seco de los juveniles de peces está compuesto de proteínas y la comida que ingieran debe aportarles una composición similar, y esto solo se puede alcanzar alimentándose de otros animales (Conceição 1997). La teoría de búsqueda de comida óptima (Optimal Foraging Theory) establece que los predadores deberían seleccionar las presas que maximizan la ganancia energética disponible en relación al coste energético de capturar, ingerir y digerir las presas (Pyke 1984). La larva decide si atacar o no a su presa, esto conlleva un coste o beneficio energético, por lo que tiene que tomar la decisión en pro del resultado deseado de este encuentro. La decisión se hace a través del reconocimiento de la presas con los órganos sensoriales (en los planctívoros, la visión en la más importante tal y como ya se ha mencionado). Mientras que los beneficios de alimentarse es alcanzar la energía suficiente para crecer y estar saludable, los costes de este proceso se producen en la búsqueda, captura, ingestión, digestión y gasto metabólico. El balance tiene que ser positivo para la supervivencia del animal.

1.3 DESARROLLO DE LAS ESTRUCTURAS RELACIONADAS CON LA ALIMENTACIÓN

Las larvas de peces emplean diversas estrategias alimentarias según el área y temporada de puesta y eclosión larvaria, y estas estrategias están relacionadas con la morfología y fisiología de las estructuras y órganos relacionados con su alimentación (Mark et al. 1987; Bremigan y Stein 1994). Es razonable pensar que si las estructuras para alimentarse aparecen en estadios tempranos y además se desarrollan a una velocidad mayor que el resto del cuerpo, las especies que sigan este patrón tendrán a priori una ventaja mayor para poder alimentarse con éxito respecto a otras más retardadas.

Entre las estructuras más relevantes involucradas en el modo de alimentación están los ojos (desarrollo de la visión), la boca y el tracto digestivo. También la natación de la larva y la flotabilidad son aspectos importantes que van a marcar la selección del alimento. Las estructuras relacionadas con la alimentación difieren para una misma especie a lo largo de la ontogenia así como entre las especies (species-dependent), y por tanto pueden actuar como un indicador de su elección de presas y de sus habilidades predatorias. Con el desarrollo de las aletas mejora la actividad natatoria, lo que contribuye a incrementar la eficiencia de la captura de sus presas, y esto se podría traducir en una especialización de presas más evasivas o por el contrario en un potencial aumento y diversificación del espectro de la dieta. Por tanto, el comportamiento alimentario va a cambiar durante la ontogenia larvaria según los cambios morfológicos que tienen lugar. No solo la actividad natatoria y maniobrabilidad van a mejorar durante el crecimiento, también el tamaño de la boca aumentara y esto conlleva una mejora en la eficiencia predadora. Sin embargo, la alimentación también es dependiente del tamaño de presa y morfología de la presa. Con el aumento del tamaño las presas mejoran su movilidad y/o sus mecanismos de defensa (como espinas) (Scharf et al. 2000). Así pues, las mejoras de los depredadores pueden estar contrarrestadas por el comportamiento de evasión de las presas.

1.3.1 EL SISTEMA VISUAL

La diferenciación del sistema visual tiene lugar rápidamente tras la eclosión. Las larvas confían en la visión para reconocer las partículas de comida y reaccionar frente a predadores (Blaxter 1986). Aunque la retina es funcional en la mayoría de las especies al comienzo de la alimentación, su ojo relativamente pequeño puede limitar la detección de las presas y por tanto la ejecución de su captura (Pankhurst 1994). Una retina formada totalmente por conos, la más frecuente en las larvas en preflexión, sugiere que en la primera alimentación las larvas están restringidas a la visión fotópica y a la alimentación en las horas diurnas. Durante el crecimiento y la ontogenia, el diámetro de la lente del ojo y la densidad de los fotorreceptores aumenta, la retina se expande y todo esto conduce a una mejora de la agudeza visual (grado de resolución

visual). Además, un tipo de células fotorreceptoras -los bastones- aparecen en el ojo de las larvas en estadio de postflexión y en los juveniles, aumentando la sensibilidad óptica (capacidad para ver bajo condiciones de poca luz (Blaxter 1986; Pankhurst 1994)). Una visión mejorada permite detectar a las presas más fácilmente gracias a un mayor volumen de búsqueda de las larvas y distancia a la que las larvas responden a un tamaño o tipo de presa determinado (Miller et al. 1993), incrementando así la disponibilidad de presas.

1.3.2 LA BOCA

La morfología de la boca tiene implicaciones importantes en el modo de alimentación. La boca ha sido a menudo identificada como la pieza morfológica que limita el tamaño de las presas en los peces que tragan a sus presas enteras (DeVries et al. 1998; Sabatés y Saiz 2000). El modo más versátil de alimentarse entre los vertebrados es la succión (Liem 1980, en Gerking 1994) que consiste en que la comida es dirigida al interior de la cavidad bucal impulsada por una corriente de agua. La succión se desarrolla cuando la cavidad bucal se expande y crea una presión negativa. Expandiendo el cono bucal (boca y cavidad bucal) se incrementa su volumen y se dirige el agua hacia el interior de la cavidad bucal. Contrayendo el cono se fuerza al agua hacia la cámara opercular desde donde se expele. La expansión del cono determinará la velocidad del flujo del agua que entra, y a una velocidad mayor habrá una eficiencia de succión mayor. La forma, el tamaño y la capacidad para modular la acción muscular de la cavidad bucal serán determinantes para manipular la comida (Liem 1990). La mayoría de las larvas de peces se alimentan de pequeñas presas vivas mediante el mecanismo de succión con la particularidad de que estas presas son ingeridas una a una (o unos pocos a la vez), con lo que recibe el nombre de alimentación particulada (particulate feeding mode). El rápido crecimiento inicial de la boca prepara a la larva para una captura exitosa de presas (Osse et al. 1997) que es crucial en el momento en el que pasa a depender de la alimentación exógena y permite incrementar el tamaño de presa capturable para abastecer la demanda de aumento de energía que le permita crecer. Pero el tamaño de la boca no es el único elemento crucial para la alimentación, el desarrollo de las estructuras asociadas (sistema de anclaje hioides-opercular) también desempeña un papel importante por limitar la ejecución de la captura e ingestión de presas durante los estadios iniciales de vida (Wittenrich et al. 2009).

1.3.3 EL TRACTO DIGESTIVO

Las diversas adaptaciones estructurales del canal alimentario de las larvas de peces y los cambios que sufren con el desarrollo son características de los diferentes ajustes funcionales a las dietas, por lo que la incidencia alimentaria puede estar relacionada con la morfología del digestivo y la digestibilidad de las presas (Conley y Hopkings 2004).

El crecimiento y supervivencia de las larvas de peces dependen de la captura, digestión y asimilación de comida (Houde y Schekter 1980, 1983). La larva con vitelo muestra un incipiente

digestivo recto, simple y no diferenciado aunque funcional, y hacia el final del periodo larvario, con la transformación a juveniles, el sistema digestivo se convierte en un complejo canal segmentado similar al de los adultos, a pesar de que la diferenciación durante la ontogenia difiere entre taxones (Govoni et al. 1986). La morfología del tracto digestivo ha sido ampliamente discutida como un factor relacionado con la forma de alimentarse de las larvas. Algunas especies de peces mantienen tubos digestivos rectos y estrechos a lo largo de toda la etapa larvaria, como los de los clupeidos y los engráulidos, especies que han sido identificadas como aquellas con menores índices de incidencia alimentaria (Arthur 1976; Pepin y Penney 2000), mientras que otras especies desarrollan poco después de la eclosión unos digestivos torsionados con varios compartimentos que favorecen la retención de las presas y con pliegues que otorgan más espacio para la absorción, especies que presentan en general altas incidencias alimentarias. La distinta dinámica del digestivo influirá por tanto en los requerimientos diarios de comida y se reflejará en los contenidos digestivos de cada especie.

1.4 FUNDAMENTO DE LA TESIS Y OBJETIVOS

El ictioplancton es la primera fase del desarrollo de los peces en la que se producen las mayores tasas de mortalidad. Los procesos que influyen sobre la supervivencia de huevos y larvas de las especies marinas están ligados a los principales mecanismos físicos de agregación y dispersión que regulan sus distribuciones y las de sus presas, y al tipo de la estrategia de vida de cada especie (tasas de crecimiento, periodo y lugar de freza, etc.).

La comprensión de los factores que afectan a la supervivencia del ictioplancton pasa por conocer como huevos y larvas se integran en el ambiente físico y trófico en el que deben desarrollarse hasta alcanzar el tamaño y capacidad natatoria suficiente (paso a la categoría de necton) que permite mejorar su autonomía en la búsqueda del hábitat y el alimento óptimos.

La dinámica de las primeras fases de desarrollo de los peces está estrechamente ligada a la de sus presas, por lo que caracterizar el entorno ambiental en el que se desarrollan es prioritario para comprobar el papel que juega el factor "cantidad, calidad y localización del alimento" sobre las distribuciones larvarias, tanto como modulador en la concentración de larvas de peces como en su efecto sobre la probabilidad de supervivencia valorada a través de su éxito alimenticio.

El objetivo general de esta tesis doctoral es analizar la ecología trófica de las larvas de 5 especies de peces importantes en las pesquerías del Mediterráneo, teniendo en cuenta su distribución, su dieta y sus capacidades depredadoras, en relación al ambiente físico y trófico durante sus principales épocas de desarrollo (primavera-verano y otoño). Estas especies son: la merluza (*Merluccius merluccius*), la anchoa (*Engraulis encrasicolus*), la sardina (*Sardina pilchardus*), la alacha (*Sardinella aurita*) y la melva (*Auxis rochei*). La hipótesis de partida fue: **existe una relación entre el éxito alimentario de las larvas de peces y la disponibilidad de presas en el ambiente, con diferencias entre las especies debidas a los diversos morfotipos de las larvas y con patrones intraespecíficos que aumentan su importancia según el grado de desarrollo.**

De las especies objetivo de esta tesis, la merluza es la que tiene mayor importancia económica en las pesquerías de todo el Mediterráneo. De las especies pelágicas, la sardina suele ser la más abundante a nivel de capturas, aunque desde el punto de vista económico la anchoa es más importante. Las otras dos especies estudiadas son la alacha, más frecuente en aguas más cálidas aunque su abundancia ha ido aumentando en el Mediterráneo noroccidental en las últimas dos décadas (Sabatés et al. 2006), y la melva, pequeño túnido común en la región y que está adquiriendo mayor relevancia en los mercados.

La investigación se plantea desde dos puntos de vista comparativos: 1) el efecto de la morfología y desarrollo de las larvas en la elección del alimento entre especies que comparten un mismo escenario ambiental en el tiempo, y 2) el efecto del entorno físico-biológico en la elección del alimento entre larvas de especies con similares características morfológicas y ontogénicas pero

que se desarrollan en épocas con condiciones oceanográficas y tróficas distintas.

En base al objetivo general de la tesis se han planteado los siguientes objetivos específicos:

a) Determinar la relación entre la concentración de presas y la abundancia y éxito alimentario de las larvas de las especies estudiadas.

- Comprobar si la concentración de larvas covaría positivamente con la concentración promedio de presas potenciales.
- Comprobar si las larvas tienen un mayor éxito alimenticio donde la concentración de presas es más elevada.

b) Determinar la relación entre el tipo de dieta y la morfología y desarrollo ontogénico en las larvas de las cinco especies.

- Identificar el tipo y tamaño de presas que cada especie ingiere a lo largo de su desarrollo.
- Evaluar la preferencia (selección) de presas en cada especie y estadio larvario.
- Describir los cambios de las estructuras relacionadas con la alimentación como son ojos (y la visión), crecimiento del aparato bucal y morfología del digestivo durante el desarrollo ontogénico de las larvas.
- Determinar si hay diferencias en los hábitos alimenticios que puedan explicarse en base a la morfología y/o ecofisiología de las estructuras relacionadas con la alimentación (sistema visual, boca y digestivo).

c) Evaluar las similitudes o divergencias interespecíficas e intraespecíficas en la dieta larvaria de las cinco especies y determinar en qué casos se da solapamiento en la dieta.

Para la consecución de los objetivos propuestos se contó con un diseño de muestreo que contempla extensas áreas de plataforma, cantil y mar abierto del mar Catalán a una resolución de mesoescala y dos periodos del estudio de conocido contraste en la composición del ictioplancton y las condiciones oceanográficas y tróficas como fueron junio y noviembre del 2005. En el primero queda reflejada una situación de verano caracterizada por la presencia de aguas continentales sobre la plataforma, alta estabilidad de la columna de agua y baja intensidad de las corrientes cerca de la costa, y en el segundo una situación otoñal caracterizada por la homogeneidad vertical de la columna de agua y la presencia de la corriente catalana intensa cercana a la costa. Además de las diferentes condiciones ambientales, este estudio también ha tenido en consideración las diferencias y similitudes morfológicas de las especies objetivo para ver como influyen en los patrones de alimentación de las larvas, y buscar bases comunes en la selección por las presas. Se ha querido así dar respuesta a preguntas como:¿afecta el tamaño de boca de la larva a la selección de presas de distinto tamaño? ¿las larvas con bocas similares comen presas de tamaño similar?, ¿hay patrones específicos de cada especie en la selección de presas?, ¿las presas más móviles son capturadas por

las larvas con morfología más robusta?, ¿las especies con digestivo rectilíneo acumulan menos presas en él?, ¿el desarrollo visual es distinto entre especies y esto condiciona la captura de presas de distinta apariencia? etc.

La investigación de esta tesis ha sido llevada a cabo en el contexto de un proyecto multidisciplinar mas amplio donde se ha investigado la estructura espacial (horizontal y vertical) de las fases planctónicas de la merluza, anchoa y sardina para determinar las pautas de distribución de sus huevos y larvas con una resolución espacial superior a la disponible hasta ese momento: escala fina (10-10^2m), gruesa ($10^2$$10^3$m) y mesoescala ($10^3$–$10^5$m), y analizar el efecto del entorno (hidrografía y plancton) sobre las mismas. El foco de este proyecto era dilucidar las estrategias de vida de las especies, y el efecto de factores físicos y biológicos como determinantes de la supervivencia larvaria. El trabajo presentado en esta tesis ha estado dirigido a obtener información básica y necesaria para interpretar los patrones de distribución y dilucidar por primera vez el efecto del tipo de ecología trófica de diversas especies de peces en la supervivencia larvaria. A las tres especies objetivo del proyecto (merluza, anchoa y sardina) se han añadido otras dos (alacha y melva) por ser interesantes desde el punto de vista comparativo de sus estrategias alimentarias. Hasta el momento no se había abordado el estudio de la ecología trófica de las larvas de estas especies en el mar Catalán en relación a la distribución de sus presas potenciales del plancton para evaluar el papel que juega el acoplamiento espacio-temporal de larvas y presas en la estrategia alimentaria. También es la primera vez que se estudia la dieta de estas especies en relación a la morfología de estructuras relacionadas con la alimentación como son el sistema visual, la boca y el tracto digestivo.

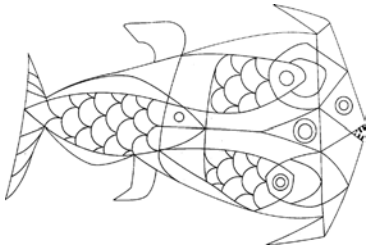
2

RESULTS

RESULTADOS

Chapter 2

2.1 TROPHIC ENVIRONMENT AND LARVAL ASSEMBLAGE



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The role of oceanographic conditions and plankton availability in larval fish assemblages off the Catalan coast (NW Mediterranean)

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ABSTRACT

In the northwestern Mediterranean, most fish species reproduce in early summer and fewer in the autumn mixing period. This study analyses and compares larval fish assemblages (LFA) in both seasons, and is the first attempt to characterize LFA structure for the autumn period. We analyze horizontal and vertical distribution of fish larvae and the micro- and mesozooplankton biomass and abundance of the main zooplankton groups. The oceanographic situation was analyzed through the study of data from CTD, Nv-Shuttle and ADCP surveys. LFA were determined by ordination analyses based on larval abundance, and the relationships between larval assemblages and environmental variables were investigated through canonical correspondence analysis. The importance of some hydrographic variables (temperature, salinity and stability of the water column), current fields (along-shelf and across-shelf transport) and the abundance of zooplankton are discussed as important factors shaping the structure of larval assemblages. In early summer, LFA were mainly structured by a combination of bathymetry and trophic components, although sea surface temperature also played a role in shaping the horizontal larval distributions. In autumn, trophic variables were the main factors influencing the shelf-dwelling species assemblage. Larvae of oceanic species, on the other hand, were not related to trophic variables but were more affected by current fields.

Key words: diversity, fish larvae, horizontal distribution, larval transport, temporal variability, vertical distribution, zooplankton

INTRODUCTION

Early ontogenetic stages of fish develop in the planktonic environment, so they are subject to the effect of major physical processes such as changes in temperature and salinity, fronts and currents, and biological processes such as food availability and the presence of predators. The variability of these processes affects the distribution and survival of fish larvae directly or indirectly, leading to great variations in the annual recruitment of species (Govoni, 2005). The study of ichthyoplankton assemblages is currently used to determine how these factors act similarly on the different species that coincide in the plankton during the early stages of development (Moser and Smith, 1993; Hare *et al.*, 2001; Keane and Neira, 2008), although the adults do not always share the same habitats. The results of this type of research are often dependent on the spatial and time scales of the sampling and on the degree of resolution in the identification of the organisms. Furthermore, the interpretation of the groups obtained tends to be constrained by the environmental information available and the type of analysis that can therefore be applied (Keane and Neira, 2008). Despite the importance of geostrophic circulation in larval distributions (Reiss *et al.*, 2000; Sánchez-Velasco *et al.*, 2006), these variables have seldom been incorporated in the matrices of hydrographic data used to explain larval fish assemblages (Boeing and Duffy-Anderson, 2008). However, it is increasingly common to use information on the physical parameters obtained with CTD surveys (Grioche *et al.*, 1999; Smith *et al.*, 1999). Furthermore, although the concentrations of microzooplankton may be critical for the survival of larvae (Cushing, 1994; Platt *et al.*, 2003), few studies include

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abundance data for the different groups of zooplankton (e.g., Munk *et al.*, 2003; Lee *et al.*, 2005), and the biomass of zooplankton and fluorescence are the variables most commonly used (Isari *et al.*, 2008).

The study area is located in the northwestern Mediterranean, a temperate and fairly oligotrophic region characterized by the SW flow of the Catalan Current (CC) along the continental slope. This current is associated with a marked salinity/density shelf-slope front, which separates the relatively low salinity waters (<37.5) of the continental shelf [influenced by Atlantic Water (AW) and by the discharges of the rivers] from the more saline open-sea waters (>38.1) (Font *et al.*, 1988). There is a clear seasonal signal in the strength of the current, with low intensity in summer and re-intensification in autumn (Pinot and Ganachaud, 1999). The CC has significant mesoscale activity, including the frontal oscillations and the succession of mesoscale anticyclonic eddies travelling over the continental shelves and modifying the local flow (Rubio *et al.*, 2005).

The biological production in the zone is mainly associated with the inputs of nutrients provided by waters of continental origin from the rivers of the region (Estrada, 1996; Salat, 1996; Cruzado *et al.*, 2002), with mesoscale phenomena in the region of the shelf-slope front, and with autumn–winter mixing processes (Salat, 1996). During the stratification period, the maximum concentrations of chlorophyll occur above 60–70 m (deep chlorophyll maximum, DCM) (Estrada, 1996). The maximum concentrations of zooplankton organisms are also observed there when they are feeding (Saiz *et al.*, 2007), with the exception of the zones influenced by waters of continental origin, where a surface chlorophyll maximum develops. The greater availability of nutrients in the photic layer during the autumn mixing period leads to a large zone of relatively high chlorophyll concentrations (Gordoa *et al.*, 2008), which contributes to the development of zooplankton populations that are more homogeneously distributed along the water column. In the NW Mediterranean there have been several studies of distribution patterns of fish larvae and larval fish assemblages in the spring-summer months (Sabatés, 1990; Sabatés and Olivar, 1996), whereas research on the autumn–winter period has focused mainly on the distribution patterns of specific species (Olivar *et al.*, 2001, 2003; Sabatés, 2004). The analyses carried out for the spring-summer period have shown that the larval fish assemblages were established in function of the bathymetry and the presence of thermohaline fronts (Sabatés, 1990; Sabatés and Olivar, 1996). The influence of more dynamic events such as the currents or the degree of instability of the water column, which can directly affect larval distributions, was not included in the analysis of the larval fish assemblages, although earlier studies indicate that the shelf-slope current circulation affects larval distributions of both oceanic and shelf species (Sabatés *et al.*, 2004; Maynou *et al.*, 2006).

In this study we assess how larval fish assemblages are structured by local hydrodynamic and biological conditions, and prove the importance of including information on physical and biological variables for the interpretation of patterns. To this end we compare larval fish assemblages and their horizontal and vertical distributions in early summer and autumn in terms of water properties (temperature, salinity, stratification of the water column and current fields), and explore the influence of biological factors such as fluorescence levels, zooplankton biomass and abundance of the main micro- and mesozooplankton components. This research was based on a multidisciplinary study in which both plankton and hydrography were analyzed at a meso-, coarse and fine scale.

MATERIAL AND METHODS

The present research is based on two oceanographic plankton surveys carried out on the southern Catalan coast (NW Mediterranean) in early summer (16–22 June) and autumn (12–16 November) 2005. We used a series of transects perpendicular to the coast, 6–7 miles apart, in which the stations were located every 2–2.5 miles. On the shelf margin, a few stations were added at shorter distances (Fig. 1). Although the grid of stations was similar in both cruises, some stations from the southernmost part were eliminated from the November cruise due to rough weather conditions (transect 6 and the coastal-most station of transect 5).

Hydrographic and biological data were obtained sequentially at each station. The basic hydrographic variables [temperature (T), salinity (S) and fluorescence (F)] in the first 300 m of the water column were obtained with a CTD SBE-25 equipped with a Sea-Tech fluorometer. The vertical profiles of measured and calculated parameters (potential temperature and Brunt–Väisälä frequency) were averaged with a 1-m interval. TS diagrams including all temperature and salinity CTD data were plotted to identify water masses. Brunt–Väisälä frequency is a measure of water stability, with higher values associated with higher stability, calculated as in Mann and Lazier (1996). In addition, the first 150 m of the water column were scanned with the 'Nv-Shuttle' (towed undulating

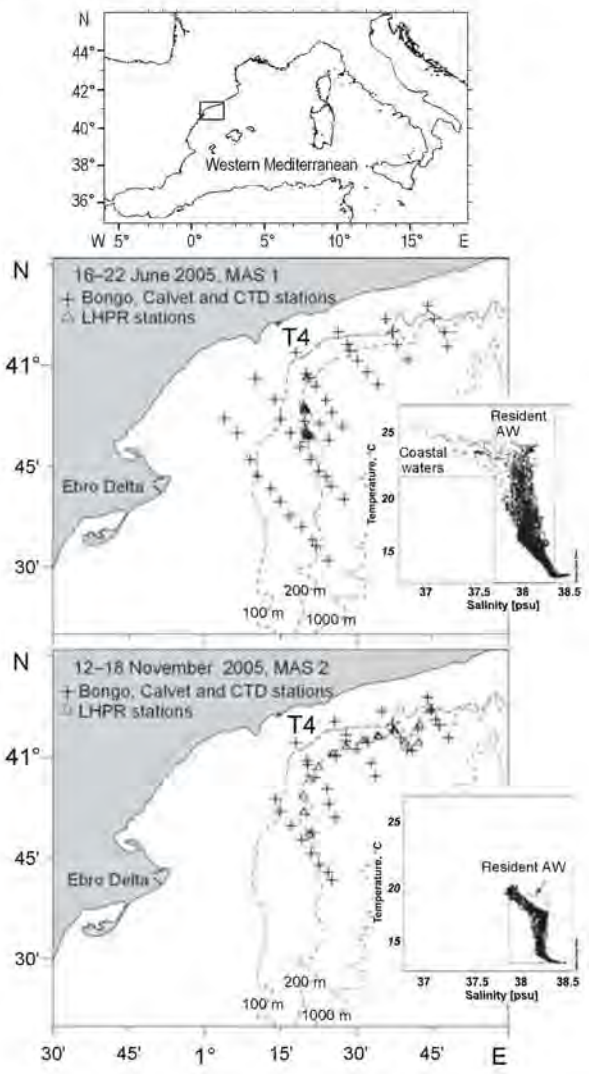


Figure 1. Study area and TS diagrams for the June (MAS1) and November (MAS2) surveys. T4: transect 4.

vehicle, Chelsea Technologies Group, West Moseley, UK), which allowed us to obtain the vertical distributions of temperature, salinity and fluorescence with high spatial resolution. Vertical cross-sections were represented using OCEAN DATA VIEW software (Schlitzer, 2008). The data from a 150-kHz vessel-mounted narrowband acoustic Doppler current profiler (ADCP) were used to estimate the direction and magnitude of the currents. The hydrographic data-gathering strategy in the study area was divided into two stages. During the first part (outward), which corresponded to the journey from Barcelona to the Ebro Delta, the work requiring stops and repositioning of the boat was carried out: the CTD casts and the plankton hauls. ADCP data were also recorded during this part of the surveys. During the second part (return) the measures were taken continuously without interruption using an undulating N*v*-Shuttle CTD system and acquisition of ADCP data. Due to technical problems, during the June survey the N*v*-Shuttle was used only in the two southern transects. With the data obtained with the N*v*-Shuttle and CTD, the potential temperature and the Brunt–Väisälä frequency were calculated. To relate the oceanographic situation and the larval distributions of the fishes, the surface level and the 60 m level were selected. The dynamics of surface waters are represented as the spatial distribution of the velocity vectors (magnitude and direction) at 16.5 m depth.

At each station vertical micro- and mesoplankton hauls were carried out using a CalVET net (Smith *et al.*, 1985) fitted with a 53- and a 200-μm mesh, respectively. The larvae were then collected using oblique Bongo hauls at a ship speed of two knots ($\sim$1 m s^{-1}) from a maximum depth of 200 m, or from 10 m above bottom to the surface. The mesh used to quantify the ichthyoplankton was 300 μm. All the nets had a flowmeter fitted in the centre of the mouth.

The samples of micro- and mesoplankton collected with the CalVET net were divided into two using a plankton splitter. Half were frozen in liquid nitrogen for later analysis of the organic matter content. Previously, to remove mesoplanktonic organisms from the >53-μm samples they were passed through a 200-μm mesh, and to remove macrozooplankton from the >200 μm samples they were passed through a 2-mm mesh.

The vertical distribution of the plankton was obtained by stratified hauls every 10 m between the surface and 150 m depth, using an Longhurst-Hardy Plankton Recorder (LHPR) net with meshes of 280 and 53 μm. The original design of the LHPR was based on the CPR (Wiebe and Benfield, 2003). In the June survey a fixed station in the wide shelf zone located to the north of the Ebro Delta was sampled over a depth of 200 m. Seven stations were analyzed to establish a vertical distribution of fish larvae and the biomass of microzooplankton (four samples during daylight hours and three at night), and two stations were analyzed during daylight hours to determine the vertical distribution of copepod nauplii and postnauplii. In the November survey, 15 stations were sampled (10 during daylight hours and five at night) along the 200-m isobath from the southern shelf to the zone off Barcelona. On this occasion, the final position of each haul was used as the initial position of the following haul. The vertical position of the copepod nauplii and postnauplii was analyzed for two of the stations sampled during daylight hours.

The plankton samples for quantification of organisms were immediately fixed in 5% borax-buffered formalin and stored for later analysis in the laboratory. All fish larvae were separated from the plankton samples and identified to the lowest taxonomic level possible. The larval abundances of the Bongo samples were calculated as number of larvae per 10 m^2 of sea surface using the volume of water filtered through the net and the maximum depth sampled at each station. The larval abundances of the LHPR samples were calculated as individuals per 1000 m^3. For each species we calculated the weighted mean depth (WMD) of larvae in each sample series as:

$$\mathrm{WMD} = \sum_{i=1}^{n} P_i Z_i \qquad (1)$$

where Z is the depth of the ith sample (at the middle of the strata), and P is the proportion of larvae at that depth (Fortier and Leggett, 1983).

For each station we calculated the diversity of the larval taxa using the Shannon–Wiener index. Larval identification was mainly done following Bertolini *et al.* (1956), Russell (1976) and Richards (2006). In some particular instances, e.g., for the Sparidae species of the June cruise, we followed Klimogianni and Kaspiris (2004) to identify *Pagellus erythrinus*. The differentiation of *Diplodus sargus* from similar larvae was based on its lack of dorsal tail pigmentation (difference with *Oblada melanura*), absence of large stellate melanophores on the lateral walls of the gut (difference with *Diplodus annularis*) and presence of a continuous series of mid-ventral series of melanophores just after the anus (difference with Centracantidae larvae).

The original plankton samples (micro- and meso-) were diluted to a volume of 100 mL and sub-sampled into aliquots after gentle mixing to distribute organisms randomly. Subsamples were analyzed in counting chambers under a binocular microscope. One or more counting chambers were analyzed from each sample until 100 individuals of the most abundant taxonomic category or 30 individuals of the three most abundant categories were counted. Zooplankton organisms were identified to species level, and to developmental stages when possible, but for the purposes of this study they were grouped into coarse taxonomic categories in most cases (tintinnids, cladocerans, etc.), and into developmental stages (eggs, nauplii, postnauplii) and subcategories (calanoid postnauplii, cyclopoid postnauplii, etc.) in the case of copepods. Postnauplii include all developmental stages from first copepodite stage to adult stage. As their vertical distribution showed their presence along the water column, the abundances were expressed per unit volume for both the CalVET net and the LHPR net. Seston biomass determinations were carried out by filtering through washed and pre-combusted (450°C, 24 h) Whatman G/FC glass-fibre filters after sampling, and immediately frozen at liquid nitrogen temperature until analyses were made. Particulate organic matter (POM) of the micro- and mesoplankton was measured gravimetrically on the pre-weighed ash-free filters in a ±0.1 μg precision microbalance after samples were consecutively dried (80°C) and burned (450°C) for 24 h.

To establish the relationship between larval fish assemblages and the environmental variables in each survey, canonical correspondence analysis (CCA) was performed (Ter Braak and Verdonschot, 1995) on the larval abundance matrices and the environmental parameter matrices. Because the June cruise has one more transect than the November one (transect 6), we performed two CCA: one using data from all the transects and a second analysis using just the common ones. Here we present results obtained using data coming from all the stations because the resulting groups of species were the same in both cases and the environmental variables with higher correlation to the canonical axis showed almost no differences using both matrices. For the density matrices the taxa that appeared at <10% of the stations and those that had an abundance of <0.1% were excluded. The environmental data matrix included the values of the oceanographic variables (both directly measured and derived) at the surface and at 60 m depth, the organic matter of the micro- and mesoplankton, the density of the main groups of zooplankton, and the depth. The current velocity and direction values were transformed into their Cartesian components: along-shore velocity (Va) and across-shelf velocity (Vc). When the correlation between pairs of variables was >0.67, one of them was eliminated. All the ordinations were performed using CANOCO version 4.5 (Ter Braak and Šmilauer, 2002). The forward selection of CCA, which is analogous to step-wise multiple regression, was used to determine the minimum number of explanatory factors that could explain statistically significant ($P < 0.05$) proportions of variation in the larval taxa data. Due to the spatial autocorrelation of the samples, we did not attempt to formally assess the significance of the environmental variables in the ordination through a permutational test (Legendre, 1993).

RESULTS

Oceanographic patterns

The TS diagram of all the CTD data of June indicates that most of the study area was occupied by resident Atlantic Waters (AW) ($13.58 < T < 23.35$; $37.82 < S < 38.28$), with a freshwater continental influence on surface layers, as denoted by the lower salinity values at 20–25°C (Fig. 1) ($S < 37.7$). The surface isotherm of 23°C divides the study area into two sectors, of which the southwestern one is warmer. The surface salinity was far lower on the whole continental shelf of this southern part, near the Ebro Delta, and the highest surface fluorescence was found in this zone (Fig. 2). Below 60 m depth the horizontal temperature and salinity distributions were fairly homogeneous.

The vertical structure (Fig. 3) showed the importance of summer stratification, with the maximum Brunt–Väisälä frequency values in the first 25 m. The main temperature and salinity gradient was also observed at this level. The vertical structure of the fluorescence was characterized by a surface peak associated with the presence of waters of the Ebro River and the typical deep fluorescence maximum between 60 and 70 m at the stations on the shelf-slope margin.

The water dynamics (ADCP maps, Fig. 4) showed weak SW current velocities of the Catalan Current along the edge of the continental shelf of the order of 5–10 cm s^{-1}, except at the shelf break in the far south, where velocities were higher. On the shelf the dynamics was more complex, with an inshore–offshore flow particularly in the northern part and evidence of an eddy in the wide shelf zone to the north of the Ebro Delta. In the days of sampling the surface current maps showed a flow towards the open sea in the south, but in the following 2 days of the second part of the survey, this flow had disappeared.

The TS diagram of the CTD data of the November survey shows more homogeneous temperature and salinity than in summer, with a range of values

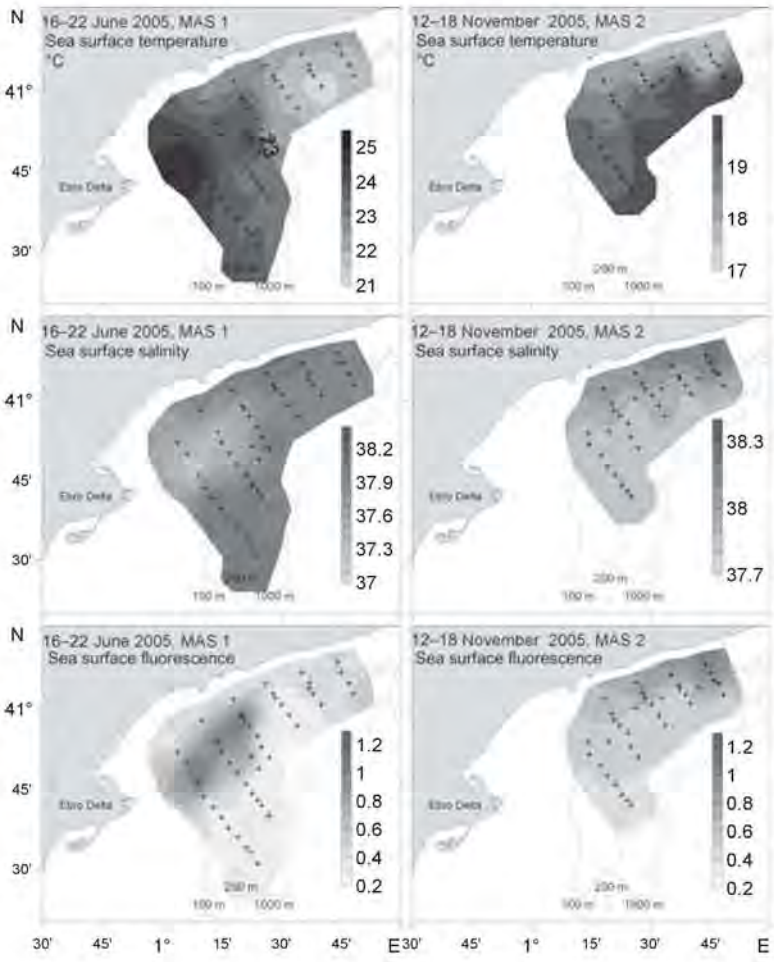


Figure 2. Horizontal distributions of sea surface temperature, salinity and fluorescence for the June and November surveys.

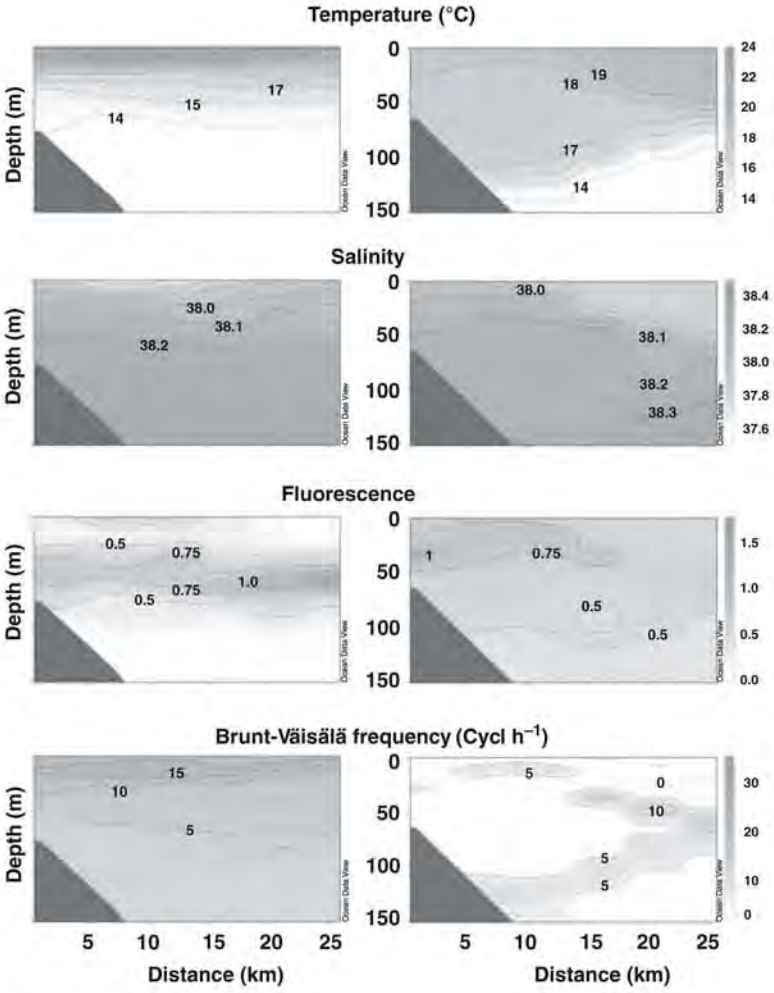


Figure 3. Cross-shelf vertical distribution of temperature, salinity, fluorescence and Brunt–Väisälä frequency in Transect 4 (indicated in Fig. 1) for the June (left) and November (right) surveys.

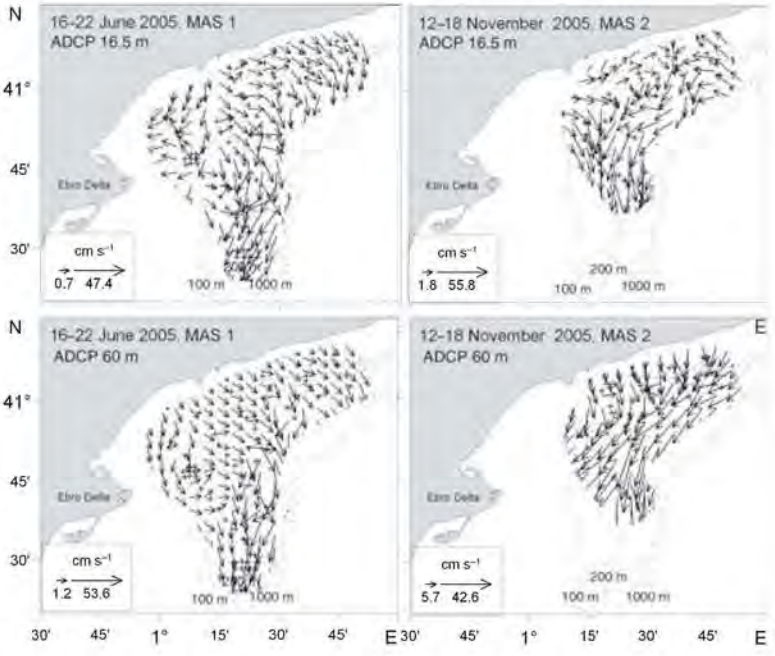


Figure 4. Distribution of horizontal ADCP currents at 16.5 and 60 m depth for the June (left) and November (right) surveys.

(13.45 < T < 19.80; 37.83 < 38.30) that indicate that the waters were resident AW influenced by the autumn conditions (lower T than in summer) and the absence of continental water influence (Fig. 1). The range of surface temperatures was narrower than in summer, only 2°C, and the lowest temperatures were observed in the coastal strip. The salinity and fluorescence showed a coast–open sea pattern with the highest values in the coastal strip (Fig. 2). At 60 m the distributions of these variables over the study area were more homogeneous.

The vertical profiles of temperature, salinity, fluorescence and Brunt–Väisälä frequency (Fig. 3) showed the start of the processes of autumn mixing and homogenization of the water column, which are more noticeable in the continental shelf area. On the ADCP maps it can be seen that a large part of the study area was under the influence of the Catalan Current, which was located nearer to the coast than in summer, with a predominant flow in the SW direction and surface velocities of the order of 30–40 cm s^{-1} (Fig. 4). To the north of the Ebro Delta the continental shelf becomes wider, implying that the Catalan Current, which flow following the shelf-break, ends up on the wide shelf zone converging with shelf waters. During this cruise, no changes were observed in the patterns of the current between the sampling dates and the two following days.

Zooplankton patterns

In both periods the maximum biomasses of micro- and mesoplankton were obtained at the stations of the continental shelf and the shelf break; they were higher in early summer than in autumn (Fig. 5a).

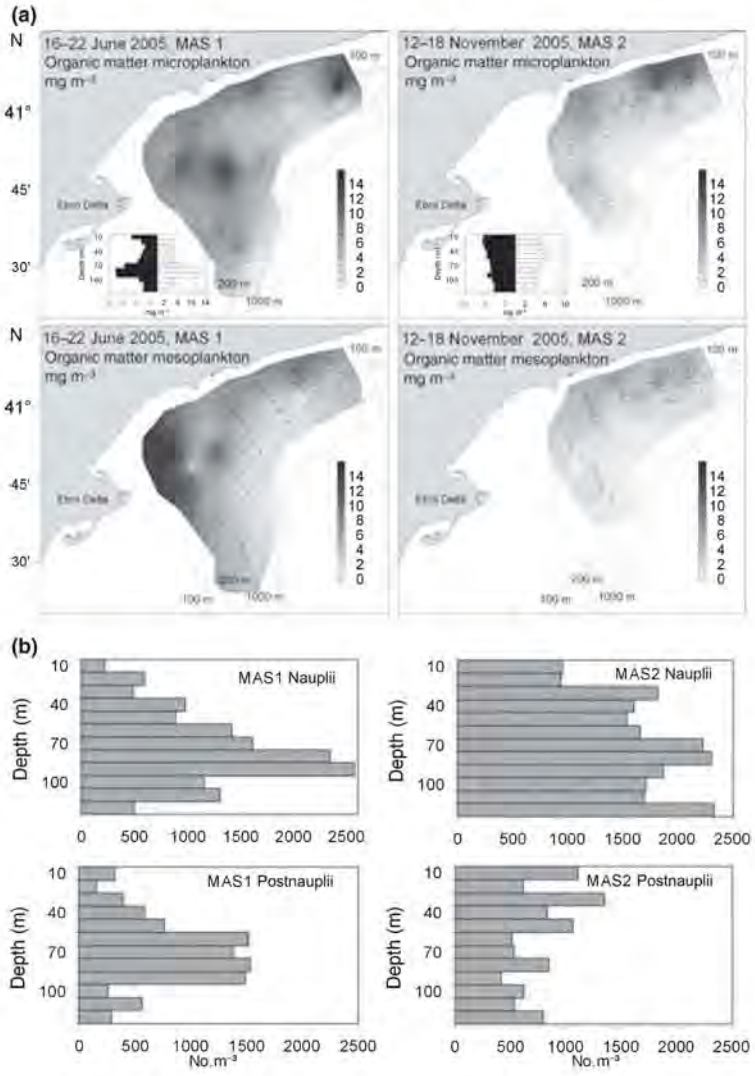


Figure 5. (a) Organic matter of micro- and mesoplankton during the June (left) and November (right) surveys; inserted graphs show vertical distribution of organic matter (day: light bars, night: dark bars). (b) Day vertical distribution of copepod nauplii and postnauplii on both cruises.

The major groups in both periods were tintinnids and dinoflagellates, followed by nauplii and copepods (Table 1). Most groups of copepods were more abundant in summer than in autumn, as were the cladocerans, which only appeared in the summer survey (Table 1). The vertical distribution of microplankton biomass in early summer showed a peak between 60 and 80 m (both during the day and at night) corresponding to the DFM, and a second peak at surface level (Fig. 5a). The vertical distributions of copepod nauplii and postnauplii also showed peaks at 60–80 m depths. In autumn, vertical distributions of microplankton biomass and abundance of copepod nauplii and postnauplii were more homogeneously distributed along the first 100 m of the water column (Fig. 5b).

Table 1. Contribution of the most common plankton groups collected with the CalVET nets during the June and November cruises and summary of main oceanographic variables. M, mean abundance as number of specimens per m^3. SE, standard error. Codes in italics indicate those variables not included in canonical correspondence analysis due to pairwise correlations with other variables higher than 0.67.

	June			November		
	Code	M	SE	Code	M	SE
Plankton groups						
Appendicularia	Ap	785.8	87.9	Ap	245.8	38.2
Bivalvia	*Biv*	211.2	21.9	*Biv*	259.8	98.6
Calanoida postnauplii	CalP	751.3	55.7	*CalP*	973.3	125.8
Cladocera	Cla	295.6	61.4	Cla	–	–
Cnidaria	*Cni*	124.3	15.1	Cni	–	–
Copepod eggs	*CopE*	2146.7	235.9	*CopE*	630	92.7
Copepod nauplii	CopN	8279.9	416.3	CopN	8496.4	789.9
Cyclopoida postnauplii	CypP	848.7	57.6	*CypP*	401.6	65.7
Dinoflagellata	*Din*	16232.8	1003.6	*Din*	18132.7	1638.4
Doliolida	*Dol*	146.5	52.2	Dol	–	–
Echinodermata	*Ech*	143.4	16.4	Ech	–	–
Foraminifera	For	453.9	40.3	*For*	889.8	92
Gastropoda	*Gast*	135.8	14	Gast	169.8	22.2
Harpacticoida postnauplii	HarP	769.4	58.1	HarP	737.5	81.5
Poecilostomatoida postnauplii	PoeP	1592.8	98.2	PoeP	937.2	73.6
Polychaeta	*Poly*	93.2	11.6	*Poly*	969.9	161.2
Radiolaria	*Rad*	95.7	23	Rad	1300.7	107.6
Tintinnida	Tint	8729.5	1348.5	*Tint*	16063.2	2102.9
Oceanographic variables						
Sea surface temperature, °C	SST	23.1	0.15	*SST*	18.9	0.1
Sea surface salinity	*SSS*	37.8	0.04	*SSS*	38	0
Sea surface fluorescence, volts	SSF	0.5	0.04	SSF	0.6	0
Surface Brunt-Väisälä frequency, cycles h^{-1}	*BvS*	14.8	0.48	BvS	3.5	0.7
Temperature 60 m	T60	14.4	0.03	T60	17.5	0
Salinity 60 m	S60	38.2	0	S60	38.2	0
Fluorescence 60 m, volts	F60	1.1	0.06	F60	0.6	0
Brunt-Väisäla frequency 60 m, cycles h^{-1}	Bv60	7.4	0.2	Bv60	3.2	0.4
Along-shore surface velocity*, cm s^{-1}	VaS	−2.4	1.67	VaS	6.2	2.7
Across-shelf surface velocity*, cm s^{-1}	VcS	2.4	2.23	VcS	5.1	2
Along-shore 60 m velocity*, cm s^{-1}	*Va60*	−1	−0.99	*Va60*	12.4	2.8
Across-shelf 60 m velocity*, cm s^{-1}	Vc60	3.3	3.32	Vc60	0.1	1.8
Depth, m	D	247.1	28.64	D	247.5	27.4
Organic matter mesoplankton, mg m^{-3}	OMes	5.5	0.42	OMes	3.6	0.3
Organic matter microplankton, mg m^{-3}	OMic	6.8	0.44	*OMic*	4.6	0.5

*positive values of along-shore component indicate direction to the Delta and positive values for across-shelf component indicate offshore direction.
–abundance < 0.3%.

Larval fish assemblages

During the summer cruise, larvae of 62 taxa belonging to 36 families (Table 2) were identified from the Bongo samples. *Engraulis encrasicolus* larvae dominated ichthyoplankton samples, appearing at all the stations and accounting for 52.6% of all the larvae, followed by *Sardinella aurita*, representing 13.6% of the larvae, and by the lanternfish *Ceratoscopelus maderensis*, which represented 6.3%. The number of taxa per station ranged from 17 to 38, with the maxima along the shelf edge, and another taxa-rich area where the continental shelf begins to widen to the north of the Ebro Delta. Diversity of the larval taxa, on the other hand, showed an increasing gradient from the coast to the open sea (Fig. 6).

In autumn, a total of 44 taxa belonging to 31 families were obtained (Table 3). *Sardina pilchardus* larvae were the most common and abundant taxa, representing 60.2% of larvae and appearing at almost all the stations (97%). The second species in order of abundance (7.4%) was the gonostomatid *Cyclothone braueri*, which was also relatively common (78% of stations), but less abundant. The number of taxa per station ranged from 12 to 24; the maxima were observed at the shelf break but the highest diversities were obtained at the stations of the SE margin of the study area.

Table 2. Contribution of the most common species collected with Bongo net during the June cruise. %D, percentage of larval density; MD, mean density (number of larvae per 10 m^2), SE, standard error of mean density; %O, percentage of occurrence; WMD, weighted mean depth (m).

						WMD (m)	
Species – June	Code	%D	%O	MD	SE	Night	Day
Engraulis encrasicolus	*Ee*	52.6	97.9	1393.4	296.6	18.5	20.9
Sardinella aurita	*Sa*	13.6	76.6	359.9	86.4	15.1	14.8
Cyclothone braueri	*Cb*	3.2	95.7	85.6	10.8	31.3	23.2
Argyropelecus hemigymnus	*Ah*	0.3	48.9	7.0	2.4	130.0	0.0
Maurolicus muelleri	*Mm*	0.3	57.4	7.2	2.2	94.3	95.0
Vinciguerria sp.	*Vin*	0.7	68.1	17.8	4.5	75.3	60.5
Paralepididae	Par	0.1	46.8	3.6	1.2	54.2	68.3
Benthosema glaciale	*Bg*	0.4	61.7	9.5	2.4	67.4	106.4
Hygophum benoiti	*Hb*	1.1	89.4	28.0	3.4	40.8	44.2
Myctophum punctatum	*Mp*	0.6	78.7	17.0	3.3	73.9	71.8
Symbolophorus veranyi	*Sv*	0.2	66.0	6.5	1.3	67.7	47.1
Ceratoscopelus maderensis	*Cer*	6.3	100.0	168.0	13.1	21.5	23.0
Lampanyctus crocodilus	*Lc*	1.1	97.9	29.2	2.8	42.6	41.9
Merluccius merluccius	*Me*	0.1	34.0	2.7	1.1	–	–
Serranus cabrilla	*Sc*	0.1	36.2	3.4	1.5	10.9	28.0
Serranus hepatus	*Sh*	1.2	48.9	33.1	17.2	23.7	24.3
Coryphaenidae	Cor	0.3	48.9	8.9	4.0	9.0	7.4
Trachurus mediterraneus	*Tm*	1.0	66.0	26.2	5.9	10.1	19.8
Trachurus trachurus	*Tt*	0.2	53.2	4.7	1.5	31.6	30.9
Diplodus sargus	*Ds*	2.8	89.4	72.9	11.2	17.7	8.3
Pagellus erythrinus	*Pe*	0.2	51.1	5.5	1.7	14.8	27.4
Mullus barbatus	*Mu*	0.6	57.4	15.6	7.6	11.7	5.0
Cepola macrophthalma	*Cm*	2.3	87.2	60.5	15.4	24.6	30.8
Coris julis	*Cj*	0.3	51.1	8.9	3.9	10.8	12.0
Trachinus draco	*Td*	0.2	44.7	4.2	1.5	15.0	20.7
Blenniidae	Bl	0.1	29.8	2.3	1.2	13.3	–
Callionymus spp.	*Call*	1.4	89.4	36.3	5.4	65.0	94.9
Gobiidae	Gob	3.9	95.7	104.5	20.4	28.1	28.7
Auxis rochei	*Aux*	2.7	76.6	71.8	20.2	9.0	8.8
Scomber japonicus	*Sj*	0.1	27.7	3.5	2.3	18.4	5.0
Scombridae A	Scom	0.2	19.1	4.9	6.8	8.4	25.0
Arnoglossus sp.	*Ar*	0.9	93.6	23.1	3.5	21.3	19.0

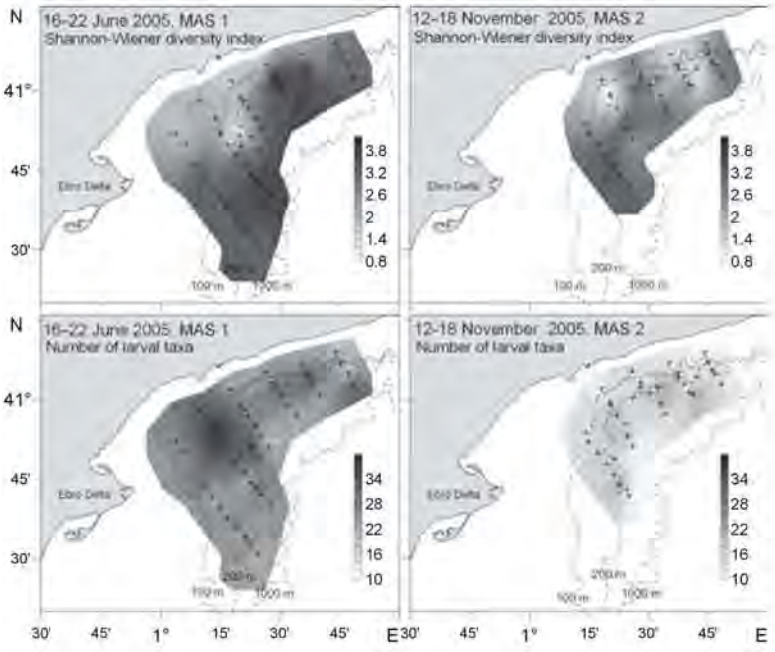


Figure 6. Diversity and number of taxa among fish larvae collected during the June (left) and November (right) surveys.

Relationships between larval fish assemblages and environment

For the early-summer cruise the original matrix of environmental data was reduced to 21 variables after the elimination of those whose pairwise correlation with other variables was higher than 0.67. The first two axes of CCA explained 61.7% of the cumulative percentage variance of species-environment relation and the correlation between species and environmental axes was 0.955 and 0.941, respectively. The first canonical axis, which explains 47.9% of the variance, was positively correlated with depth, fluorescence at 60 m and along-shore current velocity; it was negatively correlated with most biological variables, and in particular densities of cladocerans, sea- surface fluorescence and microplankton organic matter (Fig. 7). The geographical representation of the scores of each station shows that it is not only a depth axis, because on the negative side it includes the coastal stations (<100 m) and the three deep stations of the first transect (Fig. 7). Practically all the larval taxa that appear on the positive side of this axis are oceanic species and their ordination is correlated with the depth and the along-shore currents, whereas the larvae of shelf species occupied the opposite side, being more related to the biological variables, grouped at this side of the axis. The species that were located at the most extreme values of axis 1 were *Argyropelecus hemigymnus*, Paralepididae, *Maurolicus muelleri*, *Benthosema glaciale*, *Vinciguerria* sp. and *Myctophum punctatum*, which hardly appeared at the stations of the continental shelf (Fig. 8) and were absent both during the day and at night in the first 40 m of the water column (Fig. 8 and Table 2). At the opposite end of axis 1 were the larvae of coastal taxa, *Scomber japonicus*, Blenniidae, *Serranus hepatus* or Scombridae A, with the maximum abundances at stations of <100 m depth (Fig. 9). Their vertical distribution both during the day and at night was very near the surface, between 0 and 40 m (Fig. 9 and Table 2). The larvae with very wide bathymetric distributions occupied intermediate values on axis 1, although the larvae of shelf species such as *Arnoglosssus* sp., *Cepola macrophthalma*, *E. encrasicolus* and *D. sargus* had negative values, and the oceanic species such as *C. maderensis*, *C. braueri* and *Hygophum benoiti* positive values (Fig. 10). The vertical distribution covered the first 50 m of the water column and no differences between day and night were observed (Fig. 10 and Table 2).

The second axis was negatively correlated with sea surface temperature and a large number of trophic variables (Fig. 7). The representation of their scores differentiates the stations of the southern half of the study area, which had a higher sea surface temperature. The species most correlated with this axis were *Coris julis* and Coryphaenidae, which only appeared in this zone, whereas the larvae of *P. erythrinus*, *Trachinus draco* and *T. mediterraneus*, which were absent from the stations of higher temperature, occupy the opposite side of the ordination (Figs 7 and 9).

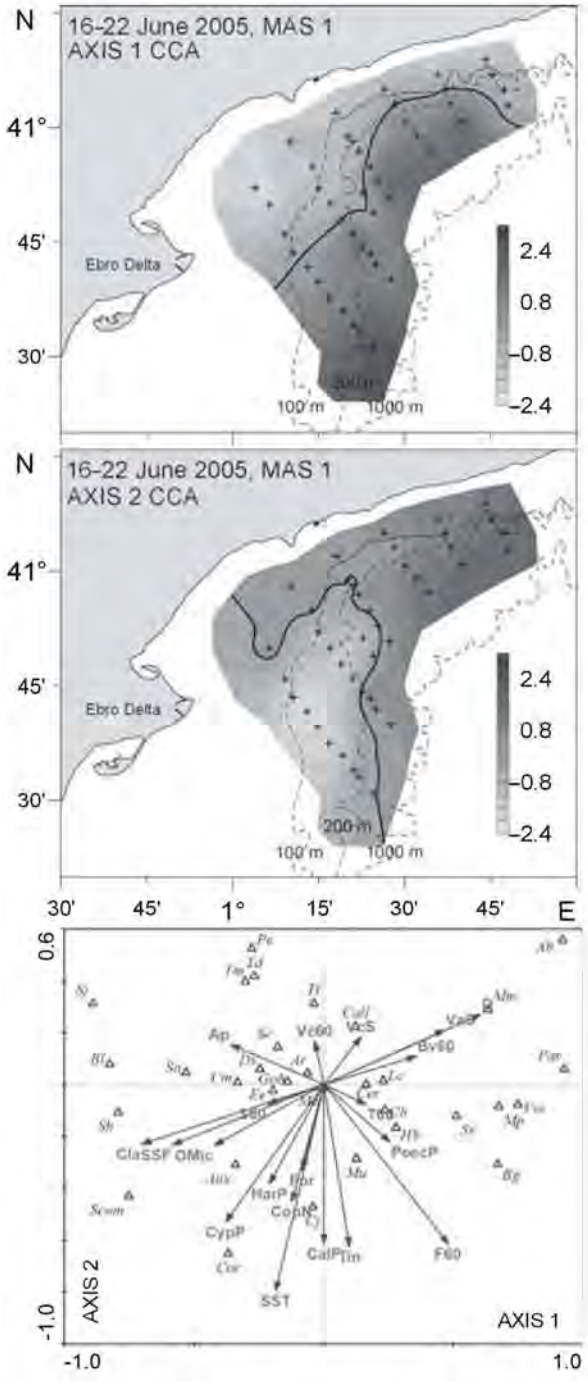
Table 3. Contribution of the most common species collected with Bongo net during the November cruise. %D, percentage of larval density. MD, mean density (number of larvae per 10 m^2). SE, standard error. %O, percentage of occurrence. WMD, weighted mean depth in m.

						WMD (m)	
Species – November	Code	%D	%O	MD	SE	Night	Day
Sardina pilchardus	*Sp*	60.2	97	277.3	52.8	49	29.7
Cyclothone braueri	*Cb*	7.4	78	34.2	7.7	37.2	36.43
Argyropelecus hemigymnus	*Ah*	2.6	58	12.1	4.1	93.6	128.94
Maurolicus muelleri	*Mm*	0.3	36.1	1.6	0.6	63.8	112.82
Vinciguerria sp.	*Vin*	0.4	25.0	1.8	1.5	–	–
Paralepididae	Par	1.8	75	8.5	2.1	96.9	65.90
Benthosema glaciale	*Bg*	1.5	64	6.9	2.3	61.8	50.96
Hygophum benoiti	*Hb*	1.8	61	8.5	3.0	31.7	30.37
Hygophum hygomii	*Hh*	0.4	30.6	2.0	1.1	–	–
Myctophum punctatum	*Mp*	0.8	47.2	3.8	1.5	27.5	52.02
Symbolophorus veranyi	*Sv*	1.5	67	7.1	2.2	63.4	68.37
Ceratoscopelus maderensis	*Cer*	0.9	42	4.3	2.2	65.9	36.91
Lampanyctus crocodilus	*Lc*	1.1	50	5.1	2.1	59.9	31.84
Lampanyctus pusillus	*Lp*	1.2	39	5.3	3.1	–	39.37
Merluccius merluccius	*Mer*	1.5	67	6.8	1.7	67	68.2
Mugilidae	Mug	3.1	78	14.2	2.7	41.0	39.06
Macroramphosus scolopax	*Ms*	0.5	44.4	2.3	0.9	34.2	56.33
Triglidae	Tri	0.2	22.2	0.9	0.7	–	39.08
Trachurus sp.	*Tra*	0.4	33.3	1.7	0.9	82.2	55.43
Sparidae A	SpA	1.4	64	6.2	2.0	–	–
Sparidae B	SpB	0.9	44	4.3	1.6	–	–
Sparidae P	SpP	1.3	67	6.0	1.5	22.6	36.23
Cepola macrophthalma	*Cm*	2.6	81	11.8	2.7	53.7	43.79
Cristallogobius linearis	*Cris*	2.2	69	10.4	2.4	93.8	100.55
Gobidae	Gob	0.7	44.4	3.0	1.1	33.2	45.10
Lepidopus caudatus	*Lep*	0.2	25.0	1.0	0.6	51.5	66.75
Arnoglossus sp.	*Ar*	0.3	19.4	1.2	0.9	–	31.42
Soleidae	Sol	0.2	19.4	0.9	0.8	–	27.16

CCA performed excluding data from the southern transect also showed the same species ordination and similar environmental correlation among canonical axis and environmental variables (figures not shown). In this case the first two axes explained 50.9% of the cumulative percentage variance and the correlation between species and environmental correlations was 0.971 and 0.944, respectively (very similar to the results with the complete data set). In this second analysis the first canonical axis was also positively correlated with depth and along-shore current velocity and negatively correlated with most biological variables; the second axis presented higher correlation to SST.

For the autumn cruise the original matrix of environmental data was reduced to 17 variables after those whose pairwise correlation was higher than 0.67 had been eliminated. The first two CCA axes for the autumn cruise explain 55.6% of the variance and the correlation between species and environmental axes is 0.899 and 0.935, respectively. The first axis of the ordination, which explains 42.9% of the variance, is negatively correlated with all the biological variables and positively correlated with depth, BV60 and along-shore and across-shelf currents (Fig. 11). The geographical representation of the scores of axis 1 shows the similarity between the slope stations and all the shelf stations and shelf-break stations in the southern transect (with positive values) (Fig. 11). The ordination of the species with regard to this axis clearly differentiates, in the positive part of the axis, larvae of oceanic species such as *C. maderensis*, *Vinciguerria* sp. and *Hygophum benoiti*, which appeared at the slope stations (>300 m) and also all along the continental shelf in the southern transect (Fig. 12). Their vertical distribution was quite variable, with species absent

Figure 7. Chart of axes 1 and 2 obtained after applying canonical correspondence analysis (CCA) for the June survey. Black line separates positive and negative values. Bottom graph: CCA plots of axes 1 and 2. Abbreviations explained in Tables 1 and 2.



from surface layers (e.g., *B. glaciale* or *Myctophum punctatum*) and others that are fairly abundant at the surface (e.g., *C. braueri*), but peak abundance was generally found at sub-superficial levels (Fig. 12 and Table 3). On the negative side of the ordination were larvae of shelf-dwelling species such as *S. pilchardus*, *C. macrophthalma*, Sparidae and *Merluccius merluccius*, with main concentrations on the continental shelf and shelf break, but with lower values or no presence in the southern transect (Fig. 13). Larvae of these species displayed a wide vertical range in the first 100 m of the water column (Fig. 13 and Table 3).

The second axis was positively correlated with BvS and along-shore velocity and negatively correlated with F60. Their geographic representation (Fig. 11) differentiated the slope stations, which were dominated by major along-shore currents. Larvae of *A. hemigymnus*, which appeared at the offshore stations, but not along the continental shelf of the southern transect, were clearly separated from the rest according to this axis (Figs 11 and 12).

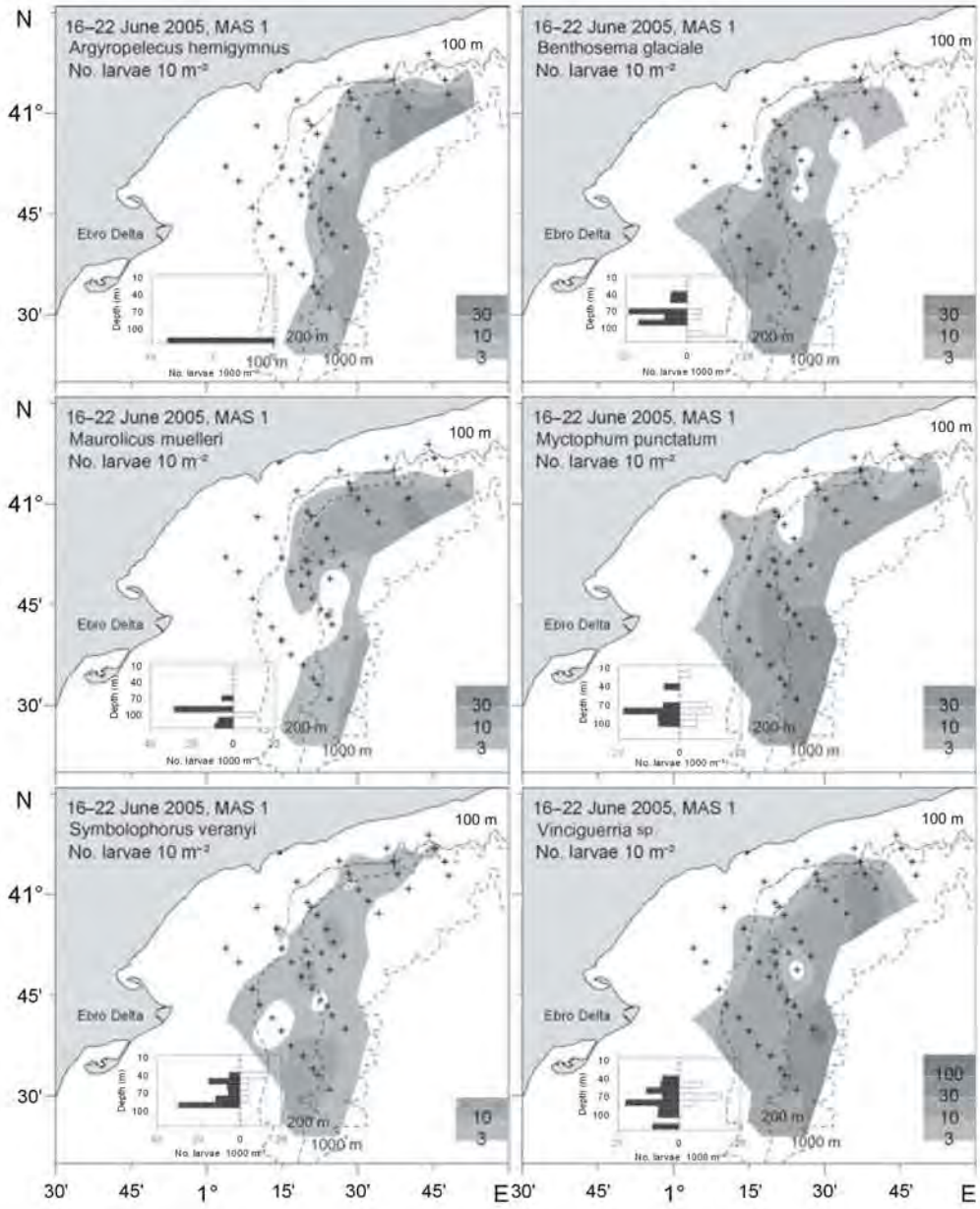
DISCUSSION

This study was carried out in two contrasting situations not only in terms of seasonality (early summer and autumn) and the related values of the hydrographical parameters, but also in relation to water stratification and current field patterns. The presence of waters of continental origin, the high stability of the water column and the slight intensity of the currents in summer showed conspicuous differences to autumn, characterized by homogeneity in horizontal and vertical distributions of hydrographical variables and the presence of an intense course of the CC, which was close to the coast. In both periods, the maximum biomasses of micro- and mesoplankton were obtained at the stations of the continental shelf and the shelf-break, although they were higher and showed a wider cross-shelf extension in early summer than in autumn. Diversity and abundance of larval fish taxa were also higher in early summer and their cross-shelf distributions were wider than in autumn.

Physical and biological environment

In terms of the water masses, the study area was relatively homogeneous. It was characterized in both periods by the presence of resident AW, though with a major influence of waters of continental origin in early summer. Although the November cruise did not survey the southernmost transect (number 6), the continental influence in the June cruise reaches up to the fourth transect (in the middle of the study area), hence any continental influence of a similar magnitude should have been observed in transects 5 and 4. The vertical distributions of the thermohaline

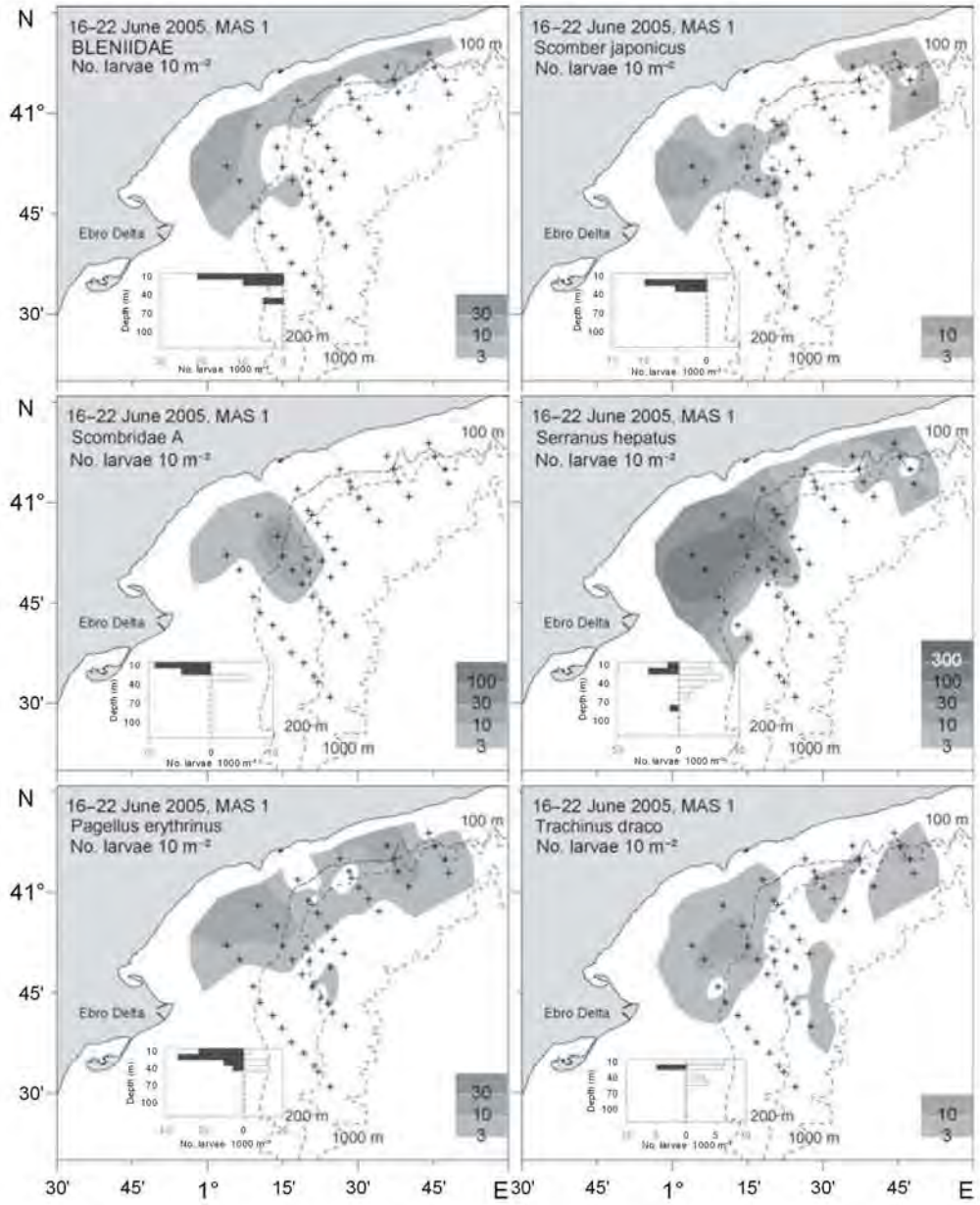
Figure 8. Horizontal larval distribution of some oceanic species for the June survey. Inserted graphs show vertical distribution of each species (day: light bars, night: dark bars).



parameters and their derivatives (Brunt–Väisälä frequency), and currents can be considered to be typical of the periods of early summer and autumn (Salat, 1996) and show the change in the vertical structure of the water column from a summer stratification in June to the autumn homogenization in November. This is particularly evident from the lower Brunt–Väisälä frequency values, and the more homogeneous vertical profile in November, particularly in the shelf area, which indicates that water mixing in this zone involves the whole water column.

In summer the temporal variability in the dynamics of the currents on the continental shelf was very high, <4 days, showing the high mesoscale activity in this period. The Catalan Current was only intense in the slope region at the southern edge of the large Ebro Delta shelf. On the continental shelf, spatial heterogeneity was the dominant pattern, with a flow towards the open sea in the northern half and signs of the presence of an eddy in the south, contributing to the wide inshore/offshore distributions of many species. However, the

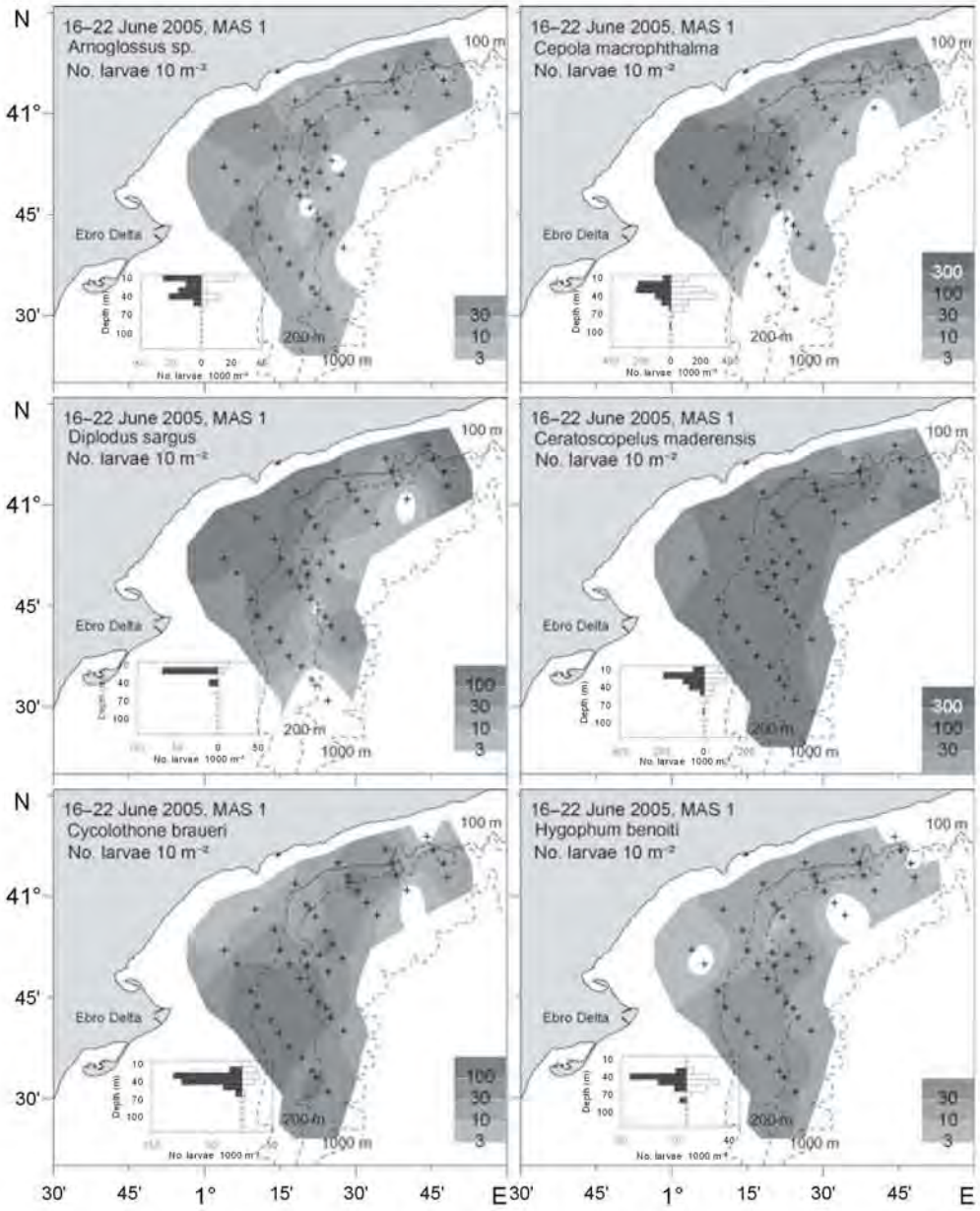
Figure 9. Horizontal larval distribution of some coastal species for the June survey. Inserted graphs show vertical distribution of each taxa (day: light bars, night: dark bars).



oceanographic conditions in autumn were characterized by the intensity of the CC and its proximity to the shelf. In the zone where the continental shelf becomes wider, to the north of the Ebro Delta, we observed an intrusion of open sea waters across the shelf edge, a phenomenon that is often reported in the region (Font *et al.*, 1990; Salat *et al.*, 2002). This meant that the along-shore and across-shelf transport associated with this current was more intense than in summer, affecting mainly the slope and shelf-break species.

As reported by Alcaraz *et al.* (2007), in the stratification period the main zooplankton peak coincided with the deep fluorescence maximum, and here we observed a second surface peak associated with the inputs of nutrients from waters with continental influence of the shelf near the Ebro Delta. This implies that in this period the horizontal distributions of zooplankton showed high values on the shelf (influenced by continental waters) and on the shelf break (where DFM was more important). The more homogeneous fluorescence patterns along the first 50 m of the water

Figure 10. Horizontal larval distribution of some shelf species for the June survey. Inserted graphs show vertical distribution of each species (day: light bars, night: dark bars).

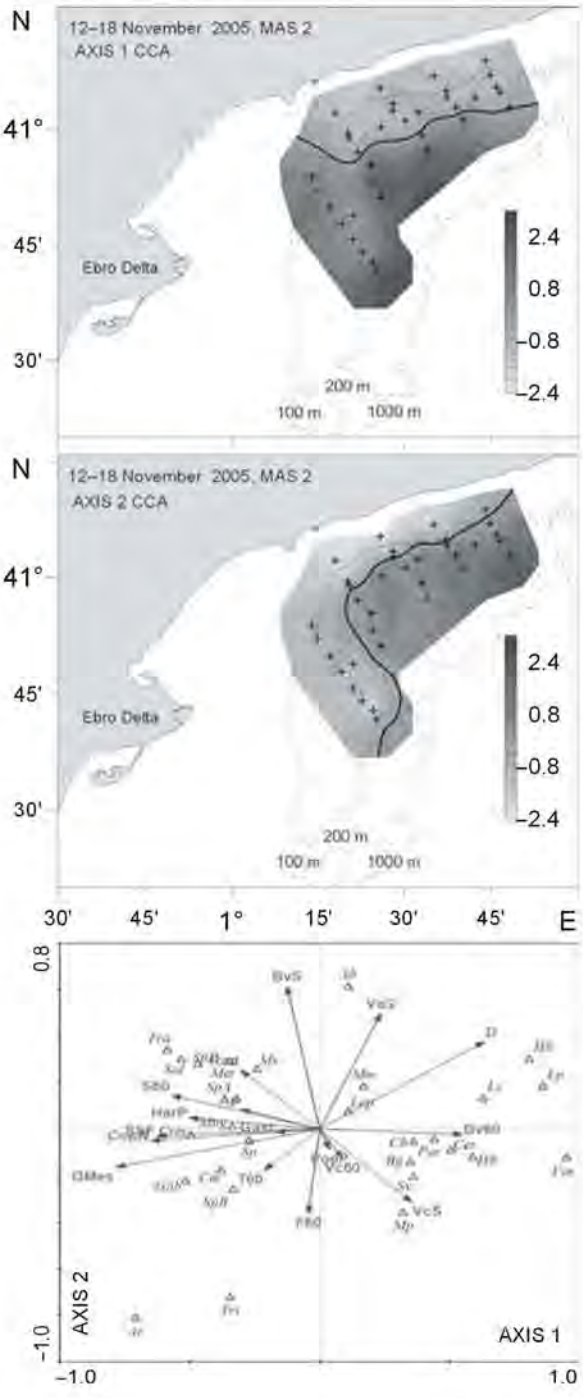


column on the shelf and shelf-break stations during the autumn mixing period (Estrada, 1996 and present data) is not closely reflected in the more evenly distributed zooplankton, which goes down to 100 m.

Fish larvae horizontal and vertical patterns

The considerable differences in number of species and in total abundance of larvae between the two periods of study show that in the NW Mediterranean only a few shelf species reproduce in autumn, and of these only a few appear exclusively in this period: *S. pilchardus*, *Macroramphosus scolopax* and Sparidae P (probably *Pagellus bogaraveo*). As has already been shown in previous studies of the summer period (see Sabatés *et al.*, 2007 for a summary), the species that dominated the ichthyoplankton in early summer were *E. encrasicolus* and *S. aurita*. Other species that only appeared in this period were *Coris julis*, *Mullus barbatus* and several species of the family Scombridae. Furthermore, the abundance of the species common to the two periods was in general far higher in June, and only hake larvae were slightly more abundant in

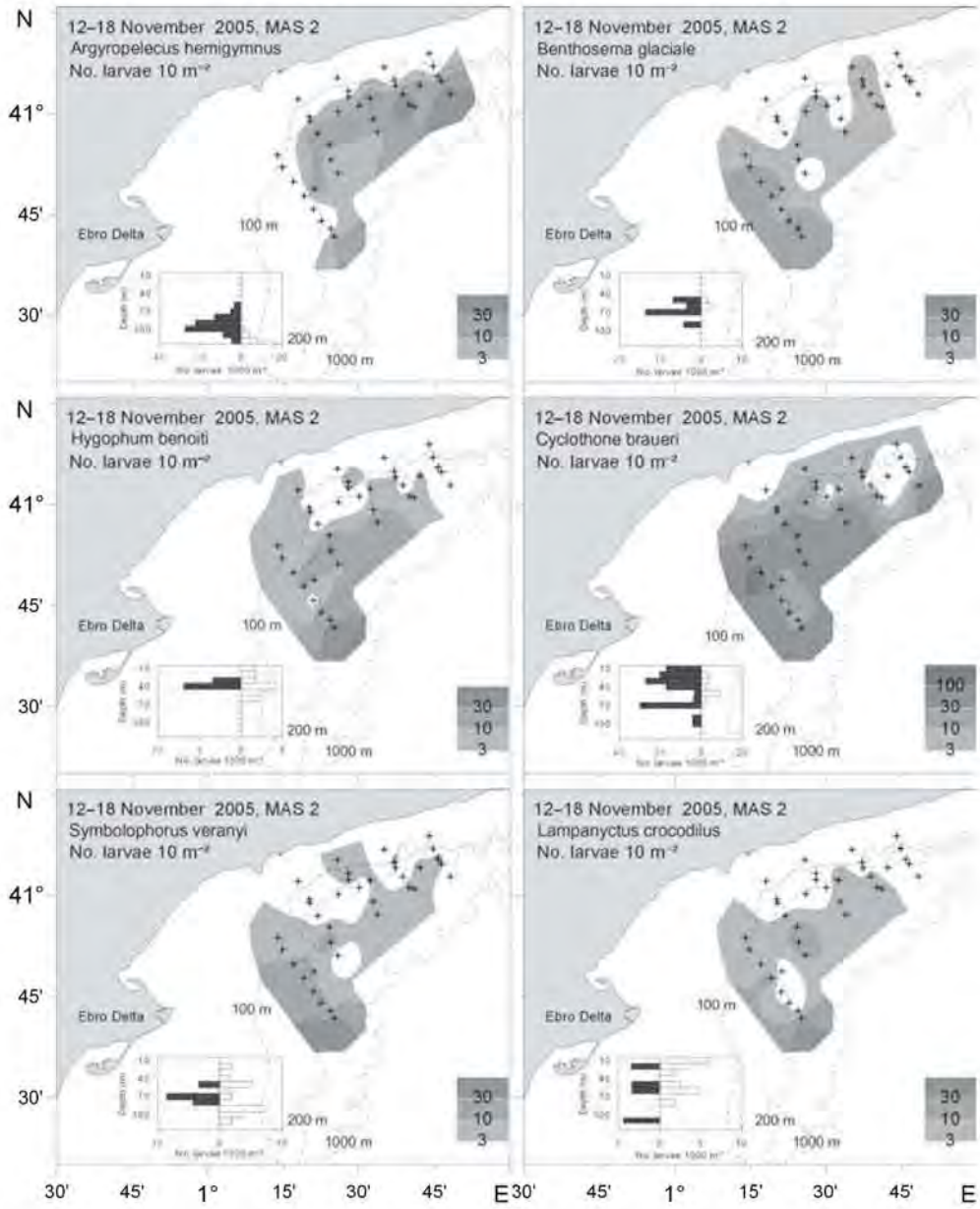
Figure 11. Chart of axes 1 and 2 obtained after applying canonical correspondence analysis (CCA) for the November survey. Black line separates positive and negative values. Bottom graph: CCA plots of axes 1 and 2. Abbreviations explained in Tables 1 and 3.



autumn, showing the peak reproduction of the species in the study area (Olivar *et al.*, 2003; Recasens *et al.*, 2008). Even when the number of species per station was considerably higher in June than in November, the diversity values showed fewer differences because in both periods the larval community in the shelf region was dominated by a few species, i.e., the larvae of clupeiforms, *E. encrasicolus* and *S. aurita*, which are extremely abundant in June, and those of *S. pilchardus*, which dominated the November samples. The differences in species/taxa composition and diversity between these two periods coincide with the observations of Koutrakis *et al.* (2004) in the eastern Mediterranean.

Despite the capacity of fish larvae to position themselves in particular water masses that are optimal for their growth and survival (Lee *et al.*, 2005), the larvae of the majority of shelf species that appeared in summer showed a very superficial vertical distribution, above the thermocline, the DFM and the maximum biomass of micro- and mesoplankton, and showed no signs of diel migrations along the water column. This means that under the circumstances in which this study was carried out, the trophic resources of the surface layers must be shared by a large number of larvae and a great diversity of species. This lack of vertical migration is certainly different from that observed for *E. encrasicolus* in periods or zones not affected by fresh water (Olivar *et al.*, 2001; Sabatés *et al.*, 2008), in which the postflexion larvae performed migrations towards the DFM during daylight hours. The high larval densities of both this species and *S. aurita* and their co-occurrence during the present study suggest that the abundance of zooplankton in the surface layers forms a favourable habitat for the nutrition, and consequently the survival, of the larvae. In fact, the concentrations of larvae of *E. encrasicolus* in the present study were far higher and showed a far more coastal horizontal distribution than on other occasions in the same zone (see Palomera *et al.*, 2007 for a summary). Interannual changes in the distribution of the larvae of this species associated with the presence of waters of continental origin have also been reported in the eastern Mediterranean (Isari *et al.*, 2008). In autumn the vertical distributions patterns of fish larvae were slightly wider, although for the species common to both periods the patterns of preference for the upper or lower layers of the water column were maintained, indicating an innate preference. However, in this period differences were observed in the daytime and night-time vertical distributions, with more superficial peaks in the daylight hours and a more dispersed distribution along the water column at night. This finding confirms the importance of the light factor in larval

Figure 12. Horizontal larval distribution of some oceanic species for the November survey. Inserted graphs show vertical distribution of each species. (day: light bars, night: dark bars).



vertical distribution (Leis, 1991; Gray and Kingsford, 2003), which is related to the daytime nutrition patterns of the majority of species (Hubbs and Blaxter, 1986; Morote *et al.*, 2008).

The total number of meso- and bathypelagic species was similar in the two periods, 17 in June and 18 in November. Although their mean abundance was higher in early summer, in relative terms they represented only 14.4% of the total larvae in June compared with 40% in November. Their vertical structuring was similar in the two periods, with a preference for the subsurface layers, as in other geographic areas with different hydrographic characteristics (Loeb, 1980; Moser and Smith, 1993; John *et al.*, 2001; Isari *et al.*, 2008) and similar to previous reports for the region (Olivar *et al.*, 1998; Sabatés, 2004). Of these species, only *C. maderensis* and *C. braueri* reached the first 20 m of the water column in both periods, and only *M. punctatum*, *B. glaciale* and *Symbolophorus veranyi* showed a maximum in the area of the DFM and a deep zooplankton maximum (DZM) in summer.

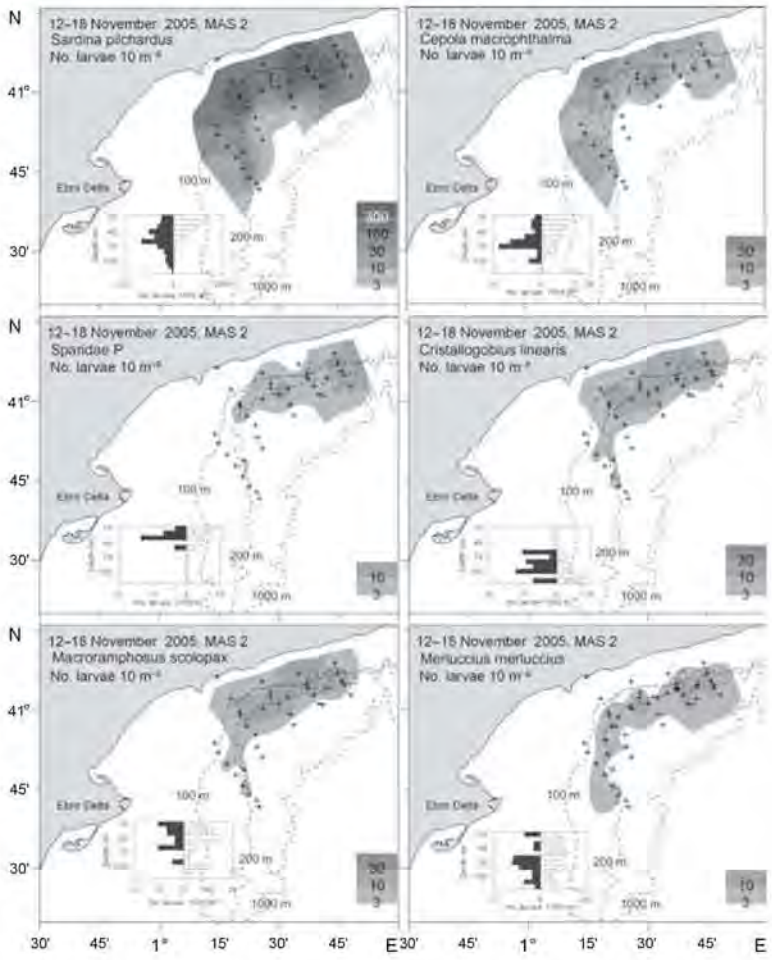


Figure 13. Horizontal larval distribution of some shelf species for the November survey. Inserted graphs show vertical distribution of each species (day: light bars, night: dark bars).

Larval fish assemblages and environment

Our results show that in the NW Mediterranean only a few species spawn in autumn, a period characterized by instability of the water column and the influence of the CC, which is nearer to the continental shelf. Ordination analysis of the dominant taxa for the two periods revealed that in addition to the importance of seasonality in shaping community structure due to the reproduction periods of the different species (Gray and Miskiewicz, 2000; Koutrakis *et al.*, 2004), other factors of their environment contribute to the differences between the two periods. Most LFA research in different regions of the world has shown the primary influence of bathymetry and therefore the influence of spawning habitats of adults on the spatial patterns (Olivar, 1990; Somarakis *et al.*, 2002; Koutrakis *et al.*, 2004; Muhling *et al.*, 2007). However, other more dynamic influences related to the particular oceanographic features, water mass distributions (Hare *et al.*, 2001; Somarakis *et al.*, 2002; Isari *et al.*, 2008; Vilchis *et al.*, 2009) and zooplankton biomass, have also been suggested (Suthers *et al.*, 2006; Brodeur *et al.*, 2008). Interfaces between LFA associated with water masses were found to be dynamic, with locations shifting temporally and spatially depending on the current or geostrophic circulation (Sánchez-Velasco *et al.*, 2006; Keane and Neira 2008). In the present study, summer LFA for the shelf-dwelling species occupied a larger cross-shelf area than in autumn, associated with the path of the CC, which was farther offshore in summer. Higher diversity of larval taxa and abundance on the continental shelf in early summer is probably a result of food availability enhanced by nutrient enrichment from the river, as well as the effect of the eddy circulation that could extend the distribution of larvae of shelf species to the shelf-break and those of slope species to the shelf. Furthermore, larvae of coastal species in the first transect of the northern zone seem to be influenced by surface cross-shelf transport towards the offshore area (see Fig. 4), favoured by its near-surface vertical distribution. The overall analysis of the environmental variables and the fish larvae showed the high

correlation between the larvae of species that reproduce on the shelf and the trophic variables (i.e., concentrations of organisms of plankton and sea surface fluorescence The oceanic species with a deep vertical distribution (e.g., *A. hemigymnus*) form an assemblage structured mainly by the bathymetry and along-shore currents. On the other hand, those distributed nearer the surface, such as *C. maderensis* and *C. braueri*, form an intermediate group between the shelf and oceanic species, showing high abundances in both zones. Their presence nearer to the coast than to adult distribution is a common situation in other parts of the Mediterranean and Atlantic (John and Zelck, 1997; Isari *et al.*, 2008), showing the extent of the transport that they have undergone and the potential use of ichthyoplankton assemblages as short-term biological tracers of hydrographic variability (Smith *et al.*, 1999). Although the analyses showed no relation to the current data in the first 60 m and these last species, bearing in mind the oceanic distribution of the adults and the fact that their larvae are found in the first 60 m of the water column, one can consider the influence of the eddy as a mechanism of entrance of these larvae from the shelf break to the shelf. Field studies on the behaviour of anticyclonic eddies in the NW Mediterranean have suggested that they play a role in shelf-slope exchanges, enhancing exchanges between shelf waters and open sea waters (Rubio *et al.*, 2005). Increased larval fish abundance related to the effect of intrusions of shelf-break and slope waters to the inner shelf have been frequently referred to in different geographical areas, one example being the Brasil Current (Franco *et al.*, 2006).

In autumn, the distribution of all shelf species (e.g., *S. pilchardus* and *M. merluccius*) is associated with the concentration of organisms of zooplankton. On the other hand, the distribution of meso- and bathypelagic species is associated with a combination of the habitat of the adults (oceanic) and the along-shore and across-shelf transport, as shown by their presence at depths of <200 m in the southern transect.

In summary, the large differences in the hydrographic variables and the vertical structure of the water column between early summer and autumn are more evident in the horizontal distribution patterns of fish larvae than in the vertical patterns, and affect both shelf and oceanic taxa. The LFA of oceanic species are similar in terms of species composition between the two periods, although it differs in the extension of the horizontal distributions. The discrimination between the LFA of shelf and oceanic species is far more apparent in autumn due to the strength and proximity of the CC to the shelf, which seems to constrain the shelf larvae to this region. During this period, larvae of a few species constitute the LFA in the shelf, the clupeiform *S. pilchardus* being the dominant component. In early summer, the very heterogeneous current field on the shelf and the CC farther offshore, contribute to the overlapping horizontal distributions of shelf and oceanic species. The summer shelf LFA were composed of a high number of taxa, although they were dominated by two clupeiforms (*E. encrasicolus* and *S. aurita*). The high number of fish larvae of both shelf and oceanic species that coincide in the horizontal and vertical fields in early summer may involve a higher competition for trophic resources, so the possibilities of survival must be strongly linked to the availability of micro- and mesoplankton (whose abundance is favoured by the enrichment by continental water). In autumn, the trophic resources also proved to be the main modulators of the distribution of the shelf species, whereas the distributions of the oceanic species, which are far less dependent on the concentrations of zooplankton, seem to be influenced mainly by current-mediated transport.

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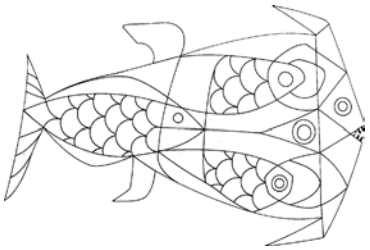
2.2 SLENDER LARVAE

(SMALL MOUTH, STRAIGHT GUT)



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A comparison of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) larvae feeding in the Northwest Mediterranean: influence of prey availability and ontogeny

Morote E, Olivar MP, Villate F, Uriarte I. J Plankton Res 30:807–816 (2008)
Diet of round sardinella, *Sardinella aurita*, larvae in relation to plankton availability in the NW Mediterranean.

A comparison of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) larvae feeding in the Northwest Mediterranean: influence of prey availability and ontogeny

Elvira Morote, María Pilar Olivar, Fernando Villate, and Ibon Uriarte

Morote, E., Olivar, M. P., Villate, F., and Uriarte, I. 2010. A comparison of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) larvae feeding in the Northwest Mediterranean: influence of prey availability and ontogeny. – ICES Journal of Marine Science, 67: 897–908.

The feeding ecology of the larvae of the two most important small pelagic species in the western Mediterranean, anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*), is investigated. Samples were collected in early summer (anchovy) and autumn (sardine). Composition of the diet and prey selectivity were compared over the course of ontogeny in anchovy (2.7–14.5 mm standard length, SL) and sardine (5.5–15.8 mm SL) larvae. Anchovy larvae begin feeding on prey items >150 μm at smaller sizes than sardine larvae, and the diets of both species are based mainly on copepod nauplii and postnauplii. Seasonal differences in the composition of the plankton influenced the contributions of prey types to the diets of the two species, e.g. the cladoceran *Evadne* spp. in anchovy and the tintinnid *Codonellopsis* sp. in sardine. Although copepod eggs are generally present in the diets of larval clupeoids, they were not major components of the diets of the species considered here. Despite morphological similarity, selection patterns were different between the species and changed through ontogeny.

Keywords: anchovy, fish larvae, larval development, plankton availability, sardine, selectivity, trophic ecology.

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Introduction

Anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) account for some 50% of the annual landings in the northwestern Mediterranean Sea and are important not only commercially (anchovy because of their high market value, sardine because of their large biomass), but also because of their ecological roles in the ecosystem. Anchovy and sardine adults and larvae feed on plankton and, being close to the base of the food chain, they derive the greatest benefit from seasonal plankton blooms, so variations in environmental processes may be a factor determining recruitment of both species in the Northwest Mediterranean (Lleonart and Maynou, 2003; Palomera *et al.*, 2007).

Survival of fish larvae and juveniles depends on their ability to find, capture, and ingest sufficient quantities of appropriate prey to avoid starvation and to ensure growth. Temporal coupling and decoupling of peak production of fish larvae and their prey organisms is one of the main sources of recruitment variability, as Cushing (1990) advanced in his "match–mismatch" hypothesis, so adverse changes in the planktonic ecosystem can result in low rates of survival of fish larvae, which can in turn lead to poor recruitment (Beaugrand *et al.*, 2003). Zooplankton is the main source of food for marine fish larvae, and are sighted visually and captured individually. Nearly all fish larvae are diurnal, particulate planktivores feeding mainly on copepods (Hunter, 1980; Last, 1980; Sampey *et al.*, 2007). Stage-specific variation in feeding habits and prey selectivity in marine fish larvae is consistent with stage-specific differences in the functional morphology of the feeding organs of fish larvae (Turingan *et al.*, 2005). Clupeoid larvae are no exception, and the diets of several species have been described as being copepod-based, in many areas shifting from copepod eggs and nauplii to copepodites and adults as the larvae grow (Govoni *et al.*, 1983; Muck *et al.*, 1989). Few studies have dealt with the diet of anchovy larvae in the Mediterranean Sea (Conway *et al.*, 1998, for the Adriatic; Tudela *et al.*, 2002, for the Catalan Sea), and no information on predator–prey relationships or selectivity is available. Information for sardine is limited to an analysis of the gut contents of postflexion larvae in a peri-estuarine region of the Gulf of Fos (near the Rhône River delta), where these larvae were described as being exclusively phytoplanktophagous (Rasoanarivo *et al.*, 1991). However, that finding is not supported by the results of other studies on sardine larvae feeding in other parts of the world, which have described their diets as being copepod-based (Arthur, 1976; Muck *et al.*, 1989; Munuera-Fernández and González-Quirós, 2006). More-detailed information on the composition of the diets and predator–prey relationships of anchovy and sardine larvae in the Mediterranean is therefore needed.

The ability of fish larvae to prey on zooplankton is affected not only by behavioural factors, but also by the hydrodynamic environment. In the Catalan Sea, anchovy and sardine larvae do

not occur concurrently in time or space, because the two species spawn at different times of the year (Palomera *et al.*, 2007): anchovy in spring/summer, when the water column is stratified and days are longer, and sardine in autumn/winter, when there is vertical mixing (Salat *et al.*, 2002) and days are shorter. Growth rates of clupeoid larvae measured in the laboratory, however, are highest at high temperatures and food densities (Blaxter and Hunter, 1982). Despite the different water temperatures experienced by the early stages of anchovy and sardine larvae in the area, growth rates for the two species fall within similar ranges (Palomera *et al.*, 2007), and sardine larvae are thought to have a specially adapted feeding strategy to compensate for the cooler water temperatures in autumn.

The object of this study was to analyse and compare the diets of anchovy and sardine larvae as they relate to the trophic environment and the larval development of each species. In addition, similarities with the diet of a third clupeoid, the round sardinella (*Sardinella aurita*), present in the region only in summer, were considered from the perspective of possible competition (diet overlap) with anchovy larvae.

Material and methods

Larvae were collected during two cruises carried out in the Catalan Sea (Northwest Mediterranean), one in summer and the other in autumn. The late spring survey was conducted from 16 to 22 June 2005 during the spawning season of *E. encrasicolus*. The autumn survey was conducted from 12 to 16 November 2005 during the spawning season of *S. pilchardus*. Sampling to obtain larval distributions and abundance was carried out along a series of transects spaced 6–7 nautical miles apart and running perpendicular to the coast, with stations every 2–2.5 miles. Some additional stations were located closer together over the shelf margin (Figure 1). Larvae were collected by oblique hauls using a Bongo net (mesh size 300 μm) from 200 m (or just above the seabed) to the surface. To avoid damaging the larvae, the codend was removed before washing the net. The larvae were immediately sorted and stored in groups of no more than 10 per vial. Vertical microplankton and mesoplankton hauls were carried out using a Calvet net fitted with mesh sizes of 53 and 200 μm. To remove mesoplanktonic organisms from the >53 μm samples, those catches were later filtered through a 200 μm mesh. Samples were preserved in 5% buffered formalin. The volume of seawater filtered was measured by flowmeters placed in the centre of the mouth opening of the net.

Laboratory analysis

Micro- and mesozooplanktonic organisms were quantified at the stations where larvae were selected for gut content analysis. Zooplankton samples were diluted to a volume of 100 ml and subsampled by aliquot. Samples counted up to 100 individuals from the most abundant taxon or 30 individuals from each of the three most abundant categories. Prey items identified were grouped by developmental stage, e.g. copepod eggs, nauplii, and postnauplii (copepodites and adult copepods), or by taxonomic category, e.g. *Evadne* spp. For each category, abundance in either the 53- or 200-μm net was measured, depending on size, e.g. copepod nauplii in the microplankton net, *Evadne* spp. in the mesozooplankton net.

Gut content analysis was carried out on 353 intact anchovy larvae and 306 intact sardine larvae selected from the Bongo samples in which they were most abundant, deemed the most representative. This allowed identification efforts to be concentrated on the zooplankton samples. Before dissection, standard length (SL) and mouth size (upper jaw length, UJL; lower jaw length, LJL; mouth width, MW) of the larvae were measured. The entire gut from each specimen was removed using a fine needle and placed in a drop of 50%-glycerine-distilled water on a glass slide, and prey organisms were teased out for identification, enumeration, and measurement. Identification of food particles in

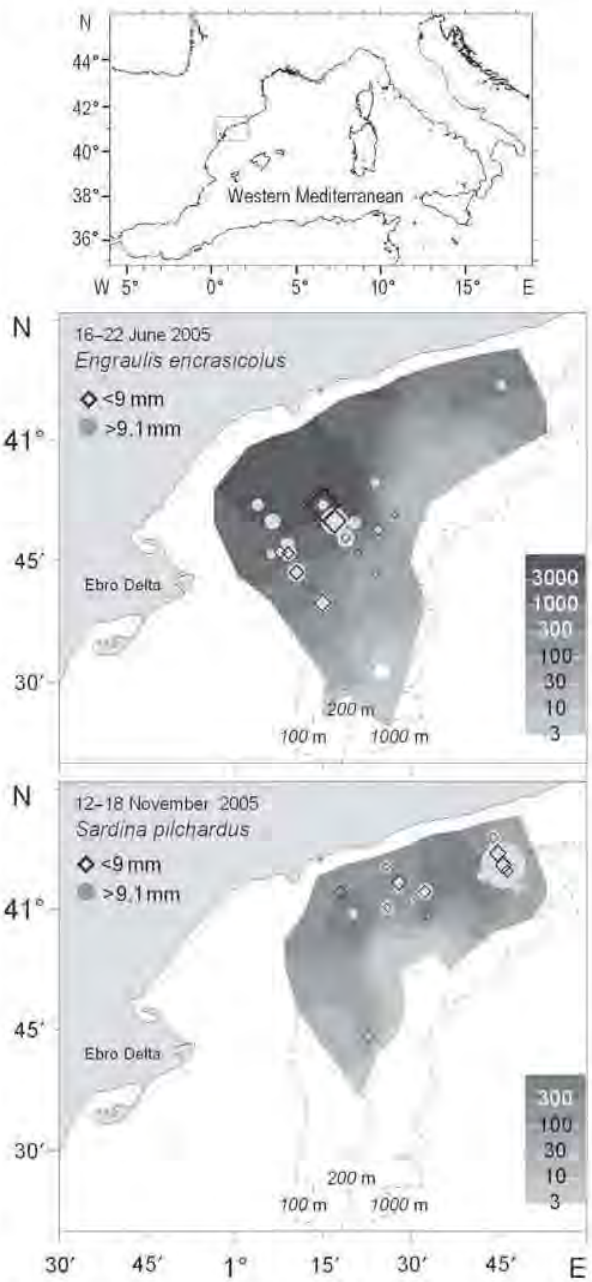

Figure 1. Distribution and abundance of anchovy and sardine larvae during the study period. Shaded contours indicate larval densities (number 10 m^{-2}) for all stations. Diamonds (small larvae) and circles (large larvae) indicate the number of larvae used for the diet analysis, with symbol size proportional to the number of larvae analysed (small, 1 larva; large, 17 larvae).

the gut was effected to the lowest taxon possible. Maximum body width of each prey item was measured under a microscope equipped with an ocular micrometer to the nearest 0.0025 mm along the widest cross-section.

To analyse trophic-niche breadth, larvae were classified by size class, selecting the intervals that would maximize the number of size classes containing three or more prey items. This turned out to be 0.12 mm SL. Following Pearre (1986), the s.d. of the $\log_{10}$-transformed prey size was taken as a measure of trophic-niche breadth.

Larvae were separated into length classes so as to be able to evaluate variations in diet and selectivity with size. As anchovy larvae are smaller at similar developmental stages, the size intervals for the two species differed. The anchovy larvae size classes were: (i) first feeding, 3.3–6.5 mm; (ii) flexion, 6.6–8.9 mm; (iii) post-flexion 1, 9–10.9 mm; and (iv) postflexion 2, 11–14.5 mm. The sardine larvae size classes were: (i) first feeding, 5.5–7.9 mm; (ii) preflexion, 8–9.9 mm; (iii) flexion, 10–11.9 mm; and (iv) late flexion–postflexion, 12–15.5 mm.

Data analysis

Feeding incidence (FI) was calculated as the percentage of the total number of larvae examined having at least one prey in the guts, by day (15 min before sunrise to 15 min after sunset) and by night. Diel-feeding activity was assessed using the gut fullness index, estimated on a scale of 0–4 (0, empty; 1, 25% full; 2, 50% full; 3, 75% full; 4, completely full; modified from Young and Davis, 1990). Feeding intensity was assessed based on the mean number of prey organisms in the gut contents of food-containing larvae over the course of development. Correlation, linear regression, and non-linear regression analysis were employed to study the different predator–prey relationships.

The quantitative importance of the different prey items in the diets was expressed using the index of relative importance (%IRI), obtained by multiplying the total number of ingested prey items examined (N) by the frequency of occurrence (F) of a prey item in larvae with food in their guts (Govoni *et al.*, 1986). Diversity of prey items in the diets was calculated using the Shannon index, $H' = -\sum_{i=1}^{m}(\mathrm{p}i \times \mathrm{Ln}\ \mathrm{p}i)$, where p$i$ is the relative abundance of prey item i in the diet and m the number of prey categories.

For the prey selectivity analysis, the alpha index, α_i (Chesson, 1978), was calculated as $\alpha_i = (r_i/p_i)\sum_{i=1}^{m}(r_i/p_i)^{-1}\ (i = 1, \ldots, m)$, where r_i and p_i are the percentage abundances of prey item i in the larval diet and in the plankton samples, respectively. Only the seven most common food organisms ingested by each species were considered, so focusing on preferences for organisms that contributed most to the larval diets, rather than on absolute prey preference (Govoni *et al.*, 1986). The value of α_i ranges from 0 to 1, with a critical value here of 1/7, higher values indicating preference and lower values rejection. As the index incorporates relative abundances of prey, it is unaffected by prey total abundance (Lechowicz, 1982). Prey selectivity was calculated for individual larvae by prey type by mean size class of larvae over 17 stations for anchovy and 16 stations for sardine. Diet overlap between the larvae of anchovy and round sardinella, another clupeoid whose larvae are also common in summer, was analysed based on data on sardinella diet reported previously (Morote *et al.*, 2008a). Diet overlap was quantified using Horn's index, R_0 (Horn, 1966), as:

$$R_0 = \frac{\left[\sum_{i=1}^{m}(p_{1i}+p_{2i})\ln(p_{1i}+p_{2i}) - p_{1i}\ln(p_{1i}) - p_{2i}\ln(p_{2i})\right]}{2\ln 2}$$
$$i = 1, \ldots, m,$$

where p_{1i} and p_{2i} are the percentage of prey item i of m prey categories in the gut contents of predator 1 (anchovy) and 2 (round sardinella), respectively. The value of this index ranges from 0 to 1, with values >0.6 indicating strong overlap (Sturdevant *et al.*, 2001).

Differences in the data were analysed by ANOVA and non-parametric (Mann–Whitney or Kruskal–Wallis) tests using the SPSS software package for Windows (SPSS Inc., version 17.0).

Results

Abundance and distribution of larvae

During the late spring survey, the distribution of anchovy larvae covered the entire study area, with the greatest concentrations (>3000 larvae 10 m^{-2}) over the broad continental shelf near the Ebro River delta. During the early autumn survey, sardine larvae were recorded over nearly the entire study area, with the greatest concentrations (>300 larvae 10 m^{-2}) over the shelf break (Figure 1). The spatial distributions of small (<9 mm SL) and large (>9 mm SL) anchovy larvae and small (<10 mm SL) and large (>10 mm SL) sardine larvae used for diet analysis were similar (Figure 1).

Diel-feeding pattern

In all, the guts of 353 *E. encrasicolus* larvae (size range 2.7–14.5 mm SL) and 306 *S. pilchardus* larvae (size range 5.5–15.8 mm SL) were examined. The smallest anchovy and sardine larvae with gut contents measured 3.3 and 5.5 mm, respectively. There were no yolk-sac larvae with ingested prey items. Naturally, the photoperiods on the two sampling surveys differed, with more hours of daylight per day in summer than in autumn.

Although the percentage of larvae with food in the gut (FI) was low (ca. 30%), the values were significantly higher by day than by night, for both species (Table 1; Mann–Whitney U-test, $p < 0.005$). This difference was more pronounced in anchovy larvae, for which the diurnal values of FI also increased significantly over the course of development ($p < 0.01$), compared with a slight decrease ($p > 0.05$) for sardine larvae. Analyses of the composition of the diet, the relative importance of food items, and prey selectivity considered samples collected only during daylight, to reduce uncertainty by excluding prey items in advanced stages of digestion.

The diel-feeding pattern was similar in small and large larvae (Figure 2). Both species fed diurnally, although the feeding periodicity for the two species differed. The diel-feeding pattern of anchovy revealed a progressive decrease in feeding activity after sunrise, when larval guts exhibited maximum fullness values, to night-time, when feeding activity was rare ($p < 0.01$). Sardine larvae, in contrast, exhibited maximum activity in the afternoon, although there was no clear trend ($r^2 = 0.010$, $p > 0.05$).

Prey number and size

The number of prey items ingested per larva was significantly lower in anchovy larvae (1.7 ± 1.07, range 0–5) than in sardine larvae (3.1 ± 2.42, range 0–10; Figure 3) for the entire size range considered (t-test, $p < 0.01$). On the other hand, no increase

Table 1. Diel FI for two size groups of anchovy and sardine larvae, shown as the number of larvae with food in the gut and the number of larvae dissected.

		Day			Night		
Species	**Size class (SL)**	**FI (%)**	**Number with food**	**Number examined**	**FI (%)**	**Number with food**	**Number examined**
Anchovy ($n = 353$)	<9 mm	24.3	45	185	2.4	1	41
	9–15 mm	38.8	45	116	0	0	11
	Total	29.9	90	301	1.9	1	52
Sardine ($n = 292$)	<10 mm	33.3	44	132	26.7	4	15
	10–16 mm	29.3	27	92	5.7	3	53
	Total	31.7	71	224	10.3	7	68

n, total number of larvae of each species examined.

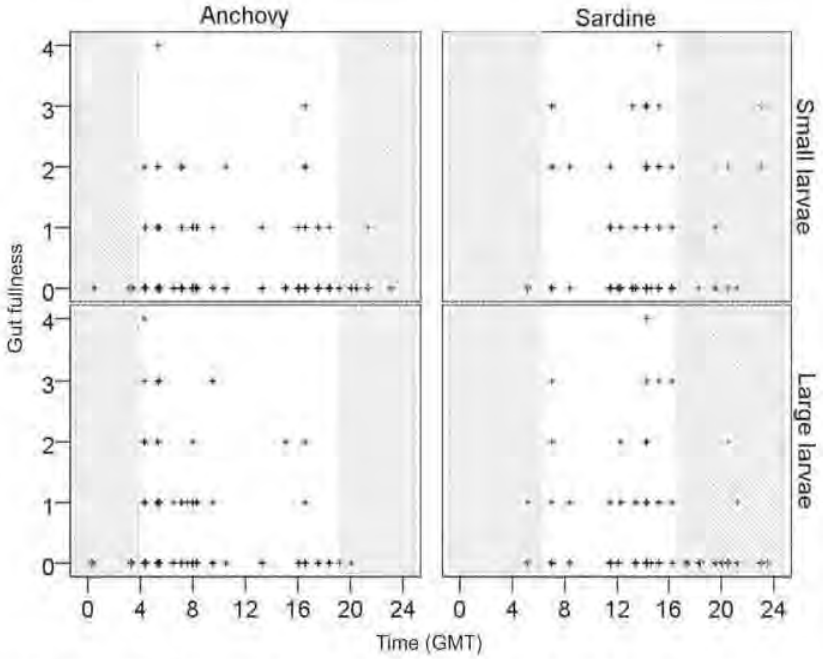

Figure 2. Diel patterns of feeding activity of anchovy (left) and sardine (right) larvae. Gut fullness ranges from 0 (empty) to 4 (full), for small (top) and large larvae (bottom) with time of day. Shadowed areas denote darkness.

in the number of prey items ingested per larva was observed for anchovy larvae over the course of larval development, whereas in sardine larvae, the number of prey items decreased with larval size, so that the number of prey items ingested by the larger larvae was similar in the two species.

The relationship of mouth size (width and length for both jaws) and SL was less than isometric in both species, indicating that mouth growth was slower than somatic growth (Table 2). A comparison of mouth size in the two species did not reveal significant differences (*t*-test, $p > 0.05$).

The mean width of prey items ingested by anchovy larvae (130.19 ± 62.74 μm) was significantly greater than that of prey items ingested by sardine larvae (97.05 ± 57.43 μm; *t*-test, $p < 0.001$). Prey size increased with larval length in both species, although the larvae continued to eat small prey items too (Figure 4). The smallest prey item recorded in anchovy was a protist (17.5 μm) in a larva measuring 9.25 mm SL, and the largest prey item was a single *Evadne* spp. (280 μm) in a larva that measured 10.5 mm SL, whereas in sardine, the smallest prey item recorded was a protist (12.5 μm) in a larva measuring 15 mm SL, and the largest prey item an *Euterpina* sp. postnauplius (310 μm) in a larva measuring 10.9 mm SL.

Large prey (>150 μm) entered the diet of anchovy larvae earlier (from 6 mm SL) and constituted 17% of the gut contents of larvae <9 mm SL, whereas they did not enter the diet of sardine larvae until 8 mm SL, and only accounted for 4.7% of the gut contents of larvae <10 mm SL. Although sardine larvae began by eating smaller prey items than anchovy larvae for a given mouth size, the rate of change in prey size in relation to mouth size was greater than in sardine ($p < 0.001$), resulting in prey of similar size to that in anchovy larvae at jaw lengths of 500 μm.

Trophic-niche breadth did not exhibit any change with length in anchovy larvae, whereas in sardine larvae, it underwent a slight but significant ($p < 0.001$) increase with increasing length, although the regression coefficient was small (Figure 5).

Composition of the diet

Diversity of the diet was slightly higher ($H' = 2.4$) in anchovy larvae than in sardine larvae ($H' = 2.2$; Table 3), with moderate

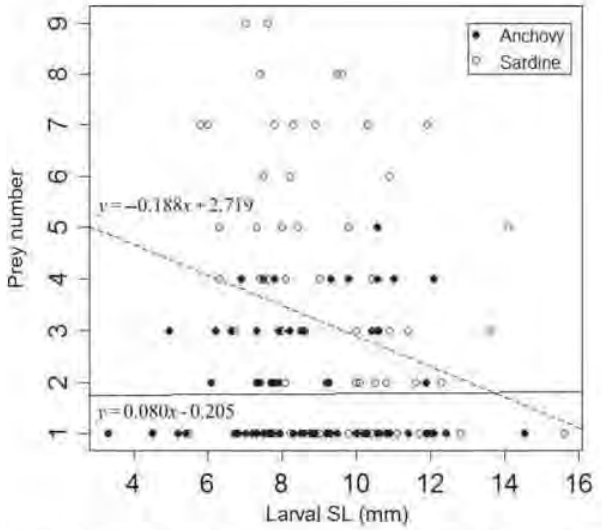

Figure 3. Number of prey items per gut plotted against anchovy and sardine larvae SL (mm). Regression analysis was significant for sardine (dashed line, $r^2 = 0.048$, $p < 0.05$) but not for anchovy larvae ($r^2 = 0.011$, $p > 0.05$).

Table 2. Regression parameters and statistics for the relationships between LJL, UJL, and MW and larval SL, with number of data points (n) for anchovy and sardine larvae, intercept (a), slope (b), 95% confidence interval (CI) and r values.

Parameter	n	a	b	CI of slope	r
Anchovy					
LJL	129	0.062	0.613	0.173	0.528
UJL	126	0.097	0.527	0.148	0.534
MW	144	0.090	0.699	0.107	0.733
Sardine					
LJL	266	0.041	0.756	0.096	0.690
UJL	263	0.051	0.785	0.085	0.749
MW	267	0.109	0.585	0.062	0.754

species richness and a uniform distribution of prey types in the diets of both species. When the percentage prey items by number was taken into account along with frequency of occurrence (IRI%), differences related to larval size were again evident between the two species (Table 3). Anchovy larvae <9 mm SL fed mainly on copepod nauplii (38%) and *Microsetella* sp. postnauplii (43%), whereas >9 mm SL larvae fed on a wide variety of prey, with *Microsetella* sp. postnauplii (44%) and the cladoceran *Evadne* spp. (37%) dominating. Sardine larvae <10 mm SL fed mainly on the tintinnid *Codonellopsis* sp. (48%) and copepod nauplii (46%), whereas the diet of the larger larvae was based on copepod nauplii (22%) and calanoid (mainly *Clausocalanus*) postnauplii (53%).

There was a tendency for the number of prey types to decrease in the largest size classes analysed, a trend evident earlier in sardine than in anchovy (Figure 6). Most of the prey items eaten by anchovy were copepods (nauplii and postnauplii) and cladocerans, other less frequent items being phytoplankton, protists (the tintinnid *Rhabdonella* sp. and a cyst), ostracods, appendicularians, unidentified eggs, pollen grains, and faecal pellets (the last two items grouped as "Others"). The main prey items eaten by sardine larvae were protists (the tintinnid *Codonellopsis* sp. and

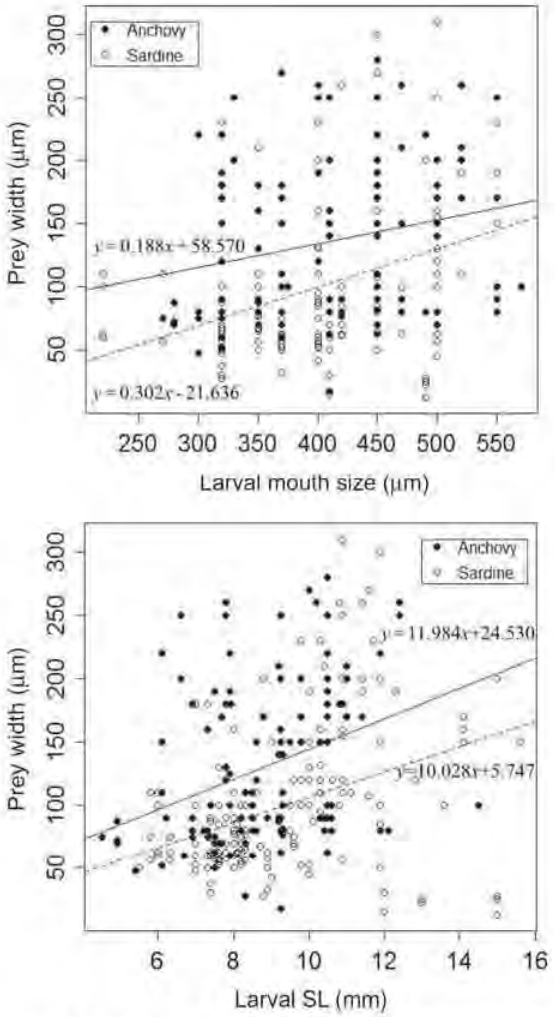

Figure 4. Relationships between prey size and (top) mouth size (width) and (bottom) SL of larvae. Regression lines (top) plotted against mouth size for anchovy ($r^2 = 0.041$, $p < 0.05$) and sardine larvae (dashed line, $r^2 = 0.152$, $p < 0.001$), and (bottom) plotted against SL for anchovy ($r^2 = 0.104$, $p < 0.001$) and sardine larvae (dashed line, $r^2 = 0.137$, $p < 0.001$).

cysts) and different copepod stages (including a small percentage of eggs). The number of other food items such as phytoplankton (identified as green remains), particulate organic matter (POM), larval polychaetes, appendicularians, and pollen grains and faecal pellets ("Others") in the gut contents of sardine larvae was low. Phytoplankton was eaten only by early-stage anchovy and sardine larvae (Figure 6).

The value of Horn's index for the two co-occurring species, anchovy and round sardinella, was slightly higher for the <9 mm SL group (0.833) than for the >9 mm SL group (0.765), but both these values indicated a high level of diet overlap between the two species.

Food availability and prey selectivity

The mean abundance of all copepod stages was comparable during the two survey periods (~10 nauplii l^{-1} and ~4 postnauplii l^{-1}; t-test, $p > 0.05$). On the other hand, availability of many of the

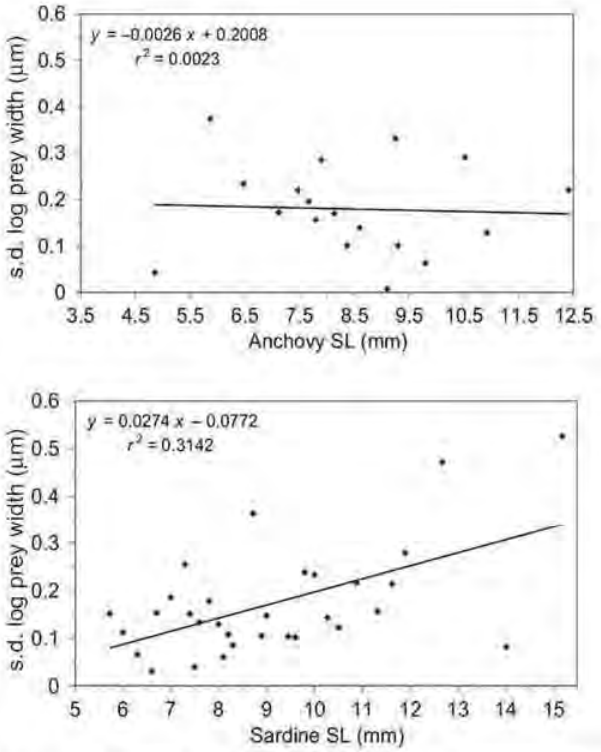

Figure 5. Relationship of trophic-niche breadth, expressed as the log s.d. of prey width, against length (SL) for anchovy (top) and sardine (bottom) larvae.

other prey items that made up the diets of anchovy and sardine larvae differed significantly between June, when anchovy larvae are found, and November, when sardine larvae dominate (Figure 7). Of the small prey items, copepod eggs were more abundant when anchovy larvae were present (*t*-test, $p < 0.0001$) and the tintinnid *Codonellopsis* sp. in the period when sardine larvae were present (Mann–Whitney *U*-test, $p < 0.0001$), with copepod nauplii and *Microsetella* sp. postnauplii displaying similar abundance levels in both periods. Of the large prey items, cladocerans (*Evadne* spp. and *Penilia avirostris*) were more abundant in the period of anchovy larval abundance, compared with ostracods, polychaetes, and *Temora* sp. postnauplii in the period of sardine larval abundance (Mann–Whitney *U*-test, $p < 0.02$). Appendicularians and cyclopoid (*Oithona* spp. and *Oncaea* spp.) postnauplii were also significantly more abundant in summer (for anchovy larvae), and postnauplii of the harpacticoid *Euterpina acutifrons* and of the main calanoid species (*Clausocalanus*, *Paracalanus*, p-*calanus*) were more abundant in autumn (sardine larvae; *t*-test, $p < 0.05$). No information on the availability of the smallest protist prey items and POM could be collected, because they were not retained by the mesh used for plankton sampling.

The seven main zooplankton taxa present in the gut contents of the anchovy and sardine larvae were examined to assess whether they were ingested selectively (Table 4), a value of $\alpha > 0.14$ indicating positive selection. There was a shift in preference towards bigger prey items as the larva size increased. Anchovy larvae <9 mm SL had a strong and nearly exclusive preference for copepod nauplii, their preference then changing towards bigger prey such as copepod postnauplii and the cladoceran *Evadne* spp. Sardine larvae <10 mm SL exhibited preferences for a wide variety of prey, whereas the selectivity in larvae >10 mm SL was pronounced but less diverse, with an exclusive preference for calanoid postnauplii.

Phytoplankton was generally avoided as food. The cladoceran *Penilia* was rejected by anchovy larvae. Copepod eggs were recorded only in sardine larvae, but tended to be avoided by the larvae. Selectivity on cyclopoid postnauplii was zero or nearly neutral in both species, even in the smallest larvae.

Discussion

This was the first study aimed at elucidating the feeding habits of sardine larvae in the Mediterranean, and comparing them with the feeding habits of anchovy larvae in the same area, though under different environmental conditions. Anchovy spawning takes place from late spring to summer, when water temperatures are warm and the water column is stratified, and anchovy larvae are then found along with the larvae of many other fish species. Sardine spawning, in contrast, takes place in autumn/winter, so their larvae inhabit colder waters during a period of vertical mixing of the water column, and they are found alongside the larvae of fewer other fish species (Sabatés *et al.*, 2007). Because there are more daylight hours in summer than in winter, and because anchovy and sardine larvae, like those of other species of fish (Last, 1980), are visual predators (Blaxter and Hunter, 1982), anchovy larvae have more foraging time. The growth rates of the larvae of the two species are similar (Palomera *et al.*, 2007), despite the different environmental conditions in which the larvae of the two species develop, so it has been suggested that sardine larvae must compensate for the lower temperatures in autumn/winter based on a more appropriate food supply (prey abundance and prey type or predation strategy; Catalán *et al.*, 2004). Nevertheless, on these two surveys, the mean abundance of copepods, the main prey organism in the diets of the larvae of both species, was similar.

The larvae of both species had similar low FI, considered a measure of a larva's ability to obtain food from the environment (Arthur, 1976), although the FI for anchovy was twice the level (15%) previously reported in the Northwest Mediterranean by Tudela *et al.* (2002). The FI of sardine larvae was similar to that reported for the same species in other regions (Conway *et al.*, 1994; Munuera-Fernández and González-Quirós, 2006). The low FI was in agreement with the findings of many other studies of larval clupeoid feeding (de Ciechomsky, 1966; Llanos *et al.*, 1996; Voss *et al.*, 2003). Regurgitation or defaecation of gut content on capture or fixing has been postulated as a reason for this low FI given the straight gut in these larvae (Hay, 1981). However, round sardinella (*S. aurita*) larvae were collected along with anchovy larvae in the June samples, and they have the same gut morphology as anchovy and sardine, yet their FI was roughly double that of anchovy larvae (Morote *et al.*, 2008a). Therefore, there must be other factors besides gut morphology, prey availability, and the method of collection that contribute to the low FI in anchovy and sardine. Therefore, our results suggest that the nutritional requirements of each species, along with predation skills, may also influence feeding activity, and that anchovy and sardine larvae have either lower energy requirements or less developed predation skills than round sardinella.

The number of prey items ingested per larva was low in both species, indicating a low level of voracity. In anchovy, this value was similar to that previously reported in the Northwest Mediterranean (Tudela *et al.*, 2002). The value for sardine larvae

Table 3. Diets for two size groups of anchovy (top) and sardine (bottom) larvae.

Prey main group	Prey taxon	N (%)	F (%)	IRI (%)	N (%)	F (%)	IRI (%)
Anchovy ($H' = 2.4$)		<9 mm SL			9–15 mm SL		
Phytoplankton	–	2.94	3.64	0.70	1.52	1.85	0.21
Protists	Cysts	0	0	0	1.52	1.85	0.21
	Tintinnids	0	0	0	1.52	1.85	0.21
Copepoda	Nauplii	26.5	21.8	37.83	4.55	5.56	1.91
	Clausocalanus sp.	1.47	1.82	0.17	3.03	3.7	0.85
	Paracalanus sp.	0	0	0	1.52	1.85	0.21
	p-calanus	0	0	0	1.52	1.85	0.21
	Unidentified calanoid postnauplii	1.47	1.82	0.17	10.61	7.41	5.95
	Oithona sp.	4.41	5.45	1.58	1.52	1.85	0.21
	Oncaea sp.	4.41	5.45	1.58	7.58	9.26	5.31
	Unidentified cyclopoid postnauplii	0	0	0	3.03	1.85	0.42
	Microsetella sp.	27.9	23.6	43.26	24.24	24.07	44.16
	Euterpina sp.	5.88	7.27	2.80	3.03	3.7	0.85
	Unidentified postnauplii	0	0	0	0	0	0
Cladocera	*Evadne* sp.	10.3	10.9	7.35	24.24	20.37	37.37
	Penilia sp.	5.88	7.27	2.80	3.03	3.7	0.85
Ostracoda	–	0	0	0	1.52	1.85	0.21
Appendicularia	*Fritillaria* sp.	1.47	1.82	0.17	0	0	0
Other eggs	–	0	0	0	1.52	1.85	0.21
Other	Pollen grains	1.47	1.82	0.17	1.52	1.85	0.21
	Faecal pellets	2.94	3.64	0.70	1.52	1.85	0.21
Crustacean remains	–	2.94	3.64	0.70	1.52	1.85	0.21
Sardine ($H' = 2.2$)		<10 mm			10–16 mm		
Phytoplankton	Green remains	4.9	8.54	2.05	0	0	0
Protists	Cysts	0	0	0	10.9	8.2	7.53
	Tintinnids	39.9	24.4	47.68	1.6	2.04	0.27
Copepoda	Copepod eggs	0.7	1.22	0.04	0	0	0
	Nauplii	32.2	29.3	46.17	18.8	14.29	22.59
	Clausocalanus sp.	3.5	4.88	0.84	20.3	22.45	38.44
	Calocalanus sp.	0.7	1.22	0.04	0	0	0
	Paracalanus sp.	0.7	1.22	0.04	1.6	2.04	0.27
	p-calanus	1.4	2.44	0.17	14.1	12.24	14.52
	Temora sp.	0	0	0	1.6	2.04	0.27
	Centropages sp.	0	0	0	1.6	2.04	0.27
	Oithona sp.	1.4	2.44	0.17	1.6	2.04	0.27
	Oncaea sp.	0	0	0	3.1	4.08	1.07
	Microsetella sp.	4.2	6.1	1.25	0	0	0
	Euterpina sp.	2.1	3.66	0.38	7.8	8.16	5.38
	Unidentified postnauplii	2.1	3.66	0.38	7.8	8.16	5.38
Polychaeta	–	0.7	1.22	0.04	0	0	0
Appendicularia	–	1.4	2.44	0.17	0	0	0
Other eggs	–	0	0	0	1.6	2.04	0.27
Other	Pollen grains	1.4	2.44	0.17	0	0	0
Particulate organic matter	–	0.7	1.22	0.04	3.1	4.08	1.08
Crustacean remains	–	2.1	3.66	0.38	4.7	6.12	2.42

H', Shannon–Wiener diversity index; N (%), frequency of abundance of prey items in percentage; F (%), frequency of occurrence in the guts in percentage; IRI = (F% × N%), expressed as a percentage.

was slightly higher than that for anchovy and was in agreement with the value for the same larvae reported in the Spanish Bay of Biscay (Conway *et al.*, 1994). This value declined with growth in tandem with the shift in prey size. An increase in prey size with length was evident in both species, but in anchovy it was progressive from the very early developmental stages (ca. 6 mm SL) and in sardine the shift in prey size with mouth size was more abrupt, taking place in the largest larvae. Arthur (1976) also observed the size of prey items in first-feeding northern anchovy to be bigger than that in first-feeding Pacific sardine, and Hunter (1977) reported that to maintain their metabolism in the laboratory, northern anchovy larvae needed large prey in addition to small prey from a size of 6 mm SL. Urostyle flexion and initial fin development take place at smaller lengths in anchovy than in sardine larvae, suggesting that anchovy larvae may be better swimmers at smaller lengths, which would help improve their ability to capture larger prey at a smaller size than sardine larvae. The more advanced development of anchovy than sardine larvae at the same length has been reported for other clupeoid larvae, with prey detection distance, the volume of water searched, and the escape response rate for engraulids all higher than for clupeids (Detwyler and Houde, 1970; Butler and

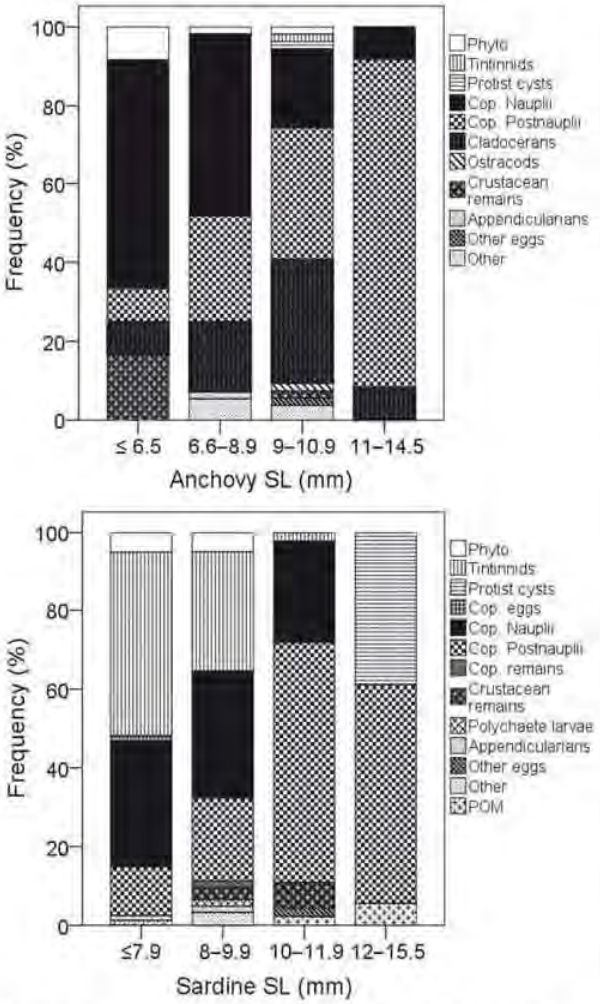

Figure 6. Percentage of the different prey types in the guts of anchovy (top) and sardine (bottom) larvae by size class.

Pickett, 1988). The different degree of development in sardine larvae at the same size as anchovy larvae can most likely be attributed to slower bone development in the feeding apparatus, which could affect feeding performance, as has been observed for other fish larvae in the laboratory (Beck and Turingan, 2007; Wittenrich *et al.*, 2009), constraining small sardine larvae to prey on smaller, less elusive prey than small anchovy larvae. The results reported here for both species suggest that the ontogenetic shift in the diet is due not to differing trophic environments for small and large larvae (both of which were taken at the same stations), but to a change in food selection patterns.

Trophic differences between the two species were also observable with respect to prey type and prey selection. Early-stage anchovy and sardine larvae had broader diets, with copepod nauplii alternating with other small organisms such as tintinnids, phytoplankton, molluscan larvae, and ciliates, as has been reported for other clupeoid larvae (Arthur, 1976; Llanos *et al.*, 1996; Morote *et al.*, 2008a). The most likely cause is that the plankton contains a greater variety of small organisms that are not highly mobile and hence are more prone to capture by larvae whose swimming ability has not yet fully developed and whose small gape restricts their choice of prey type (Hunter, 1980).

Protozoans, like tintinnids, were occasionally present in the diet of anchovy larvae, as reported previously (Conway *et al.*, 1998; Tudela *et al.*, 2002), but they were a major component, and were positively selected, in the diet of <10 mm SL sardine larvae, in agreement with other published results (Arthur, 1976; Stoecker and Govoni, 1984; Conway *et al.*, 1994; Munuera-Fernández and González-Quirós, 2006). These prey items were discernible in the gut contents because they have hard parts resistant to digestion. However, as Govoni and Chester (1990) pointed out for the sciaenid *Leiostomus xanthurus*, feeding by sardine and anchovy larvae on naked ciliates, which are undetectable under the light microscope employed in this study, cannot be ruled out, because they are very important in food chains in oligotrophic systems such as the Northwest Mediterranean (Calbet and Saiz, 2005). After analysing the fatty acids in the plankton and those of anchovy larvae in the area, Rossi *et al.* (2006) suggested that first-feeding anchovy larvae might feed on those organisms. Copepod eggs and nauplii have been described as the principal prey items in the diets of anchovy and sardine larvae in most published work, with copepod eggs gradually being replaced by copepodites with larval growth (Conway *et al.*, 1994, 1998; Llanos *et al.*, 1996; Tudela *et al.*, 2002; Munuera-Fernández and González-Quirós, 2006). Our findings were in agreement with these results, except that anchovy larvae did not feed on copepod eggs, and sardine and round sardinella larvae (Morote *et al.*, 2008a) ingested only very small numbers of copepod eggs, which were therefore negatively selected, although they were abundant in the area at both times of year.

There was a seasonal component in selectivity for certain prey species, e.g. *Evadne* spp. in late spring and *Codonellopsis* in autumn, which are preyed on and positively selected only when well represented in the plankton. However, a characteristic species-specific factor was also observed in food selection by clupeoid larvae. An example is the copepod *Microsetella* sp., which was abundant in the plankton in both periods and an important contributor to the diet of anchovy larvae but absent from the diets of sardine and round sardinella larvae (Morote *et al.*, 2008a).

Although prey size has been considered to be the principal factor contributing to selectivity patterns in larval fish, some results have reported selectivity based on prey type in clupeoid larvae (Checkley, 1982; Govoni *et al.*, 1986). Prey items are more easily spotted as they develop intense pigmentation and high activity levels, making them more conspicuous (Buskey *et al.*, 1993). In addition, species-specific larval abilities such as sensory and locomotor skills play a determining role in ensuring predation success during encounters with prey organisms (Checkley, 1982). The shift in prey-type selection by anchovy larvae to larger, more nutritious items, notwithstanding their better swimming ability, took place gradually over the course of larval development. Cladocerans (large, but poor swimmers; Drenner *et al.*, 1978; Viitasalo *et al.*, 2001) were intermediate prey items between copepod nauplii and calanoid copepods, which are large and elusive and whose capture entails substantial outlay of energy (Coughlin, 1991). In contrast, there was no such intermediate prey item for sardine larvae, and selectivity shifted directly from small prey items with low mobility, such as tintinnids and copepod nauplii, to calanoid copepods (exclusively selected by >10 mm SL larvae). The small harpacticoid *Microsetella* sp. is a readily visible prey organism on account of both its pigmentation and its poor swimming ability (Uye *et al.*,

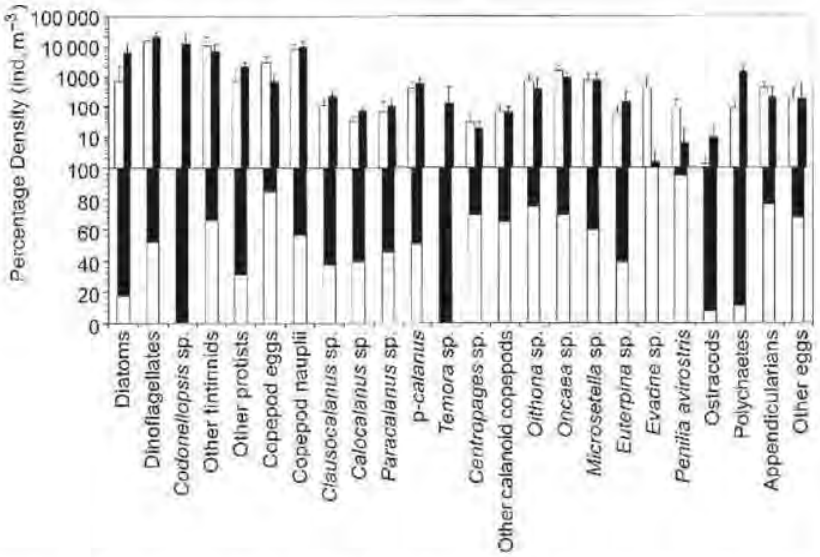

Figure 7. Planktonic organisms collected during the June (white) and November (black) cruises. Mean abundance (expressed as number m ^{3}), with standard deviations (lines; top), and the relative contribution of each category in each period (bottom).

Table 4. Mean Chesson's α-values ($\pm$ s.d.) for the most common prey items in four different size classes of anchovy (top) and sardine (bottom) larvae.

Species, SL (mm) and parameter	Phytoplankton	Copepod nauplii	Cyclopoid postnauplii	Harpacticoid postnauplii	Calanoid postnauplii	*Evadne*	*Penilia*
Anchovy <6.5							
N	5	5	5	5	5	5	5
α	0	**0.806**	0	0	0	0	0.194
s.d.	0	0.4340	0	0	0	0	0.4340
Anchovy 6.6–8.9							
N	29	29	29	29	29	29	29
α	0.039	**0.405**	0.136	0.170	0.012	0.144	0.093
s.d.	0.1866	0.4809	0.3387	0.3504	0.0658	0.3174	0.2804
Anchovy 9–10.9							
N	29	29	29	29	29	29	29
α	0.001	**0.248**	0.145	0.150	0.103	**0.324**	0.030
s.d.	0.0016	0.4332	0.3388	0.3285	0.3090	0.4651	0.1615
Anchovy 11–15							
N	9	9	9	9	9	9	9
α	0	0.111	0.009	**0.333**	**0.435**	0	0.111
s.d.	0	0.3333	0.0278	0.5000	0.5167	0	0.3333

Species, SL (mm) and parameter	Phytoplankton	Codonellopsis	Copepod eggs	Copepod nauplii	Cyclopoid postnauplii	Harpacticoid postnauplii	Calanoid postnauplii
Sardine <7.9							
N	23	23	23	23	23	23	23
α	0.016	**0.344**	0.029	**0.246**	0.043	0.172	0.149
s.d.	0.0465	0.3921	0.1394	0.3192	0.2085	0.3834	0.3350
Sardine 8–9.9							
N	19	19	19	19	19	19	19
α	0.002	0.197	0	**0.297**	0.033	**0.306**	0.165
s.d.	0.0074	0.3520	0	0.4184	0.1429	0.4637	0.3725
Sardine 10–11.9							
N	17	17	17	17	17	17	17
α	0	0	0	0.137	0.018	0.225	**0.619**
s.d.	0	0	0	0.3285	0.0762	0.4192	0.4634
Sardine 12–16							
N	6	6	6	6	6	6	6
α	0	0	0	0	0.167	0	**0.833**
s.d.	0	0	0	0	0.4082	0	0.4082

Emboldened numbers indicate strong selection.

2002), so it would seem to be a suitable intermediate prey item for sardine larvae, given that cladocerans are not available in the plankton in autumn. Nevertheless, it was not selected. Sardine larvae most likely do not prey actively on postnauplii until their feeding apparatus and locomotor skills are well developed, at which time they specialize on calanoid copepods, one way to ensure successful strikes on these more nutritious but at the same time more elusive prey. The shift in the diet results in a drop in feeding index in larger larvae, because the larvae have to learn how to attack these new, larger, more mobile prey, as Hunter (1972) observed in northern anchovy larvae in the laboratory.

The larvae of most fish species have been reported not to alter their trophic-niche breadth over the course of development (Pearre, 1986; Sabatés and Saiz, 2000), although increases (Pepin and Penney, 1997) or decreases (Morote *et al.*, 2008b; Llopiz and Cowen, 2009) have been reported for a few species. Our results were indicative of a slight increase in trophic-niche breadth in sardine larvae because of the presence of both calanoid copepods and small cysts in the diet of large larvae. Although increases in trophic-niche breadth have been observed to take place at low densities of the main prey organisms (Munk, 1995), in our opinion this is unlikely to be the cause for sardine larvae, inasmuch as plankton concentrations were observed to be similar in size to those in summer, and total fish larva abundance much lower. One reason could be that sardine larvae continue to prey upon small, slow-moving organisms as a source of easy-to-capture food to offset unfavourable environmental conditions. Protozoans are important components of the larval diets of other species (Govoni and Chester, 1990; Pepin and Dower, 2007), but additional work employing different methods would be needed to be able to ascertain the role played by protozoans in the diet of sardine larvae in the area.

The anchovy and round sardinella larvae employed a generalist foraging strategy, selecting different copepods and *Evadne* spp. This was in consonance with reports for other shelf species in the same area at the same time, e.g. white sea bream (*Diplodus sargus*) and bogue (*Boops boops*; Sánchez-Velasco and Norbis, 1997), whereas predation by the larvae of certain other spring/summer-spawning species such as scombrids (Catalán *et al.*, 2007; Morote *et al.*, 2008b) and bothids (Sánchez-Velasco, 1998) specializes on other prey organisms, e.g. appendicularians and fish larvae. Despite the extensive overlap in prey type and size in the diets of anchovy and round sardinella larvae, certain food resources were exploited by only one species, e.g. *Microsetella* sp. by anchovy larvae. In contrast, although they are similar to anchovy and round sardinella in morphology, sardine larvae employed a different strategy, specializing on calanoid copepods. Similar specialization has also been observed in the larvae of another species that occurs in the plankton along with sardine larvae, i.e. hake (*Merluccius merluccius*), which prey nearly exclusively on *Clausocalanus* sp. from first feeding (EM, unpublished data).

In summary, although the diets of anchovy and sardine larvae are similar, being based mainly on various stages of copepods, more-detailed analysis reveals differences at all stages of development. Anchovy larvae feed on larger prey from a smaller larval size than do sardine larvae, which continue to feed on small prey practically exclusively until they reach 10 mm SL. Selection of certain prey items also differs in the two species, in some cases because of seasonal effects on prey availability, e.g. cladocerans (taken by anchovy in late spring) and the tintinnid *Codonellopsis* sp. (taken by sardine in autumn), and in other cases probably because of species-specific preferences that may be linked to visual acuity, swimming ability, and ecophysiology. The feeding strategies of the larvae of these two species changed along opposite lines with ontogeny. Therefore, although the largest anchovy larvae turned into generalist predators, sardine larvae specialized on one type of prey organism.

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Diet of round sardinella, *Sardinella aurita*, larvae in relation to plankton availability in the NW Mediterranean

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The present study analyses for the first time the diet of larvae of the clupeid Sardinella aurita in the NW Mediterranean. Gut contents of larvae from first feeding (3.9 mm) to early postflexion stages (14.7 mm) were analysed. The type and abundance of ingested prey were related to the plankton composition in the environment in order to determine prey-type selectivity. The feeding incidence observed was among the highest reported for clupeid larvae, with higher values during the day (68.6%) than at night (7.7%), indicating that Sardinella aurita larvae are diurnal predators. The mean number of ingested prey was 3.3 (±2.51). The width of the prey increased from <30 to 410 μm with the development of the larvae, but the niche breadth did not vary with the size of the larvae. The diet was based on different stages of copepods (nauplii and postnauplii) and cladocerans (mainly Evadne spp.), though the preference for each type of prey varied, with higher preference for copepod nauplii in larvae <8 mm and a higher preference for Evadne spp. in larvae ≥8 mm. The copepod based diet of S. aurita larvae is very similar to that reported for Engraulis encrasicolus larvae in the Mediterranean, indicating a possible competition for food between the first-feeding larvae in situations of low prey abundance.

INTRODUCTION

Clupeiform fishes are important because of their high biomass and their role in marine ecosystems. In the Mediterranean, they constitute almost 50% of the total commercial landings (Lleonart and Maynou, 2003). In the NW Mediterranean, the most important species are the sardine, *Sardina pilchardus*, and the anchovy, *Engraulis encrasicolus*, though the commercially less valuable round sardinella, *Sardinella aurita*, is also present in lower abundance (Palomera *et al.*, 2007). *Sardinella aurita* was not abundant along the Catalan coast 20 years ago, but recent studies have shown that its distribution has moved northwards with the positive sea surface temperature anomalies of the last few decades (Sabatés *et al.*, 2006).

In the Northwestern Mediterranean, the *S. aurita* spawning period runs from June to September, when water temperature reaches the yearly maximum (23–25°C). This period is coincident with spawning of *E. encrasicolus* in the area, but the two species have some spatial segregation, the distribution of the eggs of *S. aurita* being in much shallower water (Palomera and Sabatés, 1990). Coastal areas are characterized by high environmental variability and it is likely that this species is able to survive as a result of its great plasticity and adaptability (Cury and Fontana, 1988).

Although distribution patterns and growth of clupeoid larvae in the Mediterranean have been extensively studied (Olivar *et al.*, 2001; Tsikliras *et al.*, 2005; Palomera *et al.*, 2007), larval feeding information is

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scarce for *E. encrasicolus* (Conway *et al.*, 1998; Tudela *et al.*, 2002) and sardine larvae (Rasoanarivo *et al.*, 1991) and non-existent for *S. aurita*. There is only one study on larval feeding for a closely related species, *S. brasiliensis*, in the South Atlantic (Kurtz and Matsuura, 2001).

The aim of the present work is to determine the larval feeding ecology of *S. aurita* by analysing diel feeding patterns, diet composition and selectivity as a function of prey availability. We also discuss a possible competition for prey between the larvae of this species and those of anchovy, because they co-occur during the summer period in the NW Mediterranean.

MATERIALS AND METHODS

The study area extended along the continental shelf and slope of the southern sector of the Catalan coast (Spain), from near Barcelona to the north of the Ebro River Delta. The survey was conducted from 16–22 June 2005, at the beginning of *S. aurita*'s spawning season. Larvae were collected by oblique bongo net hauls (300 μm mesh) from 200 m (or just above the bottom) to the surface. The larvae were separated immediately and stored in groups of no more than 10 per vial. The volume of sea water filtered was measured with flowmeters placed in the centre of the bongo net mouth openings.

Eight depth-stratified hauls, at 10 m depth intervals, were taken within a small area using a Longhurst Hardy Plankton Recorder (LHPR; Williams *et al.*, 1983) to determine vertical larval distribution of the different size classes. To measure the food availability, micro and mesozooplankton samples were collected just before the bongo samples at each station by vertical hauls using a Calvet net (Smith *et al.*, 1985) fitted with 53 and 200 μm mesh nets. Before preservation, microzooplankton samples were sieved through 200 μm to remove mesozooplankton, and mesozooplankton samples were filtered through 2000 μm to eliminate macroozooplankton that might have been incidentally caught. All samples were preserved in 5% buffered formaldehyde solution.

One of the bongo net samples was used to quantify the ichthyoplankton and the other for on-board sorting of the larvae for feeding studies. To avoid damage to the larvae, the cod-end-containing larvae for feeding studies was removed before the net was washed down.

Laboratory analyses

Gut content analysis was carried out on a selection of 219 intact *S. aurita* larvae collected in bongo samples from the 23 stations where they were most abundant. Before dissection, the following measurements were recorded to the nearest 0.01 mm: standard length (SL); lower jaw length (LJL), measured from the tip to the junction with the maxilla; upper jaw length (UJL), measured from the tip of the snout to the posterior end of the maxilla; and mouth width (MW), measured ventrally as the widest distance between the posterior edge of the maxillae. Dissected larvae ranged from 3.9 to 14.7 mm. The complete digestive tract was removed using a fine needle and placed in a drop of 50% glycerine distilled water on a glass slide. Gut contents were identified to the lowest taxon possible and the maximum width measured to the nearest 0.0025 mm. This is the dimension that would determine whether the larvae could ingest the organism.

Micro and mesozooplankton specimens were quantified for the 23 stations from which larvae were selected, using the Calvet net samples. Identified prey items were grouped by taxonomic categories, by developmental stage [e.g. copepod eggs, nauplii, and postnauplii (which include copepodites and adult copepods)] or by specific criteria (e.g. *Evadne* spp.), according to the prey categories identified in the diet analysis. For each category, the abundance in either the 53 or the 200 μm net was used, depending on their size (e.g. copepod nauplii in the microplankton net, *Evadne* spp. in the mesozooplankton net, etc.) (Table I).

Data analysis

The feeding incidence of *S. aurita* larvae was taken as the percentage of specimens examined with at least one prey organism and was calculated separately for larvae collected during the day (from sunrise to sunset) and at night (from sunset to sunrise). The feeding intensity was assessed as the mean number of prey organisms in the larvae. The gut fullness index was estimated on a scale of 0–4 (0 = empty; 1 = 25% full; 2 = 50% full; 3 = 75% full; 4 = completely full). The degree of prey digestion was estimated on a scale of 1–3 (1=highly digested, completely transparent; 2 = partially digested; 3 = undigested, some colour remains) (Young and Davis, 1990, modified).

In order to analyse the relationships between prey width (PW) and larval size (SL) and MW, the larvae were divided into size intervals so as to produce the maximum number of size classes containing three or more prey. To fit this requirement, an interval of 0.12 mm was used for SL and 0.01 mm for MW. The trophic niche was analysed according to Pearre (Pearre, 1986) as the standard deviation (SD) of the log 10 transformed PW.

Table I: Plankton abundance

	Microplankton			Mesoplankton		
Taxa	≤100 m	>100 m	*P*-value	≤100 m	>100 m	*P*-value
Diatoms	398.9 ± 475.9	**1127.1 ± 1275.0**	**0.026**	—	—	—
Dinoflagellates	**16 863.0 ± 6383.4**	9599.3 ± 3934.7	**<0.001**	**5814.0 ± 2621.5**	3579.6 ± 1765.5	**0.005**
Tintinnids	**12 731.7 ± 10 956.0**	5665.5 ± 5476.6	**0.019**	—	—	—
Other protists	**816.2 ± 835.6**	408.7 ± 235.4	**0.018**[a]	46.7 ± 43.7	53.9 ± 28.9	0.558
Copepod eggs	**3233.2 ± 1528.0**	1611.8 ± 1025.4	**<0.001**	—	—	—
Copepod nauplii	**9507.2 ± 2917.1**	7307.2 ± 2243.5	**0.008**	—	—	—
Harpacticoid copepod postnauplii	1330.5 ± 673.3	1536.4 ± 622.1	0.307	15.9 ± 25.4	16.0 ± 34.0	0.668[a]
Cyclopoid-Poecilostomatoid copepod postnauplii	**1898.6 ± 552.0**	1262.7 ± 548.2	**0.001**	269.9 ± **66.5**	209.0 ± 88.9	**0.027**
Calanoid copepod postnauplii	433.2 ± 341.8	488.5 ± 246.9	0.540	359.5 ± **130.4**	247.3 ± 74.3	**0.003**
Penilia avirostris (cladoceran)	—	—	—	86.0 ± **101.0**	18.1 ± 45.8	**0.002**[a]
Evadne spp. (cladoceran)	—	—	—	556.5 ± **404.8**	90.6 ± 111.6	**<0.001**
Other crustaceans	—	—	—	19.4 ± 13.7	15.5 ± 11.5	0.357
Cnidarians	22.4 ± 51.6	13.2 ± 58.6	0.338[a]	159.6 ± **89.8**	84.5 ± 55.6	**0.004**
Polychaetes	57.9 ± 58.1	82.2 ± 71.0	0.244	24.6 ± 18.8	17.1 ± 10.7	0.147
Bivalves	100.7 ± 64.5	**259.2 ± 130.7**	**<0.001**	—	—	—
Gastropods	**170.8 ± 110.2**	94.5 ± 74.4	**0.016**	31.0 ± **18.7**	17.1 ± 12.8	**0.013**
Chaetognaths	2.8 ± 12.0	0.0 ± 0.0	0.239[a]	6.3 ± 6.4	4.9 ± 4.1	0.405
Echinoderms	48.9 ± 49.4	82.6 ± 111.1	0.189	77.7 ± 42.9	72.6 ± 67.7	0.794
Appendicularians	578.0 ± 403.9	372.2 ± 356.7	0.085	438.5 ± **344.1**	248.1 ± 135.9	**0.045**
Doliolids	45.7 ± **69.6**	13.8 ± 38.6	**0.023**[a]	493.8 ± **569.7**	30.6 ± 46.0	**<0.001**[a]
Others	300.1 ± 240.3	217.7 ± 180.9	0.207	46.3 ± 45.1	33.0 ± 19.4	0.250

Mean abundance (ind m ^{3} ± SD) of taxa in the microplankton (samples >53 μm) and mesoplankton (samples >200 μm) fractions obtained at stations ≤100 m depth and >100 m depth, and *P*-values of the Student's *t*-test (normal data sets) or the Mann–Whitney *U* test (non-normal data sets) for differences between abundances on the shelf and beyond the shelf. *P*-values at the <0.05 level are shown in bold.
[a]Results of the Mann–Whitney *U* test.

Diet composition was described as the percentage frequency of occurrence (%*F*) and percentage frequency of abundance (%*N*) of prey items examined (excluding larvae from samples with empty guts). The percentage of the product of these two factors was taken as an index of relative dietary importance (%IRI) (Laroche, 1982). The Shannon–Weaver diversity index (*H'*) was adopted as a measure of diet diversity.

Relative dietary preferences were analysed using Chesson's alpha index (α) (Chesson, 1978). Alpha values were calculated for individual larvae ($n = 102$, SL range: 4.1–12.5 mm) and for each food organism and averaged for two LS classes (<8 or 8–12.5 mm SL). Eleven prey categories were considered and neutral selection resulted in a constant $\alpha = 1/11$. Alpha varied non-linearly between 0 and +1, deviated asymmetrically from the reciprocal of the number of food taxa available and was sensitive to sampling error. Alpha was zero when no individuals of a category were found in the gut, despite the presence of the item in the environment. Alpha does not change as the density of food organisms changes, unless the behaviour of the larva changes (Chesson, 1983; Govoni *et al.*, 1986).

Differences in the data were analysed through ANOVAs and non-parametric tests (Mann–Whitney or Kruskall–Wallis) with the SPSS software package for Windows (SPSS Inc, 2005).

RESULTS

Sardinella aurita distribution and abundance of potential prey

Larvae were found along the continental shelf with higher densities inshore of the 100 m isobath (Fig. 1). The LS structure at the coastal stations (<100 m) showed the highest abundance for larvae <4 mm and an exponential decrease thereafter. An increase in the modal size class was observed at the stations beyond the 100 m isobath, where all the larvae were larger than 10 mm. From the LHPR sampling, vertical distribution of *S. aurita* larvae was restricted to the top 40 m of the water column, with no differences between day and night or among size classes (Fig. 1).

Dinoflagellates, tintinnids and copepod nauplii dominated the microplankton assemblage throughout the study area, followed by copepod eggs and postnaupliar stages of cyclopoid poecilostomatoid and harpacticoid copepods (Table I). Except for postnauplii of

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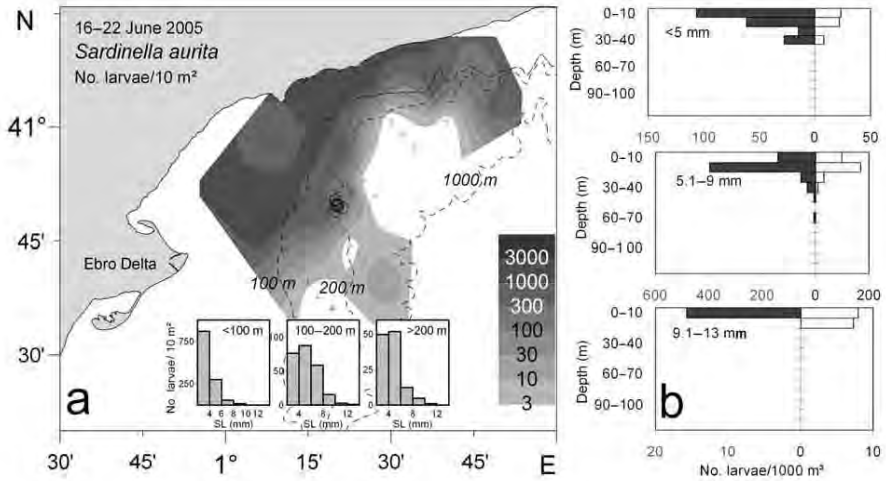

Fig. 1. (**a**) Horizontal distribution of *S. aurita* larvae and size frequency distributions by bathymetric sectors (abundances are mean density values) during the study period. Calvet and Bongo hauls (+) and LHPR hauls (O) location. (**b**) Mean larval vertical distribution by size classes during daytime (grey bars) and night-time (black bars).

harpacticoid copepods, all microplankton groups (e.g. copepod eggs and nauplii) showed a significantly higher abundance at stations of ≤100 m (Fig. 2). Armoured dinoflagellates also dominated the mesoplankton fraction at both ≤100 and >100 m, but among the mesozooplankton categories cladocerans of the genus *Evadne* was the most abundant at ≤100 m, followed by doliolids, appendicularians and calanoid postnauplii (Table I). The distribution of *Evadne* spp. and calanoid copepod postnauplii extended throughout the study area, with a continuously decreasing abundance from coastal to offshore stations (Fig. 2).

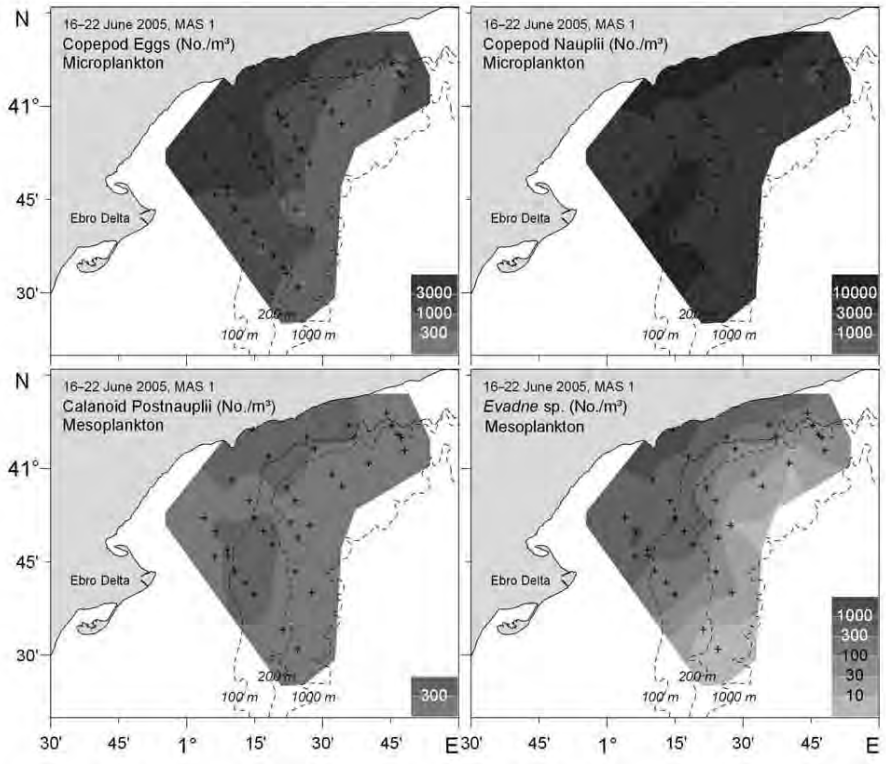

Fig. 2. Spatial distribution of the main prey of *S. aurita* larvae during the study period.

Feeding incidence and diel pattern

The midgut of the larvae contained 89.7% of the larval *Sardinella* prey, the foregut <1% and the hindgut 10%. Feeding incidence was 7.7% for night-collected larvae and 68.6% for day-collected larvae. The highest numbers of prey were recorded before sunset (19:30 h GMT) and after sunrise (4:30 h GMT) (Fig. 3) and showed a slightly lower digestion stage in the samples collected from 4:30–8 h GMT, although the differences were not significant (K–W, $P > 0.05$). Owing to the daytime feeding pattern, in subsequent analyses, only larvae collected during daylight hours and with identifiable prey items (117 larvae with a size range of 4.1–12.5 mm) were considered.

No significant differences were observed in the feeding incidence during larval development (ANOVA, $P > 0.05$), although larvae <6 mm SL showed the lowest incidence (66.6%) and larvae of the 6–7.9 mm SL size class showed the highest incidence (76.9%). The total number of prey per larva was up to 11 items, with a mean of 3.3 (SD 2.51), although significant differences in the mean prey number were observed during larval development. The number of prey items ingested decreased in larvae larger than 9.5 mm (Fig. 4).

Morphometric relationships, PW and trophic niche breadth

Sardinella aurita larvae showed a significantly negative allometric relationship between MW and SL ($b = 0.7863$, 95% confidence interval (CI) ($b = 0.0558$), whereas upper and lower jaws showed an isometric growth in relation to SL (UJL: $b = 1.0267$, 95%CI$b = 0.1009$ and LJL: $b = 0.9581$, 95%CI$b = 0.0888$).

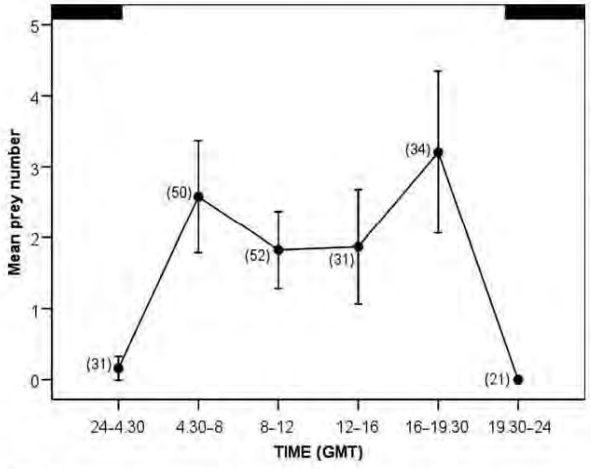

Fig. 3. Mean number of prey items per gut (±95% confidence interval) as a function of time (GMT). Values to the left of the data points indicate the number of larvae.

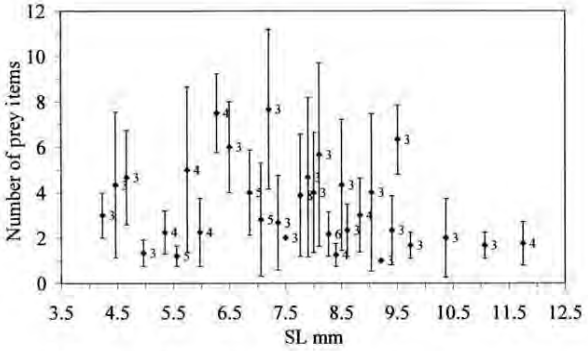

Fig. 4. Mean number of prey items per gut (±SD) in relation to larval SL. Numbers to the right of the data points indicate the number of larvae in the size class.

PW ranged from 27.5 to 410 μm. The weighted regressions for mean log width of prey items on larval SL and MW had positive slopes and significant correlations ($P < 0.001$) (Fig. 5a and b). Both mean PW and range of PW increased for larvae with an SL of 5–8 mm. MW of larvae with an SL of 4–5 mm ranged from 228 to 272 μm, and these larvae fed on prey with a width of 27.5–125 μm. Minimum PW was similar during larval development, in contrast to maximum width, which reached 400 μm in larvae larger than 8 mm SL. Smaller prey were copepod eggs and nauplii and spherical items (probably protozoan cysts), which were the smallest organisms, whereas *Penilia* sp. and copepod postnauplii were the largest. Niche breadth (SD log PW) was independent of larval length as the regression analysis showed a high dispersion pattern (a high residual sum of squares error) (Fig. 5c).

Diet and selectivity

Prey diversity (H') and prey categories were similar in larvae <8 and ≥8 mm SL, although percentages of occurrence (%F) and abundance (%N), as well as the IRI for each prey item, differed between them (Table II). Copepod nauplii were by far the most frequent and most abundant prey items in the larvae <8 mm (IRI 72.2%). *Evadne* spp. were also present in the diet of this group, but in a lower percentage. The diet of the largest larval group was composed of similar proportions of *Evadne* spp., copepod nauplii and postnauplii. Among the copepod postnauplii identified, a higher incidence of *Microsetella rosea*, *Oithona* sp. and *Clausocalanus* sp. was observed. Other prey were occasionally found, such as Amphioxus larvae (*Branchiostoma* sp.) ($n = 6$, in larvae from 5.9 to 9.9 mm

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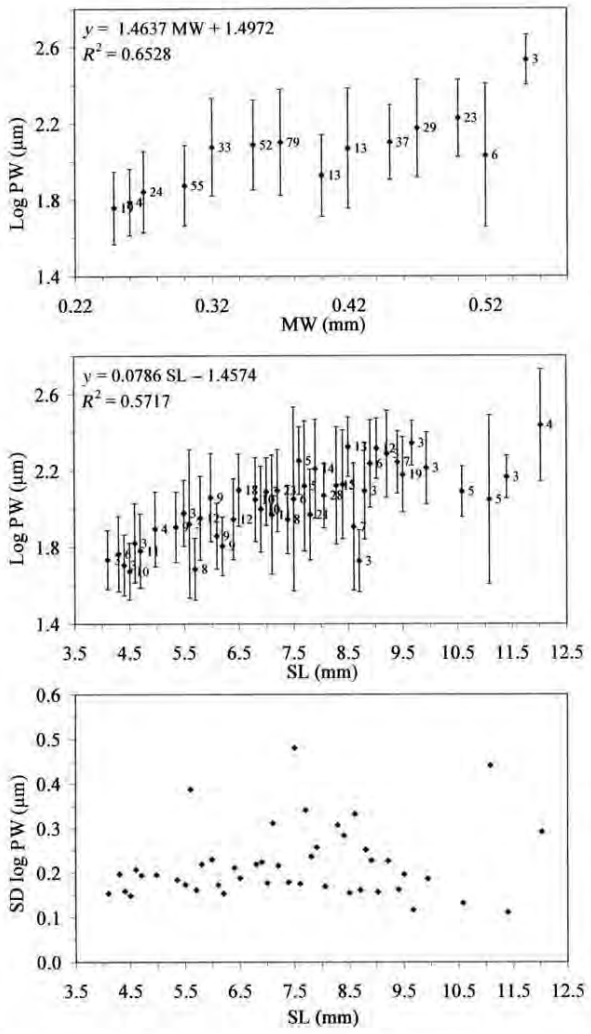

Fig. 5. (**a**) Logarithmic mean prey item width (±SD) in relation to larval MW. (**b**) Logarithmic mean prey item width (±SD) in relation to larval SL. Numbers to the right of the data points indicate the number of prey items per LS class. Weighted regressions (weighted by number of prey items per size class) are shown to the right of each point. (**c**) Niche breadth in relation to larval SL.

SL). No piscivory was observed in any of the larvae dissected.

When diet was analysed in 2 mm length larval classes, differences between first-feeding, preflexion and flexion larvae were observed. First-feeding larvae measuring <6 mm fed more on rare items; cladocerans and copepod nauplii were the basis of the diet in preflexion stages (6–9.9 mm SL), and the different stages of copepods were the only diet of flexion larvae (Fig. 6).

The prey categories for the selectivity index were phytoplankton, copepod eggs, copepod nauplii, calanoid copepod postnauplii, harpacticoid copepod postnauplii, other copepod postnauplii (cyclopoids and poecilostomata), *Evadne* spp., *Penilia* sp., gastropod larvae, polychaete larvae and appendicularians. Therefore, Chesson's index for selection resulted in an $\alpha = 0.091$. Larvae measuring <8 mm SL mainly selected copepod nauplii and *Evadne* spp., whereas other prey items such as harpacticoid copepodites, *Penilia* sp. and appendicularia had neutral selection (eaten in the same proportion as their abundance in the environment). Larvae selected against phytoplankton, copepod eggs, postnauplii of other copepods, gastropod larvae and polychaete larvae. Larvae measuring more than 8 mm SL preferred *Evadne* spp., copepod nauplii and calanoid postnauplii, showed neutral selection for *Penilia* sp. and polychaete larvae and selected against phytoplankton, copepod eggs, postnauplii of other copepods, gastropod larvae and appendicularians (Fig. 7). Although the selection was positive for copepod nauplii in both groups, this selection was stronger in the larvae <8 mm (M–W, $P < 0.05$). Calanoid postnauplii were selected positively by the largest group but not by the smallest one (M–W, $P < 0.001$).

DISCUSSION

Feeding pattern and PW

Feeding incidence is considered to be a measure of a larva's ability to obtain food from the environment (Arthur, 1976), and the reported feeding incidences for clupeoid larvae are the lowest among fish larvae, usually <40% (Arthur, 1976; Uotani, 1985; Conway *et al.*, 1994, 1998; Tudela *et al.*, 2002). A number of factors are suggested to be responsible for this, e.g. the nets tend to selectively capture dead or malnourished larvae, or larvae could regurgitate or defecate food when they are being collected, handled or put into preservatives, because of the straight shape of their guts (Schumann, 1963; Arthur, 1976; Last, 1980, and its references; Conway *et al.*, 1998). Despite this, *S. aurita* shows a much higher feeding incidence than previously reported for other clupeoids (Last, 1980; Conway *et al.*, 1994, 1998; Tudela *et al.*, 2002; Voss *et al.*, 2003; Munuera-Fernández and González-Quirós, 2006). The high values found during this study are comparable to those found in preflexion *S. brasiliensis* larvae (68%) (Kurtz and Matsuura, 2001) and herring larvae in estuarine waters (71%) (Fox *et al.*, 1999). Furthermore, the model that we have observed here for *S. aurita*, with an increase in the feeding incidence during preflexion and a maintenance or reduction in more advanced stages, is similar to that reported for several species of *Engraulis* (de Ciechomski, 1966; Arthur, 1976; Conway *et al.*, 1998), *S. pilchardus* (Munuera-Fernández

Table II: Diet of S. aurita larvae

	SL<8 mm (*N* larvae=66, *N* prey=223, *H'*=1.955)				SL≥8 mm (*N* larvae=50, *N* prey=130, *H'*=1.829)			
Prey type	%*F*	%*N*	IRI (%)	PN	%*F*	%*N*	IRI (%)	PN
Phytoplankton	4.07	3.04	0.7	0–2	2.33	1.52	0.2	0–1
Protozoa	0.81	0.43	0.0	0–1	1.16	0.76	0	0–1
Copepod eggs	4.88	3.04	0.8	0–2	1.16	0.76	0	0–1
Copepod nauplii	33.33	40.43	72.2	0–7	25.58	21.97	29.9	0–3
Copepod postnauplii	8.13	8.26	3.6	0–6	23.26	21.97	27.2	0–5
Evadne spp.	14.63	19.13	15.0	0–7	20.93	33.33	37.1	0–7
Penilia sp.	4.07	3.91	0.9	0–3	6.98	7.58	2.8	0–3
Crustacean fragments	2.44	1.74	0.2		2.33	1.52	0.2	
Appendicularians	4.88	4.35	1.1	0–3	2.33	1.52	0.2	0–1
Polychaete larvae	1.63	0.87	0.1	0–1	6.98	4.55	1.7	0–1
Gastropod larvae	4.07	2.17	0.5	0–1	1.16	0.76	0	0–1
Spherical items	8.94	7.83	3.7	0–4	1.16	0.76	0	0–1
Amphioxus	2.44	1.30	0.2	0–1	3.49	2.27	0.4	0–1
Others	5.69	3.48	1.1	0–2	1.16	0.76	0	0–1

N, number; *H'*, prey diversity index; %*F*, percentage occurrence in the gut; %*N*, percentage abundance of prey items; IRI, %*F**%*N*; PN, prey number (range).

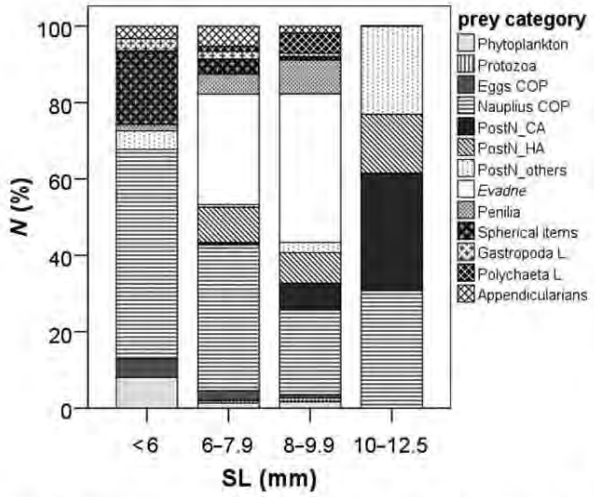

Fig. 6. Cumulative percentage composition of the diet by prey categories for *S. aurita* larvae grouped by size class intervals.

and González-Quirós, 2006) and *S. brasiliensis* (Kurtz and Matsuura, 2001). The reason for this change could be the lack of suitable food when the larvae begin a more selective type of feeding (de Ciechomski, 1966; Arthur, 1976; Conway *et al.*, 1998).

As in other species, *S. aurita* larvae show a diurnal feeding pattern (Last, 1980; Ostergaard *et al.*, 2005). The higher number of prey found after sunrise and before sunset indicates a bimodal feeding pattern of the larvae, as also reported for *E. japonica* larvae in the Pacific Ocean (Uotani, 1985). The mean prey number ingested per larvae (3.3) is high compared with that reported for *E. encrasicolus* larvae in the Mediterranean by Conway *et al.* (Conway *et al.*, 1998) (1.96–2.53) and Tudela *et al.* (Tudela *et al.*, 2002) (1.66) and for *S. pilchardus* larvae in the Cantabrian Sea [1.9–2.2, (Conway *et al.*, 1994)]. Though the number of prey ingested decreases with larval development, the maximum width and the width range of the prey increase, because though the larvae are able to eat larger prey, they also continue to eat smaller prey, as observed in other species (Last, 1980; Llanos *et al.*, 1996). However, the prey type changes from a more euryphagous small larvae to a copepod-specialized flexion larvae. This pattern is similar to that reported for *S. pichardus* in the Cantabrian Sea (Conway *et al.*, 1994; Munuera-Fernández and González-Quirós, 2006), but in contrast to that of several *Engraulis* spp. (Conway *et al.*, 1998; Tudela *et al.*, 2002) and *S. brasiliensis* (Kurtz and Matsuura, 2001), which widens the types of prey as larvae increase in size. Furthermore, the breadth of the trophic niche in *S. aurita* larvae does not vary during development, a characteristic common to many other species (Pearre, 1986; Sabatés and Saiz, 2000; Catalán *et al.*, 2007).

Diet and selectivity

Phytoplankton seems to be of little importance in the diet of *S. aurita* larvae, because they only appear in the first-feeding group and in a low percentage, as has been reported for *E. encrasicolus* larvae in the Mediterranean Sea (Conway *et al.*, 1998; Tudela *et al.*, 2002), but contrary to observations in *S. pilchardus* in the Mediterranean (Rasoanarivo *et al.*, 1991). Other small prey, such as copepod eggs, which have been considered as a main prey item for clupeoid larvae (Arthur, 1976; Conway *et al.*, 1998; Tudela *et al.*, 2002), were negatively selected by *S. aurita* larvae. Cladocerans, primarily

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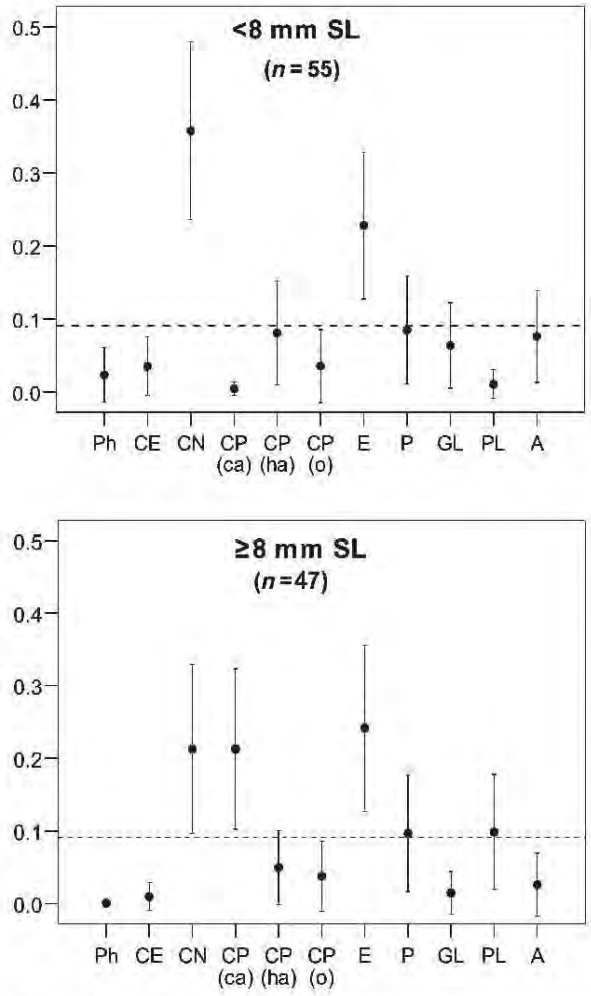

Fig. 7. Mean Chesson's α-values (±95% CI) for the most common prey items in *S. aurita* larvae <8 and ≥8 mm SL. Values above the broken line indicate positive selection. *n*, number of larvae analysed; Ph, phytoplankton; CE, copepod eggs; CN, copepod nauplius; CP, copepod postnauplii: ca [calanoids], ha [harpacticoids] or [cyclopoid-poecilostomatoid]); E, *Evadne* spp.; P, *Penilia* sp.; GL, gastropod larvae; A, appendicularians.

Evadne spp. (positive selected), are an important prey type in *S. aurita* larvae, particularly in preflexion larvae, as also happens in other species (Uotani *et al.*, 1981; Voss *et al.*, 2003; Morote *et al.*, 2008), though they are not a common prey in the diets of larvae of other clupeids (Arthur, 1976; Conway *et al.*, 1998; Tudela *et al.*, 2002). Larvae of *S. aurita* can be characterized as primarily feeders on crustaceans, especially different stages of copepods, as has been reported for many fish larvae and particularly other clupeoids (Last, 1980; Tudela *et al.*, 2002; Sampey *et al.*, 2007). Copepod nauplii were the main and most preferred prey for larvae <8 mm in length and copepod postnauplii increased in importance as the larvae increased in size. The diet comprised entirely of these prey in the largest group of larvae examined (10–12.5 mm SL), which showed improved swimming skills (notochordal flexion, developing fins and a functional swim bladder). These results agree with those for *S. brasiliensis*, which feeds selectively on copepod nauplii in the preflexion and flexion stages and later changes to feed selectively on copepodites and adult copepods (Kurtz and Matsuura, 2001). Copepod nauplii are also the most important item in the diet of *E. encrasicolus* larvae in the Mediterranean (Conway *et al.*, 1998; Tudela *et al.*, 2002), also with a positive selection. Although the selectivity index is calculated based on the relative proportions of the prey in both the digestive tracts of the larvae and the environment, it must be admitted that in studies such as the present one, in which sampling shows the abundances in the whole water column, the availability of plankton does not necessarily reflect the concentration of prey immediately around the larvae, and could therefore be a source of uncertainty.

In the northwestern Mediterranean *S. aurita* and *E. encrasicolus* have partially overlapping spawning periods, but their spatial distributions have been reported to be quite separate, with *S. aurita* larvae distributed close to the coast and *E. encrasicolus* larvae concentrated mainly near the shelf-break (Sabatés *et al.*, 2006; Palomera *et al.*, 2007). However, during the present study *S. aurita* and *E. encrasicolus* larvae showed an overlapping distribution, with the latter also having highest concentrations in the coastal zone (Olivar *et al.*, 2007). The presence of both larvae in the same area, together with their coincident vertical distribution in the upper 30 m of the water column and their morphological similarities, may result in larval competition for food.

The fact that *S. aurita* shows a higher percentage of larvae containing food and a higher feeding intensity than *E. encrasicolus* (Conway *et al.*, 1998, Tudela *et al.*, 2002) may indicate that they are either more voracious or less susceptible to voiding their guts while being caught and preserved. In order to rule out possible masking effects due to the treatment of the samples, or particular conditions of the environment during the period of study that could have led to a higher larval feeding incidence, the contents of anchovy larvae ($n = 40$, daylight collected) from the samples used for the study of *S. aurita* were examined. The results showed a feeding incidence of 30%, which is similar to those in the above studies, thus corroborating the differences in feeding strategy between these two species, and particularly the more voracious behaviour of *S. aurita* larvae. Furthermore, the copepod-based diet of *S. aurita* larvae is very similar to that reported for *E. encrasicolus* larvae in the Mediterranean Sea (Conway *et al.*, 1998; Tudela *et al.*, 2002), reinforcing the possible diet overlap between larvae of the two species.

Owing to the increasing abundance of *S. aurita* reported in recent years (Sabatés *et al.*, 2006) and the temporal and partially spatial coincidence in the distribution of the larvae of these two species, it can be predicted that first-feeding larvae could compete for the same prey and that, in situations of low prey abundance, the more successful feeder *S. aurita* may have greater chances of survival than *E. encrasicolus*.

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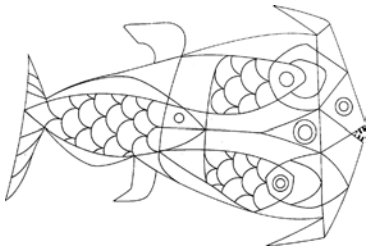
2.3 ROBUST LARVAE

(BIG MOUTH, LOOPED GUT)



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Trophic ecology of bullet tuna *Auxis rochei* larvae and ontogeny of feeding-related organs

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ABSTRACT: The bullet tuna *Auxis rochei*, Risso 1810, is a small tuna widely distributed in tropical and temperate seas. The present study reports on the first attempt to jointly monitor diet and food selection in larvae of this species and to assess the influence of the ontogenetic development of feeding-related organs on the diet. *A. rochei* larvae from 2 to 7 mm long are diurnal feeders and highly active predators, with high values of feeding incidence, gut fullness and number of ingested prey items. The rate of change in prey item size in relation to larval size was higher than in other species. Only small, non-motile prey items are eaten at the onset of feeding. Rapid mouth development and the early appearance of teeth allow larvae from 3 to 5 mm long to ingest a wide range of prey. However, niche breadth decreases at 5 mm, when larvae avoid small prey items in favour of larger ones with a higher carbon content. Chesson's selectivity index indicated that small larvae (from 2 to 3 mm long) selected a variety of small prey items, mainly copepod nauplii. Larvae measuring 3 to 5 mm selectively ate cladocerans and appendicularians, and larvae with lengths ≥5 mm preferred appendicularians and fish larvae. Precocious body development (mouth, teeth, and onset of stomach and caudal fin development) and improved visual acuity (larger lens diameter and angular cone density) are factors that contribute to the feeding success of larvae of this species.

KEY WORDS: Bullet tuna larvae · Feeding ecology · Prey selection · Ontogeny · Vision · NW Mediterranean

INTRODUCTION

The bullet tuna *Auxis rochei*, Risso 1810, is a commercially important scombrid widely distributed in tropical and subtropical waters around the world. In the NW Mediterranean Sea, the spawning period runs from June to September, with peak larval abundance when water temperature reaches the yearly maximum (Sabatés & Recasens 2001).

Scombrid larvae have some of the highest growth rates of any marine fish larvae; hence, their food requirements are high (Hunter 1981). Feeding success is a very important factor affecting survival of the early larval stages and thus subsequent recruitment strength. There is evidence that tuna larvae have high mortality levels, mainly as a consequence of starvation (Margulies 1993). More particularly, *Auxis* spp. larvae have been observed to be prone to starvation on account of their high metabolic rate and fast growth rate (Tanaka et al. 1996 and references therein). Knowledge of the trophic ecology of *Auxis rochei* larvae is essential to understanding growth and survival of these stages.

Although there have been many studies dealing with larval feeding in scombrids, only a few have actually addressed selection as it relates to prey availability in the sea. Studies on the trophic ecology of *Scomber* spp. and *Scomberomorus* spp. are relatively common (e.g.

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Conway et al. 1999), and larval feeding has been assessed for most tuna species (Uotani et al. 1981, Young & Davis 1990, Catalán et al. 2007). However, studies on the feeding habits of *Auxis* spp. larvae are limited to work by Uotani et al. (1981), who described the diet composition and prey selection of *Auxis* spp. in the SE Indian Ocean, and Sánchez-Velasco et al. (1999), who examined the diet composition of *Auxis* sp. in the Gulf of California.

Fish larvae employ different feeding strategies depending on the area and season of spawning and larval hatching, and these strategies are related to both prey availability and larval development. Prey size is one of the most important factors in prey selection; however, prey availability, prey colour, swimming behaviour and innate predator preferences can strongly affect larval selection (Hunter 1981, Govoni et al. 1986). Digestive system and sensory organ development go hand in hand with changes in larval behaviour that have major implications for feeding and predator avoidance (Hubbs & Blaxter 1986).

The object of the present study was to provide an overview of the larval feeding ecology of *Auxis rochei* by analysing diel feeding patterns, feeding incidence (FI), diet composition and selection, ontogenetic diet shift, and predator–prey relationships and to elucidate to what extent this species' larval trophic ecology depends on the ontogenetic development of organs related to feeding and foraging.

MATERIALS AND METHODS

Field collection of larvae and zooplankton. The study area was located in the NW Mediterranean Sea. The survey was carried out from 16 to 22 June 2005, during the spawning period of *Auxis rochei*. Larvae were sampled from the inshore region (50 m) to the offshore region (ca. 600 m) by means of oblique Bongo hauls (mesh size of 0.3 mm) from a depth of 200 m, or just above the bottom, to the surface. The volume of water filtered by the net was estimated using a flowmeter. The vertical larval distribution was obtained based on 8 stratified hauls (at 10 m intervals) using a Longhurst Hardy Plankton Recorder. In addition, vertical microplankton and mesoplankton hauls were carried out using a Calvet net fitted with 53 and 200 µm mesh. To remove mesoplanktonic organisms from the >53 µm samples, these catches were later filtered through a 200 µm mesh. Samples were preserved in 5% buffered formalin. Larvae for histological examination were fixed in 10% formalin in 0.1 M phosphate buffer.

Laboratory analyses. A total of 207 intact specimens of *Auxis rochei* larvae ranging from 2.0 to 6.7 mm in length were randomly selected from the samples for gut content analysis. In samples of fewer than 10 larvae, all individuals were dissected; in larger samples, a random subsample from 10 to 12 larvae was taken. Before dissection, the following measurements were recorded: standard length (SL); eye diameter (ED); lower jaw length (LJL), measured from the tip to the junction with the maxilla; upper jaw length (UJL), measured from the tip of the snout to the posterior end of the maxilla; and mouth width (MW), measured ventrally as the widest distance between the posterior edge of the maxillae. Dentary and maxillary teeth during the course of development were also studied.

The entire gut from each specimen was removed intact using a fine needle, placed in a drop of 50% glycerine-distilled water on a glass slide, and prey organisms were teased out for identification, enumeration, and measurement. Identification of food particles in the gut was effected to the lowest taxon possible. Maximum body width and body length of each prey item were measured to the nearest 0.0025 mm along the maximum cross section under a microscope equipped with an ocular micrometer. The measurements for non-crustacean prey items without exoskeletons were probably smaller than the actual size of live prey because they were partially digested and shapeless in the gut.

Prey availability was determined at 18 selected stations where the *Auxis rochei* larvae analysed were most abundant. Identified individuals were grouped by the usual coarse planktonic categories, by developmental stage (e.g. copepod eggs, nauplii, and postnauplii), or by specific criteria (e.g. *Evadne* spp. in cladocerans), using the prey categories chosen for diet analysis. The abundance of each planktonic category was calculated as ind. m^{-3} for the microplanktonic and mesoplanktonic fractions, and both values were added together to obtain the total prey abundance used to estimate the selectivity index.

Larvae used for histological examination were embedded in glycol methacrylate resin, serially sectioned (2 µm) and stained (polychrome stain). Serial transverse sections of the heads of preflexion (n = 4, SL = 2.6 to 3.8 mm), early flexion (n = 3, SL = 4 to 5 mm) and late flexion (n = 8, SL = 6 to 7 mm) larvae were used to examine changes in visual morphology. In addition, flexion stage larvae (n = 4, SL = 4.9 to 6 mm) were serially sectioned (providing tangential sections of the photoreceptors) in order to investigate the arrangement and type of cone photoreceptors in the outer nuclear layer of the retina. The gross morphology of the digestive tract in preflexion and flexion stage larvae was evaluated in whole fixed specimens. Histological evaluation of the digestive tract was performed in late flexion larvae (n = 3, SL = 6.4 to 7 mm).

Dorso-ventral eye and lens diameters were measured in largest diameter serial transverse sections in the central retina. Retinal cell counts (cone and rod photoreceptors) and cone outer segment lengths (measured from the sclerad limit of the pigmented retinal epithelium to the outer limiting membrane of the outer nuclear [photoreceptor nuclei] layer) were quantified in 1 eye from each larva using transverse histological sections with the largest lens diameter in the central retina away from the embryonic fissure and insertion point of the optic nerve. Cone cell density was determined by counting cone ellipsoids. Double cone ellipsoids could not be reliably differentiated from those of single cones in the transverse sections, with the result that single and double cones were counted as single elements. However, the presence of single and double cone photoreceptors was confirmed in tangential sections in which single and double cones could be resolved. Rod photoreceptors were determined by counting their nuclei (more densely stained and smaller than cone nuclei, with different shape and position). Retinal cell densities were determined along a single linear transect of the retina 50 µm in length in each of the dorsal, middle, and ventral retina. Small eye size and curvature of the retina limited the retinal area available for cell density determination, particularly in the smallest larvae (Browman et al. 1990, Pankhurst et al. 1993). Three measurements of cone photoreceptor outer segment length were made in each retinal region. Angular cell densities to correct for the curvature of the eye were calculated for cones and rods as cited in Poling & Fuiman (1997).

Data analysis. Differences in the data were analysed using ANOVAs and non-parametric tests (Mann-Whitney or Kruskal-Wallis) with SPSS software package for windows (SPSS 2005). FI was calculated as the percentage of larvae with gut contents out of the total number of larvae examined in the daytime and at night. The gut fullness index was estimated on a scale of 0 to 4 (0 = empty; 1 = 25% full; 2 = 50% full; 3 = 75% full; 4 = completely full), and stage of prey item digestion was estimated on a scale of 1 to 3 (1 = highly digested, completely transparent; 2 = partially digested; 3 = undigested, some color remains) (Young & Davis 1990).

In order to analyse relationships between prey width (PW) and larval size (SL and MW), the larvae were classed in size intervals to produce the maximum number of size classes containing 3 or more prey items. To fit this requirement, an interval of 0.12 mm was used for SL and 0.01 mm for MW. Relationships were estimated by linear regression analysis of $\log_{10}$-transformed PW vs. larval size (weighted by the number of prey items per larval size class) ($\log PW = b + a\,SL$). As in previous studies, the SD of the $\log_{10}$-transformed prey size was taken as a measure of trophic niche breadth (Pearre 1985). The fitted lineal model is equivalent to the exponential model: $PW = 10^b \exp[k\,SL]$, where $k = a \ln 10$. The deriv-

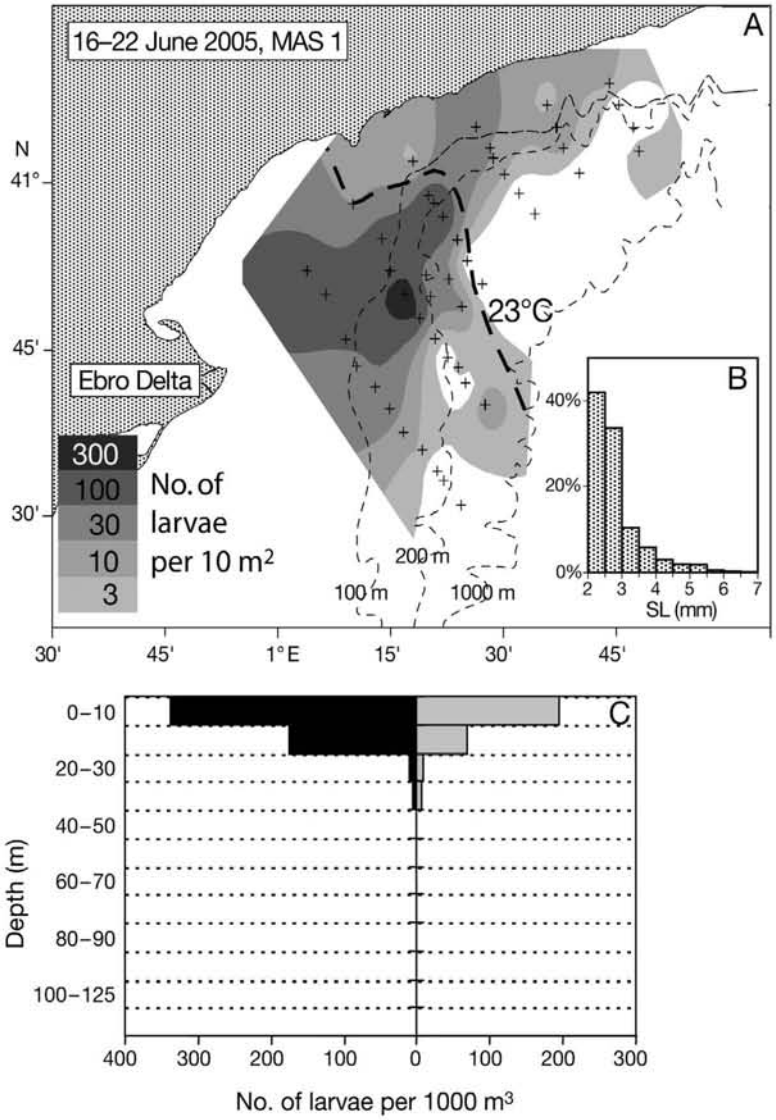

Fig. 1. *Auxis rochei* larvae. (A) Horizontal distribution during MAS 1 survey; broken black line depicts the 23°C isotherm. (B) Larval size frequency distribution; SL = standard length. (C) Mean vertical distribution obtained from Longhurst Hardy Plankton Recorder hauls during the day (grey bars) and at night (black bars). (+): sampled stations

ative of the exponential equation ($dPW/dSL = kPW$) demonstrates that k can be interpreted as the relative rate of change in prey size with respect to larval size.

The diet was described using the percentage frequency of occurrence (%F) of a diet item in larvae with food in their guts and the percentage frequency of the total number (%N) of diet items examined. The product of these 2 values was taken as the percentage index of relative importance of each diet item (%IRI) (Govoni et al. 1986). The Shannon-Weaver diversity index (H') was used as a measure of prey diversity in the gut.

Prey taxon selection by larvae (n = 92, SL = 2.1 to 6.3 mm) was determined using the alpha index (Chesson 1978) calculated for individual larvae by prey item and averaged by 1 mm larval size class, except for the final size class, which encompassed larvae ranging from 5 to 6.3 mm in length. Only the 9 most common food organisms were considered: neutral selection would thus result in a constant $\alpha = 1/9$.

The carbon equivalent mass of prey items in the gut was estimated from species-specific length–weight relationships obtained from the literature, assuming when necessary a carbon content equal to 40% of dry weight. We used equations from Menden-Deuer & Lessard (2000) for phytoplankton, Putt & Stoecker (1989) for tintinnids, Kleppel et al. (1991) for copepod eggs and other eggs, Hay et al. (1988) for copepod nauplii and postnauplii (using equations developed for *Pseudocalanus elongatus*), Catalán et al. (2007) for *Evadne* spp., and López-Urrutia et al. (2003) for appendicularians. A conservative 12 µg C per fish larva ingested was used (40% of the dry weight of a 4 mm long anchovy larva). The relationship between larval carbon content in the gut and larval size was examined. The larvae were again classed by 0.12 mm SL intervals and the size classes grouped, with cumulative gut contents of at least 3 larvae as a group-forming requirement. The mean and standard deviation for the $\log_{10}$-transformed values of total C content were calculated for each larval size class. Linear regression analysis was performed, weighted by the number of larvae.

RESULTS

Larval fish distribution and environment

Auxis rochei larvae were regularly present all along the continental shelf in the study area, with principal concentrations in the SW sector, where sea surface temperature (SST) was higher (Fig. 1A). Larval size ranged from 2 to 7 mm SL, with more than 70% of larvae measuring ≤3 mm (Fig. 1B). The vertical distribution of the larvae of this species was restricted to the top 20 m of the water column (Fig. 1C).

The microplankton in the sample tows consisted mainly of small dinoflagellates, tintinnids and copepod nauplii (Fig 2). Such meroplanktonic taxa as polychaete, bivalve and gastropod larvae were also abundant in this fraction. Copepod postnauplii and appendicularians were abundant in the micro- and mesoplankton. Cladocerans (mainly *Evadne* spp.), doliolids and cnidarians were the other abundant components of the mesoplankton.

Feeding incidence and diel pattern

The smallest larva with food in the gut measured 2.1 mm. The diel feeding pattern evidenced diurnal activity, increasing from sunrise. FI was 87.6% during the daytime, whereas only 19% of the larvae had food

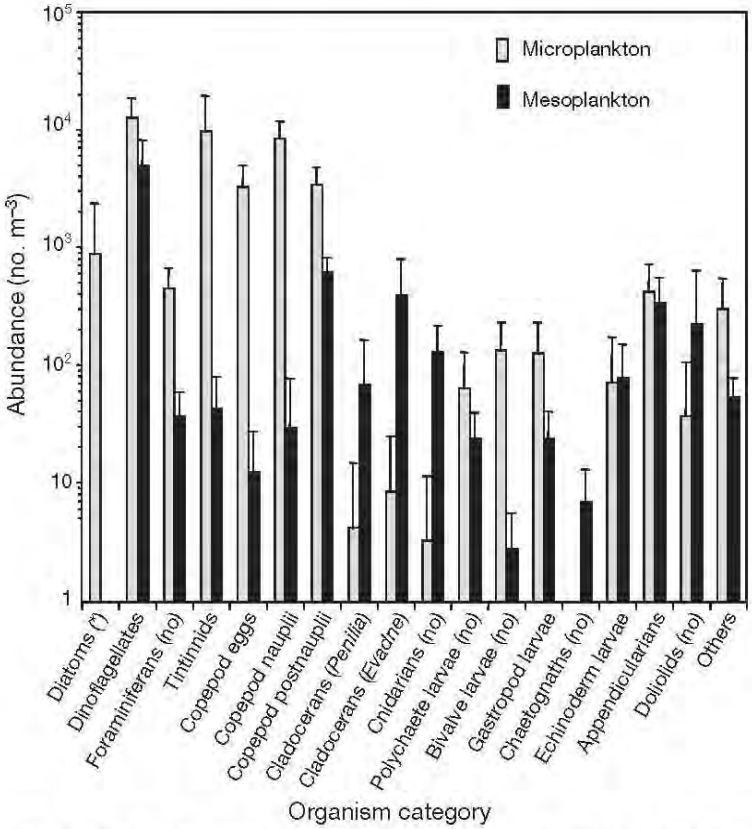

Fig. 2. Planktonic organisms. Mean abundance and standard deviation values of micro- and mesoplanktonic organisms at the 18 stations used for prey availability and larval feeding selectivity analysis. (*) = organisms not quantified in the mesoplanktonic fraction. (no) = organisms not found in larval gut contents

in their guts at night, and these prey items were in advanced stages of digestion. No differences in FI were observed with larval size either during the daytime or at night (ANOVA, $p > 0.05$). The number of prey items per larva ranged from 0 to 13 (5 ± 3.3), with the number peaking in the 14:00 to 18:00 h interval and falling off sharply after sunset (Kruskal-Wallis, $p < 0.001$) (Fig. 3). Because of this daytime feeding pattern, only larvae collected during hours of daylight with identifiable prey items (130 larvae; SL = 2.1–6.3 mm) were considered in subsequent analyses.

The highest gut fullness value, around 75%, was recorded between 14:00 and 18:00 h (ANOVA, $p < 0.001$). Gut fullness stayed at around 50% during the rest of the hours of daylight. The digestion state of prey items varied significantly only between 10:00 and 14:00 h, when prey items were in the early stages of digestion, and between 14:00 and 18:00 h, when they were in a more advanced stage of digestion (Kruskal-Wallis, $p < 0.05$).

Predator–prey relationship

The number of prey items ingested increased slightly from the early larval stage to a larval length of 4 mm SL, followed by a slight decrease (Fig. 4). PW ranged from 5 to 620 µm. In the larvae measuring <3 mm, 80% of the prey items ingested were less than 100 µm wide, and the rest of the prey items were between 200 and 300 µm. In contrast, in the largest larvae (between 5 and 7 mm), the width of 80% of the ingested prey items ranged from 100 to 500 µm (Kruskal-Wallis, $p < 0.001$) (Fig. 5).

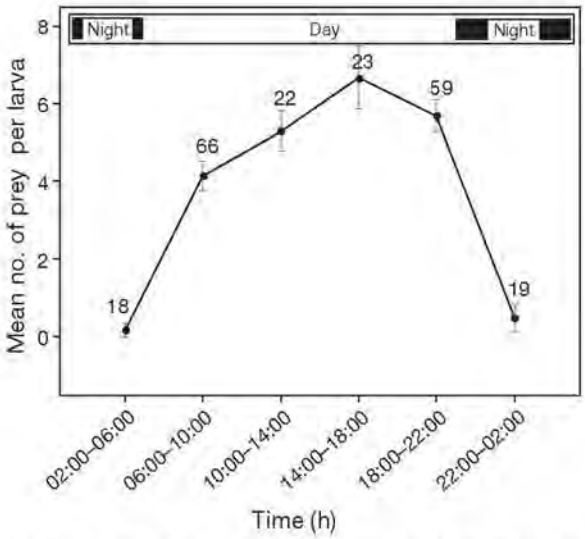

Fig. 3. *Auxis rochei* larvae. Mean number of prey per gut (±2 SE) as a function of local time (h). Values above the data points indicate number of larvae examined

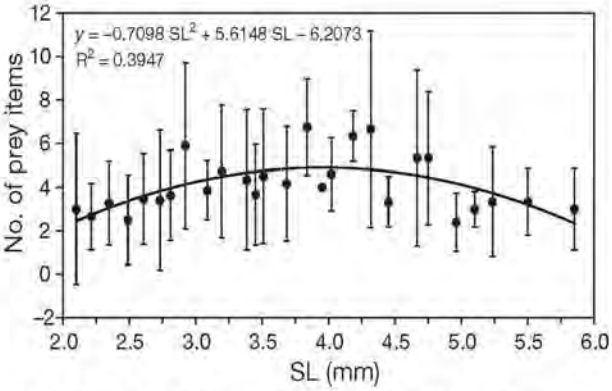

Fig. 4. *Auxis rochei* larvae. Mean number of prey items per gut (±SD) plotted against larval standard length (SL)

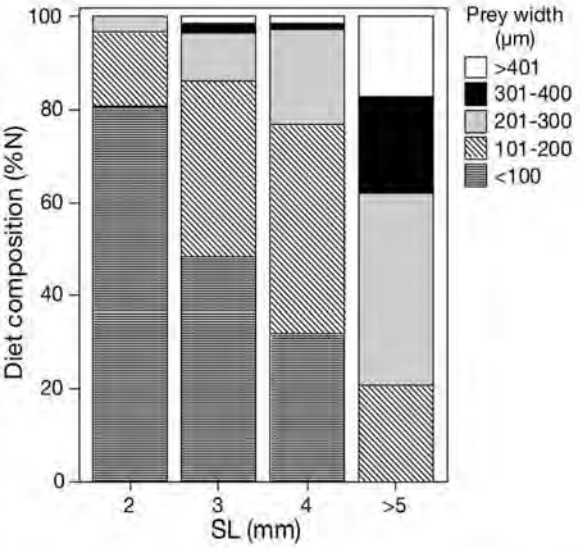

Fig. 5. *Auxis rochei* larvae. Cumulative percentage composition of the diet (%N) by prey item width class in relation to larval standard length (SL)

The weighted regressions for mean log size of prey items on larval SL and MW had positive slopes and significant correlations ($p < 0.001$) (Fig. 6A,B). The rate of change of prey size was 0.5 mm^{-1} with increasing SL and 2.6 mm^{-1} with MW. Regression analysis yielded high residual sum of squares error, but even so, the relationship between niche breadth and SL underwent a slight decrease in larvae with SL > 5 mm (Fig. 6C).

Diet and selectivity

Prey diversity (H') was highest in the larvae with SL < 4 mm (Table 1), whose gut contents included phytoplankton, tintinnids, copepod eggs, and gastropod and echinoderm larvae, none of which were recorded in

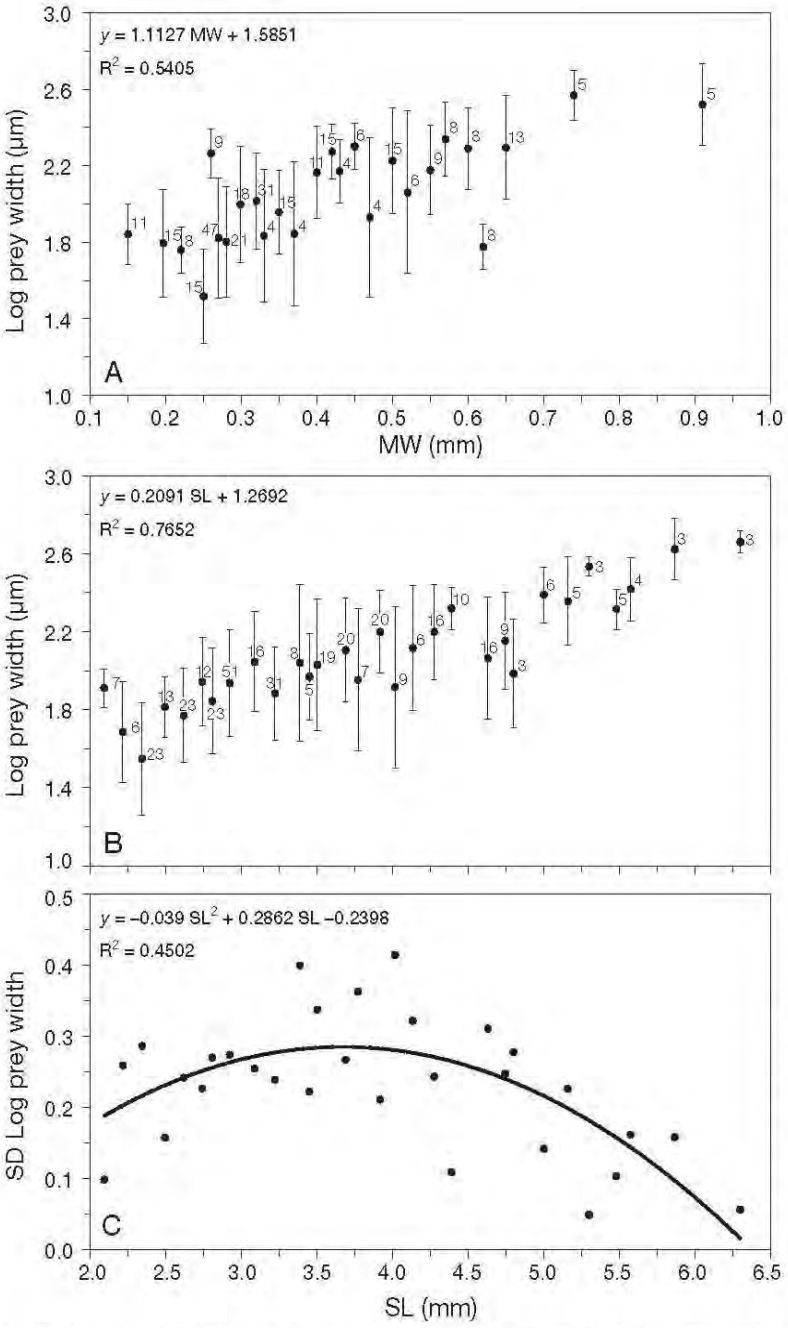

Fig. 6. *Auxis rochei* larvae. Logarithmic mean prey item width (±SD) in relation to larval (A) mouth width (MW) and (B) standard length (SL). Numbers to the right of the data points in (A) and (B) indicate number of prey items per larval size class. Weighted regressions (weighted by number of prey items per size class) shown above each plot. (C) Niche breadth, expressed as SD log of prey width, plotted against larval SL

the larger larvae. The IRI demonstrated differences between larvae with SL < 4 mm and those with SL > 4 mm. Copepod nauplii were the most frequent and most abundant prey items in larvae with SL < 4 mm (IRI 40.4%), compared with cladocerans in larvae with SL > 4 mm (IRI 52.6%) (Table 1). Larval fish prey items were observed in the larger larvae especially in larvae with SL > 5 mm.

Selectivity for particular taxa was evident when the gut content was analysed as a function of available prey. Larvae with SL < 3 mm selected mainly copepod nauplii and other small prey items like phytoplankton, gastropod larvae, copepod eggs, and small postnauplii, while selecting against tintinnids, cladocerans, appendicularians, and fish larvae. Larvae with SL from 3 to 4.9 mm preferred cladocerans and appendicularians, along with copepod nauplii to a lesser extent, and avoided other prey. The larger larvae with SL from 5 to 6.3 mm positively selected appendicularians and fish larvae, while their preference for cladocerans declined (Fig. 7).

The carbon content in the gut (log transformed) was significantly correlated with larval size. Higher increases were recorded both in the youngest larvae from the onset of feeding (with SL < 3 mm) and in larvae with SL > 5 mm (Fig. 8).

Development of feeding organs

Larvae undergo pronounced mouth development with a significant positive allometric relationship to body length (Table 2, Fig. 9). The first dentary teeth appear at around 3 mm SL and the first premaxillary teeth at 4 mm SL. The number of teeth present was higher for larvae with SL > 5 mm (5.4 ± 1.96) and pharyngeal teeth were also observed. Tooth morphology was sharp and conical, except for a few specialized hook-like dentary teeth in larvae with SL > 5 mm.

The external morphology of whole fixed larvae with SL < 3 mm revealed a digestive tract consisting of a simple tube (with fore- and hindgut regions apparently separated by a gastric valve), which was looped in larvae with SL ≥3 mm. This coincided with development of a protrusion at the end of the foregut (the presumptive incipient stomach). Histological examination of late flexion larvae confirmed that development of the stomach had commenced, with gastric glands and pyloric caecae present; however, pyloric caecae

Table 1. *Auxis rochei* larvae diet. SL = standard length, N = number, H' = prey diversity index. %F = percentage occurrence in the gut, %N = percentage abundance of prey items, IRI = (%F • %N), PN = prey number (range)

Prey items	%F	%N	IRI (%)	PN
SL < 4 mm (N = 89, H' = 2.235)				
Phytoplankton	10.29	8.12	7.11	0–3
Tintinnids	6.62	5.98	3.37	0–3
Gastropod larvae	4.41	6.41	2.40	0–6
Copepod eggs	6.62	5.56	3.13	0–2
Copepod nauplii	19.85	23.93	40.40	0–7
Copepod postnauplii	7.35	5.56	3.47	0–4
Copepod fragments	0.74	0.43	0.03	
Cladocerans	7.35	12.39	7.75	0–9
Crustacean fragments	14.71	14.10	17.63	
Appendicularians	12.50	11.97	12.72	0–4
Echinoderm larvae	0.74	0.43	0.03	0–1
Other eggs	5.15	2.99	1.31	0–4
Fish larvae	0	0	0	0
Others	3.68	2.14	0.67	0–1
SL > 4 mm (N = 40, H' = 1.619)				
Phytoplankton	0	0	0	0
Tintinnids	0	0	0	0
Gastropod larvae	0	0	0	0
Copepod eggs	0	0	0	0
Copepod nauplii	19.23	17.78	16.70	0–6
Copepod postnauplii	5.77	3.33	0.94	0–1
Copepod fragments	1.92	1.11	0.10	
Cladocerans	26.92	40.00	52.61	0–6
Crustacean fragments	15.38	12.22	9.19	
Appendicularians	19.23	18.89	17.75	0–3
Echinoderm larvae	0	0	0	0
Other eggs	1.92	1.11	0.10	0–1
Fish larvae	9.62	5.56	2.61	0–1
Others	0	0	0	0–1

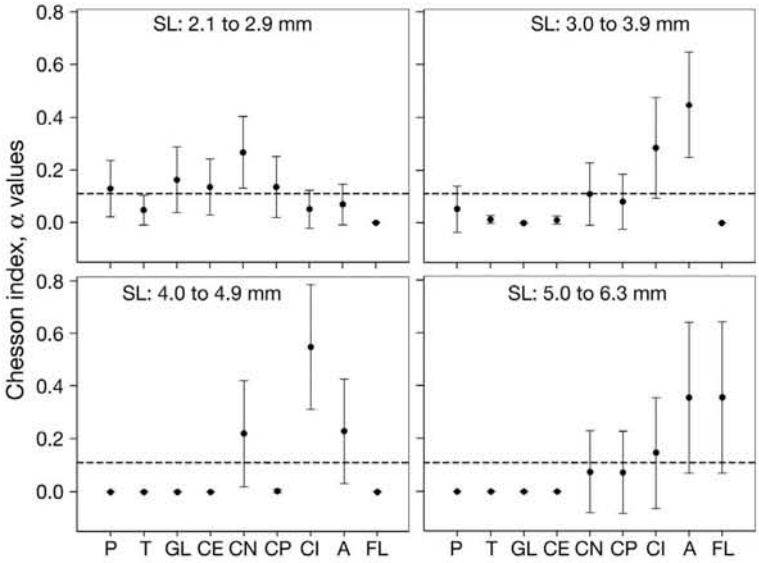

Fig. 7. *Auxis rochei* larvae. Mean Chesson's α values (±95% confidence interval) for the most common prey items in larvae of different size classes; SL: standard length. Circles above the dashed line indicate positive selection. P: phytoplankton; T: tintinnids; GL: gastropod larvae; CE: copepod eggs; CN: copepod nauplii; CP: copepod postnauplii; Cl: cladocerans – *Evadne* spp.; A: appendicularians; FL: fish larvae

were still very few in the largest larvae examined (SL = 7 mm), and digestion vacuoles were present only in the intestine and rectum but not in the developing stomach (Fig. 10).

Eye and lens diameter increased from a minimum of 0.255 and 0.095 mm, respectively, in a 2.6 mm SL larva to a maximum of 0.770 and 0.270 mm, respectively, in a 7 mm SL larva. The retina was differentiated in all larvae in the size range analysed, as evidenced by the presence of the outer nuclear layer (ONL), inner nuclear layer (INL), ganglion cell layer (G), and connecting fibre layers (the inner and outer plexiform layers and optic fibre layer) (Fig. 11A). In addition, the retinal epithelium layer (REP) was pigmented, the optic nerve connected to the optic tectum, and lens crystallization was apparent. Just single cones were present in preflexion larvae. Angular density of cones increased with larval growth (Fig. 12) (Kruskal-Wallis, $p < 0.05$), with no differences among the regions of the central retina examined. Cone photoreceptor outer segment length was higher in the dorsal region than in the ventral and middle regions, irrespective of larval stage (ANOVA, $p < 0.05$). The presence in the outer nuclear layer of dark-staining, irregularly shaped nuclei in a vitread position relative to the lighter-staining cone nuclei (presumptive rod photoreceptor precursors or nuclei) was noted in a 4.5 mm SL larva. This cell type increased during larval flexion, reaching higher densities in the dorsal retinal region (Fig. 12) (Kruskal-Wallis, $p < 0.05$). Tangential sections of a late flexion larva (SL = 6 mm) revealed the presence of both double and single cone photoreceptors arranged in an organized pattern of double cones surrounding a single cone. No double cones were observed in smaller larvae with SL from 4.9 to 5.4 mm.

DISCUSSION

Auxis rochei larvae with SL from 2 to 7 mm are diurnal feeders, a fact reflected in the small number of prey items and the advanced stage of digestion of those items in the larval gut at night and consistent with a strong reliance upon visual prey detection by these larvae. This feeding pattern is shared by many other species (Hubbs & Blaxter 1986) and agrees with the results for *Auxis* spp. in the Gulf of California published by Sánchez-Velasco et al. (1999).

Larvae of this species are voracious feeders. Not only did they feed continuously (gut fullness >50% during day-

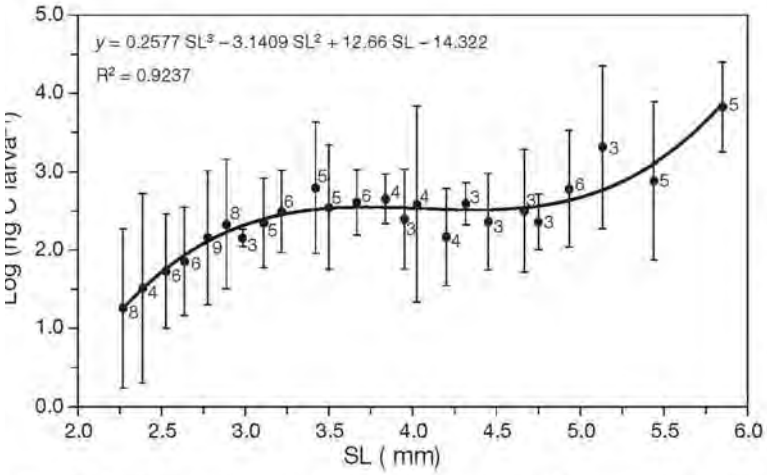

Fig. 8. *Auxis rochei* larvae. Weighted regressions of logarithmic mean carbon equivalent content in the larval gut (±SD) plotted against larval standard length (SL). Numbers to the right of the data points indicate the number of larvae in size class

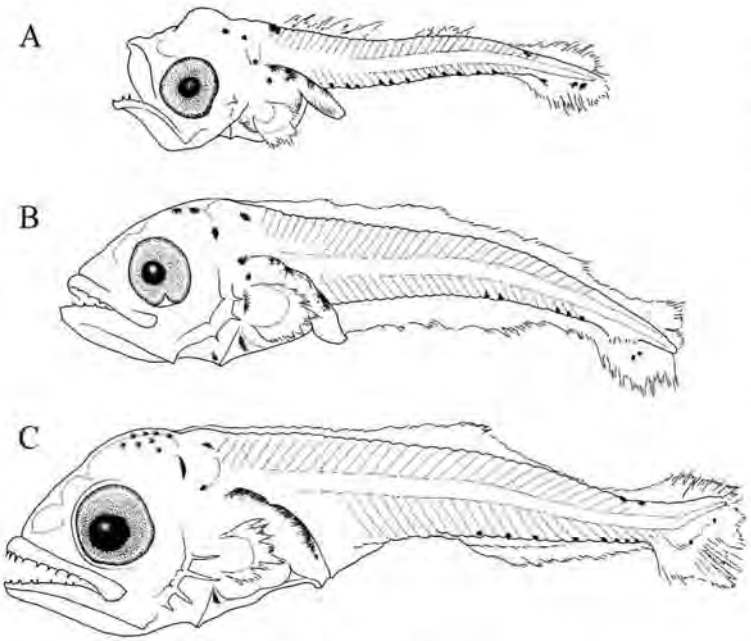

Fig. 9. *Auxis rochei* larvae. (A) Early feeding stage, SL = 2.9 mm. (B) Early flexion stage, SL = 4.9 mm. (C) Late flexion stage, SL = 7 mm

Table 2. *Auxis rochei* larvae. Regression parameters and statistics for the relationships between lower jaw length (LJL), upper jaw length (UJL), mouth width (MW), and eye diameter (ED) vs. larval standard length (SL), with r^2 values and number of data points (n). Confidence interval (CI) 95%

Model	CI of slope	r^2	n
$LJL = 0.075\ SL^{1.4203}$	0.1008	0.8154	177
$UJL = 0.0637\ SL^{1.5251}$	0.1111	0.8012	184
$MW = 0.0651\ SL^{1.3657}$	0.0998	0.8019	182
$ED = 0.1223\ SL^{0.9552}$	0.0495	0.89	181

light), but they also had much higher FI, gut fullness, and prey number values than the larvae of clupeiform and myctophiform teleost fishes (e.g. Tudela et al. 2002, Sassa & Kawaguchi 2004), and even of other tunas (Margulies 1993, Sánchez-Velasco et al. 1999). This is related to *Auxis rochei* larval morphology, i.e. a large, looped gut, large mouth, and large eyes. The looping helps retain prey, and even though the stomach was not functional in the size range studied, the presence of pinocytotic vesicles indicates that digestion was actively taking place in the gut.

Predator–prey relationship

Predator–prey relationships were analysed using log-transformed PW vs. SL or MW according to Pearre (1985). PW was used because there is evidence that prey length is not a critical factor in the ingestion of some prey items, while MW was chosen because it is closely correlated with the ability to capture prey and is a more accurate measurement than mouth gape (Hunter 1981).

Mean prey size increased with larval size, as it does in most species (Hunter 1981), but the rate of change in prey size with *Auxis rochei* larval size was higher than in other large-mouthed species like myctophids (Sabatés & Saiz 2000, Sassa & Kawaguchi 2004) and other tunas (Catalán et al. 2007), indicating that prey catching ability develops faster in this species. The greatest niche breadth was observed for the larvae with SL of ca. 4 mm and decreased in larger larvae. The reason for this is that the upper and lower limits on prey size do not increase the same way. Maximum prey size rises quite quickly up to a larval size of 4 mm SL, and then slows down. Minimum prey size holds constant until 5 mm SL and then increases. The lower niche breadth values in the larger larvae, combined with lower prey diversity, are indicative of a more selective foraging pattern, avoiding smaller prey items in favour of larger ones. Not only is this a better feeding strategy in that it concentrates on prey items with a higher energy content, but it also helps reduce competition with smaller larvae (Pearre 1985, González-Quirós & Anadón 2001).

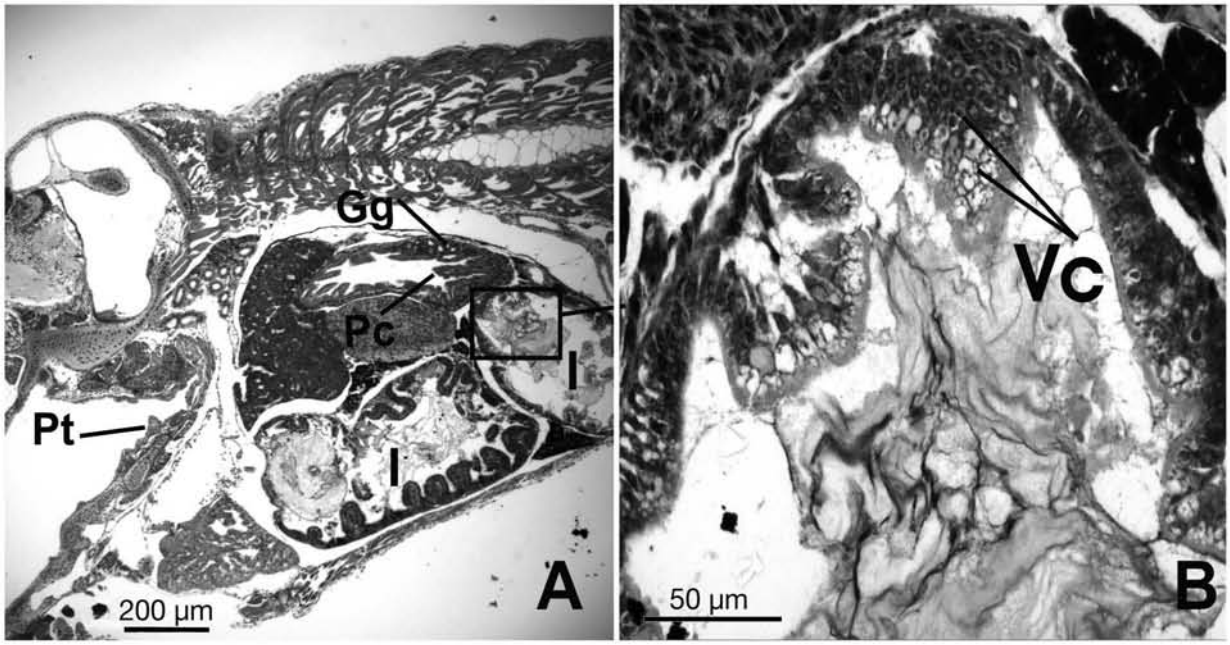

Fig. 10. *Auxis rochei* larva, SL = 7 mm. (A) Sagittal section photomicrograph of the body axis showing the digestive tract; Gg: gastric glands, Pc: pyloric caecum, Pt: pharyngeal teeth, I: intestine. (B) Detail of the intestine (I) showing different types of vacuoles (Vc)

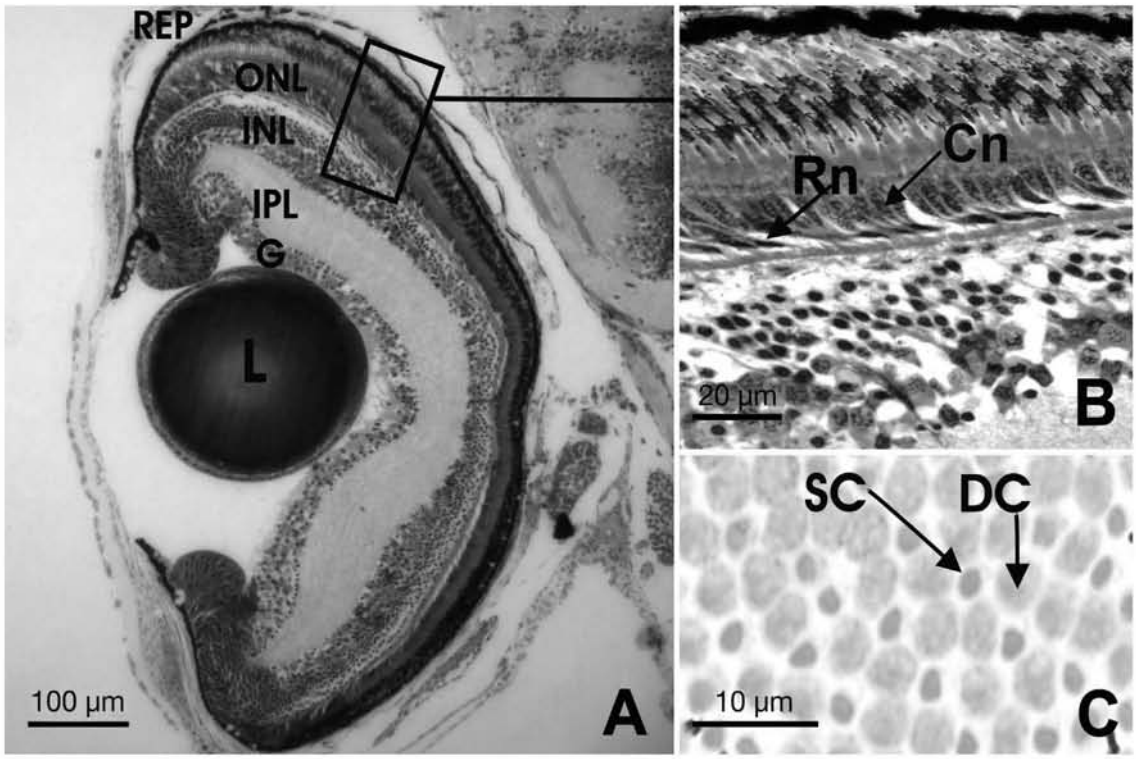

Fig. 11. Late flexion *Auxis rochei* larva. (A) Transverse section photomicrograph: layers of the eye of a 6.5 mm SL larva; REP: retinal epithelial pigment, ONL: outer nuclear layer, INL: inner nuclear layer, IPL: inner plexiform layer, G: ganglion cell layer, L: lens. Optic fibre layer not visible. (B) Outer nuclear layer of the dorsal area of the central retina of the same larva showing cone nuclei (Cn) and rod precursor nuclei (Rn). (C) Tangential section photomicrograph of the eye of a 6 mm SL larva showing double cones (DC) surrounding single cones (SC)

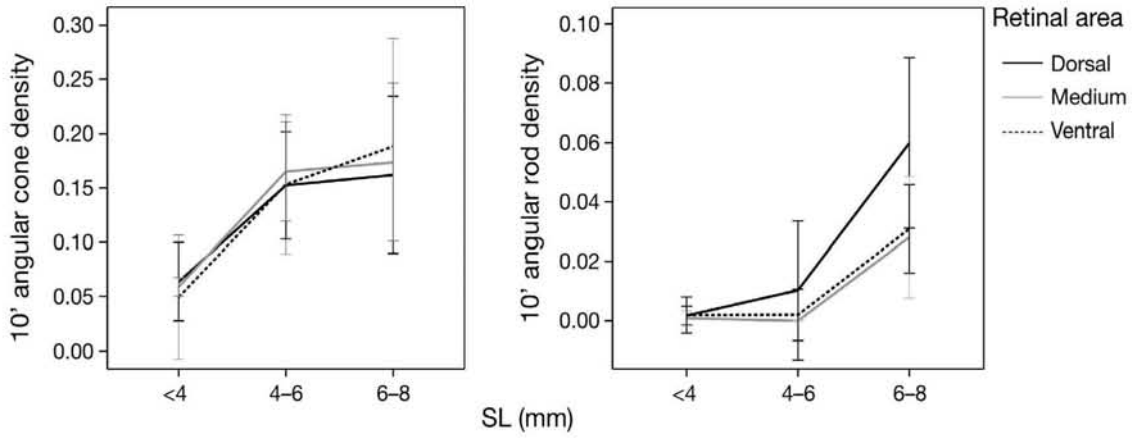

Fig. 12. *Auxis rochei* larvae. Developmental change in angular photoreceptor density (cones and rods). SL: standard length. Values are mean ± SD

Diet and selectivity

As in other species (Last 1980), *Auxis rochei* preflexion larvae are more euryphagous and eat certain types of prey that larger larvae later avoid, i.e. phytoplankton, tintinnids (above all *Undella hyalina*), gastropod larvae, and copepod eggs. In contrast, larvae with SL > 4 mm prefer larger, more mobile prey, like cladocerans (mostly *Evadne spinifera*), appendicularians, and fish larvae. Selection for cladocerans as opposed to copepods of similar size when both types of prey are available could be due to their yellow-orange color and large, pigmented eye, which makes them more visible (Govoni et al. 1986). Uotani et al. (1981) also reported positive selection for appendicularians and cladocerans (*Evadne* sp.) by *Auxis* sp. in the Indian Ocean. A finding to note was the relative absence of copepod postnauplii, a typical diet item in most marine fish postflexion larvae (Last 1980), including *Auxis* sp. larvae in the Gulf of California (Sánchez-Velasco et al. 1999) and the larvae of such other tuna species as *Thunnus alalunga* (Catalán et al. 2007) and *Thunnus maccoyii* (Young & Davis 1990).

The fact that appendicularians are important in the diet of *Auxis rochei* larvae in the Mediterranean is clear not only because of their frequency and abundance in the larval gut but also because larvae with SL > 3 mm selected for them. A number of researchers have noted the importance of soft prey items like appendicularians in the diets of the larvae of some scombrids like *Katsuwomus pelamis* (Uotani et al. 1981). Unlike the rest of the gelatinous zooplankton, the carbon content of appendicularians is similar to that of copepods (Hay et al. 1988, López-Urrutia et al. 2003). This prey species could be selected because of its slow, undulating movement, which may attract predators, and though they are large and elongate, their softness means that they are easily eaten and digested.

While piscivory appears to be widespread from the very early stages in other scombrids like *Scomber* spp. and *Scomberomorus* spp. (Conway et al. 1999, Shoji & Tanaka 2001), positive selection for larval fish was only observed in *Auxis rochei* larvae with SL > 5 mm, concomitant with greater tooth development, the appearance of specialized teeth, and flexion of the urostyle. All these attributes help make larvae more capable of both catching and handling and eating this type of prey.

The carbon content in the guts of the larvae over the course of larval development followed a rising trend, especially because of the paucity of the gut contents in some onset feeding larvae and the contribution of fish larvae to the diet in the larvae in the larger larval size intervals. Even though fish larvae were a lesser component in the diet of *Auxis rochei* larvae, their relatively high carbon content makes piscivory a determining factor in achieving high larval growth rates, because it raises the carbon content in the gut by an order of magnitude. Daily growth rates of piscivorous larvae are among the highest reported for teleost fishes (Tanaka et al. 1996 and references cited therein).

Precocious digestive systems have been observed in early piscivorous larvae like *Scomberomorus* spp. (Tanaka et al. 1996). Feeding habits and digestive system ontogeny in *Auxis rochei* are similar to *Katsuwomus* spp., i.e. an intermediate habit between strict zooplanktivory and piscivory (Uotani et al. 1981, Young & Davis 1990). The soon-to-be functional stomach in the largest *A. rochei* larvae, together with the increasing importance of fish larvae in the diet, suggests that piscivory will be important in the next developmental stages.

Vision enhancement

The retina appears functional in the smallest larvae examined, coinciding with the high FI values for these larvae during the daytime. This is consistent with a single type of retinal photoreceptor, single cones, which provide for acute visual discrimination, such as the visual detection of prey, but which limit vision to relatively high light intensity conditions in well lit 'surface' waters during the day (photopic acuity) (Hubbs & Blaxter 1986). The presence of both double and single cones in flexion stage *Auxis rochei* larvae (SL = 6 mm) provides evidence for an ontogenetic increase in visual discrimination under lower light intensity conditions in daytime (photopic sensitivity), since double cones have larger surface areas for light capture (and therefore a greater likelihood of photoreceptor stimulation) than their smaller-diameter single cone counterparts (Lythgoe et al. 2004). Photopic sensitivity is also likely to be enhanced by the ontogenetic increase in cone photoreceptor outer segment lengths, which provide a longer light path for image forming light to successfully achieve photoreceptor stimulation. Whether preflexion stage *A. rochei* larvae are capable of color vision is unknown. Previous studies have determined that the single cone retinae of many species of fish larvae have a range of single cones with different spectral absorption maxima; however, these occur later in larval development and most fish that hatch with a single cone retina have only green and possibly UV sensitivity, which is a limited color vision system (Britt et al. 2001). Ontogenetic increases in the eye and corresponding lens size result in increased visual acuity because, under optimal illumination conditions, an object viewed from a given distance by one fish with a smaller and another with a larger eye potentially stimulates more photoreceptors in the larger eye during image formation, with a resulting increase in acuity (Hairston et al. 1982). This means that given optimal visual conditions of water clarity and light brightness, larger larvae will be able to discriminate objects at greater distances (Breck & Gitter 1983). Rod photoreceptors and/or their precursors were evident in early flexion *A. rochei* larvae, with angular densities of rods increasing thereafter, thus providing for increasing visual function under conditions of dim monochromatic light at night or at depth during the daytime (scotopic sensitivity) (Hubbs & Blaxter 1986). This is consistent with another study in which rods were first noted in 7 mm SL larvae of *Auxis* spp. (Margulies 1997), where it was suggested that development of rod vision could play an important role in predator avoidance. While cone density in *A. rochei* did not vary with the retinal region examined, the fact that cone outer segment lengths and rod densities were consistently greater in the dorsal region of the central retina, irrespective of developmental stage, suggests that the visual axis for lens accommodation may be forward- and downward-projecting relative to the snout. This is consistent with the proposed visual axis of other shallow-water larval planktivores (Cobcroft & Pankhurst 2006).

Although prey size is the dominant factor in larval selection, such other factors as nutritional quality, visibility, evasion speed, and morphological makeup of potential prey are also important (Hunter 1981, Govoni et al. 1986). *Auxis rochei* larvae are highly active visual predators that have high feeding success from the onset of feeding. However increasing mouth size (jaw length and MW), enhanced vision, digestive development, and improved swimming ability with notochordal flexion lead to changes in the prey type selected, shifting from small to larger, more nutritious prey.

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ORIGINAL PAPER

Feeding selectivity in larvae of the European hake (*Merluccius merluccius*) in relation to ontogeny and visual capabilities

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Abstract Feeding ecology was analysed for the first time in the larvae of the European hake (*Merluccius merluccius*) to determine whether their diet and selectivity were constrained by environmental conditions and how these feeding characteristics were related to ontogeny, prey availability and visual capabilities. Larvae collected during both day and night were analysed, and it was found that feeding incidence was high, regardless of the time of day. Examination of the visual system corroborated the hypothesis that hake larvae should be able to cope with a wide range of photic conditions and to forage even at low light intensity. A clear preference for adult calanoid copepods and, especially, for *Clausocalanus* spp. was observed in all sizes analysed. Prey number increased with larval size, but prey size did not. This finding indicates that hake larvae behave as selective and specialist predators that consume an increasing number of prey rather than larger prey during larval growth.

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Introduction

The European hake (*Merluccius merluccius*) is widely distributed in the north-east Atlantic Ocean and the Mediterranean Sea (Alheit and Pitcher 1995). It is an important commercial gadid species, the second most important demersal fish species in landings in the Mediterranean (Sánchez et al. 2007). Fluctuations in hake recruitment occur very frequently in the Mediterranean (Oliver and Massutí 1995). Processes that determine recruitment and stock fluctuations occur during the early life stages of the fishes; predation and starvation are the highest sources of mortality during the larval phase of fishes, and they are interconnected during the planktonic phase (Bailey and Houde 1989). As larvae grow, they are less vulnerable to predation but more susceptible to starvation, so feeding success will affect larval survival and hence recruitment (Hunter 1981).

In the NW Mediterranean, hake spawning occurs over most of the year, peaking in autumn and is lowest in winter (Recasens et al. 2008). *M. merluccius* eggs and larvae are distributed over the continental shelf, with peak abundances between the 100-m isobath and the shelf edge (Olivar et al. 2003, 2010), and at a standard length of 16 mm, juveniles start to settle to the bottom (Arneri and Morales-Nin 2000). Through their position in the water column, fish larvae decrease their vulnerability to predation and search for a suitable prey field as a way to ensure their survival (Fortier and Harris 1989). This position is also a way to enhance their fitness, so they can take advantage of oceanographic conditions that benefit their growth (Fiksen et al. 2007).

Larval fish feeding success depends on several abiotic and biotic factors, such as the concentration and type of food (Hunter 1981), turbulence (MacKenzie et al. 1994), temperature (Paul 1983) and light conditions (Blaxter 1986). Environmental light conditions are important because most

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fish larvae are visual predators (Blaxter 1986). The intensity and the spectral quality of light affect larval feeding capabilities by altering prey search behaviour and reactive distances (Huse 1994). Similarly, fish larval feeding often follows a distinct diel pattern related to light intensity, and species-specific timing of peak feeding and duration of feeding periods have been observed (Hillgruber and Kloppmann 2001; Morote et al. 2008a, b). Moreover, the abundance (density and distribution), swimming behaviour of prey (Buskey et al. 1993) as well as prey conspicuousness and predator success (Govoni et al. 1986) determine the survivorship of the most vulnerable portion of the fish population. Most fish larvae do not prey indiscriminately on any prey that are around them, but are selective based on prey features (Checkley 1982; Govoni et al. 1986), becoming specialist or generalist predators depending on the strategies that each species develops.

Investigations into feeding patterns of Merluccidae larvae have been undertaken for *Merluccius productus* (Sumida and Moser 1980; Cass-Calay 2003), *M. hubbsi* (de Ciechomski and Weiss 1974), *M. gayi* (Valenzuela et al. 1995) and *M. bilinearis* (Reiss et al. 2005), but no information has been available on the larvae of *M. merluccius*. Although these studies deal with different species living in different geographical regions, the results show great similarities in diet composition, indicating that hake larvae are specialised predators.

In the present study, *Merluccius merluccius* larvae and their available prey were analysed simultaneously to determine the distribution of larvae in relation to the prey distribution and their feeding selectivity, which are aspects that have seldom been taken into account in previous studies on other hake species. Analysis of the day/night variability in larval feeding activity was also carried out. Additionally, the larval diet was analysed based on data obtained for the entire water column and data obtained for discrete layers of the water column to determine whether the results on larval feeding ecology may be biased by the sampling strategy used. The aim of this study was therefore to provide an overview of the larval feeding ecology of *Merluccius merluccius* and to elucidate its foraging strategy by analysing feeding incidence (FI), diel feeding patterns, diet composition and prey selection, ontogenetic diet shifts and feeding-related structure development (mouth size and visual system).

Materials and methods

Field sampling of larvae and zooplankton

Larvae were collected during two cruises carried out in 2005 in the Catalan Sea (north-western Mediterranean), one of which took place in summer (June), while the other was carried out in autumn (November). Sampling to obtain larval distributions and abundance was carried out both during the day and at night along a series of transects from the inshore region (50 m) to the offshore region (ca. 600 m) by means of oblique Bongo hauls (mesh size 300 μm) from a depth of 200 m, or just above the bottom, to the surface. When the net reached the deck, the codend was removed before washing the net to avoid damaging the larvae. Additionally, to determine prey availability, vertical mesoplankton hauls were carried out using a Calvet net (mesh size 200 μm) at the same stations, immediately following the Bongo hauls to achieve continuity in the sampling. Both Bongo and Calvet nets obtain samples of the whole integrated water column, as they are not closing nets. A synoptic sampling of fish larvae and zooplankton was also performed at the shelf break by means of stratified vertical hauls (at 10-m intervals) using a Longhurst Hardy plankton recorder (LHPR) with a 280-μm mesh. Microplankton samples were concurrently obtained by means of Calvet and LHPR nets with meshes of 53 μm, but the data of this fraction were not used in the present study because the prey items recorded in the gut contents of hake larvae indicated that they feed almost exclusively on mesozooplanktonic organisms. For all gears, the volume of seawater filtered was measured by flowmeters placed in the centre of the net mouth. Samples were preserved in 5% buffered formalin. Larvae for histological examination were sorted onboard, fixed in 10% formalin in 0.1 M phosphate buffer and transferred to 70° ethanol after 2–4 weeks. Fish larvae for the diet and selectivity analysis were chosen from those collected with the Bongo and LHPR nets.

Laboratory analysis

A total of 96 intact *Merluccius merluccius* larvae ranging from 2.0 to 9.0 mm standard length (SL) were selected for gut content analysis. Before gut dissection, SL and mouth width (MW, measured ventrally as the widest distance between the posterior edge of the maxillae) of the larvae were measured. The entire gut of each individual was removed using a fine needle and placed in a drop of 50% glycerine/distilled water on a glass slide, and prey organisms were teased out for identification, enumeration and measurement. The gross morphology of the digestive tract in early-stage larvae was evaluated in whole fixed specimens to determine the presence or absence of gut widening.

Food particles in the gut were identified to the lowest taxonomical level possible. The maximum body length and width of each prey item were measured along the largest cross-section under a microscope equipped with an ocular micrometre to the nearest 0.0025 mm.

Zooplankton samples were diluted to a volume of 100 ml and subsampled in aliquots. Samples were counted

up to 100 individuals from the most abundant taxon or 30 individuals from each of the 3 most abundant categories. The abundance and distribution of the main prey items identified in the larval diet were analysed. These prey items were divided into 4 categories: *Clausocalanus* spp., *Paracalanus* sp., "pCalanus" (non-distinguishable copepod stages of the genus *Clausocalanus* and *Paracalanus*) and other calanoids. The abundance of each planktonic category from the Calvet net at the same locations where larvae were analysed was calculated as individuals per m^{-3} and used to estimate the selectivity index. The plankton abundance obtained at each LHPR level was analysed and used to calculate the selectivity index of each larvae of that level.

To evaluate ontogenetic variations in the diet, larvae were separated into two length classes (2–3.9 mm (SL < 4 mm) and 4–9 mm (SL ≥ 4 mm)). For prey selectivity analysis, three length classes were differentiated (2–2.9, 3–3.9 and 4–9 mm SL) to study the potential changes of the behaviour of larvae of approximately 3 mm SL with respect to their prey in more detail. The relationships between prey size (prey length and prey width) and larval size (standard length and mouth width) were analysed for the entire size range studied.

For examination of the visual system, hake larvae were embedded in glycol methacrylate resin, serially sectioned (2 μm) and stained (Lee's methylene blue-basic fucsin stain). Serial transverse sections of the heads of larvae with SL <4 mm ($n = 4$, SL = 2.7–3.8 mm), SL = 4–5.9 mm ($n = 3$, SL = 4.3–5.65 mm) or SL = 6–7.5 mm ($n = 3$, SL = 6–7.35 mm) were used to examine ontogenetic changes in the morphology of the eye and the retina. Additionally, two small larvae (SL = 2.7 and 3.6 mm) and two large larvae (SL = 5.65 and 7.35 mm) were bisected along the mid-longitudinal plane prior to embedding, and the left side of the head was serially sectioned along a tangential plane to investigate the arrangement of cone photoreceptors in the outer nuclear layer of the retina. In sections with the largest lens diameter, the dorso-ventral eye and lens diameters were measured under a stereomicroscope using a ProgRes digital camera and Image Pro Plus analyser. Photoreceptor nuclei diameter and outer segment length (measured from the sclerad limit of the pigmented retinal epithelium to the outer limiting membrane) were measured in the central retina away from the embryonic fissure and insertion point of the optic nerve. Photoreceptor density was determined along a single linear 100-μm transect in the central retina. Small eye size and curvature of the retina limited the retinal area available for cell counting, particularly in the smallest larvae (Browman et al. 1990, Pankhurst et al. 1993). Ten measurements of photoreceptor outer segment length were taken in the same retinal region.

Data analysis

Analyses of hake larvae were performed comparing size classes, but samples of the two studied periods were pooled because of the low number of larvae collected in June.

Feeding incidence (FI) was calculated as the percentage of the total number of larvae examined having at least one prey in their gut by day (15 min before sunrise to 15 min after sunset) and by night. A brief analysis of the diel feeding activity was performed using the stage of prey digestion estimated on a scale of 1 to 3 (1 = highly digested, completely transparent; 2 = partially digested; 3 = undigested, some colour remains) (Young and Davis 1990) and the prey number in the larval guts. Despite the fact that it was not possible to sample regularly in the same stations, larvae were collected at least every 2 h covering the whole 24-h cycle.

The diversity of prey items in the diets was calculated using the Shannon index, $H' = -\sum_{i=1}^{m} (pi \times Lnpi)$, where p$i$ is the relative abundance of prey item i in the diet and m is the number of prey categories. The composition of the diet was summarised as the abundance (N) and frequency of occurrence (FO) of prey items. An estimate of the relative importance of the different prey items in the diets was calculated by multiplying the N and the FO of a prey item in larvae with food in their guts (Laroche 1982). Prey number was assessed on the basis of the mean number of prey in the gut contents of larvae over the course of development. The larvae were classified into size intervals to produce the maximum number of size classes containing 3 or more larvae.

Prey length (PL), prey width (PW) and PW in relation to larval mouth size (PW/MW) were analysed during development. Because the mouths of some larvae were disfigured by the net, it was not always possible to measure MW, and in those cases, this parameter was retrocalculated from the relationship with larval length ($r^2 = 0.86$, $P < 0.001$).

For the prey selectivity analysis, the alpha index, α_i (Chesson 1978), was calculated as $\alpha_i = (r_i/p_i) \sum_{i=1}^{m} (r_i/p_i)^{-1}$ $(i = 1, \ldots, m)$, where r_i and p_i are the percentage abundances of prey item i in the larval diet and in the plankton samples, respectively. Only the four most common food organisms ingested by the hake larvae were considered for the purpose of focussing on preferences for organisms that contributed most to the larval diets, rather than on absolute prey preference (Govoni et al. 1986). The value of α_i ranges from 0 to 1, with a limit value of $1/n$ (n = number of prey categories), with higher values indicating that the larvae eat this prey in greater proportions than they occurred in the environment. As the index incorporates the relative

abundances of prey, it is unaffected by prey total abundance (Lechowicz 1982). Selectivity towards each type of prey was calculated using prey abundance from the Calvet net and from the LHPR net to compare the effect of using data that integrate the entire water column or data that correspond to restricted water layers in the values of Chesson index.

Correlation, linear regression and non-linear regression analyses were employed to study the different predator–prey relationships that occurred during larval ontogeny. Differences in the data were analysed by Student's t test, ANOVA and non-parametric (Mann–Whitney or Kruskal–Wallis) tests using the SPSS software package for Windows (SPSS Inc., version 17.0).

Optical sensitivity (the measure of the minimum light signal that an eye can distinguish above random background noise) and resolution (how discriminately the details of an image can be seen) are two visual properties that allow the eye to see properly in a wide range of light intensities. Because the larvae of this species are distributed over the first 150 m of the water column (Olivar et al. 2010), the optical sensitivity was calculated using both the Land (1981) sensitivity equation for monochromatic light $S = (\pi/4)^2 A^2 (d/f)^2 (1 - e^{-kl})$ and the Warrant and Nilsson (1998) equation for white light $S = (\pi/4)^2 A^2 (d/f)^2 (kl/(2.3 + kl))$. These equations relate sensitivity (S) to the diameter of the eye's aperture (A), the photoreceptor diameter (d), the focal length (f), the absorption coefficient of the photoreceptor (k) and photoreceptor length (l). The eye's aperture, i.e. the diameter of the pupil, was considered equivalent to the lens diameter (Fernald 1990) because this species, like most other fish species, does not exhibit a pupil response. The focal length (f), i.e. the distance from the centre of the lens to the retina, was calculated from Matthiessen's ratio, $f = 2.55r$, where r is the radius of the lens. The absorption coefficient of monochromatic and white light is unknown in fish larvae, and therefore, the coefficient (0.035 μm^{-1}) for adult bony fish was employed (Warrant and Nilsson 1998). The optical resolution can be calculated using the inter-receptor angle $\Delta\varphi$ between two stimulated visual cell nuclei separated by an unstimulated one, in accordance with Land and Nilsson (2002) as $\Delta\varphi = s/f = l/(nf)$, where s is the photoreceptor spacing and f is the focal length. The photoreceptor spacing is the inverse of the visual cell number (n) counted in the retinal linear transect l (a single linear 100-μm transect in the present study).

Results

Feeding incidence and diet composition

A high number of larvae examined had identifiable prey in their guts, indicating a feeding incidence of 86.5% during the night and 68% during the day. No correlation was found between the time of the day when larvae were collected and the prey digestion stage (Pearson's coefficient = 0.064, $P > 0.05$) or the number of prey in the gut (Pearson's coefficient = −0.037, $P > 0.05$).

In all larval stages, the diet was mainly composed of adult calanoid copepods. There were small differences in prey diversity (Shannon–Wiener index) and richness between the gut contents of small larvae with SL <4 mm (diversity index 1.87 and 18 prey categories) and large larvae with SL ≥4 mm (diversity index 1.39 and 15 prey categories). The diet composition (NxFO) was very similar between the two size classes (t test, $P > 0.5$), and the only difference that was observed was an increase in the importance of the copepod *Clausocalanus* spp. to the largest larvae as a result of an increasing number of ingested prey together with a decrease of the other copepod types. The most important category was the copepod *Clausocalanus* spp., composing more than 80% of the percentage (Table 1). Most of the *Clausocalanus* identified were adult females. Other prey of less importance were calanoids, such as pCalanus and *Paracalanus* sp. Cyclopoid (CY) and harpacticoid (HA) copepods rarely appeared in the guts. The only noteworthy non-copepod prey category was phytoplankton (identifiable in the gut as dinoflagellates and cysts), which appeared in the guts of small hake larvae, although with a low importance in the diet (0.63% NxFO).

Predator–prey relationships

The mean prey number per larva was 4.3 ± 3.65, with a maximum of 19 prey in a 5.70-mm-SL larva. A significant increase in the number of preys with larval size was observed (Fig. 1; $r^2 = 0.86$).

The largest prey found in the larval guts corresponded to certain calanoid copepods (*Centropages* sp., *Ctenocalanus* sp.) that were not frequent in the diet. The size for the most abundant prey ranged from a width of 180 to 250 μm (Fig. 2; striped bars). Small larvae ingested prey of a wide range of sizes (prey length from 20 to 1060 μm, prey width from 15 to 450 μm), including large copepods (Fig. 3a, b). Neither the mean maximum prey length nor the maximum prey width of the ingested prey showed any significant increase with hake larval size, despite the fact that the larval mouth continued to grow (Fig. 3c). Small prey items were present at a low frequency across all larval lengths, although they tended to be fewer in number in large larvae.

The prey width/larval mouth ratio revealed a highly significant relationship with larval length (non-linear regression analysis $r^2 = 0.40$, $P < 0.001$), showing that some preflexion larvae were able to ingest prey almost as

Table 1 Diets for two size groups of hake larvae

TAXA		SL < 4 mm (n = 66)			SL ≥ 4 mm (n = 30)		
		N	FO	N × FO (%)	N	FO	N × FO (%)
PHYTOPLANKTON	UI Phytoplankton	6	3	0.63	2	2	0.12
	Cocolitophorid	–	–	–	1	1	0.03
ZOOPLANKTON	Copepod Nauplii	2	2	0.14	–	–	–
Calanoid post-N	*Clausocalanus* spp.	71	33	**81.67**	116	25	**88.50**
	Paracalanus sp.	18	10	6.27	18	8	4.39
	Ctenocalanus vanus	7	6	1.46	5	2	0.31
	pCalanus	16	14	7.81	15	10	4.58
	Calanidae	1	1	0.03	1	1	0.03
	Aetidae	1	1	0.03	–	–	–
	Candacia sp.	1	1	0.03	–	–	–
	Centropages spp.	–	–	–	2	2	0.12
	Post-N CA (UI)	4	4	0.56	8	5	1.22
Cyclopoid post-N	*Oncaea* spp.	5	3	0.52	1	1	0.03
	Oithona spp.	1	1	0.03	2	2	0.12
	Post-N CY (UI)	2	1	0.07	1	1	0.03
Harpacticoid post-N	*Euterpina acutifrons*	1	1	0.03	–	–	–
	Post-N HA (UI)	3	3	0.31	4	3	0.37
Others	UI Crustacean	1	1	0.03	2	2	0.12
	Bivalve Veliger	1	1	0.03	–	–	–
	Spherical item	3	3	0.31	1	1	0.03
	Total	144	89		179	66	

Bold value indicates the most important item

SL standard length, *n* number of larvae analysed, *N* numerical abundance of prey items, *FO* frequency of occurrence of prey items in the guts

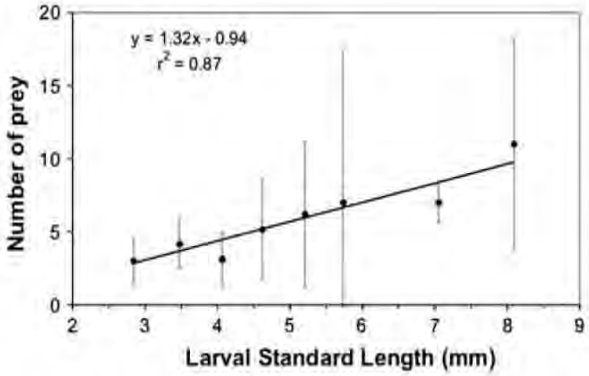

Fig. 1 Number of prey items per gut plotted against larval standard length (SL), (r^2 = 0.325)

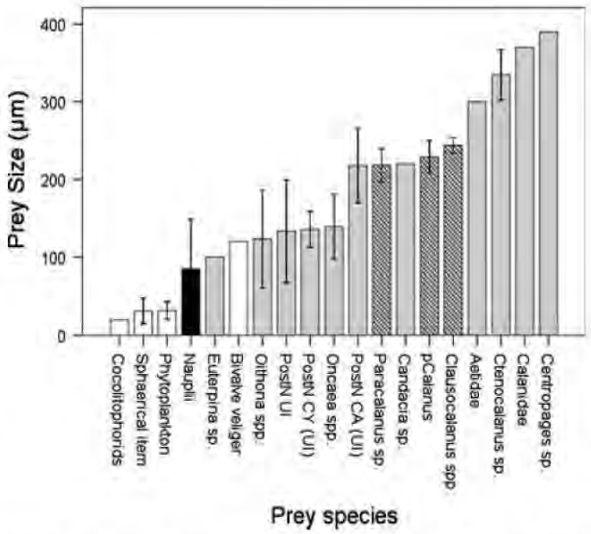

Fig. 2 Prey width (mean ± 95% CI) for each prey category. *Grey colour bars* for copepod postnauplii (adults and copepodites), *black bar* for copepod nauplii and *white bars* for non-crustacean prey. The most consumed prey are in *striped bars*

large as their maximum mouth width (Ratio = 1). However, there was no subsequent increase in prey size, even though the mouth increased in size (Fig. 3d).

Prey availability and larval selectivity

Hake larvae were sampled at 14 stations of the June cruise, while in November, they appeared at 27 stations of the same grid. The highest abundance of hake larvae was found in the areas of high abundance of their main prey, *Clausocalanus* spp. (Fig. 4a, b), with a positive correlation between the two abundances (Fig. 4c, d, $P < 0.05$).

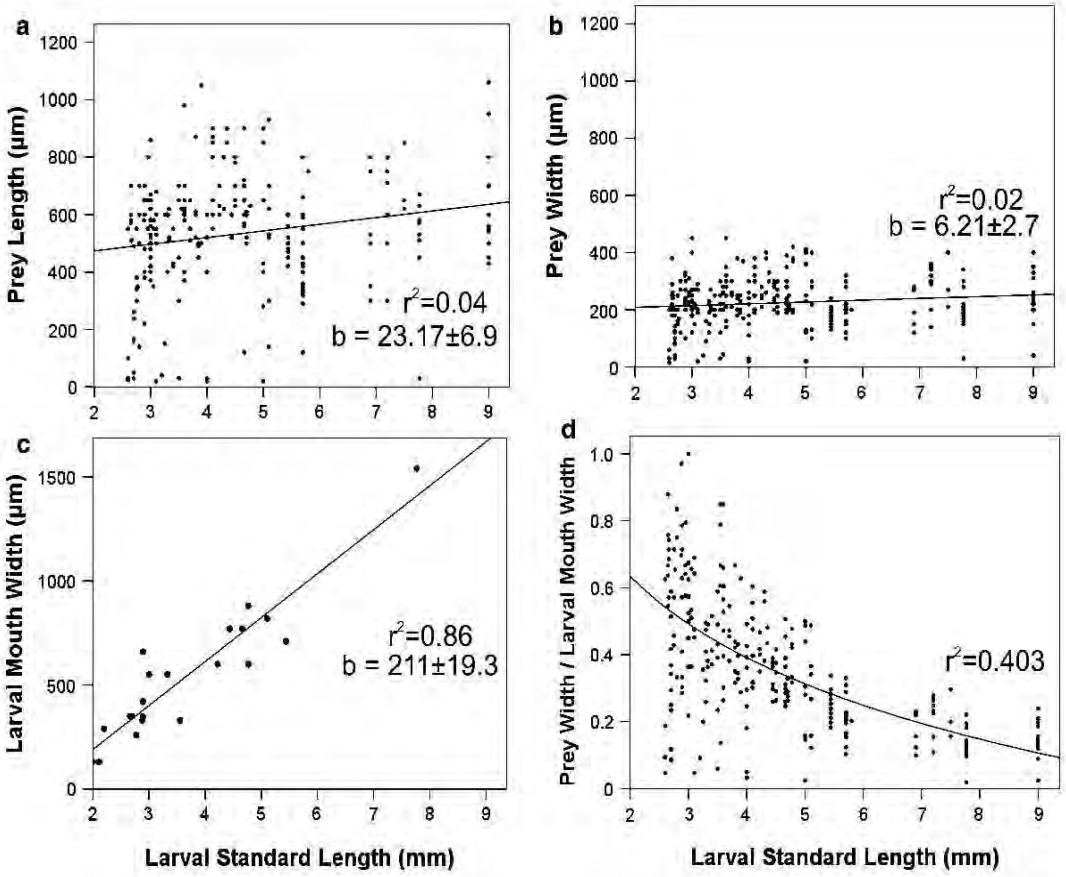

Fig. 3 Relationship between **a** prey length and larval length (SL), **b** prey width and larval length (SL), **c** larval mouth width and larval length (SL) and **d** the ratio of prey width/larval mouth and larval length (SL)

Selectivity was studied as a function of available prey. A value higher than 0.25 (1/4 prey categories) in the Chesson index indicated positive selection. Hake larvae of all stages showed a significant positive selection for *Clausocalanus* spp. (preferred prey), while the other three categories were consumed at a lower proportion than their abundance in the environment (Fig. 5). The results of the Chesson index analysis showed the same selection pattern, despite the sampling method used, indicating that the layered data obtained by the LHPR sampling method did not provide a substantial improvement compared with the depth-integrated data obtained by Calvet net sampling (Fig. 5a). When selectivity was analysed by different larval size groups, a positive selection towards *Clausocalanus* spp. was also observed. As larvae grow (larvae from 4–9 mm), they become more efficient at capturing *Clausocalanus* spp., with a decrease in the other three species of copepods, as shown by the lower selectivity index for these species. Larvae between 3 and 4 mm SL also showed a preference for *Paracalanus* sp., although the Chesson value was close to the limit value (0.25), and the difference compared with the other larval sizes was not significant (K-W, $P > 0.05$) (Fig. 5b).

Morphometric analyses of feeding-related structures

As has been found in other species of the genus *Merluccius*, the larvae of *M. merluccius* exhibit a very well-developed anterior portion of their body (head and trunk). Larval mouth width progressively increased with larval length, showing a significant positive allometric relationship with body length (allometric coefficient $b = 1.38 \pm 0.269$ (95% CI), $r = 0.744$). All of the larvae analysed had a developed digestive tract, with the mouth and anus being completely connected. In larvae of 2.6–3.2 mm SL, a widening of the gut after the foregut was observed, which is a feature that was correlated with the presence of prey in the gut (Pearson's coefficient $= 0.87$, $P < 0.01$).

With respect to the eye, the lens diameter in small larvae (SL < 4 mm) was 143.1 ± 39.2 µm; the optic nerve was centro-ventrally located; and the retina was fully differentiated (Fig. 6). However, in the smallest stage (SL = 2.4 mm), the complete functionality of the retina might be questionable, as no retinal pigment migration was observed, even in individuals caught during the day. However, retinal pigment migration was observed in individuals larger than 3 mm SL. From observation of the

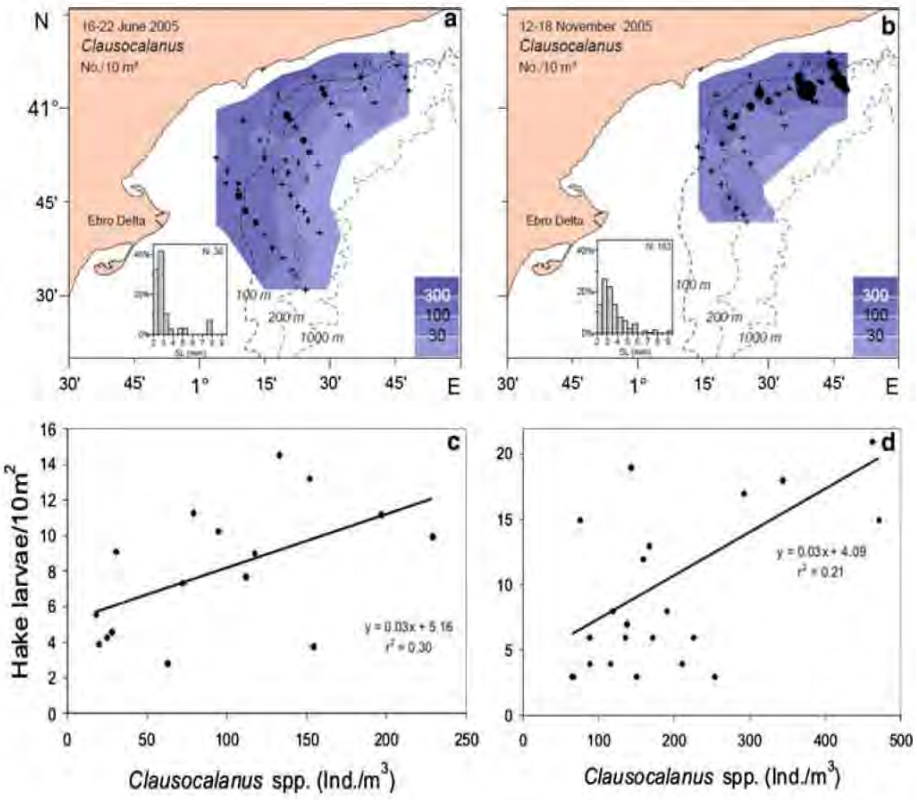

Fig. 4 Distribution and abundance of *Clausocalanus* spp. and hake larvae during the study period (**a**, **b**). *Shaded contours* indicate *Clausocalanus* spp. densities (individuals/m3). *Circles* indicate hake larval abundance, with symbol size proportional to the density of larvae (*smallest circle*, 10 larvae/10 m2; *largest circle*, 40 larvae/10 m2). Inserted graph: larval size frequency distribution. **c**, **d** Relationship between *Clausocalanus* spp. and hake larval abundance for each survey

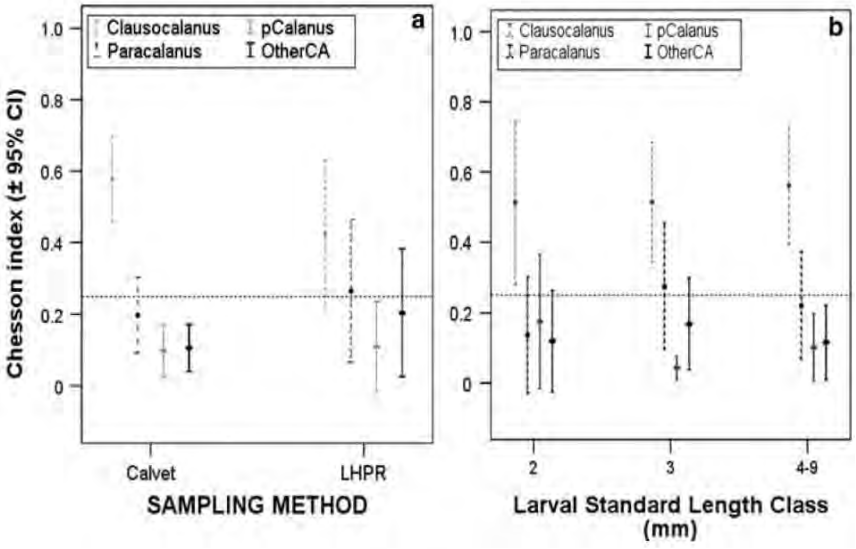

Fig. 5 Mean Chesson's α-values for the 4 most common prey items, **a** by each sampling method and **b** in three SL classes for the total hake larvae. *Bars* are ± 95% CI

photoreceptor outer segments and nuclei, a pure cone-like retina is postulated at this stage. The photoreceptor outer segment length was 14.4 ± 5.6 μm. The arrangement of the cone-like photoreceptors consisted of packed rows of single cells (Fig. 6).

In larvae >4 mm SL, the lens diameter was 253.7 ± 57.9 μm. The increase in cell numbers resulted in a progressive central displacement of the optic nerve during growth (Fig. 6a, b). The photoreceptor outer segment length was 30.7 ± 13.3 μm, and tightly packed rows of single cone-like photoreceptors were observed. In one larva of the largest size (SL = 7.35 mm), regional differentiation in cone size and packing was observed. Thinner and more densely packed photoreceptors were observed in the dorsal (2.67 ± 0.2 μm) than in the ventral (4.3 ± 2.1 μm) retina. Presumptive rod precursor nuclei in the outer nuclear layer were first observed in larvae of 7 mm SL (Fig. 6e).

The focal length increased during growth (Fig. 7a), while the inter-receptor angle decreased from the smallest

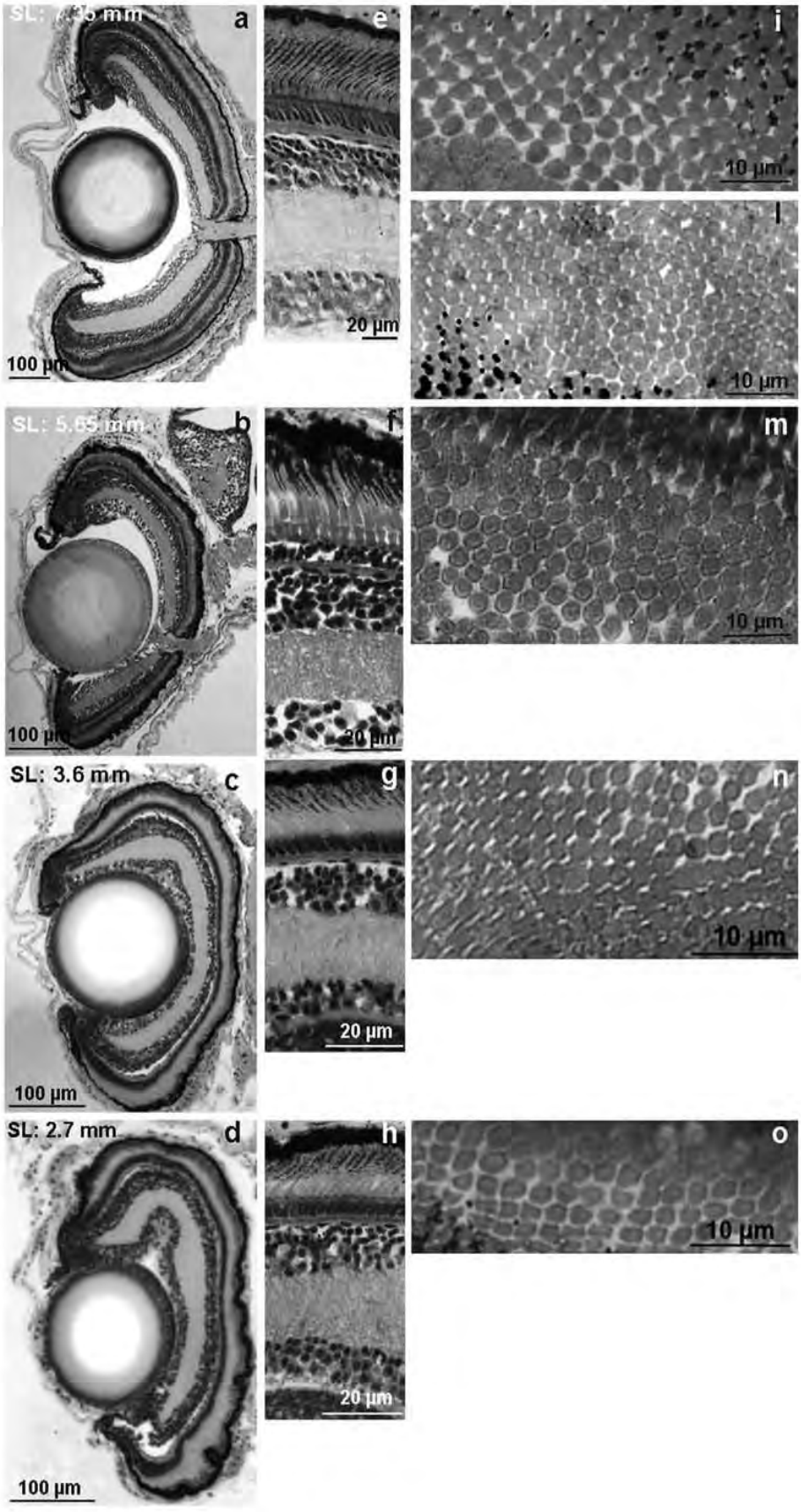

Fig. 6 Transverse (**a h**) and tangential (**i p**) sections of the eyes of hake larvae at different developmental stages. Note the change in the optic nerve position between the larvae of 5.65 and the 7.35 mm SL (*arrows*). Packed rows of single cone-like photoreceptors were observed in all stages (**i p**). Rp, rod precursor

to the medium-size larvae and remained constant in the large-size range (Fig. 7b), causing an inverse trend in the sampling frequency of the image (Fig. 7c), which represents grating in the optical resolution. The sampling frequency equation suggests that resolution can be improved by increasing the focal length, as shown in Fig. 7a, and/or by decreasing the inter-receptor angle, as shown in Fig. 7b. However, because the inter-receptor space did not change between the second and third size classes, the increase in resolution (Fig. 7c) was mainly due to the great increase in focal length observed. The enlargement of the lens and the increase in the cone-like photoreceptor diameter (from 2.0 ± 0.2 to 2.6 ± 0.3 μm) strongly determined the increase in optical sensitivity (Fig. 7d).

Discussion

Larvae of the European hake *Merluccius merluccius* living in the oligotrophic Mediterranean Sea exhibit a high feeding success, fairly similar to that observed for other species of hake larvae dwelling in highly productive areas, such as the Pacific or the Atlantic (Sumida and Moser 1980; Reiss et al. 2005). High feeding incidence (>75%) has been previously reported in other hakes as *Merluccius productus* (Sumida and Moser 1980), *Merluccius hubbsi* (de Ciechomski and Weiss 1974), *Merluccius gayi* (Valenzuela et al. 1995) and *Merluccius bilinearis* (Reiss et al. 2005). One of the factors affecting the feeding incidence in the species is related to the larval gut morphology. In this direction, the feeding incidence of larvae of non-merluccid gadoids, such as the Baltic Sea *Gadus morhua* (Voss et al. 2003), and in fish larvae of other NW Mediterranean species with similar morphology to hake larvae (looped guts) such as scombrids (*Thunnus alalunga*, Catalán et al. 2007; *Auxis rochei*, Morote et al. 2008a); and the *Callionymus* sp. (Sánchez-Velasco 1998) has been reported with high values also. In contrast, other NW Mediterranean species, such as *Engraulis encrasicolus*, *Sardina pilchardus* and *Sardinella aurita*, which have a very different larval morphology (straight guts), showed lower feeding incidences (Morote et al. 2008b, 2010). The high feeding incidence of *M. merluccius* larvae found in the present study was observed in both day- and night-collected larvae. The lack of a clear trophic diel pattern could be explained by larvae feeding throughout the day (neither a high number of empty guts nor undigested prey was found at any time). This lack could also be related to a slow prey digestion and evacuation process that causes the prey to remain in the gut for a long time period as Sumida and Moser (1980) detected in *M. productus* larvae.

Larval diet and prey size

M. merluccius larvae focused on calanoid copepods, and they did not include in their diet cyclopoids and

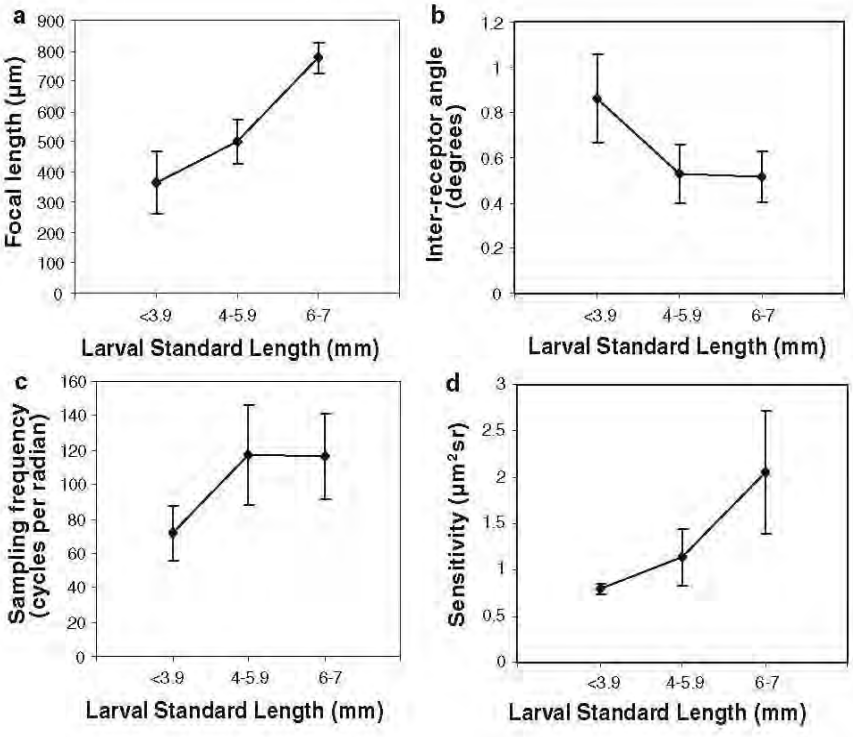

Fig. 7 Developmental changes in focal length (**a**), optical resolution (inter-receptor angle) (**b**), spatial frequency (**c**) and sensitivity (**d**). SL, larval standard length. Values are mean ± standard deviation

harpacticoid copepods, despite being abundant in the environment (Morote et al. 2010) and common items in the diets of larvae of other species, such as *Callionymus* sp. (Sánchez-Velasco 1998). The diet composition of larvae of other non-Mediterranean species of hake shows similarity to that obtained in the present study, with a highly specialised diet based on calanoid copepods. This group of copepods is nutritionally beneficial due to their high levels of highly unsaturated fatty acids in the form of phospholipids (Bell et al. 2003), which is probably an adaptation for success in the deep, cold habitats of hake species which take them through their diet (Sumida and Moser 1980). Copepods of the genera *Clausocalanus*, *Paracalanus* and *Pseudocalanus* are the reported prey found in the larvae of *M. hubbsi* (de Ciechomski and Weiss 1974), *M. productus* (Sumida and Moser 1980) and *M. bilianiaris* (Reiss et al. 2005), while they were found not to be important for *M. gayi* larvae, for which 60% of the diet was phytoplankton (Valenzuela et al. 1995). The larvae of *M. merluccius* do not exhibit ontogenetic changes in the type or size of prey. Additionally, from the onset of exogenous feeding, they ingest mainly adult *Clausocalanus* spp., particularly females (probably because they are bigger and more abundant in the water than males (Morote, pers. obs.)), and are even more selective than other species of the same genus (Sumida and Moser 1980; Reiss et al. 2005). The diet of hake larvae seems to be much more restricted than that of larvae of other gadoids, such as Baltic Sea *Gadus morhua* or North Sea *Trisopterus esmarkii*, *Melanogrammus aeglefiniis*, *Merlangius merlangus*, *Gadus morhua* and *Pollachius virens*, which begin feeding on copepod eggs and nauplii and then shift towards copepodits and adult copepods during their ontogeny (Economou 1991; Voss et al. 2003).

The width of prey ingested by the larvae of *M. merluccius* (100–450 μm) has an intermediate range compared with those ingested by similar size larvae of other hake species (70–200 μm for *M. productus* (Sumida and Moser 1980) or 500–800 μm for *M. hubbsi* (de Ciechomski and Weiss 1974)).

Thus, in contrast to the commonly observed behaviour in first-feeding larvae of many other fish species, which feed on small prey at the onset of feeding and replace them with larger prey as the larvae grow (Last 1980; Economou 1991), *M. merluccius* larvae prey on relatively large and mobile prey from the onset of feeding, which is similar behaviour to that observed in the larvae of marine cottid species, e.g. *Myoxocephalus aenaeus* (Laroche 1982). This prey size trend contrasts with that of other species that co-occur with hake in the same distribution range, such as myctophids (Sabatés and Sáiz 2000) and clupeids (Morote et al. 2008b; Morote et al. 2010), which have a first-feeding stage based on nauplii.

Selectivity

A suitable prey field is essential to determine feeding success and larval abundance (e.g. Robert et al. 2008); therefore, the foraging environment of a species must also be taken into account when analysing trophic ecology. In the present study, in which the diet of *M. merluccius* larvae was composed mainly of one prey type, we observed a high spatial coincidence between the horizontal distribution of the larvae and that of their main prey, *Clausocalanus* spp., which a priori indicates a suitable prey field. This prey type is practically the only type selected positively, regardless of the size class of the larvae and the sampling method employed. It is clearly selected in preference to other copepods in the environment, and hence, it is likely that there are factors (colour or behaviour) that influence the encounter rate. The vulnerability of these copepods is a function of their visibility and catchability. The individuals of the *Clausocalanus* genus are not brightly coloured. *Clausocalanus* is also a genus with little lipid storage in comparison with other calanoid copepods (Kattner and Hagen 2009), so its members do not exhibit lipid drops which could increase their visibility in certain light conditions. The catchability of *Clausocalanus* spp. probably depends on their swimming characteristics and the predation skills of the larvae. The survival capacity of a copepod against a predator usually depends on its capacity to avoid attacks. The swimming characteristics of *Clausocalanus*, which are rapid but aimless (Mazzocchi and Paffenhöfer 1999), make them an easy prey for *M. merluccius*. Furthermore, when swimming, these copepods do not create any currents that may provide them with information on their environment, such as the presence of predators, which must contribute to this species being more vulnerable to predation (Mazzocchi and Paffenhöfer 1999). For *Pseudocalanus* species, experiments and foraging models have proven that these copepods are more vulnerable to predation than other species because of their poor escape response (Viitasalo et al. 2001; Petrik et al. 2009). Similarly, *Paracalanus* have been described as nutritious and easy to capture because of their poor escape response (McLaren and Avendaño 1995). Larval swimming skills also influence predator–prey encounters. Therefore, the good swimming capability of *M. merluccius* larvae, as shown by the strong and early development of the caudal peduncle (Palomera et al. 2005), surely assists during predation activities.

The development of larvae is usually accompanied by new swimming and sensory skills that allow them to select larger or more nutritious prey necessary for an optimal growth of the larvae. The larvae examined in this study were within a size range in which the major ontogenetic changes have not yet taken place, which may explain why

their diet is so homogeneous. Postflexion and juvenile larvae of *M. merluccius* move to shallower zones (Maynou et al. 2003) and only settle to the bottom when they are between 11 and 16 mm TL (Arneri and Morales-Nin 2000). This migration is reflected in changes in diet. The examination of a 20-mm specimen captured in a zone located at north of the study area indicated a diet based on euphausiids, in addition to amphipods, ostracods and cumaceans (Morote, pers. obs.). Similarly, other studies carried out in the same area (Bozzano et al. 1997), as well as in the Balearic Islands (Cartés et al. 2009), have found that juvenile hake have a more varied diet than their larvae, in which copepods are alternated with larger prey, such as euphausiids.

Larval development and visual capabilities

As in other species, the yolk-sac larvae of *Merluccius merluccius* exhibit scant development. However, although growth in length is almost null for larvae younger than 12 days (up to 3 mm SL), the development of feeding-related structures (mouth and gut) takes place mainly during this period (Palomera et al. 2005). At approximately 3 mm SL, gut widening takes place, which is a factor as important as mouth size for determining the ingestion of prey. As larval development advances, folds appear in the gut, providing additional space for accumulating prey and favouring food retention. The presence of folds in the gut of larvae over 3 mm SL has also been reported in *M. hubbsi* and *M. productus* larvae (de Ciechomski and Weiss 1974; Sumida and Moser 1980).

Another aspect of larval development related to the feeding success of *M. merluccius* is its well-developed visual acuity from its earliest stages, which must help it to detect a wide size range of prey types, or prey of the same size from a greater distance. A prey of size O at a distance U from the larva's eye forms an angle of $\alpha = O/U$ radians at the centre of the eye (Land and Nilsson 2002). The size of the most important prey type (*Clausocalanus* spp., for which mean size is approximately 500 μm) and the inter-receptor angle in the larva's eye in good light condition result in a minimum distance at which the copepods can be detected of approximately 3 cm for small-size larvae and 5.5 cm for large larvae. Similar distances were obtained for larvae of the sunfish *Lepomis* spp., but at sizes of 8 and 10 mm, respectively (Walton et al. 1994).

As the main prey item of hake larvae is the relatively transparent *Clausocalanus* spp., analysing the visual capabilities that enable transparent prey detection, such as polarisation and UV sensitivity, would provide important information about prey detection in these larvae. Transparency enables some pelagic organisms that cannot hide to avoid detection by visual predators (Shashar et al. 1998). No information is currently available on these optical characteristics in hake larva retina, and it would be of great interest to investigate them to explain the high feeding preference of this species for a single type of copepod.

M. merluccius larvae feed not only during the day but also at night, and nocturnal feeding activity is unusual in fish larvae (Last 1980). Taking into account that large eyes and lenses provide increased sensitivity, a comparison of hake lenses with the lens size of other species of larvae of similar size ranges demonstrates that hake larvae are better equipped for vision at low light levels, providing support for the hypothesis of nocturnal feeding behaviour in *M. Merluccius* larvae. In fact, even in the small range size investigated (2–2.9 mm SL), the lens diameter of hake larvae was 50% greater than that of *Acanthopagrus butcheri* (Shand et al. 1999) and 20% larger than that of *Auxis rochei* (Morote et al. 2008a). In the medium-size larvae (4–5.9 mm SL), the hake lens was 15% larger than that of *Lampanyctus crocodilus* (Bozzano et al. 2007), and at larger size (7–8 mm SL), it was 25% larger than that of *Auxis rochei* (Morote et al. 2008a). Additionally, the values of optical sensitivity found in the present study (above 1) indicate that this species is crepuscular rather than diurnal (Land and Nilsson 2002).

In summary, this study showed that European hake larvae in the NW Mediterranean are selective feeders and feed almost exclusively on adult *Clausocalanus*. This pattern is evident from the early stages in this species and shows no change as larvae grow, indicating that they are skilled predators due to the high degree of development of their feeding-related structures. The remarkable similarity observed in the food type, prey size and selectivity of hake larvae as they grow is in accordance with previous studies of merluccid larvae in other regions and shows that the genus *Merluccius* can be described as a very selective predator that focuses on calanoid copepods, regardless of the study area.

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