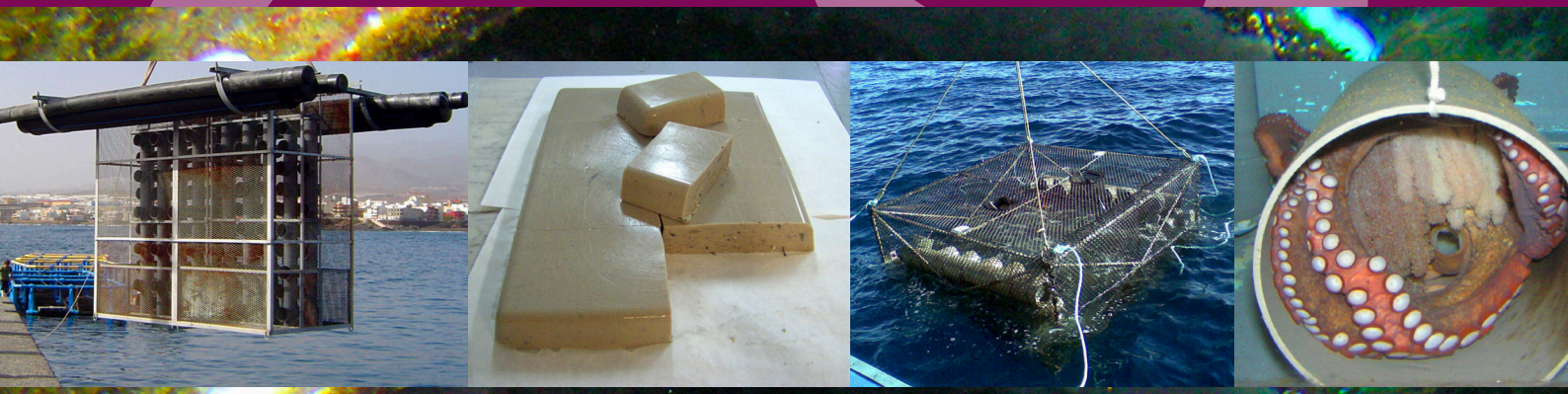
TESIS DOCTORAL

Optimización de las condiciones de engorde y avances en el conocimiento de los requerimientos nutricionales de pulpo común *Octopus vulgaris* (Cuvier, 1797)



Juan A. Estefanell Ucha

Las Palmas de Gran Canaria - 2012

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Departamento de Biología

IUSA Instituto Universitario de Sanidad Animal

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UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

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Anexo I

Dª MARÍA SORAYA DÉNIZ SUÁREZ, SECRETARIA DEL INSTITUTO UNIVERSITARIO DE SANIDAD ANIMAL Y SEGURIDAD ALIMENTARIA DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA.

CERTIFICA

Que el Consejo de Doctores del Departamento en su sesión de fecha 21 de diciembre de 2011 tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada: **"Optimización de las condiciones de engorde y avances en el conocimiento de los requerimientos nutricionales de pulpo común *Octopus vulgaris* (Cuvier,1797)"**, presentada por el doctorando D. Juan Estefanell Ucha, dirigida por los Dr. D. Juan A. Socorro Cruz y Dr. D. Javier Roo Filgueira.

Y para que así conste, y a efectos de lo previsto en el Artº 73.2 del reglamento de Estudios de Doctorado de esta Universidad, firmo la presente en Las Palmas de Gran Canaria, a veintidós de diciembre de dos mil once.

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Anexo II

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Departamento: Instituto Universitario de Sanidad Animal y Seguridad Alimentaria

Programa de Doctorado: Acuicultura: Producción controlada de animales acuáticos

Título de la Tesis

"Optimización de las condiciones de engorde y avances en el conocimiento de los requerimientos nutricionales de pulpo común *Octopus vulgaris* (Cuvier, 1797)".

Tesis Doctoral presentada por **D. Juan A. Estefanell Ucha**

Dirigida por **el Dr. D. Juan A. Socorro Cruz y por el Dr. D. Javier Roo Filgueira**

El Director, **El Director,** **El Doctorando,**

Juan A. Socorro Cruz **Javier Roo Filgueira** **Juan A. Estefanell Ucha**

Las Palmas de Gran Canaria, a 7 de diciembre de 2011

Optimización de las condiciones de engorde y avances en el conocimiento de los requerimientos nutricionales de pulpo común *Octopus vulgaris* (Cuvier, 1797)

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Doctorado en Acuicultura: Producción controlada de animales acuáticos. Grupo de Investigación en Acuicultura (GIA), Instituto Universitario de Sanidad Animal y Seguridad Alimentaria (IUSA) e Instituto Canario de Ciencias Marinas (ICCM)

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Abreviaturas

A-FCR - Apparent Food Conversión Rate
AGR - Absolute Growth Rate
ANOVA - Analysis normal variance
APROMAR - Asociación Empresarial de productores de cultivos marinos
ARA - Arachydonic acid (20: 4n-6)
BI - Biomass Increment
BI_S - Standarized Biomass Increment
DGI - Digestive Gland Index
DHA - Docosahexaenoic acid (22: 6n-3)
DW - Dry weight (peso seco)
EPA - Eicosapentaenoic acid (20: 5n-3)
FAO - Food and Agriculture Organization
FCR - Feed Conversion Ratio
FE - Feed Efficiency
GSI - Gonadosomatic Index
H - Sexual maturity Hayashi modificado por Guerra (1975)
ICCM - Instituto Canario de Ciencias Marinas
n-3 HUFA - n-3 series highly unsaturated fatty acid (20 or more carbons)
PER - Protein Efficiency Ratio
PIT – Passive Integrated Transponder
PPV - Protein Productive Value
PVC – Polivynil Chloride (policloruro de vinilo)
S - Survival
SD - Standard Deviation
SEI - Specific Energy Intake
SFI - Specific Feed Intake
SGR - Specific Growth Rate
SLI - Specific Lipid Intake
SPI - Specific Protein Intake
WD - Weight Dispersión
WW - Wet Weight (peso húmedo)

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Parece que fue ayer (marzo de 2007) cuando Juan Socorro me comentó la posibilidad de contratarme por un proyecto JACUMAR ("Optimización del engorde de pulpo *Octopus vulgaris*", 2007-2009). Me dijo: "*con este proyecto te sacas la tesis, dos por uno*". Han sido unos años fantásticos llenos de vivencias y aprendizajes. Me gustaría agradecer a todas las personas que han contribuido de una u otra manera a la realización de esta tesis doctoral y a hacerme pasar tan buenos ratos en la isla.

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Resumen

El pulpo común *Octopus vulgaris* es una especie de rápido crecimiento y alto valor de mercado, cuyas pesquerías se encuentran en declive. Por estos motivos, el sector científico y empresarial la ha identificado como candidata para diversificar la acuicultura marina. La presente tesis se planteó con 2 objetivos fundamentales: optimizar las condiciones de cultivo de pulpo común y avanzar en el conocimiento de los requerimientos nutricionales de esta especie.

En el "Estudio 1" se estableció un protocolo de anestesiado mediante inmersión en agua de mar a temperatura ambiente con un 1.5% de etanol (96%). A su vez, se estableció un procedimiento de marcaje individual con chips (PIT), insertado a nivel subcutáneo en la parte superior del brazo izquierdo III. Tanto el anestesiado como el marcaje no tuvieron un efecto negativo sobre el crecimiento ni la supervivencia de los ejemplares.

En el "Estudio 2" se estudió la maduración gonadal del pulpo bajo condiciones de cultivo, constatándose que los procesos reproductivos incrementan la mortalidad tanto en machos como en hembras. La madurez sexual se evaluó macroscópicamente, mediante índices sexuales y mediante cortes histológicos, y además el proceso de degradación post-reproductivo se estudió también en la glándula digestiva.

En el "Estudio 3" se realizó una comparación entre un sistema de cultivo grupal (tradicional) en jaulas flotantes y otro individual en jaulas de 80 L alojadas dentro de cada jaula en la parte superior. Este estudio permitió demostrar que el aislamiento limita la mortalidad, sin afectar a la composición bioquímica de los ejemplares, información que se tuvo en cuenta en los sucesivos estudios de nutrición de la tesis.

En el “Estudio 4” se utilizaron tanques compartimentados en 4 (400 L/pulpo, N = 4 por dieta) y tuvo una duración de 4 semanas. Se testaron 4 dietas basadas en boga *B. boops*: de origen salvaje (sub-producto de la pesca local) y de origen de descarte de granjas marinas (sub-producto de la acuicultura), en formato fresco y en formato pienso. Tanto las dietas, como el músculo y la glándula digestiva (inicial y final), fueron analizadas bioquímicamente. Los resultados mostraron que la dieta de boga de acuicultura tiene un elevado contenido lipídico (19-26 % en peso seco), especialmente rico en ácido oleico (23-24% del total de ácidos grasos) y linoleico (16-17% del total de ácidos grasos), y con bajo contenido en ARA (<1% del total de ácidos grasos) en comparación con los tejidos de pulpo inicial salvaje (14% del total de ácidos grasos). Por el contrario la boga salvaje presentó un contenido lipídico similar al músculo de pulpo (5-6% en peso seco), con un perfil de ácidos grasos rico en n-3 HUFA (37% del total de ácidos grasos). A pesar de ello, tanto el crecimiento (1.9-1.5%/d) como la retención proteica fueron más elevados en pulpo alimentados con boga de origen de acuicultura que con la de origen salvaje, hecho que sugiere la eficiente utilización de los lípidos de esta especie. Los pulpos alimentados con piensos experimentales mostraron una buena aceptación y crecimientos de 1.1-1.5%/d. El contenido lipídico y perfil de ácidos grasos de la dieta se vio reflejado en la glándula digestiva, mientras que el músculo presentó una composición muy estable.

En el “Estudio 5” se utilizaron tanques compartimentados en 8 (200 L/pulpo, N = 8 por dieta) y tuvo una duración de 8 semanas. Se testaron 5 dietas frescas: cangrejo blanco *Plagusia depressa*, cangrejo azul *Portunus pelagicus*, boga *B. boops* (sub-producto de acuicultura), dieta mixta cangrejo blanco – boga y dieta mixta cangrejo azul – boga. Los pulpos alimentados con monodietas de cangrejo y boga presentaron

un crecimiento similar (0.9 – 1.1 %/día). El crecimiento fue mayor en pulpos alimentados con la dieta mixta de cangrejo azul – boga (1.4%/d) respecto a los alimentados con boga (0.9%/d). Las dietas ricas en lípidos (boga y dietas mixtas) presentaron una mayor retención proteica en comparación con las dietas bajas en lípidos (cangrejos). Los lípidos de la dieta se reflejaron cuantitativa y cualitativamente en la glándula digestiva, mientras que el músculo presentó un perfil más estable. En concreto el bajo contenido en ARA de la boga se vio reflejado en todos los tejidos.

En el "Estudio 6" se utilizaron tanques compartimentados en 4 (400 L/pulpo, N = 4 por dieta) y tuvo una duración de 8 semanas. Se testaron 5 dietas: pienso de boga (subproducto de acuicultura), pienso de boga y carne de cangrejo azul, pienso de boga y carne de cangrejo rojo (*Grapsus grapsus*), pienso de harina de boga y cangrejo rojo y una dieta mixta troceada de boga y cangrejo azul (control). Los pulpos alimentados con el pienso de harina presentaron un crecimiento negativo, mientras que no se observaron diferencias en crecimiento entre ejemplares alimentados con los otros piensos experimentales y la dieta control (0.8-0.9%/d). Estos resultados sugieren que las harinas convencionales comúnmente utilizadas en la fabricación de piensos de peces son inadecuadas para el pulpo común, y plantea la necesidad de buscar nuevos sistemas de desecación y procesado de las materias primas para la elaboración de dietas formuladas para cefalópodos.

En el "Estudio 7" se evaluó el efecto de una dieta única de boga (sub-producto de la acuicultura) sobre el crecimiento y la mortalidad en machos y hembras de pulpo bajo condiciones industriales de cultivo (grupal) en jaulas flotantes. Como dieta control se utilizó una dieta mixta (cangrejo azul – boga). Ambas dietas fueron testadas en 2 experiencias de engorde consecutivas, con densidad inicial de 10 kg/m^3. En la primera

experiencia (N = 30 por dieta, 918 ± 125 g, sex ratio 1:1), los pulpos alimentados con la dieta mixta presentaron un crecimiento ligeramente superior (1.9-2.0%/d) que los alimentados con boga sola (1.8-1.9%/d), independientemente del sexo, y la mortalidad fue del 3% en ambos tratamientos. En la segunda experiencia (N = 32 por dieta, 1483 ± 269 g, sex ratio 1,4:1), el crecimiento fue mayor en los machos alimentados con dieta mixta (1.8%/d), seguido de los machos alimentados con boga (1.4%/d), y finalmente las hembras independientemente de la dieta (1.1-1.3%/d). Además, la mortalidad fue del 22-28% en ambos tratamientos. Tanto el bajo crecimiento de las hembras como la mayor mortalidad en la segunda experiencia se relacionaron con fenómenos reproductivos. Este estudio confirma el valor nutricional de la boga de descarte de acuicultura como dieta única para el engorde de pulpo común en la fase adulta, al menos hasta los 2 meses de cultivo.

En el "Estudio 8", se testaron 2 sistemas de cultivo para pulpo común: el tradicional en jaulas flotantes y uno experimental en jaulas bentónicas, situadas a 27 m de profundidad sobre fondos arenosos. Las características rígidas de la jaula flotante limitan su uso a zonas de aguas tranquilas, y la baja disponibilidad de zonas costeras resguardadas compite con intereses turísticos. Además, el sistema bentónico presenta otras posibles ventajas en relación al flotante: impacto visual mínimo y mayor estabilidad de temperatura y salinidad que en superficie. Ambos sistemas se testaron en 2 experimentos y se evaluó el crecimiento, la mortalidad y la composición bioquímica utilizando boga (sub-producto de la acuicultura) como dieta única. No se encontraron diferencias en crecimiento (1.8-1.9%/d), mortalidad (3–9%) ni composición bioquímica, por lo que se concluye que el sistema bentónico es adecuado para el cultivo del pulpo común.

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1. INTRODUCCIÓN GENERAL

1.1. La acuicultura

La *acuicultura* abarca la producción, engorde y comercialización de organismos acuáticos, animales o vegetales. Aunque ya era practicada en la antigüedad (2500 a. C.) el verdadero desarrollo de la acuicultura tuvo lugar después de la 2ª Guerra Mundial y fue consecuencia de un aumento exponencial de la demanda mundial de pescado, motivado por la reducción de las pesquerías por agotamiento de los stocks naturales. La FAO predijo en el año 2000 que el consumo mundial de proteínas acuáticas alcanzaría una demanda de 150-160 millones de toneladas a lo largo del siglo XXI, cifras que ya se han superado en 2009 (FAO, 2011).

1.1.1. Estado actual de la pesca y la acuicultura.

La producción mundial de la pesca está estancada en 90 millones de toneladas desde finales de los años 80, por lo que se considera que los océanos están explotados al límite de su capacidad (Caddy y Rodhouse, 1998). Por lo tanto, para compensar la creciente demanda de productos acuáticos, la producción mundial de la acuicultura ha aumentado de 0,6 millones de t en 1950 (3,2% del total de productos acuáticos) a 73,0 millones de t en 2009 (44,8% del total de productos acuáticos), con una tasa media de incremento anual del 8,5% (FAO, 2011), la mayor de todos los sectores de producción animal (Tacon, 2010). Esta expansión de la acuicultura es la llamada “revolución azul” (Costa-Pierce, 2002).

La acuicultura en Asia representó el 91,3% del total mundial en 2009, seguido de América (3,6%) y Europa (3,4%). China es el primer productor, con 45,3 millones de t en 2009 (62% de la producción mundial). Entre los 10 principales productores tan sólo aparece un país no asiático, Noruega (puesto 10º) (FAO, 2011). Entre las especies

principales producidas en 2009 destaca la presencia de un alga como especie mayoritaria, además de varias especies de peces herbívoras o detritívoras y dos especies de moluscos filtradores (Fig. 1.1). Tan sólo aparece una especie carnívora (puesto 7º), el langostino blanco (Litopenaeus vananmei, Bonne 1931), que sin embargo generó el mayor valor de producción (FAO, 2011).

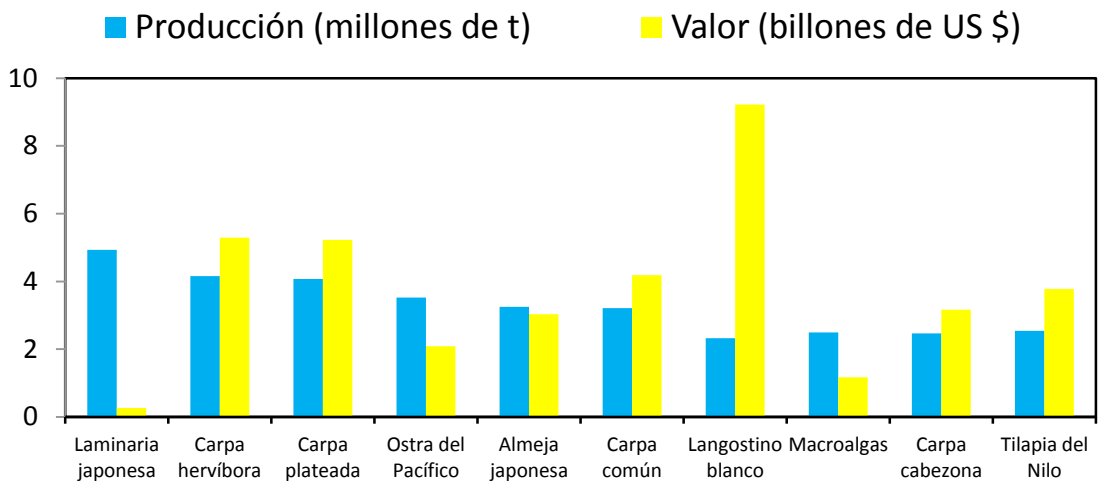


Fig. 1.1: Producción en cantidad (millones de t) y en valor (billones de US $) de las 10 especies principales cultivadas en el mundo en 2009 (FAO, 2011).

La pesca en Europa alcanzó su máximo en 1997 (20 millones de t), reduciéndose desde entonces hasta las 15,9 millones de t en 2009. La producción de la acuicultura no ha conseguido compensar el descenso de la pesca, sin embargo ha mostrado una importancia creciente hasta el 2009 tanto en valores absolutos de producción (2,5 millones de t) como en importancia relativa (15,7% del total de productos acuáticos). El productor mayoritario es Noruega (38,7%), seguido de España (10,7%) y Francia (9,4%) (FAO, 2011). Entre las 10 especies principales producidos en Europa en 2009 sobresale el salmón atlántico (*Salmo salar* L. 1758) como especie mayoritaria, seguida del mejillón (*Mytilus spp*) (Fig. 1.2). En comparación con la

acuicultura mundial, destaca la abundancia de especies de peces carnívoras de alto valor de mercado.

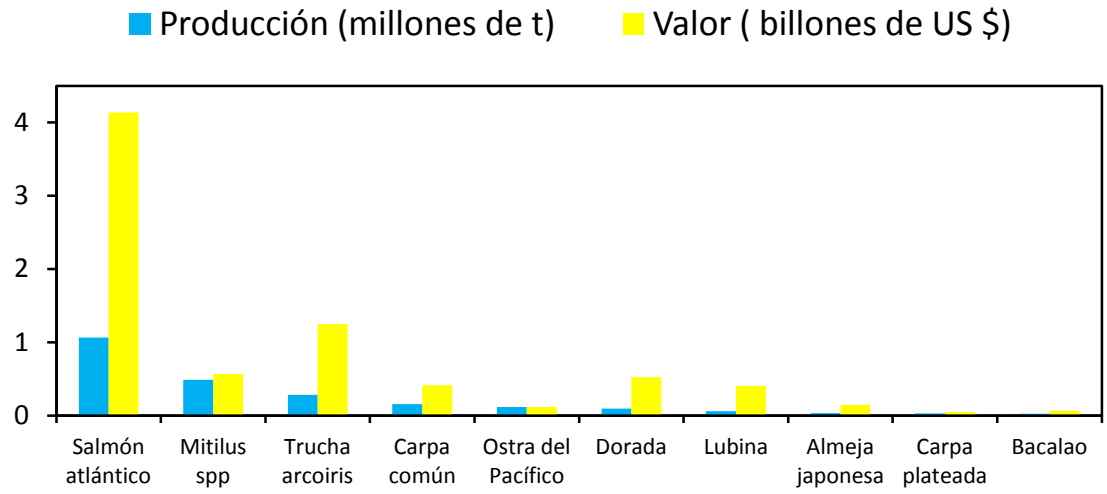


Fig. 1.2: Producción en cantidad (millones de t) y en valor (billones de US $) de las 10 especies principales cultivadas en Europa en 2009 (FAO, 2011).

España presenta un patrón similar al resto de Europa. La producción de productos acuáticos alcanzó su máximo a finales de los 90 (1,6 millones de t), reduciéndose hasta 1,17 millones de t en 2009. La importancia relativa de la acuicultura en comparación con la pesca es de 20-25% desde el año 2000, superior a la media europea. Sin embargo, la producción de la acuicultura se ha reducido desde las 318.185 t en 1999 a 266.479 en 2009. A pesar de ello, España es el Estado Miembro de la UE con una mayor producción en acuicultura, ocupando el puesto 19º a nivel mundial (FAO, 2011). Al igual que en las últimas décadas, la mayor parte de la producción de la acuicultura en España se basó en el mejillón (*Mytilus edulis* L. 1758, *Mytilus galloporvincialis* Lammark, 1819), representando más del 74% del total. Le siguen de lejos la dorada (*Sparus aurata* L. 1758) (8,7%), la trucha arcoíris (*Onchorinkus mykiss* Walbaum, 1792) (6,9%), la lubina (*Dicentrarchus labrax* L. 1758) (4,7%), el

rodaballo (*Psetta máxima* L. 1758) (2,7%) y la corvina (*Argyrosomus regius* Asso 1801) (0,5%).

1.1.2. Reducción de la tasa de crecimiento de la acuicultura

A pesar de ser una actividad consolidada, la acuicultura está sufriendo una desaceleración en su ritmo de crecimiento a nivel mundial, especialmente en Europa. De hecho, analizando únicamente los datos de producción de la UE los años 2000-2009 el crecimiento es negativo (-1,0%). Particularmente en España, la acuicultura presenta una tendencia similar al resto de la UE (-0,6%) (Fig. 1.3).

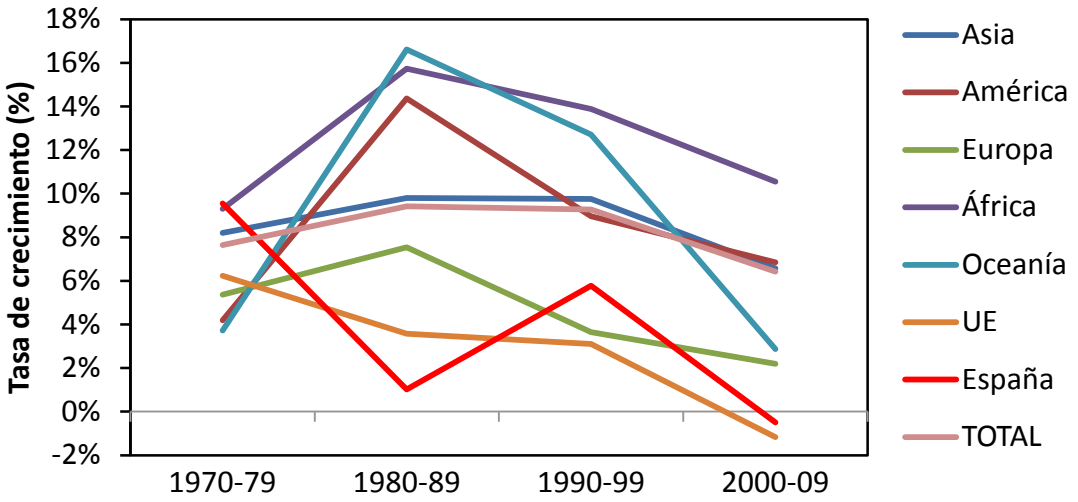


Fig. 1.3: Comparativa de la tasa de crecimiento de la acuicultura (%) en cada continente, en la Unión Europea y en España desde la década de los 70 hasta la actualidad (FAO, 2011)

Son varias las razones que explican esta situación. En primer lugar, la crisis económica actual está provocando una reducción en el consumo de pescado y el precio está ganando peso como factor de compra, favoreciendo a productos sustitutivos más baratos (Ojeda, 2009). Además, algunos mercados tradicionalmente muy rentables (mejillón, dorada) parecen estar saturados por una cierta sobreproducción que ha reducido la rentabilidad del sector (Vaz Pires *et al*., 2004). Esta situación tiene un impacto mayor sobre la acuicultura europea, ya que está

basada en un reducido número de especies. Por ejemplo, en 2009 la especie principal en Asia representa el 7,4% del total (Laminaria japonesa), en América el 17,3% (langostino blanco), en Europa el 42,8% (salmón) y en España el 74,5% (mejillón). Considerando la importancia de las 5 especies principales cultivadas en cada región, en Asia representan el 29,8%, en América el 56,7%, en Europa el 75,5% y en España el 97,6% del total de la producción.

Por otro lado, la acuicultura en los países desarrollados suele estar basada en especies de peces carnívoras, cuya producción representa la mayor parte del valor de mercado (FAO, 2011). Los piensos comerciales para estas especies deben incluir necesariamente un porcentaje de harina y aceite de pescado, que unido al creciente precio de dichas materias primas, limita la rentabilidad y crecimiento del sector (Sargent y Tacon, 1999; Tacon y Metian, 2008).

1.1.3. Soluciones para el estancamiento de la acuicultura en la UE

Para solventar el estancamiento de la acuicultura en la UE es necesario, en primer lugar, adecuar la producción a la demanda de pescado, evitando así situaciones de saturación de mercado y la pérdida de rentabilidad del sector. Por otro lado, la búsqueda de materias primas alternativas, como por ejemplo de origen vegetal, ha conseguido reducir el contenido de harina y aceite de pescado en los piensos de peces (Izquierdo *et al*., 2005; Montero *et al*., 2008; Naylor *et al*., 2009), rebajando costes y aumentando la sostenibilidad del cultivo de especies carnívoras (Tacon, 2010). Una fuente de materia prima alternativa de origen de pescado son los subproductos de la propia acuicultura: cabezas, vísceras, espinas o piel (Sathivel *et al*., 2003; Sun *et al*., 2006; Grahl-Nielsen y Glover, 2010). Otra fuente alternativa de origen de pescado, hasta ahora inexplotada por el sector, es la biomasa de especies de peces que es

accidentalmente “cultivada” en las granjas marinas. La especie más numerosa es la boga, y en ocasiones representa más del 5% de la biomasa total producida en jaulas de dorada (Estefanell, 2006). La disponibilidad de este recurso, unido a su escaso interés para consumo humano, sugiere su uso potencial como dieta de especies carnívoras en acuicultura, ya sea en forma directa o procesada (harina, aceite). Por otro lado, los sectores científico y empresarial deben aunar fuerzas para diversificar la oferta, generando nuevos productos comerciales a partir de las mismas especies (fileteados, precocinados, etc.) y sobretodo mediante el cultivo de nuevas especies (APROMAR, 2010).

Estas medidas imitan el exitoso ejemplo de Noruega, que a pesar de basar su producción en una sola especie, el salmón atlántico (89,7% del total), ha conseguido ser el 10º productor en cantidad y el 7º en valor a escala mundial (FAO, 2011). El éxito del cultivo del salmón se basa en el perfecto conocimiento de la biología de la especie, tanto a nivel de ciclo reproductivo como de requerimientos nutricionales. Estos avances han permitido sustituir hasta un 20-50% de las materias primas de origen de pescado por materias de origen vegetal (Tacon y Metian, 2008; Bendiksen *et al*., 2011), de modo que por unidad de salmón cultivado se necesita menos de una unidad de materia prima de origen de pescado, proporcionado por la pesca (Crampton *et al*., 2010). Además el salmón se comercializa de muchas maneras: fresco, ahumado, en conservas, etc., que aumenta su consumo a nivel mundial. En cuanto a la diversificación en el cultivo de nuevas especies, la acuicultura noruega ha implementado el bacalao (*Gadus morhua* L. 1758), cuya producción no superaba las 200 toneladas anuales en el año 2000, y que ha alcanzado su máximo provisional en el 2009 con 20.924 toneladas (FAO, 2011). Estos datos resaltan la importancia de la

diversificación en la acuicultura, como método de limitar situaciones de saturación de mercado, aumentando la rentabilidad y estabilidad del sector.

1.1.4. Selección de especies candidatas para diversificar la acuicultura

Las características principales que inicialmente deben tener las especies candidatas para diversificar la acuicultura en Europa, de acuerdo con el sector científico y empresarial, son un elevado valor de mercado y un rápido crecimiento. El elevado coste de producción en Europa, motivado entre otras cosas por los mayores salarios en comparación con Asia, hace prácticamente inviable producir especies de bajo coste a un precio competitivo a escala global. Otro criterio importante en la selección de especies es que sus pesquerías se encuentren en declive, hecho que garantizaría una adecuada demanda de mercado a corto y medio plazo.

El sector científico europeo ha desarrollado la tecnología para el cultivo de varias "nuevas" especies, sin embargo su implantación a gran escala está limitada por diversas causas. Como ejemplo puede citarse la corvina (*Argyrosomus regius*, Asso 1801), especie de mayor crecimiento que los espáridos y fácil adaptación a los sistema de cultivo actuales (Fernández-Palacios *et al*., 2009; Roo *et al*., 2009a). Su producción ha aumentado de 3 t en 2003 a 1.348 t en 2009 (FAO, 2011) y se prevé una mayor expansión en los próximo años. El lenguado (*Solea senegalensis* Kaup, 1858) o el bocinegro (*Pagrus pagrus* L., 1758) son especies de alto precio de mercado que ya se cultivan en varios países del Mediterráneo, sin embargo son muy delicadas en cuanto al manejo, por lo que la aparición de patologías limita la producción industrial (Imsland *et al*., 2003; Roo *et al*., 2009b). En una fase preliminar de investigación se encuentran varias especies de peces de rápido crecimiento, que despiertan gran interés a nivel europeo y mundial, como por ejemplo el medregal (*Seriola dumerili* Risso, 1810), el

medregal negro (*Seriola rivoliana* Valenciennes, 1833), el atún rojo (*Thunnus thynnus* L., 1758) o el jurel dentón (*Pseudocaranx dentex* Bloch y Schneider, 1801) (Papandroulakis *et al*., 2005; Roo *et al*., 2007, 2009c, 2010; Ortega *et al*., 2009).

En cuanto a especies herbívoras, la acuicultura europea se ha centrado en el cultivo de moluscos. La especie que actualmente despierta mayor interés es la oreja de mar (*Haliotis tuberculata* L., 1758) gasterópodo ramoneador de alto precio de mercado que sin embargo hasta la fecha presenta un lento crecimiento bajo condiciones de cultivo (Viera *et al*., 2005; Bilbao *et al*., 2010). En una situación similar se encuentra la navaja (*Solen marginatus* Pennánt, 1777) (Da Costa y Martínez-Patiño, 2009).

Dentro de los moluscos, los cefalópodos son un grupo de gran interés para la diversificación de la acuicultura marina, ya que presentan un crecimiento excepcionalmente elevado en comparación con especies de peces de tamaño similar (Saville, 1987). La información disponible sobre el cultivo de varias especies de cefalópodos en las últimas décadas proviene de su utilidad como modelos de experimentación biomédica y biología pesquera (Hanlon, 1987). Sin embargo, el declive en las pesquerías de varias especies bentónicas costeras, muy apreciadas por los consumidores de cada región, han impulsado la realización de estudios sobre crecimiento, desarrollo larvario y nutrición de algunas especies de cefalópodos, de cara a evaluar sus posibilidades de cultivo. Dentro de este grupo destaca la *Sepia officinalis* (L. 1758) (Sykes *et al*., 2006; Baeza Rojano *et al*., 2010) y varias especies de octópodos: el *Octopus maya* (Voss y Solis Ramirez 1966) en Méjico (Rosas *et al*., 2007; Briceño *et al*., 2010), el *Octopus mimus* (Gould 1852) en Chile (Cortez *et al*., 1999; Carrasco y Guisado, 2010) y el *Octopus vulgaris* (Cuvier 1797) en Europa

(principalmente España, Grecia e Italia) (Iglesias *et al*., 2000; Miliou *et al*., 2005; Prato *et al*., 2010).

1.2. El pulpo común *O. vulgaris*: candidato para diversificar la acuicultura europea

El pulpo común *Octopus vulgaris* presenta varias características biológicas que lo hacen atractivo para el cultivo industrial: elevado crecimiento, muy superior a las especies de peces habitualmente cultivadas (Fig. 1.4), elevado factor de conversión, gran fecundidad potencial y rápida adaptación a las condiciones de cultivo (Mangold y Boletzky, 1973; Mangold, 1987; Guerra, 1992; Iglesias *et al*., 2000).

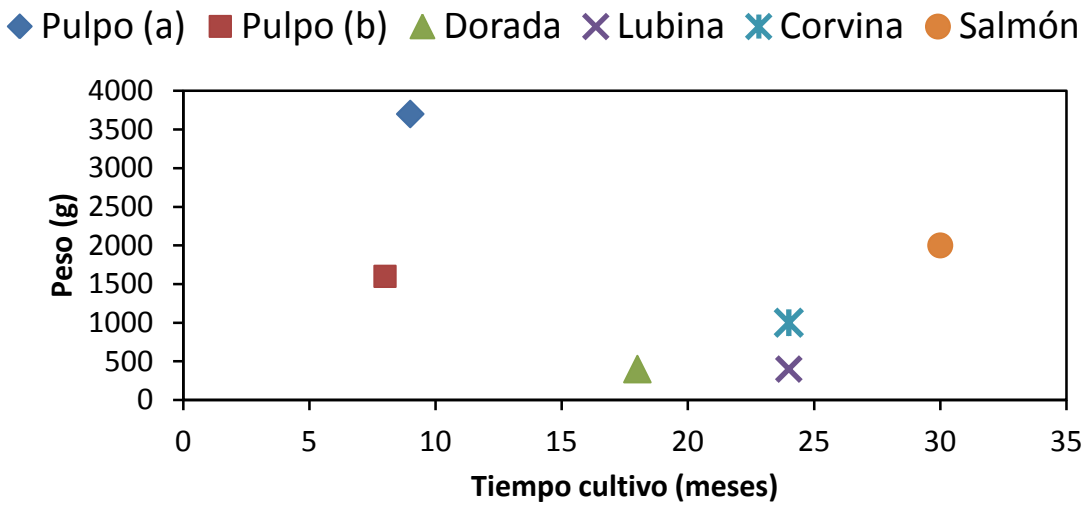


Fig. 1.4: Peso comercial y tiempo de cultivo para dorada, lubina, corvina y salmón (FAO, 2005-2012) y para pulpo común *O. vulgaris* (a, Smale y Buchan, 1981; b, Iglesias *et al*., 2004).

Además es una especie muy apreciada en el área Mediterránea (España, Portugal, Italia), en Asia (Japón, Corea) y Latinoamérica (México, Perú) (Vaz-Pires *et al*., 2004), y sus pesquerías se encuentran en constante recesión desde hace algunos años (Fig. 1.5) (FAO, 2011). Su precio de mercado es elevado, próximo a los 9 €/kg

(Mercamadrid, 2006-2010), y al carecer de esqueleto su índice de aprovechamiento es superior al 90% de su peso.

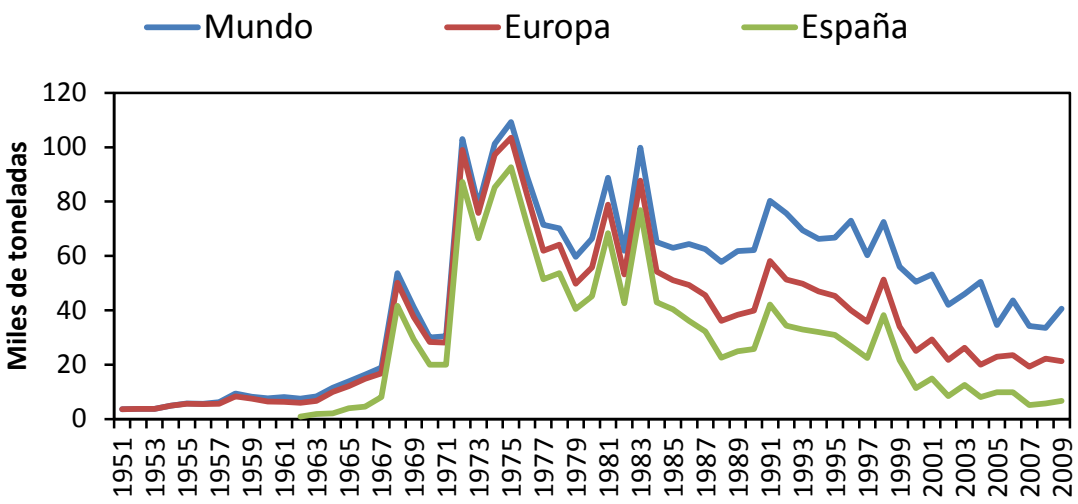


Fig. 1.5: Evolución de la pesquería mundial de pulpo común desde 1950 hasta 2009 (FAO, 2011)

1.2.1 Generalidades: clasificación, morfología, hábitat y distribución

El pulpo común *O. vulgaris*, también llamado pulpo de roca, es un molusco cefalópodo perteneciente al orden de los octópodos y su característica fundamental es la presencia de 8 brazos musculosos, con 2 filas de ventosas, y una cabeza globosa. Es un animal simétrico y los brazos se numeran en posición dorsal, con el pulpo mirando al frente, de modo que poseen 4 brazos izquierdos y 4 brazos derechos (Fig. 1.6). Habitualmente se pescan ejemplares de más de 5 kg (Guerra, 1992), llegando a alcanzar los 12,3 kg en cautividad (Iglesias *et al*., 2000). Es una especie dioica con claro dimorfismo sexual a nivel del tercer brazo derecho. Dicho brazo se denomina hectocótilo en los machos, funciona a modo de órgano copulador y se caracteriza por su extremo con forma de cuchara, denominada lígula, y sirve para diferenciar a simple vista machos de hembras.

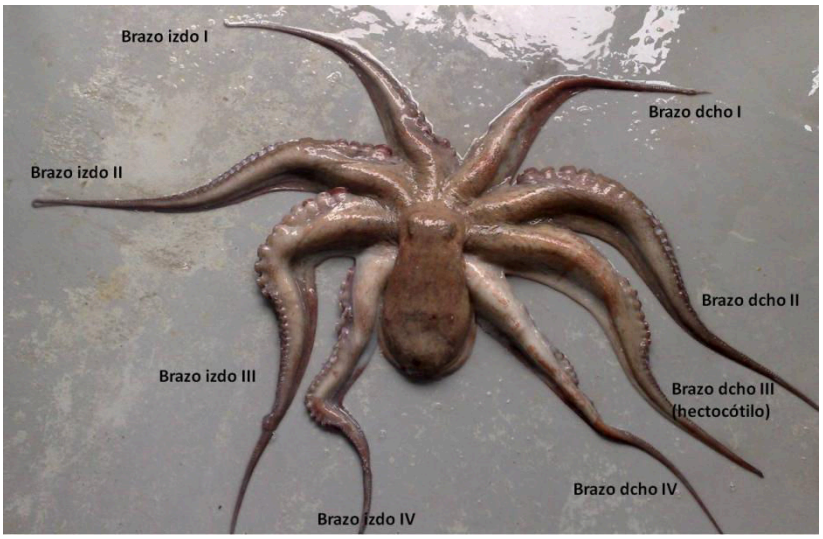


Fig. 1.6: Imagen de un pulpo adulto, vista dorsal con 4 brazos derechos y 4 izquierdos.

El *O. vulgaris* es una especie marina merobentónica de aguas cálidas tropicales, subtropicales y templadas, que aparece típicamente desde los 0 hasta los 200 m de profundidad (Guerra, 1992). Aparece tanto en fondos rocosos como coralinos, praderas de fanerógamas y fondos planos fangosos o arenosos (Mangold, 1983). Es una especie tolerante a los cambios de temperatura (7-33ºC), aunque su óptimo para el crecimiento se sitúa en torno a los 18-21ºC en el Mediterráneo (Aguado Giménez y García García, 2002). Por el contrario, es muy poco tolerante a los descensos de salinidad (Boletzky y Hanlon, 1983), por lo que pequeñas fluctuaciones por proximidad a ríos o lluvias intensas puede resultar mortal (Chapela *et al*., 2006).

Debido a que en la mayor parte del mundo se llama “pulpo común” al pulpo más abundante de cada región existe confusión sobre su verdadera distribución. Tradicionalmente se considera que el *O. vulgaris* se encuentra en el mar Mediterráneo y en el océano Atlántico, este y oeste (Mangold, 1998), distribución aceptada por la FAO. Estudios genéticos han ampliado su distribución a algunos puntos del Pacífico (Taiwán, Japón) (Warnke *et al*., 2004) y del Índico (Sudáfrica, Islas de San Pablo, Isla de

Ámsterdam) (Teske *et al*., 2007; Guerra *et al*., 2010), y sin embargo lo han diferenciado de especies de pulpo presentes en el Pacífico Este (Méjico, Perú, Chile), el *Octopus mimus* (Gould 1852) y el *Octopus maya* (Söller *et al*., 2000; Pérez Losada *et al*., 2002).

1.2.2. Anatomía

El sistema nervioso de los cefalópodos es el más grande y evolucionado de los moluscos, presentando rasgos comparables con los vertebrados (Hanlon y Messenger, 1996). Particularmente, el cerebro del pulpo común contiene 1-2 x 10^8 células nerviosas y se extiende por todo el cuerpo en masas de ganglios periféricos. Por ejemplo, en los brazos se estima que hay 3 x 10^8 células nerviosas que confieren a cada brazo un cierto grado de autonomía de movimiento (Nixon y Young, 2003). La capacidad de aprendizaje de esta especie ha sido demostrada mediante la reducción en el tiempo de solución de un mismo problema (Fiorito *et al*., 1990). De hecho, conclusiones recientes apuntan a que una parte importante del comportamiento individual del pulpo común viene condicionada por su experiencia (aprendizaje) individual (Boyle y Rodhouse, 2005). Entre los órganos de los sentidos destacan los mecanorreceptores, que dan información sobre orientación y los movimientos compensatorios de la cabeza, ojos y movimientos del cuerpo (Arkhipking y Bizikov, 2000); los quimiorreceptores, células ciliadas capaces de captar señales químicas, tanto por contacto directo como a distancia (olfato) (Boyle, 1986a); y los fotorreceptores, muy desarrollados en cefalópodos y uno de los rasgos más característicos del grupo. Los ojos tienen una estructura superficial similar a la de los vertebrados (Jagger y Sands, 1999), con cristalino que les confiere una gran agudeza visual. La visión en *O. vulgaris* ha sido estudiada en profundidad, constatando su capacidad para distinguir entre objetos según la forma, el brillo, la orientación vertical

u horizontal y el plano de polarización (Messenger, 1981). Sin embargo, se ha demostrado que la mayoría de las especies de cefalópodos son incapaces de distinguir colores (Hanlon y Messenger, 1996). Además, el pulpo *O. vulgaris* y los cefalópodos en general pueden adoptar una gran variedad de colores corporales, únicos dentro del reino animal, que le sirven a modo de camuflaje o señalización. La coloración corporal está mediada por la acción de los cromatóforos, células pigmentadas (negro, marrón, rojo, naranja, amarillo) activadas por acción muscular, en combinación con células reflectoras de luz (leucóforos e iridióforos) (Hanlon y Messenger, 1996; Boyle y Rodhouse, 2005).

El sistema circulatorio del pulpo es cerrado, y se basa en un corazón arterial y dos corazones branquiales (Smith y Boyle, 1983). Las branquias están bien vascularizadas dentro de la cavidad del manto. La sangre de los cefalópodos es de color azul debido a la presencia de hemocianina, pigmento respiratorio que contiene cobre es su estructura. El consumo de oxígeno en octópodos es de 10-100 ml O_2/kg h, alterado principalmente por la actividad (ataque, escape) o la digestión del alimento (Wells y Clarke, 1996). Bajo condiciones de cultivo a 17-20ºC el óptimo de saturación de oxígeno en *O. vulgaris* es de 65-100% (Cerezo Valverde y García García, 2005). El amonio es el principal producto de desecho del metabolismo del pulpo y la cantidad de amonio excretado depende de la temperatura, del tamaño del animal y de la dieta (Katsanevakis *et al*., 2005a,b; Mazón *et al*., 2007).

Octopus vulgaris es un una especie carnívora estricta y su sistema digestivo ha sido descrito en detalle por Boucaud-Camou y Boucher-Rodoni (1983). La masa bucal del pulpo común es una estructura grande y compleja. Se encuentra en posición ventral, en el centro de los brazos, y su función es trocear e ingerir alimento. Está

formada por el pico, la rádula y las glándulas salivares. El pico es quitinoso y está asociado a fuertes grupos musculares. La rádula es una cinta dentada que sirve para taladrar conchas de bivalvos o exoesqueleto de crustáceos. Las secreciones de las glándulas ayudan a inmovilizar a las presas (contienen cefalotoxinas) y provocan una digestión enzimática extracelular (proteasas, quitinasas) que facilita la extracción de los tejidos de las presas. El alimento troceado atraviesa el esófago y llega al estómago, donde tiene lugar la primera parte de la digestión. En ocasiones, cuando el estómago se llena, el alimento puede ser almacenado en el buche, hasta que la primera parte de la comida es digerida. La digestión en el estómago se produce mediante la acción mecánica del propio órgano y está catalizada por enzimas, segregadas por la glándula digestiva. Posteriormente, el jugo resultante pasa al ciego, donde la digestión continúa, y finalmente llega al intestino, donde se produce la absorción de los nutrientes. La fracción indigerible pasa desde el estómago directamente al intestino. Allí se unen con restos sólidos del ciego y productos excretados de la glándula digestiva, formando las heces (cordones mucosos) que son eliminadas a través del ano. La glándula digestiva tiene otras funciones relacionadas con la absorción y acumulación de nutrientes, mientras que el intestino y el ciego participan en la absorción y en la regulación iónica (Boucher-Rodoni *et al*., 1987).

1.2.3. Biología reproductiva

El pulpo común presenta un ciclo de vida corto, posiblemente inferior a 18-24 meses (Mangold, 1983; Guerra, 1992). La falta de un sistema de datación fiable impide verificar esta afirmación, aunque se cree que puede ser incluso más corto, de 8 a 15 meses (Smale y Buchan, 1981; Raya y Hernández-González, 1998; Domain *et al*., 2000; Hernández López *et al*., 2001; Boyle y Rodhouse, 2005; Katsanevakis y Verriopoulos,

2006). Es una especie semélpara, con una única reproducción que marca el final de su ciclo de vida. La proporción de machos y hembras en el medio natural es de 1:1 en distintas áreas geográficas del Atlántico y del Mar Mediterráneo (Quetglas *et al.,* 1998; Oosthuizen y Smale, 2003; Rodríguez de la Rúa *et al*., 2005; Otero *et al*., 2007; Jiménez Badillo *et al*., 2008). Sin embargo, Hernández García *et al.* (2002) encontraron un sex-ratio de machos-hembras de 1:0.5 en aguas de las Islas Canarias, posiblemente influenciados por la mayor susceptibilidad de los machos a ser capturados mediante artes de pesca de tipo "nasas" (Hernández López, 2000).

La maduración sexual en *Octopus vulgaris* se inhibe cuando la intensidad de luz o el fotoperiodo son elevados (Mangold, 1987), lo que sugiere que está regulada por la glándula óptica (Wells y Wells, 1975). La temperatura del medio es otro factor que afecta a la maduración sexual del pulpo, de modo que a mayor temperatura se acelera la maduración, posiblemente asociado a un tamaño determinado (Van Heukelem, 1976). La talla de maduración sexual en el medio natural, entendida como la talla en la que el 50% de los ejemplares son maduros, es mayor en hembras que en machos y varía ligeramente según la zona geográfica. Las hembras alcanzarían la madurez entre 1200-1788 g y los machos entre 700-1125 g en aguas litorales de Andalucía, Canarias, Galicia y Méjico (Mangold, 1983; Hernández García *et al.,* 2002; Rodríguez de la Rúa *et al*., 2005; Otero *et al*., 2007; Jiménez Badillo *et al*., 2008). La presencia de ejemplares maduros de *O. vulgaris* durante todo el año indica que el período de puesta de esta especie abarca todo el ciclo anual. Sin embargo, en la mayoría de las regiones se observan 2 picos de puesta, el principal en primavera y otro secundario en otoño (Hernández García *et al.,* 1998, 2002; Rodríguez de la Rúa *et al*., 2005; Katsanevakis y Verriopoulos, 2006; Jiménez Badillo *et al*., 2008). En Galicia no se observa el pico de

otoño, posiblemente asociado a la presencia del afloramiento costero en septiembre, que genera temperaturas de agua muy bajas (Otero *et al*., 2007).

El comportamiento reproductor se inicia con una migración de los individuos hacia aguas más someras (Guerra, 1992). La fecundación se caracteriza por la introducción de los espermatóforos por parte del macho a través del hectocótilo en la cavidad paleal de la hembra, donde permanece almacenado durante semanas o meses (Hanlon y Messenger, 1996). Las hembras realizan la puesta llevando a cabo la "autofecundación" de los huevos a la vez que producen los racimos, que son fijados en el interior de grietas u otro tipo de cavidades. Este proceso puede durar días o incluso semanas, en función del número de huevos y de la temperatura (Mangold, 1983). En el caso de tanques de cultivo dichos racimos se fijan a los tubos de PVC que sirven de guaridas (Iglesias *et al.,* 2000). Se estima que esta especie pone entre 42.000 y 790.000 huevos por hembra, con un tamaño medio de 2 mm (Fig. 1.7) (Mangold, 1983; Iglesias *et al.,* 2000; Oosthuizen y Smale, 2003).

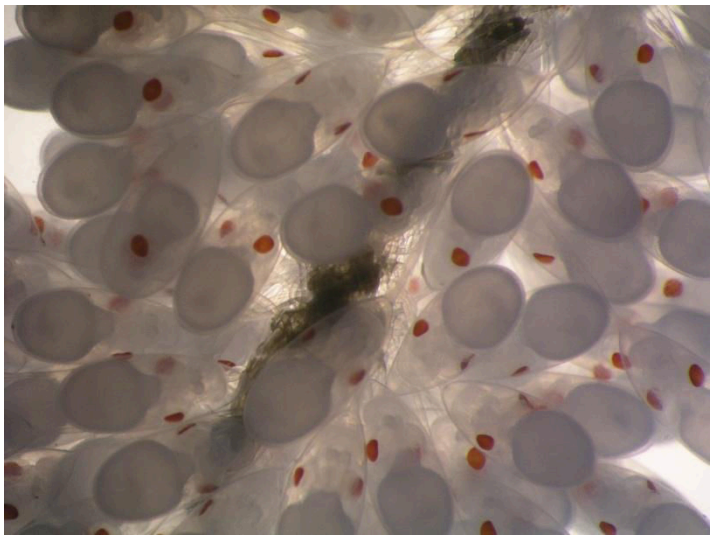


Fig. 1.7: Racimo de huevos fecundados de pulpo común en avanzado desarrollo embrionario.

Las hembras cuidan de los huevos solas, limpiando y oxigenando los racimos, protegiendo la puesta sin abandonar nunca la guarida (Fig. 1.8) (Mangold, 1987; Guerra, 1992). Dejan de alimentarse durante el proceso llegando a perder entre el 30 y el 65% de su peso corporal (Iglesias *et al.,* 2000; Chapela *et al*., 2006), y una vez que se produce la eclosión mueren (Guerra, 1992; Hernández García *et al.,* 2002). La duración del desarrollo embrionario varía con la temperatura, de 20 días a 25ºC hasta los 135 días a 13ºC (Guerra, 1992; Villanueva, 1995; Iglesias *et al.,* 2000), y las diferentes fases del desarrollo embrionario han sido descritas en profundidad por Naef (1928). Las larvas de pulpo se denominan "paralarvas", ya que no presentan metamorfosis (Young y Harman, 1988).

Fig. 1.8: Hembra de pulpo cuidando los racimo de huevos, fijados en una guarida de PVC bajo condiciones de cultivo. La variación de tonalidad de los racimos indica distintas fases del desarrollo embrionario.

El conocimiento sobre esta fase inicial del ciclo de vida de *O. vulgaris* proviene fundamentalmente de experiencias de laboratorio. En el momento de la eclosión, las paralarvas miden entre 3.0 y 4.8 mm de longitud total (Itami *et al*., 1963; Mangold,

1983; Seixas *et al*., 2010a), mostrando los brazos poco desarrollados (Fig. 1.9). Esta variabilidad en la talla de las paralarvas se debe a la temperatura de incubación y al tamaño de la hembra (Sakaguchi *et al*., 2002; Pecl *et al*., 2004; Forsythe, 2004). Su sistema nervioso es excepcionalmente grande en comparación con el resto del cuerpo (Nixon y Mangold, 1996).

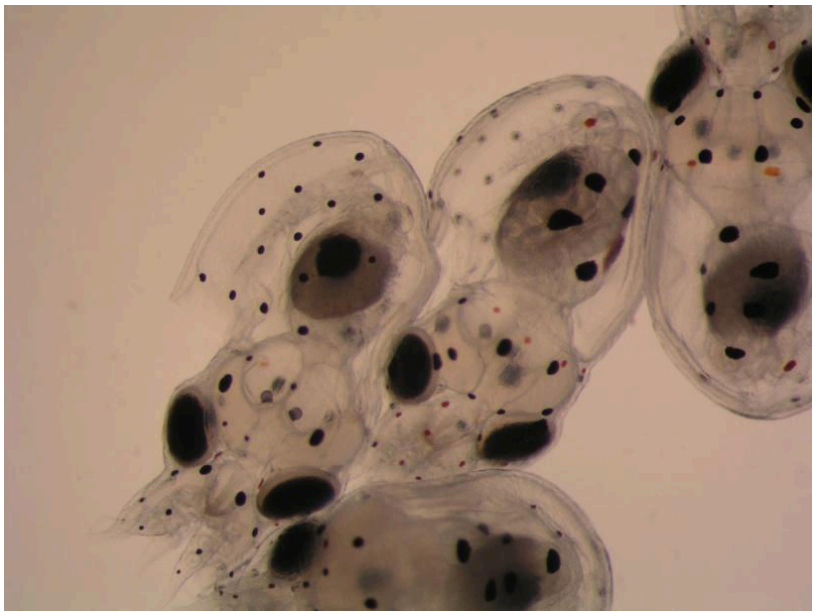


Fig. 1.9: Paralarvas de *O. vulgaris* recién eclosionadas, destacan los ojos, los cromatóforos y la glándula digestiva.

Tras la eclosión, las paralarvas de *O. vulgaris* pasan por una fase de vida planctónica, con movimiento a propulsión tipo jet, que puede durar entre 33 días y 3 meses en función de la temperatura (Itami *et al*., 1963; Villanueva, 1995; Mangold, 1997). Se produce un cambio morfológico gradual, con el desarrollo de los brazos, el aumento del número de ventosas y del número de cromatóforos en la zona dorsal (Nixon y Mangold, 1996; Villanueva *et al.,* 1996). La siguiente fase es el asentamiento en el fondo, iniciándose su vida bentónica. Según varios autores, al cambio a la vida bentónica es independiente de la edad o la temperatura, y sucede cuando los pulpos adquieren la talla crítica de 10-15 mm de longitud total del manto (aproximadamente

0.2 g de peso en peso húmedo) (Itami *et al*., 1963; Villanueva, 1995; Villanueva *et al*., 1996). Otros autores obtuvieron juveniles bentónicos de 0.05-0.1 g (peso húmedo) con una longitud total del manto de 6.5 mm (Iglesias *et al*., 2004; Carrasco *et al*., 2006). En esta fase, los juveniles presentan la misma morfología que los adultos, con brazos largos y una densa capa de cromatóforos.

1.2.4. Ecología de paralarvas y juveniles de *O. vulgaris*

A pesar de su tamaño, las paralarvas aparecen muy raramente en muestreos de plancton costero y, cuando lo hacen, son muy difícilmente identificables a nivel de especie (Rocha *et al*., 1999; González *et al*., 2005; Vidal *et al*., 2010). Por lo tanto, los datos asociados a su ecología son muy escasos. Datos recientes sugieren que las paralarvas de *O. vulgaris* en el medio natural se encuentran en superficie durante la noche y en profundidad durante el día (Otero, 2006). Se sabe que las hembras fijan los huevos en zonas someras, y es en esta franja donde presumiblemente tiene lugar la fase plantónica y la etapa juvenil del pulpo común. Por lo tanto se encuentran en una zona rica en fitoplancton y zooplancton, con una gran variedad de presas (Villanueva y Norman, 2008). Durante los primeros días de la fase plantónica la energía proviene de reservas endógenas y de la digestión de presas capturadas (Boletzky, 2003). Teniendo en cuenta que las paralarvas no ingieren las presas enteras (Hernández García *et al*., 2000), el análisis de contenidos estomacales para obtener información sobre sus presas potenciales es de poca utilidad. Tras el cambio a la vida bentónica, la dieta cambia a crustáceos bentónicos y moluscos (Villanueva, 1994; Boyle y Rodhouse, 2005). Sin embargo, la ecología de los juveniles al inicio de su vida bentónica es poco conocida, debido a que su pequeño tamaño y su gran capacidad de camuflarse los vuelve prácticamente invisibles. De hecho, es probablemente su capacidad de no ser

vistos lo que aumenta las probabilidades de los juveniles bentónicos de *O. vulgaris* de llegar a la fase adulta (Hanlon y Messenger, 1996).

1.2.5. Ecología de sub-adultos

Como ya hemos mencionado, el pulpo es un animal solitario y únicamente se agrupan en períodos reproductivos. Es un depredador visual, muy versátil y oportunista (Guerra, 1978). La secuencia de ataque, descrita bajo condiciones de laboratorio, tiene tres fases: levanta la cabeza, se acerca lentamente a la vez que cambia de color y finalmente se abalanza sobre la presa utilizando su sistema de propulsión a chorro (Mather, 1993). Parece ser que tanto el movimiento de la presa (Wells, 1962) como su aspecto (Boyle, 1986b) provocan la reacción de ataque en el pulpo. Las referencias de pulpos alimentándose *in situ* en el medio natural son escasas, por lo que la mayoría de la información deriva del análisis de los contenidos estomacales, complementada por datos de restos de presas alrededor de las guaridas (Nixon, 1987). Algunos autores encontraron un predominio de bivalvos en la dieta de pulpo (Smale y Buchan, 1981; Ambrose y Nelson, 1983), mientras que otros autores sugirieron que los crustáceos son los mayoritarios, representando el 65-80% de la dieta natural de pulpos (Guerra, 1978; Quetglas *et al*., 1998; Smith, 2003). Ejemplares capturados en las Islas Canarias presentaron una gran variabilidad de restos en el estómago, con un predominio de peces (hasta un 53%), seguido de crustáceos (10-24%) y por último otros cefalópodos-canibalismo (3-29%) (Hernández-López, 2000). Se encontraron diferencias en la cantidad de bivalvos (1-16%) y peces (15-53%) en el contenido estomacal de los octópodos en 3 puntos de la costa portuguesa (Rosa *et al*., 2004). Estos datos ponen de manifiesto que el pulpo común *O. vulgaris* se alimenta

mayoritariamente de las presas disponibles en su entorno, las cuales a su vez dependen de la características oceanográficas y geomorfológicas del hábitat (Boyle, 1990; Hanlon y Messenger, 1996).

1.2.6. Crecimiento en *O. vulgaris*

Para describir el crecimiento en cefalópodos se han usado medidas de longitud (total o del manto) y de peso, tradicionalmente asociados a estudios de campo y de laboratorio, respectivamente. Sin embargo, el peso es una medida más sensible y dinámica que la longitud. Los cefalópodos muestran una fase de crecimiento inicial exponencial y una segunda fase logarítmica (Forsythe y Van Heukelem, 1987; Semmens *et al*., 2004; Boyle y Rodhouse, 2005). En *O. vulgaris*, los datos de crecimiento en las primeras etapas de vida son escasos y fueron obtenidos en experiencias de laboratorio. Así, la tasa de crecimiento estándar desde la eclosión hasta el asentamiento en el fondo es de 7-10% diario (Itami *et al*., 1963; Villanueva, 1995; Iglesias *et al*., 2004; Carrasco *et al*., 2006). La fase exponencial se alarga hasta los 100-150 g, punto en el cual se inicia la fase logarítmica, con valores iniciales de crecimiento estándar superiores al 5% diario que van disminuyendo progresivamente hasta el 1% hacia el fin del ciclo su vida (Mangold, 1987; Hernandez López, 2000; Iglesias *et al*., 2004). La gran variabilidad en el crecimiento de *O. vulgaris* parece ser una característica inherente a la especie, observada bajo estrictas condiciones de laboratorio (Wells *et al*., 1983; O'Dor *et al*., 1983). Sin embargo, son varios los factores que explican la gran variabilidad en el crecimiento entre diferentes áreas geográficas, tanto en el medio natural como en condiciones de laboratorio (Semmens *et al*., 2004): la alimentación es el factor que más decisivamente afecta al crecimiento en esta especie, tanto en fase larvaria como juvenil y adulta (Villanueva, 1994, 1995; Iglesias *et*

al., 2000). Este hecho explica el mayor crecimiento observado bajo condiciones de laboratorio, donde los ejemplares son generalmente alimentados a saciedad (Katsanevakis y Verriopoulos, 2006). En Canarias en condiciones naturales el crecimiento de subadultos es de 200-250 g/mes (Hernández López, 2000), muy por debajo de los 750-1000 g/mes observados bajo condiciones de cultivo en jaulas flotantes (Socorro *et al*., 2005). El siguiente factor que más afecta al crecimiento de *O. vulgaris* es la temperatura. En general, a mayor temperatura mayor crecimiento, lo cual puede condicionar el crecimiento de las paralarvas y subadultos en función de la época del año en que se produce la eclosión (Forsythe, 1993; Villanueva, 1995; Leporati *et al*., 2007). Además, los ejemplares más jóvenes presentan un crecimiento más acelerado, por lo que varios autores han indicado que el peso o la talla podrían no ser indicadores fiables de la edad de los pulpos, muy influenciada por la tasa de alimentación y la temperatura (Mangold y Boletzky, 1973; Smale y Buchan, 1981).

1.3. El cultivo de pulpo

En general el pulpo común se adapta fácilmente a condiciones de cultivo y acepta una gran variedad de alimentos, tanto frescos como congelados (Mangold y Boletzky, 1973; Iglesias *et al*., 2000). Para su mantenimiento en tanques tan sólo es necesario mantener una buena calidad de agua, ya sea en circuito cerrado o abierto, de modo que el nivel de oxígeno no baje del 60-80% de saturación (Cerezo Valverde *et al*., 2005). Como adaptación al estilo de vida del pulpo, se incluyen guaridas en los tanques, generalmente tubos de PVC (Villanueva, 1995; Hanlon y Messenger, 1996). Bajo condiciones de cultivo los pulpos presentan un marcado comportamiento jerárquico y territorialista, con habituales disputas ya sea por el alimento, por las

mejores guaridas o por fecundar a las hembras, que frecuentemente deriva en agresiones, mutilaciones y cierto grado de mortalidad (García García *et al*., 2009; Domingues *et al*., 2010). La dosis diaria de alimentación, generalmente proporcionada en una sola toma, es del 5-10% de la biomasa/día (Aguado Giménez y García García, 2002; Socorro *et al*., 2005).

1.3.1. Engorde artesanal del pulpo en Galicia (España)

El gran potencial del cultivo del pulpo ha llevado a un grupo de empresas en Galicia a ser pioneras en el desarrollo de esta actividad. El sistema de producción se basa en la captura de ejemplares salvajes de 750-1000 g, para su posterior engorde en jaulas flotantes (Fig. 1.10). Estas jaulas suelen tener 10 m^3 y en torno a 200 guaridas (generalmente "T" de PVC de 160 mm de diámetro). Están compuestas de un armazón de acero recubierto de malla metálica de 2 cm de luz.

Fig. 1.10: Jaula flotantes de 10 m^3 (3 x 3 x 1.5 m), utilizada para el engorde de pulpos en el muelle de Taliarte (Telde).

Como dieta de engorde se utilizan distintas especies, todos ellos descartes de la pesca de bajura: cangrejos ("cangrejo atlántico o verde", *Carcinus maenas*, L. 1758*;*

"patexo", *Polybius henslowi*, Leach 1820), mejillones (*Mytilus sp*.), cefalópodos *(*"pota voladora" *Illex coindetii*, Verany 1839*)* o peces ("jurel", *Trachurus trachurus*, L. 1758; "bacaladilla", *Micromesistius poutassou*, Risso 1826; "sardina europea", *Sardina pilchardus*, Walbaum 1792*;* "boga", *Boops boops*, L. 1758; "mugil o lisa", *Mugil cephalus*, L. 1758; "caballa del Atlántico", *Scomber scombrus*, L. 1758) (Rama-Villar *et al.*,,,, 1997; Luaces Canosa y Rey Méndez, 1999; Tuñón *et al*., 2001, 2002).

Bajo estas condiciones, la producción de pulpo es de tipo artesanal, y ha sido relativamente baja e inestable a lo largo de los últimos años (Fig. 1.11). Estudios de analítica de costes indican que se trata de un negocio de rentabilidad baja y de alto riesgo (García García *et al*., 2004a, 2004b, 2010; García García y García García, 2011). Estos autores calcularon un precio mínimo de venta del pulpo, en función del nº de ciclos anuales, de 5.80-6.61 €/kg. El precio máximo de adquisición de juveniles sería de 2.22-4.58 €/kg y la producción mínima para su rentabilidad de 39-116 toneladas anuales.

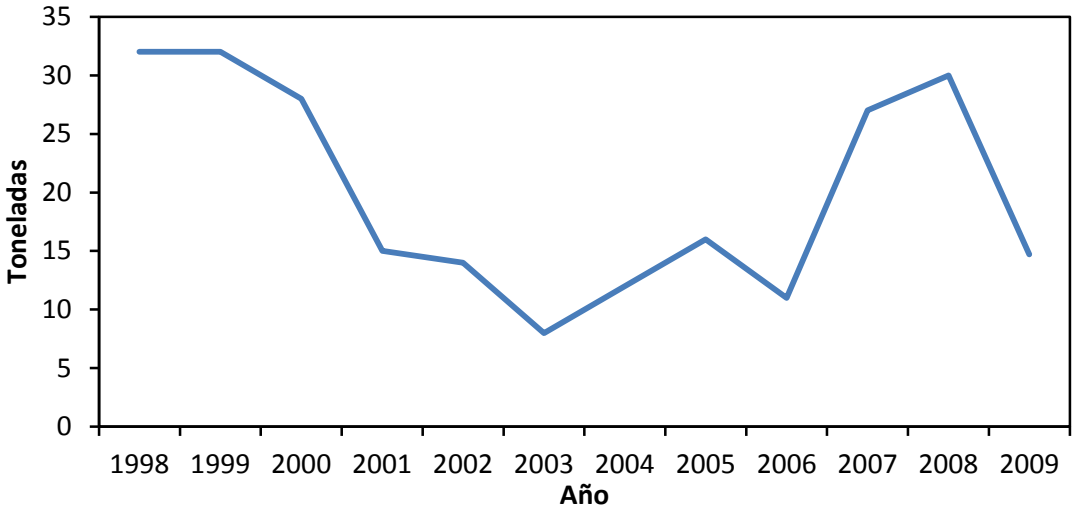


Fig. 1.11: Producción de la acuicultura de pulpo en España desde 1998-2009 (FAO, 2011)

1.3.2. Factores que afectan al éxito de la fase de engorde en *O. vulgaris*

Para mejorar la rentabilidad del engorde de pulpo una parte del sector científico se ha centrado en optimizar las condiciones de engorde, mediante experiencias en tanques y en jaulas flotantes. En general, los ejemplares jóvenes, con talla inicial inferior a 1 kg, presentan un crecimiento elevado (0.5-1 kg/mes) y altas tasas de supervivencia bajo condiciones de cultivo (70-90%), en función de varios factores:

a) Dieta: los crustáceos (especialmente cangrejo), como base o complemento de la dieta, generan mayores crecimientos en esta especie en comparación con una dieta basada únicamente en peces (Cagnetta y Sublimi, 1999; Aguado Giménez y García García, 2002; Tuñón *et al*., 2002; García García y Cerezo Valverde, 2006). Sin embargo, estudios recientes obtuvieron crecimientos similares en pulpo alimentados con crustáceos, peces o una combinación de ambos (Biandolino *et al*., 2010; Prato *et al*., 2010). Por otro lado, el uso de calamar como dieta control de pulpo común también ha generado buenos resultados de crecimiento (Miliou *et al*., 2005, 2006; Quintana *et al*., 2008; Domingues *et al*., 2010; García Garrido *et al*., 2011). Sin embargo, tanto el cangrejo como el calamar son costosos y poco abundantes en algunas zonas (García García *et al*., 2004b, García García y García García, 2011), por lo que su uso sólo es aplicable a escala experimental. Por este motivo, y dentro del desarrollo del cultivo del pulpo, se han ensayado diferentes dietas frescas alternativas para su engorde. Una dieta alternativa debe ser barata, abundante y no tener interés para el consumo humano. Dentro de este grupo destacan los descartes de las granjas marinas. Las jaulas off-shore atraen un gran número de especies de peces (Dempster

et al., 2002). Al final de cada ciclo de engorde, varias de estas especies (*Boops boops*, *Sardina spp.*, *Sarpa salpa* o *Mugil cephalus*) aparecen frecuentemente dentro de las jaulas, debiendo ser descartadas tras el despesque final. Estas especies tienen tallas muy variables, de entre 20 y 600 g, pudiendo entrar en la jaula a través de la luz de malla en la fase juvenil o bien a través de agujeros accidentales en la red, engordando junto con la especie objetivo (dorada, lubina) durante meses. La boga *Boops boops* es la más abundante entre los descartes, representando en ocasiones el 5% de la producción total de jaulas de dorada en granjas marinas del Mediterráneo y el Atlántico oriental. Ensayos de engorde en jaulas flotantes donde los pulpos fueron alimentados exclusivamente con boga de descarte dieron resultados prometedores (Socorro *et al*., 2005).

b) Comportamiento: los sistemas de cultivo individual minimizan la mortalidad (Miliou *et al*., 2005; García García y Cerezo Valverde, 2006; Biandolino *et al*., 2010; García Garrido *et al*., 2011; Prato *et al*., 2010), incluso en inanición (García Garrido *et al*., 2010). Debido al comportamiento agresivo jerárquico-territorialista de esta especie en el cultivo grupal, los estudios de nutrición en sistemas de cultivo individual minimizan la interacción entre los ejemplares, reduciendo el "factor comportamiento" durante el cultivo.

c) Densidad: existe una cierta disparidad en los resultados en cuanto a la densidad inicial más adecuada para el cultivo de *O. vulgaris*, en función de la zona geográfica y el sistema de cultivo. En tanques se ha observado una densidad óptima de 4 kg/m^3 en Huelva (Domingues *et al*., 2010), mientras que en Galicia ascendería a 10 kg/m^3 (Iglesias *et al*., 2000). En pulpos cultivados en jaulas, algunos autores observaron un mayor crecimiento cuando la densidad inicial es de 10 kg/m^3 (Rodríguez *et al*.,

2006), mientras que otros autores no observaron un efecto de la densidad inicial sobre el incremento de biomasa (Iglesias *et al*., 2007b).

d) Dispersión de talla: el comportamiento jerárquico y territorialista del pulpo común bajo condiciones de cultivo, que deriva ocasionalmente en canibalismo, se ve potenciado por la dispersión de talla inicial (Otero *et al*., 2001; Socorro *et al*., 2005; Rodríguez *et al*., 2006). En cualquier caso, el incremento de la dispersión de tallas a lo largo del ciclo de engorde es algo habitual, independientemente de la dosis de alimento, por lo que se recomienda partir de tallas homogéneas (dispersión menor al 20%) y realizar clasificaciones periódicas (García García *et al*., 2009).

e) Salinidad: la salinidad es un factor importante a considerar, dado que se ha observado una mortalidad del 100% en jaulas flotantes en las Rías Gallegas como consecuencia de un descenso de la salinidad superficial, provocado por fenómenos de escorrentía superficial tras lluvias copiosas (Chapela *et al*., 2006).

f) Temperatura: en subadultos el rango de temperatura que maximiza el crecimiento, determinado bajo condiciones de cultivo, es de 18-21ºC (Aguado Giménez y García García, 2002). Posteriormente Miliou *et al*. (2005) completó estos resultados, observando que los juveniles (50-150 g) necesitan una temperatura de cultivo un poco más elevada (25ºC) en comparación a tallas más grandes (15-20ºC), probablemente reflejando sus hábitats naturales. Estos resultados son similares a los obtenidos con otras especies de octópodos (André *et al*., 2008).

g) Hidrodinamismo: el sistema de cultivo en jaulas flotantes en zonas expuestas, afectadas por el oleaje, tiene consecuencias negativos sobre el crecimiento y mortalidad del pulpo, posiblemente asociado a un aumento del stress bajo

condiciones de cultivo o dificultando la captura del alimento (García García *et al*., 2009).

h) Maduración sexual: los machos y hembras de *O. vulgaris* presentan un crecimiento similar hasta la maduración sexual. En este punto las hembras realizan una mayor inversión de la energía ingerida en el desarrollo gonadal, hecho que no se aprecia en los machos (Forsythe y Van Heukelem, 1987). En general, bajo condiciones de cultivo los machos presentan generalmente un crecimiento superior a las hembras (Iglesias *et al*., 2000; Rey Méndez *et al*., 2003), aunque en ocasiones no se obtuvieron diferencias estadísticas (Aguado Giménez y García García, 2002; Chapela *et al*., 2006). La mortalidad en ambos sexos está ligada al ciclo reproductivo (Guerra, 1992; Hernández García *et al*., 2002). Sin embargo, se han obtenido resultados positivos en ciclos de engorde en jaulas con machos y hembras (Socorro *et al*., 2005; Rodríguez *et al*., 2006; García García *et al*., 2009), por lo que el beneficio de la separación de sexos bajo condiciones de cultivo todavía debe ser evaluado.

1.3.3. Tasa de ingesta e índice de conversión bajo condiciones de cultivo

La tasa de ingesta depende de la temperatura y del tipo de alimento disponible. En general aumenta con la temperatura hasta los 20-23ºC y es superior en pulpos alimentados con cangrejos y calamar (6-8% de su peso/día) que con peces o mejillones (2-4% de su peso/día) (Aguado Giménez y García García, 2002; García García y Cerezo Valverde, 2006; Biandolino *et al*., 2010; Domingues *et al*., 2010; Prato *et al*., 2010). Estos autores observaron una tasa de conversión del alimento elevada, superiores en ejemplares alimentados con peces y calamar (40-50%) que con crustáceos (18-30%).

1.3.4. Principales limitaciones del cultivo del pulpo a escala industrial

Los dos aspectos del cultivo del pulpo que más urgentemente deben optimizarse de cara a la implantación industrial de esta actividad son la baja supervivencia durante la fase larvaria y la falta de una dieta inerte formulada específica para esta especie.

A pesar de la gran facilidad de obtención de puestas en cautividad, la baja supervivencia de las paralarvas durante la fase planctónica es el principal cuello de botella de cara al desarrollo del cultivo industrial de esta especie, debido a que la artemia y los sistema de enriquecimiento tradicionales no son adecuados para el cultivo de paralarvas (Iglesias *et al*., 2007a). Varios grupos de investigación han obtenido ocasionalmente juveniles bentónicos, utilizando zoeas de diferentes especies de crustáceos como complemento de la dieta (Itami *et al*., 1963; Imamura, 1990; Villanueva, 1994, 1995; Iglesias *et al*., 2004; Carrasco *et al*., 2006). La gran dificultad de obtención de zoeas de crustáceos limita su uso a escala experimental. Sin embargo, estos resultados han permitido identificar el factor nutricional como el más determinante de cara al éxito del cultivo de paralarvas (Iglesias *et al*., 2007a).

La otra gran limitación al desarrollo industrial del cultivo del pulpo es la falta de una dieta inerte formulada que cubra los requerimientos nutricionales de esta especie, maximizando el crecimiento. Además, un pienso tiene otras grandes ventajas, como son la regularidad del suministro y de la composición, un fácil almacenamiento y conservación, la reducción del riesgo de transmisión de enfermedades y un menor impacto ambiental de las granjas (O'Dor y Wells, 1987; Lee, 1994). Estudios sobre costes de producción indican que el uso de dietas preparadas en substitución de dietas vivas o naturales pueden reducir los costes en un 40-80% (Hanlon *et al*., 1991). De

hecho, la falta de este tipo de piensos limita el desarrollo industrial de otras especies de cefalópodos con desarrollo embrionario directo, como la *Sepia officinalis* o el *Octopus maya* (Domingues *et al*., 2005, 2007b, 2008; Rosas *et al*., 2007, 2008).

1.3.5. Experiencias previas con piensos experimentales en cefalópodos

Los primeros piensos experimentales fueron rechazados por *Octopus bimaculoides* (Pickford y McConnaughey, 1949) y *S. officinalis* (Castro, 1991; Lee *et al*., 1991). Nuevamente con *S. officinalis* se obtuvo una buena aceptación de un pienso experimental y se observó un crecimiento positivo por primera vez (Castro *et al*., 1993; Castro y Lee, 1994). Sin embargo, en ambos casos el crecimiento fue bajo en relación al obtenido habitualmente con dietas naturales, lo cual fue atribuido a una menor ingesta (Domingues *et al*., 2005, 2008). El sector científico está de acuerdo en que tanto la textura como la palatabilidad son dos características muy importantes en un pienso para pulpo. La textura debe ser gomosa, firme y cohesiva, para adaptarse al modo de alimentación del pulpo, que manipula y trocea el alimento antes de ingerirlo. De hecho, los piensos granulados, extrusionados y semihúmedos tal y como se elaboran para peces no son utilizados para el pulpo, ya que la manipulación del alimento previo a la ingesta provoca una elevada disgregación (García García y Cerezo Valverde, 2006).

Recientemente se han obtenido resultados prometedores con dietas experimentales en dos especies de pulpo, *O. maya* y *O. vulgaris* (Estefanell *et* al., 2007b, 2011a; Rosas *et al*., 2008; Cerezo Valverde *et al*., 2008; Quintana *et al*., 2008). En general estos autores utilizaron la fracción comestible de las dietas húmedas comúnmente empleadas en el laboratorio (boga, cangrejo, calamar, langostinos), aglutinadas con distintos componentes (gelatina, goma guar, alginato y calcio),

obteniendo en todos los casos resultados de crecimiento positivos. En general los resultados de crecimiento fueron inferiores a los observados con las dietas naturales, lo que sugiere una disminución de la digestibilidad o palatabilidad de la dieta. Sin embargo, algunos autores incluso observaron un crecimiento similar al generado por la dieta natural, de 1.4-1.9%/día (Quintana *et al*., 2008; Rosas *et al*., 2008). Por otro lado, el uso de materias primas termo-tratadas como base o complemento de la dieta parece afectar negativamente a la palatabilidad del pienso en ambas especies de pulpo (Aguila *et al*., 2007; Domingues *et al*., 2007b, 2008; Rosas *et al*., 2007; García Garrido et al., 2011).

Estos resultados plantean la necesidad de buscar nuevos sistemas de procesado y atractantes que aseguren una elevada ingesta del pienso. En todo caso, la elaboración de estos piensos húmedos de composición nutricional conocida permitirá avanzar en el conocimiento de los requerimientos nutricionales de los cefalópodos.

1.3.6. Herramientas para la investigación: anestesiado y marcaje individual

Para facilitar el manejo, varias sustancias anestésicas han sido probadas en *O. vulgaris* con resultados satisfactorios: agua fría a 3-6ºC (Fuentes, 2004), inmersión en agua de mar con uretano, etanol o cloruro de magnesio (Messenger, 1968, Andrews y Tansey, 1981; O'Dor *et al*., 1984; Messenger *et al*., 1985). Más recientemente se evaluó el efecto del aceite de clavo sobre *Octopus minor* (Sasaki 1920), con resultados positivos tras varios minutos de inmersión (Seol *et al*., 2007).

El marcaje individual es una herramienta de gran utilidad para obtener información sobre parámetros biológicos bajo condiciones de cultivo, como el crecimiento, la longevidad o la mortalidad asociada a una talla o a un sexo determinado. Un sistema de marcaje adecuado debe ser barato y fácil de aplicar,

presentar una alta tasa de retención y no influir negativamente en el crecimiento o la mortalidad de los pulpos. Entre los sistemas de marcaje más comúnmente empleados en *O. vulgaris* destaca las marcas externas de tipo visible, empleadas habitualmente en estudios de ecología y pesquerías, que sin embargo causan un cierto daño a los ejemplares y las tasas de retención no superan el 50% (Watanuki e Iwashita, 1993; Nagasawa *et al*., 1993; Domain *et al*., 2000, 2002). Las marcas externas no visibles (radio transmisor) presentan altas tasas de retención pero son muy costosas (Mather *et al*., 1985), por lo que su utilización es poco habitual. Otro tipo de marcas diseñadas para reconocer cohortes y no ejemplares individualizados son el "visual implant elastomer" o VIE, testado con éxito en calamares (Zeeh y Wood, 2008), y el marcaje de tipo químico, diseñado específicamente para paralarvas (Fuentes *et al*., 2000). Un sistema de marcaje interno no visual es el "passive integrated transponder" o PIT, muy sencillo de utilizar y barato. Ha sido utilizado con éxito en dos especies de octópodos (Anderson y Babcock, 1999). Rey Méndez *et al*. (2003) utilizó PIT en *O. vulgaris* para estudiar el comportamiento de los ejemplares bajo condiciones de cultivo.

1.4. Aproximación a los requerimientos nutricionales en cefalópodos

En análisis bioquímico de los tejidos corporales y de las presas naturales más habituales, así como estudios de digestibilidad e inanición, han permitido realizar una primera aproximación a los requerimientos nutricionales de las tres especies de cefalópodos bentónicos con mayor interés para el cultivo: *O. vulgaris*, *O. maya* y *S. officinalis*.

1.4.1. Proteínas

En general se trata de especies carnívoras estrictas, y su composición corporal y la de sus presas naturales más frecuentes (crustáceos) presentan un elevado contenido de proteínas, superior al 70% en peso seco (O'Dor y Wells, 1987; Lee, 1994; Domingues, 1999; Villanueva *et al*., 2004; García García y Cerezo Valverde, 2006; Prato *et al*., 2010). Estos datos reflejan fundamentalmente la composición del músculo de cefalópodos, que representa el 90-95% del cuerpo, y contiene más de un 80% de proteínas en peso seco (Rosa *et al*., 2004; Zlatanos *et al*., 2006; Cerezo Valverde *et al*., 2008). Además, las proteínas presentan una digestibilidad muy alta (O'Dor *et al*., 1984; Lee, 1994; Mazón *et al*., 2007; Rosas *et* al., 2007; Sanchez *et al*., 2009; Seiça Neves *et al*., 2010), gracias a una eficaz maquinaria enzimática para su digestión (Boucher-Rodoni, 1982; Caruso *et al*., 2004; Hamdan *et al*., 2007), ya observada desde la fase de paralarva en octópodos (Villanueva *et al*., 2002; Solorzano *et* al., 2009). Estos datos sugieren que el metabolismo de los cefalópodos, particularmente del género *Octopus*, es fundamentalmente proteico (O'Dor y Wells, 1987) y el requerimiento en aminoácidos para la producción de las mismas es muy alto (Houlihan *et al*., 1990). Además, la pérdida de peso en períodos de inanición está asociada mayoritariamente al catabolismo de las proteínas del músculo (O'Dor *et al*., 1984; Villanueva y Norman, 2008; George-Zamora *et al*., 2011).

En general, los aminoácidos libres predominantes en los tejidos de cefalópodos bentónicos son la prolina, la arginina y la octopina; formando parte de las proteínas son la leucina, la lisina y la arginina como esenciales, y entre los no esenciales el glutamato y el aspartato (Domingues, 1999; Rosa *et al*., 2002, 2004; Villanueva *et al.,* 2004; Aguila *et al*., 2007; Cerezo Valverde *et al*., 2009). Sin embargo, estudios de

inanición han demostrado la utilización de treonina, alanina, serina y glutamato en *O. maya* o prolina y alanina en *S. officinalis*, lo que sugiere el uso de estos aminoácidos como fuente de energía (Domingues, 1999; George-Zamora *et al*., 2011). Por último, la maduración gonadal es otro factor que parece afectar a los requerimientos de proteínas, ya que se ha observado un aumento de proteína en el ovario en pulpos maduros, que afecta especialmente al perfil de aminoácidos esenciales, proporcionados directamente a través de la dieta (Rosa *et al*., 2004).

1.4.2. Lípidos

En comparación con las proteínas, los lípidos representan una fracción minoritaria en los cefalópodos, del 5-10% en peso seco (Lee, 1994; Navarro y Villanueva, 2000; García García y Cerezo Valverde, 2006; Zlatanos *et al*., 2006). Su importancia relativa es mayor en la fase de paralarva (10-12% en peso seco), y se reduce progresivamente al aumentar de tamaño en la fase adulta (4-5% en peso seco) (Navarro y Villanueva, 2003). Esto sugiere que posiblemente los lípidos son componentes mayoritarios de los sistemas nervioso y visual, excepcionalmente desarrollados en las paralarvas de cefalópodos. Los primeros estudios sobre metabolismo en cefalópodos atribuyeron una digestibilidad baja a los lípidos (Ballantyne *et* al., 1981; Mommsen y Hochachka, 1981), que se asimilan lenta e ineficientemente en *O. vulgaris* (O'Dor *et al*., 1984). Por estos motivos, la visión tradicional sobre nutrición de cefalópodos considera que los requerimientos de lípidos son bajos. Sin embargo, estudios más recientes encontraron una gran variabilidad en la digestibilidad lipídica (75-97%) en *S. officinalis* y *O. vulgaris*, en función del tipo de lípido y la composición nutricional de la dieta, de modo que en general la digestibilidad de los lípidos es más baja en dietas ricas en grasa (Lee, 1994; Mazón *et al*., 2007;

Sánchez *et al*., 2009; Seiça Neves *et al*., 2010). A pesar de ello, en estudios recientes se ha observado un crecimiento elevado en pulpos alimentados con dietas de alto contenido lipídico (*Boops boops*), incluso en comparación ejemplares alimentados con dietas mixtas que incluyeron crustáceos (Biandolino *et al*., 2010; Prato *et al*., 2010). Estos autores utilizaron un sistema de cultivo en tanques compartimentados y sus resultados contrastan con los obtenidos por otros autores utilizando dietas similares (peces de alto contenido lipídico: *Boop boops*, *Sardina pilchardus*, *Engraulis encrasicolus*) en pulpos mantenidos individualmente en volúmenes más pequeños (García García y Aguado Giménez, 2002; Petza *et al*., 2006), lo que sugiere que el sistema de cultivo podría afectar a la utilización de los nutrientes de la dieta.

En cualquier caso, la utilización de los lípidos ha sido confirmada por la existencia de actividad lipasa a lo largo del sistema digestivo de *O. vulgaris*, especialmente en la glándula digestiva (Caruso et al., 2004; Hamdan et al., 2007). Este es además el único órgano en los cefalópodos que presenta un elevado contenido lipídico (30-50% en peso seco) (Phillips *et al*., 2001; Rosa *et al*., 2004, 2005; Moltschanlwskyj y Johnston, 2006; Sieiro *et al*., 2006; Cerezo Valverde *et al*., 2008). Recientemente, la drástica reducción del contenido lipídico en la glándula digestiva en *O. vulgaris* mantenidos en inanición demuestra el uso de este órgano como reserva de energía (García Garrido *et al*., 2010). El uso de lípidos como fuente de energía también ha sido observado en otras especies de cefalópodos (Castro *et al*., 1992; Semmens, 1998; Moltschaniwskyj y Johnston, 2006).

En general, la fracción lipídica del pulpo común, al igual que en otros cefalópodos, es rica en fosfolípidos y colesterol. Dentro de los ácidos grasos, los mayoritarios son el ácido palmítico (16:0), ARA (20:4n-6), EPA (20:5n-3) y DHA (22:6n-

3) (Koueta *et al*., 2002; Navarro y Villanueva, 2003; Rosa *et al*., 2005; Almansa *et al*., 2006; Zlatanos *et al*., 2006; Miliou *et al*., 2007; Estefanell *et* al., 2008; Prato *et al*., 2010; Cerezo Valverde *et al*., 2011). Sin embargo, existen marcadas diferencias en *O. vulgaris* entre los diferentes tejidos, tanto a nivel cuantitativo como cualitativo. Así, el músculo de brazos y manto muestra un perfil similar, caracterizado por un bajo contenido lipídico rico en fosfolípidos, particularmente fosfatidil-serina, fosfatidil-inositol y fosfatidil-etanolamina, y colesterol, con predominio de ácidos grasos saturados (32-33%), particularmente 16:0 (18-20%), y n-3 y n-6 HUFA (55-60%), especialmente ARA (3-4%), EPA (19-21%) y DHA (29-33%); la glándula digestiva presenta un elevado contenido lipídico, muy abundante en triglicéridos y ácidos grasos de tipo monoenos (23-25%), especialmente el ácido oleico (18:1n-9) (8-10%), y también n-3 y n-6 HUFA (45-50%), con predominio de ARA (4-6%), EPA (14-16%) y DHA (25-30%); el ovario presenta valores intermedios de lípidos, con predominio de fosfolípidos y ácidos grasos n-3 y n-6 HUFA (50-55%), particularmente ARA (5-9%), EPA (13-16%) y DHA (25-30%); el testículo en general tiene un bajo contenido lipídico, similar al músculo, sin embargo destaca el alto contenido en ARA (14%), EPA (13-14%) y DHA (18-20%) (Sieiro *et* al., 2006; Rosa *et al*., 2004; García Garrido *et al*., 2010; Cerezo Valverde *et al*., 2011).

El estado de maduración sexual es un factor que afecta al contenido lipídico en tejidos de octópodos, de modo que éste aumenta tanto en gónada como en glándula digestiva en ejemplares maduros con independencia del sexo (Rosa *et al*., 2002, 2004). Asimismo, el perfil de ácidos grasos también se ve alterado, afectando de diferente forma a machos y hembras. En músculo de hembras maduras se aprecia un aumento en monoenos y n-3 HUFA, particularmente el DHA, mientras que los machos presentan

un perfil más estable. En la glándula digestiva se aprecia un aumento en monoenos en ambos sexos con la madurez sexual, mientras que sólo las hembras aumentan sus reservas de ácidos grasos de tipo HUFA, llegando a duplicar los contenidos de ARA, EPA y DHA en este órgano. En las gónadas se aprecia un aumento general en los HUFA totales, especialmente drástico en el ovario, que duplica los valores de los ácidos grasos principales (ARA, EPA, DHA). Además, el contenido de colesterol aumenta con la madurez en músculo y ovario de hembras mientras que disminuye en la glándula digestiva y músculo de machos (Rosa *et al*., 2004).

Recientemente, García Garrido *et al*. (2010) evaluaron el uso de la fracción lipídica en músculo y glándula digestiva en *O. vulgaris* mantenido en inanición. Los lípidos totales en músculo se mantuvieron constantes durante inanición prolongada (21 días), reduciendo *in extremis* la fracción neutra (triglicéridos, esterol ésters) y sin efecto sobre ningún ácido graso. En cuanto a la glándula digestiva, su peso se redujo progresivamente hasta un 85% al final del período de estudio, afectando fundamentalmente a la fracción lipídica, particularmente al contenido de triglicéridos y esterol ésters. Es de destacar la disminución de monoenos, 16:0, EPA y DHA y la retención selectiva de ARA al final de la experiencia, hecho que sugiere la esencialidad de este ácido graso en *O. vulgaris*.

1.4.3. Minerales

El contenido mineral en los octópodos representa en torno a un 2% del peso total del cuerpo, y juegan un papel fundamental en la formación de estructuras esqueléticas, mantenimiento del equilibrio osmótico, múltiples procesos metabólicos o incluso como componentes de enzimas y hormonas (Lall, 2002). A pesar de ello, los datos disponibles sobre contenidos de minerales en tejidos de cefalópodos son

escasos. Algunos autores observaron abundancia de cobre, azufre, calcio y estroncio en paralarvas, juveniles (0.3-14 g) y sus presas más habituales, y un aumento de varios minerales desde el huevo hasta la paralarva, lo que sugiere la absorción de determinados elementos desde el agua (Miramand *et al.*, 2006; Villanueva y Bustamante, 2006). Particularmente el estroncio parece ser esencial para la formación del otolito durante la fase de paralarva, y deficiencias en este mineral provoca problemas de coordinación y dificultades para nadar y cazar, por lo que las paralarvas mueren de hambre (Hanlon *et al*., 1989). Los resultados aportados por el Plan Nacional "Optimización del engorde de pulpo" de JACUMAR (2007-2009) han revelado en el caso concreto del pulpo *O. vulgaris* que el sodio y potasio son mayoritarios en los tejidos, especialmente en músculo, y en sus dietas naturales preferentes. Entre los microminerales esenciales cabe destacar el elevado contenido en cobre, sin embargo, éste es exclusivo de la glándula digestiva, donde además se concentran el hierro, el zinc y metales pesados como el cadmio y el plomo. Estos resultados destacan la función de la glándula digestiva como órgano de reserva de minerales. Finalmente el zinc, con independencia de la glándula digestiva, mostró valores excepcionalmente elevados en la gónada del pulpo en comparación con el resto de tejidos. Estos resultados son similares respecto a micro-elementos en músculo y glándula digestiva en pulpos capturados en la costa portuguesa (Napoleao *et al*., 2005). Estos autores además destacan el alto contenido de vanadio, níquel y molibdeno en los corazones branquiales.

1.4.4. Carbohidratos

El contenido de carbohidratos en cefalópodos representa una fracción minoritaria en comparación con proteínas y lípidos (Lee, 1994). En el caso concreto del

O. vulgaris, los carbohidratos constituyen un 2-5% en peso seco en tejidos (músculo, glándula digestiva) y un 0.5% en peso seco en dietas naturales (crustáceos) (Rosa *et al*., 2005; Prato *et al*., 2010; Morillo Velarde *et al*., 2011). Los primeros estudios sobre utilización de nutrientes en esta especie demostraron que los carbohidratos son eficientemente digeridos (96%) pero tan solo una pequeña parte se acumula en el músculo, donde son utilizados como fuente de energía en momentos de ejercicio intenso (escape, caza) (O'Dor *et al*., 1984; Wells y Clarke, 1996). Estudios recientes demostraron la reducción en el contenido de carbohidratos en músculo de pulpo tras 4-8 días de inanición, a un ritmo de 0,02 g/100 g por día (Morillo Velarde *et al*., 2011), representando una fracción mínima de la pérdida de peso del animal, fundamentalmente relacionada con el catabolismo proteico en músculo y lipídico en la glándula digestiva (Lee, 1994; García Garrido *et al*., 2010).

1.4.5. Vitaminas

Hay muy poca información disponible sobre los requerimientos de vitaminas en cefalópodos. Los primeros estudios consideraron que dichos requerimientos estaban cubiertos si los ejemplares eran alimentados con dietas frescas, aunque alertaban de que la situación cambiaría con el desarrollo de piensos formulados (Lee, 1994). Algunas vitaminas medidas en tejidos de cefalópodos son: la vitamina B12 (1,5-15 µg/100 de tejido en peso húmedo), vitamina C (5 mg/100 de tejido en peso húmedo), vitamina B1 (10-150 µg/100 de tejido en peso húmedo), vitamina B2 (50-350 µg/100 de tejido en peso húmedo), vitamina B9 (10-40 µg/100 de tejido en peso húmedo), vitamina B3 (1.5-4 mg/100 de tejido en peso húmedo) (Sidwell, 1981). Recientemente se determinó el contenido de vitamina A y E en juveniles (0,9-14 g) de *O. vulgaris*, con

valores de 1.5-3 y 300-400 µg/g en peso seco, respectivamente (Villanueva *et al*., 2009).

1.5. Consideraciones sobre la sostenibilidad del cultivo del pulpo

La baja supervivencia en la fase planctónica de *O. vulgaris* y la falta de un pienso específico provoca una dependencia de la pesca, tanto para la obtención de juveniles como de diversas especies de peces y crustáceos para su alimentación. En estas condiciones, el engorde de pulpo plantea un serio debate sobre su sostenibilidad. Para superar los cuellos de botella del cultivo de esta especie es fundamental contar la implicación del sector científico (Seixas *et al*., 2010b). Particularmente la ausencia de dietas formuladas específicas para cefalópodos es actualmente la principal limitación para el desarrollo industrial de especies con fase larvaria bentónica, como el *O. mimus* y la *S. officinalis*. A pesar de algunos resultados prometedores, el crecimiento generado por las dietas experimentales aún no puede compararse en muchos casos con las dietas naturales. Sin embargo, estos resultados no difieren de los observados en las primeras experiencias de alimentación en peces tras el cambio de alimento fresco a dietas manufacturadas (Dabrowski *et al*., 1978; Lindberg y Doroshov, 1986).

Algunos autores proponen un sistema de "cultivo integrado" para los cefalópodos, donde una parte de las paralarvas o juveniles producidos sean liberados al medio para reponer el stock (Boyle y Rodhouse, 2005). De hecho, el ciclo de vida corto de muchos cefalópodos sugiere que prácticamente no hay solapamiento anual, por lo que el tamaño de la población depende mayoritariamente del éxito del reclutamiento anual, muy influenciado por la variabilidad ambiental de cada zona (Dawe *et al*., 2000; Waluda *et al*., 2001; Garofalo *et al*., 2010) y también por la

sobrepesca (Hernández García *et al*., 1998; Hernández López, 2000). Por lo tanto, los cultivos en mar abierto podrían suponer un importante input de paralarvas al medio natural, que potencialmente podría incrementar el reclutamiento. De hecho, las hembras capturadas por las artes de pesca tradicionales (nasas, poteras, etc.) no se han reproducido, ya que este hecho marca el fin de su ciclo de vida. Por lo tanto, el cultivo de pulpos salvajes en jaulas en mar abierto facilitaría los eventos reproductivos, hecho que potencuialmente podría incrementar el reclutamiento anual.

2. OBJETIVOS

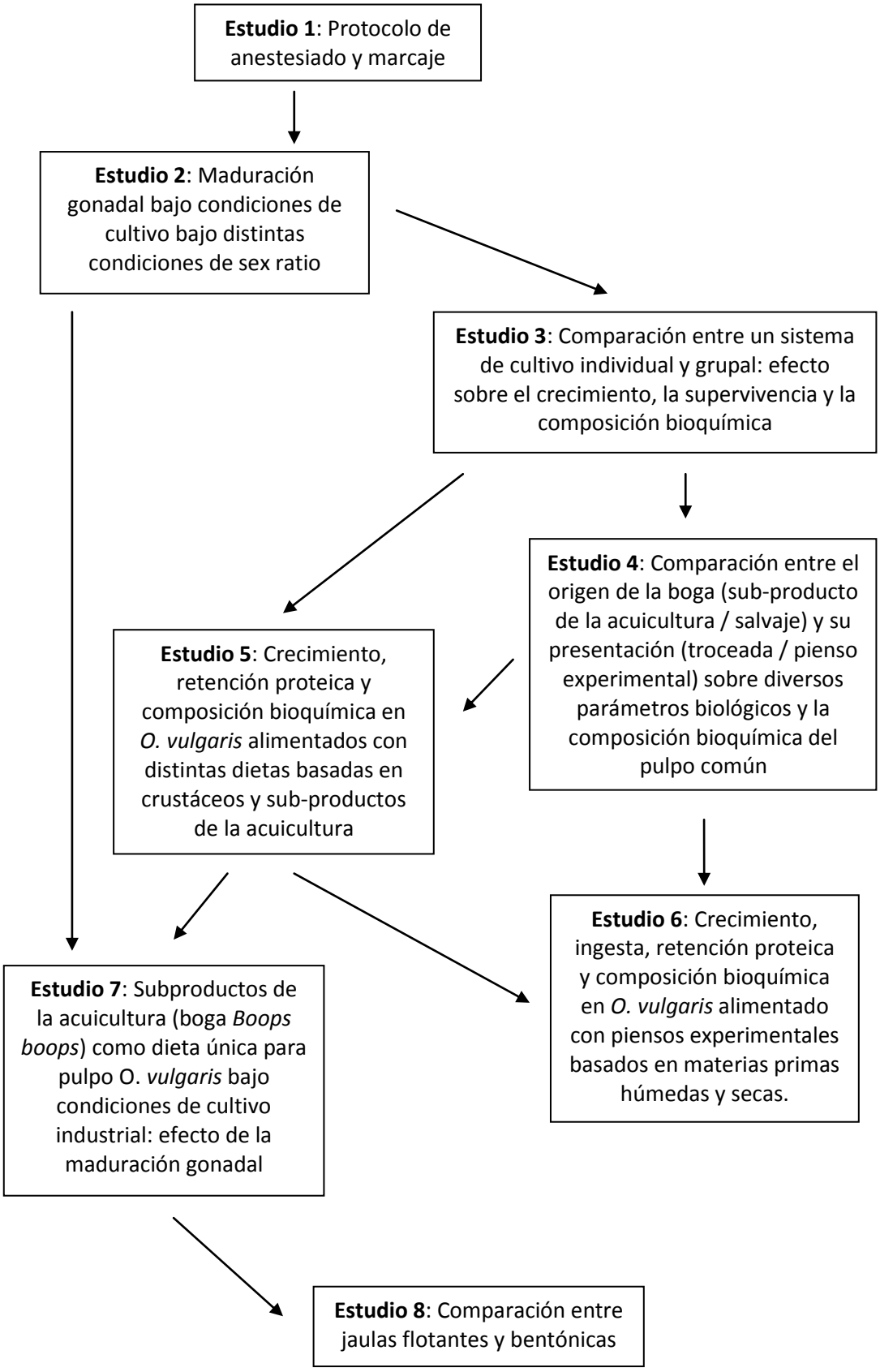
El objetivo general de esta tesis es establecer un protocolo de cultivo a nivel experimental y escala semi-industrial utilizando, entre otros, productos de descarte de la acuicultura, incrementando a su vez el conocimiento de los requerimientos nutricionales del pulpo común *Octopus vulgaris*. Para ello, se establecieron los siguientes objetivos particulares:

1. Establecimiento de un protocolo de anestesiado y marcaje para pulpo común
2. Evaluación de la maduración gonadal del pulpo bajo distintas condiciones de sex ratio, distintos sistemas de cultivos y su efecto sobre el crecimiento y la mortalidad en machos y hembras en presencia / ausencia de fenómenos reproductivos
3. Comparación del crecimiento, la mortalidad y la composición bioquímica en pulpos en un sistema de cultivo individual y grupal
4. Evaluación del crecimiento, la mortalidad y la composición bioquímica en pulpos alimentados con distintas dietas frescas: aprovechamiento de subproductos de pesca y acuicultura
5. Evaluación del crecimiento, la mortalidad y la composición bioquímica en pulpos alimentados con distintas formulaciones de piensos experimentales
6. Comparación del crecimiento, la mortalidad y la composición bioquímica en pulpos cultivados en jaulas flotantes y en jaulas bentónicas

Para abordar estos 6 objetivos se diseñaron 8 estudios dentro de la presente tesis. El objetivo 1 fue abordado exclusivamente en el estudio 1. El objetivo 2 fue abordado en los estudios 2 y 7, aunque también se incluyeron datos de maduración

gonadal en los estudios 3 y 8. El objetivo 3 fue abordado exclusivamente en el estudio 3. El objetivo 4 fue abordado en los estudios 4, 5, 6, 7 y 8. El objetivo 5 fue abordado en los estudios 4 y 6. Finalmente, el objetivo 6 fue abordado exclusivamente en el estudio 8.

3. ESQUEMA DE LA TESIS



4. MATERIALES Y MÉTODOS

4.1. Captura, transporte y aclimatación de los pulpos

Los pulpos fueron capturados por pescadores profesionales mediante nasas cilíndricas, de 2 m de diámetro, 1 m de altura y con malla metálica de 31,6 mm de luz (Fig. 4.1), fondeadas en aguas litorales del sur de Gran Canaria (España), entre 20 y 50 m de profundidad.

Fig. 4.1: Nasa artesanal utilizada por pescadores profesionales en Canarias.

Los pulpos recién capturados se mantuvieron en los tanques vivero de barcos de pesca en el Puerto de Mogán (Gran Canaria), diseñados para mantener cebo vivo para la pesca del atún, mediante un sistema de circuito abierto (Fig. 4.2). Con este sistema se agrupó un stock mínimo de 10 ejemplares, pudiendo permanecer entre 1 y 4 días en función de la disponibilidad, y siendo alimentados por los pescadores con descartes de la pesca.

El transporte desde el puerto de Mogán hasta las instalaciones del Instituto Canario de Ciencias Marinas se realizó por carretera y tuvo una duración de 60-70 minutos. Se utilizó un camión equipado con 3 tanques de PVC de 500 L provistos de

tapa y un sistema de oxigenación auxiliar, de modo que el oxígeno disuelto no bajase de los 10-12 mg/L. En estas condiciones se transportaron entre 10 y 40 kg/m^3 de pulpo, en función del número de ejemplares. A lo largo de la fase experimental de la tesis se realizaron 45 desplazamientos a Mogán, transportando 1.006 pulpos, con un peso medio de 1061 ± 423 g. El traslado de los pulpos desde los tanques vivero a los tanques de transporte, y posteriormente hasta los tanques de aclimatación en el ICCM se realizó mediante salabres (Fig. 4.3).

Fig. 4.2: Tanques vivero donde se mantienen los pulpos desde que se pescan hasta el transporte al ICCM

Fig. 4.3: Transporte de los pulpos por carretera, en un camión equipado con tanques de 500 L y un sistema de oxigenación auxiliar.

Ocasionalmente, se contrataron pescadores adicionales en épocas de escasez de pulpo, que capturaron los ejemplares de manera manual, mediante buceo. En este caso el número de pulpo no superó los 5-7 ejemplares y fueron transportados directamente al ICCM en un tanque de 250 litros.

La aclimatación de los ejemplares a condiciones de cautividad se realizó en 6 tanques rectangulares de fibra de vidrio de 2 m^3 (Fig. 4.4). Como adaptación a la biología de esta especie se incluyeron guaridas de PVC y malla de sombreo (Hanlon y Messenger, 1996). Como guaridas se utilizaron tubos de PVC de 160 mm de diámetro, insertados en una estructura metálica rectangular con 6 guaridas que optimiza el espacio y facilita la limpieza (Fig. 4.5).

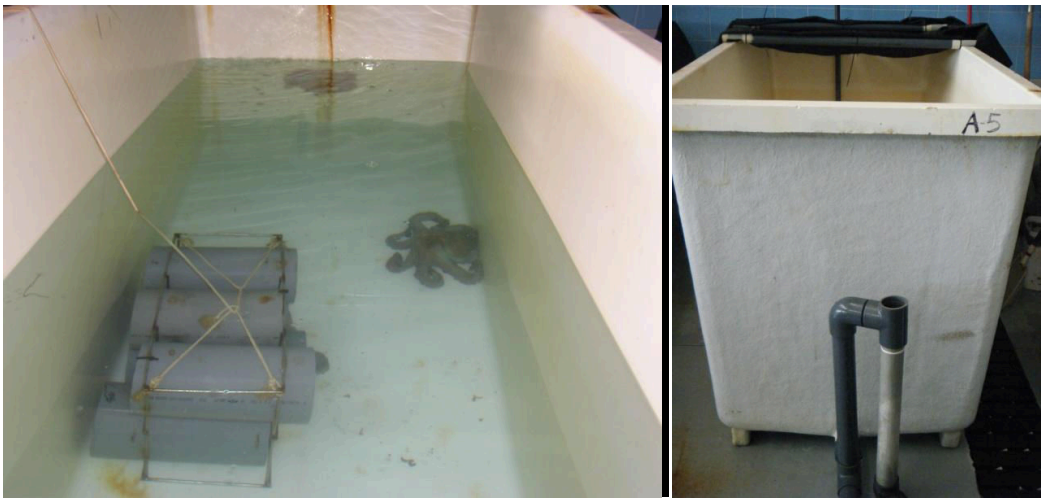


Fig. 4.4: Tanques de aclimatación de 2 m^3, utilizados hasta el 75% de su capacidad, con control externo del nivel del agua.

El número de guaridas disponibles en cada tanque fue siempre superior al número de ejemplares. Además, experiencias previas recomendaron utilizar tan sólo el 75% del volumen de los tanques, dejando un espacio de seguridad entre la superficie del agua y borde del tanque, a fin de evitar escapes durante los experimentos (Estefanell, 2006). Cada tanque estaba equipado con una única entrada de agua y con

control del nivel de agua externo, mantenido en circuito abierto a una renovación completa a la hora, de modo que la concentración de oxígeno disuelto no bajase del 80% de saturación (Cerezo Valverde y García García, 2005). Excepcionalmente se utilizaron tanques circulares de 1 m^3 para la aclimatación, a los que se les incorporó una malla de PVC de 2 cm de luz en todo el borde a fin de evitar escapes (Fig. 4.6).

Fig. 4.5: Estructura de 6 tubos de PVC de 160 mm de diámetro, utilizadas a modo de guaridas en los tanques de aclimatación.

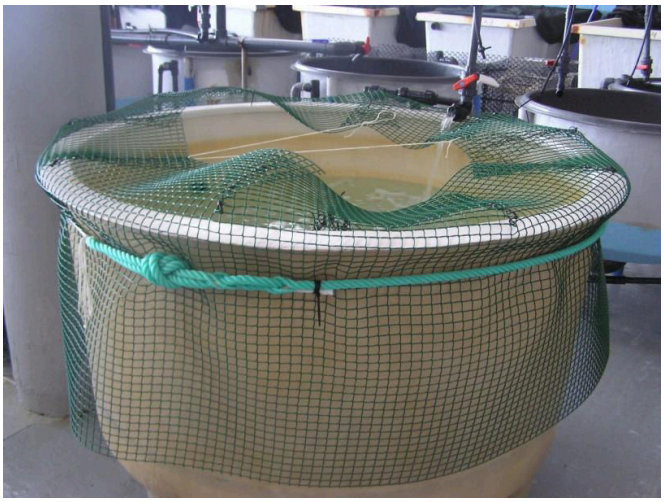


Fig. 4.6: Tanque circular utilizado ocasionalmente para la aclimatación de los pulpos, con una malla anti escape en el borde superior.

En general, la densidad inicial de cultivo durante la aclimatación fue de 5 kg/m^3, seleccionando ejemplares de talla similar en cada tanque y separando machos de hembras. Esta fase de aclimatación tuvo una duración de una semana previa a cada experiencia, y los animales fueron alimentados a saciedad con una dieta mixta a base de boga *Boops boops* (L. 1758) y cangrejo (*Portunus pelagicus* L. 1758), añadidos en formato troceado en días alternos 6 días por semana.

4.2. Protocolo de anestesiado

Tras varias pruebas realizadas con 2 sustancias anestésicas a distintas concentraciones (ver Estudio 1), finalmente se estableció la siguiente disolución anestésica para pulpo común *O. vulgaris*: 1.5% de etanol (96%) en agua de mar a temperatura ambiente (Fig. 4.7). El efecto narcótico es rápido, con tiempo de inmersión de 60 – 90 segundos, en función del peso corporal. La recuperación a los 5 minutos es total y la supervivencia del 100% a las 24 horas.

Fig. 4.7: Pulpos en cubeta de anestesiado, mantenido en inmersión en agua de mar con la dosis de anestésico establecida.

4.3. Protocolo de marcaje con chip subcutáneo (PIT)

Los chips utilizados fueron de tipo *Passive Integrated Transponders* (PIT) (Trovan, Douglas,UK), de 0,096 ± 0.001 g y 2,05x11 mm, asociado a un código de 15 dígitos, detectado por un lector *ARE H5* (Trovan) (Fig. 4.8). Previamente al marcaje, todos los pulpos fueron anestesiados según el protocolo descrito en el apartado 4.2. Se realizaron varios experimentos de marcaje para determinar el mejor punto de inserción (brazo izquierdo III o entre los ojos), el efecto del marcaje sobre el crecimiento y mortalidad en comparación con pulpos no marcados y el posible incremento de la tasa de retención mediante la aplicación de un punto de sutura (ver Estudio 1). El protocolo final de marcaje de *O. vulgaris* con PIT subcutáneo fue el siguiente: los PIT, mantenidos en alcohol, fueron introducidos a nivel subcutáneo en la parte superior del brazo izquierdo III, mediante una aguja hipodérmica (Trovan). El procedimiento de implantación fue rápido y sencillo, con una duración no superior a 10 segundos.

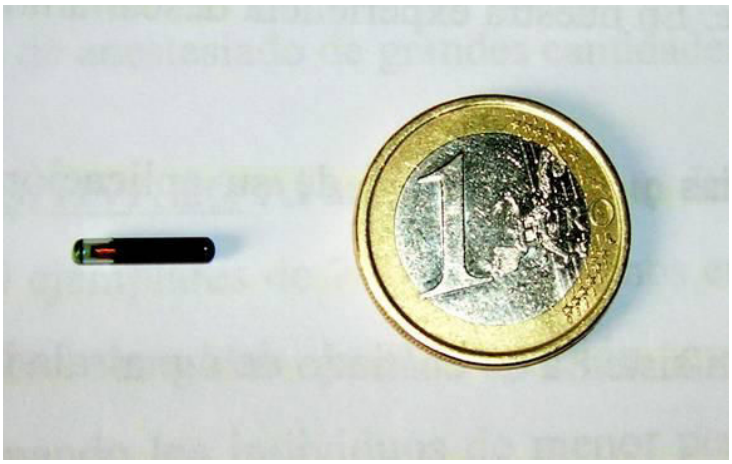


Fig. 4.8: Imagen comparativa del tamaño de un *Passive Integrated Transponder* (PIT)

4.4. Dietas para el engorde de pulpo común

Se probaron distintas dietas naturales (cangrejos) y subproductos de la pesca y la acuicultura (peces), además de elaborar diferentes piensos semihúmedos experimentales basados en dichas materias primas húmedas (filete de pescado, carne de cangrejo) o secas (harinas de las dietas frescas habitualmente ingeridas por el pulpo común). En general la dosis diaria inicial de cangrejo/pienso y pescado fue del 10% y 6% de la biomasa del tanque/día, respectivamente, ajustando la dosis posteriormente según demanda. Los pulpos en las sucesivas experiencias de engorde de la tesis fueron alimentados a saciedad una vez al día 6 veces por semana.

4.4.1. Dietas frescas naturales y subproductos de la pesca/acuicultura

4.4.1.1. Cangrejos

Como dietas frescas naturales se probaron dos especies de cangrejo. Inicialmente se utilizó el cangrejo blanco (*Plagusia depressa*, Fabricius 1775) (Fig. 4.9), especia característica del archipiélago canario que fue proporcionado vivo por pescadores locales. El tamaño de los especímenes fue de 20 - 70 g.

Fig. 4.9: Cangrejo blanco *Plagusia depressa*

Otra especie de crustáceo evaluada como alimento para pulpo fue el cangrejo azul (*Portunus pelagicus*, L. 1758) (Fig. 4.10), adquirido congelado a una empresa local importadora de marisco. El tamaño de los especímenes fue de 300 - 700 g.

Fig. 4.10: Cangrejo azul *Portunus pelagicus*

4.4.1.2. Peces

Como subproductos de la pesca y de la acuicultura se utilizó la boga *Boops boops* (Fig. 4.11), especie muy abundante en el medio natural en el archipiélago canario, que presenta un bajo valor comercial y escaso interés para el consumo humano. La elección de una especie de pescado blanco como la boga fue debido a la escasa aceptación observada en pulpos alimentados con pescado azul como la sardina o la caballa (Socorro, J., Com. Pers.), y además es la principal especie de descarte de las granjas marinas. La boga salvaje presentó un tamaño de 300-400 g mientras que la de descarte de granjas marinas o "boga de cultivo" fue muy variable, con pesos de 50 a 400 g.

4.4.1.3. Dietas mixtas

También se probaron dietas mixtas cangrejo – boga, incluyendo un 60 % de cangrejo y un 40% de boga, suministrados en días alternos (Fig. 4.12).

Fig. 4.11: Boga *Boops boops*, de origen salvaje (debajo) y de origen de descarte de jaulas de acuicultura (arriba).

Fig. 4.12: Dieta mixta de boga y cangrejo azul.

4.4.2. Piensos semihúmedos experimentales

4.4.2.1. Materias primas

Las materias primas utilizadas fueron de tipo húmedo (partes blandas de boga y cangrejo) y de tipo seco (harina comercial y harina fabricada en las instalaciones del ICCM a partir de materias primas húmedas).

En cuanto a las materias primas húmedas, con una humedad del 70-80%, se utilizaron partes blandas de boga *B. boops*, incluyendo filetes y piel, sin escamas ni espinas, tanto de origen salvaje como de descarte de jaulas de acuicultura. Las partes blandas de 2 especies de cangrejo fueron cuidadosamente extraídas, excluyendo vísceras, en concreto de las especies *Portunus pelagicus* y cangrejo "Moro" *Grapsus grapsus* (L. 1758) (Fig. 4.13).

Fig. 4.13: Cangrejo moro *Grapsus grapsus*

Las materias secas, con una humedad del 6-8%, fueron de tipo harina. Inicialmente se utilizó una harina comercial de pescado y posteriormente se decidió elaborar en nuestras instalaciones harina a partir de boga de descarte de acuicultura y

de cangrejo moro. El protocolo de elaboración fue el siguiente: se trituró cada materia prima entera en una moledora industrial (K3, SAMMIC, España) hasta obtener una textura homogénea. Seguidamente se secó en las estufa a 40ºC durante 24 horas y posteriormente fue procesado mediante un molino de grano (3874, SEVERIN, España) hasta obtener una textura muy fina, adecuada para la elaboración de los piensos.

4.4.2.2. Protocolo de elaboración del pienso experimental

El primer paso es homogeneizar las materias primas (filete de boga, carne de cangrejo), mediante una minipimer industrial (TRBM350, SAMMIC, España), hasta obtener un tamaño de partícula fino y homogéneo. Se utilizó un aglutinante comercial basado en alginato y calcio (Pokel Merl + Pokel Cals, Productos del Sur, S.A., Murcia), previamente utilizado con buenos resultados en un pienso de pulpo (Cerezo Valverde *et al*., 2008). La cantidad de cada aglutinante se calcula en base al total del pienso a preparar, y fue un 2% de Pokel Merl y un 1% de Pokel Cals. Cada componente se diluyó en un volumen independiente de agua. El volumen total de agua disponible se calculó de modo que la mezcla final tuviese una humedad cercana al 80%. La mayor parte del agua añadida es utilizada para disolver el Pokel Merl y es necesario utilizar una minipimer doméstica para garantizar completa disolución, que es inmediatamente mezclada con las materias primas.

Para garantizar su homogeneizado se debe utilizar nuevamente la minipimer industrial. Acto seguido se añade la disolución de Pokel Cals, que debe mezclarse rápidamente con el resto de la mezcla, también utilizando la minipimer industrial. Finalmente, se vierte el contenido en una bandeja y se guarda en la nevera a 4ºC durante 24 horas. Al día siguiente la mezcla ha gelificado (Fig. 4.14), y normalmente se

envasa al vacío en porciones diarias de alimentación. Las porciones se almacenan congeladas a -20ºC hasta su uso.

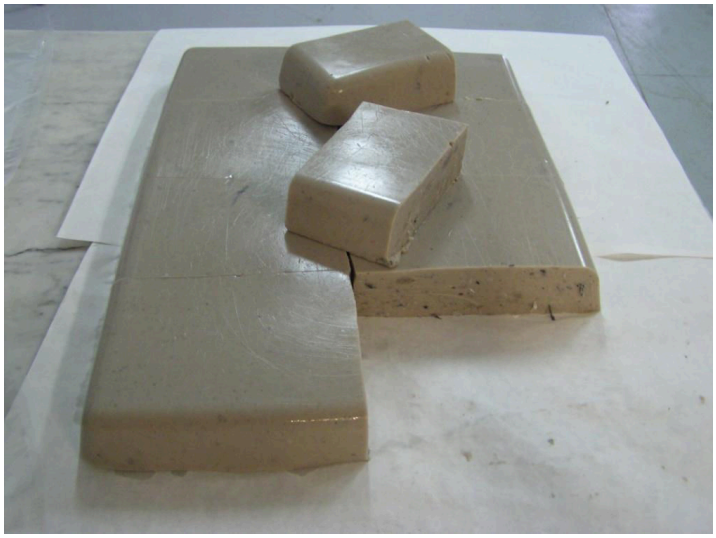


Fig. 4.14: Pienso semihúmedo experimental tras 24 h a 4ºC.

4.5. Sistemas de cultivo

La investigación con especies bentónicas territoriales de alto crecimiento como el caso del pulpo común *Octopus vulgaris* plantea el problema de continuas disputas, ya sea por el alimento, por fecundar a las hembras o por establecerse en determinadas guaridas, que generalmente provocan agresiones, mutilaciones e incluso episodios de mortalidad. Tras varias experiencias previas de cultivo grupal donde raramente se mantenía el 100% de supervivencia (Estefanell *et al*., 2007b, 2008) se diseñó un sistema de cultivo individual.

4.5.1. Tanques compartimentados

Se diseñaron compartimentos de malla para mantener individualmente a los pulpos dentro de los tanques rectangulares de agrupación de stock. En función de la disponibilidad de pulpos se trabajó con tanques compartimentados en 4 (400 L/pulpo) o en 8 (200 L/pulpo) (Fig. 4.15). La malla utilizada fue de PVC negro de 1 cm de luz.

Para evitar escapes se cosió un falso techo en la parte superior. El sistema de cultivo fue en circuito abierto a una renovación completa a la hora y en cada compartimento se incluyó una "T" de PVC (160 mm de diámetro) a modo de guarida.

Fig. 4.15: Tanque de compartimentado en 4, con 400 L por compartimento, diseñado para minimizar la interacción entre los pulpos en los experimentos de nutrición.

4.5.2. Jaulas en el mar

4.5.2.1. Jaulas flotantes (grupal)

Se utilizaron 2 jaulas flotantes de 10 m^3, compuestas por un armazón de acero galvanizado de 3 x 3 x 1,5 m de longitud, profundidad y anchura, respectivamente, y malla metálica de 2 cm de luz. En función de la disponibilidad de pulpos, cada jaula fue dividida en 2 sub-jaulas de 5 m^3 o en 4 sub-jaulas de 2,5 m^3 de volumen útil, utilizando malla negra de PVC de 2 cm de luz (Fig. 4.16). Como adaptación a la biología del pulpo se añadieron guaridas de PVC (T de 160 mm de diámetro) y se forró la parte superior con malla de sombreo. El número de guaridas fue de 180, 90 y 42 en la jaula de 10 m^3, 5 m^3 y 2,5 m^3, respectivamente. Dichas jaulas se fondearon en el muelle de Taliarte

(Telde, Las Palmas). La densidad inicial de cultivo fue de 10 kg/m^3 (Rodríguez *et al*., 2006; Estefanell *et al*., 2009b)

Fig. 4.16: Jaula flotante de 3 x 3 x 1,5 m, de 10 m^3 de volumen útil de cultivo, fondeadas en el muelle de Taliarte (Telde, Las Palmas).

4.5.2.2. Jaulas flotantes (individual)

Además, se probaron jaulas individuales alojadas dentro de las mismas jaulas flotantes, con el fin de evaluar un posible efecto positivo del confinamiento sobre la mortalidad (Fig. 4.17). Para ello se insertaron jaulas individuales de 35 x 30 x 75 cm, de 80 L de volumen, en la cara interna superior de cada sub-jaula de 5 m^3. En total se adecuaron 2 filas de 4 jaulas individuales, cada una con una T de PVC de 160 mm de diámetro a modo de guarida.

4.5.2.3. Jaulas bentónicas

Por último, se evaluó la posibilidad de cultivar pulpo en jaulas bentónicas, fondeadas a 27 m de profundidad sobre fondo arenoso a 3 km del muelle de Taliarte. En este caso, la jaula tuvo unas dimensiones de 2 x 2 x 1 m, dividida en 2 sub-jaulas de 2 m^3, con 26 guaridas cada una (T de PVC de 160 mm de diámetro) (Fig. 4.18).

Fig. 4.17: Compartimentos individuales, alojados en la cara interna superior de cada sub-jaula.

Fig. 4.18: Jaula bentónica de 2x2x1 m, dividida en 2 sub-jaula de 2 m^3, fondeada a 27 m de profundidad.

4.6. Parámetros biológicos

En todos los experimentos de la presente tesis se calcularon el crecimiento individual y la mortalidad.

- Tasa de crecimiento absoluto ("Absolute Growth Rate"):

$AGR = (P_f - P_i) / t$

- Tasa de crecimiento específico ("Specific Growth Rate"):

$SGR = (Ln\ P_f - Ln\ P_i) * 100 / t$

- Mortalidad (“Mortality”):

$M = 100 * (1 – (n_f / n_i)$

Los siguientes parámetros biológicos se calcularon únicamente en los experimentos realizados en tanques individuales o compartimentados:

- Tasa de ingesta específica (“Specific Feed Intake”):

$SFI = (AI / t) * 100 / P_m$

- Ingesta proteica específica (“Specific Protein Intake”):

$SPI = (IP / t) * 100 / P_m$

- Ingesta lipídica específica (“Specific Lipid Intake”):

$SLI = (IL / t) * 100 / P_m$

- Ingesta energética específica (“Specific Energy Intake”):

$SEI = ((AI / t) * GE / 1000) / P_m$

- Eficiencia proteica (“Protein Efficiency Ratio”):

$PER = (P_f$ en peso seco - P_i en peso seco) / IP

- Valor productivo de la proteína (“Protein Productive Value”):

$PPV = 100 * ((P_f * Pr_f - P_i * Pr_s) / IP)$

- Índice de conversión (“Feed Conversion Ratio”):

$FCR = AI / (P_f - P_i)$

- Eficiencia del alimento (“Feed Efficiency”):

$FE = (P_f - P_i) * 100 / AI$

(Donde P_f = Peso final (en g); P_i = Peso inicial (en g); P_m = Peso medio (en g); t = tiempo total (en días); n_f = Nº final de ejemplares; n_i = Nº inicial de ejemplares; AI = alimento ingerido (en g); IP = proteína ingerida (en g); IL = lípidos ingeridos (en g); GE = energía bruta (kJ/g de alimento); Pr_f = porcentaje final de proteína en músculo (peso

húmedo); y Pr_s = porcentaje medio de proteína en músculo en pulpos salvaje (peso húmedo)).

Para estimar AI se secaron los restos hasta peso constante en una estufa a 105ºC para luego inferir el peso húmedo inicial y calcular así la ingesta. En otras ocasiones, por imprevistos técnicos, fue necesario aplicar la siguiente fórmula: $AI = A_a - 0,9 * A_r$; donde AI es alimento ingerido (g); A_a es el alimento añadido (g); A_r es el alimento retirado (g). Dicha fórmula fue obtenida tras pesar 10 muestras de cada dieta antes y tras 24 horas en agua de mar en las mismas condiciones que en los tanques experimentales. En los experimentos donde se probaron piensos experimental se sifonaron los restos del fondo cada 2 días, posteriormente fueron secados en una estufa a 105ºC hasta peso constante, y una vez inferido el peso húmedo inicial, substraído proporcionalmente del alimento ingerido por cada pulpo.

En los experimentos realizados en jaulas se calcularon los siguientes parámetros biológicos:

- Índice de conversión aparente ("Apparent Food Conversión Rate"):

$A\text{-}FCR = AA / (B_f - B_i)$

- Dispersión de tallas ("Weight Dispersión"):

$WD = DE / P_m$

- Incremento de biomasa ("Biomass Increment"):

$BI = (B_f - B_i) / B_i$

(Donde AA es el alimento añadido (g); B_f es la biomasa final (g); B_i es la biomasa inicial (g); DE es la desviación estándar del peso medio (g); y P_m es el peso medio (g)).

Además, en la mayoría de los casos los pulpos fueron diseccionados al final del experimento para extraer la glándula digestiva (Fig. 4.19) y las gónadas, de modo que se calcularon los siguientes índices individualmente:

- Índice de la glándula digestiva ("Digestive Gland Index"):

$DGI = P_{gd} / P_f$

- Índice gonadosomático ("Gonadosomatic Index"):

$GSI = P_g / P_f$

- Índice de madurez sexual de Hayashi modificado por Guerra (1975):
  - En machos: $H_M = P_N / (P_N + P_T)$
  - En hembras: $H_F = P_{GO} / (P_{GO} + P_O)$.

(Donde P_{gd} = peso de la glándula digestiva (g); P_f = peso final (en g); P_g = peso de la gónada (en g); P_N = peso de la glándula de Needhman + saco espermático (g); P_T = peso del testículo (in g); P_{GO} = peso de la glándula oviductal (in g); P_O = peso del ovario (g)).

Fig. 4.19: Glándula digestiva de pulpo *O. vulgaris*, con la bolsa de tinta en la parte superior

Además, el estado de madurez sexual fue evaluado macroscópicamente según la escala de Dia y Goutschine (1990): I, inmaduro; II, en maduración; III, maduro; y IV, post-reproductivo.

4.7. Toma de muestras: dietas y tejidos de pulpo

En general, las dietas evaluadas en cada uno de los experimentos de la presente tesis fueron analizadas entre 3 y 4 veces durante el período de estudio. Cada muestra se tomó de un pull formado al azar por 6 ejemplares, en ocasiones de partes blandas y en otras de dieta entera. Los pulpos fueron sacrificados mediante inmersión en agua helada, para ser inmediatamente pesados y diseccionados. La muestra de músculo se tomó del brazo izquierdo II, la glándula digestiva se extrajo entera y se separó de la bolsa de tinta; de la gónada se analizó separadamente el testículo o el ovario. En función del objetivo del estudio se analizaron muestras individuales o un pull de varios individuos. Asimismo, se tomaron muestras iniciales de tejidos de pulpo, varias veces a lo largo de la tesis, directamente llegados del medio natural. Las muestras de bioquímica se guardaron en bolsas individuales envasadas al vacío a -80ºC hasta su procesado en el laboratorio. Las muestras de histología se guardaron individualmente en recipientes de 100 mL con formol tamponado (10%).

4.8. Análisis bioquímicos

La composición proximal de las dietas y de los tejidos de pulpo fueron analizados siguiendo procedimiento estándares (AOAC, 1997). La humedad se determinó mediante secado de la muestra en un estufa a 105ºC hasta peso constante; las cenizas mediante quemado en un horno mufla a 600ºC durante 12 horas; el

contenido proteico fue determinad por el método Kjeldahl (N × 6.25) y los lípidos totales por el método de Folch *et al*. (1957). Los ácidos grasos fueron extraídos de los lípidos totales por transmetilación del modo descrito por Christie (1982) y separados por cromatografía de gas según lo descrito por Izquierdo *et al*. (1992). Los análisis de cada muestra fueron realizados por triplicado.

4.9. Análisis histológicos

Los análisis histológicos fueron realizados en gónada y glándula digestiva. Las muestras fueron mantenidas en formol tamponado (10%). Posteriormente, cada muestra fue deshidratada en alcohol en un procesador de tejidos (Histokinette 2000, Leica, Nussloch, Alemania). Acto seguido, cada muestra fue incluida en parafina (dispensador Jung Histoembedder, Leica, Nussloch, Alemania) y se aplicó un protocolo de tinción con hematoxilina-eosina. Finalmente, se realizaron secciones de 5 micras en un micrótomo (Leica, RM2135, Leica Instruments, Nussloch, Alemania) para su observación en el microscopio Olympus CX41 (Olympus, Hamburgo, Alemania), según el procedimiento descrito por Martoja y Martoja-Pierson (1970). Las fotografías fueron realizadas con una cámara Olympus XC30 (Olympus, Hamburgo, Alemania). Las imágenes obtenidas fueron procesadas mediante el software de fotografía CellB® (Olympus, Hamburgo, Alemania).

4.10. Análisis estadísticos

Para el tratamiento de los datos se utilizó el programa estadístico SPSS v15 (SPSS, Chicago, IL, USA) y se aplicaron los métodos estadísticos tradicionales (Sokal y Rolf, 1995; Fowler *et al*., 1998). Los datos presentados como media ± desviación

estándar fueron sujetos a un test de normalidad (Kolmogorov–Smirnoff) y homogeneidad de varianzas (Levene). En algunos casos los datos fueron transformados (arcoseno de la raíz cuadrada), en particular cuando fueron presentados como porcentaje. En los casos donde no se obtuvo normalidad u homogeneidad de varianzas se aplicaron tests no paramétricos (Kruskal–Wallis, Games-Howell). Los diseños estadísticos de la presente tesis contaron con 1 o 2 factores, aplicándose un modelo lineal general:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \beta X_{ij} + \varepsilon_{ij}$$

Donde μ es la media, α_i es el efecto fijo del primer factor, β_j es el efecto fijo del segundo factor, $\alpha\beta_{ij}$ es la interacción entre los factores, βX_{ij} la regresión de cada dato sobre el peso inicial en función de cada factor (covariable), y ε_{ijk} el error residual. El uso de la covariable fue posible gracias a la técnica de marcaje individual, y permitió incluir el efecto del peso inicial sobre las variables respuestas en los diferentes estudios de la tesis. En los estudios 5 y 6, algunos parámetros biológicos fueron comparados mediante un ANOVA de medias repetidas, ya que fueron medidos en el mismo grupo de individuos durante todo el período de estudio. En todos los estudios de la presente tesis se consideró que había diferencias estadísticas entre las variables respuesta cuando $P < 0,05$. Para establecer diferencias específicas entre los tratamientos, se aplicó un test *a posteriori* de *Bonferroni*, estableciendo la diferencia estadística cuando $P < 0,05$. En aquellos diseños estadísticos donde no se pudo incluir el peso inicial como convariable, por ejemplo al comparar la composición bioquímica de las dietas, se aplicó un test *a posteriori* de *Tukey*, estableciendo la diferencia estadística cuando $P < 0,05$.

En algunas ocasiones, cuando únicamente se utilizaron dos dietas, estas fueron comparadas mediante un modelo t de Student y nuevamente los tratamientos se consideraron diferentes cuando $P < 0,05$.

Cuando los datos de mortalidad pertenecían ejemplares individualizados o a una sola réplica (jaulas) la mortalidad fue transformada (0 = supervivientes; 1 = bajas) y comparada mediante modelos no paramétricos (chi-cuadrado, Kruskal–Wallis) y se consideraron diferentes cuando $P < 0,05$.

Para visualizar las afinidades en el perfil de ácidos grasos de la dieta y de la glándula digestiva al final de cada período de estudio, una ordenación nm-MDS fue realizada sobre los datos no transformados según una matriz de disimilitud de Bray-Curtis. Para confirmar un posible solapamiento de perfiles, el coeficiente de correlación de Spearman (ρ) fue calculado entre ambas matrices de disimilitud. Además, este mismo coeficiente de correlación ρ se calculó en el estudio 8 entre el peso final y el índice H en machos y hembras. En ambos casos la correlación se consideró significativa cuando la $P < 0,05$.

Por último, el coeficiente de correlación de Pearson (r) se calculó en el estudio 4 para determinar la relación entre la ingesta proteica y lipídica y el contenido de lípidos y proteínas en el músculo y la glándula digestiva de pulpo, respectivamente. Se consideró que la correlación era significativa cuando la $P < 0,05$.

5. Estudio 1: "Evaluation of two anaesthetic agents and PIT tagging system in *Octopus vulgaris* (Cuvier 1797)"

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5.1. Abstract

Octopus vulgaris is a market demanded species with potential to diversify European aquaculture. However, this species develops complex social interactions under culture conditions which may have negative effects on growth, survival and profitability. In order to understand its behaviour under such conditions, individual tagging systems allow careful evaluation of biological parameters, such as growth and longevity. The present work describes a combined protocol (anaesthetic and tagging) for implanting subcutaneous Passive Integrated Transponder tags (PIT). The effect of

two anaesthetic agents to facilitate octopus handling is assessed, clove oil at 20-40-100 mg/L and ethanol (96%) at 1-1.5-2%. The most suitable body location of PIT tags, its effect on growth and mortality, the addition of a stitch and PIT retention rate after 2 months in floating cages were evaluated. It was concluded that immersion in sea water with 1.5% of ethanol at 22.3 ± 0.5ºC is a suitable anaesthetic for this species. Results showed that the best selected PIT body location was upper left arm III. No effect of PIT tagging system on growth and survival was found when tagged and untagged octopuses were compared. The stitch did not increment retention rate and 81-100% tag retention regardless of dietary treatment was observed after 2 months.

Key words: Octopus vulgaris, PIT tags, Anesthetics, Retention

5.2. Introduction

The common octopus (*Octopus vulgaris*, Cuvier 1797) shows high market prices and an increasing demand in European, South American and Asian countries (Vaz Pires *et al*., 2004). Rapid growth rate (Mangold, 1983) and easy adaptation to culture conditions (Iglesias *et al*., 2000) confers this species great farming potential (Socorro *et al*., 2005; Chapela *et al*., 2006; Rodríguez *et al*., 2006; García García *et al*., 2009). Despite unsolved problems in larval rearing (Iglesias *et al*., 2007a), a few companies based in Spain (Galicia) have been pioneers in octopus farming. Annual production has fluctuated from 7 to 32 tons/year from 1998 to these days, using floating cages technology and low price species as feed.

In wild populations, this species has a solitary life, mating only for reproductive porpoises (Mangold, 1983; Guerra, 1992). Under culture conditions, *O. vulgaris*

develops complex social relations and interactions which may have a negative effect on growth and survival. It seems that interaction between animals in group rearing tends to generate a conflict of dominance (Rey Méndez *et al*., 2003; García García *et al*., 2009) and understanding this hierarchical behaviour appears to be essential to guarantee octopus culture profitability. In this manner, the physical tagging is a precise and effective method for obtaining individual data, allowing careful evaluation of biological parameters such as growth or longevity.

An appropriate tagging system for *O. vulgaris* must prove several characteristics like high retention rate, be economical and easy to apply and have no effect on growth and survival. Several external-visual tags have been tested in cephalopods for fisheries management and ecology studies (Watanuki and Iwashita, 1993; Nagasawa *et al*., 1993; Domain *et al*., 2000, 2002; Fuentes *et al*., 2006). External systems have the advantages of being economical, easy to apply and do not require sophisticated equipment (Moffett *et al*., 1997) but may incur in some damage to the organism (Nagasawa *et al*., 1993). External-non visual tags (sonic and radio transmitters) have been used with some success to track adult *Octopus dofleini* (Mather *et al.*, 1985), although they are expensive which limits the number of individuals that can be tracked. An internal-visual tag, Visible Implant Elastomer (VIE), has been tested in squid *Sepioteuthis sepioidea* and no effect on growth or survival was found (Zeeh *et al*., 2008), although this tagging system is designed to mark cohorts and individual identification is complex, especially for a great number of animals. Regarding chemical tagging in cephalopods, Fuentes *et al*. (2000) described an efficient tagging system for *Octopus vulgaris* paralarvae. In order to tag cephalopods sub-adults, internal non-visual tags (Passive Integrated Transponders or PIT tags) are unequivocal, easy to apply

and appear to have no effect on growth and survival in *Octopus tetricus* and *Octopus maorum* (Anderson and Babcock, 1999). This tagging system was used in *Octopus vulgaris* by Rey Méndez *et al*. (2003) to study this species behaviour in floating cages.

To facilitate handling a few anaesthetics agents have been tested in *Octopus vulgaris*. Immersion in sea water with 1-3% of urethane (Messenger, 1968) or 2% of ethanol (Andrews and Tansey, 1981; O'Dor *et al*., 1984) has proved effective. Cold water anaesthesia (3-6ºC) was used by Fuentes (2004) and Andrews and Tansey (1981) with exposure time up to 5 minutes. Messenger *et al*. (1985) evaluated the anaesthetic effect of magnesium chloride on this species. On the other hand, Seol *et al*. (2007) assessed the narcotic effect of clove oil in O*ctopus minor*. Clove oil is a natural anaesthetic and has been widely used in aquaculture (García-Gómez *et al*., 2002; Hoskonen and Pirhonen, 2004; Bilbao *et al*., 2007; Otero Ferrer *et al*., 2007).

However, tagging systems need to be tested for each species because of differences in susceptibility to anesthesia and manipulation, capacity to recovery, growth rate and morphology (Navarro *et al*., 2006). In this way, the present work describes PIT tagging procedure and evaluates its effect on growth and mortality in *Octopus vulgaris* on two body locations and confronted with untagged individuals. Also, the addition of a stitch on the insertion point and PIT retention after 2 months in floating cages under 2 dietary treatments was tested. On the other hand, the effect of two anaesthetics agents was also assessed in this species: ethanol and clove oil.

5.3. Materials and Methods

5.3.1. Capture and acclimation of the stock

Octopuses were caught at sea in Mogán (Gran Canaria, Spain). Local fishermen

used cylindrical trawls (1.5 m diameter, 0.4 m high with metallic net of 31.6 mm mesh) located at 20-30 m depth. Octopuses were kept on board in open flow-throw sea water reservoirs and transport by truck to the laboratory was performed in 500 L square tanks. This operation took 60-80 minutes, oxygen flow was provided so oxygen level would not be limiting (above 12 mg/L), mean temperature was 22.9 ± 0.7ºC and a 100% survival was recorded to handling and transport at arrival and after 24 hours. Acclimatization lasted ten days in rectangular 1.5 m^3 tanks with open flow-throw sea water system (1500 L/h). PVC tubes as shelters and shadowing nets were provided (Hanlon and Messenger, 1996). A control diet, based on "discarded" bogue (*Boops boops*, L. 1758) and de-frozen crab (*Portunus pelagicus*, L. 1758), was provided on alternate days once a day 6 times per week. Food ration was provided to station and photoperiod was natural. The bogue used in these trials was supplied by local fish farms as "discarded" species from off shore sea bream cages. After 10 days, those octopuses who were unwounded, regularly took and ingested food, were selected for the following experiments.

5.3.2 Anaesthetic agents and dose

After acclimation, octopuses where placed in 40 L aerated sea water tanks and treated with different concentrations of anaesthetic. Ethanol (96%) was tested at 1-1.5-2% and clove oil at 20-40-100 mg/L. Water temperature during the experiments was 22.3 ± 0.5ºC. Exposure time, up to 6 minutes, was evaluated separately in 3 octopuses per treatment (1268 ± 291 g), who were immediately transferred into 500 L tanks with open flow-throw sea water system to assess recovery time and evaluate mortality within 24 h. A description of anaesthesia-recovery stages observed in this species is shown in Table 5.1. Finally, the most suitable anaesthetic agent identified

was tested in 5 octopuses weighting between 700 and 1130 g in order to find a relationship between octopus weight and exposure time to become anaesthetised.

5.3.3. Tagging procedure

Passive Integrated Transponders (PIT, Trovan Ltd., UK) were 0.096 ± 0.001 g weight, 2.05 × 11 mm size and were associated to a 15 digit code detected by ARE H5 reader (Trovan Ltd., UK). All octopuses were anaesthetised by immersion in seawater with 1.5% of ethanol (96%) prior to tagging, and PIT tags were previously immersed in alcohol and then introduced at subcutaneous level with a hypodermic needle (Trovan Ltd., UK). Each tag was inserted away from the hypodermic insertion point to avoid tag ejection. Tagging procedure was quick and simple with tags being implanted within 10 seconds. PIT retention rate and its effect on growth and mortality were evaluated in 4 trials. Each experimental tank was provided with PVC tubes as shelters and open flow-throw sea water system was adjusted so oxygen levels would be above the 80% saturation (Cerezo Valverde y García García, 2005). Water temperature and oxygen levels were measured once a day. Food was supplied to satiation 6 times per week and photoperiod was natural.

- Trial 1: the objective of this experiment was to determine a suitable body location for PIT tags in *Octopus vulgaris*. 12 octopuses (2633 ± 271 g), all males, were selected and two body locations were tested. Accordingly, 6 animals were tagged in upper left arm III (PIT-A) and another 6 individuals were tagged in between the eyes (PIT-E). The assay lasted 10 days and both tagging treatments were kept separately in 2 m^3 tanks. The control diet was provided to station once a day. Mean water temperature was 19.4 ± 0.1ºC.

- Trial 2: the objective of this experiment was to determine whether PIT tagging

had an effect on growth and survival confronted with untagged animals, under two dietary treatments. 24 octopuses (442 ± 115 g) were selected. A triplicate of 4 octopuses for each treatment, sex ratio 1:1, was placed in 400 L tanks. One tank was PIT tagged in upper left arm III so biological parameters could be confronted with the remaining two untagged tanks. The assay lasted 4 weeks and PIT tags were read every week to evaluate retention. The control diet (PIT-A control diet, Untagged control diet) and a moist diet (PIT-A moist diet, Untagged moist diet) were provided to station once a day. Mean water temperature was 21.5 ± 0.7ºC.

- Trial 3: the objective of this experiment was to determine whether a stitch on the syringe insertion point could improve PIT retention. 18 octopuses (2207 ± 494 g.) were PIT tagged in left arm III (PIT-A), and on half of the individuals an "X" shaped stitch, made of thin fishing line, was added on the syringe insertion point (PIT-S). A triplicate of 3 octopuses from each treatment, sex ratio 2:1, was placed in 1 m^3 tanks. The assay lasted 4 weeks and PIT tags were read every week to evaluate retention. Bogue, discarded from fish farms, was provided to station once a day. Mean water temperature was 23.5 ± 0.3ºC.

- Trial 4: the objective of this experiment was to evaluate PIT tag retention in floating cages in 2 successive on-growing cycles under 2 dietary treatments. This floating cage was situated in Taliarte Harbour (Telde, Las Palmas, Spain). It was made of galvanized stainless steel, approximately 10 m^3 of total volume (3x3x1.5 m) and 2x2 cm mesh, divided in 2 sub-cages. Initial biomass was 9.2-9.9 k/m^3 and the assays lasted 2 months. PIT tags were read after 30 days to evaluate retention. Dietary treatments were the same tested in trial 2 and were provided to station once a day. In the 1st cycle, 64 octopuses (1483 ± 269 g) were selected, separated in 2 groups (sex ratio

1.5:1) and placed in each sub-cage (PIT-A control 1, PIT-A bogue 1). Mean water temperature was 18.3 ± 0.3ºC. In the 2nd cycle, 42 octopuses, all males (2280 ± 305 g) were selected, separated in 2 groups and placed in each sub-cage (PIT-A control 2, PIT-A bogue 2). Mean water temperature was 21.0 ± 1.3ºC.

5.3.4. Biological parameters

All individuals were weighted at the beginning and when PIT digits were read until the end of the experimental period. The following parameters were calculated individually: Absolute growth rate: AGR = $(W_f-W_i) / t$; PIT tag retention: R = $(N_t-N_l)*100 / N_t$, where W_f = final weight in g.; W_i = initial weight in g., t = time (days), N_t = number of animals that were initially tagged; N_l = number of individual who lost tags. Mortality was evaluated every day and expressed as accumulated percentage. Mortality within the 1st week (M_{w1}) and final mortality (M_T) were calculated.

5.3.5. Statistical analysis

Means and standard deviations were calculated for each parameter measured. All data were tested for normality and homogeneity of variances. Every index from each diet fed group was subjected to F-test to compare standard deviations. When $P \geq 0.05$ means were compared using a Students "t" and significant differences were considered at $P < 0.05$ (Sokal and Rolf, 1995).

5.4. Results

5.4.1. Anesthetic

Table 5.1 shows a description of anaesthesia and recovery stages observed in this species. Anaesthesia starts with hyperventilation (stage I), followed by losing of muscle tone and flaccid arms (stage II). Losing of sucking activity and weak breathing

(stage III) was observed prior to full anaesthesia (stage IV) when chromatophores relax and skin becomes white. Once animals are placed back on clean seawater, sucking activity (stage I) and chromatophores activity (stage II) show that the octopus is recovering. Recuperation of breathing movements (stage III) to regular breathing and normal activity (stage IV) indicates full recovery. Clove oil, regardless of concentration and exposure time, hardly reached stage I of anaesthesia, showing poor narcotic effect in *O. vulgaris* in the conditions described. On the other hand, Ethanol always reached level IV of anaesthesia regardless of concentration. Ethanol 2% had the lowest exposure time to full anaesthesia (Fig. 5.1). In all cases recovery was quick, 3-6 minutes. Survival was 100% in both ethanol and clove oil treatments.

Table 5.1: Description of Anaesthesia and Recovery Stages observed in *O. vulgaris*

Stages	Anaesthesia	Recovery
I	Hyperventilation	Resurgence of sucking activity
II	Muscle tone disappears. Flaccid arms	Recovery of chromatophores activity
III	Weak breathing and loss of sucking intensity	Recuperation of breathing movements
IV	Chromatophores relax (skin becomes white)	Recovery of activity, regular breathing

The most suitable anaesthetic agent identified was a 1.5% of ethanol (96%) in sea water and was tested in octopuses weighting range between 700 and 1130 g. Fig. 5.2 shows the relationship between exposure time to become anaesthetized and octopus weight. As can be observed, heavier octopuses need more exposure time, and weight explained the 90% of exposure time.

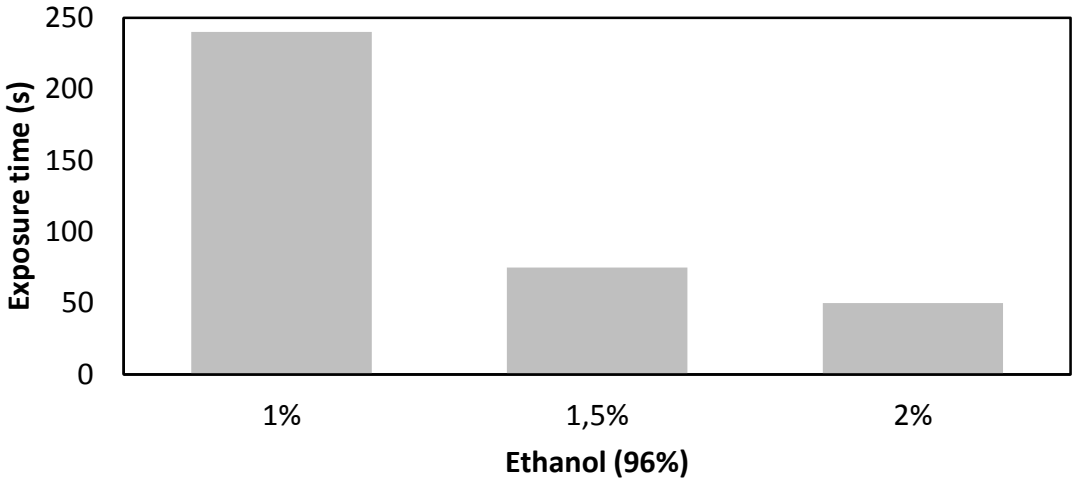

Fig. 5.1: Exposure time (s) to reach full anaesthesia in sea water with different % of ethanol (96%) in *O. vulgaris.*

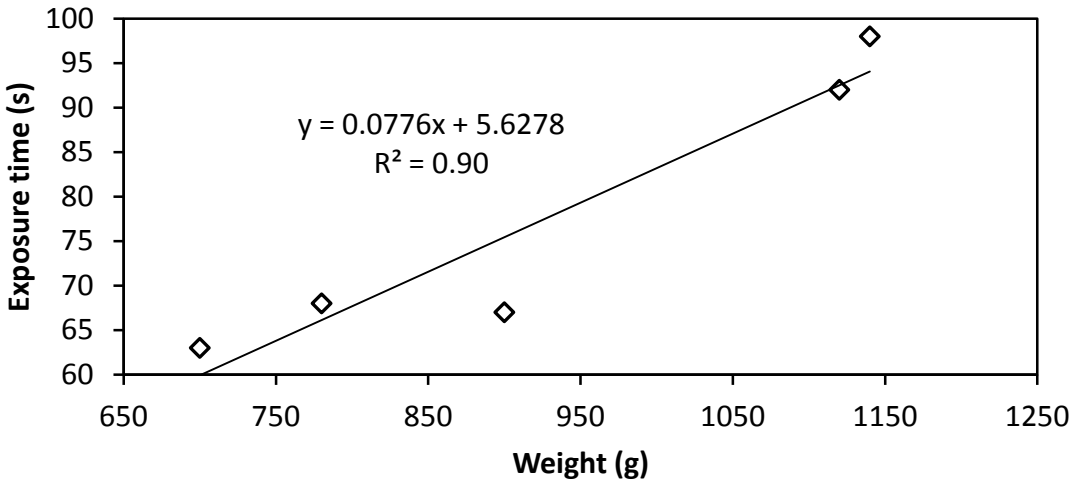

Fig. 5.2: Relationship between exposure time in sea water with 1.5% of ethanol (96%) and octopus initial weight.

5.4.2. Tagging:

Results obtained in tagging trials are summarized in Table 5.2.

- Trial 1: one PIT tag was lost from each treatment and 100% survival was recorded after 10 days. Despite no statistical difference in growth, higher values were obtained in octopuses tagged in left arm III.

- Trial2: PIT tag retention was 100% regardless of dietary treatment. No statistical difference was found in terms or growth between tagged and untagged individuals. Nevertheless, one tagged octopus died under the control diet treatment and another untagged octopus died under the moist diet treatment.

- Trial 3: the addition of the stitch on the syringe insertion point did not increment tag retention rate. In fact, only one PIT tag recorded belonged to the stitch treatment and all stitches were lost by the end of the experimental period. Besides, no difference in growth was observed between treatments. Regarding mortality, one individual tagged in the arm without the stitch died the last day of the experimental period.

- Trial 4: PIT retention rate was 81-100% regardless of dietary treatment. Mortality, on the other hand, occurred towards the end of the experimental period (6-8th week).

Table 5.2: Number of individuals (N), rearing time (t, days), PIT Retention (%), Mortality within the 1st week (M_{w1}), Total Mortality (M_T) and AGR obtained in trials 1-4.

Trial	Tag (treatment)	t (d)	Retention (%)	M_{w1} (%)	M_T (%)	AGR (g/d)
1	PIT-A (Arm)	10	83.3	0	0	51.1 ± 14.7
	PIT-E (Eye)		83.3	0	0	37.0 ± 7.8
2	PIT-A (control diet)	28	100	0	25.0	14.9 ± 4.1
	Untagged (control diet)		-	0	0	20.1 ± 6.4
	PIT-A (moist diet)		100	0	0	5.4 ± 1.1
	Untagged (moist diet)		-	0	12.5	4.7 ± 2.3
3	PIT-A	28	100	0	11.1	17.8 ± 8.0
	PIT-S (stitch)		88.9	0	0	16.1 ± 5.6
4	PIT-A control 1	60	84.4	0	28.0	37.3 ± 21.3
	PIT-A bogue 1		81.3	0	21.9	30.4 ± 12.8
	PIT-A control 2		100	0	35.0	47.0 ± 18.9
	PIT-A bogue 2		100	0	63.6	16.2 ± 12.4

5.5. Discussion

In the present study octopuses showed high resistance to handling so anesthesia was required prior to tagging. In contrast, some authors claim that tagging in octopods (*Octopus tetricus* and *Octopus maorum*) can be performed without anaesthesia (Anderson and Babcock, 1999). Immersion in sea water with 2% of ethanol (96%) generated the lowest anaesthetic time although the drastic change to white colour in the 3 individuals tested suggests that its effect could be lethal if prolonged. In fact, 2% concentration has been used to anaesthetize cephalopods prior to dissection (Boyle, 1981; O'Dor *et al*., 1984; Gleadall *et al*., 1993). On the other hand, this anaesthetic agent was satisfactory tested prior to surgery in *O. vulgaris* (Andrews and Tansey ,1981). Cold water anaesthesia (Andrews and Tansey, 1981; Fuentes, 2004) and immersion in sea water with magnesium chloride (Messenger *et al*., 1985) have proved effective in this species although longer exposure times to reach narcotic effects were recorded. Recently, Seol *et al*. (2007) concluded that clove oil is an effective anaesthetic agent in O*ctopus minor* at higher concentrations than the ones evaluated here. In the present study, clove oil was ineffective while immersion in sea water with 1.5% of ethanol (96%) proved quick anaesthetic time without the drastic effects showed by the higher concentration.

Regarding the PIT tagging trials, left arm III was selected as a possible PIT body location because it was opposite to the hectocotylus in males so the least interference with reproductive processes was expected. Also, PIT tagging between the eyes was selected because it was considered to be an easy point to locate. In the present study, no difference in terms of retention, growth or mortality were found between body locations, unlike Fuentes *et al*. (2006) and Domain *et al*. (2002) who found lower

retention in the mantle independently of the arm tested. In the present experiment PIT tagging in upper left arm III is recommended since it is considered to be a safer tagging location. On the other hand, Anderson and Babcock (1999) selected the dorsal crown at the 1st left arm pair for tag insertion since they intended to read the PIT without removing the animal from the shelter in ecology studies.

In this study the tag retention rate was lower than the obtained by Anderson and Babcock (1999) with other species of octopods (*Octopus tetricus* and *Octopus maorum*), but higher than in some other trials where external visual tags were tested in several cephalopods species (Domain *et al*., 2000, 2002; Fuentes *et al*., 2006). In Rey Méndez *et al*. (2003) PIT retention rate was not evaluated. Regarding PIT tagging system in other marine organisms, it has been widely used in several species of fish (Baras *et al*., 1999, 2000; Roussel *et al*., 2000; Bubb *et al*., 2002; Navarro *et al*., 2006; Soula *et al*., 2006) and in sea turtles (Kamezaki *et al*., 1998; Godley *et al.,* 1999) with retention rates close to 100%.

PIT tagging showed no effect on growth and survival when compared with untagged individuals (Anderson and Babcock, 1999). In fact, mortality in all trials was observed towards the end of the experimental period and could be related to increased size dispersion under culture conditions (García García *et al*., 2009) or high rearing temperature (Aguado Giménez and García García, 2002; Miliou *et al*., 2005; García García *et al*., 2009). In fact, in trial 2, there was not mortality in tagged octopuses fed on the moist diet, while there was mortality in untagged octopuses in the same diet.

In trial 3 only one tag loss was recorded in an individual whose left arm III was wounded prior to tagging. It is therefore advisable to avoid tagging in wounded arms since regenerative processes may have a negative effect on PIT retention.

In conclusion, immersion in sea water with 1.5% of ethanol (96%) and PIT tagging at subcutaneous level are simple and effective methods to facilitate handling and evaluate individual biological parameters in *Octopus vulgaris*, with no effect on growth and survival and retention rates of 81-100% regardless of dietary treatment.

5.6. Acknowledgments

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6. Estudio 2: "Gonad maturation in *Octopus vulgaris* during ongrowing, under different conditions of sex ratio"

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6.1. Abstract

Octopus vulgaris is a suitable candidate for aquaculture, but there are problems with breeding in captivity, such as aggressive behaviour among males and the frequent death of females after the eggs hatch. To avoid these problems and further understand sexual maturation of common octopus in captivity, males and females were reared together and separately under similar culture conditions. In all trials, the initial rearing density was 10 kg/m^3. Females (N = 15, sex ratio 0:1) and males (N = 11, sex ratio 1:0) were kept in circular tanks, and a mixed group (N = 209, sex ratio 4:1) in

floating cages. Trials started in November 2008 and octopuses from each treatment were examined macroscopically and histologically in December and January to assess sexual maturation. All the males matured, regardless of the sex ratio during rearing, as did all females in the mixed group. In contrast, a large proportion of the females kept isolated from males were still immature in December and January. Although maturation was successful in floating cages, there was 76% mortality there, in contrast to the zero mortality in tanks. Moreover, most of the dead octopuses from the cages were in post-reproductive condition, with a low digestive gland index, suggesting that this was natural post-reproductive mortality. Therefore, sex segregation is deemed advantageous to avoiding early mortality.

Keywords: digestive gland, gonad maturation, mortality, *Octopus vulgaris*.

6.2. Introduction

The common octopus (*Octopus vulgaris*) is a merobenthic species that lives from the coast to the outer edge of the continental shelf, up to 200 m deep (Guerra, 1992), all along western and eastern Atlantic coasts, in the Mediterranean Sea, and in the Northwest Pacific, around Taiwan and Japan (Söller *et al*., 2000; Warnke *et al*., 2004). It has been proposed as a candidate for diversification of European aquaculture, the further development of which is constrained by market saturation because of the low number of species produced commercially (Vaz Pires *et al.*, 2004). The common octopus commands high market prices and there is increasing demand for it in Europe, South America, and Asia (Vaz Pires *et al.*, 2004). It grows rapidly (Mangold, 1983) and easily adapts to culture conditions (Iglesias *et al*., 2000), so would seemingly have an

excellent potential for culture (Socorro *et al*., 2005; Chapela *et al*., 2006; Rodríguez *et al*., 2006; García García *et al*., 2009). Despite unsolved problems in rearing the larvae (Navarro and Villanueva, 2000, 2003; Villanueva *et al.*, 2002, 2004, 2009; Iglesias *et al*., 2004, 2006, 2007b; Villanueva and Bustamante, 2006), a few companies based in Spain (Galicia and the Canary Islands) already ongrow wild subadults in floating cages, using low price species as feed.

One factor influencing the industrial development of octopus culture is sexual maturation under rearing conditions. Male octopuses die after mating, and females die immediately after the eggs hatch (Guerra, 1992; Hernández-García *et al*., 2002) and can lose as much as 30–60% of their initial body weight during egg-laying (Iglesias *et al*., 2000). Although there have been several successful ongrowing experiments with males and females reared together (Socorro *et al*., 2005; Rodríguez *et al*., 2006; García García *et al*., 2009), the effect of sex segregation during broodstock maturation has not been studied well, and results are contradictory. Whereas Chapela *et al*. (2006) stated that both males and females grow at similar rates even though 60% of females spawn during summer, Rey Méndez *et al*. (2003) documented slower growth and greater survival in females than in males.

Sexual maturation of the common octopus in wild populations has been extensively studied (Fernández Nuñez *et* al., 1996; Quetglas *et al*., 1998; Hernández García *et al*., 2002; Silva *et al*., 2002; Rodríguez de la Rúa *et al*., 2005; Otero *et al*., 2007), but little attention has been paid so far to evaluating sexual maturation under rearing conditions (Mangold and Boletzky, 1973; Cerezo *et al*., 2007). The aim of the present work was to study gonad maturation in males and females reared together and separately under similar culture conditions, compared with animals taken from

local fisheries. The effect of sexual maturation on the digestive gland index (DGI, an indicator of condition; Cerezo Valverde *et al*., 2008) and under two dietary treatments is also discussed.

6.3. Material and methods

6.3.1. Capture and acclimation of the stock

Octopuses were caught in Mogán (Gran Canaria, Canary Islands). Local fishers used cylindrical trawls (1.5 m diameter, 0.4 m high, with a metallic net of 31.6 mm mesh) placed 20–30 m deep. Octopuses were kept on board in open flow-through seawater reservoirs and transported to the laboratory by truck in 500 L square tanks. This operation took 60–80 min and oxygen was provided so that its concentration would not be limiting (>12 mg/L).

Acclimatization was in rectangular 1.5 m^3 tanks, and (previously frozen) blue crab (*Portunus pelagicus*) was provided *ad libitum* once daily. After one week, the octopuses were PIT tagged subcutaneously on left arm III, using immersion in seawater with 1.5% ethanol (96%) as anaesthetic, following the procedures described by Estefanell *et al*. (2007a). One week later, tagging was verified as successful by reading the PIT digits in all octopuses, prior to the start of the experiment.

6.3.2. Experimental design

For males and females reared separately, octopuses were kept in circular tanks of 1 m^3 and the water level was kept up to 500 L to prevent the octopuses from escaping. The initial density in these tanks was 9.9 ± 1.2 kg/m^3 of water. A total of 11 males were split between two tanks (M_1, M_2) and 16 females among 3 tanks (F_1, F_2, F_3; Table 6.1). Where males and females were being reared together ($M{:}F_{control}$, $M{:}F_{fish}$),

two galvanized stainless-steel floating cages (3 m long, 1.5 m wide, and 3 m high) with 20 mm mesh size and a total internal volume of ~10 m^3 were used. More than 100 octopuses, male:female sex ratio 4:1, were placed in each cage and the initial density was 10.9 ± 0.2 kg/m^3 of water (Table 6.1).

Table 6.1: The rearing system used for the experiment, treatment name, number of tanks or cages ($N_{tanks/cages}$), total number of octopuses (N_T), number of males (N_M), number of females (N_F), and initial body weight of males (W_M) and females (W_F) (mean ± SD)

Rearing system	Treatment	$N_{tanks/cages}$	N_T	N_M	N_F	W_M (g)	W_F (g)
Tank	M_n	2	11	11	0	1122 ± 267	–
	F_n	3	15	0	15	–	865 ± 226
Floating cage	$M:F_{control}$	1	109	88	21	1041 ± 249	916 ± 322
	$M:F_{fish}$	1	105	84	21	1060 ± 326	888 ± 267

Different rearing systems were used for practical reasons. The relative scarcity of females provided by the local fishers (natural sex ratio 4:1), along with the difficulty gathering the octopuses, led us to place the single-sex groups in tanks.

6.3.3. Rearing conditions

Regardless of rearing system, shelters (PVC "T" shaped tubes, 160 mm diameter) and shadowing nets were provided (Villanueva, 1995; Hanlon and Messenger, 1996). In tanks, an open flow-through seawater system was adjusted to 500 L/h. All treatments were fed *ad libitum* 6 d per week under the natural photoperiod. Tank treatments and one floating cage ($M:F_{control}$) were fed a "control" diet based on a 40–60% bogue–crab mix on alternate days (García García and Cerezo Valverde, 2006). The other floating cage ($M:F_{fish}$) was fed a monospecific diet of bogue (*Boops boops*). Daily feeding rate was 6% of initial biomass for bogue, and 10% for crab. The bogue used in the trials were provided by local farms as discard species from offshore sea bream cages. The blue crab was purchased from a local trader. The trials

lasted 11 weeks in the floating cages (1 November 2008 – 22 January 2009) and 8 weeks in the tanks (26 November 2008 – 24 January 2009). Mean water temperature and oxygen levels, measured once a day, were 18.4 ± 0.4ºC and 7.1 ± 0.2 mg/L in tanks, and 19.0 ± 1.0ºC and 7.0 ± 0.3 mg/L in floating cages, respectively. Light intensity, measured at the water surface with a portable luxometer (Model HT170N; Italy), was 30–50 lx in the tanks and 250–300 lx in the floating cages. Octopuses were observed by scuba three times per week; dead animals were removed daily.

6.3.4. Sampling procedure

One tank per treatment (N = 5) was randomly sampled in December, and all the tanks were sampled in January. From each cage, six octopuses were sampled randomly in December (by scuba), and all surviving animals at the end of experimental period. We also dissected any dead octopuses collected each month from the cages that showed no signs of cannibalism. Animals were sacrificed by immersion in ice-cold seawater. Finally, during the experiment, 11 males and 1 female from the local fishery (wild group) were sacrificed after catching in order to assess the biological parameters in wild populations.

6.3.5. Biological parameters

Mortality was recorded daily. After sacrifice, all animals were weighed and the sex was determined. The following indices were calculated, and data were expressed in relation to a monthly mean value of each parameter.

(i) Reproductive status in males (where W_N is the Needham's complex + spermatophoric sac weight in g, W_T the testis weight in g, and W is the octopus weight in g):

- Hayashi index, *H*, as modified by Guerra (1975), i.e. $H_M = W_N/(W_N + W_T)$.

• Gonadosomatic index, GSI (Otero *et al*., 2007): $GSI_M = 100 \times W_N/(W - W_N)$.

(ii) Indices for the reproductive status of females (where W_{OG} is the oviducal gland weight in g, and W_O is the ovary weight in g):

• Hayashi index, H, as modified by Guerra (1975), i.e. $H_F = W_{OG}/(W_{OG}+W_O)$.

• Gonadosomatic index, GSI (Otero *et al*., 2007): $GSI_F = 100 \times W_O/(W - W_O)$.

(iii) Digestive gland index: $DGI = (W_{DG}/W) \times 100$, where W_{DG} is the digestive gland weight in g.

In addition, a macroscopic maturation stage (Dia and Goutschine, 1990) was assigned to every octopus collected (I, immature; II, maturing; III, mature; IV, post-reproductive), for both males and females.

6.3.6. Histological and statistical analyses

Histological studies were performed on gonad and digestive gland from sacrificed octopuses obtained from the floating cages. The organs were fixed in 10% neutral-buffered formalin, embedded in paraffin, then stained with haematoxylin and eosin (HandE) for optical examination (García del Moral, 1993). Micrographs of gonads were taken from the paraffin sections using a Nikon Microphot-FXA microscope and an Olympus DP50 camera.

In terms of the statistics, means and standard deviations were calculated for initial body weight, initial rearing density, and temperature. Data from each treatment were submitted to a Levene's test, and when that showed no difference in variance among groups ($p \geq 0.05$), individual means were compared using one-way ANOVA along with a Tukey's test for multiple comparisons (Sokal and Rolf, 1995).

6.4. Results

No differences in initial body weight (Table 6.1) and rearing temperature were found among treatments. The mean values for each index calculated for males and females are listed in Table 6.2. Daily observations revealed mating from the start of the experiment. In females, H_F decreased with maturity, but then increased sharply after spawning. In males, H_M increased with maturity. In females, both GSI and DGI increased with maturity, but collapsed after egg-laying. In males no immature or maturing stages were found regardless of treatment. The DGI decreased after mating and the GSI remained static. No mortality was recorded in any of the male-only or female-only treatments, but in cages, mortality reached 76% and 77% for $M:F_{control}$ and $M:F_{fish}$, respectively.

Table 6.2: Sexual maturity and condition indices calculated in this study for female and male of *Octopus vulgaris*: the Hayashi index modified by Guerra (H), the gonadosomatic index (GSI), and the digestive gland index (DGI).

Sex	Maturity stage	Description	H	GSI (%)	DGI (%)
Female	I	Immature	0.21 ± 0.06	0.20 ± 0.09	4.07 ± 0.48
	II	Maturing	0.11 ± 0.02	1.06 ± 1.15	4.21 ± 1.70
	III	Mature	0.05 ± 0.02	3.45 ± 2.05	4.52 ± 1.35
	IV	Post-reproductive	0.47 ± 0.06	0.46 ± 0.14	0.98 ± 0.32
Male	III	Mature	0.54 ± 0.08	0.39 ± 0.10	2.71 ± 1.25
	IV	Post-reproductive	0.78 ± 0.05	0.58 ± 0.17	1.14 ± 0.58

The numbers of individuals dissected per treatment, either sacrificed or collected dead, final weight, H index, GSI values, and DGI values are listed in Table 6.3 for males and in Table 6.4 for females. Macroscopic maturity stages in males and females from all treatments are shown in Fig. 6.1 and 6.2, respectively. Males were consistently mature throughout the experiment, and octopuses collected dead, regardless of sex and diet, were mainly in the post-reproductive stage (IV). In contrast,

a smaller proportion of the males sacrificed were at stage IV.

Table 6.3: The number of male *Octopus vulgaris* dissected (sacrificed or collected dead, N_M), weight, Hayashi index modified by Guerra (H_M), gonadosomatic index (GSI_M), and digestive gland index (DGI) in December and January (mean ± SD).

Month	How collected	Treatment	N_M	Weight (g)	H_M	GSI_M (%)	DGI (%)
December	Sacrificed	M_1	5	1648 ±529	0.52± 0.12	0.43 ± 0.08	2.93 ± 1.61
		$M:F_{control}$	6	2943 ± 1369	0.66 ± 0.16	0.37 ± 0.10	1.89 ± 1.64
		$M:F_{fish}$	6	2117 ± 596	0.65 ± 0.16	0.44 ± 0.06	1.74 ± 1.16
		Wild	4	1187 ± 78	0.43 ± 0.09	0.38 ± 0.17	4.39 ± 1.42
	Collected dead	$M:F_{control}$	3	1400 ± 453	0.76 ± 0.07	0.61 ± 0.13	1.52 ± 0.86
		$M:F_{fish}$	8	1624 ± 609	0.77 ± 0.10	0.65 ± 0.26	1.20 ± 0.40
January	Sacrificed	M_2	6	2168 ± 777	0.50 ± 0.09	0.45 ± 016	3.77 ± 1.15
		$M:F_{control}$	14	3021 ± 1373	0.65 ± 0.12	0.36 ± 0.07	1.70 ± 0.83
		$M:F_{fish}$	12	2957 ± 930	0.59 ± 0.11	0.41 ± 0.10	2.52 ± 1.62
		Wild	7	1883 ± 344	0.41 ± 0.04	0.33 ± 0.09	3.46 ± 0.68
	Collected dead	$M:F_{control}$	4	2358 ± 1250	0.73 ± 0.10	0.52 ± 0.14	1.28 ± 0.70
		$M:F_{fish}$	2	1697 ± 514	0.80 ± 0.06	0.57 ± 0.09	1.06 ± 0.37

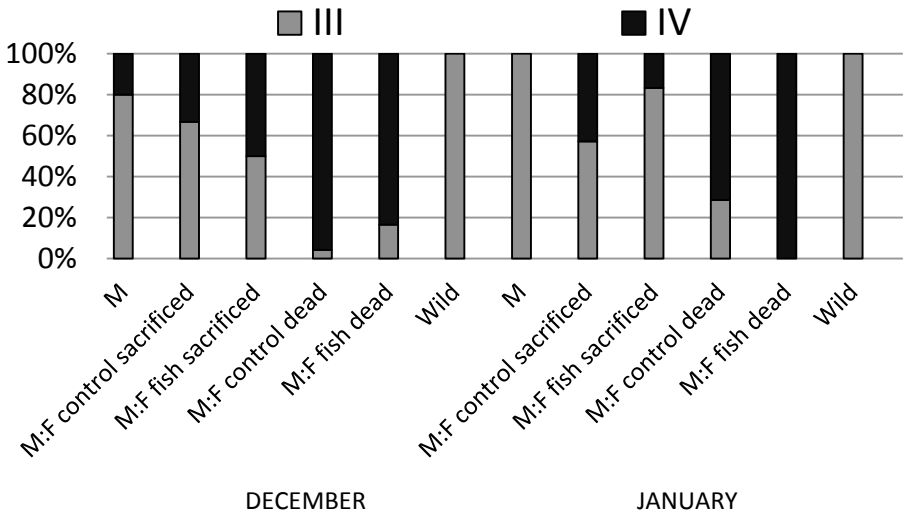

Fig. 6.1. Macroscopic maturation of male *Octopus vulgaris* in December and January.

In terms of females, data were absent from random sampling in floating cages in December, and wild specimens (those dissected immediately after capture) were scarce throughout the experimental period. The females sampled from the cages in January were mainly at stage IV, in contrast to females kept separately from males in

tanks. In fact, in the tank treatment, immature females represented 40% and 80% of those sampled in December and January, respectively.

Table 6.4: The number of female *Octopus vulgaris* dissected (sacrificed or collected dead, N), weight, Hayashi index modified by Guerra (H_F), gonadosomatic index (GSI_F), and digestive gland index (DGI) in December and January (mean ± SD).

Month	How collected	Treatment	N	Weight (g)	H_F	GSI_F (%)	DGI (%)
December	Sacrificed	F_1	5	1348 ± 349	0.12 ± 0.11	2.99 ± 2.89	4.73 ± 0.44
		$M:F_{control}$	0	–	–	–	–
		$M:F_{fish}$	0	–	–	–	–
		Wild	0	–	–	–	–
	Collected dead	$M:F_{control}$	4	831 ± 163	0.24 ± 0.18	1.53 ± 1.57	1.23 ± 0.62
		$M:F_{fish}$	4	861 ± 285	0.37 ± 0.21	1.42 ± 2.07	0.97 ± 0.40
January	Sacrificed	$F_{2–3}$	0	2009 ± 188	0.10 ± 0.03	0.87 ± 0.73	4.92 ± 0.64
		$M:F_{control}$		800	0.42	0.75	1.28
		$M:F_{fish}$	1	1120	0.45	0.49	0.61
		Wild	1	1192	0.08	0.65	4.71
	Collected dead	$M:F_{control}$	5	923 ± 123	0.44 ± 0.04	0.35 ± 0.07	0.99 ± 0.23
		$M:F_{fish}$	3	745 ± 176	0.55 ± 0.03	0.56 ± 0.10	1.24 ± 0.56

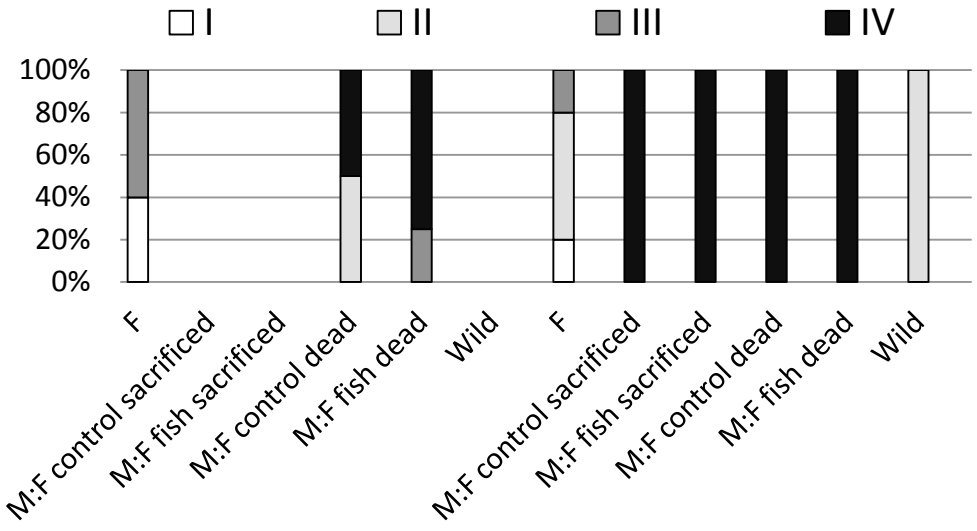

Fig. 6.2: Macroscopic maturation of female *Octopus vulgaris* in December and January.

The DGI (%) of octopuses fed on the control diet was significantly lower in those at the post-reproductive stage (Fig. 6.3), both males and females. The H index was correlated with DGI in males and females fed the control diet (Fig. 6.4). In females, the

DGI increased with sexual maturation, then collapsed after spawning, and in males, it decreased from mature to post-reproductive animals.

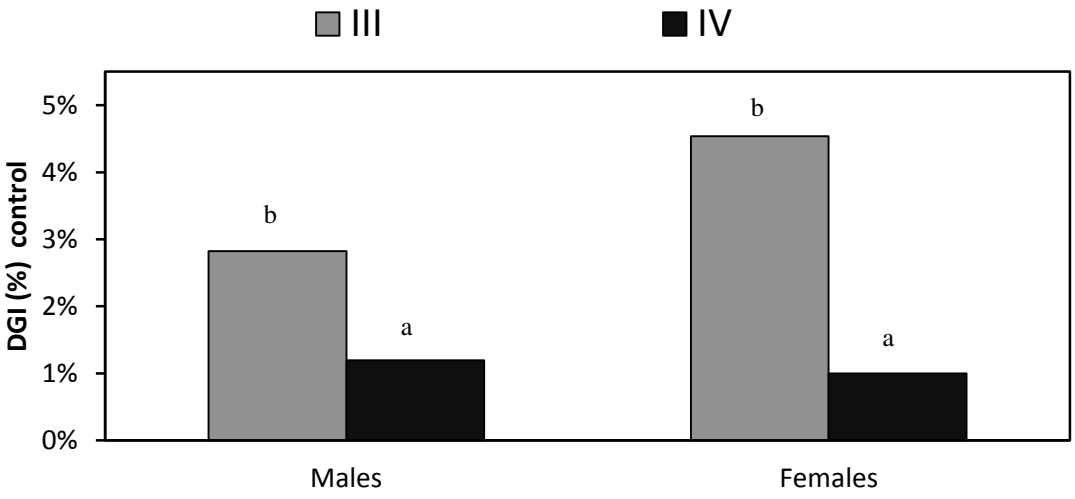

Fig. 6.3: Digestive gland index (DGI, %) in octopuses fed the control diet at the stages mature (III) and post-reproductive (IV).

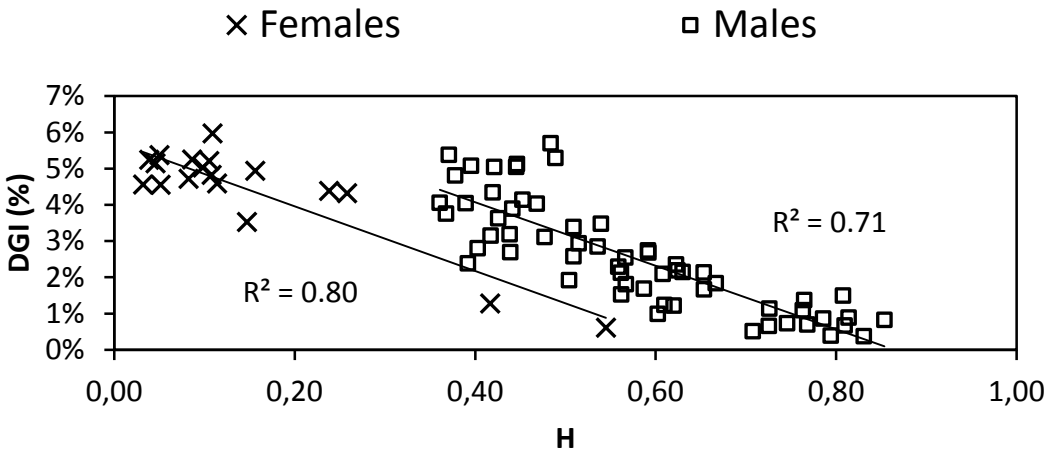

Fig. 6.4: Relationship between the digestive gland index (DGI) and the Hayashi index modified by Guerra (H) in sacrificed males and female *Octopus vulgaris*, from all treatments.

In terms of the histology, Fig. 6.5 shows a mature and a post-reproductive testis and digestive gland. The lumen was full of spermatozoa in the mature testis (Fig. 5a), whereas few spermatozoa and abundant empty spaces in the lumen were indicative of

the spermatozoa having been expelled in the post-mating state (Fig. 5b). Histological examination revealed evidence of spermatogonia, spermatocytes, spermatids, and spermatozoa in mature testes. In the digestive gland, octopuses in the post-reproductive state showed degenerative vacuolization in the parenchyma (Fig. 5d) compared with that of mature animals (Fig. 6.5c).

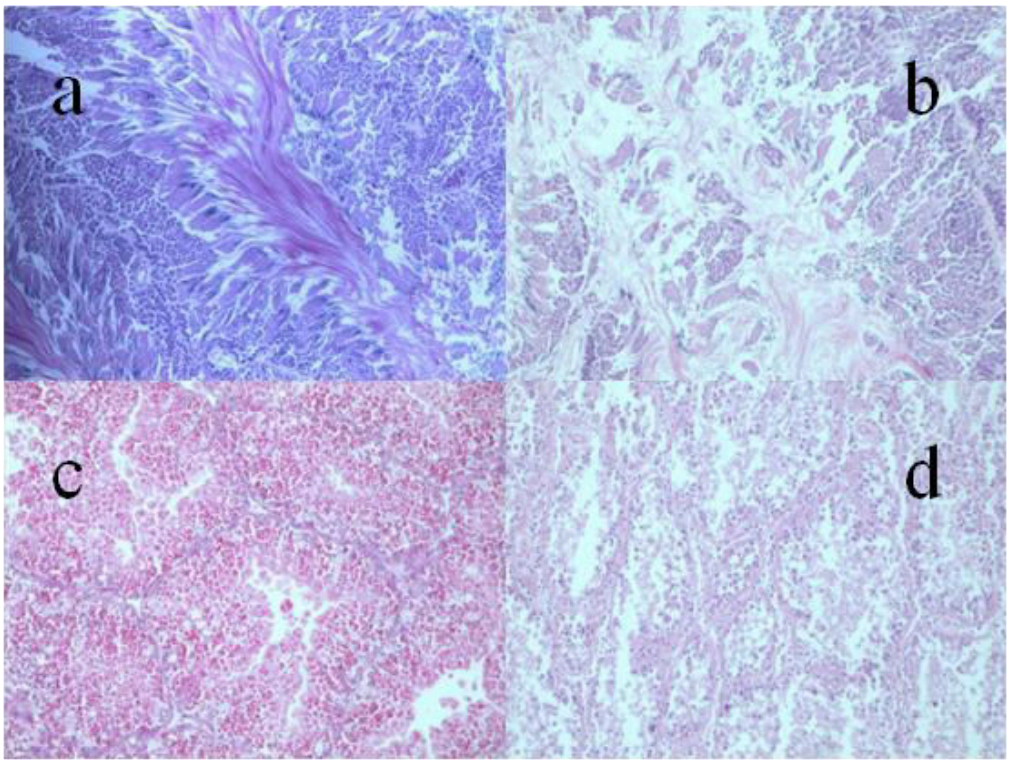

Fig. 6.5: Transverse sections (×10) of testis and digestive gland at different stages of maturity. (a) Mature testis; (b) post-reproductive testis; (c) mature digestive gland; (d) post-reproductive digestive gland.

6.5. Discussion

Under rearing conditions, *O. vulgaris* frequently breed regardless of the time of year (Mangold and Boletzky, 1973; Iglesias *et al*., 2000; Estefanell, 2006). In the Canary Islands, *O. vulgaris* in the wild breed all year round, with two periods of main activity, one from January to July, with a peak in April, and a second in autumn (October–

November; Hernández García *et al*., 2002). In the current experiment, males were constantly mature throughout the period of sampling, irrespective of treatment (Fig. 1), a finding that supports data obtained under rearing conditions (Mangold and Boletzky, 1973; Cerezo *et al.*, 2007) and several previous reports from wild populations in the Atlantic Ocean (Guerra, 1979; Caverivière *et al.*, 2002; Silva *et al*., 2002; Carvalho and Sousa Reis, 2003; Oosthuizen and Smale, 2003; Rodríguez de la Rúa *et al*., 2005; Otero *et al*., 2007) and Mediterranean (Quetglas *et al*., 1998). In contrast, a high proportion of the females kept isolated from males remained immature or maturing (Fig. 6.2), suggesting that the presence of males may promote female sexual maturation. Indeed, sex steroid hormones have been reported in *O. vulgaris* of both sexes (D'Aniello *et al*., 1996; Di Cosmo *et al*., 2001), and they could act as pheromones if released to the water, coordinating maturation in both sexes. Therefore, in sex-segregated females, the absence of hormones from the opposite sex could postpone maturation. On the other hand, females matured in cages where the light intensity at the surface was nine times higher than in tanks inside the experimental facilities, contradicting the statement of Mangold (1987) that high light intensity can retard sexual maturation and that low light intensity can stimulate gonad development in aquarium-held octopuses.

The few females caught in random sampling in cages during December was probably the result of breeding activity then, because females remain in their dens cleaning, ventilating, and caring for the egg strings (Guerra, 1992). It may also explain the few females caught in the wild before and during the experiment. Progressively lower values of H_F were recorded from the start of winter to spring in females living in the wild around Gran Canaria (Hernández García *et al*., 2002), and deviations from a

1:1 sex ratio were attributed to reproductive behaviour or the fishing strategy (Silva *et al*., 2002).

In the cages, high proportions of octopuses in post-reproductive state were found (Fig. 6.1 and 6.2) contrary to the findings of Cerezo *et al*. (2007). Daily observations showed mating behaviour throughout the experiment, which agrees with the findings of Iglesias *et al*. (2000). Occasionally, two males were seen introducing a hectocotylus into the same female at the same time. Most octopuses collected dead from either treatment were at the post-reproductive stage, which demonstrates a clear relationship between mating processes under rearing conditions and mortality, recorded here for the first time in floating cages, supporting earlier observations in tanks (Hernández García *et al*., 2002).

Histological examination confirmed the maturity stages obtained from macroscopic observation and the Hayashi index. Accordingly, the presence of spermatogonia/spermatocytes in the tubular wall and spermatids/spermatozoa in the central lumen are evidence of maturing and mature stages in male *O. vulgaris*, and empty spaces in the lumen indicate that spermatozoa have been expelled after mating (Rodríguez de la Rúa *et al*., 2005). On the other hand, the presence of cells at all stages of maturation suggests the different phases of gonad development at the various maturity stages of the testes as found in the testis of maturing or mature *Octopus maya* (Avila-Poveda *et al*., 2009).

In the present experiment, female GSI and DGI increased with sexual maturation, but this was not the case for males (Table 6.2; Silva *et al*., 2002; Rodríguez de la Rúa *et al*., 2005; Otero *et al*., 2007). The significantly higher DGI in mature than in post-mating *O. vulgaris* (Fig. 6.3) supports other authors' findings (Otero *et al*., 2007).

A decrease in DGI in post-mating *Octopus mimus* (Cortez *et al*., 1995) and *Sepia officinalis* (Castro *et al*., 1992) has been documented.

In contrast to the findings of Otero *et al*. (2007), the correlation between H and GSI was very poor, probably related to the fact that few females in an immature or maturing state were analysed here, and that few post-reproductive animals were available in Otero *et al.* (2007). On the other hand and according to this author, DGI and H are inversely correlated in both sexes (Fig. 6.4).

For egg production, *O. vulgaris* uses energy directly from food, rather than from stored resources (Rosa *et al*., 2004; Otero *et al*., 2007). In our work, sexual maturation of both males and females did not seem to be influenced by diet, because most specimens, irrespective of diet, were in a mature or post-reproductive stage.

To conclude, under the experimental conditions described, there was a clear increase in mortality attributable to the reproductive process. Deterioration of the gonads was confirmed by histological examination (Fig. 6.5), and by a massive decrease in digestive gland weight in post-reproductive animals. Consequently, further work needs to evaluate the benefits of segregation by sex under rearing conditions if mortality reduction and an increase in profitability of octopus culture is desired.

6.6. Acknowledgments

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7. Estudio 3: "Comparison between individual and group rearing systems in *Octopus vulgaris* (Cuvier, 1797)"

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7.1. Abstract

Recently most research on cephalopod culture has focused on the development of new specific enrichments for paralarvae and compound feeds for juveniles and sub-adults. However, little research has been conducted in order to test new rearing systems, specifically designed to meet the particularities of these species. The present experiment was set to compare the biological performance of Octopus vulgaris reared under traditional group conditions in floating cages (5 m^3) and

individually in net cages (80 L), in two successive ongrowing trials. Octopuses (1565 ± 263 g) were fed a mixed diet containing crab and fish during 60 days.

In general, higher mortality was observed in octopus reared under group conditions (28.1-36.7%) rather than individually (0-12.5%), related to breeding behaviour and to weight dispersion along both trials. This led to highest biomass increment in octopus reared individually. However, the group rearing system had a positive effect on growth, reflecting in higher biomass increment and food conversion rates until 40-50 days of rearing. Accordingly, in order to maximize profitability of traditional group ongrowing, periodic grading and selection of males during the reproductive period are recommended. In addition, no difference in proximate composition and fatty acid profile was found in muscle regardless of rearing system.

7.2. Introduction

Cephalopods are preferred species to diversify the aquaculture industry since they show rapid growth, high fecundity, low food conversion rate and high market price (Iglesias *et al.*, 2000; Semmens *et al.*, 2004; Vaz-Pires *et al.*, 2004). Currently, the scientific sector is focusing on the development of cephalopod specific enrichments and compound feeds, to increase survival and growth both at paralarval and juvenile phase in a few cephalopod species, such as *Octopus maya*, *Octopus vulgaris* and *Sepia officinalis*) (Domingues *et al.*, 2001; Navarro and Villanueva, 2003; Iglesias *et al.*, 2007a; Rosas *et al.*, 2009; Seixas *et al.*, 2010a). Indeed, promising results with manufactured feeds have been attained, and on some occasions growth was even comparable to that observed in cephalopods fed fresh diets (Rosas *et al.*, 2007; Cerezo Valverde *et al.*, 2008; Quintana *et al.*, 2008; Estefanell *et al.*, 2009a). The potential of

cephalopod farming, particularly *O. vulgaris*, led a few pioneer companies in Galicia (Spain) to start rearing wild juveniles in floating cages in 1998, with annual production fluctuation between 7 and 32 tons (FAO, 2011).

However, very little research has been done on specific rearing systems for cephalopods. Indeed, octopus industrial farming is carried out in floating cages provided with shelters (Chapela *et al.*, 2006; Rodríguez *et al.*, 2006). Particularly in *O. vulgaris*, mortality under rearing conditions is associated to hierarchical behaviour, cannibalism and reproductive processes (García García *et al.*, 2009; Estefanell *et al.*, 2010b). In contrast, high survival rates were observed in octopuses reared in confinement in individual tanks (Petza *et al.*, 2006; García García *et al.*, 2006; Miliou *et al.*, 2007; García Garrido *et al.*, 2011) or in individual net cages placed inside tanks (400-1000 l) (Biandolino *et al.*, 2010; Estefanell *et al.*, 2009a; Prato *et al.*, 2010).

The present study intends to evaluate growth and mortality in *O. vulgaris* reared under traditional group rearing conditions confronted with an individual rearing system for 60 days. In addition, the effect of both rearing systems on proximate composition and fatty acid profile in muscle was also assessed.

7.3. Material and methods

7.3.1. Capture and acclimatization of the stock

Wild octopuses were caught by local fishermen using cylindrical trawls (1.5 m diameter, 0.4 m high with metallic net of 31.6 mm mesh) placed at 20-30 m depth in the coast of Mogán (Canary Islands, Spain). Octopuses were transported to lab facilities in three 0.5 m^3 square tanks provided with pure oxygen. Acclimatization period lasted 1 week and was carried out in rectangular 1.5 m^3 tanks, provided with

PVC tubes as shelters, shadowing nets and open flow-through seawater system (1500 L/h). One week after arrival each octopus was PIT tagged on upper left arm III (Estefanell *et al.*, 2011b). During this period octopus were fed ad libitum once a day with a mixed diet containing de-frozen blue crab, *Portunus pelagicus* (L. 1758) and bogue, *Boops boops* (L. 1758), supplied on alternate days.

7.3.2. Rearing Conditions

Rearing trials were performed in one stainless steel floating cage (1.5x3x1.5 m length, height and width, respectively), with approximately 5 m^3 of water capacity, enclosed with galvanized steel net of 2 cm mesh. Shadowing net on the top external side and 90 "T" shaped PVC tubes (160 mm diameter) were added to the cage. In addition, eight individual cages made of PVC net of 2 cm mesh (35x30x75 cm, approx. 80 L of total volume), were fixed laterally to the top internal side of the cage, in two rows of four (Fig. 7.1). One PVC "T" shaped tube was fixed to the bottom of each individual cage. This floating cage was anchored in Taliarte Harbour (Telde, Canary Islands, Spain). The assays were performed under natural photoperiod.

7.3.3. Experimental Design

- Trial 1: after acclimatization, 40 specimens (1523 ± 296 g), 26 males (1607 ± 298 g) and 14 females (1439 ± 298 g), were selected and transferred to each rearing system. Initial number of individuals was 32 for the grouped (18 males and 14 females) and 8 (all males) for the individual rearing system, respectively. Initial biomass was 11.9 kg/m^3. The experimental period lasted 60 days (February 26th – April 27th) and octopuses were weighted after 30 days of rearing. Mean water temperature and oxygen levels were 18.2 ± 0.2ºC and 7.0 ± 0.2 mg/L, respectively.

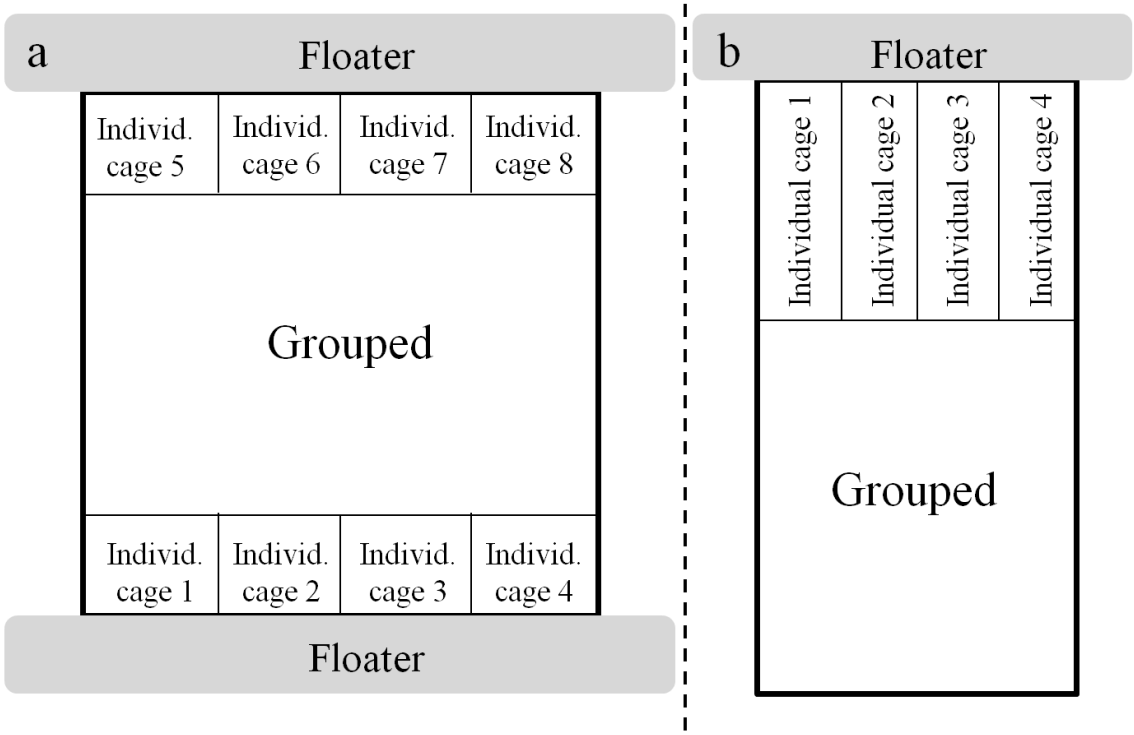

Fig. 7.1: Floating cage (1.5x3x1.5 m^3), with 8 individual net cages attached to the upper internal side of each flank: a) Upper view; b) Lateral view.

- Trial 2: After acclimatization, 38 wild octopuses (1605 ± 220 g), all males, were selected and transferred to each rearing system. Initial number of individuals was 30 for the grouped and 8 for the individual rearing system, respectively. Initial biomass was 12.2 kg/m^3. The experimental period lasted 60 days (April 16th – June 15th) and octopuses were weighted after 30 days of rearing. Mean water temperature and oxygen levels were 19.2 ± 0.8 C and 6.7 ± 0.4 mg/L, respectively.

7.3.4. Dietary Treatment

Octopuses were fed a diet containing a 60% blue crab, *P. pelagicus*, and a 40% bogue, *B. boops*, on alternate days. Crab was purchased from a local fish trade company and main carapace and walking legs were removed. Bogue was supplied by local fish farms, as discarded species that are accidentally reared along with target species (sea bream, sea bass). Octopuses were fed ad libitum 6 days per week (8:00

am). Initial food ratio was 8% of initial biomass, adjusted according to remaining food in the cage along the experimental period.

7.3.5. Biological Parameters

The following parameters were calculated individually:

Specific Growth Rate: SGR = (LnW_f - LnW_i)*100/t; Digestive gland index: DGI = (W_{DG} / W_f); Sexual maturity "Hayashi Index" as modified by Guerra (1975) for males: $H_M = W_N / (W_N + W_T)$, and females: $H_F = W_{OG} / (W_{OG} + W_O)$.

The following parameters were calculated per rearing system:

Weight dispersion: WD = (Standard deviation / W_{af}); Mortality (%): dead octopuses were collected daily by scuba-diving; Biomass Increment: BI = (B_f - B_i) / B_i; Apparent Food Conversion Rate: A-FCR = PF / (W_{af} - W_{ai}); Where: W_f = Final weight (g); W_i = Initial weight (g); W_{af} = Final average weight (g); W_{ai} = Initial average weight (g); t = total time (d); B_f = Final biomass (g); B_i = Initial biomass (g); PF is total food provided (g); W_{DG} = digestive gland weight (g); W_N = Needham's complex + spermatophoric sac weight (g); W_T = Testis weight (g); W_{OG} = Oviducal gland weight (g); and W_O = Ovary weight (g).

Individual growth data and daily mortality allowed the calculation of octopus biomass along the rearing period on each trial and rearing system, so "biomass increment" and "apparent food conversion rate" were estimated every 10 days of rearing.

In addition, a macroscopic maturation stage was assigned to every octopus that had retained PIT tags by the end of each trial (I, immature; II, maturing; III, mature, and IV, post-reproductive) (Dia and Goutschine, 1990).

7.3.6. Sampling Procedure

Each feed was analysed three times during the experimental period. Samples were taken from a pull of 6 individuals. Only the edible fraction from crab was included in the pull, while whole discarded bogue was homogenized. At the end of the experimental period, all octopuses were sacrificed by immersion in ice-cold sea water, prior to being weighted. Three octopuses per rearing system, all males, were randomly selected and a sample of muscle was taken (whole left arm II), which was analysed individually.

7.3.7. Biochemical Analysis

Proximate composition from diets and octopus muscle from each treatment were analysed following standard procedures of (AOAC, 1997). Moisture was determined after drying the sample in an oven at 105 C to constant weight; ash by combustion in a muffle furnace at 600ºC for 12 hours; protein content (N × 6.25) was determined by Kjeldahl method and crude lipid was extracted following the method described by Folch et al. (1957). Fatty acids methyl esters from total lipids were extracted by transmethylation as described by Christie (1982) and separated by gas chromatography under the conditions described by Izquierdo et al. (1992). All analyses were conducted in triplicate.

7.3.8. Statistical Analysis

All data, presented as mean ± standard deviation, were tested for normality and homogeneity of variances. Data (biological parameters, muscle proximate composition and fatty acids profile) were analyzed with the statistical computer package SPSS v15 (SPSS, Chicago, IL, USA), using the following General Linear Model:

$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \beta X_{ij} + \varepsilon_{ij}$; where μ is the population mean, α_i the fixed

effect of the rearing system (grouped-individual), β_j the fixed effect of the rearing period (trial 1 or trial 2), $(\alpha\beta)_{ij}$ the interaction between the factors, βX_{ij} is regression of each biological parameter on initial weight within rearing system and rearing period factors, and ε_{ij} the residual error.

Particularly, proximate composition of food items were compared with only on fixed factor ("dietary treatment"). Mortality data was transformed (0, survivors; 1, dead) and compared according to rearing system using a chi-squared test. Other biological parameters calculated per rearing system (WD, BI, A-FCR) corresponded to one single replicate so could not be compared statistically.

7.4. Results

7.4.1. Biological Parameters

In trial 1, SGR and final weight were twofold in males (1.8 ± 0.4 %/d, 4467 ± 1160 g) than in females (0.9 ± 0.6 %/d, 2590 ± 630 g) reared under group conditions. Regarding biological performance in octopus males, specimens reared under group conditions showed higher SGR and final weight than those reared individually in both trials. Also, males reared in "Trial 1" showed higher growth, final weight and DGI than those reared in "Trial 2" (Table 7.1).

In both trials, weight dispersion increased in grouped and decreased in individually reared octopus (Fig. 7.2). Mortality showed a similar tendency in both trials, with significantly higher values observed in group reared octopus (28.1-36.7%) in comparison with those reared individually (0-12.5%) (Fig. 7.3). By the end of the experimental period, biomass increment was higher in individually than in group reared octopus males. However, group reared males in both trials maintained higher

biomass increment rates until 40-50 days of rearing (Fig. 7.4). Similarly, A-FCR showed lowest values in individually reared octopus at the end of both trials, however this parameter showed better values in group reared octopus until the 50th day of rearing (Fig. 7.5). By the end of the experimental period, all octopuses that had retained PIT tags (89.5-92.5%) were sexually matured regardless of sex and rearing system in "Trial 1", while 33-37% were in post-reproductive stage in "Trial 2" (Table 7.2).

Table 7.1: Biological parameters calculated in males after 60 days of rearing in Trial 1 and Trial 2 (mean ± SD)

	Trial 1		Trial 2	
	Grouped	Individual	Grouped	Individual
W_i (g)	1607 ± 298	1502 ± 286	1604 ± 221	1608 ± 231
W_{int} (g)	3323 ± 544 $^{B}{}_{b}$	2360 ± 339 $^{A}{}_{b}$	2540 ± 604 $^{B}{}_{a}$	2183 ± 199 $^{A}{}_{a}$
W_f (g)	4467 ± 1160 $^{B}{}_{b}$	3350 ± 541 $^{A}{}_{b}$	3333 ± 894 $^{B}{}_{a}$	2603 ± 255 $^{A}{}_{a}$
SGR (%/d)	1.8 ± 0.4 $^{B}{}_{b}$	1.3±0.4 $^{A}{}_{b}$	1.2 ± 0.6 $^{B}{}_{a}$	0.8 ± 0.3 $^{A}{}_{a}$
DGI (%)	2.8 ± 1.0 $_{b}$	3.2±1.3 $_{b}$	1.8 ± 0.8 $_{a}$	2.0 ± 0.7 $_{a}$

Different superscript capital letters within a row denote significant difference according to rearing system ($P < 0.05$). Different subscript minuscule letters within a row denote significant difference according to rearing period ($P < 0.05$).

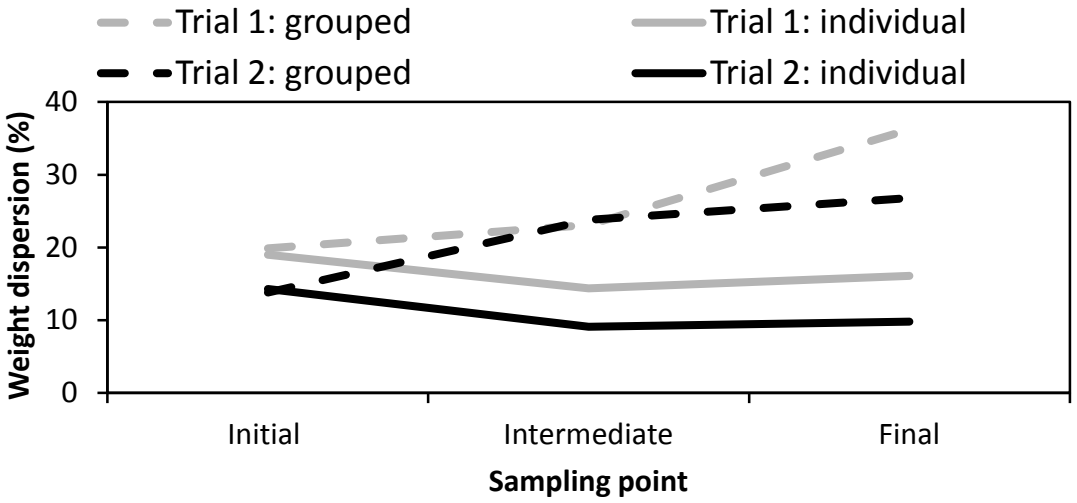

Fig. 7.2: Weight dispersion calculated in grouped and individually reared *O. vulgaris* during the experimental period in Trial 1 and Trial 2.

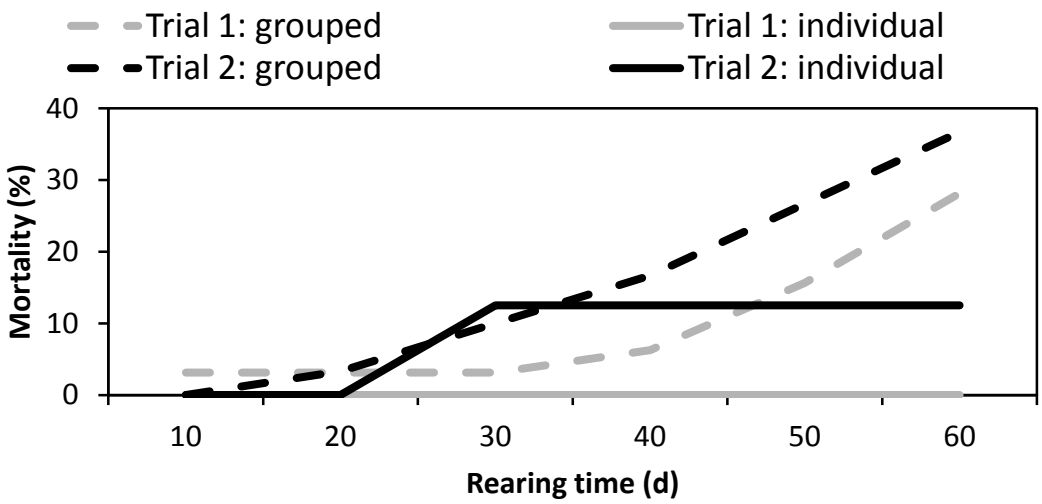

Fig. 7.3: Accumulated daily mortality observed in grouped and individually reared *O. vulgaris* during the experimental period in Trial 1 and Trial 2.

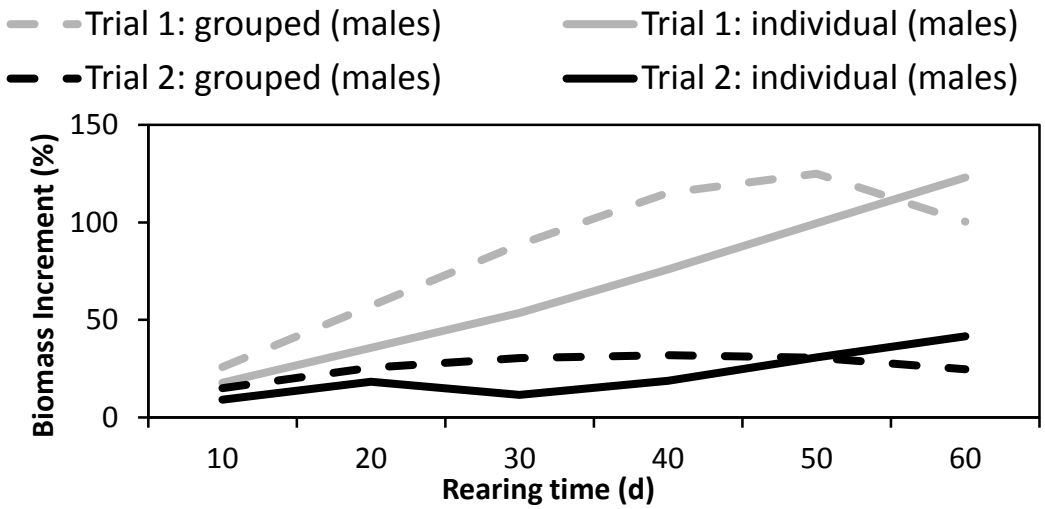

Fig. 7.4: Accumulated biomass increment observed in male and female *O. vulgaris* reared under group and individual conditions during the experimental period in Trial 1 and Trial 2.

7.4.2. Proximate Composition: Diet

Bogue, *B. boops*, showed higher lipid and lower protein content than blue crab, *P. pelagicus* (Table 7.3).

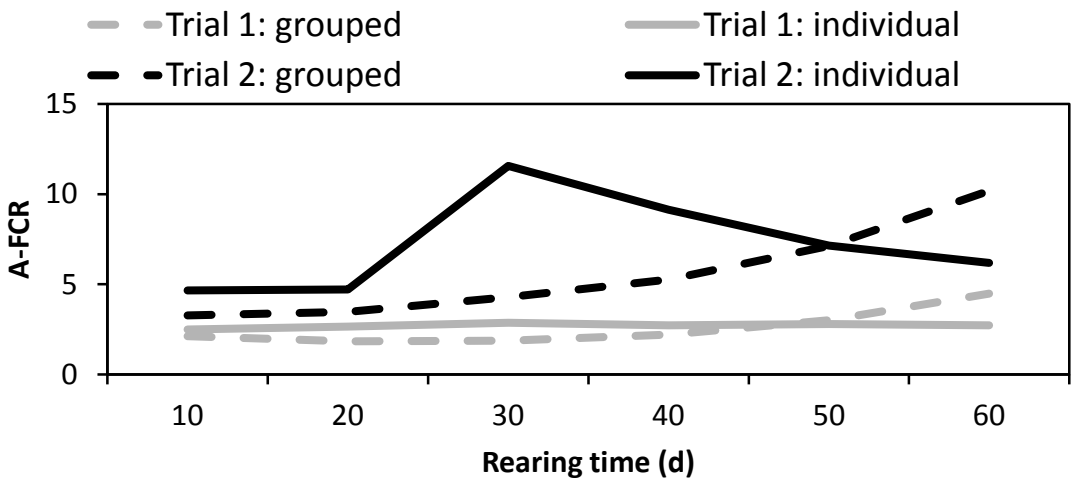

Fig. 7.5: Apparent feed conversion rate calculated in *O. vulgaris* reared under group and individual conditions during the experimental period in Trial 1 and Trial 2.

Table 7.2: Sexual maturity data in reared octopus who maintained PIT tags after 60 days of rearing in Trial 1 and Trial 2.

					Macroscopic maturity stage	
			N	H	Mature	Post-reproductive
Trial 1	Grouped	Females	9	0.03 ± 0.01	100%	0%
	Grouped	Males	11	0.50 ± 0.05	100%	0%
	Individual	Males	8	0.52 ± 0.06	100%	0%
Trial 2	Grouped	Males	16	0.60 ± 0.11	63%	37%
	Individual	Males	6	0.55 ± 0.08	67%	33%

Table 7.3: Proximate composition of each food item (% dry substance) and gross energy (GE, kJ/100 g food wet weight) in Trial 1 and Trial 2 (mean ± SD, N = 3).

	Trial 1		Trial 2	
	P. pelagicus	*B. boops*	*P. pelagicus*	*B. boops*
Lipids (%)	5.7 ± 1.0 ^{a}	43.8 ± 6.1 ^{b}	5.2 ± 0.2 ^{a}	48.7 ± 4.6 ^{b}
Proteins (%)	85.0 ± 1.4 ^{b}	46.7 ± 5.8 ^{a}	82.9 ± 1.4 ^{b}	40.6 ± 3.8 ^{a}
Moisture (%)	79.1 ± 1.6 ^{b}	62.3 ± 3.8 ^{a}	80.1 ± 1.5 ^{b}	59.9 ± 0.4 ^{a}
Ash (%)	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	1.9 ± 0.1

Different superscript letters within a row indicate significant differences (P < 0.05).

7.4.3. Proximate Composition and Fatty Acid Profile in Muscle.

Proximate composition and fatty acid profile in male octopus muscle was not affected by rearing system. However, octopuses reared in “Trail 2” showed lower

protein and higher lipid, moisture and ash in this organ than those reared in “Trial 1” (Table 7.4). Also, octopus reared in “Trial 2” showed higher ARA (20:4n-6) and DHA (22:6n-3) and lower EPA (20:5n-3) in muscle than specimens reared in “Trial 1” (Table 7.5).

Table 7.4: Weight and proximate composition (% dry substance) in male *O. vulgaris* muscle after 60 days of rearing in Trial 1 and Trial 2 (mean ± SD, N = 3)

	Trial 1		Trial 2	
	Grouped	Individual	Grouped	Individual
Weight (g)	$4807 \pm 133^{B}{}_{b}$	$3420 \pm 347^{A}{}_{b}$	$2602 \pm 525^{B}{}_{a}$	$2407 \pm 524^{A}{}_{a}$
Lipids (%)	$3.5 \pm 0.3_{a}$	$3.8 \pm 0.5_{a}$	$5.2 \pm 0.3_{b}$	$5.2 \pm 0.3_{b}$
Proteins (%)	83.9 ± 2.7	82.9 ± 4.7	77.4 ± 1.9	82.2 ± 0.8
Moisture (%)	$80.5 \pm 1.3_{a}$	$79.8 \pm 1.6_{a}$	$85.5 \pm 1.2_{b}$	$83.4 \pm 0.3_{b}$
Ash (%)	$1.9 \pm 0.1_{a}$	$1.8 \pm 0.1_{a}$	$2.3 \pm 0.1_{b}$	$2.1 \pm 0.0_{b}$

Different superscript capital letters within a row denote significant difference according to rearing system ($P < 0.05$). Different subscript minuscule letters within a row denote significant difference according to rearing period ($P < 0.05$)

7.5. Discussion

In the present study, higher biomass increments and lower A-FCR induced by the individual rearing system in both trials were related to higher survival than in group reared octopuses. These results underline the positive effect of individual rearing on octopus survival, also observed in *O. vulgaris* reared individually in tanks (Petza *et al.*, 2006; Miliou *et al.*, 2007; Cerezo Valverde *et al.*, 2008; García Garrido *et al.*, 2011) and in net cages inside tanks (Estefanell *et al.*, 2009a; Biandolino *et al.*, 2010; Prato *et al.*, 2010). For this reason, the use of individual rearing is a common practice in other high price and highly cannibalistic farmed species, such as crab *Portunus sanguinolentus* and lobster, *Homarus gammarus* (Nicholson *et al.*, 2008; Perez *et al.*, 2010).

Table 7.5: Main fatty acids profile (% of total fatty acids) in male *O. vulgaris* muscle after 60 days of rearing in Trial 1 and Trial 2 (mean ± SD, N = 3).

	Trial 1		Trial 2	
	Grouped	Individual	Grouped	Individual
14:0	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.3	0.9 ± 0.3
16:0	18.0 ± 0.5	18.2 ± 0.5	17.6 ± 0.2	17.4 ± 1.2
16:1n-7	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.0
18:0	6.3 ± 0.2	6.8 ± 0.9	5.5 ± 0.7	6.1 ± 0.3
18:1n-9	11.0 ± 1.0	11.5 ± 0.3	10.4 ± 0.0	10.8 ± 1.0
18:1n-7	2.9 ± 0.3	3.8 ± 1.4	2.8 ± 0.3	2.8 ± 0.1
18:1n-5	1.9 ± 0.1 ^{b}	2.0 ± 0.2 ^{b}	1.5 ± 0.1 ^{a}	1.5 ± 0.0 ^{a}
18:2n-6	2.0 ± 0.5 ^{b}	1.4 ± 0.2 ^{b}	0.8 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}
18:3n-3	0.3 ± 0.1 ^{b}	0.2 ± 0.1 ^{b}	0.1 ± 0.0 ^{a}	0.1 ± 0.0 ^{a}
20:1n-9	3.9 ± 0.2 ^{b}	4.2 ± 0.2 ^{b}	3.3 ± 0.4 ^{a}	3.4 ± 0.2 ^{a}
20:2n-6	1.0 ± 0.2	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.2
20:4n-6	6.2 ± 2.5 ^{a}	5.5 ± 2.1 ^{a}	9.9 ± 1.7 ^{b}	9.6 ± 1.6 ^{b}
20:5n-3	14.9 ± 2.1 ^{b}	15.6 ± 1.0 ^{b}	12.7 ± 0.7 ^{a}	13.3 ± 1.2 ^{a}
22:4n-6	0.7 ± 0.1 ^{b}	0.5 ± 0.1 ^{b}	0.1 ± 0.0 ^{a}	0.1 ± 0.0 ^{a}
22:5n-6	0.6 ± 0.2 ^{b}	0.5 ± 0.2 ^{b}	1.0 ± 0.1 ^{a}	0.9 ± 0.1 ^{a}
22:5n-3	1.7 ± 0.1 ^{a}	1.6 ± 0.1 ^{a}	1.9 ± 0.2 ^{b}	1.8 ± 0.2 ^{b}
22:6n-3	23.7 ± 0.3 ^{a}	22.7 ± 0.9 ^{a}	25.6 ± 0.9 ^{b}	25.3 ± 0.5 ^{b}
∑ Saturated	26.6 ± 0.4 ^{b}	27.1 ± 0.9 ^{b}	24.7 ± 0.1 ^{a}	24.9 ± 1.2 ^{a}
∑ Monoenes	21.0 ± 0.8	22.8 ± 1.8	20.2 ± 1.1	20.4 ± 0.9
∑ n-3	41.1 ± 1.9	40.6 ± 2.9	41.5 ± 0.9	41.2 ± 1.1
∑ n-6	10.9 ± 3.2	8.9 ± 2.1	12.7 ± 1.9	12.5 ± 1.3
∑ n-9	15.2 ± 1.1 ^{b}	16.0 ± 0.2 ^{b}	13.9 ± 0.4 ^{a}	14.3 ± 0.9 ^{a}
∑ n-3 HUFA	40.6 ± 1.9	40.2 ± 3.0	40.4 ± 1.4	40.4 ± 1.0

There were no significant differences according to rearing system ($P < 0.05$). Different superscript letters within a row denote significant difference according to rearing period ($P < 0.05$).

Particularly in “Trial 1”, low growth rates observed in females and the presence of egg masses evidenced reproductive behaviour (Iglesias *et al.*, 2000), which is associated to the end of *O. vulgaris* life cycle in both sexes (Estefanell *et al.*, 2010b). Also, different growth rate and final weight between sexes led to high weight dispersion, which also increases mortality in *O. vulgaris* under rearing conditions, due to hierarchical behaviour and cannibalism (Socorro *et al.*, 2005; García García *et al.*, 2009). As a result, mortality in this trial could have been related to reproductive

processes, to interaction among octopuses or to a combination of both. In order to clarify this, only males were selected in "Trial 2", confirming a positive effect of individual rearing on survival and thus biomass increment, related to low interaction and low weight dispersion among octopus. These results are in agreement with this species solitary lifestyle and the lack of schooling behaviour during its benthic phase (Guerra, 1992).

Even though octopus in both trials had similar initial size, size-at-age is not clear in cephalopods, since growth largely depends on availability of food and water temperature in the natural environment (Semmens *et al.*, 2004; Katsanevakis and Verriopoulos, 2006; Leporati *et al.*, 2007). Accordingly, low growth and high mortality observed in "Trial 2" was probably related to longevity. *O. vulgaris* in Canarian waters shows a peak of reproductive activity in April (Hernández García *et al.*, 2002), so most adult octopuses in early summer may be facing the end of their life cycle. This is in agreement with the number of post reproductive specimens found at the end of "Trial 2" in both rearing systems. Prior to the beginning of this trial, males and females were separated upon arrival to our facilities, so sexual interaction probably occurred in nature or most likely in the trawl, where octopus may be kept for a few days until transport to lab facilities. Also, low DGI in comparison with octopus reared in "Trial 1" is related to poor condition (Cerezo Valverde *et al.*, 2008; Estefanell *et al.*, 2010b).

Despite the negative effect on survival, the traditional group rearing system had a positive effect on growth and final weight, especially noticeable in males, probably related to the stimulation of food intake, producing better biomass increment and A-FCR than individually reared specimens until the 40-50th day of rearing. This finding underlines that traditional group rearing system could be more

profitable than individual rearing if mortality can be reduced, for example with periodic grading, which should reduce weight dispersion during the ongrowing cycle (Socorro *et al.*, 2005; García García *et al.*, 2009). Also, present results underlined the negative effect of breeding behaviour on *O. vulgaris* biological performance, especially in females. Accordingly, the utilization of individual trawls and the selection of males appear to be optimal solutions to prevent sexual interaction and avoid early mortality.

In general, growth was similar to previous studies where octopuses were fed comparable mixed diets (García García and Cerezo Valverde, 2006; Biandolino *et al.*, 2010; Prato *et al.*, 2010). In the present study, high growth observed in octopus fed high lipid bogue suggests efficient lipid utilization by *O. vulgaris* (Estefanell *et al.*, 2009a). Even though first works on cephalopods nutrition reported a mainly protein based metabolism (O'Dor *et al.*, 1984; Lee, 1994), recent studies agreed that lipid utilization in cephalopods is affected by lipid source and nutrient composition (Mazón *et al.*, 2007; Sánchez *et al.*, 2009; Seiça Neves *et al.*, 2010). Furthermore, the use of lipids as energy source has been suggested in several cephalopod species (Castro *et al.*, 1992; Semmens, 1998; Moltschaniwskyj and Johnston, 2006; García Garrido *et al.* 2010).

In the present study, proximate composition and fatty acid profile in muscle was not affected by rearing system, which underlined the adequacy of individual rearing in *O. vulgaris*. In addition, high lipid content in the diet did not reflect on muscle proximate composition, which showed similar profile in comparison to previous data in cephalopods, with low lipid and high protein content (Rosa *et al.*, 2004; Ferreira *et al.*, 2009). Low muscle protein content in octopus reared in "Trial 2" was again related to poor physiological state, and suggests decreasing feeding rates as

this species approaches the end of its life cycle. Slight differences on muscle lipid content between trials may be related to different final weight. Indeed, a decrease on lipid content associated to increasing size was also observed in wild and reared *O. vulgaris* (Navarro and Villanueva, 2003; Biandolino *et al.*, 2010). Main fatty acids in octopus muscle are in agreement with previous data (Navarro and Villanueva, 2003; Rosa *et al.*, 2004; Miliou *et al.*, 2007; Prato *et al.*, 2010). Decrease in ARA and DHA in octopus reared in "Trial 1" may be related to higher demand of these fatty acids induce by high growth rates in comparison to specimens reared in "Trial 2". Indeed, a reduction in ARA in best growing octopus was previously reported (García Garrido *et al.*, 2011), and was also related to increasing EPA levels in muscle (Estefanell *et al.*, 2009a). In any case, muscle quality in terms of fatty acid profile was not affected by rearing system.

As a conclusion, *O. vulgaris* culture in individual cages maximizes survival and thus biomass increment, showing a similar proximate composition and fatty acid profile in muscle in comparison to traditional group reared specimens after 60 days of rearing.

7.6. Acknowledgments

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8. Estudio 4: "Efficient utilization of dietary lipids in *Octopus vulgaris* (Cuvier 1797) fed fresh and agglutinated moist diets based on aquaculture by-products and low price trash species"

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8.1. Abstract

The aim of this study was to evaluate growth, biochemical composition and dietary nutrients utilization in *O. vulgaris* fed on four diets based on bogue *Boops boops*, from different origin and in two presentations: fresh discarded bogue (aquaculture by-product) (DB-f), fresh wild bogue (low price trash species) (WB-f), discarded bogue agglutinated moist diet (DB-m) and wild bogue agglutinated moist diet (WB-m). Diets based on DB showed higher lipid content (19-26% dw) than those

based on WB (5-6% dw). Octopuses fed on DB based diets showed higher growth (1.5-1.9%/d) and higher protein efficiency ratio (0.64-0.69) than those fed on WB based diets (1.1-1.5%/d and 0.36-0.37, respectively), which suggests good utilization of dietary lipids and also a possible protein sparing effect by lipids in *O. vulgaris*. Octopuses fed on diets presented fresh showed a higher growth (1.9-1.5%/d) and a higher feed efficiency (62-65%) than those fed on agglutinated diets (1.1-1.5%/d and 52-60%, respectively). Regarding fatty acids, the digestive gland clearly reflected dietary lipids and fatty acid profile, while muscle showed a more stable composition. Low dietary ARA content reflected in octopus tissues, especially in specimens fed on DB based diets, which did not seem to affect growth during the experimental period.

Keywords: Octopus, Dietary lipids, Agglutinated diets, Fatty acids, ARA, Growth.

8.2. Introduction

Octopus vulgaris is a preferred species to diversify aquaculture for its fast growth, low food conversion ratio and wide market demand (Vaz-Pires *et al.,* 2004). Despite several promising results, paralarvae rearing still constrains octopus industrial development (Iglesias *et al*., 2007a). According to these authors, high mortality in early stages is related to the nutritional imbalance of live prey and enrichments commonly used in fish larval rearing, so specific experimental enrichments and microdiets are currently being developed and tested. In contrast, the ongrowing of wild octopus juveniles has shown promising results (Rodríguez *et al*., 2006; García García *et al*., 2009) and a few companies in Spain have been pioneers in octopus farming, using low price trash species as food (provided by fisheries), which raises an issue regarding the

sustainability of this activity. Nowadays the aquaculture industry has an increasing demand for fish meals and fish oil (Sargent and Tacon, 1999) and, consequently, several alternative sources (vegetable and animal) have been tested as partial replacement in aqua-feeds (Izquierdo *et al*., 2005; Montero *et al*., 2008). An alternative source must be available, cheap and do not interfere with human markets. In this manner, little attention has been paid to aquaculture by-products. Indeed, juveniles from several fish species (*Boops boops*, *Sardina spp.*, *Sarpa salpa* or *Mugil cephalus*) come into fish farms cages through the net mesh or occasional net holes, feeding on manufactured compound diets and growing along with the target species (*Sparus aurata*, *Dicentrarchus labrax*) until the end of the growing cycle. Bogue (*Boops boops*, L. 1758) is the most abundant "discarded" species in Mediterranean and Eastern Central Atlantic fish farms, which can reach at least 2-5% of final biomass in gilthead sea bream production cages. These fishes, which are discarded at harvesting, have no market value and no interest for human consumption, and could be used directly (fresh/frozen) or as potential source of fish origin raw materials in the development of aqua-feeds.

In addition, testing natural and alternative diets allowed the compilation of several conclusions regarding the nutritional requirements of *O. vulgaris*. For instance, O'Dor *et al*. (1984) suggested that protein sources are the main energy source in *O. vulgaris* feeds, whereas lipids are poorly digested and inefficiently utilized. Contrarily, Mazón *et al*. (2007) observed higher dietary lipid digestibility in this species than previous studies, related to the low lipid content in the tested diets. Indeed, crustaceans (main natural preys) (Quetglas *et al*., 1998) and octopus muscle contain low levels of lipids (5-10% dw) (Rosa *et al*., 2004; Cerezo Valverde *et al*., 2008), which

suggests a low requirement for dietary lipids (Phillips *et al*., 2001). However, high lipid content in digestive gland confers this organ a key role in *O. vulgaris* metabolism, acting as lipid and energy storage (García Garrido *et al*., 2010). Moreover, a reduction in lipid content in digestive gland, associated to a decrease in triglycerides and sterol esters has been observed in starved *O. vulgaris*, while lipid composition in muscle was more stable (García Garrido *et al*., 2010). Besides, other authors suggested that dietary lipids for cephalopods must be rich in phospholipids and cholesterol (Navarro and Villanueva, 2000). Regarding fatty acid profile in octopods, several authors underlined the importance of polyunsaturated fatty acids (PUFA), especially EPA (20:5n-3) and DHA (22:6n-3) (Navarro and Villanueva, 2000; 2003; Miliou *et al*., 2006; 2007; García Garrido *et al*., 2010, 2011). These studies suggest a certain utilization of dietary lipids by *O. vulgaris*, despite the importance of dietary protein as the main energy source.

The lack of an octopus specific compound diet has restricted the research on nutrient utilization in this species. Indeed, *Octopus vulgaris* displays a complex pre-handling behaviour (Fiorito and Gherardi, 1999), which disaggregates feeds limiting ingestion rates. Recently, the use of different binders added to raw materials has contributed to solve this problem in *O. vulgaris* (Cerezo Valverde *et al*., 2008; Quintana *et al*., 2008; Rosas *et al*., 2008).

Therefore, the aim of the present study was to evaluate growth, survival, biochemical composition and dietary nutrients utilization in *O. vulgaris* fed bogue, discarded from fish farms. In order to evaluate the nutrient utilization of high lipid discarded bogue, this treatment was confronted with low lipid wild bogue. Finally, a binder was tested on feeds based on both types of bogue, in order to select a base raw material towards the development of octopus compound diets.

8.3. Materials and Methods

8.3.1. Capture and acclimatization of the stock

Wild octopuses were caught by local fishermen using cylindrical trawls (1.5 m diameter, 0.4 m high with metallic net of 31.6 mm mesh) placed at 20-30 m depth in the coast of Mogán (Canary Islands, Spain). Octopuses were transported to GIA facilities in three 0.5 m^3 square tanks provided with pure oxygen. One week acclimatization period was carried out in rectangular 1.5 m^3 tanks, provided with PVC tubes as shelters, shadowing nets and open flow-through seawater system (1500 L/h). During this period octopus were fed *ad libitum* once a day with a mixed diet containing de-frozen crab (*Portunus pelagicus*, L. 1758) and wild and discarded bogue, supplied on alternate days.

8.3.2. Rearing conditions

Rearing trials were performed in 4 rectangular tanks (1.5 m^3 capacity). In order to minimize interaction between animals, each tank was internally divided with PVC nets (2 cm mesh) into 4 compartments (0.8x1x0.5 m, 0.4 m^3), to keep each octopus individually. Each compartment was provided with a PVC tube (160 mm diameter) as a shelter and all tanks were covered with shadowing nets. The assays lasted 4 weeks (October 6th - November 3rd) under natural photoperiod and open flow-through sea water system (1.5 m^3/h). Mean water temperature and oxygen levels, measured once a day, were 21.9 ± 0.6ºC and 6.5 ± 0.2 mg/L, respectively.

8.3.3. Experimental design and diets

A total of 16 octopuses (initial weight: 886 ± 150 g, N = 4 per diet) were acclimatized to the individual rearing system for another week prior to the beginning

of the experiment. In order to avoid reproductive processes during the trial, only males were selected (Estefanell *et al*., 2010b). Each diet was tested per quadruplicate, that is, on four individually reared octopuses. All tested diets were based on the use of bogue, either discarded from local fish farms (aquaculture by-product) or wild provided by local fishermen (low price trash species). Both types of bogue were also used as agglutinated moist diets combined with a binder. Thus, the following four diets were tested:

1. Discarded bogue moist diet (DB-moist)
2. Wild bogue moist diet (WB-moist)
3. Fresh discarded bogue (DB-fresh)
4. Fresh wild bogue (WB-fresh)

Octopuses were fed to satiation 6 days per week (10:00 am) removing uneaten food the day after (8:00 am).

8.3.4. Agglutinated moist diets

Commercial alginate and calcium were used as binders in agglutinated moist diets (Pokel Merls and Pokel Cals, Productos del Sur, S. A., Murcia, Spain) (Cerezo Valverde *et al*., 2008). Both alginate and calcium were dissolved separately in distilled water as shown in Table 8.1. Fish fillets, cleaned from bones and scales, were carefully blended and mixed firstly with the alginate and finally with the calcium solution. The mixture was then poured into a plastic tray (50x30x7cm) and left for 24 h to solidify at 4ºC. Portions of 400 g were vacuum-packed and stored at -20ºC. Single portions of DB-moist (90 ± 16 g) and WB-moist (86 ± 15 g) were provided to each treatment.

Table 8.1: Composition (g/kg) of discarded bogue moist diet (DB-m) and wild bogue moist diet (WB-m)

		DB-m	WB-m
Alginate solution	Alginate (g)	20	20
	Water (mL)	280	280
Calcium solution	Calcium (g)	10	10
	Water (mL)	90	90
	Fish (fillets) (g)	600	600

8.3.5. Fresh bogues

Head, tail and viscera were removed from fresh bogues.. Single portions of DB-fresh (84 ± 14 g) or WB-fresh (81 ± 20 g) were provided daily to each octopus.

8.3.6. Biological parameters

All individuals were weighted at the beginning and weekly until the end of the experimental period. The following parameters were calculated individually per octopus:

- Specific Growth Rate: $SGR = (LnW_f - LnW_i)*100/t$
- Specific Feed Intake: $SFI = (FI/t)*100/W_a$
- Specific Protein Intake: $SPI = (IP/t)*100/W_a$
- Specific Lipid Intake: $SLI = (IL/t)*100/W_a$
- Specific Energy Intake: $SEI = ((FI/t)*GE/1000)/W_a$
- Protein Efficiency Ratio: $PER = (W_f$ in dry weight - W_i in dry weight)/IP
- Protein Productive Value in muscle: $PPV_M = 100*((W_f*P_f - W_i*P_w)/IP)$
- Feed Conversion Ratio: $FCR = FI/(W_f - W_i)$
- Feed Efficiency: $FE = (W_f - W_i)*100/FI$

(Where W_f = final weight (g); W_i = initial weight (g); W_a = average weight between sampling (g); t = total time (d); FI = feed intake per octopus (g); P_f = final %

protein in muscle (wet weight) for each octopus; P_w = average % protein in muscle (wet weight) in wild octopuses; IP = ingested protein (g) and GE = gross energy (kJ/g of feed)).

To estimate FI, the following formula was applied: $FI = F_p - 0.9*F_r$; where FI is ingested food (g); F_p is food provided (g); F_r is removed food (g). This formula was obtained after weighting 10 times each food item before and after 24 hours immersion in sea water, in the same conditions as the experimental tanks. Remaining feed fragments were removed by water vacuum every 2 days, dried in an oven at 105ºC to constant weight and subtracted proportionally from each octopus total ingested food.

Additionally, digestive gland index (DGI) was individually measured at the end of the experimental period ($DGI = W_{DG} / W_f$) where W_{DG} was digestive gland total weight (g) and W_f is the final weight (g). Mortality was evaluated every day.

8.3.7. Sampling procedure

The edible fraction from each type of food was sampled three times per week in order to obtain a pull from each week. In this manner, each diet would be analysed four times during the experimental period. On the other hand, at the beginning of the experimental period four wild octopuses males (weight: 1124 ± 246 g) and, at the end, all reared octopuses from each dietary treatment were sacrificed by immersion in ice-cold sea water. A sample from muscle and digestive gland was taken from each individual. Ink bag was previously emptied and muscle sample was taken from whole left arm II. All individual samples were homogenized separately and immediately frozen at -80ºC until biochemical determinations.

8.3.8. Biochemical analysis

Proximate composition from diets and octopus tissues from each treatment

were analysed following standard procedures of AOAC (1997). Moisture was determined after drying the sample in an oven at 105ºC to constant weight; ash by combustion in a muffle furnace at 600ºC for 12 hours; protein content (N × 6.25) was determined by Kjeldahl method and crude lipid was extracted following the method described by Folch *et al*. (1957). Fatty acids methyl esters from total lipids were extracted by transmethylation as described by Christie (1982) and separated by gas chromatography under the conditions described by Izquierdo *et al*. (1992). Gross energy was estimated using the Miglavs and Jobling (1989) energy coefficients: protein 23.6 kJ/g, lipid 38.9 kJ/g and carbohydrate 16.7 kJ/g. All analyses were conducted in triplicate.

8.3.9. Statistical analysis

All data, presented as mean ± standard deviation, were tested for normality (asymmetry, kurtosis) and homogeneity of variances (Levene test). Data (biological parameters, macronutrient composition and fatty acids profile from diets, muscle and digestive gland) were analyzed with the statistical computer package SPSS v15 (SPSS, Chicago, IL, USA), using the following General Linear Model with "bogue origin" (discarded vs wild) and "diet presentation" (fresh bogue vs moist diets) as fixed factors (two way ANOVA):

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \beta X_{ij} + \varepsilon_{ij}$$

Where μ is the population mean, α_i the fixed effect of the bogue origin, β_j the fixed effect of the diet presentation, $(\alpha\beta)_{ij}$ the interaction between the factors, βX_{ijk} is regression of each biological parameter on initial weight within origin and presentation factors, and ε_{ijk} the residual error. βX_{ijk} was removed from the model to compare initial weight and macronutrient composition and fatty acids profile from each diet. Two way

ANOVA significant differences were expressed in a right margin extra column on each table. When data from wild octopuses was included in the model, only “dietary treatment” as fixed factor was applied (one way ANOVA) and significant difference were expressed by different letter within a row. In this case, when differences were found, a *post-hoc* Bonferroni test was used to determine the homogeneous subsets.

The Pearson correlation coefficient (r) was calculated between:

1) SPI and the final absolute protein content in digestive gland and muscle.

2) SLI and the final absolute lipid content in digestive gland and muscle.

(Final protein or lipid content in digestive gland = digestive gland weight (g) * protein or lipid content in digestive gland (% wet weight); Final protein or lipid content in muscle = eviscerated whole animal weight (excluding the beak) * protein or lipid content in muscle (% wet weight)). The correlation was considered significant when $P < 0.05$.

To visualizate affinities in the fatty acid profiles, for both the diet and the digestive gland according to the origin and presentation, a nm-MDS ordination was carried out on untransformed data calculated from Bray-Curtis dissimilarities. To determine whether the fatty acid profile from the digested gland overlapped the fatty acid profile from the diet, we estimated the ρ correlation coefficient between both dissimilarity matrices. The correlation was considered significant when $P < 0.05$.

8.4. Results

8.4.1. Proximate composition of the diets

Discarded bogue origin and moist presentation showed higher lipid and GE, together with a lower protein content than wild bogue origin and fresh presentation

(Table 8.2). Finally, wild bogue origin and moist presentation showed higher ash and moisture content when confronted with discarded origin and fresh presentation.

Table 8.2: Macronutrient composition of each diet (% dry substance) and gross energy (GE, kJ/100 g food wet weight) (mean ± SD, N = 4).

Diets	DB-moist	WB-moist	DB-fresh	WB-fresh	P < 0.05
Lipids (%)	26.3 ± 1.3	6.2 ± 2.0	18.7 ± 2.0	4.6 ± 1.8	O-P
Proteins (%)	59.1 ± 2.7	77.7 ± 4.5	75.2 ± 2.8	88.0 ± 2.5	O-P
Moisture (%)	77.2 ± 0.7	83.0 ± 0.6	73.5 ± 0.5	78.2 ± 1.6	O-P
Ash (%)	2.2 ± 0.1	2.4 ± 0.2	1.3 ± 0.0	1.6 ± 0.2	O-P
GE (KJ/100 g)	571 ± 21	362 ± 24	669 ± 19	495 ± 54	O-P

"O" and "P" indicates significant difference according to Origin and Presentation of the bogue respectively (P < 0.05).

8.4.2. Biological parameters

Survival rate was 100% and all diets were well accepted by octopuses. Diets based on discarded bogue induced significantly higher SFI, SLI and PER values than those based on wild bogue regardless of diet presentation. Diets based on discarded bogue and fresh presentation induced significantly higher SPI, SEI and PPV_M than those based on wild bogue and moist presentation, respectively. Subsequently, octopuses fed on discarded bogue origin and fresh presentation diets showed significantly higher SGR and final weight than octopus fed on wild bogue origin and moist presentation diets, respectively (Table 8.3). Fresh diets induced higher FE and lower FCR than the agglutinated moist diets regardless of bogue origin.

Finally, DGI was higher in octopuses fed on discarded than wild bogue based diets, regardless of presentation. This parameter was positively correlated with SGR (R^2=0.7). DGI in wild octopuses (3.0 ± 0.6%) was similar to DGI calculated in reared octopuses.

Table 8.3: Effect of bogue origin and diet presentation on biological parameters of octopus after four weeks of feeding (mean ± SD, N = 4)

	DB-moist	WB-moist	DB-fresh	WB-fresh	P<0.05
Initial weight (g)	918 ± 241	882 ± 130	864 ± 185	889 ± 118	-
Final weight (g)	1396 ± 316	1190 ± 221	1473 ± 278	1348 ± 190	O-P
SGR (%/d)	1.5 ± 0.2	1.1 ± 0.2	1.9 ± 0.2	1.5 ± 0.0	O-P
SFI (%/d)	2.6 ± 0.2	2.4 ± 0.3	3.2 ± 0.5	2.2 ± 0.5	O
SPI (%/d)	0.30 ± 0.05	0.30 ± 0.04	0.61 ± 0.09	0.41 ± 0.09	O-P
SLI (%/d)	0.13 ± 0.04	0.02 ± 0.00	0.15 ± 0.02	0.02 ± 0.00	O
SEI (J/g d)	142 ± 14	84 ± 15	206 ± 35	106 ± 25	O-P
PER	0.69 ± 0.13	0.37 ± 0.12	0.64 ± 0.06	0.36 ± 0.10	O
PPV_M (%)	32.3 ± 4.0	18.1 ± 6.7	45.9 ± 7.1	27.8 ± 7.8	O-P
FCR	1.7 ± 0.1	1.9 ± 0.1	1.6 ± 0.1	1.6 ± 0.2	P
FE (%)	60.3 ± 2.3	51.6 ± 3.5	61.5 ± 4.4	64.5 ± 7.8	P
DGI (%)	3.4 ± 0.5	2.6 ± 0.9	4.3 ± 0.5	2.6 ± 0.6	O

"O" and "P" indicates significant difference according to Origin and Presentation of the bogue respectively ($P < 0.05$)

8.4.3. Proximate composition of muscle and digestive gland

Proximate composition of digestive gland was affected by bogue origin but not by diet presentation. Digestive gland in octopuses fed on discarded bogue based diets showed higher lipid and lower protein, moisture and ash content than octopuses fed on wild bogue based diets (Table 8.4). In comparison to wild specimens, reared octopuses showed lower ash content in digestive gland, while lipid and protein content in this organ were similar to wild bogue fed octopuses.

In contrast, muscle proximate composition was affected by diet presentation but not by bogue origin. Octopuses fed on moist diets showed higher lipid and lower protein content than octopuses fed on fresh bogue based diets. In comparison to wild specimens, reared octopuses showed lower ash content in muscle, while in fresh bogue fed animals lipid content was higher than in wild and moist diets fed treatments (Table 8.4).

Table 8.4: Proximate composition (% dry substance) in digestive gland and muscle of *O. vulgaris* after four weeks of feeding (mean ± SD, N = 4)

		Wild	DB-moist	WB-moist	DB-fresh	WB-fresh	P < 0.05
Digestive gland	Lipids (%)	26.7 ± 4.3^{a}	46.2 ± 6.2^{b}	28.3 ± 3.5^{a}	61.6 ± 6.1^{c}	22.2 ± 5.6^{a}	O
	Proteins (%)	65.3 ± 5.1^{b}	44.9 ± 6.2^{a}	73.2 ± 3.2^{b}	37.5 ± 6.4^{a}	72.9 ± 7.5^{b}	O
	Moisture (%)	63.8 ± 1.2^{ab}	63.6 ± 4.0^{ab}	67.4 ± 3.1^{b}	60.7 ± 2.9^{a}	64.7 ± 1.8^{ab}	O
	Ash (%)	1.8 ± 0.0^{c}	1.5 ± 0.1^{b}	1.4 ± 0.2^{ab}	1.2 ± 0.1^{a}	1.6 ± 0.2^{bc}	O
Muscle	Lipids (%)	6.1 ± 0.2^{b}	5.5 ± 0.5^{b}	6.1 ± 0.6^{b}	4.4 ± 0.7^{a}	4.3 ± 0.3^{a}	P
	Proteins (%)	83.4 ± 4.1	82.8 ± 2.2	82.0 ± 2.5	88.4 ± 2.7	83.3 ± 4.3	P
	Moisture (%)	79.2 ± 1.8^{a}	83.4 ± 1.7^{b}	82.6 ± 1.1^{ab}	80.7 ± 1.2^{ab}	83.8 ± 1.9^{b}	-
	Ash (%)	2.1 ± 0.2^{b}	1.7 ± 0.1^{a}	1.7 ± 0.1^{a}	1.6 ± 0.1^{a}	1.9 ± 0.2^{a}	-

Different letters within a row denotes significant differences (one way ANOVA including wild octopus) (P < 0.05). "O" and "P" indicates significant difference according to Origin and Presentation of the bogue, respectively (P < 0.05).

8.4.4. Correlations between final nutrient content in octopus tissues and nutrient intake

All correlations were positive and lineal. By the end of the study, the final absolute lipid content in digestive gland (g) and the final absolute protein content in muscle (g) were significantly correlated with SLI (r = 0.83) and SPI (r = 0.67), respectively. On the contrary, final absolute protein content in digestive gland (g) and final absolute lipid content in muscle (g) were not significantly correlated with SPI (r = 0.42) and SLI (r = 0.20), respectively.

8.4.5. Fatty acids profile: diets, muscle and digestive gland

In the present experiment most abundant fatty acids in wild octopus tissues were considered, accounting for a 91.0-96.4% of total fatty acids in the samples analysed.

Wild bogue based diets showed higher relative contents of saturated fatty acids (particularly 16:0, palmitic acid and 18:0, stearic acid), n-3 HUFA (20:5n-3, EPA; 22:6n-3, DHA) and 20:4n-6 (ARA) than discarded bogue based ones (Table 8.5). However, in absolute values n-3 HUFA contents were similar between the two types of bogue, since

discarded bogue had four times higher lipid content. Discarded bogue based diets were more abundant in monoenes and n-9 (16:1n-7, palmitoleic acid; 18:1n-9, oleic acid; and 20:1n-9, eicosenoic acid), n-6 (18:2-n6, linoleic acid), and 18:3n-3 (linolenic acid) than wild bogue based diets. DHA/EPA ratio was higher and EPA/ARA lower in wild bogue origin and fresh presentation than discarded origin and moist presentation, respectively. DHA/ARA was higher in discarded bogue based diets regardless of diet presentation.

In digestive gland, main fatty acids in wild specimens in order of abundance were: 16:0, 18:1n-9, DHA, ARA and EPA (Table 8.6). In comparison, a decrease in ARA content and an increase in 18:2n-6 was observed in reared octopuses. In general, most fatty acids in digestive gland reflected the profile of the diet (Fig. 8.1), ρ correlation coefficient was 0.72 ($p=0.04$), denoting the influence of bogue origin in this organ. Accordingly, 18:1n-9 and 18:2n-6 showed higher values in octopuses fed on discarded bogue based diets, while 16:0, ARA and DHA showed higher values in octopuses fed on wild bogue based diets. EPA recorded similar values regardless of treatment. All fatty acid ratios showed lower values in wild than in reared octopuses. Octopuses fed on DB-fresh showed the highest DHA/ARA and EPA/ARA, while DHA/EPA was higher in wild bogue based diets regardless of presentation.

Table 8.5: Fatty acids profiles from each diet (% of total fatty acids) (mean ± SD, N = 4)

	DB-moist	WB-moist	DB-fresh	WB-fresh	P < 0.05
14:0	3.1 ± 0.1	2.4 ± 0.6	3.1 ± 0.2	1.8 ± 0.9	O
16:0	15.8 ± 1.6	18.3 ± 0.7	14.1 ± 0.4	19.2 ± 1.1	O
16:1n-7	4.4 ± 0.2	2.9 ± 0.6	4.3 ± 0.2	2.3 ± 0.7	O
16:2n-4	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.2	-
17:0	0.4 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	O
18:0	4.5 ± 0.7	6.7 ± 0.5	3.8 ± 0.1	6.8 ± 0.1	O
18:1n-9	23.6 ± 1.0	13.0 ± 2.7	23.4 ± 0.6	9.4 ± 2.1	O
18:1n-7	2.3 ± 0.1	2.2 ± 0.0	2.4 ± 0.1	2.0 ± 0.2	O
18:1n-5	0.2 ± 0.0	0.6 ± 0.3	0.2 ± 0.0	0.7 ± 0.4	O
18:2n-6	15.8 ± 0.7	8.5 ± 0.8	16.8 ± 0.5	8.5 ± 1.3	O
18:3n-3	2.3 ± 0.1	0.9 ± 0.3	2.3 ± 0.1	0.7 ± 0.3	O
20:1n-9	4.0 ± 0.4	1.8 ± 0.8	3.7 ± 0.1	1.8 ± 1.1	O
20:2n-6	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	-
20:4n-6	0.5 ± 0.0	2.6 ± 0.9	0.6 ± 0.0	2.8 ± 0.5	O
20:5n-3	3.3 ± 0.1	4.7 ± 0.5	3.4 ± 0.3	4.5 ± 1.4	O
22:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	O
22:5n-6	0.2 ± 0.0	2.1 ± 0.8	0.2 ± 0.0	2.4 ± 1.1	O
22:5n-3	1.6 ± 0.0	2.0 ± 0.2	1.8 ± 0.1	1.7 ± 0.4	-
22:6n-3	8.1 ± 0.2	24.1 ± 4.7	10.3 ± 0.7	30.1 ± 6.0	O-P
∑ Saturated	24.6 ± 2.4	28.8 ± 1.1	22.0 ± 0.5	29.2 ± 2.4	O
∑ Monoenes	39.4 ± 2.1	21.7 ± 3.9	38.2 ± 0.7	17.2 ± 3.4	O
∑ n-3	17.0 ± 0.4	32.9 ± 3.6	19.7 ± 1.0	37.4 ± 3.4	O-P
∑ n-6	18.0 ± 0.8	14.8 ± 1.2	19.2 ± 0.6	12.6 ± 3.2	O
∑ n-9	28.3 ± 1.4	15.2 ± 3.6	27.7 ± 0.6	11.7 ± 3.1	O
∑ n-3 HUFA	13.7 ± 0.3	31.3 ± 4.2	16.3 ± 0.9	36.8 ± 4.7	O-P
DHA/EPA	2.5 ± 0.1	5.3 ± 1.4	3.0 ± 0.3	7.4 ± 1.4	O-P
DHA/ARA	16.7 ± 0.4	10.0 ± 2.3	18.7 ± 0.8	10.9 ± 1.1	O
EPA/ARA	6.7 ± 0.1	1.7 ± 0.3	6.2 ± 0.5	1.3 ± 0.2	O-P

"O" and "P" indicates significant difference according to Origin and Presentation of the bogue respectively (P < 0.05)

Table 8.6: Fatty acids profile (% of total fatty acids) in digestive gland of *O. vulgaris* after four weeks of feeding (mean ± SD, N = 4)

	Wild	DB-moist	WB-moist	DB-fresh	WB-fresh	P < 0.05
14:0	2.2 ± 0.6^{a}	3.0 ± 0.3^{b}	2.4 ± 0.2^{ab}	2.7 ± 0.1^{ab}	2.6 ± 0.1^{ab}	O
16:0	18.1 ± 1.8^{ab}	16.0 ± 4.3^{ab}	21.2 ± 3.8^{ab}	12.3 ± 0.8^{a}	17.8 ± 0.4^{b}	O
16:1n-7	3.7 ± 1.4^{ab}	4.2 ± 0.5^{ab}	3.2 ± 0.4^{ab}	4.1 ± 0.2^{b}	3.3 ± 0.3^{a}	O
16:2n-4	0.7 ± 0.3^{b}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	-
17:0	2.2 ± 0.5^{c}	0.4 ± 0.1^{a}	0.9 ± 0.2^{b}	0.4 ± 0.0^{a}	0.9 ± 0.1^{b}	O
18:0	8.2 ± 2.3^{ab}	5.5 ± 1.9^{ab}	9.1 ± 1.7^{b}	3.9 ± 0.2^{a}	7.7 ± 0.7^{b}	O
18:1n-9	13.3 ± 2.2^{a}	16.1 ± 2.0^{ab}	13.3 ± 1.7^{a}	19.4 ± 0.7^{b}	13.4 ± 1.5^{a}	O
18:1n-7	4.2 ± 0.9^{b}	1.8 ± 0.2^{a}	2.1 ± 0.2^{a}	2.2 ± 0.1^{a}	2.4 ± 0.3^{a}	O-P
18:1n-5	2.4 ± 0.9^{b}	0.5 ± 0.2^{a}	1.0 ± 0.5^{a}	0.3 ± 0.1^{a}	1.1 ± 0.3^{a}	O
18:2n-6	1.2 ± 0.0^{a}	15.9 ± 2.2^{c}	6.8 ± 0.9^{b}	18.6 ± 1.0^{c}	5.1 ± 1.5^{b}	O
18:3n-3	0.8 ± 0.5^{a}	1.7 ± 0.3^{b}	0.8 ± 0.1^{a}	2.0 ± 0.1^{b}	0.8 ± 0.1^{a}	O
20:1n-9	3.2 ± 0.3^{ab}	3.0 ± 0.3^{a}	2.6 ± 0.1^{a}	4.0 ± 0.4^{bc}	4.2 ± 0.5^{c}	P
20:2n-6	1.2 ± 0.4^{a}	1.9 ± 0.3^{a}	1.6 ± 0.2^{a}	3.1 ± 0.9^{b}	1.9 ± 0.3^{a}	O-P
20:4n-6	10.6 ± 2.5^{d}	1.9 ± 0.3^{b}	3.5 ± 1.0^{bc}	1.1 ± 0.1^{a}	3.8 ± 0.7^{c}	O
20:5n-3	5.4 ± 1.8	6.1 ± 0.5	5.6 ± 0.5	5.0 ± 0.5	6.2 ± 0.5	-
22:4n-6	0.0 ± 0.0^{a}	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	-
22:5n-6	1.3 ± 0.4^{b}	0.4 ± 0.0^{a}	1.0 ± 0.0^{b}	0.3 ± 0.0^{a}	1.2 ± 0.2^{b}	O
22:5n-3	1.2 ± 0.2^{a}	1.9 ± 0.2^{b}	1.4 ± 0.1^{a}	1.9 ± 0.2^{b}	1.4 ± 0.2^{a}	O
22:6n-3	11.0 ± 3.4^{a}	13.8 ± 1.1^{a}	18.1 ± 1.5^{b}	11.4 ± 1.1^{a}	19.7 ± 1.5^{b}	O
∑ Saturated	32.1 ± 4.2^{b}	25.4 ± 6.6^{ab}	34.6 ± 6.0^{b}	19.7 ± 1.1^{a}	30.1 ± 1.1^{b}	O-P
∑ Monoenes	29.7 ± 4.6^{ab}	28.1 ± 3.2^{ab}	23.6 ± 2.5^{a}	33.7 ± 1.7^{b}	26.1 ± 2.0^{a}	O-P
∑ n-3	20.0 ± 3.9^{a}	24.7 ± 1.5^{ab}	27.1 ± 2.0^{b}	21.7 ± 1.8^{a}	29.5 ± 1.7^{b}	O
∑ n-6	16.0 ± 3.1^{a}	20.7 ± 2.1^{b}	13.7 ± 1.7^{a}	23.8 ± 1.4^{b}	13.3 ± 1.1^{a}	O
∑ n-9	17.2 ± 3.0^{a}	19.7 ± 2.4^{ab}	16.7 ± 1.8^{a}	24.4 ± 1.1^{b}	18.5 ± 1.9^{a}	O-P
∑ n-3 HUFA	18.3 ± 3.7^{a}	22.4 ± 1.3^{ab}	25.8 ± 2.0^{bc}	19.1 ± 1.7^{a}	28.0 ± 1.7^{c}	O
DHA/EPA	1.6 ± 0.2^{a}	2.3 ± 0.3^{b}	3.2 ± 0.1^{c}	2.3 ± 0.2^{b}	3.2 ± 0.3^{c}	O
DHA/ARA	0.9 ± 0.1^{a}	7.5 ± 1.4^{c}	5.4 ± 0.9^{b}	10.8 ± 0.8^{d}	5.2 ± 0.7^{b}	O-P
EPA/ARA	0.5 ± 0.1^{a}	3.3 ± 0.7^{c}	1.7 ± 0.3^{b}	4.7 ± 0.4^{d}	1.6 ± 0.3^{b}	O-P

Different letters within a row denotes significant differences (one way ANOVA including wild octopus) (P < 0.05). “O” and “P” indicates significant difference according to Origin and Presentation of the bogue, respectively (P < 0.05).

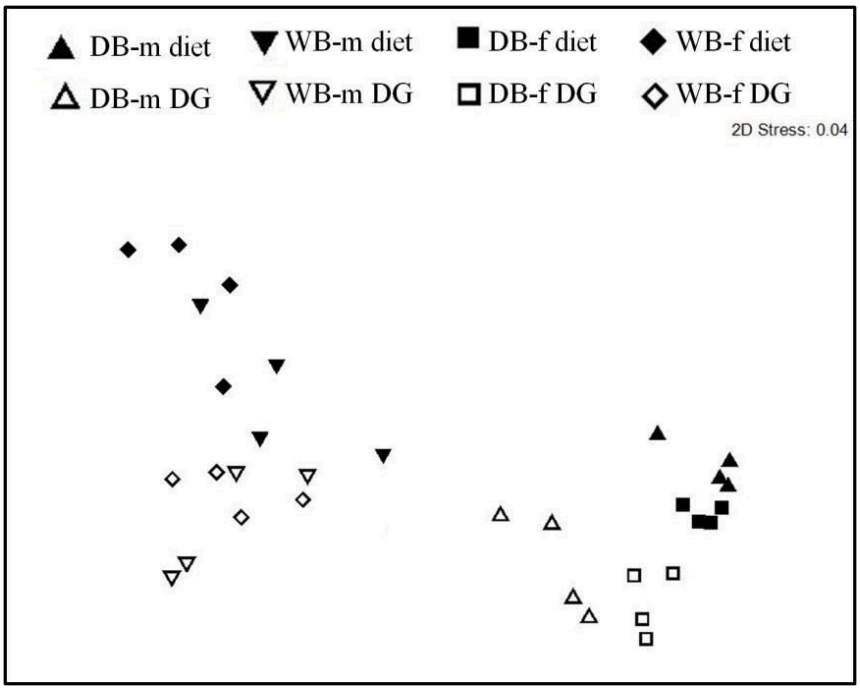

Fig. 8.1: Ordination (multidimensional scaling, MDS) of fatty acid profiles found in the diet and in the digestive gland of *O. vulgaris* after 4 weeks of feeding (DB-m = discarded bogue moist; WB-m = wild bogue moist; DB-f = discarded bogue fresh; WB-f = wild bogue fresh t; DG = digestive gland).

In muscle, main fatty acids in wild specimens in order of abundance were: DHA, 16:0, ARA, EPA and 18:1n-9 (Table 8.7). Reared octopuses showed a lower content of ARA and n-6, especially in discarded bogue based diets. EPA, DHA and 18:1n-9 contents were not affected by dietary treatment. Saturated content was higher in muscle from octopuses fed on fresh than on moist diets regardless of bogue origin. DHA/EPA showed similar values in this organ regardless of treatment. Octopuses fed on discarded bogue origin and fresh presentation diets showed higher levels of DHA/ARA and EPA/ARA ratios than their confronting treatments, all higher than wild specimens.

Table 8.7: Fatty acids profile (% of total fatty acids) in muscle of *O. vulgaris* after four weeks of feeding (mean ± SD, N = 4)

	Wild	DB-moist	WB-moist	DB-fresh	WB-fresh	P < 0.05
14:0	0.5 ± 0.0^{a}	0.7 ± 0.2^{ab}	0.6 ± 0.1^{ab}	1.0 ± 0.3^{b}	0.7 ± 0.1^{ab}	-
16:0	15.8 ± 0.5^{a}	18.2 ± 1.0^{abc}	17.5 ± 1.5^{ab}	20.4 ± 1.8^{c}	19.9 ± 0.1^{bc}	P
16:1n-7	0.6 ± 0.0^{c}	0.4 ± 0.1^{ab}	0.3 ± 0.1^{a}	0.5 ± 0.1^{bc}	0.3 ± 0.0^{a}	O
16:2n-4	0.2 ± 0.0^{a}	0.8 ± 0.2^{b}	0.7 ± 0.2^{ab}	0.5 ± 0.1^{a}	0.8 ± 0.0^{ab}	-
17:0	1.9 ± 0.0^{c}	1.6 ± 0.2^{bc}	1.5 ± 0.3^{bc}	1.2 ± 0.4^{b}	1.8 ± 0.2^{c}	-
18:0	7.1 ± 0.2^{b}	5.8 ± 0.1^{a}	6.1 ± 0.8^{a}	5.5 ± 0.3^{a}	5.6 ± 0.2^{a}	-
18:1n-9	8.4 ± 0.9	10.3 ± 0.5	10.5 ± 1.5	9.7 ± 0.4	9.7 ± 0.7	-
18:1n-7	2.2 ± 0.0^{a}	2.5 ± 0.5^{ab}	2.2 ± 0.1^{a}	3.0 ± 0.3^{b}	2.3 ± 0.2^{a}	O
18:1n-5	1.8 ± 0.1^{b}	1.2 ± 0.1^{a}	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}	1.3 ± 0.2^{a}	-
18:2n-6	0.8 ± 0.1^{a}	0.9 ± 0.1^{ab}	0.7 ± 0.1^{a}	1.1 ± 0.1^{b}	0.8 ± 0.1^{a}	O-P
18:3n-3	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	-
20:1n-9	2.8 ± 0.1^{a}	3.2 ± 0.4^{ab}	3.0 ± 0.3^{ab}	3.5 ± 0.3^{b}	3.1 ± 0.2^{ab}	-
20:2n-6	0.8 ± 0.1^{a}	0.9 ± 0.2^{ab}	0.8 ± 0.1^{ab}	1.2 ± 0.2^{b}	0.9 ± 0.1^{ab}	P
20:4n-6	14.3 ± 0.9^{d}	9.4 ± 0.5^{b}	11.4 ± 0.5^{c}	6.9 ± 1.0^{a}	10.0 ± 0.9^{bc}	O-P
20:5n-3	11.3 ± 0.5	11.7 ± 0.9	10.4 ± 2.0	11.9 ± 1.2	10.3 ± 0.6	-
22:4n-6	0.1 ± 0.0^{a}	1.4 ± 0.2^{bc}	1.9 ± 0.8^{c}	1.0 ± 0.2^{b}	1.5 ± 0.3^{bc}	O
22:5n-6	1.3 ± 0.1^{b}	1.0 ± 0.1^{ab}	1.3 ± 0.3^{b}	0.7 ± 0.2^{a}	1.4 ± 0.2^{b}	O
22:5n-3	1.8 ± 0.1	1.8 ± 0.2	1.9 ± 0.3	1.7 ± 0.2	1.6 ± 0.1	-
22:6n-3	25.0 ± 1.5	24.0 ± 1.4	24.1 ± 1.2	24.2 ± 2.0	23.0 ± 0.6	-
∑ Saturated	25.8 ± 0.4	26.7 ± 0.9	26.1 ± 1.5^{a}	28.5 ± 2.2	28.4 ± 0.3	P
∑ Monoenes	16.6 ± 0.7^{a}	19.7 ± 1.3^{ab}	18.9 ± 2.4^{ab}	20.0 ± 1.0^{b}	19.0 ± 0.7^{ab}	-
∑ n-3	39.3 ± 0.7	38.1 ± 2.3	36.9 ± 2.9	38.6 ± 2.5	35.5 ± 0.6	-
∑ n-6	17.5 ± 1.2^{d}	14.0 ± 0.6^{b}	16.4 ± 0.9^{cd}	11.1 ± 1.3^{a}	14.8 ± 1.1^{bc}	O-P
∑ n-9	11.4 ± 1.2	13.6 ± 0.5	13.6 ± 1.8	13.3 ± 0.6	13.0 ± 0.8	-
∑ n-3 HUFA	38.6 ± 0.8	37.8 ± 2.3	36.7 ± 2.8	38.3 ± 2.5	35.2 ± 0.7	-
DHA/EPA	2.2 ± 0.2	2.0 ± 0.1	2.4 ± 0.4	2.0 ± 0.2	2.2 ± 0.2	-
DHA/ARA	1.8 ± 0.2^{a}	2.6 ± 0.2^{b}	2.1 ± 0.2^{ab}	3.5 ± 0.4^{c}	2.3 ± 0.2^{b}	O-P
EPA/ARA	0.8 ± 0.0^{a}	1.3 ± 0.1^{b}	0.9 ± 0.2^{ab}	1.7 ± 0.3^{c}	1.0 ± 0.1^{ab}	O-P

Different letters within a row denotes significant differences (one way ANOVA including wild octopus) (P < 0.05). "O" and "P" indicates significant difference according to Origin and Presentation of the bogue, respectively (P < 0.05).

8.5 Discussion

Fish farms attract multi-species schools of pelagic fish (Dempster *et al*., 2002), being bogue *Boops boops* one of the most abundant (Boyra *et al.*, 2004; Arechavala López *et al*., 2011). Accordingly, bogue is the most frequent "discarded" species in Mediterranean aquaculture, and represents a potential source of fish origin raw

materials for the production of aqua-feeds. In the present experiment, high growth observed in octopus fed on discarded bogue was similar to other studies feeding diets containing crustaceans, known to promote high growth in *O. vulgaris* (García García and Cerezo Valverde, 2006; Prato *et al*., 2010), and even higher than those containing other fish species (García García and Aguado Giménez, 2002; Aguado Giménez and García García, 2002; Petza *et al*., 2006).

Despite high energy content in discarded bogue, octopus fed this food item showed a high feed intake which could be related to the lipid composition of discarded bogue. The type or amount of lipid contained in this food item, which probably reflects the ingestion of commercial compound diets from fish farms, may act as feed stimulant for *O. vulgaris*, since these nutrients are responsible for taste in food (Mottram, 1998; De Roos, 2005). Also, high lipid content in discarded bogue, together with fast growth, suggests a good utilization of dietary lipids in *O. vulgaris*. Recent data showed that during starvation *O. vulgaris* effectively catabolises sterol esters, triglycerides and monoenes (García Garrido *et al*., 2010), suggesting their mobilization as energy sources for metabolic maintenance. Indeed, discarded bogue is rich in monoenes (40% of total fatty acids), reported as energy source in marine organisms (Sargent *et al*., 1995). Also, the use of lipids as a potential energy source has been suggested in other cephalopods species (Semmens, 1998; Moltschaniwskyj and Johnston, 2006).

Moreover, increase in dietary lipids in octopus fed discarded bogue markedly increased PER and PPV denoting a better utilization of dietary protein and suggesting the sparing effect of proteins. In this manner, the catabolism of dietary lipids would partially cover the energy requirements of *O. vulgaris*, allowing the utilization of

dietary proteins for a faster growth in octopus fed high lipid bogue. The sparing effect of proteins by dietary lipids has been widely described in fish and other vertebrates (Vergara *et al*., 1996).

Both fresh and moist diets induced higher feed efficiencies than previous studies (Cerezo Valverde *et al*., 2008; Quintana *et al.*, 2008; Biandolino *et al*., 2010; García Garrido *et al*., 2011). This underlines the good nutritional value of bogue as a valuable ingredient for formulated feeds for this species. Growth rates observed with discarded bogue moist diets showed higher values than previous studies using alginate as binder (Cerezo Valverde *et al*., 2008; García Garrido *et al*., 2011) and close to those using gelatine (Quintana *et al.*, 2008). The agglutinant effect of alginate and gelatine seem to largely depend on the characteristics of the raw materials (Cerezo Valverde *et al*., 2008; García Garrido *et al*., 2011), although an adverse effect of alginate on nutrient digestibility has been recently reported (Rosas *et al*., 2008; Seiça Neves *et al*., 2010). Nevertheless, the low growth observed in this study in octopuses fed on moist diets in comparison to those fed on fresh diets seems to be related with their lower energy content in relation to fresh ones, which lead to a lower energy intake. Thus, since different dietary GE was not compensated by SEI, and opposite to what happens in vertebrates (Lupatch *et al*., 2003), feed intake does not seem to be regulated by dietary energy content in *O. vulgaris*. Indeed, high feed intake has been observed in octopuses fed on crustaceans, which show low energy content (García García and Cerezo Valverde, 2006), suggesting that certain nutrients stimulate feed intake in *O. vulgaris*.

Lipid deposition in digestive gland and DGI increased according to dietary lipid content and SLI, which together with higher growth and accumulation of saturated and

monounsaturated fatty acids (55-60% of total fatty acids), denotes the effective utilization of digestive gland as an energy storage organ. In other cephalopods species the digestive gland reflected as well the fatty acid profile of the diet while muscle composition was more stable (Phillips *et al*., 2001; Stowasser *et al*., 2006; Fluckiger *et al*., 2008). High levels of linoleic and linolenic acids in digestive gland from octopus fed discarded bogue based diets is probably related to the inclusion of vegetable oils in commercial feed. Indeed, similar fatty acids were found in *Trachurus mediterraneus* and bogue aggregated to fish farms and fed on lost pellets (Fernández Jover *et al*., 2007; Arechavala López *et al*., 2011). On the contrary, the final protein content in digestive gland did not correlate with dietary input, while the final protein content in muscle was positively correlated with SPI, confirming the efficient dietary protein utilization in *O. vulgaris*. This is in agreement with fast protein digestion and mobilization to somatic growth found in previous studies (O'Dor and Webber, 1986).

Macronutrient content in octopus muscle was more constant than in digestive gland, which is in agreement with previous reports in cephalopods (Almansa *et al*., 2006; Ferreira *et al*., 2009). High growth rates in this species produced a decrease in lipid content in muscle, also noted in reared *O. vulgaris* (Biandolino *et al*., 2010). Macronutrient composition from wild juveniles showed a decrease in whole body lipid content as they increased weight (Navarro and Villanueva, 2003).

Palmitic acid, DHA and EPA were among the most abundant fatty acids in octopus tissues, while higher ARA content in wild specimens was found in the present experiment than in previous reports (Rosa *et al*., 2004; Miliou *et al*., 2007; Prato *et al*., 2010). ARA reduction in reared octopus tissues could be related to low dietary ARA. Thus, high growth rates observed in the present experiment emphasized ARA dietary

imbalance, which nevertheless did not seem to affect growth along the experimental period. A possible explanation would be the substitution of ARA by EPA during phospholipids' esterification, as occurs in fish (Bell *et al*., 1995). Indeed, ARA is mainly related to egg quality in fish (Fernández-Palacios et al., 1995; Izquierdo *et al*., 2001), which remains to be confirmed in *O. vulgaris* in further experiments. Regarding previous rearing trials, specific retention of this fatty acid in *O. vulgaris* after feeding deficient diets (Navarro and Villanueva, 2000; 2003) or after long term starvation (García Garrido *et al.*, 2010) underlined its importance for this species. On the other hand, increase in DHA/ARA and EPA/ARA in reared octopuses was higher than data reported by García Garrido *et al*. (2011).

In summary, high growth and feed intake observed in octopuses fed discarded bogue gives additional value to this by-product of the aquaculture industry. Present results suggest good dietary lipid utilization in *O. vulgaris*. Promising results induced by agglutinated moist diets should allow specific research on the nutritional requirements of this species. Digestive gland clearly reflected dietary lipid and fatty acid content, while octopus muscle was more stable, showing a slight decrease in lipid content together with increasing final weight. DHA and EPA content were similar in muscle between wild and reared specimens. Only ARA decreased in octopus tissues, probably related to the low dietary ARA content, which nevertheless did not affect growth during the experimental period.

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9. Estudio 5: "Growth, protein retention and biochemical composition in *Octopus vulgaris* fed on different diets based on crustaceans and aquaculture by-products"

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9.1. Abstract

The octopus, *Octopus vulgaris*, is one of the main targets for aquaculture diversification in Mediterranean countries. However, the development of octopus farming is limited by the lack of information regarding nutritional requirements of this species during its life cycle. In this study, five diets were tested on the biological performance (growth, protein retention and biochemical composition) of individually reared octopuses (N = 8 per diet), including three single diets constituted by: an endemic crab (the white crab, *Plagusia depressa*), a commercial crab imported frozen

(the blue crab, *Portunus pelagicus*), and bogue (*Boops boops*) discarded from fish farms (aquaculture by-product), as well as two mixed diets, containing a 60-40% of blue crab-bogue and white crab-bogue, respectively. The rearing period lasted 8 weeks. Octopuses that fed on a mixed diet constituted by blue crab-bogue showed a higher growth than those feeding on bogue as a single food item. No significant differences in growth were observed among individuals feeding on single food items. Highest protein retention was observed in octopuses fed on diets containing discarded bogue, associated with a high lipid and monoenes content in this food item, underlying the use of lipid as energy source in *O. vulgaris*. However, discarded bogue was deficient in ARA in comparison with octopus tissues, which did not seem to affect growth during the experimental period. These findings underline the potential of aquaculture by-products, particularly bogue, as an adequate diet for culturing *O. vulgaris.*

Keywords: *Octopus vulgaris*, Crab, Fish, Growth, Culture, By-product.

9.2. Introduction

Aquaculture production rose to ~73 million t in 2009, representing nearly 45% of total aquatic products. However, aquaculture growth worldwide has slowed down over the last decade, and in some areas, like the European Union, a negative growth has been observed (FAO, 2011). The low number of species cultured at industrial scale is partially responsible, among other factors, for the relative market saturation in the EU. Accordingly, diversification is one of the main targets of European aquaculture for the next decades.

Cephalopods are potential candidates for the aquaculture industry for a number of reasons: they have short life cycles, fast growth and efficient conversion rates, high prices and increasing market demand in several countries (Semmens *et al*., 2004; Vaz-Pires *et al.* 2004; Sykes *et al*., 2006). Particularly, the octopus, *O. vulgaris* (Cuvier, 1797), is one of the main targets for aquaculture diversification in Mediterranean countries (Iglesias *et al*., 2000). Currently, the development of octopus farming is limited by the lack of specific enrichments and compound diets, which would maximize growth and survival along the life cycle of this species (Garcia Garcia and Cerezo Valverde, 2006; Iglesias *et al*., 2007a). Indeed, high mortality in paralarval rearing is related to nutritional deficiencies of enriched *Artemia*, commonly used in fish larval rearing, especially in n-3 HUFA (Navarro and Villanueva, 2000, 2003). The ongrowing of wild sub-adults in floating cages using low price trash species as food (discarded from fisheries) has shown promising results (Rodriguez *et al*., 2006; Garcia Garcia *et al*., 2009), and a few companies in Spain have been pioneers in octopus farming. This activity, however, has showed a low profitability in comparison with other farmed species, due to the high cost of octopus juveniles and food (Garcia Garcia *et al*., 2004). The first experimental moist diets have been well accepted by octopods, producing a positive growth, although an octopus specific compound feed is not available yet (Rosas *et al*., 2007; Cerezo Valverde *et al*., 2008; Quintana *et al*., 2008; Estefanell *et al*., 2009a). A temporary solution to decrease production costs during octopus farming lays on the identification of low-price food items, easily available and with no interest for human markets, which may promote high growth rates in this species. In this sense, little attention has been given to aquaculture by-products, which represent a potential source of fish origin raw materials for the aquaculture industry.

This is especially interesting nowadays, as a result of the increasing demand of such raw materials in the production of aqua-feeds (Sargent and Tacon, 1999). Large aggregations of small pelagic fishes are typically found around off-shore cages (Dempster *et al*,. 2006), and some species can get into the cages through the net mesh or occasional net holes, feeding on commercial compound diets together with target species until harvesting. Along the Mediterranean and eastern Atlantic, the bogue, *Boops boops* (L. 1758), is the most abundant discard, representing at least a 2-5% of total production in sea bream cages (Socorro *et al*., 2005; Estefanell *et al*., 2009a, 2010b).

Biochemical analyses of octopus tissues and diets could provide valuable information regarding nutritional requirements for *O. vulgaris* and nutritional quality changes for human consumption purposes (Fluckiger *et al.*, 2008). Both octopus muscle (90-95% of total body weight) and those preys that have promoted a high growth (e.g. crab, squid) show high protein and low lipid content (Rosa *et al*., 2004; Cerezo Valverde *et al*., 2008; Garcia Garrido *et al*., 2010). These findings are in agreement with the mainly protein-based metabolism and the low capacity to oxidise lipids, which have been observed in cephalopods (O'Dor *et al*., 1984; Lee, 1994). However, the use of dietary lipids as an energy source has been observed in *O. vulgaris*, through a decrease in lipid content in digestive gland in unfed octopuses (Garcia Garrido *et al*., 2010), and by a high protein retention in octopus fed on a high lipid diet (Estefanell *et al*., 2009a). Also, several works have suggested that lipid digestibility in *O. vulgaris* largely depends on the quantity and quality of dietary lipids (Mazón *et al*., 2007; Sánchez *et al*., 2009; Seiça Neves *et al.*, 2010).

The aim of this work was to evaluate the biological performance (growth,

feeding rate and mortality rate) of octopuses feeding on three single diets constituted by: an endemic crab (the white crab, *Plagusia depressa* Fabricius 1775), a commercial crab (the blue crab, *Portunus pelagicus*, L. 1758) and discarded bogue (*B. boops*), the most abundant aquaculture by-product along the Mediterranean and eastern Atlantic. To test the effect of mixed diets, both crab species were also supplemented with discarded bogue. Diets, muscle and digestive gland from wild and reared animals were also analysed, so changes on proximate composition and fatty acids profile between initial (wild) and final (reared) individual were assessed.

9.3. Material and methods

9.3.1. Capture and acclimatization of individuals

Wild octopuses were caught by local fishermen using cylindrical trawls (1.5 m diameter, 0.4 m high), placed at 20-30 m depth in the coast of Mogán (Canary Islands, Spain). Octopuses were transported to lab facilities in three 0.5 m^3 square tanks provided with pure oxygen. Individuals were acclimatizated for one week in rectangular 1.5 m^3 tanks, where PVC tubes were provided as shelters. Sea water circulation was open flow-through (1500 L/h) and each tank was covered with a shadowing net. During this period, octopuses were fed *ad libitum* once a day on a mixed diet containing blue crab (*P. pelagicus*), white crab (*P. depressa*) and discarded bogue (*B. boops*), supplied on alternate days.

9.3.2. Rearing conditions

Rearing trials were performed in 5 rectangular tanks (1.5 m^3). In order to minimize interactions between animals, each tank was internally individualized using a PVC net (2 cm mesh) into 8 compartments (each ca. 0.4x1x0.5m, 0.2 m^3), so each

octopus was kept individually. Each compartment was provided with a T-shaped PVC tube (160 mm diameter) as a shelter, and all tanks were covered with shadowing nets, since benthic octopods spend most of their daily cycle out of light in dens in the natural environment (Hanlon and Messenger, 1996). The assays lasted 8 weeks (March 4th – April 29th) under natural photoperiod (approx. 12.5 hours of light and 11.5 hours of dark) using an open flow-through sea water system (1500 L/h). Mean water temperature and oxygen levels, measured once a day with a portable oxymeter (Oxiguard Handy, Point tour Systems Inc., Canada), were 20.3 ± 0.3ºC and 6.2 ± 0.3 mg/L (85 ± 5%), respectively. Salinity was 35‰.

9.3.3. Experimental design and diets

A total of 40 octopuses (1176 ± 230 g, N = 8 per diet) were PIT-tagged (Estefanell *et al*., 2011b) and acclimatized to the individual rearing system for another week prior to the beginning of the experiment. In order to avoid reproductive processes during the experiment, only males were selected (Estefanell *et al*., 2010b). The following five diets were tested:

1.White crab, *P. depressa* (WC)

2.Blue crab, *P. pelagicus* (BC)

3.Bogue, *B. boops*, discarded from fish farms (DB)

4.White crab, *P. depressa* + discarded bogue, *B. boops* (60-40% of total food supplied) (WC+DB)

5.Blue crab, *P. pelagicus* + discarded bogue, *B. boops* (60-40% of total food supplied) (BC+DB)

Octopuses were fed *ad libitum* 6 days per week (aprox. at 10:00 am), removing uneaten food every two days (aprox. at 8:00 am). In mixed diets, crab and fish were

provided on alternate days. White crab (55 ± 18 g), an endemic crab in the Canarian archipelago, was provided fresh by professional fishermen. Blue crab (517 ± 111 g), an indo-west Pacific crab, was provided frozen by a local fish trade company. Bogue, *B. boops* (166 ± 32 g), was provided by a local fish farm as a discarded species. Walking legs and main carapace were removed from crabs, while bogues were provided as a whole piece.

9.3.4. Biological parameters

All individuals were weighted at the beginning, at two intermediate points (3rd and 6th week) and at the end of the experimental period. The following parameters were calculated individually:

- Specific Growth Rate: $SGR = (LnW_f - LnW_i)*100/t$
- Daily Biomass Increment: $DBI = (B_f-B_i)/(B_i*t)$
- Specific Feed Intake: $SFI = (FI/t)*100/W_a$
- Specific Protein Intake: $SPI = (IP/t)*100/W_a$
- Specific Lipid Intake: $SLI = (IL/t)*100/W_a$
- Specific Energy Intake: $SEI = ((FI/t)*GE/1000)/W_a$
- Protein Efficiency Ratio: $PER = (W_f$ in dry weight - W_i in dry weight$)/IP$
- Protein Productive Value in muscle: $PPV_M = 100*((W_f*P_f - W_i*P_w)/IP)$
- Feed Efficiency: $FE = (W_f - W_i)*100/FI$
- Digestive Gland Index: $DGI = W_{DG} / W_f$

(Where W_f = Final weight (g); W_i = initial weight (g); B_f = final biomass (g); B_i = initial biomass (g); FI = feed intake per octopus (g); W_a = average weight between sampling (g); t = total time (d); IP = ingested protein (g); IL = ingested lipid (g); GE = gross energy (kJ/g of feed); P_f = final % protein in muscle (wet weight) for each

octopus; P_w = average % protein in muscle (wet weight) in wild octopuses; W_{DG} = digestive gland total weight (g).

To estimate ingested food (FI), uneaten food was removed 3 times per week and dried in an oven at 105ºC to constant weight. In mixed diets, crab and fish were weighted separately. The following formula was applied: $FI = F_p – (100*F_r/(100-M))$; where F_p is food provided (g); F_r is dried removed food (g); M is the diet moisture. Crumbs were also removed every week by water-vacuum the tanks, and were mainly small carapace bits, fish spines or fish scales. In mixed diets, those crumbs that were unequivocal from crab (carapace bits) or from fish (spines, scales) were also separated and quantified. Crumbs initial weight was estimated after drying in an oven at 105ºC to constant weight and subtracted proportionally from each octopus total ingested food. Mortality was evaluated every day.

9.3.5. Sampling procedure

The edible fraction from each type of food was sampled three times during the experimental period. Each sample was obtained from a pool of 6 individuals, randomly selected every 2-3 weeks of feeding. After transportation from the sea, eight wild octopuses (males, weight: 1187 ± 277 g) and all reared octopuses from each dietary treatment at the end of the experimental period, were sacrificed by immersion in ice-cold sea water. A sample from muscle and digestive gland was taken from each octopus. Muscle samples were taken from whole left arms II. From each tissue, three pools of 3-3-2 specimens were homogenized from each dietary treatment and stored at -80ºC until biochemical analysis. In those treatments were mortality occurred during the experimental period, at least 2 samples were included on each pool.

9.3.6. Biochemical analysis

Biochemical analysis followed standard procedures of AOAC (1997). Moisture was determined after drying the sample in an oven at 105ºC to constant weight; ash by combustion in a muffle furnace at 600ºC for 12 hours; protein content (N × 6.25) was determined by Kjeldahl method and crude lipid was extracted following the method described by Folch *et al*. (1957). Fatty acids from total lipids were prepared by transmethylation, as described by Christie (1982), and separated by gas chromatography under the conditions described by Izquierdo *et al*. (1992). Gross energy was estimated using the Miglavs and Jobling (1989) energy coefficients. All analyses were conducted in triplicate of pools. Proximate composition and gross energy in mixed diets were estimated from the profile of each single food item.

9.3.7. Estimation of proximate composition and fatty acid profile in mixed diets

The proximate compositions of mixed diets were estimated as a weighted average of the biochemical composition of each single food item and their contribution to total ingested food in the corresponding mixed diet. The fatty acid profiles of mixed diets were estimated as a weighted average of the fatty acid profile of each single food item and the contribution of each food item to total lipid content of each pool of mixed diet. Accordingly, the following formulas were used:

- $PC_{WC+DBi} = PC_{WCi} * (IF_{WC}/(IF_{WC}+IF_{DB1}) + PC_{DBi} * (IF_{DB1}/(IF_{WC}+IF_{DB1})$, i = pool 1, 2 or 3.

- $PC_{BC+DBi} = PC_{BCi} * (IF_{BC}/(IF_{BC}+IF_{DB2}) + PC_{DBi} * (IF_{DB2}/(IF_{BC}+IF_{DB2})$, i = pool 1, 2 or 3.

- $FA_{WC+DBi} = FA_{WCi} * ((L_{WCi} * (IF_{WC}/(IF_{WC}+IF_{DB1}))/L_{WC+DBi}) + FA_{DBi} * ((L_{DBi} * (IF_{DB1}/(IF_{WC}+IF_{DB1})) / L_{WC+DBi})$, i= pool 1, 2 or 3.

•FA_{BC+DBi} = FA_{BCi} * ((L_{BCi} * (IF_{BC}/(IF_{BC}+IF_{DB2}))/L_{BC+DBi}) + FA_{DBi} * ((L_{DBi} * (IF_{DB2}/(IF_{WC}+IF_{DB2})) / L_{BC+DBi}), i= pool 1, 2 or 3.

Where "PC_{WC+DBi}" is the proximate composition of the "white crab – discarded bogue" mixed diet (% dw), "PC_{WCi}" is the proximate composition of the white crab (% dw), "IF_{WC}" is average total ingested white crab (g ww), "IF_{DB1}" is average total ingested discarded bogue in white crab – discarded bogue mixed diet (g ww), "PC_{DBi}" is the proximate composition of the discarded bogue (% dw), "PC_{BC+DBi}" is the proximate composition of the "blue crab – discarded bogue" mixed diet (% dw), "PC_{BCi}" is the proximate composition of the blue crab (% dw), "IF_{BC}" is the average total ingested blue crab (g ww), "IF_{DB2}" is the average total ingested discarded bogue in the blue crab – discarded bogue mixed diet, "FA_{WC+DBi}" is the fatty acid profile of the white crab – discarded bogue mixed diet (% of total fatty acids), "FA_{WCi}" is the fatty acid profile of the white crab (% of total fatty acids), "L_{WCi}" is the lipid content of the white crab (% dw), "L_{WC+DBi}" is the total lipid content of the white crab – discarded bogue mixed diet (% dw), "FA_{DBi}" is the fatty acid profile of the discarded bogue (% of total fatty acids), "L_{DBi}" is the lipid content of the discarded bogue (% dw), "FA_{BC+DBi}" is the fatty acid profile of the blue crab - discarded bogue mixed diet (% of total fatty acids), "FA_{BCi}" is the fatty acid profile of the blue crab (% of total fatty acids), "L_{BCi}" is the lipid content in the blue crab (% dw) and "L_{BC+DBi}" is the lipid content of the blue crab – discarded bogue mixed diet (% dw).

9.3.8. Statistical analysis

All data, presented as mean ± standard deviation, were tested for normality with the one-sample Kolmogorov–Smirnoff test as well as for homogeneity of variances (Levene's test). When necessary, arcsin square root transformation of the

data was carried out, particularly when data was presented as % (Fowler *et al*., 1998). When normality or homogeneity of variances was not achieved, non parametric tests were used. Significant differences were considered when $p<0.05$.

Data were analyzed with the statistical computer package SPSS v15 (SPSS, Chicago, IL, USA), using a General Linear Model, where "diet" was established as a fixed factor. Significant differences were considered when $P < 0.05$. Some data (initial weight, intermediate weights, final weight, SGR, SFI) were submitted to repeated measures ANOVA, since all these parameters were measured 3 times (3, 6 and 8 weeks) on the same experimental groups along the trial. Other data (SPI, SLI, SEI, PER, PPVm, FE, DGI, proximate composition and fatty acids profiles from diets, muscle and digestive gland from each treatment) were calculated at the end of the rearing period, and compared via a one way ANOVA. The weight of each individual at the start of the experimental period was considered as a covariate, to remove the potential effect of differences in the weight of individuals at the start of the experimental period on response variables. When differences were found, a *post-hoc* Bonferroni test was used to determine the homogeneous subsets ($P < 0.05$). Survival was compared on transformed data (1 = survivors; 0 = deaths) by a Kruskal-Wallis non parametric test, and significant differences were considered when $P < 0.05$.

To visualizate affinities in the fatty acid profiles (% of total fatty acids) between the diet and the digestive gland corresponding to each diet at the end of the experimental period, a nm-MDS ordination was carried out on untransformed data calculated from Bray-Curtis dissimilarities. The nm-MDS is robust to deviations from linear responses, because it uses ranks to visualizate similarities among treatments. To determine whether the fatty acid profile from the digestive gland overlapped the fatty

acid profile from the diet, the ρ correlation coefficient was estimated between both dissimilarity matrices.

9.4. Results

9.4.1. Biochemical composition of the diets

9.4.1.1. Proximate composition of the diets

The highest lipid and energy content was observed in the discarded bogue diet, followed by mixed diets and single crab diets. In contrast, the highest protein and moisture content were observed in the single crab diets, followed by mixed diets and discarded bogue (Table 9.1).

Table 9.1: Proximate composition of each diet (% dry substance) and gross energy (GE, kJ/100 g food wet weight) (mean ± SD, N = 3)

	WC	BC	DB	WC+DB	BC+DB
Lipids (%)	9.1 ± 1.8^{a}	7.0 ± 0.4^{a}	46.5 ± 1.6^{c}	26.3 ± 1.1^{b}	24.8 ± 1.0^{b}
Proteins (%)	79.1 ± 2.0^{c}	80.8 ± 2.3^{c}	46.5 ± 1.9^{a}	64.1 ± 1.0^{b}	65.4 ± 1.8^{b}
Moisture (%)	76.4 ± 0.6^{c}	79.4 ± 1.5^{c}	63.0 ± 2.8^{a}	70.2 ± 1.0^{b}	72.0 ± 1.6^{b}
Ash (%)	2.4 ± 0.0	2.4 ± 0.2	2.2 ± 0.5	2.3 ± 0.2	2.3 ± 0.3
GE (KJ/100 g)	530 ± 20^{a}	451 ± 42^{a}	1084 ± 83^{c}	784 ± 28^{b}	736 ± 47^{b}

Different superscript letters within a row denotes significant differences ($P < 0.05$).

9.4.1.2. Fatty acid profile of the diets

In this study, the most abundant fatty acids in wild octopus tissues were considered, accounting for a 91.2-93.2% of total fatty acids in the samples analysed. Different lipid content among diets led to differences in the fatty acid profile expressed in relative or absolute terms. In relative terms, higher 20:4n-6 (ARA) and n-3 HUFA content were observed in both single crab diets than in diets containing discarded bogue (Table 9.2). The high contribution of DB to total lipid content in mixed diets reflected in a similar fatty acid profile in comparison to DB, with the exception of ARA

which showed higher values in mixed diets than in DB (Table 9.2).

Table 9.2: Fatty acids profiles from each diet (% of total fatty acids) (mean ± SD, N = 3)

	WC	BC	DB	WC+DB	BC+DB
14:0	1.9 ± 0.1^{b}	0.8 ± 0.1^{a}	4.6 ± 0.2^{d}	4.1 ± 0.2^{c}	4.0 ± 0.2^{c}
16:0	18.5 ± 1.4^{b}	14.8 ± 0.9^{a}	17.3 ± 0.4^{b}	17.5 ± 0.3^{b}	16.9 ± 0.4^{b}
16:1 n-7	3.5 ± 0.6^{a}	5.5 ± 0.4^{b}	6.6 ± 0.3^{c}	6.0 ± 0.2^{bc}	6.5 ± 0.3^{bc}
18:0	6.5 ± 0.3^{b}	8.6 ± 0.8^{c}	4.7 ± 0.2^{a}	5.0 ± 0.1^{a}	5.3 ± 0.0^{a}
18:1 n-9	9.8 ± 0.6^{a}	13.2 ± 1.0^{b}	17.7 ± 0.1^{c}	16.2 ± 0.3^{c}	17.0 ± 0.3^{c}
18:1 n-7	2.5 ± 0.2^{a}	3.0 ± 0.4^{ab}	3.2 ± 0.0^{b}	3.1 ± 0.9^{b}	3.2 ± 0.1^{b}
18:1n-5	1.2 ± 0.2^{c}	2.5 ± 0.1^{d}	0.8 ± 0.0^{a}	0.9 ± 0.1^{ab}	1.1 ± 0.1^{bc}
18:2 n-6	2.6 ± 0.2^{b}	1.8 ± 0.1^{a}	15.7 ± 0.7^{d}	13.1 ± 0.9^{c}	13.5 ± 0.8^{c}
18:3 n-3	1.3 ± 0.3^{b}	0.4 ± 0.1^{a}	1.8 ± 0.1^{c}	1.7 ± 0.1^{c}	1.6 ± 0.1b^{c}
20:1 n-9	0.9 ± 0.2	0.5 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
20:2n-6	1.3 ± 0.4^{b}	0.9 ± 0.1^{b}	0.3 ± 0.0^{a}	0.5 ± 0.0^{a}	0.4 ± 0.0^{a}
20:4 n-6	13.5 ± 1.6^{c}	10.9 ± 1.3^{c}	0.7 ± 0.0^{a}	3.2 ± 0.4^{b}	2.3 ± 0.2^{b}
20:5 n-3	14.9 ± 1.2^{b}	9.9 ± 1.2^{a}	7.8 ± 0.7^{a}	9.2 ± 0.6^{a}	8.1 ± 0.5^{a}
22:4 n-6	0.5 ± 0.2^{b}	1.4 ± 0.3^{c}	0.2 ± 0.0^{a}	0.2 ± 0.0^{ab}	0.4 ± 0.1^{ab}
22:5 n-6	0.2 ± 0.1^{a}	1.1 ± 0.2^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{a}
22:5 n-3	0.6 ± 0.1^{a}	1.8 ± 0.3^{b}	2.1 ± 0.1^{b}	1.8 ± 0.1^{b}	2.1 ± 0.1^{b}
22:6 n-3	12.0 ± 1.3^{b}	14.0 ± 0.6^{c}	8.4 ± 0.1^{a}	9.1 ± 0.2^{a}	9.3 ± 0.1^{a}
∑ Saturated	30.0 ± 1.7^{b}	27.0 ± 0.9^{a}	27.9 ± 0.1^{ab}	28.3 ± 0.5^{ab}	27.7 ± 0.2^{ab}
∑ Monoenes	19.1 ± 1.4^{a}	26.9 ± 0.5^{b}	30.1 ± 0.3^{d}	28.1 ± 0.2^{bc}	29.6 ± 0.2^{cd}
∑ n-3	31.0 ± 2.4^{c}	27.4 ± 1.3^{b}	22.4 ± 0.9^{a}	24.1 ± 0.4^{ab}	23.2 ± 0.6^{a}
∑ n-6	18.9 ± 2.2	16.6 ± 0.8	18.1 ± 0.7	18.2 ± 0.5	17.8 ± 0.8
∑ n-9	10.7 ± 0.6^{a}	14.2 ± 0.4^{b}	19.0 ± 0.1^{d}	17.5 ± 0.3^{c}	18.2 ± 0.2^{cd}
∑ n-3 HUFA	28.0 ± 2.0^{b}	26.0 ± 1.5^{b}	19.0 ± 0.9^{a}	20.8 ± 0.5^{a}	20.1 ± 0.6^{a}
DHA/EPA	0.8 ± 0.1^{a}	1.4 ± 0.1^{c}	1.1 ± 0.1^{b}	1.0 ± 0.1^{ab}	1.1 ± 0.1^{b}
DHA/ARA	0.9 ± 0.2^{a}	1.3 ± 0.1^{a}	12.2 ± 0.2^{d}	2.9 ± 0.3^{b}	4.1 ± 0.3^{c}
EPA/ARA	1.1 ± 0.1^{b}	0.9 ± 0.0^{a}	11.2 ± 0.7^{e}	2.9 ± 0.2^{c}	3.6 ± 0.3^{d}

Different superscript letters within a row denote significant differences ($P < 0.05$).

In absolute terms, the highest n-3 HUFA content was observed in DB, followed by mixed diets and finally both crab species. ARA showed the lowest value in DB, followed by mixed diets and BC, and WC showed the highest value (Table 9.3). In comparison with crab species, particularly high values of monoenes and n-9 (16:1n-7, palmitoleic acid; 18:1n-9, oleic acid), 18:2-n6 (linoleic acid) and 18:3n-3 (alpha-linolenic acid) were observed in DB (Table 9.3). Also, higher absolute values in ARA and EPA

were observed in WC than in BC.

Table 9.3: Main fatty acids from each diet and estimation of mixed diets (mg fatty acids/g substance dw) (mean ± SD, N = 3)

	WC	BC	DB	WC+DB	BC+DB
14:0	0.17 ± 0.05^{a}	0.06 ± 0.01^{a}	2.13 ± 0.10^{c}	1.03 ± 0.05^{b}	0.99 ± 0.04^{b}
16:0	1.70 ± 0.49^{a}	1.03 ± 0.10^{a}	7.93 ± 0.25^{c}	4.44 ± 0.38^{b}	4.14 ± 0.16^{b}
16:1 n-7	0.32 ± 0.14^{a}	0.39 ± 0.01^{a}	3.05 ± 0.17^{c}	1.52 ± 0.11^{b}	1.58 ± 0.07^{b}
18:0	0.58 ± 0.11^{a}	0.60 ± 0.09^{a}	2.15 ± 0.13^{c}	1.27 ± 0.11^{b}	1.30 ± 0.02^{b}
18:1 n-9	0.88 ± 0.19^{a}	0.92 ± 0.08^{a}	8.11 ± 0.20^{b}	4.10 ± 0.22	4.16 ± 0.04
18:1 n-7	0.23 ± 0.05^{a}	0.21 ± 0.04^{a}	1.48 ± 0.04^{c}	0.78 ± 0.05^{b}	0.78 ± 0.03^{ab}
18:1n-5	0.11 ± 0.04^{a}	0.18 ± 0.02^{ab}	0.36 ± 0.02^{d}	0.22 ± 0.30^{bc}	0.26 ± 0.02^{c}
18:2 n-6	0.23 ± 0.05^{a}	0.13 ± 0.00^{a}	7.22 ± 0.44^{c}	3.31 ± 0.19^{b}	3.32 ± 0.20^{b}
18:3 n-3	0.12 ± 0.04^{a}	0.03 ± 0.01^{a}	0.84 ± 0.04^{c}	0.43 ± 0.04^{b}	0.39 ± 0.02^{b}
20:1 n-9	0.08 ± 0.04^{a}	0.04 ± 0.02^{a}	0.46 ± 0.02^{c}	0.25 ± 0.03^{b}	0.23 ± 0.02^{b}
20:2n-6	0.12 ± 0.02^{bc}	0.06 ± 0.01^{a}	0.15 ± 0.01^{c}	0.13 ± 0.01^{bc}	0.10 ± 0.01^{b}
20:4 n-6	1.21 ± 0.24^{c}	0.76 ± 0.07^{b}	0.32 ± 0.01^{a}	0.82 ± 0.14^{b}	0.56 ± 0.03^{b}
20:5 n-3	1.34 ± 0.23^{b}	0.69 ± 0.13^{a}	3.57 ± 0.28^{d}	2.32 ± 0.23^{c}	1.98 ± 0.11^{c}
22:4 n-6	0.04 ± 0.03^{a}	0.10 ± 0.03^{b}	0.08 ± 0.01^{ab}	0.06 ± 0.02^{ab}	0.09 ± 0.02^{b}
22:5 n-6	0.02 ± 0.01^{a}	0.07 ± 0.01^{bc}	0.11 ± 0.00^{d}	0.06 ± 0.00^{b}	0.09 ± 0.01^{cd}
22:5 n-3	0.05 ± 0.01^{a}	0.13 ± 0.03^{a}	0.97 ± 0.07^{c}	0.45 ± 0.04^{b}	0.51 ± 0.05^{b}
22:6 n-3	1.09 ± 0.22^{a}	0.98 ± 0.05^{a}	3.87 ± 0.14^{c}	2.31 ± 0.18^{b}	2.28 ± 0.05^{b}
∑ Saturated	2.75 ± 0.82^{a}	1.89 ± 0.20^{a}	12.81 ± 0.40^{c}	7.18 ± 0.62^{b}	6.80 ± 0.20^{b}
∑ Monoenes	1.75 ± 0.55^{a}	1.88 ± 0.17^{a}	13.82 ± 0.38^{c}	7.10 ± 0.46^{b}	7.26 ± 0.14^{b}
∑ n-3	2.80 ± 0.59^{a}	1.91 ± 0.09^{a}	10.30 ± 0.45^{c}	6.10 ± 0.49^{b}	5.69 ± 0.14^{b}
∑ n-6	1.70 ± 0.35^{a}	1.16 ± 0.05^{a}	8.30 ± 0.47^{c}	4.60 ± 0.25^{b}	4.37 ± 0.19^{b}
∑ n-9	0.97 ± 0.23^{a}	0.99 ± 0.09^{a}	8.73 ± 0.20^{c}	4.42 ± 0.25^{b}	4.47 ± 0.06^{b}
∑ n-3 HUFA	2.52 ± 0.45^{a}	1.81 ± 0.07^{a}	8.73 ± 0.44^{c}	5.25 ± 0.42^{b}	4.93 ± 0.20^{b}

Different superscript letters within a row denote significant differences ($P < 0.05$).

9.4.2. Biological parameters

Similar SGR and W_f were observed in octopuses that fed on single crab diets and DB (Table 9.4, Fig. 9.1). In general, a higher SGR was observed in octopuses that fed on mixed diets (crab-fish) in comparison to those fed on single diets, although significant differences were only found between octopuses that fed on BC+DB and those fed on DB.

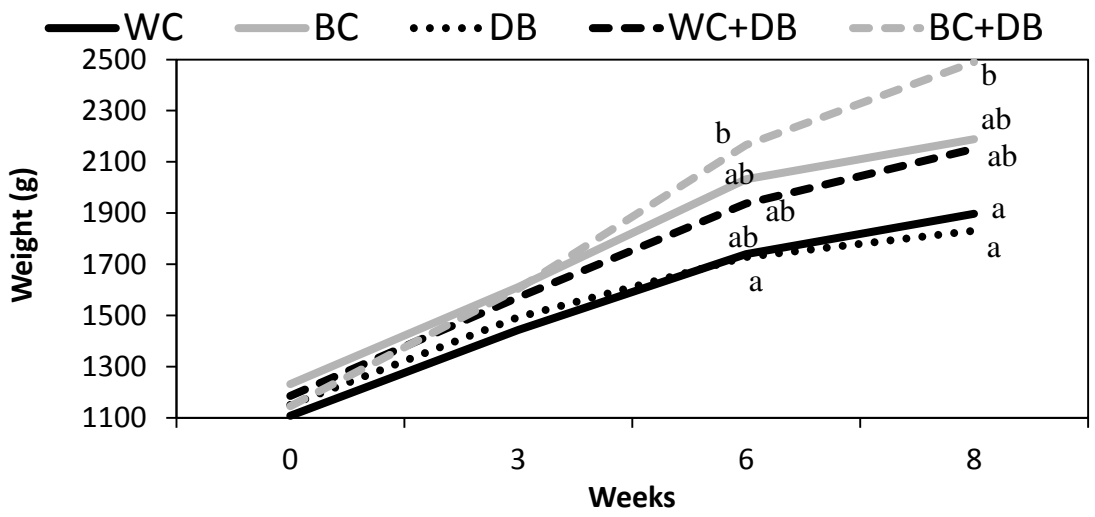

Fig. 9.1: Change in mean weight of individuals fed on each diet (N = 8) during the experimental period (8 weeks). Different letters denote significant differences at 6 and 8 weeks ($P < 0.05$).

Survival was high and similar among treatments, with mortality only registered in individuals that fed on diets containing white crab (Table 9.4). From total ingested food, the percentage of crab-bogue ingested was 44-56 ± 4% for those that fed on WC+DB and 45-55±4% for octopuses that fed on BC+DB. A higher SFI was observed in octopuses that fed on diets containing blue crab than for those that fed on diets containing white crab and discarded bogue. Regarding SPI, highest values were observed in octopus fed on BC, followed by those fed on WC and finally those fed on DB, while specimens fed on mixed diets showed intermediate values. Also, a higher SLI and SEI was observed in octopuses that fed on DB than for those that fed on single crab diets, while mixed diets showed intermediate values (Table 9.4). Feeding rates were reduced towards the end of the experimental period for all treatments (Fig 9.2). Higher PER and PPV_M were observed in octopuses fed on diets containing discarded bogue in comparison to those fed in single crab species, although results were only significant between mixed diets and BC for PER and diets containing discarded bogue

and BC for PPVm. A lower FE was observed in octopuses that fed on BC, in comparison with those that fed on diets containing discarded bogue. DGI in (initial) wild specimens was 2.5 ± 0.7%, a similar value to those of reared octopuses (Table 9.4).

Table 9.4: Initial weight, final weight and biological parameters in *O. vulgaris* after eight weeks of feeding (mean values ± SD, N = 8)

	WC	BC	DB	WC+DB	BC+DB
Initial weight (g)	1107±241	1232±303	1150±182	1185±281	1145±167
Final weight (g)	1898±255^{a}	2189±316^{ab}	1831±360^{a}	2152±451^{ab}	2490±565^{b}
SGR (%/d)	1.0±0.3^{ab}	1.1±0.3^{ab}	0.9±0.3^{a}	1.1±0.3^{ab}	1.4±0.4^{b}
Survival (%)	87.5	100	100	87.5	100
DBI (%/d)	1.3	1.4	1.1	1.5	2.1
SFI (%/d)	2.45±0.34^{a}	3.36±0.69^{b}	2.06±0.46^{a}	2.28±0.31^{a}	3.04±0.20^{b}
SPI (%/d)	0.46±0.06^{b}	0.56±0.11^{c}	0.35±0.08^{a}	0.41±0.06^{ab}	0.51±0.03^{bc}
SLI (%/d)	0.05±0.01^{a}	0.05±0.01^{a}	0.36±0.08^{d}	0.20±0.03^{b}	0.27±0.02^{c}
SEI (J/g d)	130±18^{a}	152±31^{a}	223±49^{c}	172±23^{ab}	214±14^{bc}
PER	0.31±0.07^{ab}	0.26±0.04^{a}	0.36±0.12^{ab}	0.40±0.09^{b}	0.41±0.12^{b}
PPV (%)	23.1±5.6^{ab}	16.0±3.3^{a}	30.2±10.2^{b}	34.3±7.2^{b}	35.5±9.9^{b}
FE (%)	39.7±6.9^{ab}	30.3±4.6^{a}	46.6±9.6^{b}	45.9±9.2^{b}	44.9±9.4^{b}
DGI (%)	2.4±1.1	2.5±1.0	2.2±0.9	1.9±0.5	1.7±0.8

Different superscript letters within a row denote significant differences (P < 0.05).

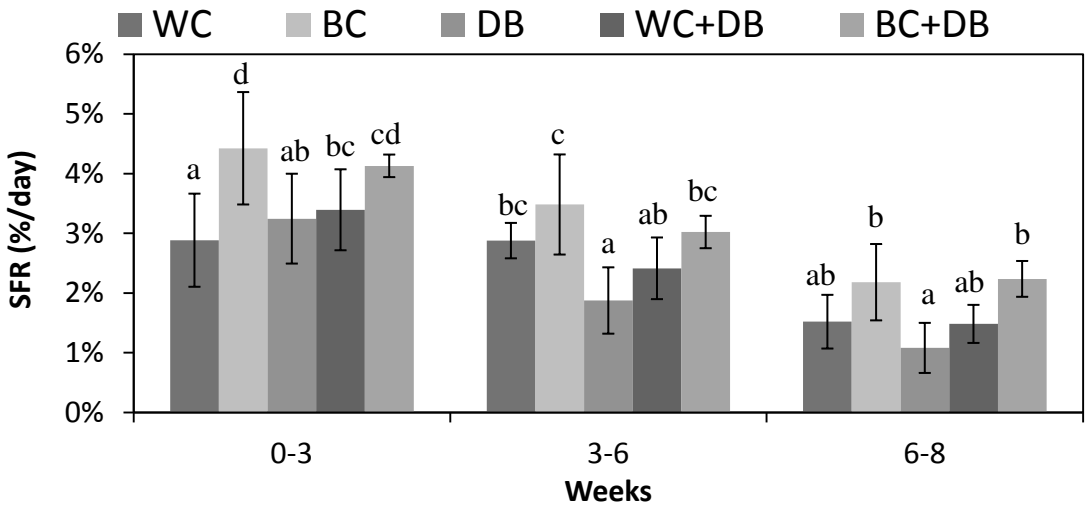

Fig. 9.2: Standard feeding rate (%/d) of individuals fed on each diet (N = 8) during the experimental period (8 weeks). Different letters denote significant differences (P < 0.05).

9.4.3. Biochemical composition of muscle and digestive gland

9.4.3.1. Proximate composition of muscle and digestive gland

A higher lipid and lower protein content was detected in the digestive gland of octopuses that were fed on diets containing discarded bogue, either as a main item or mixed with crabs, than in initial wild octopuses (Table 9.5). Regarding the muscle, a higher protein content was observed in individuals that fed on single discarded bogue and blue crab - bogue mixed diet, confronted with initial wild octopuses and those that fed on blue crab as a single food item (Table 9.5).

Table 9.5: Proximate composition (% dry weight) in digestive gland and muscle of wild (initial) and reared *O. vulgaris* after eight weeks of feeding (mean ± SD, N = 3)

		Wild (initial)	WC	BC	DB	WC+DB	BC+DB
Digestive gland	Lipids (%)	19.9 ± 4.2^{a}	27.6 ± 4.3^{ab}	19.5 ± 0.6^{a}	45.5 ± 6.1^{c}	45.5 ± 8.8^{c}	42.6 ± 9.1^{bc}
	Proteins (%)	68.7 ± 4.2^{b}	60.5 ± 5.8^{b}	67.8 ± 2.0^{b}	42.3 ± 5.5^{a}	40.9 ± 7.7^{a}	44.7 ± 6.3^{a}
	Moisture (%)	71.5 ± 5.5	67.9 ± 2.4	69.2 ± 3.9	64.7 ± 3.7	60.8 ± 7.4	60.3 ± 7.7
	Ash (%)	1.8 ± 0.1^{ab}	1.7 ± 0.2^{ab}	2.0 ± 0.1^{b}	1.6 ± 0.2^{ab}	1.5 ± 0.1^{a}	1.5 ± 0.2^{a}
Muscle	Lipids (%)	5.5 ± 0.2	5.8 ± 0.3	5.7 ± 0.2	5.2 ± 0.5	5.4 ± 0.2	5.4 ± 0.3
	Proteins (%)	77.8 ± 3.2^{a}	81.9 ± 1.5^{ab}	78.3 ± 0.9^{a}	84.0 ± 0.6^{b}	82.7 ± 2.1^{ab}	83.9 ± 0.4^{b}
	Moisture (%)	83.6 ± 2.2	85.2 ± 0.6	85.9 ± 0.4	84.7 ± 1.0	84.1 ± 0.6	84.0 ± 0.9
	Ash (%)	1.8 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.0

Different superscript letters within a row denote significant differences ($P < 0.05$).

9.4.3.2. Fatty acids profile in muscle and digestive gland

A lower level of DHA in the digestive gland was detected in reared octopuses at the end of the rearing period, in comparison with initial wild octopus. In general, the fatty acids profile of the diet was reflected in relative terms in the digestive gland (Fig. 9.3); in turn, a significant correlation ($\rho = 0.86$, $p=0.05$) between the fatty acid profile on the diet and the digestive gland was detected. An increase in 18:2n-6, EPA and DHA, and a decrease in ARA, was observed in octopuses that fed on diets containing discarded bogue, in comparison with octopuses that fed on single crab diets (Table

9.6). Particularly, high values of saturated (16:0, palmitic acid; 18:0, stearic acid) and monoenes (18:1n-9, 20:1n-9, linolenic acid) were detected in octopuses that fed on blue crab.

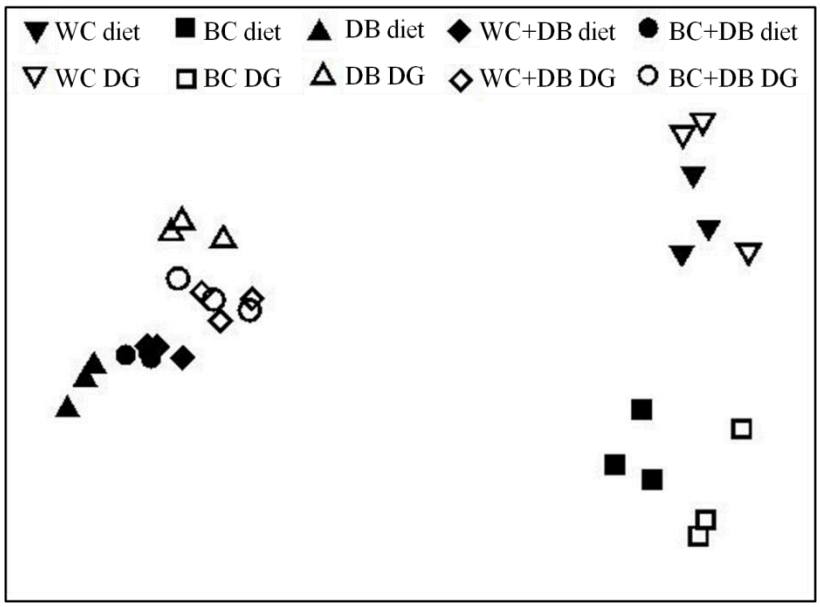

Fig. 9.3: Ordination (multidimensional scaling, MDS) of fatty acid profiles found in the diet and in the digestive gland of *O. vulgaris* after 8 weeks of feeding (stress = 0.04).

Regarding muscle, a decrease in saturated (16:0, 17:0) was observed in octopuses that fed on crab diets in comparison to the initial wild octopus fatty acid profile. Also, a decrease in ARA and an increase in EPA content was detected in animals that fed on discarded bogue, either alone or supplemented with blue crab. DHA was not affected by the diet (Table 9.7).

Table 9.6: Fatty acids profile (% of total fatty acids) in digestive gland of wild (initial) and reared *O. vulgaris* after eight weeks of feeding (mean ± SD, N = 3)

	Wild (initial)	WC	BC	DB	WC+DB	BC+DB
14:0	1.3 ± 0.4^{a}	1.9 ± 0.0^{a}	1.7 ± 0.3^{a}	3.7 ± 0.2^{b}	4.2 ± 0.1^{b}	3.9 ± 0.2^{b}
16:0	14.3 ± 0.1^{a}	16.2 ± 0.8^{bc}	17.7 ± 0.5^{c}	14.7 ± 1.1^{ab}	15.2 ± 0.5^{ab}	14.3 ± 0.5^{a}
16:1 n-7	2.8 ± 0.6^{a}	4.9 ± 0.6^{b}	6.0 ± 0.8^{b}	5.3 ± 0.2^{b}	6.2 ± 0.6^{b}	6.0 ± 0.4^{b}
18:0	7.8 ± 0.9^{bc}	6.4 ± 0.4^{ab}	9.4 ± 1.7^{c}	4.9 ± 0.7^{a}	4.6 ± 0.6^{a}	4.9 ± 0.5^{a}
18:1 n-9	10.7 ± 0.4^{b}	7.9 ± 0.3^{a}	12.1 ± 0.5^{bc}	11.8 ± 0.4^{bc}	12.5 ± 0.8^{c}	12.7 ± 0.7^{c}
18:1 n-7	3.1 ± 0.1^{bc}	3.3 ± 0.1^{c}	4.5 ± 0.4^{d}	2.6 ± 0.1^{a}	2.8 ± 0.0^{a}	2.8 ± 0.1^{ab}
18:1n-5	2.2 ± 0.2^{b}	2.1 ± 0.6^{b}	3.4 ± 0.2^{c}	0.7 ± 0.3^{a}	0.7 ± 0.2^{a}	0.8 ± 0.2^{a}
18:2 n-6	1.3 ± 0.3^{a}	2.9 ± 0.1^{b}	1.4 ± 0.2^{a}	13.5 ± 1.1^{c}	12.4 ± 0.3^{c}	12.8 ± 0.6^{c}
18:3 n-3	0.6 ± 0.1^{a}	1.6 ± 0.3^{b}	0.2 ± 0.1^{a}	1.2 ± 0.1^{b}	1.4 ± 0.1^{b}	1.3 ± 0.1^{b}
20:1 n-9	2.5 ± 0.1^{d}	1.5 ± 0.1^{b}	2.2 ± 0.1^{c}	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	1.2 ± 0.0^{a}
20:2n-6	0.9 ± 0.1^{a}	2.3 ± 0.3^{bc}	1.8 ± 0.3^{b}	3.3 ± 0.5^{c}	2.1 ± 0.7^{ab}	2.3 ± 0.4^{bc}
20:4 n-6	9.9 ± 2.5^{b}	18.9 ± 1.2^{d}	13.8 ± 1.6^{c}	2.2 ± 0.3^{a}	4.9 ± 0.7^{a}	3.9 ± 1.0^{a}
20:5 n-3	6.5 ± 0.9^{a}	11.7 ± 1.5^{b}	5.6 ± 0.3^{a}	11.8 ± 0.5^{b}	10.7 ± 0.5^{b}	10.6 ± 0.9^{b}
22:4 n-6	1.4 ± 0.3^{c}	1.0 ± 0.1^{b}	0.0 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}
22:5 n-6	1.8 ± 0.1^{c}	0.3 ± 0.1^{a}	1.0 ± 0.1^{b}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.0^{a}
22:5 n-3	1.8 ± 0.2^{b}	1.1 ± 0.1^{a}	1.2 ± 0.1^{a}	2.3 ± 0.1^{c}	2.1 ± 0.1^{c}	2.3 ± 0.2^{c}
22:6 n-3	23.2 ± 0.1^{d}	9.0 ± 1.1^{a}	10.4 ± 0.7^{a}	14.9 ± 0.3^{c}	12.1 ± 0.2^{b}	13.1 ± 0.2^{b}
∑ Satured	25.9 ± 1.7^{a}	26.3 ± 0.9^{a}	31.1 ± 1.5^{b}	24.1 ± 1.8^{a}	25.0 ± 1.0^{a}	24.1 ± 1.1^{a}
∑ Monoenes	23.3 ± 0.2^{bc}	21.1 ± 0.5^{a}	30.1 ± 0.3^{d}	22.4 ± 0.4^{ab}	24.4 ± 1.2^{c}	24.5 ± 0.9^{c}
∑ n-3	33.2 ± 1.0^{e}	25.0 ± 1.2^{b}	18.3 ± 0.7^{a}	31.0 ± 1.1^{de}	28.2 ± 1.0^{c}	29.1 ± 1.3^{cd}
∑ n-6	16.1 ± 2.5^{a}	26.5 ± 1.1^{c}	18.2 ± 1.5^{ab}	20.2 ± 0.7^{b}	20.9 ± 1.4^{b}	20.7 ± 1.1^{b}
∑ n-9	13.6 ± 0.6^{bc}	9.5 ± 0.3^{a}	14.8 ± 0.5^{c}	13.3 ± 0.4^{b}	14.0 ± 0.7^{bc}	14.2 ± 0.7^{bc}
∑ n-3 HUFA	32.0 ± 0.7^{e}	22.7 ± 0.7^{b}	17.5 ± 0.7^{a}	29.7 ± 1.0^{d}	25.7 ± 0.8^{c}	26.7 ± 1.0^{c}
DHA/EPA	3.6 ± 0.5^{c}	0.8 ± 0.2^{a}	1.9 ± 0.2^{b}	1.3 ± 0.0^{ab}	1.1 ± 0.0^{a}	1.2 ± 0.1^{ab}
DHA/ARA	2.4 ± 0.6^{b}	0.5 ± 0.0^{a}	0.8 ± 0.1^{a}	6.7 ± 1.0^{c}	2.5 ± 0.4^{b}	3.5 ± 0.9^{b}
EPA/ARA	0.7 ± 0.3^{a}	0.6 ± 0.1^{a}	0.4 ± 0.1^{a}	5.4 ± 0.9^{c}	2.3 ± 0.4^{b}	2.9 ± 1.0^{b}

Different superscript letters within a row denote significant differences ($P < 0.05$).

Table 9.7: Fatty acids profile (% of total fatty acids) in muscle of wild (initial) and reared *O. vulgaris* after eight weeks of feeding (mean ± SD, N = 3)

	Wild (initial)	WC	BC	DB	WC+DB	BC+DB
14:0	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.1
16:0	17.8 ± 0.9^{b}	15.3 ± 0.7^{a}	15.4 ± 0.9^{a}	18.2 ± 0.5^{b}	17.0 ± 0.8^{ab}	17.0 ± 0.7^{ab}
16:1 n-7	0.7 ± 0.1^{c}	0.4 ± 0.1^{a}	0.5 ± 0.1^{bc}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{ab}
18:0	6.0 ± 0.5^{ab}	7.0 ± 0.1^{b}	6.3 ± 0.5^{ab}	5.9 ± 0.4^{a}	6.6 ± 0.3^{ab}	6.6 ± 0.3^{ab}
18:1 n-9	9.3 ± 0.7^{a}	11.6 ± 0.3^{b}	11.0 ± 0.4^{ab}	10.6 ± 0.8^{ab}	12.2 ± 0.7^{b}	11.8 ± 0.7^{b}
18:1 n-7	2.5 ± 0.4	2.6 ± 0.4	2.7 ± 0.5	2.4 ± 0.1	2.4 ± 0.2	2.5 ± 0.3
18:1n-5	1.6 ± 0.0	1.4 ± 0.2	1.4 ± 0.3	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.2
18:2 n-6	0.6 ± 0.2	0.8 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.8 ± 0.0	0.9 ± 0.1
18:3 n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:1 n-9	3.0 ± 0.4	3.3 ± 0.1	3.2 ± 0.3	3.4 ± 0.3	3.3 ± 0.4	3.3 ± 0.3
20:2n-6	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	1.0 ± 0.1
20:4 n-6	13.0 ± 0.9^{c}	14.7 ± 0.9^{c}	13.6 ± 0.1^{c}	9.3 ± 1.6^{a}	12.3 ± 1.5^{bc}	10.1 ± 0.5^{ab}
20:5 n-3	10.1 ± 1.4^{a}	10.6 ± 0.8^{ab}	11.0 ± 0.5^{abc}	13.4 ± 1.2^{c}	12.0 ± 0.8^{abc}	13.3 ± 1.1^{bc}
22:4 n-6	1.8 ± 0.2	1.8 ± 0.2	1.3 ± 1.0	1.6 ± 0.5	1.5 ± 0.3	1.3 ± 0.1
22:5 n-6	1.4 ± 0.1^{b}	0.9 ± 0.1^{a}	1.2 ± 0.1^{ab}	1.1 ± 0.2^{ab}	0.9 ± 0.2^{a}	0.9 ± 0.0^{a}
22:5 n-3	1.5 ± 0.2	1.8 ± 0.0	1.8 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.2
22:6 n-3	23.3 ± 1.9	22.1 ± 1.0	23.8 ± 0.9	25.0 ± 1.2	22.6 ± 0.8	23.1 ± 1.3
∑ Saturated	26.7 ± 1.2^{b}	23.6 ± 0.5^{a}	23.1 ± 0.3^{a}	25.1 ± 0.6^{ab}	24.6 ± 1.0^{ab}	24.8 ± 0.6^{ab}
∑ Monoenes	18.9 ± 1.7	21.0 ± 1.1	20.8 ± 0.1	19.5 ± 0.8	21.2 ± 1.6	21.1 ± 1.7
∑ n-3	35.9 ± 3.6^{ab}	35.2 ± 1.8^{a}	37.4 ± 1.5^{ab}	41.0 ± 2.5^{b}	37.2 ± 0.3^{ab}	39.1 ± 1.7^{b}
∑ n-6	17.6 ± 1.4	19.3 ± 1.1	17.9 ± 1.2	13.9 ± 2.1	16.5 ± 1.9	14.3 ± 0.5
∑ n-9	12.5 ± 1.1^{a}	14.9 ± 0.3^{b}	14.3 ± 0.4^{ab}	14.1 ± 0.7^{ab}	15.6 ± 1.1^{b}	15.2 ± 1.1^{b}
∑ n-3 HUFA	35.4 ± 3.6	34.8 ± 1.8	36.9±1.5	40.5 ± 2.4	36.6 ± 0.3	38.5 ± 1.8
DHA/EPA	2.3 ± 0.1^{c}	2.1 ± 0.1^{abc}	2.2 ± 0.1^{bc}	1.9 ± 0.1^{ab}	1.9 ± 0.2^{ab}	1.7 ± 0.2^{a}
DHA/ARA	1.8 ± 0.3^{a}	1.5 ± 0.2^{a}	1.7 ± 0.1^{a}	2.8 ± 0.6^{b}	1.9 ± 0.2^{a}	2.3 ± 0.1^{ab}
EPA/ARA	0.8 ± 0.2^{a}	0.7 ± 0.1^{a}	0.8 ± 0.0^{a}	1.5 ± 0.1^{b}	1.0 ± 0.2^{ab}	1.3 ± 0.2^{b}

Different superscript letters within a row denote significant differences ($p<0.05$).

9.5. Discussion

In this study, octopuses that fed on a blue crab-bogue mixed diet showed a higher growth than those that fed exclusively on bogue. No significant differences in growth, however, were observed among diets constituted by a single food item. This result contrasts with previous works, where diets exclusively constituted by crabs had promoted the highest growth for *O. vulgaris* (Aguado Giménez and García García,

2002; García García and Cerezo Valverde, 2006; Prato *et al*., 2010). Our results underline, therefore, the potential of rich-lipid bogue discarded from fish farms, as an adequate diet for the ongrowing of *O. vulgaris* (Socorro *et al*., 2005), especially in comparison with other rich-lipid fish (García García and Aguado Giménez, 2002; Petza *et al*., 2006). However, growth, feeding rates and FE induced by a diet constituted by discarded bogue were lower than previous experiments using the same food item under comparable rearing conditions (Estefanell *et al*., 2009a). Such a difference could be explained by the different proximate composition in discarded bogue, with more than a threefold lipid and nearly half protein content in the bogue used in this study. Recent data on octopus ongrowing performed in off-shore sea cages, also providing rich-lipid discarded bogue (45% dry weight) as single diet, showed high growth rates (1.8-1.9%/d) (Estefanell *et al*., 2010a). This is suggesting that individual rearing in a reduced space (0.2 m^3 cages) could have a negative effect on growth, perhaps related to the lack of caloric burn or stress due to strict confinement.

Despite similar proximate composition, the blue crab induced a higher feed intake (SFI, SPI) than the white crab, either as a single or mixed diet. This outcome suggests its value as a potential diet for octopus culture. A high crab intake in *O. vulgaris* was previously observed, related to adequate palatability or to an effort to compensate lipid and amino-acid deficiencies relative to fish (García García and Cerezo Valverde, 2006; Cerezo Valverde *et al*., 2008, 2009). Also, mortality was observed in tanks where octopuses were fed on diets containing white crab. The presence of the coccidian parasite, *Aggregata octopiana,* has been recently described for both wild and reared *O. vulgaris* in the Canary Islands (Betancor *et al*., 2010). Since this parasite requires crustaceans as intermediary hosts, mortality in octopus that fed on local fresh

white crab may be related to this parasite, which causes the so-called “malabsorption syndrome” in octopuses (Gestal *et al*., 2002). The combination of blue crab and fish (discarded bogue) maximized growth rates in *O. vulgaris*, suggesting that mixed diets may better cover the nutritional requirements of octopuses (Smale and Buchan, 1981; Cagnetta and Sublimi, 1999). Indeed, octopus natural diet is based on a wide variety of prey, including crustaceans, fish and other molluscs (Quetglas *et al*., 1998; Rosa *et al*., 2004).

With respect to nutrient utilization, octopuses that fed on diets containing discarded bogue (highest lipid and energy intake) also showed the highest values of PER, PPVm and FE, which demonstrates the use of lipids as energy source in this species, allowing a more efficient utilization of dietary protein (Estefanell *et al*., 2009a). Indeed, discarded bogue is very abundant in monoenes, reported as energy substrates in marine organisms (Sargent *et al*., 1995). Other works have also showed the use of lipids in *O. vulgaris* as an energy source during starvation (García Garrido *et al*., 2010). In this sense, recent findings support that lipid digestibility in *O. vulgaris* depends on the quantity and quality of dietary lipids (Mazón *et al*., 2007; Sánchez *et al*., 2009; Seiça Neves *et al*., 2010).

The edible fraction of crabs and octopus muscle showed a similar lipid and fatty acid profile, with high levels of ARA, EPA and DHA (Rosa *et al*., 2004; Miliou *et al*., 2007; Prato *et al*., 2010). In contrast, the fatty acid profile in bogue discarded from fish farms was very abundant in oleic and linoleic acid, since fish reflect the fatty acid profile of diets, and commercial aqua-feeds have increased levels of these fatty acids (Izquierdo *et al*., 2005). Also, bogue showed very low levels of ARA, in agreement with previous findings in wild bogue (Prato *et al*., 2010).

Lipid and fatty acid profiles in the digestive gland clearly reflected diets, denoting the use of this organ as a lipid and energy storage (Estefanell *et al*., 2009a; García Garrido *et al*., 2010). Octopuses that fed on mixed diets showed a similar lipid and fatty acid profile in the digestive gland in comparison with those that fed exclusively on discarded bogue, which suggests that this organ has a limited capacity to accumulate dietary lipids, and also the major contribution of bogue to the total lipid content in mixed diets. In other cephalopod species, the digestive gland also reflected the fatty acid profile of the diet (Stowasser *et al*., 2006; Fluckiger *et al*., 2008). The low n-3 HUFA absolute content in blue crab was reflected in the digestive gland after 8 weeks of feeding. However, n-3 HUFA reserves in this organ maintained n-3 HUFA levels in muscle at the end of the rearing period. This finding suggests that n-3 HUFA optimal content in octopus diets must be above 1.8 mg/g of lipid (dw). Proximate composition in octopus muscle was more constant than in the digestive gland, which is in agreement with previous reports in cephalopods (Almansa *et al*., 2006; Ferreira *et al*., 2009; García Garrido *et al*., 2011). However, the low content of ARA in discarded bogue reflected in octopus muscle, which apparently did not have a negative effect on growth, perhaps was associated to its substitution by EPA during phospholipids' esterification, as occurs in fishes (Bell *et al*., 1995). ARA is the main precursor of eicosanoids, involved in several physiological functions, such as immune response, neural function and reproduction (Tocher, 2003). Regarding previous rearing trials, specific retention of ARA in *O. vulgaris* after feeding with deficient diets (Navarro and Villanueva, 2000, 2003), or after long term starvation (García Garrido *et al.*, 2010), underlined its importance for this species. Finally, low lipid content in crabs, despite its adequate fatty acid profile, was reflected in low saturated content in reared octopus

muscle. This suggests the importance of saturated in muscle, perhaps for energy production.

In summary, these results have confirmed the potential of aquaculture by-products, particularly bogue, as an adequate diet to feed *O. vulgaris*, either as a single diet or supplemented with the blue crab *Blue crab*. The nutritional characteristics of a mixed diet constituted by blue crab-bogue should be taking into account in the development of octopus specific compound feeds.

9.6. Acknowledgments

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10. Estudio 6: "Growth, food intake, protein retention and fatty acid profile in *Octopus vulgaris* (Cuvier 1797) fed agglutinated moist diets containing fresh and dry raw materials based on aquaculture by-products"

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10.1. Abstract

The lack of specific compound diets for cephalopods is limiting the industrial development of some species. In this study, four agglutinated moist diets were tested in individually reared *Octopus vulgaris* (979 ± 151 g) for 8 weeks. All diets were based on bogue *Boops boops*, accidentally reared in fish farms (aquaculture by-product), and agglutinated with alginate and calcium. One diet was based exclusively on bogue fillets, two on bogue fillets complemented with meat from two crab species (*Portunus pelagicus*, *Grapsus grapsus*) and the last one on bogue and *G. grapsus* meal. As a

control diet, bogue and *P. pelagicus* were supplied fresh on alternate days. All diets induced similar feed intake (2.1-2.6% day^{-1}). However, the meal based diet induced negative growth in comparison with the control and the other agglutinated diets (0.80-0.85% day^{-1}). Higher lipid content in agglutinated diets (28-30% dw) in comparison with the control diet (16% dw) led to higher protein retention in muscle. These results underline the inadequacy of traditional meals in diets for cephalopods and that the inclusion of crab meat did not increase feeding rates and growth in *O. vulgaris*. The dietary fatty acid, with high levels of oleic and linoleic acid and low levels of ARA, clearly reflected in digestive gland, while only decreasing ARA and increasing EPA levels were observed in muscle, with no apparent negative effect on growth.

Keywords: *Octopus vulgaris*, Agglutinated moist diet, Growth, Food intake, protein retention, ARA.

10.2. Introduction

Total capture of cephalopods has been steadily declining in some areas over the last few years (FAO, 2011). In addition, cephalopods show fast growth, have a wide market demand and therefore have been suggested as potential candidates to diversify the aquaculture industry (Hanlon, 1987; Semmens *et al*., 2004; Vaz-Pires *et al*., 2004). For these reasons, research has been carried out on a few benthic coastal species (*O. vulgaris*, *Octopus maya* Voss and Solis Ramirez 1966, *Sepia officinalis* L. 1756) in order to determine their rearing potential. In general, these species can be easily maintained under rearing conditions, feeding on a wide variety of prey (García García and Cerezo Valverde, 2006; Sykes *et al*., 2007). Despite the low survival of the

paralarvae after the planktonic phase (Iglesias *et al*., 2007), Spain (Galicia, NW) has been pioneer in *O. vulgaris* farming. Fishermen associations select small octopuses (less than 1 kg) from their daily catch and rear them in floating cages for 3-4 months, using low price trash species as food, discarded from their daily fishing activity. Octopus farming in the conditions described gives wild females another chance to reproduce which potentially could increase recruitment (Boyle and Rodhouse, 2005). However, total octopus production has been low, from 7 to 32 tons per year since 1998 (FAO, 2011), mainly affected by the lack of an octopus specific compound feed which would meet its nutritional requirements maximizing growth. Indeed, this is the main factor limiting large scale culture of some holobenthic species with direct embryonic development (*O. maya*, *S. Officinalis*), who have been cultured throw multiple generations under lab conditions (Sykes *et al*., 2006; Rosas *et al*., 2009). In addition, the use of inert compound feeds could reduce costs in 40-80% in comparison with fresh diets (Hanlon *et al*., 1991), which currently represent the major expenditure in octopus farms (García García and García García, 2011). Also, compound feeds have other advantages in comparison with fresh diets, such as regular supply and standardized composition, easy storage and conservation, low risk of disease transmission and decreasing environmental impact from farms (O'Dor and Wells, 1987; Lee, 1994).

Firsts works with cephalopods (*S. officinalis*) fed experimental compound diets showed low growth in comparison with natural diets (Castro *et al*., 1993; Castro and Lee, 1994), which was related to low feeding rates (Domingues *et al*., 2005). Indeed, in cephalopods both diet palatability and texture have to be adequate to warrantee acceptance and minimize disaggregation due to the particular feeding system of these

species (Domingues *et al*., 2005; García García and Cerezo Valverde, 2006). In recent works, compound feeds based on the edible fraction of fresh raw materials (fish fillets, crab meat, squid meat) promoted high acceptance and growth in octopods (*O. vulgaris*, *O. maya*), sometimes even comparable with those induced by natural diets (Rosas *et al*., 2008; Cerezo Valverde *et al*., 2008; Quintana *et al*., 2008; Estefanell *et al*., 2011a). However, when dry raw materials were included in compound feeds, growth was generally negative or very low, underlying its inadequacy for cephalopods (Aguila *et al*., 2007; Domingues *et al*., 2007; Rosas *et al*., 2007; García Garrido *et al*., 2011).

In the present study, main component of experimental compound diets is bogue *Boops boops* (L., 1758), provided by fish farms as "discarded" species. Indeed, aquaculture by-products are available, cheap and do not interfere with human markets, so could represent a potential source of fish origin raw material to produce aqua feeds. Particularly bogue showed high level of lipids, very abundant in triglycerides (Cerezo Valverde *et al*., 2011), promoting high growth in *O. vulgaris* when provided fresh in sea cages (Socorro *et al*., 2005; Estefanell *et al*., 2012b). Other studies showed that the high lipid content in this aquaculture by product induced higher protein retention in octopus muscle in comparison with low lipid diets (crab, wild bogue), either in fresh or in agglutinated moist presentation (Estefanell *et al*., 2011a; Estefanell *et al*., 2011C). Two crab species (*Portunus pelagicus*, *Grapsus grapsus*) were also selected as ingredients in this study, since crustaceans are considered natural preys of *O. vulgaris* (Guerra, 1978; Quetglas *et al*., 2003) and have also induced the highest feeding and growth rates under rearing conditions in this species (García García and Cerezo Valverde, 2006; Prato *et al*., 2010).

This study intended to compare whether two combinations of crab-bogue

agglutinated moist diets (with two different crab species) may increase feeding rates and thus growth in comparison to an agglutinated moist diet based on bogue (aquaculture by-product) as a single ingredient (Estefanell *et al*., 2011a) . The percentage of crab meat in the moist diets was 10-11% of total ingredients, in agreement with stomach content data reported from wild specimens in the Canarian Archipelago (Hernández López, 2000). In addition, another diet based on dry raw materials (bogue and crab meal), produced in our facilities from fresh food items normally accepted by the octopuses, was also tested. The main advantages of dry raw materials are related to their standardized composition and availability. Data on protein retention, conversion rates, proximate composition and fatty acid profile of diets and octopus tissues (muscle, digestive gland) were also obtained and discussed.

10.3. Materials and Methods

10.3.1. Capture and acclimatization of the stock

Wild octopuses were caught by local fishermen using cylindrical trawls (1.5 m diameter, 0.4 m high with metallic net of 31.6 mm mesh) placed at 20-30 m depth in the coast of Mogán (Canary Islands, Spain). Octopuses were transported to lab facilities in three 0.5 m^3 square tanks provided with pure oxygen. One week acclimatization period was carried out in rectangular 1.5 m^3 tanks, provided with PVC tubes as shelters, shadowing nets and open flow-through seawater system (1500 L/h). During this period octopus were fed to satiation once a day with a mixed diet containing de-frozen crab (*Portunus pelagicus*, L. 1758) and bogue (*B. boops*), supplied on alternate days.

10.3.2. Rearing conditions

Rearing trials were performed in 5 rectangular tanks (1.5 m^3 capacity). In order to minimize interaction between animals and prevent early mortality (Estefanell *et al*., 2012a), each tank was internally divided with PVC net (2 cm mesh) into 4 compartments (Estefanell *et al*., 2011a). In this study, each compartment was 0.8x1x0.5 m (approx. 0.4 m^3), to facilitate the daily cleaning of the tank. Each compartment was provided with a T-shaped polyvinyl chloride tube (160 mm diameter) as a shelter, and all tanks were covered with shadowing nets, since benthic octopods spend most of their daily cycle out of light in dens in the natural environment (Hanlon and Messenger, 1996). The experimental period lasted 8 weeks (December 8th – February 2nd) under natural photoperiod (approx. 10.75 hours of light and 13.25 hours of dark) and open flow-through sea water system (1.5 m^3 h^{-1}). Mean water temperature and oxygen levels, measured once a day with a portable oxymeter (OXIGUARD HANDY, Point tour Systems Inc., Canada), were 18.4 ± 0.4ºC and 7.1 ± 0.2 mg L^{-1}, respectively.

10.3.3. Experimental design

A total of 20 octopuses (initial weight: 979 ± 151 g, N = 4 per diet) were acclimatized to the individual rearing system for another week prior to the beginning of the experiment. In order to avoid reproductive processes during the trial, only males were selected (Estefanell *et al*., 2010b). Octopuses were fed to satiation 6 days per week (10:00 am) removing uneaten food the day after (8:00 am).

10.3.4. Diets

The following five diets were tested:

1. “Discarded” bogue and blue crab, fresh (Control diet)

2. “Discarded” bogue agglutinated moist diet (DB-moist)
3. “Discarded” bogue + blue crab agglutinated moist diet (DB+BC-moist)
4. “Discarded” bogue + red rock crab agglutinated moist diet (DB+RC-moist)
5. Meal agglutinated moist diet: “discarded” bogue meal + red rock crab meal (M-moist)

Four diets were presented as agglutinated moist diets. Three of them were based on the edible fraction of fresh raw materials, particularly bogue *B. boops*, “blue crab” *P. pelagicus* and “red rock crab” *Grapsus grapsus* (L. 1758). A fifth diet was entirely based on fish and crab meal, produced in our facilities from those fresh raw materials (bogue, red rock crab). Formulation of each agglutinated moist diet is shown in Table 10.1, and was calculated to obtain moist diets of similar moisture content. Bogue was provided by fish farms, as the main aquaculture by-product that is normally discarded from off-shore production cages (Estefanell *et al*., 2011a). Blue crab was purchased frozen from a local fish trade company. Red rock crab was supplied live by professional fishermen.

Table 10.1: Formulation of “Discarded” bogue moist diet (DB-m), “Discarded bogue” and blue crab moist diet (DB+BC-m), “Discarded bogue” and red rock crab moist diet (DB+RC-m), and meal moist diet (F-m) (g/kg).

		DB-m	DB+BC-m	DB+RC-m	M-m
Alginate solution	Alginate[1] (g)	20	20	20	60
	Water (mL)	280	265	270	540
Calcium solution	Calcium[2] (g)	10	10	10	30
	Water (mL)	90	75	80	170
Bogue fillets (g)		600	520	520	-
Blue crab meat (g)		-	110	-	-
Red rock crab meat (g)		-	-	100	-
Bogue meal (g)		-	-	-	160
Red rock crab meal (g)		-	-	-	40

[1] POKEL MERL; [2] POKEL CALS (Productos del Sur, S.A., Spain)

10.3.4.1. Control diet

A 40% bogue and a 60% blue crab were supplied fresh on alternate days (Estefanell *et al*., 2011c). Bogue was provided eviscerated, without head or tail. Blue crab was provided eviscerated, without walking legs and main carapace. Initial daily food ratio (% of octopus weight) was 10% for crab and 6% for bogue.

10.3.4.2. Fresh and dry (meal) raw materials

Only the edible fractions of fresh raw materials were used in agglutinated moist diets. Accordingly, bogue fillets were selected (excluding viscera, scales and bones). Regarding blue crab and red rock crab, only meat was selected (excluding viscera and carapace bits). Each raw material was homogenized separately. In order to elaborate meal, both whole bogue and whole red rock crab were homogenized and dried in an oven at 40ºC for 24 hours, prior to being grinded.

10.3.4.3. Agglutinated moist diets

Commercial alginate and calcium were used as binders (Pokel Merls and Pokel Cals, Productos del Sur, S. A., Murcia, Spain) (Cerezo Valverde *et al*., 2008). Both alginate and calcium were dissolved separately in distilled water as shown in Table 10.1. Raw materials well homogenised were carefully mixed with the alginate solution and finally with the calcium solution. The mixture was then poured into a plastic tray (50x30x7cm) and left for 24 h to solidify at 4ºC. Portions of 400 g were vacuum-packed and stored at -20ºC. Initial daily food ratio (% of octopus weight) was 10% for each agglutinated moist diet.

10.3.5. Biological parameters

All individuals were weighted at the beginning and weekly until the end of the experimental period. Mortality was evaluated daily. The following parameters were calculated individually per octopus:

- Specific Growth Rate: $SGR = (LnW_f - LnW_i) * 100 / t$
- Specific Feed Intake: $SFI = (FI/t) * 100 / W_a$
- Specific Protein Intake: $SPI = (IP/t) * 100 / W_a$
- Specific Lipid Intake: $SLI = (IL/t) * 100 / W_a$
- Specific Energy Intake: $SEI = ((FI/t) * GE / 1000) / W_a$
- Protein Efficiency Ratio: $PER = (W_f$ in dry weight - W_i in dry weight) / IP
- Protein Productive Value in muscle: $PPV_M = 100 * ((W_f * P_f - W_i * P_w) / IP)$
- Feed Efficiency: $FE = (W_f - W_i) * 100 / FI$
- Digestive Gland Index: $DGI = W_{DG} / W_f$

(Where W_f = Final weight (g); W_i = initial weight (g); W_a = average weight (g); t = total time (d); FI = Feed Intake per octopus (g); P_f = final % protein in muscle (wet weight) for each octopus; P_w = average % protein in muscle (wet weight) in wild octopuses; IP = Ingested Protein (g); IL = Ingested Lipid (g); GE = gross energy (kJ/g of feed); and W_{DG} = digestive gland total weight (g))

To estimate FI, the following formula was applied: $FI = F_p - 0.9*F_r$; where FI is ingested food (g); F_p is food provided (g); F_r is removed food (g). This formula was obtained after weighing 10 times each food item before and after 24 hours immersion in sea water, in the same conditions as the experimental tanks. Remaining feed fragments were removed by water vacuum every day, dried in an oven at 105ºC to constant weight to estimate the initial wet weight and subtracted proportionally from each octopus total ingested food.

10.3.6. Sampling procedure

A pool from each diet was obtained four times during the experimental period, from 6 samples randomly taken every two weeks of feeding. In addition, four wild octopuses (all males, 1019 ± 46 g) after transportation from the sea, and all reared octopuses at the end of the rearing period, were sacrificed by immersion in ice-cold sea water, following the recommendation of the European directive 2010/63 on animal welfare, that included cephalopods. A sample from muscle and digestive gland was taken from each individual. The muscle sample was taken from whole left arm II. The digestive gland sample was taken from whole digestive gland. Each sample was homogenised individually and stored at -80ºC until biochemical determinations.

10.3.7. Biochemical analysis

Proximate composition from diets and octopus tissues from each treatment were analysed following standard procedures of AOAC (1997). Moisture was determined after drying the sample in an oven at 105ºC to constant weight; ash by combustion in a muffle furnace at 600ºC for 12 hours; protein content (N × 6.25) was determined by Kjeldahl method and crude lipid was extracted following the method described by Folch *et al*. (1957). Fatty acids methyl esters from total lipids were extracted by transmethylation as described by Christie (1982) and separated by gas chromatography under the conditions described by Izquierdo *et al*. (1992). Gross energy was estimated using the Miglavs and Jobling (1989) energy coefficients. All analyses were conducted in triplicate.

10.3.8. Estimation of proximate composition and fatty acid profile in the control diet

The proximate composition of the control diet was estimated as a weighted

average of the biochemical composition of each pool of single food item and their contribution to total ingested food. The fatty acid profile of the control was estimated as a weighted average of the fatty acid profile of each single food item and the contribution of each food item to total lipid content of each pool in the mixed diet. Accordingly, the following formulas were used (Estefanell *et al*., 2011c):

- $PC_{Ci} = PC_{BCi} * (IF_{BC}/(IF_{BC}+IF_{DB}) + PC_{DBi} * (IF_{DB}/(IF_{BC}+IF_{DB})$, i = pool 1, 2, 3 or 4.
- $FA_{Ci} = FA_{BCi} * ((L_{BCi} * (IF_{BC}/(IF_{BC}+IF_{DB}))/L_{BC+DBi}) + FA_{DBi} * ((L_{DBi} * (IF_{DB2}/(IF_{WC}+IF_{DB})) / L_{BC+DBi})$, i= pool 1, 2, 3 or 4.

Where "PC_C" is the proximate composition of the control diet pool i (% dw), "PC_{BCi}" is the proximate composition of the blue crab pool i (% dw), "IF_{BC}" is the average total ingested blue crab (g ww), "IF_{DB}" is the average total ingested discarded bogue in the control diet, "PC_{DBi}" is the proximate composition of the discarded bogue pool i (% dw), "FA_{Ci}" is the fatty acid profile of the control diet pool i (% of total fatty acids), "FA_{BCi}" is the fatty acid profile of the blue crab pool i (% of total fatty acids), "L_{BCi}" is the lipid content in the blue crab pool i (% dw) and "L_{BC+DBi}" is the lipid content of the blue crab pool i + lipid content of the discarded bogue pool i (% dw), "FA_{DBi}" is the fatty acid profile of the discarded bogue pool i (% of total fatty acids), "L_{DBi}" is the lipid content of the discarded bogue pool i (% dw).

10.3.9. Statistical analysis

All data, presented as mean ± standard deviation, were tested for normality with the one-sample Kolmogorov–Smirnoff test as well as for homogeneity of variances (Levene's test). When necessary, and arcsin transformation of the data was carried out, particularly when data was presented as %. When normality or homogeneity of variances was not achieved, non parametric tests were used.

Significant differences were considered when $P < 0.05$. Data was analyzed with the statistical computer package SPSS v15 (SPSS, Chicago, IL, USA), using a General Linear Model with "diet" as fixed factor. The weight of each individual at the start of the experimental period was considered as a covariate, to remove the potential effect of differences in the weight of individuals at the start of the experimental period on response variables. Weight data (initial, intermediates, final) were submitted to repeated measures ANOVA, since all these parameters were measured several times on the same experimental groups along the trial. All biological parameters and biochemical data were submitted to a one way ANOVA analysis. When differences were found ($P < 0.05$), a *post-hoc* Bonferroni test was used to determine the homogeneous subsets ($P < 0.05$). Survival was compared on transformed data (1 = survivors; 0 = deaths) by a Kruskal-Wallis non parametric test, and significant differences were considered when $P < 0.05$. To determine whether the fatty acid profile of the diets reflected on the fatty acid profile of the digestive glands, the ρ correlation coefficient was estimated between both profiles. The correlation was considered significant when $P < 0.05$.

10.4. Results

10.4.1. Biochemical composition of the diets

10.4.1.1. Proximate composition of the diets

The final average proportion of crab and bogue ingested in the control diet was 50-50 ± 4.1%, respectively. Similar energy content was observed among diets. In the control diet, a lower lipid and higher protein content was observed in comparison with the agglutinated moist ones. Similar lipid, energy and moisture content were observed

in the agglutinated diets. In the M-moist diet, a lower protein and higher ash content was detected in comparison with the other agglutinated moist diets (Table 10.2).

Table 10.2: Macronutrient composition of each diet (% dry substance) and gross energy (GE, kJ/100 g food wet weight) (mean ± SD, N = 4)

	Control	DB-m	DB+BC-m	DB+RC-m	M-m
Lipids (%)	15.5 ± 1.8 [a]	27.7 ± 0.1 [b]	30.0 ± 2.8 [b]	30.2 ± 2.1 [b]	28.2 ± 2.3 [b]
Protein (%)	77.9 ± 0.9 [c]	56.4 ± 0.6 [b]	56.4 ± 2.0 [b]	54.5 ± 1.7 [b]	39.8 ± 1.9 [a]
Moisture (%)	75.9 ± 0.3	77.5 ± 0.3	77.5 ± 1.0	77.4 ± 1.5	76.2 ± 0.6
Ash (%)	1.4 ± 0.1 [a]	2.3 ± 0.2 [b]	2.2 ± 0.0 [b]	2.1 ± 0.1 [b]	4.4 ± 0.3 [c]
GE (KJ/100 g)	591.0 ± 16.5	564.0 ± 7.6	576.2 ± 38.6	579.1 ± 47.0	539.3 ± 34.1

Different superscript letters within a row denote significant difference ($P < 0.05$).

10.4.1.2. Fatty acid profile of the diets

In this study, most abundant fatty acids in wild octopus tissues were considered, accounting for a 92-96% of total fatty acids in the samples analysed. In general, a similar fatty acid profile was observed in all moist diets, with high levels of 18:2-n6 (linoleic acid) and 18:1n-9 (oleic acid), and low levels of 20:4n-6 (ARA) in comparison with the fatty acid profile estimated for the control diet. A decrease in DHA was detected in the M-moist diet in relation to the DB-moist diet. Similar DHA/EPA ratios were observed in the control and M-moist diets, and the highest DHA/EPA ratios were detected in moist diets based on fresh raw materials. The lowest DHA/ARA and EPA/ARA were observed in the control diet, while both ratios showed higher values in moist diets, especially in DB-moist diet (Table 10.3).

Table 10.3: Fatty acids profiles from each diet (% of total fatty acids) (mean ± SD, N = 4)

	Control	DB-m	DB+BC-m	DB+RC-m	M-m
14:0	2.8 ± 0.1 ^{a}	3.5 ± 0.0 ^{b}	3.6 ± 0.1 ^{bc}	3.5 ± 0.1 ^{bc}	3.7 ± 0.0 ^{c}
16:0	15.1 ± 0.5	15.0 ± 0.2	15.6 ± 0.0	15.5 ± 0.3	15.7 ± 0.1
16:1n-7	4.5 ± 0.0 ^{a}	4.5 ± 0.0 ^{a}	4.7 ± 0.0 ^{ab}	4.6 ± 0.1 ^{ab}	4.9 ± 0.3 ^{b}
17:0	0.6 ± 0.0 ^{c}	0.3 ± 0.0 ^{a}	0.3 ± 0.0 ^{ab}	0.3 ± 0.0 ^{b}	0.4 ± 0.0 ^{b}
18:0	5.9 ± 0.3 ^{b}	3.9 ± 0.0 ^{a}	4.2 ± 0.0 ^{a}	4.2 ± 0.1 ^{a}	4.2 ± 0.2 ^{a}
18:1n-9	20.6 ± 0.5 ^{a}	23.8 ± 0.5 ^{b}	23.8 ± 0.6 ^{b}	23.4 ± 0.9 ^{b}	22.5 ± 1.2 ^{ab}
18:1n-7	3.0 ± 0.1 ^{b}	2.7 ± 0.0 ^{a}	2.8 ± 0.0 ^{a}	2.8 ± 0.1 ^{a}	2.9 ± 0.1 ^{ab}
18:1n-5	0.7 ± 0.0 ^{b}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}
18:2n-6	14.6 ± 1.4 ^{a}	18.3 ± 0.2 ^{b}	18.8 ± 0.5 ^{b}	18.6 ± 0.4 ^{b}	20.1 ± 1.5 ^{b}
18:3n-3	1.9 ± 0.1 ^{a}	2.5 ± 0.0 ^{b}	2.5 ± 0.0 ^{b}	2.5 ± 0.1 ^{b}	2.6 ± 0.0 ^{b}
20:1n-9	2.2 ± 0.3	3.0 ± 0.1	2.9 ± 0.2	2.8 ± 0.2	2.3 ± 0.6
20:2n-6	0.7 ± 0.1 ^{b}	0.5 ± 0.0 ^{a}	0.6 ± 0.0 ^{a}	0.6 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}
20:4n-6	2.5 ± 0.2 ^{b}	0.5 ± 0.0 ^{a}	0.6 ± 0.0 ^{a}	0.6 ± 0.0 ^{a}	0.6 ± 0.0 ^{a}
20:5n-3	6.3 ± 0.4 ^{b}	3.7 ± 0.2 ^{a}	3.6 ± 0.3 ^{a}	3.8 ± 0.4 ^{a}	4.2 ± 0.4 ^{a}
22:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:5n-6	0.4 ± 0.0 ^{b}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}
22:5n-3	1.7 ± 0.1	1.8 ± 0.0	1.6 ± 0.1	1.6 ± 0.2	1.6 ± 0.0
22:6n-3	9.5 ± 0.4 ^{c}	7.9 ± 0.2 ^{b}	6.6 ± 0.3 ^{ab}	7.0 ± 1.0 ^{ab}	6.3 ± 0.0 ^{a}
∑ Saturated	25.2 ± 0.6 ^{b}	23.7 ± 0.3 ^{a}	24.7 ± 0.1 ^{ab}	24.5 ± 0.6 ^{ab}	24.9 ± 0.3 ^{b}
∑ Monoenes	33.8 ± 1.1 ^{a}	38.1 ± 0.7 ^{b}	38.1 ± 1.0 ^{b}	37.5 ± 1.4 ^{ab}	35.8 ± 2.2 ^{ab}
∑ n-3	21.0 ± 0.7 ^{b}	17.7 ± 0.5 ^{a}	16.0 ± 0.7 ^{a}	16.7 ± 1.7 ^{a}	16.6 ± 0.4 ^{a}
∑ n-6	19.0 ± 1.1 ^{a}	20.0 ± 0.2 ^{ab}	20.4 ± 0.4 ^{ab}	20.5 ± 0.4 ^{ab}	21.9 ± 1.5 ^{b}
∑ n-9	23.4 ± 0.8 ^{a}	27.5 ± 0.6 ^{ab}	27.5 ± 0.7 ^{ab}	27.0 ± 1.1 ^{ab}	25.5 ± 1.9 ^{b}
∑ n-3 HUFA	18.1 ± 0.9 ^{b}	14.1 ± 0.4 ^{a}	12.5 ± 0.6 ^{a}	13.1 ± 1.6 ^{a}	12.8 ± 0.3 ^{a}
DHA/EPA	1.5 ± 0.1 ^{a}	2.1 ± 0.0 ^{c}	1.8 ± 0.1 ^{bc}	1.9 ± 0.1 ^{c}	1.5 ± 0.2 ^{ab}
DHA/ARA	3.8 ± 0.1 ^{a}	15.9 ± 0.4 ^{c}	11.2 ± 0.5 ^{b}	10.9 ± 1.9 ^{b}	10.1 ± 0.5 ^{b}
EPA/ARA	2.5 ± 0.1 ^{a}	7.5 ± 0.4 ^{c}	6.1 ± 0.5 ^{b}	5.9 ± 0.9 ^{b}	6.7 ± 0.3 ^{bc}

Different superscript letters within a row denote significant difference ($P < 0.05$).

10.4.2. Biological parameters

A 75% survival was observed in octopus that fed on the M-moist diet, while no mortality was observed in the other treatments after 8 weeks of feeding. All diets were well accepted by the octopuses and weight evolution along the rearing period is shown in Fig. 10.1. Disaggregation, expressed as % of crumbs from total ingested food, was 2.3% in octopuses fed on the control diet, 22.9% in octopus fed on the DB-moist, 23.0 in octopuses fed on the DB+BC-moist, 22.1 in octopuses fed on the DB+RC-moist and

23.9% in octopuses fed on the M-moist diet. In octopuses fed on the control diet and on agglutinated moist diets based on fresh raw materials a similar SGR (0.80-0.85%/d), final weight (1495-1678 g) and FE (34-43%) were observed, significantly higher than specimens fed on the M-moist diet. Similar SFI, SPI and SEI were calculated among treatments, while SLI was in general significantly higher in octopus fed on agglutinated diets in comparison with those fed on the control diet. Higher PER and PPV_M were obtained in octopus fed on agglutinated moist diets based on fresh raw materials in comparison with specimens that fed on the control diet, while the lowest protein retention was observed in octopus fed the M-moist diet. Finally, DGI in those specimens sacrificed at the beginning of the experimental period was 2.5 ± 0.7 %, a similar value to those observed in reared octopuses after 8 weeks of feeding (Table 10.4).

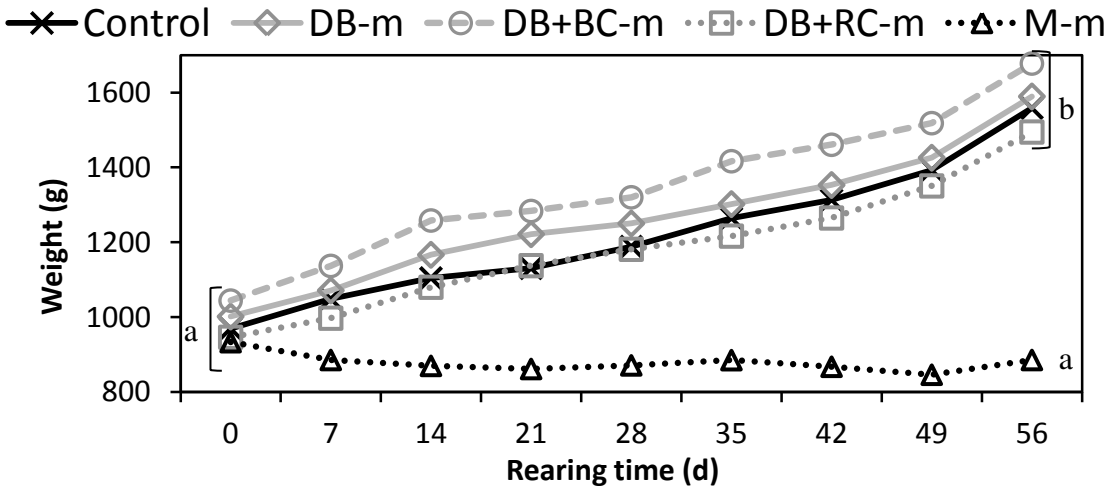

Fig. 10.1: Weight evolution along the experimental period in octopus fed each diet (N = 4)

Table 10.4: Initial weight (W_i), final weight (W_f) and biological parameters calculated in octopuses after 8 weeks of feeding (mean ± SD, N = 4)

	Control	DB-m	DB+BC-m	DB+RC-m	M-m
W_i (g)	970 ± 150	1002 ± 241	1044 ± 130	946 ± 185	933 ± 128
W_f (g)	1559 ± 204 ^{b}	1590 ± 312 ^{b}	1678 ± 321 ^{b}	1495 ± 356 ^{b}	886 ± 175 ^{a}
SGR (%/d)	0.85 ± 0.14 ^{b}	0.83 ± 0.15 ^{b}	0.83 ± 0.12 ^{b}	0.80 ± 0.09 ^{b}	-0.11± 0.13 ^{a}
SFI (%/d)	2.06 ± 0.57	2.43 ± 0.35	2.09 ± 0.19	2.52 ± 0.62	2.65 ± 0.51
SPI (%/d)	0.39 ± 0.11	0.31 ± 0.04	0.27 ± 0.02	0.31 ± 0.08	0.25 ± 0.05
SLI (%/d)	0.08 ± 0.02 ^{a}	0.15 ± 0.02 ^{ab}	0.14 ± 0.01 ^{ab}	0.17 ± 0.04 ^{b}	0.18 ± 0.03 ^{b}
SEI (J/g d)	122 ± 34	137 ± 20	121 ± 11	146 ± 36	143 ± 28
PER	0.42 ± 0.06 ^{b}	0.62 ± 0.07 ^{c}	0.79 ± 0.10 ^{c}	0.70 ± 0.10 ^{c}	0.05 ± 0.09 ^{a}
PPV_M (%)	35.8 ± 4.8 ^{b}	56.9 ± 7.3 ^{c}	65.4 ± 5.6 ^{c}	58.2 ± 11.0 ^{c}	8.8 ± 6.4 ^{a}
FE (%)	43.3 ± 5.0 ^{b}	35.0 ± 5.6 ^{b}	39.5 ± 4.4 ^{b}	34.2 ± 5.5 ^{b}	-5.0 ± 6.6 ^{a}
DGI (%)	3.0 ± 1.0	3.0 ± 0.6	3.8 ± 0.4	3.7 ± 0.9	3.5 ± 1.1

Different superscript letters within a row denote significant difference (P < 0.05).

10.4.3. Biochemical composition of digestive gland and muscle

10.4.3.1. Proximate composition of digestive gland and muscle

In digestive gland, a higher lipid and a lower protein and moisture content was observed in octopuses fed on the control and agglutinated moist diets based on fresh raw materials, in comparison with initial specimens and those fed on the M-moist diet (Table 10.5). In muscle, higher lipid content was observed in initial specimens confronted with those fed on the control and single DB-moist diet (Table 10.5).

Table 10.5: Proximate composition (% dry substance) in digestive gland and muscle of initial (wild) and reared *O. vulgaris* after 8 weeks of feeding (mean ± SD, N = 4)

		Wild (initial)	Control	DB-m	DB+BC-m	DB+RC-m	M-m
Digestive gland	Lipids (%)	20.6 ± 2.6^{a}	52.8 ± 9.1^{b}	44.1 ± 5.3^{b}	53.4 ± 2.7^{b}	50.3 ± 12.1^{b}	22.2 ± 6.3^{a}
	Proteins (%)	68.6 ± 3.9^{c}	37.1 ± 7.0^{a}	45.9 ± 5.9^{ab}	32.4 ± 1.1^{a}	36.8 ± 9.6^{a}	61.4 ± 4.2^{bc}
	Moisture (%)	73.2 ± 5.4^{b}	52.9 ± 3.1^{a}	61.1 ± 2.4^{a}	56.4 ± 1.9^{a}	60.2 ± 2.3^{a}	75.2 ± 4.5^{b}
	Ash (%)	1.5 ± 0.3	1.3 ± 0.1	1.7 ± 0.0	1.4 ± 0.1	1.3 ± 0.1	1.8 ± 0.3
Muscle	Lipids (%)	5.4 ± 0.7^{b}	3.3 ± 0.5^{a}	3.4 ± 0.5^{a}	4.1 ± 0.7^{ab}	3.9 ± 0.7^{ab}	4.2 ± 0.3^{ab}
	Proteins (%)	81.5 ± 4.4	83.0 ± 2.6	86.7 ± 1.4	82.1 ± 1.8	83.8 ± 1.1	85.7 ± 0.6
	Moisture (%)	84.0 ± 2.6	83.2 ± 2.9	81.6 ± 0.6	80.5 ± 1.0	80.6 ± 0.8	82.2 ± 1.2
	Ash (%)	1.8 ± 0.2	2.1 ± 0.3	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1

Different superscript letters within a row denote significant difference (P < 0.05).

10.4.3.2. Fatty acid profile in digestive gland

In initial wild specimens, main fatty acids in digestive gland in order of abundance were: DHA, 16:0 (palmitic acid), oleic acid, ARA and EPA (Table 10.6). A significant correlation ($\rho = 0.92$, $P < 0.01$) was observed between the fatty acid profile of the diet and the digestive gland. After 8 weeks of feeding, a decrease in saturated (mainly associated to 16:0 and 18:0, stearic acid), some n-6 HUFA (ARA, 22:4n-6, adrenic acid, 22:5n-6, osbond acid) and DHA, and an increase in monoenes and n-9 (mainly associated to 18:1n-9), 18:3n-3 (ALA), 18:2n-6 and 20:2n-6 (eicosadienoic acid) were detected in octopuses fed on the control and moist diets based on fresh raw material. In octopuses fed on the M-moist diet, a decrease in DHA, 22:4n-6, 22:5n-6 and an increase in 18:2n-6, were observed, while levels of ARA, saturated (18:0) and monoenes (18:1n-9) were similar to initial (wild) values in digestive gland. A decrease in 20:1n-9 was observed in octopuses fed on the M-moist diet in comparison with initial wild and the other reared octopuses. A decrease in DHA/EPA ratio was observed in reared octopus in relation to initial ones. Also, an increase in DHA/ARA and EPA/ARA ratios were detected in reared octopuses in comparison with initial ones, with the exception of those fed on the M-moist diet that showed similar values. The highest DHA/ARA ratio was observed in those individuals fed on DB-moist diet, followed by those fed on moist diets containing crab meat and finally by those fed on the control diet. The highest EPA/ARA ratio was observed in octopuses fed on DB-moist diet, with specimens fed on diets including crab showing intermediate levels and the lowest values were detected in initial wild individuals and those fed on the M-moist diet (Table 10.6).

Table 10.6: Fatty acids profile (% of total fatty acids) in digestive gland of initial (wild) and reared *O. vulgaris* after 8 weeks of feeding (mean ± SD, N = 4)

	Wild (initial)	Control	DB-m	DB+BC-m	DB+RC-m	M-m
14:0	1.8 ± 0.1^{a}	3.0 ± 0.1^{b}	2.8 ± 0.3^{b}	2.6 ± 0.2^{b}	2.8 ± 0.1^{b}	2.7 ± 0.2^{b}
16:0	15.9 ± 1.9^{b}	12.7 ± 1.3^{ab}	12.0 ± 0.6^{a}	11.1 ± 0.3^{a}	12.3 ± 0.8^{ab}	14.7 ± 1.8^{ab}
16:1n-7	2.9 ± 0.6^{a}	4.5 ± 0.2^{b}	3.7 ± 0.3^{ab}	3.9 ± 0.3^{ab}	4.1 ± 0.3^{b}	3.9 ± 0.5^{ab}
17:0	0.5 ± 0.1^{b}	0.3 ± 0.0^{ab}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.2^{b}
18:0	7.3 ± 1.3^{b}	4.2 ± 0.2^{a}	3.8 ± 0.2^{a}	3.7 ± 0.2^{a}	3.8 ± 0.4^{a}	8.7 ± 1.5^{b}
18:1n-9	9.4 ± 2.3^{a}	18.9 ± 1.3^{b}	15.8 ± 1.1^{b}	16.3 ± 0.4^{b}	17.2 ± 0.2^{b}	12.1 ± 1.8^{a}
18:1n-7	3.4 ± 0.5^{b}	2.6 ± 0.2^{a}	2.1 ± 0.1^{a}	2.2 ± 0.0^{a}	2.4 ± 0.0^{a}	2.0 ± 0.0^{a}
18:1n-5	1.9 ± 0.5^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
18:2n-6	1.4 ± 0.4^{a}	19.3 ± 0.7^{bc}	22.2 ± 1.1^{c}	22.0 ± 0.6^{c}	21.0 ± 1.1^{c}	16.5 ± 1.7^{b}
18:3n-3	0.7 ± 0.2^{a}	2.0 ± 0.0^{c}	1.9 ± 0.2^{c}	1.8 ± 0.0^{c}	1.9 ± 0.0^{c}	1.2 ± 0.3^{b}
20:1n-9	2.3 ± 0.4^{b}	2.8 ± 0.2^{b}	2.3 ± 0.2^{b}	2.7 ± 0.1^{b}	2.6 ± 0.1^{b}	1.6 ± 0.2^{a}
20:2n-6	1.1 ± 0.3^{a}	2.1 ± 0.6^{abc}	3.9 ± 0.4^{c}	3.7 ± 0.5^{c}	3.2 ± 1.0^{bc}	1.6 ± 0.4^{ab}
20:4n-6	8.6 ± 1.5^{b}	2.0 ± 0.1^{a}	1.2 ± 0.2^{a}	1.5 ± 0.0^{a}	1.6 ± 0.2^{a}	5.2 ± 1.4^{b}
20:5n-3	7.9 ± 2.7	6.0 ± 0.7	6.4 ± 1.0	6.9 ± 0.5	6.4 ± 0.4	7.6 ± 0.6
22:4n-6	1.5 ± 0.3^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}
22:5n-6	1.7 ± 0.2^{b}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
22:5n-3	1.9 ± 0.3^{ab}	2.0 ± 0.1^{ab}	2.1 ± 0.1^{ab}	2.2 ± 0.1^{b}	2.1 ± 0.1^{ab}	1.7 ± 0.2^{a}
22:6n-3	22.4 ± 1.5^{b}	10.0 ± 0.7^{a}	12.8 ± 1.7^{a}	12.4 ± 0.3^{a}	11.8 ± 0.5^{a}	12.3 ± 1.4^{a}
∑ Saturated	26.5 ± 1.1^{b}	21.2 ± 1.6^{a}	19.4 ± 0.9^{a}	18.6 ± 0.1^{a}	19.9 ± 1.3^{a}	27.2 ± 3.1^{b}
∑ Monoenes	22.1 ± 2.4^{a}	32.0 ± 1.5^{c}	26.4 ± 1.9^{b}	27.9 ± 0.4^{bc}	29.0 ± 0.6^{bc}	21.4 ± 1.8^{a}
∑ n-3	34.6 ± 2.7^{b}	21.3 ± 1.3^{a}	24.6 ± 2.2^{a}	24.6 ± 0.5^{a}	23.6 ± 0.8^{a}	23.7 ± 2.3^{a}
∑n-6	15.0 ± 1.6^{a}	24.3 ± 2.1^{b}	28.2 ± 1.4^{b}	28.1 ± 0.3^{b}	26.6 ± 1.9^{b}	24.0 ± 0.8^{b}
∑ n-9	12.2 ± 2.6^{a}	22.6 ± 1.4^{b}	18.9 ± 1.3^{b}	19.9 ± 0.3^{b}	20.7 ± 0.2^{b}	14.5 ± 1.4^{a}
∑ n-3 HUFA	32.9 ± 1.8^{b}	18.6 ± 1.2^{a}	22.1 ± 2.4^{a}	22.3 ± 0.5^{a}	21.0 ± 0.8^{a}	22.0 ± 1.9^{a}
DHA/EPA	3.1 ± 1.1^{b}	1.7 ± 0.2^{a}	1.9 ± 0.1^{a}	1.8 ± 0.1^{a}	1.8 ± 0.0^{a}	1.6 ± 0.2^{a}
DHA/ARA	2.6 ± 0.5^{a}	5.1 ± 0.4^{b}	11.2 ± 1.5^{d}	8.2 ± 0.2^{c}	7.4 ± 0.7^{c}	2.5 ± 0.8^{a}
EPA/ARA	1.0 ± 0.4^{a}	3.1 ± 0.5^{b}	5.6 ± 0.4^{d}	4.5 ± 0.2^{cd}	4.0 ± 0.4^{bc}	1.6 ± 0.5^{a}

Different superscript letters within a row denote significant difference ($P < 0.05$).

0.4.3.3. Fatty acid profile in muscle

In initial wild specimens, main fatty acids in muscle in order of abundance were: DHA, 16:0, ARA, EPA and 18:1n-9 (Table 10.7). After 8 weeks of feeding, a decrease in ARA and an increase in EPA content were observed in muscle, with the exception of those individuals fed on the control diet, who showed similar values to initial ones. A decreased in 22:4n-6 was observed in all reared specimens in relation to initial wild samples. Particularly, an increase in oleic acid was detected in octopuses fed on DB-moist diet in comparison with initial samples. A decrease in DHA/EPA ratio was observed in reared octopus, except in those fed on the control diet that showed similar values in relation to initial wild samples. An increase in DHA/ARA ratio was detected in octopuses fed on DB-moist and DB+BC-moist diets in comparison with initial wild samples. Finally, an increase in EPA/ARA ratio was observed in octopuses fed on moist diets based on fresh raw materials, in comparison with initial samples (Table 10.7).

Table 10.7: Fatty acids profile (% of total fatty acids) in muscle of initial (wild) and reared *O. vulgaris* after 8 weeks of feeding (mean ± SD, N= 4)

	Wild (initial)	Control	DB-m	DB+BC-m	DB+RC-m	M-m
14:0	0.8 ± 0.2	1.0 ± 0.4	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.0
16:0	17.9 ± 0.7	16.4 ± 1.5	19.2 ± 1.4	18.0 ± 1.0	17.7 ± 0.3	16.9 ± 0.4
16:1n-7	0.8 ± 0.1	0.5 ± 0.2	0.3 ± 0.0	0.7 ± 0.2	0.7 ± 0.4	0.6 ± 0.2
17:0	1.8 ± 0.0	1.6 ± 0.2	1.9 ± 0.4	1.7 ± 0.6	1.9 ± 0.3	1.6 ± 0.0
18:0	6.0 ± 0.5	6.4 ± 0.3	6.5 ± 0.3	6.4 ± 0.6	5.6 ± 0.2	6.6 ± 0.2
18:1n-9	9.1 ± 1.1^{a}	9.9 ± 1.1^{a}	12.5 ± 1.1^{b}	10.6 ± 1.3^{ab}	9.8 ± 0.8^{a}	10.4 ± 0.2^{ab}
18:1n-7	2.5 ± 0.4	3.0 ± 0.6	2.7 ± 0.3	2.9 ± 0.2	2.7 ± 0.3	3.0 ± 0.2
18:1n-5	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.6 ± 0.3	1.2 ± 0.3	1.3 ± 0.1
18:2n-6	0.6 ± 0.2	1.2 ± 0.4	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.3	1.0 ± 0.1
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20:1n-9	3.0 ± 0.3	3.7 ± 0.3	3.3 ± 0.4	3.7 ± 0.3	3.3 ± 0.2	3.3 ± 0.0
20:2n-6	0.7 ± 0.2^{a}	1.0 ± 0.3^{ab}	1.0 ± 0.2^{ab}	1.2 ± 0.1^{b}	1.0 ± 0.2^{ab}	0.8 ± 0.0^{ab}
20:4n-6	13.3 ± 0.4^{b}	10.2 ± 2.2^{ab}	7.2 ± 1.2^{a}	6.5 ± 0.4^{a}	7.9 ± 1.8^{a}	9.0 ± 1.8^{a}
20:5n-3	9.7 ± 0.8^{a}	11.2 ± 1.0^{ab}	12.5 ± 1.2^{b}	13.2 ± 0.9^{b}	13.1 ± 0.6^{b}	13.0 ± 0.6^{b}
22:4n-6	2.0 ± 0.2^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
22:5n-6	1.5 ± 0.1^{b}	1.2 ± 0.1^{ab}	0.8 ± 0.2^{a}	0.8 ± 0.2^{a}	1.0 ± 0.3^{ab}	1.0 ± 0.1^{ab}
22:5n-3	1.5 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	1.6 ± 0.4	2.0 ± 0.2	1.6 ± 0.1
22:6n-3	22.7 ± 1.0	24.9 ± 1.5	23.6 ± 0.7	24.2 ± 1.0	23.5 ± 0.4	24.1 ± 1.3
∑ Saturated	27.0 ± 1.0^{ab}	25.8 ± 1.4^{a}	28.8 ± 1.0^{b}	27.4 ± 0.9^{ab}	26.4 ± 0.6^{ab}	26.2 ± 0.7^{ab}
∑ Monoenes	19.2 ± 1.4	20.1 ± 1.0	21.8 ± 0.6	21.8 ± 1.8	19.8 ± 1.0	20.6 ± 0.2
∑ n-3	34.8 ± 1.8^{a}	38.9 ± 2.1^{ab}	38.7 ± 0.7^{ab}	40.1 ± 2.2^{b}	39.7 ± 0.4^{b}	39.6 ± 2.1^{b}
∑ n-6	18.2 ± 0.8^{c}	13.8 ± 1.9^{b}	10.3 ± 1.1^{a}	9.9 ± 0.2^{a}	12.0 ± 1.2^{ab}	12.1 ± 1.8^{ab}
∑ n-9	12.3 ± 1.5^{a}	14.6 ± 0.8^{ab}	15.9 ± 0.8^{b}	15.7 ± 1.3^{b}	14.2 ± 0.8^{ab}	14.9 ± 0.2^{ab}
∑ n-3 HUFA	34.3 ± 1.8^{a}	38.3 ± 2.1^{ab}	38.1 ± 0.7^{ab}	39.4 ± 2.4^{b}	39.0 ± 0.4^{ab}	39.0 ± 2.1^{ab}
DHA/EPA	2.3 ± 0.1^{c}	2.2 ± 0.2^{bc}	1.9 ± 0.2^{ab}	1.8 ± 0.1^{a}	1.8 ± 0.1^{a}	1.9 ± 0.0^{ab}
DHA/ARA	1.7 ± 0.1^{a}	2.5 ± 0.5^{ab}	3.4 ± 0.6^{b}	3.7 ± 0.0^{b}	3.1 ± 0.6^{ab}	2.8 ± 0.7^{ab}
EPA/ARA	0.7 ± 0.1^{a}	1.1 ± 0.3^{ab}	1.8 ± 0.4^{bc}	2.0 ± 0.0^{c}	1.7 ± 0.4^{bc}	1.5 ± 0.4^{abc}

Different superscript letters within a row denote significant difference ($P < 0.05$).

10.5. Discussion

In this study, octopus fed on moist diets based on fresh raw materials showed similar growth rates and final weight in comparison with specimens fed on the control diet, in agreement with previous works testing similar agglutinated moist diets in octopods (Rosas *et al*., 2008; Quintana *et al*., 2008; Estefanell *et al*., 2011a). Even though *O. vulgaris* natural diet includes crustaceans (Guerra, 1978; Quetglas *et al*., 1998; Smith, 2003), which represent at least 10% of total diet in the Canarian Archipelago (Hernández-López, 2000; Hernández-López *et al*., 2004), octopus fed on agglutinated moist diets containing a 10% of crab meat did not induce higher feeding rate or growth confronted with specimens fed on the DB-moist diet. This finding suggests that either the species tested or the percentage of inclusion does not have a positive effect on diet palatability in *O. vulgaris*.

Regarding the M-moist diet, fish and crab meal were elaborated from fresh raw materials commonly accepted by octopus (Socorro *et al*., 2005; Estefanell *et al*., 2011c, 2012b). The transformation of aquaculture by-products into fish meals should facilitate its use by the aquaculture industry. The inclusion of crab meal intended to increase the diet palatability and stimulate feed intake, as previously observed in fishes and crustaceans (Goytortúa-Bores *et al*., 2006; Romero García *et al*., 2010). However, octopuses fed on the M-moist diet showed negative growth, despite inducing similar feed intake in comparison with the other diets. This is in agreement with previous reports in octopods fed on agglutinated compound diets including commercial dry raw materials (Aguila *et al*., 2007; Domingues *et al*., 2007; Rosas *et al*., 2007; García Garrido *et al*., 2011). Some authors reported that the thermal treatment had a negative effect on the nutritional quality of the diet in *S. officinalis*, associated to adulteration,

denaturalization or loss of proteins/aminoacids and oxidation of lipids (Domingues *et al*., 2009*).* Also, the high ash content in fish and crab meals may have decreased the digestibility of the M-moist diet. Indeed, both the low lipid accumulation in the digestive gland and the low protein retention (PER, PPV_M) observed in octopuses fed on the M-moist diet evidenced that this diet was not properly assimilated, contrary to the observed in the digestive gland of octopuses fed on the other diets. In addition, some authors observed a negative effect of alginate on nutrient digestibility (Rosas *et al*., 2008; Seiça Neves *et al*., 2010; García Garrido *et al*., 2011), and in this study the M-moist diet required 3 times more alginate and calcium than the other moist diets to obtain a similar texture. These results suggest that alternative drying system and manufacturing methods should be investigated to elaborate cephalopod specific compound feed, having the enormous advantages of using dry raw materials in comparison to fresh ones (O'Dor and Wells, 1987; Lee, 1994).

In general, the biological performance (growth rates, feeding rates, feed conversion rates) in this study were lower than in previous trials where *O. vulgaris* were fed on similar agglutinated moist and mixed fresh diets under comparable rearing conditions (Estefanell *et al*., 2011a, 2011c). This could be related to the rearing temperature observed during the trial, abnormally low for the time of the year in the Canarian Archipelago, which is normally 20-21ºC (Fernández-Palacios, H., Pers. Comm.), since *O. vulgaris* metabolism is strongly influenced by the rearing temperature (Aguado Giménez and García García, 2002; Milliou *et al*., 2005).

Regarding nutrient utilization, the higher lipid content in agglutinated moist diets based on fresh raw materials induced higher protein retention (PER, PPV_M) confronted with octopus fed on the control diet. This finding underlines the efficient

lipid utilization as energy source, saving dietary proteins for somatic growth, in agreement with previous observations in *O. vulgaris* (Estefanell *et al*., 2011a, 2011c). Also, FE calculated in octopus fed agglutinated moist diets based on fresh raw materials showed higher values than previous data reported for *O. vulgaris* fed on similar agglutinated moist diets (Cerezo Valverde *et al*., 2008; Quintana *et al*., 2008). The use of lipids as energy source was also reported in starved *O. vulgaris* (García Garrido *et al*., 2010) and other cephalopod species (Castro *et al*., 1992; Semmens, 1998; Moltschaniwskyj and Johnston, 2006). Contrary to what occurs in fishes, rich-lipid diets did not reflect on increasing lipid content in muscle (Izquierdo *et al*., 2005). What is more, a decrease in lipid content was observed in octopus muscle after 8 weeks of feeding, which was associated to increasing size (Biandolino *et al*., 2010; Navarro and Villanueva, 2003).

Similar fatty acid profiles in the diets probably reflected the high contribution of discarded bogue to total lipid content in all diets. Decrease in DHA/ARA and EPA/ARA ratios in both bogue-crab moist diets in comparison to DB-moist diet reflected high ARA content in crab meat in comparison to fish (Prato *et al*., 2010; Estefanell *et al*., 2011c). As in previous studies in *O. vulgaris* and other cephalopods, the dietary fatty acid reflected in the digestive gland profile (Stowasser *et al*., 2006; Fluckiger *et al*., 2008; Estefanell *et al*. 2011a, 2011c). Those differences observed in the fatty acid profile between octopuses fed on the M-moist diet and the other reared specimens are mainly explained by the low diet assimilation. In particular, the decrease in 20:1n-9 in digestive gland suggests that this fatty acid could be used as preferred energy substrate in *O. vulgaris*.

The fatty acid profile in muscle showed a more stable composition than

digestive gland regardless of the diet, in agreement with data observed in cephalopods (Almansa *et al*., 2006; Ferreira, Marquez, Andrade, Lorenzo & Domingues 2009; Estefanell *et al*., 2011a, 2011b). However, low ARA content in the diets reflected in this tissue at the end of the rearing period, with no apparent negative effect on growth (Estefanell *et al*., 2011a, 2011b, 2012b). This may be related with the substitution of ARA by EPA during phospholipids' esterification, as occurs in fishes (Bell *et al*., 1995). Specific retention of ARA in *O. vulgaris* after long term starvation (García Garrido *et al.,* 2010) suggests its importance for this species. Indeed, ARA is the main precursor of eicosanoids, involved in several physiological functions, such as immune response, neural function and reproduction (Tocher, 2003).

Despite the high availability of 18:2n-6 and 20:2n-6 in the diets, both largely accumulated in the digestive gland, no increase in ARA or its intermediate metabolites were observed (22:4n-6, 22:5n-6), which suggests that ARA biosynthesis does not occur in *O. vulgaris*, contrary to the observed in this species by Milliou *et al*. (2006). Other authors recently demonstrated by gene expression the presence of an enzyme that participates in the endogenous production of ARA (and EPA) (Monroig *et al*., 2012). However, these authors also underlined that both fatty acids are probably required in the diet of this species, due to low dietary availability of immediate biosynthesis precursors (20:4n-3, 20:3n-6), and the lack of specific enzymes to biosynthesize them from 18:2n-6 and 18:3n-3. Our results also show that ARA's utilization decreased in low growing specimens, which would explain the higher levels of ARA in digestive gland of octopuses fed on the M-moist diet. A similar finding was observed in low growing *O. vulgaris* paralarvae, which maintained initial ARA levels after feeding on deficient diets (Navarro and Villanueva, 2000, 2003).

In summary, no positive effect on feeding and growth rates were observed in octopuses by including a 10% of crab meat in a fish based agglutinated moist diet. The use of fish and crab meal, dried by traditional methods, induced negative growth and mortality after 8 weeks of feeding, underlying the need of testing alternative drying systems and manufacturing methods to elaborate diets for cephalopods.

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11. Estudio 7: "Aquaculture by products (bogue *Boops boops*) as a single diet for *Octopus vulgaris* under industrial rearing conditions: effect of sexual maturation processes"

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11.1. Abstract

Octopus vulgaris is considered a potential candidate to diversify marine farming. Since an octopus specific formulated diet is not available yet, several low price fresh diets, mostly fisheries by-products, have been tested as temporary diets. Recently, the potential of aquaculture by products, in particularly bogue *Boops boops*, was investigated in *O. vulgaris* males reared individually. This fish is accidentally reared in commercial off shore cages. Some authors suggested that a single fish diet was inadequate for octopus ongrowing, and indeed most growth trials under industrial

rearing conditions in *O. vulgaris* used mixed diets containing crustaceans. This study intends to evaluate growth, mortality and sexual maturity data in males and females of *O. vulgaris* in two consecutive growing cycles under industrial rearing conditions in floating cages. Specimens were fed on a single diet of bogue (aquaculture by-product) (44% of lipids dry weight), and confronted with a mixed diet containing a 60-40% of crab-bogue (control diet). All octopuses were PIT tagged in order to obtain individual data. The rearing period lasted 2 months and initial rearing density was 10 kg/m^3 in both trials. Trial 1 started in January (N = 30 per diet, 918 ± 125 g, sex ratio 1:1), growth rates were higher in octopuses fed on the mixed diet (1.9-2.0%/d) than in those fed on the bogue diet (1.8-1.9%/d) irrespective of sex, and mortality was 3% regardless of sex and diet. In this trial, no egg masses were found, and a high percentage of females were still maturing, with average gonadosomatic index of 2.0–3.6% regardless of diet. Trial 2 started in March (N = 32 per diet, 1483 ± 269 g, sex ratio 1.4:1), growth rates were higher in males fed on the mixed diet (1.8%/d) than in males fed on the bogue diet (1.4%/d), both higher than females (1.1-1.3%/d), and mortality was 22-28% regardless of sex and diet. In this trial, egg masses were found irrespective of diet and most females were mature, with average gonadosomatic index of 8.8-11.4% regardless of diet. These results show that sexual maturation has a negative effect on growth in females and that reproductive processes increase mortality in both sexes, which should be taken into account towards the successful commercial ongrowing of *O. vulgaris*. In addition, octopuses fed on aquaculture by-products showed acceptable growth, close to 1 kg/month in males, underlying the value of these by-products as a single diet for this species.

Keywords: *Octopus vulgaris*, growth, mortality, sexual maturation, aquaculture by-products, lipids

11.2. Introduction

Octopus vulgaris is potential candidate to diversify the aquaculture industry for a number of reasons: rapid growth and low food conversion rates, a wide market demand and decreasing landings in some areas over the last few years (Iglesias *et al*., 2000; Vaz-Pires *et al.*, 2004; FAO, 2011). For these reasons, despite unsolved problems in the paralarvae rearing (Iglesias *et al.*, 2007) and the lack of octopus specific compound diets (Cerezo Valverde *et al.*, 2008; Quintana *et al.*, 2008; Estefanell *et al*., 2011a), a few fishermen associations in Spain (Galicia, NW) have been pioneers in *O. vulgaris* farming. Wild juveniles are reared in floating cages feeding on low price trash species, provided by local fisheries (Chapela *et al.*, 2006; Rodríguez *et al.*, 2006). This rearing system is in agreement with the "integrated aquaculture" model proposed for cephalopods (Boyle and Rodhouse, 2005). Indeed, octopuses caught by fisheries are unlikely to have reproduced (Estefanell *et al*., 2010b), so the ongrowing of males and females in sea cages could provide an input of paralarvae to the environment.

Growth in cephalopods is mainly affected by the quantity and quality of food (Semmens *et al*., 2004). Best growth rates in *O. vulgaris* have been observed in specimens fed on marine crustaceans or squid (Miliou *et al*., 2005; García García and Cerezo Valverde, 2006; Domingues *et al*., 2010; Prato *et al*., 2010); however, these food items are expensive and its use is restricted to research purposes. The identification of a potential natural diet for cephalopods, which should be cheap, available and promote high growth in this species, could represent a temporary

solution for the implementation of cephalopod farming. Also, testing natural fresh diets under different rearing conditions could provide valuable information regarding the nutritional requirements of cephalopods for the development of compound feeds.

Aquaculture by-products, particularly those small pelagic fish species that are accidentally reared in sea cages, represent a potential source of fish origin raw material for the aquaculture industry. Bogue *Boops boops* is the most abundant discarded species, and has induced high growth and survival rates as a single diet in individually reared males of *O. vulgaris*, comparable to those induced by single diets of crab species (Estefanell *et al*., 2011a, 2011c). However, some authors pointed out that a single diet of fish increased cannibalism and mortality in *O. vulgaris* reared under group conditions, probably associated to some nutritional deficiencies (Aguado *et al*., 2001; Tuñón *et al*., 2002). For this reason, studies on *O. vulgaris* performance under industrial rearing conditions in sea cages have used mixed diets containing crustaceans (Chapela *et al*., 2006; Rodríguez *et al*., 2006; García García *et al*., 2009). Indeed, previous trials performed in our facilities with octopuses reared under group conditions and fed on single fish based diets showed mortality rates ranging from 9 to 56% (Estefanell *et al*., 2007b; Socorro *et al*., 2005).

Another factor that affects the biological performance of *O. vulgaris* is the sexual maturation. First studies reported that males and females showed similar growth rates until females became sexually mature, when they showed decreasing growth rates associated to a higher energy investment in gonad development than males (Forsythe and Van Heukelem, 1987). Other studies in *O. vulgaris* under culture conditions reported highest growth in males (Iglesias *et al*., 2000) or similar growth irrespective of sex (Aguado Giménez and García García, 2002; Chapela *et al*., 2006).

Increased mortality associated to reproductive processes has been observed under rearing conditions in both sexes (Estefanell *et al*., 2010b). In contrast, several authors reported high biological performance in males and females of *O. vulgaris* reared together in floating cages (Rodríguez *et al*., 2006; García García *et al*., 2009).

Following recent results in individually reared males of *O. vulgaris* using bogue as single diet (aquaculture by-product) (Estefanell *et al*., 2011a, 2011cc), this study intends to evaluate the effect of the same food item on growth and mortality in males and also females reared under industrial grouped conditions in floating cages. As a control diet, a mixed diet containing crustaceans and bogue was used. Both diets were tested in two consecutive ongrowing trials and data on sexual maturation was obtained in males and females prior and at the end of each trial.

11.3. Material and Methods

11.3.1. Capture and acclimatization of the stock

Wild octopuses were caught by local fishermen using cylindrical trawls (1.5 m diameter, 0.4 m high with metallic net of 31.6 mm mesh) placed at 20-30 m depth in the coast of Mogán (Canary Islands, Spain). Octopuses were transported to lab facilities in three 0.5 m^3 square tanks provided with pure oxygen. Acclimatization period lasted two weeks and was carried out in rectangular 1.5 m^3 tanks, provided with PVC tubes as shelters, shadowing nets and open flow-through seawater system (1500 L/h). One week after arrival each octopus was PIT tagged on upper left arm III (Estefanell *et al*., 2011b). During this period octopus were fed to satiation once a day with a mixed diet containing de-frozen crab (*Portunus pelagicus*) and bogue (*B. boops*) supplied on alternate days. Water temperatures during the acclimatization periods

were 18-20ºC and oxygen levels were kept above the 80% saturation (Cerezo Valverde *et al*., 2005).

11.3.2. Diets

Two diets were tested: bogue *B. boops* as a single diet and a mixed diet containing a 60% of crab *P. pelagicus* and a 40% of bogue (Estefanell *et al*., 2011c). Bogue was supplied by local fish farms as "discarded species" (aquaculture by-product). Crab was purchased from a local fish trade company and main carapace and walking legs were removed. Octopuses were fed to satiation 6 days per week (0800 h). Daily food ratio (% of biomass) was 3-6% for bogue and 5-10% for crab, provided in at least 3 portions of food per octopus, adjusted along each rearing period according to intermediate weight measurements, mortality rates and remaining food in the cage, checked visually by scuba diving three times per week. In order to reproduce industrial rearing conditions, food remains were not removed from the bottom of the cage. Dead octopuses were collected daily from the bottom of the cage to avoid any interference in the growth performance due to cannibalism.

11.3.3. Rearing conditions and experimental design

Rearing trials were performed in one stainless steel floating cage (1.5x3x3 m length, height and width, respectively) with 20 mm mesh size and 10 m^3 of total internal volume (Socorro *et al*., 2005). Shadowing net and dens (T-shaped PVC tubes, 160 mm diameter) were added to the cage (Hanlon and Messenger, 1996). The cage was anchored in Taliarte Harbour (Telde, Las Palmas, Canary Islands, Spain) (27º 59'20.2'' N, 15º 22' 6.8'' W) and the assays were performed under natural photoperiod. Initial rearing density was 10 kg/m^3 (Rodríguez et al. 2006). Water temperature and oxygen levels were measured once a day with a portable oxymeter

(Oxiguard Handy, Point tour Systems Inc., Canada).

In “trial 1”, the floating cage was divided into 4 sub-cages (1.5x3x0.75 m length, height and width, respectively) of approx. 2.5 m^3 of total internal volume each and only two were used. Forty two dens were added to each sub-cage. Sixty octopuses (918 ± 125 g), male:female sex ratio 1:1, were selected and transferred proportionally to each sub-cage (N = 30). Initial rearing density was 10.1 and 10.9 kg/m^3 for the cage fed on the bogue and on the mixed diet, respectively. Initial rearing conditions are shown in Table 2. The rearing period lasted 61 and 63 days for the cage fed on the bogue and on the mixed diet, respectively (January-February, approx. 11 h of light and 13 h of dark). Octopuses were weighted at the beginning, after 35 days and at the end of the rearing period. Mean water temperature and oxygen levels were 20.5 ± 0.4 ºC and 6.1 ± 0.4 mg/L, respectively.

In “trial 2”, the floating cage was divided into 2 sub-cages (1.5x3x1.5 m length, height and width, respectively) of approx. 5 m^3 of total internal volume each. Sixty-four octopuses (1483 ± 269 g), male:female sex ratio 1.4:1, were selected and transferred proportionally to each cage (N = 32). Initial rearing density was 9.2 and 9.7 kg/m^3 for the cage fed on the bogue and on the mixed diet, respectively. Initial rearing conditions are shown in Table 2. The rearing period lasted 60 days (March-April, approx. 12.25 h of light and 11.75 h of dark). Octopuses were weighted at the beginning, after 32 days and at the end of the rearing period. Mean water temperature and oxygen levels were 18.3 ± 0.3 ºC y 7.0 ± 0.2 mg/L, respectively.

11.3.4. Biological parameters

The following biological parameter was calculated individually: “Standard Growth Rate”, SGR = 100 (LnW_f - LnW_i) / t, where W_f = individual final weight (g); W_i =

individual initial weight (g); t = total rearing time (d).

The following biological parameters were calculated per cage: “Weight dispersion”, WD = SD / W_{af}; “Mortality”, M = (N_f – N_i) / n_i (%); “Biomass Increment”, BI = (B_f - B_i) / B_i (%) (this parameter was standardized to 60 days of rearing, BI standardized: BI_S = 60 BI / t); “Apparent Feed Conversion Ratio”, A-FCR = FP / (W_{af} - W_{ai}), where SD = standard deviation; W_{af} = final average weight (g); N_f = final number of specimens; N_i = initial number of specimens; B_f = final biomass (g); B_i = initial biomass (g); W_{ai} = initial average weight (g). Daily mortality data and individual growth data allowed the estimation of biomass on a daily basis, which was represented as accumulated BI_S every 10 days of rearing.

The following sexual maturity and condition indices were calculated: “Sexual maturity *Hayashi Index* as modified by Guerra (1975)”, for males: H_M = W_N / (W_N + W_T), and females: H_F = W_{OG} / (W_{OG} + W_O); “Gonadosomatic Index”, for males: GSI_M = W_N / (W_f - W_N), and females: GSI_F = W_O / (W_f - W_O) (Otero et al. 2007); “Digestive gland index”, DGI = W_{DG} / W_f (Cerezo Valverde et al. 2008), where W_N = Needham’s complex + spermatophoric sac weight (g); W_T = testis weight (g); W_{OG} = oviducal gland weight (g); W_O = ovary weight (g); W_{DG} = digestive gland weight (g); W_f = individual final weight (g).

Also, a macroscopic maturation stage was assigned to every octopus dissected (I, immature; II, maturing; III, mature, and IV, post-reproductive) (Dia and Goutschine, 1990) and egg masses were counted per cage at the end of the experimental period.

11.3.5. Sampling procedure

Each food item was analyzed three times along the experimental period. Samples were taken from a pool of 6 individuals randomly selected every three weeks

of feeding. Only the edible fraction from crab was included in the pool, while whole discarded bogue was homogenized. All octopuses were sacrificed by immersion in ice-cold sea water, prior to being weighted and dissected. Initial sexual maturity data was obtained from wild octopuses sacrificed upon arrival to our facilities. In particular, 12 octopuses were sacrificed at the beginning of trial 1 (N = 3 males, 1079 ± 145 g; N = 9 females, 1003 ± 142 g) and 6 octopuses were sacrificed at the beginning of trial 2 (N = 3 males, 1581 ± 281 g; N = 3 females, 1549 ± 422 g).

11.3.6. Biochemical analysis

Diets proximate compositions were analyzed following standard procedures of AOAC (1997). Moisture was determined after drying the sample in an oven at 105ºC to constant weight; ash by combustion in a muffle furnace at 600ºC for 12 hours; protein content (N × 6.25) was determined by Kjeldahl method and crude lipid was extracted following the method described by Folch et al. (1957). All analyses were conducted in triplicate.

11.3.7. Estimation of the proximate composition of the mixed diet

The following formula was applied to estimate the "proximate composition of the mixed diet": $PR_{MDi} = 0.45\ PR_{Bi} + 0.55\ PR_{Ci}$, (i= pool number 1, 2 or 3), where PR_{Bi} is the proximate composition of the bogue i (% dw) and PR_{Ci} is the proximate composition of the crab i (% dw). This formula was obtained from one previous studies performed in our facilities where the feeding rates of octopuses fed the same mixed diet (60-40% crab-bogue) were accurately calculated (Estefanell *et al*., 2011c).

11.3.8. Statistical analysis

All data, presented as mean ± standard deviation, were tested for normality with the one-sample Kolmogorov–Smirnoff test as well as for homogeneity of

variances (Levene's test). When necessary, an arcsin transformation was carried out, particularly when data was presented as % (Fowler *et al*., 1998). When normality or homogeneity of variances was not achieved, non parametric tests were used. Significant differences were considered when $p<0.05$.

Data were analyzed with the statistical computer package SPSS v15 (SPSS, Chicago, IL, USA), using the following General Linear Model, where "diet" (mixed diet, bogue diet) and "sex" (male, female) were established as fixed factors to test for differences at the end of the experimental period via a two way ANOVA..The weight of each octopus at the start of the experimental period was considered as a covariate, to remove the potential effect of differences on initial octopus weight on the response variables. When initial (wild) data was included in the model the "diet" factor was replaced by the "treatment" factor (wild, bogue diet, mixed diet). In particular, some data (weight and SGR) were analyzed using a repeated measures ANOVA, using "diet" and "sex" as fixed factors; other data (H, GSI and DGI) were analyzed using a one way ANOVA, with only "treatment" as fixed factor independently to each sex; the biochemical composition of crab, bogue and the mixed diet were compared using "treatment" as fixed factor (one way ANOVA). Significant differences were considerer when $p<0.05$. When there were more than 2 data, a *post-hoc* Bonferroni test was used to determine the homogeneous subsets ($p<0.05$). Mortality data was transformed (0, survivors; 1, dead) and compared according to diet and sex using a chi-squared test. Significant differences in the statistical analysis were considered when $p<0.05$. Finally, other biological parameters calculated per cage (WD, BI, A-FCR) corresponded to one single replica and could not be compared statistically.

11.4. Results

11.4.1. Proximate composition: diets

A similar proximate composition was observed in bogue and the mixed diet in trial 1 and trial 2 (Table 11.1). A lower protein and a higher lipid and energy content were detected in the bogue diet in comparison with the mixed diet in both trials (Table 11.1).

Table 11.1: Proximate composition (% dry substance) and gross energy (GE) (kJ/100 g food wet weight) of bogue *B. Boops* discarded from fish farms and estimation of the mixed diet in trial 1 and in trial 2 (mean ± SD, N = 3)

		B. boops	Mixed diet
Trial 1	Lipids (%)	44.2 ± 4.4 ^{b}	23.5 ± 1.6 ^{a}
	Proteins (%)	50.2 ± 4.0 ^{a}	67.8 ± 1.8 ^{b}
	Moisture (%)	65.0 ± 4.0 ^{a}	72.5 ± 1.7 ^{b}
	Ash (%)	1.6 ± 0.2 ^{a}	2.0 ± 0.2 ^{b}
	GE (KJ/100 g)	979 ± 126 ^{b}	719 ± 58 ^{a}
Trial 2	Lipids (%)	43.8 ± 6.1 ^{b}	22.8 ± 2.4 ^{a}
	Proteins (%)	46.7 ± 5.8 ^{a}	67.8 ± 2.8 ^{b}
	Moisture (%)	62.3 ± 3.8 ^{a}	71.5 ± 1.3 ^{b}
	Ash (%)	2.1 ± 0.1	2.2 ± 0.1
	GE (KJ/100 g)	1090 ± 146 ^{b}	745 ± 51 ^{a}

Different superscript letters within a row denote significant differences ($P < 0.05$)

11.4.2. Biological parameters

In trial 1, a higher intermediate weight, final weight and SGR were observed in octopuses fed on the mixed diet in comparison with those fed on bogue diet, irrespective of sex (Table 11.2). In trial 2, a higher intermediate weight, final weight and SGR were observed in males than in females in both diets. Also, a higher intermediate weight, final weight and SGR were observed in males fed on the mixed diet than in those fed on the bogue diet (Table 11.2).

Table 11.2: Initial number of octopuses (N_i), initial weight (W_i), intermediate weight (W_{INT}), final weight, (W_f), standard growth rate calculated during the 1st half (SGR_1), during the 2nd half (SGR_2) and total (SGR_T) in trial 1 and in trial 2 (mean ± SD)

		Bogue diet		Mixed diet	
		Females	Males	Females	Males
Trial 1	N_i	14	16	15	15
	W_i (g)	875 ± 101 ^{a}	872 ± 97 ^{a}	933 ± 167 ^{b}	988 ± 101 ^{b}
	W_{INT} (g)	1839 ± 323 ^{a}	1909 ± 337 ^{a}	2076 ± 511 ^{b}	2322 ± 256 ^{b}
	W_f (g)	2544 ± 448 ^{a}	2681 ± 562 ^{a}	3160 ± 423 ^{b}	3459 ± 862 ^{b}
	SGR_1 (%/d)	2.0 ± 0.5 ^{a}	2.3 ± 0.4 ^{a}	2.4 ± 0.2 ^{b}	2.4 ± 0.4 ^{b}
	SGR_2 (%/d)	1.2 ± 0.5 ^{a}	1.3 ± 0.4 ^{a}	1.5 ± 0.5 ^{b}	1.3 ± 0.6 ^{b}
	SGR_T (%/d)	1.8 ± 0.3 ^{a}	1.9 ± 0.3 ^{a}	2.0 ± 0.3 ^{b}	1.9 ± 0.5 ^{b}
Trial 2	N_i	13	19	14	18
	W_i (g)	1367 ± 247	1478 ± 188	1439 ± 298	1586 ± 278
	W_{INT} (g)	2340 ± 223 $_{A}$	2669 ± 381 $_{B}$	2393 ± 373 $_{A}$	3323 ± 544 $_{B}$
	W_f (g)	2721 ± 341 $_{A}$ ^{a}	3583 ± 756 $_{B}$ ^{b}	2590 ± 630 $_{A}$ ^{a}	4467 ± 1160 $_{B}$ ^{c}
	SGR_1 (%/d)	1.5 ± 0.3 $_{A}$	1.8 ± 0.3 $_{B}$	1.6 ± 0.5 $_{A}$	1.9 ± 0.4 $_{B}$
	SGR_2 (%/d)	0.4 ± 0.6 $_{A}$	1.1 ± 0.5 $_{B}$	0.3 ± 0.3 $_{A}$	1.3 ± 0.3 $_{B}$
	SGR_T (%/d)	1.1 ± 0.3 $_{A}$ ^{a}	1.4 ± 0.3 $_{B}$ ^{b}	0.9 ± 0.6 $_{A}$ ^{a}	1.8 ± 0.4 $_{B}$ ^{c}

Different superscript letters within a row denote significant difference according to diet (P<0.05); Different subscript capital letters within a row denote significant difference according to sex (P<0.05).

An increase in weight dispersion was found in reared octopuses along the experimental period, especially in those specimens that fed on the mixed diet in trial 2 (36%) (Fig. 11.1).

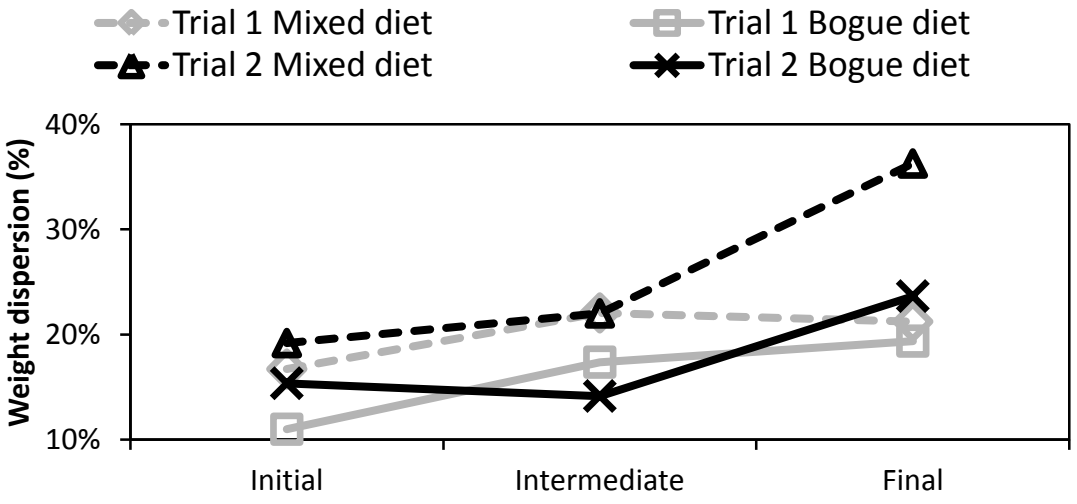

Fig. 11.1: Weight dispersion (%) in *O. vulgaris* in trial 1 and trial 2

Mortality was not affected by diet or sex. A low mortality was observed in trial 1 (3%) in comparison with trial 2 (22-28%) (Fig. 11.2), regardless of sex and diet. In trial 1, one female died per diet. In trial 2, 4 females and 5 males died in the cage that was fed on the mixed diet, while 4 females and 3 males died in the cage that was fed on the bogue diet.

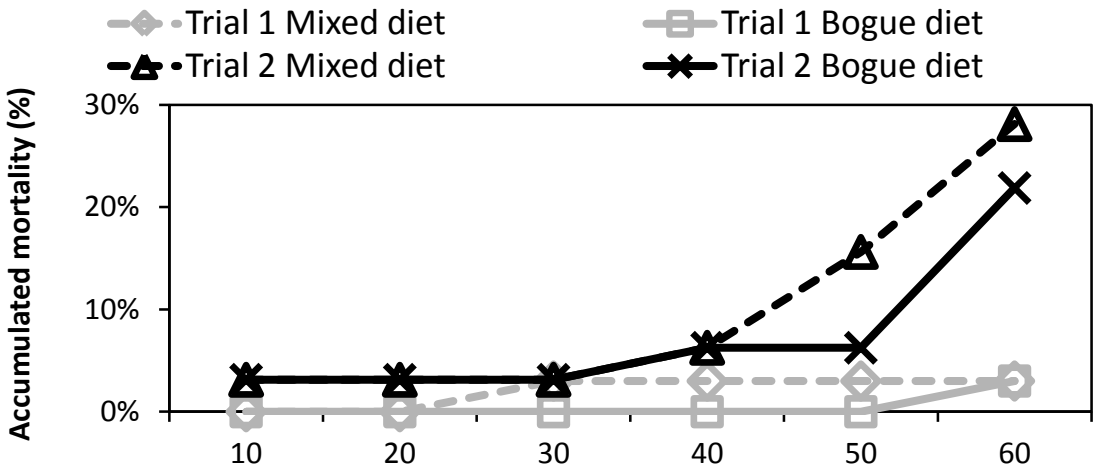

Fig. 11.2: Accumulated mortality (%) in *O. vulgaris* in trial 1 and trial 2

A higher biomass increment was detected in trial 1 (189-234%) in comparison with trial 2 (69-75%) (Fig. 11.3). A lower A-FCR was calculated in trial 1 (2.6 and 2.9 in octopuses fed on the bogue and on the mixed diet, respectively) in comparison with trial 2 (3.7 and 4.5 in octopuses fed on the bogue and on the mixed diet, respectively).

Sexual maturity and condition data was not affected by the diet. All males dissected at the beginning and at the end of the rearing period were sexually mature according to H_M, GSI_M (Table 11.3) and macroscopic evaluation (stage III) in both trials. A decrease in GSI_M was observed from initial (wild) to reared octopus males in both trials. In contrast, a similar H_M and increasing DGI values were observed between initial and reared males in trial 1, and increasing H_M values and similar DGI were

detected between initial and reared octopus males in trial 2 (Table 11.3).

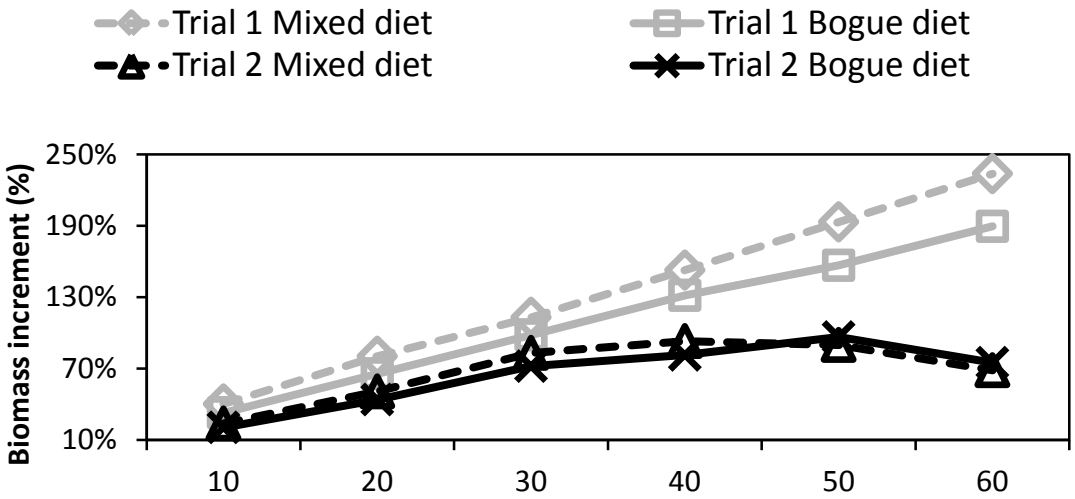

Fig. 11.3: Accumulated biomass increment (%) in *O. vulgaris* in trial 1 and trial 2

Table 11.3: Number of octopuses dissected (N), sexual maturity "Hayashi Index" as modified by Guerra (1975) (H), gonadosomatic index (GSI) and digestive gland index (DGI) in initial (wild) and reared males and females in trial 1 and 2 (mean ± SD).

			Initial (wild)	Bogue diet	Mixed diet
Trial 1	Males	N	3	15	16
		H_M	0.43 ± 0.05	0.46 ± 0.04	0.46 ± 0.07
		GSI_M (%)	0.5 ± 0.1 ^{b}	0.3 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}
		DGI (%)	1.9 ± 0.4 ^{a}	3.5 ± 0.7 ^{b}	3.8 ± 0.9 ^{b}
	Females	N	9	14	13
		H_F	0.10 ± 0.03 ^{b}	0.05 ± 0.02 ^{a}	0.03 ± 0.01 ^{a}
		GSI_F (%)	0.6 ± 0.6 ^{a}	2.0 ± 1.4 ^{ab}	3.6 ± 1.63 ^{b}
		DGI (%)	2.4 ± 0.7 ^{a}	4.6 ± 0.8 ^{b}	5.2 ± 0.7 ^{b}
Trial 2	Males	N	3	16	13
		H_M	0.42 ± 0.03 ^{a}	0.49 ± 0.06 ^{b}	0.51 ± 0.05 ^{b}
		GSI_M (%)	0.4 ± 0.1 ^{b}	0.3 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}
		DGI (%)	3.1 ± 0.9	2.9 ± 0.9	2.7 ± 1.0
	Females	N	3	9	10
		H_F	0.08 ± 0.01 ^{b}	0.03 ± 0.01 ^{a}	0.03 ± 0.01 ^{a}
		GSI_F (%)	1.3 ± 0.4 ^{a}	8.8 ± 3.6 ^{b}	11.4 ± 2.5 ^{b}
		DGI (%)	4.0 ± 0.8 ^{b}	2.7 ± 1.1 ^{ab}	2.6 ± 0.9 ^{a}

Different superscript letters within a row denote significant difference (P<0.05).

A different pattern was observed in females in both trials (Fig. 11.4). According to macroscopic evaluation and H index, a 67% of initial samples in trial 1 were still immature, while the other 33% were already maturing. By the end of that trial, no immature females were found regardless of diet, with up to 46-54% of females fed on bogue and 86-14% of females fed on the mixed diet in maturing-mature stage, respectively. In contrast, all females (100%) were still maturing at the beginning of trial 2 and most were already mature by the end of it (100% of females fed on the mixed diet and 89% of females fed on the bogue diet). A decrease in H_F and an increase in GSI_F were observed in both trials. GSI_F showed highest values in trial 2, where the ovary represented up to 15% of total body weight in some females. No egg masses were observed at the end of trial 1, contrary to trial 2, where 3 and 5 egg masses were found in the cage that fed on the mixed diet and on the bogue diet, respectively. Increased DGI was observed between initial and reared females in trial 1, while this parameter tended to decrease at the end of trial 2.

Fig. 11.4: Macroscopic sexual maturity stages (%) (Dia and Goutschine, 1990) in wild and reared females in trial 1 and trial 2 (I: Immature; II: Maturing; III: Mature)

11.5. Discussion

In this study, the potential of bogue (aquaculture by product) as a single diet for *O. vulgaris* was confirmed under industrial rearing conditions in sea cages, where octopuses showed high biological performance, in agreement with previous trials performed in individually reared males (Estefanell *et al*., 2011a, 2011c). Indeed, this food item induced higher growth rates in *O. vulgaris* than several low price fish species (García García and Aguado Giménez, 2002; García García and Cerezo Valverde, 2006; Petza *et al*., 2006), and similar to diets including marine crustaceans or squid (Cerezo Valverde *et al*., 2008; Domingues *et al*., 2010; Prato *et al*., 2010). In this study, the mixed diet containing crustaceans induced higher SGR than bogue as a single diet, suggesting its better nutritional profile in comparison with bogue as a single diet (Cagneta and Sublimi, 1999; Estefanell *et al*., 2011c). This information should be taken into account in the formulation of octopus specific compound feeds.

Also, fast growth observed in *O. vulgaris* fed on high lipid bogue (44% dw) suggests efficient lipid utilization (Estefanell *et al*., 2011a, 2011c). First studies on *O. vulgaris* nutrition suggested that lipid digestibility was slow and inefficient (O'Dor *et al*., 1984) and the low lipid content in octopus muscle and natural preys (crustaceans) suggests that small amounts are needed for physiological processes (Navarro and Villanueva 2003). Indeed, octopuses fed on high lipid fish species showed low growth rates (García García and Aguado Giménez, 2002; Petza *et al*., 2006). However, several authors observed that lipid digestibility varies greatly depending on lipid source and nutrient composition (Mazón et al. 2007; Sánchez et al. 2009; Seiça Neves et al. 2010) and the use of lipids was recently reported in *O. vulgaris* during starvation (García Garrido et al. 2010).

The different biological performance between trial 1 and trial 2 were related to differences in sexual maturation. Contrary to trial 1, reproductive processes were evidenced in trial 2 by the presence of egg masses at the end of the rearing period. Also in trial 2, the increase in H_M in reared males suggests that spermatophores' transfer had started, while the drastic increase in GSI_F and the decrease in DGI in reared females imply that brooding behaviour was occurring (Guerra, 1975; Mangold, 1987; Otero *et al*., 2007; Estefanell *et al*., 2010b). Even though H_F and macroscopic evaluation underlined that sexual maturity was reached in some females in trial 1, lower GSI_F values in comparison with those observed in trial 2 suggests that the ovary was still growing, denoting that full sexual maturity had not been reached. This finding underlines the importance of using several sexual indices to assess the maturity stage in females of *O. vulgaris*.

Accordingly, similar SGR between sexes in trial 1 was related to the lack of sexual maturity in females, and low SGR observed in females in trial 2 was explained by the higher energy investment for gonad development than in males (Forsythe and Van Heukelem, 1987; Otero *et al*., 2007). Different stages of sexual maturation probably explains the controversy in growth reported between males and females of *O. vulgaris* under rearing conditions (Iglesias *et al*., 2000; Aguado Giménez y García García, 2002; Chapela *et al*., 2006). The relatively high mortality observed in trial 2 was also related to reproductive processes, which marks the end of *O. vulgaris* life cycle both in males and females (Hernández García *et al*., 2002; Estefanell *et al*., 2010b). The probable different age between the octopuses reared in trial 1 and in trial 2, suggested by the lower initial size and higher proportion of immature specimens recorded at the beginning of trial 1, would also explain the different biological performance between

trials, since younger cephalopods show higher growth rates and lower mortalities than older ones (Semmens *et al*., 2004; Miliou *et al*., 2005; Leporati *et al*., 2007). Also, increasing weight dispersion during the experimental period was another factor increasing mortality (Socorro *et al*., 2005; García García *et al*., 2009), especially noticeable in octopuses fed on the mixed diet in trial 2. In trial 2, mortality and low growth in females limited BI and increased A-FCR in comparison with trial 1. Both BI and A-FCR in trial 1 showed the best values ever recorded in *O. vulgaris* under industrial rearing conditions (Socorro *et al*., 2005; Rodríguez *et al*., 2006), underlying the nutritional value of both diets and also the potential of this species for the diversification of marine aquaculture. In addition, these results also suggest that sex segregation, at least during the reproductive period of *O. vulgaris*, and periodic grading, should minimize mortality during the ongrowing of this species (García García *et al*., 2009; Estefanell *et al*., 2010b).

In summary, bogue discarded from fish farms appears to be an adequate diet for *O. vulgaris* adults at least during 2 months of rearing. The biological performance was drastically affected by sexual maturation and reproductive processes, reducing growth rates in females and increasing mortality in both sexes. High biomass increment prior to reproductive processes underlined de potential of *O. vulgaris* for aquaculture diversification.

11.6. Acknowledgments

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12. Estudio 8: "Benthic cages *versus* floating cages in *Octopus vulgaris*: biological performance and biochemical composition feeding on *Boops boops* discarded from fish farms"

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12.1. Abstract

Some benthic cephalopods are considered potential candidates to diversify marine aquaculture, as they show fast growth and high market price. Most research on cephalopod culture is currently focusing on the development of specific enrichments and compound feeds, while little research has been conducted in order to test new rearing systems for cephalopods. The rigid characteristic of the floating cages commonly used for the ongrowing of *Octopus vulgaris* has restricted their use to calm water conditions (estuaries and harbors). Such sites are scarce and highly demanded,

especially by the tourism industry; therefore the development of *Octopus vulgaris* grow out at these locations competes with touristic interests. The present study was set to compare the biological performance of *Octopus vulgaris* reared in a benthic cage (2 m^2) as opposed to the traditional floating cage (2.5 m^2), during two ongrowing trials. Initial rearing density was 10 kg/m^3 and octopuses (892 ± 125 g) were fed on bogue *Boops boops*, discarded from fish farms, for 60-67 days. High growth (1.8-1.9%/d) and high survival (91-97%) were observed, regardless of the rearing system, and led to best biomass increment (178-212%) and food conversion rates (2.3-2.6) ever recorded for *O. vulgaris* under industrial rearing conditions. These results underline the adequacy of the benthic cage for the ongrowing of this species, and also the potential of aquaculture discarded fishes, particularly bogue, as a single diet for this species. High growth rates obtained and the high lipid content of bogue (44% dry weight) suggest efficient lipid utilization in this species. Proximate composition and fatty acid profile in octopus muscle was not affected by the rearing system. High dietary lipid content was not reflected in muscle proximate composition, which showed high protein (87% dw) and low lipid content (5% dw) by the end of the experimental period. Farmed octopus showed high levels of n-3 HUFA (42%), which should enhance its value for the consumers.

Keywords: Octopus, benthic cages, growth, survival, aquaculture by-products, lipids.

12.2. Introduction

Some benthic cephalopod species are potential candidates to diversify marine farming as they show high growth rates and wide market demand (Hanlon, 1987; Semmens *et al*., 2004; Vaz-Pires *et al.* 2004; Iglesias *et al*., 2007). These species have short life cycles, have been successfully cultured through multiple generations under lab conditions (*Octopus maya* Voss & Solis Ramirez 1966, *Sepia officinalis* L. 1758) (Sykes *et al*., 2006; Rosas *et al*., 2009) and can be fed on low price unfrozen trash species (*Octopus vulgaris* Cuvier 1797) (Rodríguez *et al*., 2006; García García *et al*., 2009). In order to lower the costs and increase the sustainability of the cephalopod rearing industry, new larval rearing techniques (Navarro and Villanueva, 2003; Iglesias *et al*., 2007) and specific compound feeds are currently being developed (Domingues *et al*., 2005; Rosas *et al*., 2007; Cerezo Valverde *et al*., 2008; García Garrido *et al*., 2009; Estefanell *et al*., 2011a). In Spain, despite the low survival after the planktonic phase of the local common octopus *O. vulgaris* (Iglesias *et al*., 2004), high market demand led a few fishermen associations to become pioneers in octopus farming. This activity is based on the ongrowing of wild juveniles in floating cages. The associations obtain both the octopus juveniles (less than 1 kg) and the trash species, used as food, (fisheries discards) from their daily fishing activity. Octopus farming in these conditions described is in agreement with the "integrated aquaculture model" proposed for cephalopods (Boyle and Rodhouse, 2005). Indeed, during each growing cycles lasting 3-4 months, the octopuses are likely to reproduce and potentially increase the annual recruitment.

In a previous paper, the potential of fish farm discards used as a single diet for *O. vulgaris* was investigated (Estefanell *et al*., 2011a) and the growth and low mortality

observed were similar to the ones obtained with diets containing crustaceans, known to promote best growth in this species (García García and Cerezo Valverde, 2006; Biandolino *et al*., 2010; Prato *et al*., 2010; Estefanell *et al*., 2011c). Several small pelagic fish species are accidentally reared in floating cages in fish farms, one of them, the bogue *Boops boops* (L. 1758), is the main discarded species in Mediterranean and Eastern Central Atlantic fish farms and represents at least 2-5% of sea bream production cages. Such by product is of interest for animal nutrition as it is readily available and of no commercial interest for human consumption.

Commercial cage aquaculture is considered the fastest growing sector in aquaculture (Tacon and Halwart, 2007). The main advantage of cage culture is that it uses existing water bodies, which implies that both the initial capital investment and operational costs are considerably lower than in land-based facilities, which rely on pumped water (Beveridge, 2004). More than 150 fish species and a dozen of crustacean species have been cultured in cages at commercial or experimental scale (Chua and Tech, 2002). Indeed, in recent years the common octopus *O. vulgaris* has also been cultured in floating cages, kept afloat by polyethylene or fiberglass tubes (Socorro *et al*., 2005; Rodríguez *et al*., 2006; García García *et al*., 2009; Estefanell *et al*., 2012a, 2012b) or suspended from mussel rafts (Chapela *et al*., 2006). In general, the frame is made of stainless steel or galvanized iron enclosed with metallic net (normally 20x20 mm mesh) and has different sizes (from 4 to 27 m^3). Since benthic cephalopods spend most of their day cycle out of light in dens (Hanlon and Messenger, 1996), those were included in the different designs (10-25 dens/m^3, normally PVC T-shaped tubes of 160 mm diameter). The rigid characteristics of these floating cages require calm water conditions, such as estuaries ("Rías" in Galicia, NW Spain) (Chapela *et al*., 2006) or

harbors (Socorro *et al*., 2005; Rodríguez *et al*., 2006). The finite numbers of these sheltered areas and the competition with touristic interests affects the whole sea cage aquaculture industry, and suggests moving further offshore where wave action is the main concern (Pérez *et al*., 2003). Indeed, high water hydrodynamism in exposed coasts has been identified as a negative factor on octopus growth (García García *et al*., 2009). These authors also concluded that rearing temperatures above 21ºC had a negative effect on octopus biological performance in floating cages. High water temperatures in the sea are particularly critical at surface level and in sheltered areas, so octopus farming in floating cages in some areas like the Mediterranean would be restricted to 7-8 months of the year (October to June). Another factor decreasing octopus survival at sea surface level is related to occasional salinity drops associated to rainfall in winter months, where 100% mortality was reported (Chapela *et al*., 2006).

The present study intends to evaluate growth and survival in *O. vulgaris* reared in benthic cages confronted with traditional floating cages. The benefits of benthic rearing system are discussed in terms of ethology of benthic cephalopods and rearing environment (temperature, salinity, hydrodynamism), as well as visual impact in comparison to surface level rearing methods. Finally, the biochemical composition of muscle (90-95% of total body weight), obtained at the end of each trial is analyzed to study the effect of each rearing system on flesh quality.

12.3. Material and Methods

12.3.1. Capture and acclimatization of the stock

Wild octopuses were caught by local fishermen using cylindrical trawls (1.5 m diameter, 0.4 m high with metallic net of 31.6 mm mesh) placed at 20-30 m depth out

of the coast of Mogán (Canary Islands, Spain). Octopuses were transported to laboratory facilities in three 0.5 m^3 square tanks provided with pure oxygen. The specimens were acclimatized to captive conditions during one week in rectangular 1.5 m^3 tanks, provided with PVC tubes as shelters, shadowing nets and open flow-through seawater system (1500 L/h). Each octopus was PIT tagged on the upper left arm III (Estefanell *et al*., 2011b). During this period octopus were fed to satiation once a day with a mixed diet containing unfrozen crab (*Portunus pelagicus*, L. 1758) and bogue (*B. boops*) supplied on alternate days.

12.3.2. Rearing system

Grow-out trials were performed in floating and benthic cages (Fig. 12.1). Main structure of both systems was made of stainless steel enclosed in black PVC net of 2 cm mesh. The benthic cage was 2 x 1 x 2 m length, height and width respectively, divided into two subunits of 2 m^3 water capacity each. Twenty six dens (T-shaped PVC tubes of 160 mm diameter) were added to each subunit. The cage was placed on sandy sea bed at 27 m depth, underneath a local fish farm in Tufia (Telde, Gran Canaria, Spain) (27º 57' 31.7'' N, 15º 22' 22.5'' W). This area is regarded as highly exposed, submitted to wave height of 0.4 – 2.1 m and winds of 0.2 – 16 m/s, mainly NE, during the rearing period (network of state ports, Spanish Government, http://www.puertos.es). The floating cage was 1.5 x 3 x 1.5 m length, height and width respectively, divided into two subunits of 2.5 m^3 water capacity each. Forty two dens (T-shaped PVC tubes of 160 mm diameter) were added to each subunit and shadowing nets were attached to the top external side. The cage was anchored in a sheltered area in Taliarte Harbour (Telde, Gran Canaria, Spain) (27º 59'20.2'' N, 15º 22' 6.8'' W).

The assays were performed under natural photoperiod from December to

February (approx. 11:13 ± 0.5 hours of light:dark). In the benthic cage mean water temperature, measured once per day with a crystal thermometer, was 20.4 ± 0.5 ºC. In the floating cage, mean water temperature and oxygen levels, measured once a day with a portable oxymeter (Oxiguard Handy, Point tour Systems Inc., Canada), were 20.5 ± 0.4 ºC and 6.1 ± 0.4 mg/L, respectively.

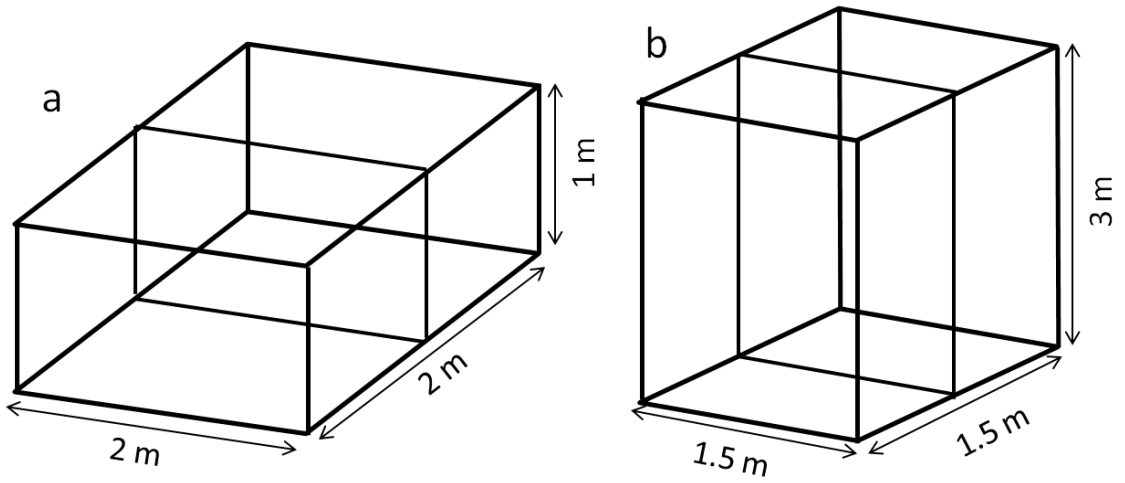

Fig. 12.1: Diagram of the sea cages used in this study: (a) benthic cage; (b) floating cage.

12.3.3 Diet

During this study octopuses were fed on whole bogue *B. Boops* as a single diet, supplied by local fish farms as "discarded species" (aquaculture by-product) of average size 88±54 g. Octopuses were fed to satiation 6 days a week (8:00 am). Initial daily food ratio represented 8% of octopus biomass and was provided in at least 3 portions of food per octopus (28 ± 4 g each). Octopuses in the benthic cage were fed by a professional diver. Daily food ratio was adjusted along the rearing period according to mortality rates and remaining food in the cage, checked visually three times per week by a member of our staff. In order to reproduce industrial rearing conditions, food remains were not removed from the bottom of the cage. Dead octopuses were

removed from the cages in order to avoid cannibalism which would interfere with the diet utilization.

12.3.4. Experimental design

The assays were run during 60-67 days (December-February) and initial rearing density was 10 kg/m^3 (Rodríguez *et al.*, 2006). Octopuses were weighted at the beginning and at the end of the experimental period. Initial rearing conditions are shown in Table 12.1.

- Trial 1: Octopuses were transferred to the benthic cage (N = 22) and to the floating cage (N = 30). This trial lasted 67 days in the benthic cage and 61 days in the floating cage. Male:female sex ratio was 1:1.

- Trial 2: Octopuses were transferred to the benthic cage (N = 22) and to the floating cage (N = 30). This trial lasted 62 days in the benthic cage and 60 days in the floating cage. In this trial only males were selected (sex ratio 1:0)

12.3.5. Biological parameters

12.3.5.1. Biological parameter calculated individually

- Specific Growth Rate: $SGR = (LnW_f - LnW_i)*100 / t$ (%/d)

12.3.5.2. Biological parameters calculated per treatment

- Initial weight dispersion: WD_i = (Standard deviation / W_{ai}); final weight dispersion: WD_f = (Standard deviation / W_{af}) (%).

- Initial density: $D_i = (B_i$ / cage volume); Final density: $D_f = (B_f$ / cage volume) (kg m^{-3}).

- Survival: $S = (n_f / n_i)$ (%)

- Biomass Increment: $BI = (B_f - B_i) / B_i$ (%). This parameter was standardized to 60 days of rearing (BI standardized: $BI_s = BI*60 / t$)

- Apparent Feed Conversion Ratio: A-FCR = PF / (W_{af} - W_{ai})

12.3.5.3. Condition and sexual maturity data

- Digestive gland index: DGI = (W_{DG} / W_f) (%)

- Sexual maturity "Hayashi Index" as modified by Guerra (1975) for males: H_M = W_N / (W_N + W_T), and females: H_F = W_{OG} / (W_{OG} + W_O).

- Gonadosomatic Index for males: GSI_M = W_N / (W_f - W_N), and females: GSI_F = W_O / (W_f - W_O) (%) (Otero *et al*., 2007)

- Macroscopic maturation (I, immature; II, maturing; III, mature, and IV, post-reproductive) (Dia and Goutschine, 1990)

- Egg masses were counted per treatment at the end of the trial.

Where: W_f = Final weight (g); W_i = Initial weight (g); t = Total time (d); W_{ai} = Initial average weight (g); W_{af} = Final average weight (g); B_f=Final biomass (g); B_i =Initial biomass (g); n_f =Final number of octopuses; n_i =Initial number of octopuses; PF is total food provided (g); W_N = Needham's complex + spermatophoric sac weight (g); W_T = Testis weight (g); W_{OG} = Oviducal gland weight (g); W_O = Ovary weight (g) and W_{DG} = digestive gland weight (g).

12.3.6. Sampling procedure

Bogue, discarded from fish farms, was analyzed at three different periods along the trials. Samples were taken from a pool of 6 whole individuals randomly selected every 3 weeks of feeding. At the end of each trial, octopuses were sacrificed by immersion in ice-cold sea water, prior to being weighted and dissected. Samples of muscle were taken from the whole left arm II and 3 pools of four octopuses were randomly selected per cage and trial. Each pool was homogenized and stored at -80ºC until biochemical analysis.

12.3.7. Biochemical analysis

Proximal composition of diets and octopus tissues from each treatment were analyzed following standard procedures of AOAC (1997). Moisture was determined after drying the sample in an oven at 105ºC until reaching constant weight; ash was determined by combustion in a muffle furnace at 600ºC for 12 hours; protein content (N × 6.25) was determined by Kjeldahl method and crude lipid was extracted following the method described by Folch *et al*. (1957). Fatty acids methyl esters from total lipids were extracted by transmethylation as described by Christie (1982) and separated by gas chromatography under the conditions described by Izquierdo *et al*. (1992). Only major fatty acids in bogue and octopus muscle are presented in this study. All analyses were conducted in triplicate.

12.3.8. Statistical analysis

Data, presented as mean ± standard deviation, was tested for normality (asymmetry, kurtosis) and homogeneity of variances (Levene's test). When necessary, arcsin transformation of the data was carried out, particularly when data was presented as %. When normality or homogeneity of variances was not achieved, non-parametric tests were used. Data (initial weight, final weight, SGR, DGI, GSI, H, proximate composition and fatty acids profile in muscle) were analyzed with the statistical computer package SPSS v15 (SPSS, Chicago, IL, USA), using the following General Linear Model, where "rearing system" was established as a fixed factor to test for differences at the end of each trial (one way ANOVA):

$$Y_i = \mu + \alpha_i + \beta X_i + \varepsilon_i$$

Where μ is the population mean, α_i the fixed effect of the diet, βX_i is regression of each biological parameter on initial weight within "rearing system" factor, and ε_i the

residual error. The weight of each individual at the beginning of the experimental period was considered as a covariate, in order to remove the potential effect of weight differences between individuals at the start of the experimental period on response variables. Survival data was transformed (0, survivors; 1, dead) and compared according to rearing system using a chi-squared test. The final weight of the females in trial 1, according to their sexual maturity stage (maturing or mature), was compared irrespective of the rearing system using a Student t test. The ρ correlation coefficient (Spearman) was calculated between final weight and H in males and females. Significant differences were considered when $P < 0.05$ throughout the manuscript. Biological parameters, calculated per cage, (weight dispersion, density, biomass increment and apparent food conversion rate) could not be compared statistically as they related to a single replica.

12.4. Results

12.4.1. Biological parameters calculated individually

No significant differences were observed in initial weight between rearing systems in trial 1 and 2. Octopuses reared in the benthic and in the floating cage showed significantly equal final weight and SGR in both trials (Table 12.1). In trial 1, males and females presented significantly equal SGR regardless of rearing system (1.8%/d).

12.4.2 Biological parameters calculated per cage

In general, high survival rates (91-97%) and high BI (178-212%) were observed in all cages irrespective of rearing system. Mortality equally affected males and females (one male and one female died in the benthic cage and only one female died

in the floating cage) during trial 1. An increase in rearing density, from 10 to 29-32 kg/$m^{3,}$ and weight dispersion (approximately 10%) were observed by the end of the rearing period in both trials regardless of the rearing system. Very low A-FCR was estimated in both rearing systems (2.3-2.6) (Table 12.2).

Table 12.1: Initial rearing conditions and individual growth data in the benthic and floating cage in both trials (mean ± SD)

		Benthic cage	Floating cage
Trial 1 (sex ratio 1:1)	N_i	22	30
	W_i (g)	932 ± 157	873 ± 96
	W_f (g)	3067 ± 813	2615 ± 506
	SGR (%/d)	1.8 ± 0.3	1.8 ± 0.4
Trial 2 (sex ratio 1:0)	N_i	22	30
	W_i (g)	921 ± 130	862 ± 115
	W_f (g)	3081 ± 821	2652 ± 599
	SGR (%/d)	1.9 ± 0.4	1.8 ± 0.4

Different superscript letters within a row denote significant differences according to rearing system ($P < 0.05$).

Table 12.2: Biological parameters calculated per cage in trial 1 and in trial 2.

	Trial 1		Trial 2	
	Benthic cage	Floating cage	Benthic cage	Floating cage
WD_i (%)	16.8	11.0	14.1	13.4
D_i (kg/m^3)	10.3	10.1	10.1	9.9
WD_f (%)	26.5	19.4	26.2	22.6
D_f (kg/m^3)	30.7	29.2	32.4	29.6
S (%)	90.9	96.7	95.5	96.7
BI_s (%)	178.3	186.4	212.2	197.5
A-FCR	2.4	2.6	2.3	2.5

12.4.3. Condition and sexual maturity data

A significantly equal DGI in males and females was observed in octopuses reared in the benthic and in the floating cage (Table 12.3). In this study no octopus was found in immature or post reproductive stage. All males dissected (N = 55) were sexually mature according to sexual indices and macroscopic evaluation in both rearing

systems. In contrast, a higher proportion of mature females were found in the benthic cage (80%), which also showed higher GSI_F in comparison to those reared in the floating cage (14%) (Fig. 12.2, Table 12.3). When analyzing data of sexual maturity in females, irrespective of rearing system, a significantly higher final weight was observed in those mature (N = 10, 8 from the benthic and 2 from the floating cage, 3006 ± 497 g,) than in those maturing (N = 14, 2 from the benthic and 12 from the floating cage, 2489 ± 471 g). Indeed, H_F was significantly correlated with final weight in females (ρ = 0.56), while H_M was not significantly correlated with final weight in males (ρ = 0.19). It is noteworthy that a significantly equal SGR was observed between mature females (1.8 ± 0.2%/d) and maturing females (1.7 ± 0.3%/d), regardless of rearing system. No egg masses were found in the dens at the end of the rearing period.

Table 12.3: Condition and sexual maturity data calculated at the end of the rearing period.

		Benthic cage	Floating cage
Males*	n_f	21	34
	W_f (g)	3112 ± 904	2662 ± 580
	DGI (%)	3.5 ± 1.1	3.6 ± 0.7
	H_M	0.48 ± 0.05	0.46 ± 0.05
	GSI_M (%)	0.3 ± 0.1	0.4 ± 0.1
Females	n_f	10	14
	W_f (g)	2840 ± 626	2545 ± 448
	DGI (%)	4.5 ± 1.0	4.6 ± 0.8
	H_F	0.03 ± 0.01	0.05 ± 0.03
	GSI_F (%)	4.0 ± 1.7 ^{b}	2.0 ± 1.4 ^{a}

(* Data including males reared in trial 1 and in trial 2). Different superscript letters within a row denote significant differences according to rearing system ($P < 0.05$).

12.4.4. Proximate composition and fatty acid profile in bogue (aquaculture by-product)

The proximate composition of bogue (N = 3) underlined the high lipid content of this food item (43.6 ± 5.2% dw), a protein content representing a 49.7 ± 5.9% dw, moisture a 66.4 ± 3.4% and ash a 1.8 ± 0.7%. Regarding the fatty acid profile, discarded

bogue was abundant in monoenes and n-9 (16:1n-7, palmitoleic acid; 18:1n-9, oleic acid) and n-6 (18:2n-6, linoleic acid), and showed low 20:4n-6 content (ARA) (Table 12.4).

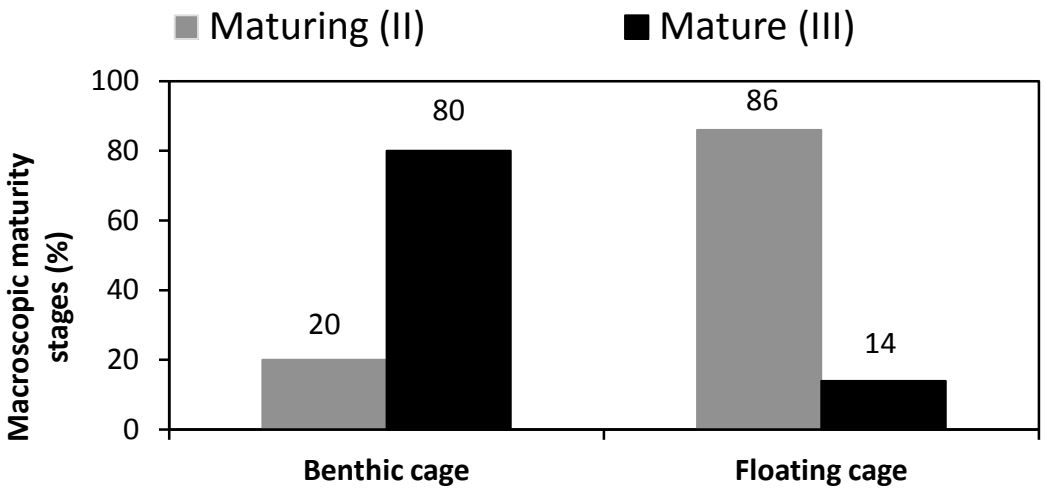

Fig. 12.1: Macroscopic sexual maturity stages (%) (Dia and Goutschine, 1990) in females reared in the benthic (N = 10) and in the floating cage (N = 14).

Table 12.4: Selected fatty acids in bogue, discarded from fish farms (% of total fatty acid) (mean ± SD, N = 3)

	Bogue
14:0	4.5 ± 0.0
16:0	17.5 ± 0.5
16:1 n-7	6.9 ± 0.1
18:0	4.8 ± 0.2
18:1 n-9	18.6 ± 0.3
18:1 n-7	3.0 ± 0.1
18:2 n-6	15.7 ± 0.5
20:4 n-6	0.7 ± 0.0
20:5 n-3	7.5 ± 0.2
22:6 n-3	7.2 ± 0.4
∑ Saturates	28.4 ± 0.7
∑ Monoenes	31.5 ± 0.1
∑ n-3	19.6 ± 1.1
∑ n-6	18.0 ± 0.5
∑ n-9	20.1 ± 0.2
∑ n-3 HUFA	17.2 ± 0.3
DHA/EPA	1.0 ± 0.1
DHA/ARA	10.6 ± 0.6
EPA/ARA	11.0 ± 0.5

12.4.5. Proximate composition and fatty acid profile in muscle

A significantly equal proximate composition was observed in muscle at the end of the rearing period, with low lipid and high protein content irrespective of rearing system (Table 12.5). The fatty acid profile in this tissue was not affected by rearing system, and was particularly abundant in 16:0 (palmitic acid), 18:1n-9 (oleic acid) and n-3 HUFA (20:5n-3, EPA; 22:6n-3, DHA) (Table 12.6).

Table 12.5: Proximate composition in muscle (% dry substance) after two months of feeding in the benthic and in the floating cage (mean ± SD, N = 6)

	Benthic cage*	Floating cage*
Lipids (%)	5.2 ± 0.2	5.5 ±0.4
Proteins (%)	86.8 ± 2.2	86.9 ± 2.7
Moisture (%)	81.6 ± 0.9	80.8 ± 0.9
Ash (%)	1.8 ± 0.1	1.7 ± 0.2

(* Data including octopuses reared in trial 1 and in trial 2). Different superscript letters within a row denote significant differences according to rearing system ($P < 0.05$).

12.5. Discussion

The results of this study underline the potential of the benthic cage for octopus ongrowing. Even though octopuses showed a similar biological performance in comparison to those reared in the floating cage in the harbor, low availability of these sheltered areas and competition with touristic interests demand moving sea cages further offshore, a situation that is affecting the entire sea cage aquaculture industry (Pérez *et al*., 2003). In order to adapt to offshore conditions, fish farms are currently using flexible cages, designed to operate in highly exposed coasts (Beveridge, 2004). However, the rigid characteristics of floating cages commonly used in octopus ongrowing makes them especially vulnerable to wave action. Indeed, recent reports observed that even relatively low wave height (0.4 - 1.2 m) had a negative effect on *O. vulgaris* growth in the Mediterranean (García García *et al*., 2009). Also, the use of

benthic cages would provide a more stable rearing environment than at surface level (Oppedal *et al*., 2011), since both high water temperature in the summer and decreasing salinity in the winter have been related with low growth and mortality in *O. vulgaris* (Chapela *et al*., 2006; García García *et al*., 2009). Also, even though in this study the octopuses were hand fed by a professional diver, the technology to overcome this practical limitation is already available (Dempster *et* al., 2009; Doxa *et al*., 2011).

Table 12.6: Selected fatty acids in octopus muscle (% of total fatty acids) after two months of feeding in the benthic and in the floating cage (mean ± SD, N = 6)

	Benthic cages*	Floating cages*
14:0	0.8 ± 0.1	0.9 ± 0.2
16:0	18.8 ± 0.4	18.9 ± 0.5
16:1 n-7	0.6 ± 0.1	0.7 ± 0.1
18:0	5.1 ± 0.2	5.2 ± 0.1
18:1 n-9	12.4 ± 0.6	12.5 ± 0.6
18:1 n-7	2.9 ± 0.5	2.6 ± 0.3
18:2 n-6	1.1 ± 0.3	0.9 ± 0.2
20:4 n-6	5.0 ± 0.5	5.4 ± 0.7
20:5 n-3	15.8 ± 1.4	15.4 ± 0.6
22:6 n-3	23.9 ± 0.7	23.8 ± 0.8
∑ Saturated	26.0 ± 0.6	26.3 ± 0.4
∑ Monoenes	22.7 ± 1.4	22.9 ± 0.9
∑ n-3	42.4 ± 1.5	41.8 ± 1.0
∑ n-6	8.4 ± 0.5	8.5 ± 0.9
∑ n-9	16.2 ± 0.7	16.4 ± 0.6
∑ n-3 HUFA	41.9 ± 1.5	41.4 ± 1.0
DHA/EPA	1.5 ± 0.1	1.5 ± 0.1
DHA/ARA	4.8 ± 0.4	4.5 ± 0.8
EPA/ARA	3.2 ± 0.4	2.9 ± 0.4

(* Data including octopuses reared in trial 1 and in trial 2). Different superscript letters within a row denote significant differences according to rearing system ($P < 0.05$).

With regards to the estimation of cephalopods grow-out performances, values can be compared between different grow-out structures and settings. Several cephalopod species have been maintained, reared or cultured in tanks and aquariums with high levels of success (Hanlon, 1987; Vaz Pires *et al*., 2004; Sykes *et al*., 2006).

From a biological point of view, similar performance has been reported in *O. vulgaris* reared in-land and in sea cages under different conditions (Milliou *et al*., 2005; García García and Cerezo Valverde, 2006; Domingues *et al*., 2008; Biandolino *et al*., 2010; Prato *et al*., 2010). However, in-land facilities require higher initial investment and operational costs than sea cage facilities (Beveridge, 2004). In particular, the profitability of *O. vulgaris* farming was evaluated in the Mediterranean, concluding that the minimal selling price for viability was 6.41€ in sea cages and 8.06€ in in-land closed water system facilities (García García *et al*., 2004, 2010). Based on these financial differences, the development of octopus farming is very likely to occur in offshore cages. Other cephalopods also reared at large scale in floating cages and ponds were the squid *Sepioteuthis lessoniana* (Férussac 1831) and the cuttlefish *Sepia lycidas* (Gray 1849), with high levels of success (Hanlon, 1987). The benthic cage described in this study could also be tested for other cephalopods with great potential for large scale culture, such as the *O. maya*, the *Octopus bimaculoides* (Pickford & McConnaughey 1949) and the *S. officinalis* (Hanlon, 1987; Sykes *et al*., 2006; Rosas *et al*., 2009).

In this study, high growth and low mortality led to best biomass increment (178-212%) and A-FCR (2.3-2.6) ever recorded in *O. vulgaris* ongrowing in sea cages (Socorro *et al*., 2005; Chapela *et al*., 2006; Rodríguez *et al*., 2006; García García *et al*., 2009; Estefanell *et al*., 2012a, 2012b). High survival observed in both rearing systems was related to the lack of reproductive processes, associated to increasing mortality under rearing conditions for both sexes (Estefanell *et al*., 2010b). Indeed, no post-reproductive octopus was found, confirmed by sexual maturity data and also by the absence of egg masses in trial 1 at the end of the rearing period. This could be related

to the time of the year, since *O. vulgaris* in the Canary Islands shows a peak of brooding behaviour in spring (Hernandez García *et al*., 2002). Also, males and females showed similar growth rates regardless of sexual maturity stage. This differs from previous findings, where females showed decreasing growth rates after sexual maturation (Forsythe and Van Heukelem, 1987). It is noteworthy that even though 80% of females were sexually mature according to the H_F index and to macroscopic evaluation in the benthic cage, GSI_F showed lower values than previous data observed in full mature females of *O. vulgaris* under rearing conditions, which can reach up to 13% (unpublished data). This observation suggests that perhaps full sexual maturity in females was not reached and if the experimental period had been extended for another month significant differences in growth between sexes would have been found and reproductive behaviour would have probably occurred. High correlation between H_F and females weight suggests that different sexual maturity observed in females was related to differences in size rather than the rearing system, although the presumably higher light intensity at surface level than at 27 m depth is one of the factor decreasing sexual maturation in *O. vulgaris* (Mangold, 1987).

Other factors that explain the high biological performance observed in this study were the adequate temperature range (20-21ºC) and the low initial weight dispersion (<20%) (García García *et al*., 2009). Final biomass reached up to 32 kg/m^3, a value higher than the ones observed in *O. vulgaris* reared in tanks at similar temperature range (Domingues *et al*., 2008), probably as a consequence of the high water quality in sea cages. Finally, growth rates observed in this study were comparable to those observed in octopuses fed on crustaceans, known to promote best growth in *O. vulgaris* (García García and Cerezo Valverde, 2006; Biandolino *et al*.,

2009; Prato *et al*., 2010; Estefanell *et al*., 2011c). These results emphasized the potential of bogue as a single diet for this species, suggesting a profitable use of fish farm discards (aquaculture by-products).

High lipid content and the abundance of particular fatty acids in discarded bogue were in agreement with the profile obtained from farm-aggregated bogue (Arechavala Lopez *et al*., 2010), and probably a consequence of accidental feeding on commercial compound diets, normally high in lipids and containing vegetable oils as partial replacement of fish oils. High growth observed in octopus fed on bogue presenting high lipid content suggests efficient lipid utilization by *O. vulgaris* (Estefanell *et al.*, 2011a). While first works on cephalopods nutrition reported a low capacity to utilize dietary lipids (O'Dor *et al.*, 1984), other studies agreed that lipid utilization in cephalopods was affected by the type and quantity of dietary lipids (Lee, 1994; Mazón *et al.*, 2007; Sánchez *et al.*, 2009; Seiça Neves *et al.*, 2010). Furthermore, the use of lipids as energy source has been reported in *O. vulgaris* after long term starvation (García Garrido *et al.*, 2010).

Biochemical composition in muscle was not affected by rearing system, which underlines the adequacy of the benthic cage for large scale ongrowing of this species. In addition, high lipid content in the diet was not reflected in muscle proximate composition, which showed similar profile to the ones of other cephalopods, with low lipid and high protein content (García García and Cerezo Valverde, 2006; Prato *et al*., 2010). Also, main fatty acids in octopus muscle are in agreement with previous data, showing high 16:0, 18:1n-9, ARA, EPA and DHA content (Navarro and Villanueva, 2003; Miliou *et al.*, 2007; Prato *et al.*, 2010). Low ARA content in bogue did not have a negative effect on octopus growth, being consistent with similar observations in a

previous study (Estefanell *et al*., 2011a, 2011c). High n-3 HUFA content in reared octopus muscle should enhance its value for the consumer.

As a conclusion, the benthic cage has several advantages in comparison with the traditional floating cage that suggests its use for large-scale ongrowing of *O. vulgaris* and other benthic cephalopod species. High biological performance observed in this study was mainly related to the lack of reproductive behaviour and the nutritional value of bogue, provided as a single food item, which also suggest a profitable alternative to fish farm discards. Both proximate composition and fatty acid profile in octopus muscle were not affected by the rearing system.

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13. CONCLUSIONES

1. La inmersión en agua de mar con un 1.5% de etanol (96%) es adecuado para el anestesiado del pulpo común *Octopus vulgaris*. El efecto narcótico es rápido, entre 60 y 100 segundos de inmersión, y la recuperación es total a los 5 minutos.
2. El marcaje individual con PIT subcutáneo es un proceso sencillo y rápido, con tasas de retención próximas al 100% y sin efecto negativo sobre el crecimiento y la supervivencia.
3. La maduración sexual en los machos no se ve afectada por el sistema de cultivo, el sex ratio o la presencia de hembras. Por el contrario, la maduración sexual en las hembras se acelera por la presencia de machos bajo condiciones de cultivo.
4. La madurez sexual en las hembras tiene un claro efecto negativo sobre su crecimiento. Además, se observa un incremento de la mortalidad en ambos sexos asociado a los fenómenos reproductivos.
5. El sistema de cultivo grupal en jaulas flotantes genera un mayor crecimiento en pulpo en comparación con el sistema individual.
6. El cultivo de pulpos en jaulas individuales minimiza la dispersión de tallas y la mortalidad en comparación con el sistema grupal, sin afectar a la composición bioquímica de los ejemplares.
7. La boga, procedente de descartes de la acuicultura, presenta un elevado contenido lipídico rico en ácido oleico y linoleico, y pobre en ARA en comparación con el pulpo.
8. La boga salvaje presenta un bajo contenido lipídico, similar al músculo de

pulpo, con altos valores de n-3 HUFA, pero un bajo contenido de ARA.

9. Las especies de cangrejos evaluadas presentan un bajo contenido lipídico, similar al músculo de pulpo, con altos valores de n-3 HUFA y ARA.
10. El mayor crecimiento y retención proteica registrado en pulpos alimentados con boga de cultivo en comparación con ejemplares alimentados con boga salvaje implica una eficiente utilización de los lípidos de la dieta como fuente de energía.
11. El crecimiento observado en pulpos alimentados con boga de cultivo es similar al obtenido en ejemplares alimentados con monodietas de cangrejos. La dieta mixta de un 60% de cangrejo azul y un 40% de boga de cultivo genera el crecimiento más elevado, superior al obtenido en una dieta única de boga.
12. Los ensayos de cultivo industriales confirman el potencial del uso de la monodieta de boga procedente de subproductos de la acuicultura para el engorde de pulpo, con tasas de crecimiento de 800-1000 g/mes.
13. La glándula digestiva refleja el perfil lipídico de la dieta, tanto a nivel cuantitativo como cualitativo, mientras que el músculo presenta un perfil más estable.
14. El bajo contenido en ARA en la boga se ve reflejado en el músculo de pulpo desde las 4 semanas de alimentación, sin efecto aparente sobre el crecimiento hasta las 8 semanas de cultivo.
15. Los piensos semihúmedos basados en filetes de boga fueron aceptados e ingeridos por los pulpos, observándose un crecimiento de 300-500 g/mes en tanques.
16. La inclusión de un 10% de carne de cangrejo en un pienso de filetes de boga no

mejora la ingesta ni el crecimiento del pulpo.

17. Las harinas testadas en la preparación de los piensos para pulpo generan un crecimiento negativo, siendo necesario profundizar en la búsqueda de ingredientes y sistemas de elaboración de los piensos específicos para pulpo.
18. Los pulpos cultivados en la jaula bentónica presentan un crecimiento y una supervivencia similar a los cultivados en jaulas flotantes.
19. Las jaulas bentónicas para el cultivo de pulpo presentan varias ventajas: no necesitan zonas resguardadas del oleaje, no tienen impacto visual y proporcionan un ambiente estable en cuanto a temperatura y salinidad.

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