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Laboratorio de Biología Marina
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Uso de los ANFÍPODOS (Crustacea: Peracarida: Amphipoda) en acuicultura

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Use of amphipods (Crustacea: Peracarida: Amphipoda) in aquaculture

Pablo Jiménez Prada

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INFORMAN:

Que esta Memoria de Investigación, titulada "Uso de los anfípodos (Crustacea: Peracarida: Amphipoda) en acuicultura", fue realizada por Pablo Jiménez Prada bajo su dirección, en el Departamento de Zoología de la Universidad de Sevilla. Considerando que reúne las condiciones necesarias para constituir un trabajo de Tesis Doctoral, autorizan su defensa ante los miembros del Tribunal para optar al titulo de Doctor.

Sevilla, a 09 de mayo del 2018

Director (1)

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Agradecimentos

> - Hola, buenas, soy alumno de la universidad de La Laguna, estuve en la planta de visita y me gustaría poder hacer prácticas de empresa con los pulpos.
>
> - Vale, ¿Cuándo terminas los exámenes? - Dijo una voz ronca al otro lado del teléfono.
>
> - El 6…
>
> - Po'vente el 7. – Sentenció con acento *andalú.*

De esta forma, hace casi una década conocí a Eduardo. A él le doy las gracias por abrirme las puertas de la investigación, por tratarme como a un biólogo más sin haber terminado la carrera, por darme la oportunidad de ejercer mi actual profesión e ilusión, y mandar mi primer póster que sería después ¡mi primer artículo! Y, por supuesto, que me llevara al chiringuito del puerto a comer bocadillos de mejillones, pollito y atún ¡qué sería Tenerife sin ellos! ¡O sin Ricardo! "El sureño más internacional del globo", de risa contagiosa y conversación interminable, con quien mejor me lo he pasado en el trabajo y volveré a pasarlo, estoy seguro. No puedo hablar de Tenerife sin acordarme de "my lab-Darling", mi querida Sarita, "mi niña" de sonrisa y lágrima fácil que, junto a su mini-familia (Ana, Cova, José, Lupe y Marga), me hicieron sentir como uno más en todo momento.

Si hablamos de familia, al entrar en el departamento encontré una. Cómo no mencionar a mi *Pili pili*, la mujer que puso su vida en mis manos saltando al vacío y de la que heredé su mesa; Maca con sus eternas anécdotas; y, como no, "La Grinch" del departamento que tantas buenas tardes me hizo pasar, quién me diría que después su tesis sería mi biblia ¿verdad, Elena? No haré ningún comentario sobre la Beca de Colaboración y cómo algún "hombre de poca fe" siempre me omitió de todos los emails al respecto, pero no dudó en darme la oportunidad de poder escribir estos agradecimientos. Al entrar en el departamento tuve la suerte de conocer a Sara que, aunque nos abandonara por "sus honguitos", no duda en sumarse a una peli con pizza (lo de la peli es secundario) y, sin estar, siempre será mi compi de departamento preferida. Con Carlos, conseguí el título de "Rey de lo Tanaidaceos" durante las tardes interminables mientras separabamos bichos en los ratos libres que teníamos entre ver videos de Youtube y comer galletas de dinosaurios. A estos dos individuos solo les puedo decir: os quiero. La familia siguió creciendo y llegó Gemma, mi estómago gemelo y fiel seguidora de nuestro lema THINK IN FAT. Paco y Altai, "The Couple", con sus increíbles fotos submarinas; Martita, "la reina del

Power Point"; Semperepedia, "el maestro Jedi de la estadística"; Rafa, "The Ginger Slave"; y, por último, el imperturbable hombre que todo lo hace bien: Free. Ellos/as son los/as que han hecho sentirme como en casa y les debo gran parte de esta tesis. Gracias, familia.

Lo bonito de la etapa predoctoral es que la tesis se vuelve de todos, de los más cercanos, no es algo propio, no se puede hacer sólo, intervienen en el proceso cada persona que conoces, incluso las que se van, pero hay alguien que siempre ha estado desde mucho antes de saber siquiera que es un doctorado, ella es Elvira, esa niña de carácter cortante que desde los 14 años ha ido perfeccionado la habilidad de no insultarme (aunque lo parezca mucho, la verdad) sino de calificarme objetivamente para saber como soy y que, desde la distancia, siempre ha sido un hombro para mí. Anita y Valeria me hicieron descubrir que tenía una hermanita en Sevilla que, aunque no es de sangre, lo es de corazón, y que las uruguayas son las más "dramaqueen" del planeta (sin exagerar, lo prometo) pero las mejores amigas también. Belén hizo que los rincones de Cádiz para mí dejaran de tener nombre y pasaran a tener fechas de cafés y charlas. Haciendo uso de un término muy biológico, gracias a la serendipia, "descubrimiento o hallazgo realizado por accidente, casualidad, inesperado y afortunado, de cosas que no se están buscando ni investigando, pero que son la solución para otro problema que se tenía", conocí a Eva, mi cojita escenógrafa cortacuentos que, aparte de ser la diseñadora de la magnífica portada, en tan poco tiempo ha conseguido ser tan importante en mi vida. Gracias, Pequeña.

Para terminar, agradecerles a mis directores por confiar en mí para este proyecto. A Ismael, por todo lo que me ha enseñado del lipídico mundo de la acuicultura, y a Jose… ¡qué no agradecerle a Jose! Si le dedico a él más líneas que a ninguna otra persona en los agradecimientos, perdonad. Pero cómo se le agradece a un director de tesis que se acuesta a las 2 de la madrugada porque te ha hecho una tarta de cumpleaños, que te llama a cualquier hora del día para continuar la conversación que dejasteis a media, que incluso enfadado transmita cercanía haciéndote mejorar día a día como investigador pero, sobre todo, como persona, y por miles de detalles que lo hacen, sin duda alguna, la mejor persona que conozco. Me siento afortunado de haber sido doctorando tuyo, pero más aun de ser tu amigo.

Y, por supuesto, a la persona que me ha enseñado todo lo que sé de la vida, a la que me ha enseñado que con esfuerzo y trabajo se consigue todo, la que me enseñó a reír llorando y a llorar de risa, la mujer de mi vida: mi **MADRE.**

Roads go ever ever on,
Over rock and under tree,
By caves where never sun has shone,
By streams that never find the sea;
Over snow by winter sown,
And through the merry flowers of June,
Over grass and over stone,
And under mountains in the moon.

The Road goes ever on and on
Down from the door where it began.
Now far ahead the Road has gone,
And I must follow, if I can,
Pursuing it with eager feet,
Until it joins some larger way
Where many paths and errands meet.
And whither then? I cannot say.

Song of Bilbo Baggins

Índice

Resumen general

La protección costera, el ciclo de nutrientes, el control de la erosión, la purificación del agua y el secuestro de carbono son servicios ecosistémicos proporcionados por los esteros. Además, las salinas ofrecen un hábitat de cría para peces proporcionando además abundantes invertebrados, como anfípodos, potencialmente útiles como recurso en la acuicultura. La harina de pescado y el aceite de pescado son recursos alimentarios necesarios para apoyar la acuicultura de especies carnívoras debido a sus ácidos grasos poliinsaturados de cadena larga (LC-PUFA) omega-3 y omega-6. Actualmente, la acuicultura depende de pesquerías y piensos limitados con niveles elevados de LC-PUFA n-3, pero es necesario desarrollar fuentes de alimentos más sostenibles. Los anfípodos son crustáceos con una alta calidad nutricional y un gran potencial para criarlos como alimento para especies de interés acuícola, juegan un rol fundamental en el intercambio energético de la cadena trófica como recurso de los depredadores, y parecen ser un recurso alternativo potencial de alta calidad para la acuicultura.

La presente tesis trata de explorar las siguientes preguntas: i) ¿Cuál es la importancia de los anfípodos en la dieta en especies de interés comercial en acuicultura; ii) ¿Qué composición nutricional tienen las especies de anfípodos de esteros?; iii) ¿Cómo se cultiva y qué análisis nutricional tienen los anfípodos alimentados con diferentes dietas en condiciones de laboratorio? y iv) ¿Es posible utilizar anfípodos como recurso alternativo de alimentos con especies de interés comercial?.

Por ello, se realizó un estudio nutricional para varias especies principales de anfípodos -*Microdeutopus gryllotalpa, Monocorophium acherusicum, Gammarus insensibilis, Melita palmata* y *Cymadusa filosa*- en esteros del sur de España. Estas especies mostraron un alto contenido de proteína (hasta 40%), altos niveles de PUFA n-3 y fosfolípidos, altos niveles de fosfatidilcolina (PC), fosfatidiletanolamina (PE) y triacilgliceroles (TAG). Isoleucina, glicina y alanina fueron los aminoácidos dominantes en todas las especies. Además, los anfípodos recolectados en estanques mostraron bajos niveles de metales pesados.

Por lo tanto, los anfípodos de esteros son buenos candidatos para ser utilizados como alimento y se proponen como un nuevo recurso económico sostenible para ser utilizado en la acuicultura. De las especies estudiadas, *G. insensibilis* puede ser la mejor para el cultivo intensivo como recurso de alimentación alternativo porque muestra: 1) composición adecuada de PUFA n-3 y fosfolípidos; 2) altos niveles de glicina, alanina, tirosina, isoleucina y lisina; 3) altas densidades naturales; 4) tamaño grande (≥1 cm) y 5) alta concentración de calcio. Además, una acuicultura combinada de anfípodos y peces en los estanques de esteros parece una forma prometedora y ambientalmente sostenible de desarrollar un sistema de Acuacultura Multitrófica Integrada (IMTA) en estos ecosistemas.

Se utilizaron dos modelos de anfípodo para el cultivo, los caprélidos *Caprella equilibra* y *C. scaura* y el gammarideo *Gammarus insensibilis*. En ambos casos, se ensayaron distintas dietas, *Artemia*, fitoplancton y detritus (consistente en heces y resto de pienso, deshechos de tanques de acuicultura). Además, se añadió *Ulva* en el experimento de *G. insensibilis*. Los caprélidos adultos alimentados con detritus mostraron una tasa de supervivencia significativamente más alta, y aquellos alimentados con fitoplancton y detritus fueron más ricos en ácidos grasos poliinsaturados, especialmente DHA. Curiosamente, los caprélidos alimentados con detritus también fueron una fuente rica de LA (18:2-n6), considerada como un ácido graso esencial en los vertebrados. Se encontró que los desechos basados principalmente en heces y restos de pienso no consumidos pueden considerarse un alimento adecuado para los caprélidos adultos, proporcionando una fuente de ácidos grasos omega-3 (DHA) y omega-6 (LA). En el estudio con gammarideos, *G. insensibilis* se cultivó durante 21 días. Tanto los alimentados con detritus y *Ulva* mostraron tasas de supervivencia interesantes y contribuyeron a altas concentraciones de ácido palmítico (16: 0), ácido oleico (18: 1n9), ácido araquidónico (20: 4n6) (ARA), ácido eicosapentaenoico (20: 5n3) (EPA) y ácido docosahexaenoico (22: 6n3) (DHA). Además, *Gammarus insensibilis* podría desempeñar un papel clave como biorremediador siendo cultivado con detritus y *Ulva* obteniendo una composición bioquímica adecuada para ser utilizada en acuicultura. Este anfípodo podría reemplazar parcial o totalmente la dieta formulada con la consiguiente reducción de los costes económicos.

Finalmente, se realizaron dos experimentos con larvas de *Seriola dumerili* (modelo de especie comercial acuícola) de 22 y 44 días después de la eclosión. Se alimentaron con el anfípodo *Gammarus insensibilis*, procedente de esteros, y con una dieta formulada. Se midieron supervivencia, biometría y perfil nutricional de las larvas. En el primer experimento, el tratamiento con *G. insensiblis* mostró una mejor supervivencia, aunque los otros parámetros no fueron diferentes. Por otro lado, en el segundo experimento la supervivencia fue similar, el crecimiento fue mejor en la dieta comercial, pero los alimentados con el anfípodo tuvieron un mejor perfil químico. El tratamiento con *G. insensibilis* mostró valores más altos de ARA (5,53 ± 0,18%) y DHA (19,07 ± 0,19%), niveles bajo de TAG (2,18 ± 0,5%) y una coloración similar a la de los juveniles silvestres de *S. dumerili*.

Como conclusión, la presente tesis muestra el alto valor nutricional de los anfípodos tanto en el medio natural como cultivados, y su gran potencial para poder ser usado como recurso trófico en acuicultura. Es interesante explorar futuras iniciativas en el contexto de la Acuicultura Multitrófica Integrada, donde los anfípodos parecen ser un recurso muy adecuado, pues puede combinarse su uso como biofiltros y una producción a gran escala con un coste muy reducido a partir de los desechos de cultivos asociados

General summary

Coastal protection, nutrient cycling, erosion control, water purification, and carbon sequestration are ecosystem services provided by salt marshes. Additionally, salt ponds offer coastal breeding and a nursery habitat for fishes and they provide abundant invertebrates, such as amphipods, which are potentially useful as a resource in aquaculture. Fishmeal and fish oil are necessary food resources to support aquaculture of carnivorous species due to their omega-3 and omega-6 long-chain polyunsaturated fatty acids (LC-PUFA). Currently aquaculture depends on limited fisheries and feed with elevated n-3 LC-PUFA levels, but the development of more sustainable food sources is necessary. Amphipods are crustacean with high nutritional quality and great potential to be reared as food for species of aquaculture interest. They play a fundamental rol in energetic exchange of trophic chain as predators' resource. They appear to be a potential high quality alternative feed resource for aquaculture.

The present thesis tries to explore the next questions: i) How important are amphipods in diet of species of commercial interest in aquaculture; ii) What nutritional composition have the amphipods species terrestrial ponds?; iii) How can be cultured and what nutritional value have the amphipods fed with different diets under laboratory conditions? and iv) Is it possible use the amphipod as alternative food recourse with species of commercial interest?.

Hence, a nutritional study was carried out for several main amphipod species -*Microdeutopus gryllotalpa*, *Monocorophium acherusicum*, *Gammarus insensibilis*, *Melita palmata* and *Cymadusa filosa*- in terrestrial ponds in the South of Spain. These species showed high protein content (up to 40%), high n-3 PUFA and phospholipid levels, and high levels of phophatidylcholine (PC), phosphatidylethanolamine (PE) and triacylglycerols (TAG). Isoleucine, glycine and alanine were the dominant amino acids in all species. In addition, amphipods collected from ponds showed low levels of heavy metals. Therefore, pond amphipods are good candidates to be used as feed and are proposed as a new sustainable economic resource to be used in aquaculture. Of the five studied species, *G. insensibilis* may be the best for intensive culture as an alternative feed resource because it shows: 1) adequate n-3 PUFA and phospholipids composition; 2) high levels of glycine, alanine, tyrosine, isoleucine and lysine; 3) high natural densities; 4) large body size (≥1 cm), and 5) high concentration of calcium. Moreover, a combined culture of amphipods and fishes in these marsh ponds seems a promising and environmentally sustainable way to develop Integrate Multi-Trophic Aquaculture (IMTA) in these ecosystems.

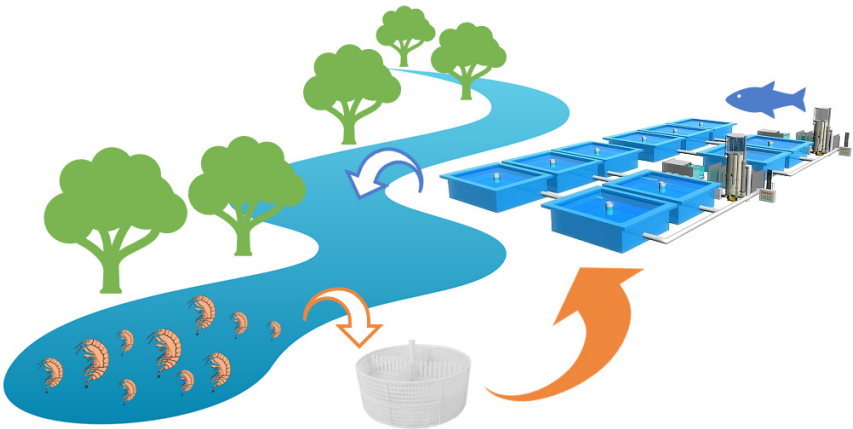
Two models of amphipod were used to be cultured, caprellids (*Caprella equilibra* and *C. scaura*) and gammarideans (*Gammarus insensibilis*). In both experiment *Artemia*, Phytoplankton, Detritus were used as food. In addition, *Ulva* was added in *G. insensibilis* experiment. Caprellids showed an adult survival rate significantly higher for caprellids fed with Detritus, and those fed with Phytoplankton and Detritus were richer in polyunsaturated fatty acids, especially DHA. Interestingly, caprellids fed with Detritus were also a rich source of LA (18:2-n6), considered to be an essential fatty acid in vertebrates. It was found that detritus based mainly on fish faeces and uneaten feed pellets can be considered an adequate food for adult caprellids, providing a source

of both omega-3 (DHA) and omega-6 (LA) fatty acids. In the study with gammarideans, *G. insensibilis* was cultured for 21 days. When fed with Detritus and *Ulva* gammarideans also showed interesting survival rates and contributed to high concentrations of palmitic acid (16:0), oleic acid (18:1n9), arachidonic acid (20:4n6) (ARA), eicosapentaenoic acid (20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA). Furthermore, *Gammarus insensibilis* could play a key role as biorremediator being cultured with detritus and *Ulva* getting an adequate biochemical composition to be used in aquaculture. This amphipod could replace partial or totally the formulated diet with two consequences, no dependence of fish oil and reduction of economic costs.

Finally, two experiments were done with *Seriola dumerili* larvae (model of commercial aquaculture species) of 22 and 44 days after hatchery. They were feed with the amphipod *Gammarus insensibilis*, collected from a terrestrial pond, and one formulated diet. Survival, biometry measures and chemical profile were measured. In the first experiment, *G. insensiblis* treatment showed better survival, although the other parameters were no different. On the other hand, in the second experiment the survival was similar, the growth was better in formulated diet but those fed with amphipod had the best chemical profile. Juveniles fed with *G. insensibilis* showed higher values of ARA (5.53±0.18%) and DHA (19.07±0.19%), low level of TAG (2.18±0.5%) and a coloration more similar to wild juveniles of *S. dumerili*.

In conclusion, this thesis shows the high nutritional value of amphipods both in the wild and cultivated, and its great potential to be used as new trophic resource in aquaculture. It is interesting to explore future initiatives in the context of Integrated Multitrophic Aquaculture, where amphipods appear to be a very suitable resource, since their use as biofilters and a large-scale production can be combined at a very low cost.

Capítulo
1 Introducción general



1. Introducción general

La relevancia de la acuicultura radica en la importancia del consumo de productos acuáticos en las dietas equilibradas al ofrecer proteína de alta calidad, contener todos los aminoácidos esenciales y ser fácilmente digeribles (Kaushik and Seiliez, 2010). Además, son especialmente indicados por su alto contenido en ácidos grasos esenciales omega3 (EPA y DHA) y omega6 (ARA) (Tocher, 2010), vitaminas (D, A y B) (NRC, 2011) y minerales (calcio, iodo, zinc, hierro y selenio) (Watanabe et al., 1988,1997). Tradicionalmente la totalidad de los productos acuícolas han procedido de la actividad extractiva, pero desde los años 80 se estabilizó en torno a los 90 millones de toneladas anuales, techo definitivo que se había vaticinado para esta actividad (FAO, 2016). Fue precisamente desde la década de los 80's, cuando se produce el despegue definitivo de la acuicultura.

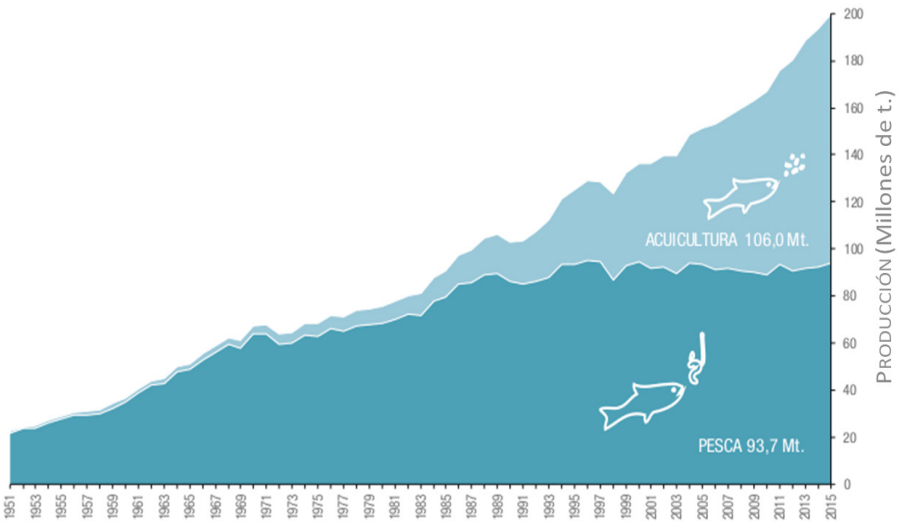


Figura 1. Producción de productos acuícola en el periodo 1950-2015.
Fuente: APROMAR (2017).

Tal como se muestra en la figura 1 el máximo se alcanza en 2015, produciéndose 106 millones de toneladas y superando en 12,3 millones a la cantidad extraída por la actividad pesquera la cual ese mismo año la actividad extractiva alcanzó también su máximo (93,7 millones de toneladas) (FAO, 2016).

Por lo que, lejos que la acuicultura sea un complemento de la actividad pesquera es su evolución natural, al igual que la ganadería reemplazó a la caza. La acuicultura presenta cinco grandes ventajas frente a la ganadería terrestre (APROMAR, 2017):

- El 79% de la superficie terrestre es agua.
- No consume agua dulce.
- Tasas de reproducción de varios ordenes de magnitud superior a los vertebrados terrestres.
- Al flotar en su medio tienen tasas de conversión del alimento superiores.
- Ahorro de energía en el calor corporal al ser homeotermos.

De la totalidad de la producción acuícola (53.9 % es de agua marina y el 46.1% de agua dulce) el 49% son peces (Fig. 2) con un valor de mercado cercano a los 80 millones de euros (FAO, 2016)..

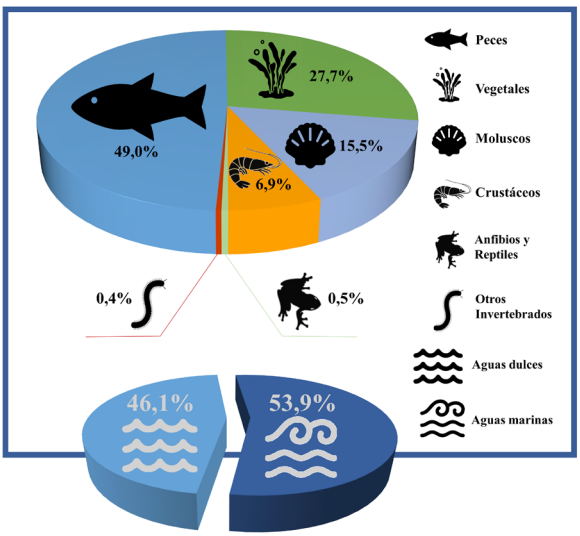


Figura 2. Distribución porcentual de la producción de acuicultura mundial. FAO (2016)

Por ello, en el marco de los programas de investigación innovadores en acuicultura, actualmente hay dos áreas de interés creciente:

1) La búsqueda de organismos alternativos de alimentación en vivo:

Muchos de los esfuerzos de la acuicultura de peces marinos, particularmente para larvas o etapas juveniles de peces, van dirigidos a los cultivos auxiliares que utilizan una gama limitada de organismos vivos como: *Artemia*, rotíferos, copépodos y/o misidáceos (ver Woods, 2009). Aunque las dietas formuladas se están desarrollando para reemplazar estos organismos (y así reducir costes de producción), los organismos vivos como alimento siguen siendo vitales en la acuicultura ya que de su uso se obtienen mejores resultados (Conceição et al., 2010; Hamre et al., 2013; Baeza-Rojano et al., 2014). En consecuencia, existe una necesidad urgente de explorar e investigar el potencial de nuevos organismos acuáticos como alimento vivo en la acuicultura, por ejemplo, los anfípodos.

2) El progreso en acuicultura multi-trófica integrada.

La Acuicultura Multi-Trófica Integrada (IMTA, por sus siglas en inglés, "Integrated Multi-trophic Aquaculture") (Fig. 3) permite que especies de dos o más niveles tróficos crezcan simultáneamente en el mismo sistema, y el desperdicio de uno alimente al otro (Cruz-Suárez et al., 2010). Estos desperdicios pueden ser consumidos de forma directa por animales o algas como la *Ulva*, que crecen en los tanques o esteros que se usen para ello. Estas algas, a su vez, pueden directamente formar parte de piensos de especies herbívoras u omnívoras (Shpigel et al., 2017) o ser consumidas por organismos que después serán usados como alimento.

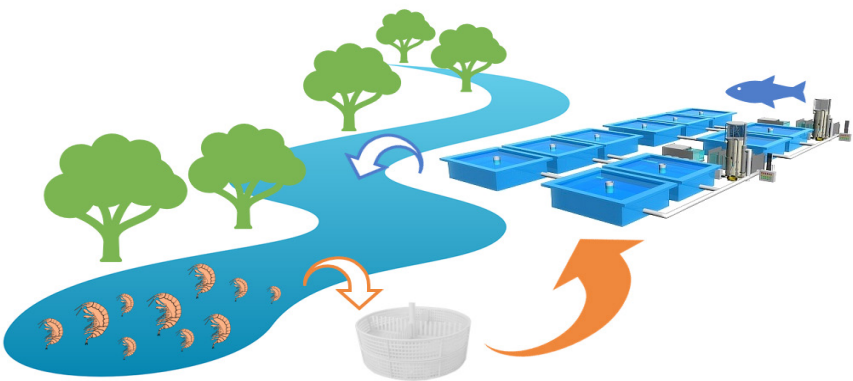


Figura 3. Esquema Teórico de un sistema IMTA

La zona de marismas situada al este y al sur del Parque Natural Bahía de Cádiz (PNBC), en el sur de España (Fig. 4), se caracteriza por un complejo sistema de canales de marea y arroyos que suministran agua de

mar a estanques salobres de peces situados a lo largo de sus cursos. La mayoría de los estanques permanecen permanentemente inundados durante la mayor parte del año y constituyen un ecosistema de lagunas seminaturales explotadas para la cría de peces de forma extensiva y semi-intensiva (Arias y Drake, 1994). Las marismas salinas son sistemas acuáticos que ofrecen multitud de servicios ecosistémicos que surgen a raíz de las funciones y procesos del propio ecosistema, como la protección costera, el ciclo de nutrientes, el control de la erosión, la purificación del agua y el secuestro de carbono (Barbier et al., 2011). Hoy en día, aproximadamente el 50% (y aumentando) de las marismas sufre deterioro debido a las actividades humanas (Barbier et al., 2011). Curiosamente, algunas marismas, como las ubicadas en el sur de España, requieren actividades antropogénicas (control hidráulico, conservación de muros, eliminación de sedimentos, etc.) para ser económicamente sostenibles en el contexto de la acuicultura (Arias y Drake, 1999). Los esteros son ecosistemas muy productivos gracias a su particular hidrología y morfología, que permiten un uso óptimo de la alta cantidad de luz y nutrientes disponibles. Su elevada productividad primaria está asociada a la carga de nutrientes y a la capacidad de inundación desde tierras adyacentes (Cañavate et al., 2015). La comunidad de macroinvertebrados de las marismas salinas es una fuente principal de alimento para peces criados de forma no intensiva (Arias y Drake, 1994, 1999). Estas marismas salinas modificadas proporcionan, por lo tanto, servicios como criaderos costeros y hábitat de cría, que son utilizados por la población local para practicar una especie de acuicultura extensiva que genera actividad económica.

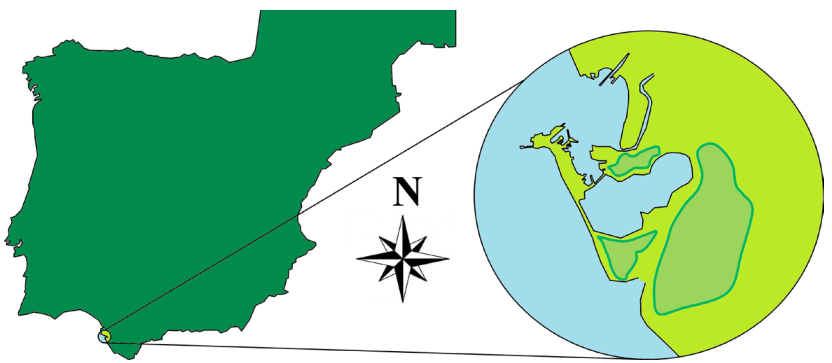


Figura 4. Marismas del Parque Natural Bahia de Cádiz

Por estas cualidades, los esteros son un ecosistema que propicia la integración de la acuicultura extensiva en un sistema IMTA para producir de forma paralela cultivo de peces y un cultivo o una extracción sistemática de anfípodos para ser usado como alimento vivo o pienso para peces. En cuanto a los anfípodos (Crustacea: Peracarida: Amphipoda) son el grupo más diverso de crustáceos respecto a forma de vida, hábitat, tamaño y tipo de alimentación (De Broyer y Jazdzewski, 1996), capaces de alimentarse de detritus, animales (crustáceos, poliquetos, oligoquetos, kinorrincos, hidroideos), macroalgas, microalgas, dinoflagelados y foraminíferos (Guerra-García et al., 2014). Es un grupo con una alta abundancia, gran riqueza de especies y amplia distribución, por lo que juega un papel relevante en la ecología de hábitats rocosos y fondos blandos (de la Ossa-Carretero et al., 2011). Forman parte importante del intercambio energético de la cadena trófica como recurso de muchos depredadores, siendo un vínculo entre los productores primarios y secundarios y niveles tróficos superiores como peces, aves y mamíferos (Legezynska et al., 2012). Son piezas claves en las comunidades del macrobentos, consumidos por multitud de especies, algunas especializadas en anfípodos (O'Gorman et al., 2008; Serrano et al., 2003), y altamente depredados en praderas de *Zostera marina* (Nelson, 1978, 1979; Caine, 1991) especialmente por peces de pequeño tamaño cuyo nivel de importancia en sus dietas puede ir modificándose a lo largo del desarrollo ontogenético de los peces depredadores (Woods, 2009).

La composición química de gammarideos y caprélidos del sur de la Península Ibérica ha sido estudiada por Baeza-Rojano et al. (2014) mostrando niveles altos de proteínas (37,9 – 44,6%) y cenizas (29,3 – 39,7%), y bajos niveles de carbohidratos (3,1 – 9,1%) y lípidos (5,1 – 9,6%). En cuanto a los lípidos fueron estudiados en mayor profundidad (Guerra-García et al, 2004 y Baeza-Rojano et al., 2014) observándose un alto contenido en ácidos grasos poliinsaturados (38,3%), con alta dominancia en 20:4 n6 (ARA), 20:5 n3 (EPA), 22:6 n3 (DHA), seguido de los ácidos grasos saturados con un 31,7% y monoinsaturados con el 24,4%. Tanto caprélidos como gammarideos se caracterizan por tener tasas de crecimiento rápidas, maduración sexual temprana al alcanzar el mes de vida, reproducción continuada desde la maduración y aumento progresivo del número de juveniles emergidos con la longitud corporal de la hembra (Baeza-Rojano, 2012). Estas características hacen que en la

naturaleza logren alcanzar densidades de hasta 319.000 ind/m^2 (Ashton, 2006) y de hasta 10.000 ind/m^2 cultivados en condiciones controladas (Baeza-Rojano et al., 2013).

Diversos estudios han demostrado la viabilidad del cultivo de diferentes especies de anfípodos, lo cual permitiría minimizar costes para un posible uso a gran escala (Baeza-Rojano et al., 2011; Baeza-Rojano y Guerra-García, 2013). Los gammarideos a su vez presentan más resistencia que los caprélidos a situaciones de estrés siendo mejores candidatos (Grabowski et al., 2007). Por estas propiedades, los anfípodos han sido considerados de interés en acuicultura, tanto para su uso como alimento vivo de peces (Woods, 2009) y cefalópodos (Baeza-Rojano et al., 2010; Baeza-Rojano et al., 2013b), como para la fabricación de harinas para la elaboración de dietas inertes para acuicultura (Moren et al., 2006) y ser integrante de los sistemas de Acuicultura Multitrófica Integrada alimentándose de los desechos procedentes del cultivo de los peces (González- Silvera et al., 2015). El objetivo de esta tesis es demostrar la viabilidad del uso de los anfípodos como fuente de alimentación en acuicultura, a través del estudio de su composición nutricional y la aplicación directa en cultivos de especies de interés acuícola.

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Objetivos generales

Los objetivos del presente estudio se han abordado en forma de capítulos, con los siguientes contenidos:

- Capítulo 2. **Revisión bibliográfica de la importancia de los anfípodos en especies de interés comercial en la acuicultura:** Se ha revisado la dieta de varias especies de interés acuícola, centrándonos en la importancia de los anfípodos, con el objetivo de evaluar su uso como alimento para estas especies, bien como alimento vivo o como componente de dietas inertes.
- Capítulo 3. **Descripción de la composición nutricional de diferentes especies de anfípodos de esteros:** Análisis bioquímico de cinco especies de anfípodos, describiéndose la composición general (proteínas, lípidos, carbohidratos, y cenizas) así como análisis de su composición especifica de aminoácidos, clases lipídicas y ácidos grasos, con el objetico de elegir la especie más adecuada para el uso en acuicultura teniendo en cuenta además su tamaño, abundancia y su accesibilidad.
- Capítulo 4. **Ensayo de cultivo y análisis nutricional de anfípodos alimentados con diferentes dietas en condiciones de laboratorio para explorar su potencialidad en Acuicultura Multitrófica Integrada.** Se llevaron a cabo dos experiencias diferentes, una con caprélidos y otra con gammarideos:

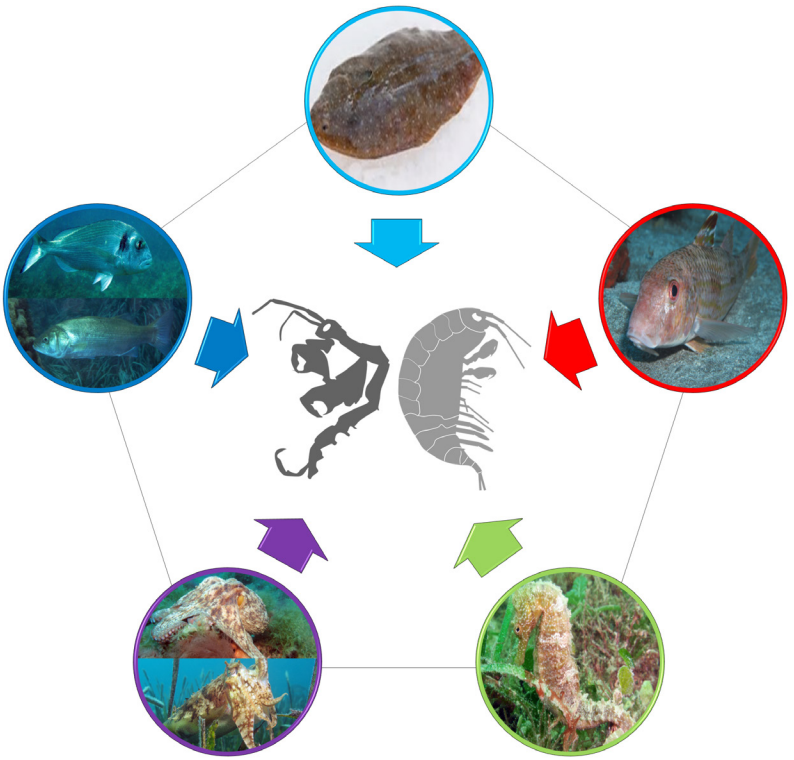
  Capítulo 4.1. Cultivo de especies de los caprélidos *Caprella scaura* y *Caprella equilibra*.

  Capítulo 4.2. Cultivo del gammarideo *Gammarus insensibilis*.

- Capítulo 5. **Aplicación y comparación del anfípodo *Gammarus insensibilis* con dietas comerciales en especies de interés comercial. *Seriola dumerili* como modelo de estudio.** Comparación de la calidad nutricional en dos etapas del desarrollo, larvas y juveniles, de *S. dumerili* alimentados con liofilizado de *G. insensibilis* precedente de los esteros y pienso comercial.

Capítulo 2

Importancia de los anfípodos en la dieta de especies de interés acuícola del litoral andaluz.



Adaptado de: Jiménez-Prada P, Hachero-Cruzado I y GuerraGarcía JM. 2015. Importancia de los anfípodos en la dieta de especies de interés acuícola del litoral andaluz. Zoologica baetica, 26: 3-29

Autores de las fotografias de este capítulo:
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Resumen

Los anfípodos son crustáceos con un alto valor nutricional y gran potencial para ser cultivados como alimento en especies de interés acuícola. Juegan un papel fundamental en el intercambio energético de la cadena trófica como recurso de muchos depredadores. Para evaluar la importancia de este grupo, se han revisado los estudios sobre alimentación de 10 especies (ocho peces y dos cefalópodos) del litoral andaluz cultivadas o con interés en acuicultura. Los anfípodos están presentes en la dieta de especies ya consolidadas en acuicultura como *Sparus aurata*, *Dicentrarchus labrax* y *Solea senegalensis*, aunque no se usan en ninguna de las fases de su cultivo intensivo. Existen otras muchas especies en las que los anfípodos podrían jugar un papel fundamental en su cultivo, como son *Sepia officinalis*, *Octopus vulgaris*, *Solea solea*, *Mullus surmuletus* y *M. barbatus*, además del género *Hippocampus*, de gran interés en acuariofilia. Es necesario intensificar la investigación de estos crustáceos para evaluar adecuadamente su importancia como presas en el medio natural y para cuantificar su valor nutricional como fuente alternativa de alimento.

Abstract

Amphipods are crustacean with high nutritional quality and great potential to be reared as food for species of aquaculture interest. They play a fundamental rol in energetic exchange of trophic chain as predators' resource. To evaluate the importance of this group, the feeding habits of 10 species (eight finfish and two cephalopods) of Andalusian coast have been reviewed. Amphipods are present in diet of consolidated species in aquaculture like *Sparus aurata*, *Dicentrarchus labrax* and *Solea senegalensis*, althought they are not used in any production step. There are a lot of other species where amphipods could play an important rol in their intensive cultures, like *Sepia officinalis*, *Octopus vulgaris*, *Solea solea*, *Mullus surmuletus* and *M. barbatus*, in addition to the genus *Hippocampus*, with great interest in the field of fishkeeping. It is necessary to intensify research of these crustacean to evaluate correctly their importance as preys in the wild and to quantify their nutritional values as an alternative food source.

Importancia de los anfípodos en la dieta de especies de interés acuícola del litoral andaluz

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Este capítulo tiene como objetivo dar respuesta a la pregunta inicial: ¿pueden ser los anfípodos un buen alimento para los peces y cefalópodos? Para ello, se realizó una revisión bibliográfica sobre la dieta de diferentes especies marinas, con especial atención a las especies con interés acuícola del litoral andaluz, sur de España.

1. Especies actualmente en producción acuícola

- *Dicentrarchus labrax, Linneo 1758 (Fig. 1)*

  Nombre común: Lubina, robalo.

  Interés comercial e importancia en la acuicultura

D.labrax es una de las especies comerciales más abundantes en las costas atlánticas. De hecho, la lubina y la dorada (*Sparus aurata*) son las dos especies en las que se basa la acuicultura del Mediterráneo. Después del salmón, la lubina fue la primera especie en ser cultivada, debido a su comportamiento en etapas juveniles, habitando estuarios y lagunas costeras donde se podían recolectar los ejemplares y dejar crecer confinados en estructuras naturales o creadas por el hombre, como por ejemplo los esteros de Cádiz. Aunque son Francia e Italia las que en la década de los 60 se implicaron en poder producir esta especie a gran escala (Moretti et al., 1999). Su cultivo intensivo se inició en la década de los 80, pero fue a finales de la década siguiente cuando la producción piscícola sufrió una aceleración debido al impulso de la Unión Europea mediante ayudas a la investigación y creación de empresas (Büke, 2002). Actualmente la extracción pesquera supone un volumen constante del 7

u 8% del consumo global (8.000–12.000 t/anual), siendo la acuicultura la encargada de suministrar más del 90% restante de la demanda mundial, alcanzando una producción mundial de 137.723 toneladas en el año 2013, de las cuales 3.777 toneladas fueron producidas en Andalucía (APROMAR, 2014), con un valor aproximado de 30 millones de euros (CAP,2013).

Hábitat y distribución

La lubina habita la mayor parte de su vida (reproducción y etapas juveniles) en estuarios y aguas litorales con fondos rocosos y arenosos de poca profundidad; sin embargo, en invierno los adultos emigran a aguas abiertas más profundas donde su alimentación es casi exclusivamente piscívora (Spitz et al., 2013). La distribución se extiende por las costas del Atlántico Este desde el Mar del Norte, Mar Báltico y Mar de Irlanda a las costas de Marruecos, y todo el Mediterráneo (Barnabé, 1991; Aguilera et al., 2009).

Importancia de los anfípodos en la dieta

Los anfípodos cobran importancia en las tallas más pequeñas de la lubina, especialmente en zonas de cría como son las marismas y estuarios. Selleslagh y Amara (2014) describen *D.labrax* (87,0 ± 1,6 mm) en el estuario de Canche (Francia) como especie especialista dirigida hacia dos especies de invertebrados, *Gammarus duebani,* Liljeborg 1852 (anfípodo), con una frecuencia de aparición en los estómagos del 47,7% y *Hediste diversicolor,* Müller 1776 (poliqueto) con un 6,2%, a pesar de no ser los más abundantes en el medio. La abundancia de ambos es 14,4 y 27,2 ind/m^2 respectivamente, muy inferior a la abundancia que presentan en primavera los oligoquetos (539,7 ind/m^2) y el anfípodo *Bathyporeia sarsi,* Watkin 1938 (195,9 ind/m^2). *Dicentrarchus labrax* también presenta este comportamiento especialista en las marismas (Laffaille et al., 2000), consumiendo preferentemente gammáridos (*Orchestia gammarellus,* Pallas 1766 y *Corophium volutator,* Pallas 1766) y misidáceos (*Neomysis integer,* Leach 1814). *O. gammarellus* es un anfípodo semi-terreste con baja capacidad natatoria, lo que le hace una presa potencial para los individuos de tallas menores (26 ± 6 mm en julio, hasta 60 ± 9 mm en octubre) que habitan las marismas, formando parte de la dieta de la lubina de un 11% al 46%, en verano, y del 22,4% al 71,1% en otoño, según la zona muestreada (Laffaille et al. 2000). Esta

variación es debida a la alta correlación entre *O. gammarellus* y el arbusto *Atriplex portulacoides*, que al ser desplazado por otras especies vegetales como *Elymus athericus* (Laffaille et al. 2005), produce un cambio en la dieta de la lubina, pasando del 78,9% al 22,8% la frecuencia de aparición de *O. gammarellus* y la desaparición del consumo de *C.volutator*, junto con el aumento al 81,0 % del misidáceo *Neomysis integer* (entre los años 1998 al 2002). No en todas las áreas de distribución los anfípodos son la presa principal, pero sí están presentes como parte fundamental de la dieta. En el SE de Inglaterra aparecen en el 20% de los estómagos, siendo la tercera presa más importante, después de decápodos y poliquetos (Fonseca et al., 2011), y un 12 % en el golfo de Cádiz superado sólo por misidáceos y decápodos (Arias, 1980).

- *Solea senegalensis, Kaup 1858 (Fig. 2)*

  Nombre común: Lenguado senegalés

  Interés comercial e importancia en la acuicultura

Especie de fácil adaptación a ambientes salinos, siendo de gran importancia junto con la dorada y la lubina en los esteros del sur y este de la península Ibérica (Arias y Drake, 1990; Castelo et al., 2010) donde presenta un crecimiento rápido en cultivos extensivos (Drake et al., 1984). Para poder conseguir cultivos intensivos de esta especie se han tenido que superar tres grandes dificultades: la alta mortalidad asociada a patologías (Suquet et al., 2009), el control de la reproducción (Cañavate, 2005) y su comportamiento alimenticio (Imsland et al., 2003; Castelo et al., 2010). Por ello actualmente existen muchos estudios dirigidos a conocer y potenciar el cultivo de esta especie (Dinis et al., 1999; Imsland et al., 2003; Villalta, 2007; Carazo, 2012; Boglino, 2013), viéndose reflejado en el aumento de producción. Sólo en Andalucía se han llegado a producir 16,4 t en el año 2013 cuyo valor aproximado es de 140.000 euros.

Hábitat y distribución

Se distribuye por el Atlántico este, desde Senegal al Golfo de Vizcaya (Lagardére et al., 1979) y todo el Mediterráneo (Rodríguez y Rodríguez, 1980), en hábitat de fondos rocosos y arenosos entre los 100 y 200 metros de profundidad (Teixeira y Cabral, 2010). En los meses de marzo a junio, durante la época de puesta, ocupan marismas y estuarios, pasando las fases de larva y juvenil dentro de éstos (Dinis et al., 1999).

Importancia de los anfípodos en la dieta

Según los estudios realizados en el Mediterráneo por Molinero y Ros (1992) y García-Franquesa et al. (1996), *S. senegalensis* consume principalmente crustáceos, poliquetos y bivalvos. García-Franquesa et al. (1996) describen que los crustáceos tienen una frecuencia de aparición en los estómagos del 58,32%, siendo los anfípodos el grupo más consumido apareciendo en más del 20% de los individuos muestreados, seguido por el grupo de los poliquetos (35,4%). En las costas portuguesas, *S. senegalensis* también consume crustáceos (42,7%) como presa principal, apareciendo los anfípodos en el 14,8 % de los estómagos, seguidos por los poliquetos (37,7%) (Teixeira y Cabral, 2010). Sin embargo, otros estudios muestran como los anfípodos pueden no aparecer (Arias y Drake, 1990) o ser escasamente consumidos en la dieta del lenguado senegalés (Cabral, 2000; Sá et al., 2003). Vinagre et al. (2009) asocian la distribución de *S. senegalensis* en las marismas del estuario del rio Tajo a la presencia de anfípodos, mientras que Cabral y Costa (1999) determinan que en su distribución sólo influye la presencia de poliquetos. Aunque la baja variabilidad de presas consumidas puede indicar una alimentación especialista (Sá et al, 2003), Cabral (2000) expone que la dieta de *S. senegalensis* cambia según la abundancia y diversidad de las especies del medio, variando la importancia de los anfípodos en la dieta según el hábitat.

- *Sparus aurata, Linneo 1758 (Fig. 3)*

  Nombre común: Dorada.

  Interés comercial e importancia en acuicultura

  La dorada es una de las especies con mayor importancia económica de la producción acuícola tanto en jaulas *off-shore* como en instalaciones en tierra. La pesquería mundial de las doradas desembarca anualmente entre 7.000 y 8.500 toneladas, tan sólo el 4,9% del consumo mundial. La producción acuícola es la encargada de abastecer la demanda mundial, produciendo más de 150.000 toneladas anuales en Europa (APROMAR, 2014), de las cuales Grecia produce el 41,7%, seguido de Turquía (23,2%) y España (9,3%), donde Andalucía aporta 1.786 toneladas de doradas (APROMAR, 2014), cuyo valor asciende a los 8 millones de euros (CAP, 2013).

Hábitat y distribución

La dorada es un espárido, comúnmente encontrada en fondos arenosos y praderas de *Posidonia*. Se distribuye por el Atlántico noreste, presente desde las costas de Gran Bretaña a Senegal, el Mar Mediterráneo e incluso en el mar Negro. Debido a sus hábitos eurihalinos y euritérmicos, la especie se encuentra tanto en ambientes marinos como salobres, tales como lagunas costeras y áreas estuarinas, en particular durante las etapas iniciales de su ciclo de vida (Moretti et al., 1999). Los juveniles (< 20 cm) suelen migrar a principios de la primavera hacia las aguas costeras protegidas, donde pueden encontrar abundantes recursos tróficos y temperaturas más suaves, y permanecen en áreas relativamente poco profundas (hasta 30 m), mientras que los adultos pueden alcanzar aguas más profundas, generalmente sin superar los 50 m.

Importancia de los anfípodos en la dieta

Múltiples estudios (Arias, 1980; Ferrari y Chieregato, 1981; Russo et al., 2007) en toda la cuenca del Mar Mediterráneo indican que los anfípodos son consumidos por esta especie desde etapas tempranas. Según Russo et al. (2007) son consumidas desde las últimas fases de la larva (2,5 cm) hasta adultos. Arias (1980) muestreó individuos desde los 2,1 cm hasta los 18,0 cm de longitud en los esteros de la provincia de Cádiz, donde los anfípodos presentan una frecuencia de aparición en los estómagos del 6,9%, reduciéndose en adultos. Pita et al. (2002) en Ría Formosa estudiaron tallas intermedias (8,5 cm a 44,4 cm) apareciendo los anfípodos en el 22,6% de los estómagos, pudiendo alcanzar más del 50% (Ferrari and Chieregato, 1981) para tallas entre 2,15 cm y 7,8 cm en el Delta del Po. Tener un rango de distribución amplio y ser una especie oportunista hace que la dieta de la dorada pueda variar según la zona de estudio (Pita et al., 2002). Por otra parte, a diferencia de otros grupos consumidos por la dorada, el consumo de anfípodos es estacional, siendo los meses de verano los que presentan los mayores valores (Ferrari y Chieregato, 1981; Pita et al., 2002).

2. Otras especies con interés en acuicultura

- *Mullus barbatus, Linneo 1758 (Fig. 4)*

  Nombre común: Salmonete, Salmonete de fango.

Interés comercial e importancia en acuicultura

En el mar, el salmonete es uno de los principales peces de extracción pesquera. Según la FAO (2014) se han desembarcado unas 17.000 toneladas anualmente de media durante los últimos 10 años. Tiene gran importancia económica en Andalucía al ser una especie altamente capturada por artes de pesca artesanal (enmalle y trasmallo) (Voliani, 1999; Tserpes et al., 2002; Esposito et al., 2014), presentando un alto consumo local y un alto valor comercial. Por estas razones es objeto de investigación para el cultivo de nuevas especies en la acuicultura.

Hábitat y distribución

El salmonete de fango es una especie demersal que habita desde zonas rocosas de las costas someras a fondos fangosos y arenosos en profundidades de más de 200 metros (Tserpes et al., 2002). Se reproduce en aguas someras entre 10 y 55 metros de profundidad entre los meses de abril y agosto. Su distribución abarca todo el Mediterráneo, incluido el Mar Negro, además del Atlántico Este de África y Europa (Esposito et al., 2014).

Importancia de los anfípodos en la dieta

Tanto su tamaño (no superior a los 30 cm, Planas and Vives (1956)) como su ecología, convierten a esta especie en un potencial depredador de los anfípodos. Esposito et al. (2014) describen la alimentación del salmonete a diferentes profundidades, desde la "zona de olas" (rompiente) hasta los 30 metros de profundidad, en los cuales el porcentaje de presencia de anfípodos en los estómagos varía del 55,8% en la zona de olas, al 83,6% en los 10 metros de profundidad, representados en casi su totalidad por el suborden *Gammaridea*. La importancia de los anfípodos es mayor en los meses de invierno, pero son consumidos durante todo el año pues su abundancia relativa en el medio es elevada. Es en verano cuando los misidáceos tienen su cenit en bundancia, razón por la que sustituyen a los gammáridos como presa mayoritaria. Según Cherif et al. (2011), los

anfípodos son el segundo grupo más importante en la dieta (25,3%) después de los decápodos con un 29,74% (en aguas de Túnez), e incluso en otros estudios no se encuentran entre los principales grupos consumidos por la especie (Vassilopoulou y Papaconstantinou, 1993; Labropoulou and Elefheriou, 1997; Machias and Labropoulou, 2002). Los valores registrados difieren de los obtenidos para otras especies mencionadas anteriormente, como la dorada. El consumo fluctuante de gammáridos a lo largo del año es debido a la variabilidad de su abundancia temporal (Vassilopoulou et al., 2001). Además, al ser una especie oportunista, el comportamiento alimenticio del salmonete de fango presenta variaciones según la zona de muestreo (Esposito et al., 2014).

- *Mullus surmuletus, Linneo 1758 (Fig. 5)*

  Nombre común: Salmonete de roca.

  Interés económico e importancia en la acuicultura

Aunque empezó siendo una especie accesoria, fue a partir de la década de los 90 cuando pasó a ser especie objetivo debido al pronto incremento en la captura en el Canal de la Mancha, Mar Del Norte (Mahe et al., 2014) y costas españolas. Los artes de pesca utilizados para su captura son tanto el arrastre, como artes de pesca tradicionales (enmalle o trasmallo), por lo que juega un papel económico importante a escala local, tanto en el Atlántico Norte como en todo el Mar Mediterráneo. Al igual que *Mullus barbatus,* debido a su alto consumo y valor comercial, es una especie objetivo para futuras investigaciones y diversificación de la acuicultura, aunque actualmente no se encuentra en ninguna fase de estudio.

Hábitat y distribución

Es una especie bentónica de fondos arenosos que vive en aguas profundas, preferentemente templadas y de alta salinidad (ICES, 2010). Su distribución ocupa todo el Mar Mediterráneo, incluido el Mar Negro, y aguas del Atlántico Norte desde el sur del Estrecho de Gibraltar al sur de Noruega y norte de Escocia, con una alta concentración en el Canal de la Mancha en los meses de invierno (Labroupoulou et al., 1997).

Importancia de los anfípodos en la dieta

El salmonete de roca se alimenta de decápodos, anfípodos, poliquetos, misidáceos y peces (Labroupoulou et al., 1997; Mazzola et al., 1999). En los estudios de Labroupoulou et al. (1997) en aguas del Mediterráneo Oeste los estómagos del salmonete de roca mostraban una alta preferencia por dos grupos, los decápodos y los anfípodos. Ambos explican el 90,06% del IRI (Índice relativo de importancia; Hacunda, 1981). Como grupo, los decápodos son los más consumidos (45,3% IRI), seguidos de los anfípodos (40,1%), pero a nivel de especie son dos anfípodos los más frecuentes en los estómagos del salmonete, *Apherusa chiereghinii,* Giordani- Soika 1949 (45.9%) y *Dexamine spinosa,* Montagu 1813 (44.5%). La abundancia relativa de ambos grupos en el medio según la estación del año influye en la frecuencia de aparición. Aunque en el cómputo anual los decápodos son los más importantes, los anfípodos son más consumidos en invierno (51% IRI) y primavera (73,2 % IRI), y los decápodos en verano, coincidiendo con el periodo de reclutamiento de estos (Robertson, 1984). No sólo influye la abundancia relativa en la selección de la presa, ya que en el medio natural los grupos más representativos son los poliquetos y moluscos, y no son tan consumidos, por lo que tamaño, comportamiento, densidad y la abundancia relativa de la presa influyen en la selectividad (Labropoulou et al., 1997).

- *Solea solea, Linneo 1758 (Fig. 6)*

  Nombre común: Lenguado.

  Interés comercial e importancia en la acuicultura

  Especie muy consumida y valorada nutricional y económicamente. La captura mundial en el 2012 fue de 32.746 toneladas, pudiéndose considerar constante desde el año 2000 (FAO, 2014). *Solea solea* es una especie que actualmente se cultiva en Grecia y España, cuya producción acuícola del año 2010 alcanzó las 125 toneladas, aunque en la actualidad dicha producción ha bajado bruscamente, no llegando a las 50 toneladas en el 2012 (FAO, 2014) exclusivamente en España.

Hábitat y distribución

Es una especie bentónica de fondos arenosos y fangosos, desde aguas superficiales hasta los 300 metros de profundidad, produciéndose la puesta entre los 50 y 100 metros de profundidad entre los meses de enero y abril (Koutsikopoulos et al., 1989), por lo que las larvas habitan desde febrero a mayo en las zonas de cría, esteros y aguas costeras poco profundas (Amara et al., 2001). Los juveniles (hasta 2 años de edad) se quedan en zonas someras hasta pasar al estado adulto en el que emigran a mar abierto (Nicolas et al., 2007). Se distribuye por las costas del Atlántico Este desde los fiordos noruegos a las costas senegalesas y el Mar Mediterráneo (Teixeira y Cabral, 2010).

Importancia de los anfípodos en la dieta

Los estudios realizados en el estuario del Ebro por Molinero y Flos (1992) describen una alimentación que varía según las estaciones del año en *S. solea*. Los crústaceos son las presas principales, excepto en otoño que son sustituidos por los poliquetos. Dentro de los crustáceos, los decápodos son las presas más consumidas en invierno, y son los anfípodos los más consumidos en primavera, verano y otoño, consumidos preferentemente por lenguados de 2 y 3 años de edad. En las aguas del Canal de la Mancha, al alcanzar tallas superiores a 50 mm, los juveniles reemplazan los copépodos harpacticoides por poliquetos y anfípodos, estos últimos con una frecuencia ascendente de aparición en los estómagos del 29 al 50% (Amara et al., 2001). Un cambio similar ha sido observado en las costas atlánticas francesas por Castel y Lasserre (1982) y Marchand y Masson (1989). En aguas portuguesas el lenguado en fase adulta consume crustáceos, poliquetos y bivalvos, siendo los anfípodos los crustáceos más consumidos y con mayor importancia en la dieta en primavera y verano (Teixeira y Cabral, 2010), comportamiento alimenticio similar al descrito por Molinero and Flos (1992) en el estuario del Ebro.

- *Sepia officinalis, Linneo 1758 (Fig. 7)*

  Nombre común: Choco, Jibia, Sepia.

Interés comercial e importancia en acuicultura

Debido a su gran distribución, la sepia es una de las especies más capturadas; sólo en el año 2012 se capturaron un total de casi 30.000 toneladas (FAO, 2014). La importancia de la sepia radica en ser una especie capturada tanto por pesquerías de alta mar como por flota artesanal, teniendo gran impacto en la economía local e industrial. En la década de los 90, la sepia empezó a ser investigada como especie objetivo, junto con otras especies de cefalópodos, para ser cultivada en acuicultura (Fuentes y Iglesias, 2001; Sykes et al 2006). Túnez ha sido el único país que logró producir sepia a gran escala, produciendo 23 toneladas entre los años 1990 y 1991 (FAO, 2014),

Sin proseguir con la producción de sepia después de ellos. Baeza-Rojano et al. (2010) cultivaron sepia con el uso de gammarideos y caprélidos como alimentación en etapas tempranas del desarrollo de esta especie, con lo que demostraron el posible uso de los gammarideos como fuente de alimento, a falta de experimentar la viabilidad de producción a gran escala.

Hábitat y distribución

Sepia officinalis es una especie necto-bentónica que ocupa un amplio rango de profundidad desde pocos metros hasta 200 m, en fondos fangosos y arenosos, con mayor abundancia en los 100 metros de profundidad. Tanto la puesta como el crecimiento de los juveniles tienen lugar en aguas poco profundas, donde la hembra fija los huevos al sustrato en verano, desplazándose los juveniles a aguas más profundas en otoño e invierno. En cuanto a su distribución geográfica, ocupa todo el Mar Mediterráneo, y aguas del Atlántico Este, desde Mauritana hasta el sur de Noruega y norte de Reino Unido (Guerra, 2006).

Importancia de los anfípodos en la dieta

Los anfípodos son parte de la dieta de la sepia, pero exclusivamente de las etapas tempranas. Según Pinczon et al. (2000) los anfípodos son la presa mayoritaria durante los tres primeros meses de vida de *S.*

officinalis (tallas comprendidas entre 1,46 ± 0,47 a 4,70 ±,04 cm), formando parte de la dieta en un 59 %, 80%, y 23% del primer al tercer mes, desapareciendo de la dieta en meses posteriores y siendo sustituidos por *Carcinus maenas* Linnaeus 1758. Este cambio en la dieta también ha sido descrito por otros autores como Guerra (1985) y Le Mao (1985). Blanc y Daguzan (2000), describen las especies más consumidas por *S. offinalis* desde junio (mes delas primeras puestas) a septiembre; éstas son principalmente una especie de isópodo (*Cyathura carinata,* Krøyer 1847) y 5 especies de anfípodos, 3 gammáridos (*Ampelisca bevicoris,* Costa 1853, *Marinogammarus marinus,*Leach 1815 y *Dexamine spiros,* Montagu 1813), un talítrido (*Orchestia gammarellus*) y un caprélido (*Phtisica marina,* Slabber 1769), siendo esta última la más consumida. En las Ría de Vigo, Castro y Guerra (1990) describen el consumo de presas según el tamaño de la sepia, apareciendo los anfípodos exclusivamente en las tallas menores de 65 mm de longitud de manto, aunque en proporciones más bajas que lo citado anteriormente (4,5%). La desaparición de los anfípodos en la dieta puede ser debido a que dejen de ser rentables desde un punto de vista energético por el aumento de tamaño de la sepia (Pinczon et al., 2000).

- *Octopus vulgaris*, Cuvier 1797 (Fig. 8)

  Nombre común: pulpo común.

  Interés comercial e importancia en la acuicultura

  Especie muy importante en las pesquerías mundiales debido a su distribución (Roper et al., 1984), cuyas capturas han ido descendiendo en las dos últimas décadas (80.247 toneladas desembarcadas en 1991) hasta alcanzar 40.453 toneladas en el año 2012 (FAO, 2014). Junto al descenso de capturas mundial, la gran demanda comercial (Vaz-Pires et al., 2004), su rápido crecimiento y su alta tasa de conversión (Iglesias et al., 2000) hacen del pulpo una especie con gran interés para la acuicultura. Se han realizado multitud de ensayos respecto a la alimentación de las paralarvas (Iglesias et al., 2007) teniendo como factor común el uso de *Artemia* y zoeas de crustáceos. Únicamente los trabajos de Iglesias et al. (2004) y Carrasco et al. (2006) lograron completar el ciclo de vida de *O. vulgaris*. El Plan Nacional de Cultivo del Pulpo (Planes JACUMAR), llevado a cabo desde 2001 al 2004, reveló que las mayores dificultades que presenta el cultivo de pulpo son la alta mortalidad y bajo crecimiento de las paralarvas, atribuido a la poca estandarización del cultivo y el

uso de presas con bajos valores de ácidos grasos insaturados, entre otros factores (Iglesias et al., 2007). Navarro and Villanueva (2000, 2003) han demostrado la importancia de estos ácidos grasos (EPA y DHA) en las paralarvas y su alimentación, lo que convierte a los anfípodos en presas potenciales interesantes.

Hábitat y distribución

Especie bentónica de fondos rocosos, arenosos y de fango, que habita desde la línea costera al borde de la plataforma continental. Comúnmente considerada una especie de distribución cosmopolita de aguas templadas debido a la existencia de especies crípticas, su distribución estricta se considera el Mar Mediterráneo y este del Océano Atlántico (Jereb et al., 2014).

Importancia de los anfípodos en la dieta

El pulpo es un gran depredador que se alimenta en su fase adulta principalmente de moluscos, crustáceos y peces (Mather, 1991). Existe un gran desconocimiento de la dieta del pulpo en su primera fase de vida, ya que su digestión es externa, por lo que los estudios clásicos de contenidos estomacales son muy difíciles de realizar (Roura et al., 2012). Para conocer qué tipo de animales consumen, Roura et al. (2012) identificaron las presas de las paralarvas utilizando *primers* específicos de la región 16S de ADN ribosómico de crustáceos para realizar una PCR de las presas potenciales. Se añadieron *primers* específicos para los copépodos, ya qeu los generales no los amplificaron; quizás la ausencia de bandas correspondientes a los anfípodos pudiera ser debida a esa misma causa. Aun así se han encontrado anfípodos en estómagos de individuos adultos (Smith, 2003).

3. Especies con interés en acuarofilia (género *Hippocampus*)

- *Hippocampus spp. (Fig. 9)*

  Nombre común: Caballito de mar.

  Interés comercial e importancia en la acuicultura

La extracción/producción de especies marinas tiene como objetivo la alimentación, ornamentación, uso industrial o medicinal. Los caballitos de mar, todos pertenecientes al género *Hippocampus*, son usados en acuariofilia y en la medicina tradicional china (MTC), la cual consume más de 45 toneladas en peso seco anuales de caballitos de mar. Esto crea una demanda cubierta en la actualidad exclusivamente por la extracción del medio, lo que provoca una sobrexplotación en muchas especies de caballitos de mar (Koldewey y Martin-Smith, 2010). La producción a gran escala de caballito de mar empezó a desarrollarse en Estados Unidos, Australia y Nueva Zelanda, con objeto de sustituir parte de la demanda de la MTC y el uso en acuarios. Pero las dificultades para ser competitivo económicamente radican en el bajo coste de los individuos extraídos del medio natural como captura accesoria. Actualmente, las tres prioridades para su cultivo a gran escala consisten en mejorar los tratamientos de salud y enfermedades, mantener poblaciones con independencia de capturas de individuos salvajes y reducir los costes de alimentación, ya que pueden llegar a ser un tercio del coste de producción (Koldewey and Martin-Smith, 2010). En este sentido, se han ensayado dietas congeladas sin resultados positivos, por lo que la búsqueda de presas vivas de bajo coste es de gran relevancia (Woods, 2003). Actualmente el valor de los caballitos de mar vivos varía entre los 100 y 750 dólares la unidad (http://seahorse.com), por lo que el conocimiento de las dietas naturales de estos peces es fundamental para el crecimiento y producción intensiva de los mismos, no sólo para conseguir un crecimiento más acelerado, sino para evitar mortalidad, deformaciones o mala pigmentación que devalúen el producto. Es el caso de la alta mortalidad de juveniles por aparición de burbujas en los estómagos, asociada a la alimentación con *Artemia* enriquecida con productos comerciales (Palma et al., 2014). En España, el Instituto de Investigaciones Marinas de Vigo está llevando a cabo la cría y reproducción de varias especies del género *Hippocampus* a través del proyecto "*Hippocampus*", que tiene como objetivo la repoblación de caballitos de mar en el medio natural. Para su alimentación están usando copépodos y *Artemia* (Planas, 2012); ambas presas no son consumidas en el medio natural (Koldewey and Martin-Smith 2010). Esto conlleva problemas en la alimentación y supervivencia en los primeros días de vida (Planas, 2012), probablemente por el déficit en ácidos grasos poliinsaturados omega-3.

Hábitat y distribución

El género *Hippocampus* tiene una distribució global asociada a aguas cálidas y templadas. En Andalucía podemos encontrar dos especies, *Hippocampus guttulatus* Cuvier, 1829 e *Hippocampus hippocampus* Linnaeus, 1758 (Fig. 9). Son especies de escasa capacidad natatoria, cuya estrategia ofensiva es el camuflaje, por lo que suelen estar asociados a un tipo de sustrato, al que imitan, adheridos por la cola, para acercarse a las presas o esperar que entren en el rango de ataque (Woods, 2002).

Importancia de los anfípodos en la dieta

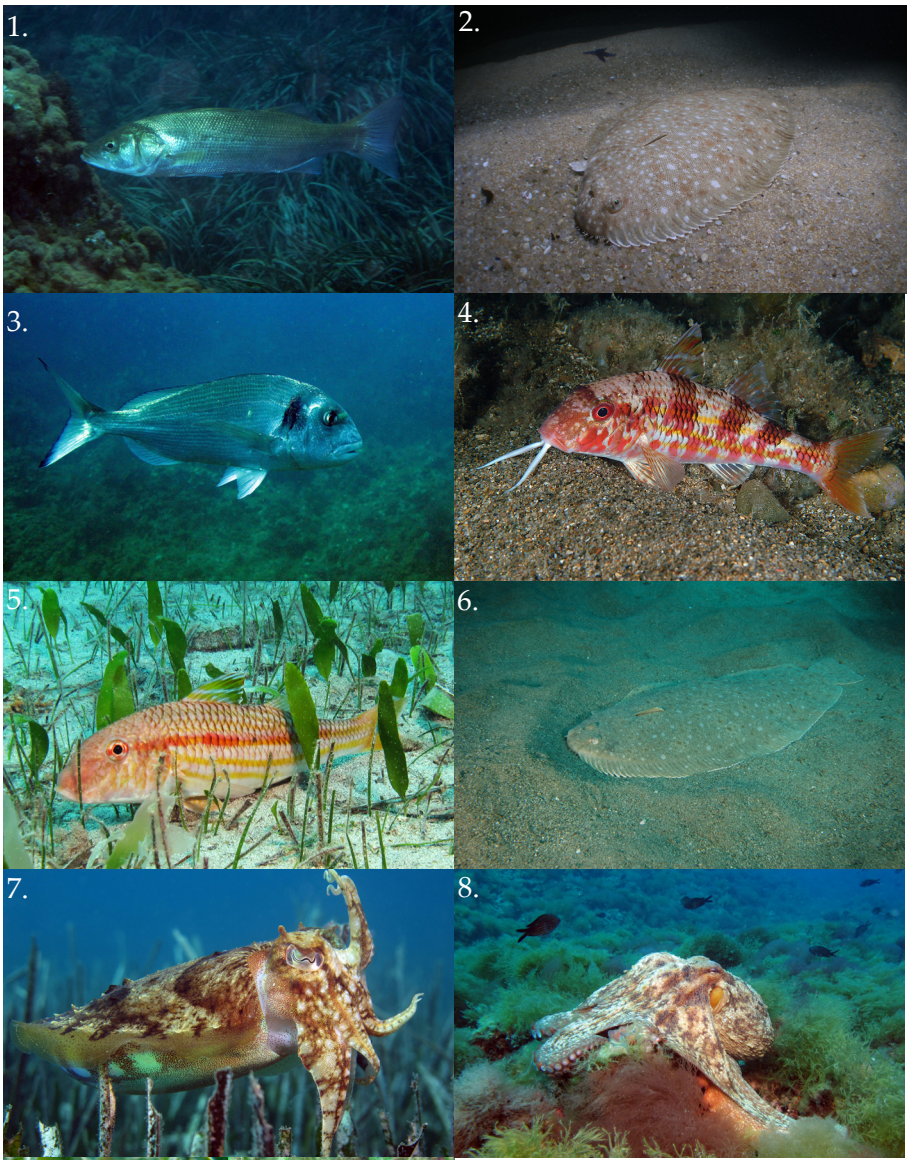
Tienen una dieta diversa, principalmente constituida por anfípodos, decápodos, y misidáceos (Koldewey and Martin-Smith, 2010). En cuanto a las dos especies de Andalucía, *H. guttulatus* consume mayoritariamente decápodos (zoeas), misidáceos y anfípodos, con una frecuencia de aparición en los estómagos del 100%, 85,71% y 47,62% respectivamente (Kitsos et al., 2008; Gurkan et al., 2011). Por su parte, *H. hippocampus* tiene una dieta más diversa, ocupando los anfípodos el segundo puesto de importancia (frecuencia del 21,05%) detrás de los decápodos (larvas) (frecuencia del 26,32%) (Kitsos et al. 2008; Gurkan et al., 2011). Los anfípodos son de gran importancia en varias especies alóctonas de caballitos de mar que podrían ser cultivadas en circuitos cerrados, junto con las especies autóctonas de Andalucía. Por ejemplo, los estudios sobre *H. abdominalis* (Lesson, 1827) describen una dieta basada en crustáceos. A nivel de grupo son los peracáridos en general y anfípodos en particular los más frecuentes (52,5 y 49,2% respectivamente), y a nivel de especie *Caprella equilibra* (Say, 1818) ocupa el primer puesto (25.5%). Los anfípodos son más importantes en la dieta de los juveniles que en adultos, y son especialmente consumidos en primavera e invierno (Woods, 2002).

También para *Hippocampus japonicus* (Kaup, 1856) los anfípodos son la principal fuente de alimentación, ya que el 88,4% de la masa total encontrada en su dieta son gammáridos, siendo fundamentales en todo el ciclo de vida, seguidos en orden de importancia por los caprélidos con un 5,9% (Kwak et al., 2004). La revisión de Koldewey y Martin-Smith (2010) aporta más información respecto al papel de los anfípodos en la dieta de otras especies de éste género. El uso de los anfípodos en la dieta de los caballitos de mar podría mejorar la alimentación y crecimiento de estas

especies pues cumplen con los requisitos nutricionales necesarios y son presas altamente consumidas en el medio natural. Por ello, podría llegarse a cubrir la demanda de estos animales para repoblación, acuariofília o usos en la medicina tradicional china.

4. Conclusiones

Las especies de interés acuícola en Andalucía consumen frecuentemente anfípodos en el medio natural, aunque el consumo puede variar a lo largo del desarrollo y estacionalmente. En algunas especies, la frecuencia de aparición de anfípodos en el tracto digestivo puede llegar a superar el 70%, como es el caso de *D.labrax*, *S. senegalensis*, *M. barbatus*, *M. surmuletus* y *S. officinalis*. La mayor parte de los trabajos de dieta suelen identificar las presas a nivel de grandes grupos, y son muy pocos los estudios que alcanzan la resolución taxonómica de género o especie. Son necesarios, por tanto, estudios más detallados de las especies de anfípodos más consumidos para orientar adecuadamente los estudios aplicados en el uso de estos crustáceos como recurso alternativo en acuicultura. El uso de los anfípodos, directamente como alimento vivo o formando parte de piensos y harinas, puede generar nuevas líneas de investigación, no sólo por ser consumidos en el medio natural sino por su alto valor nutritivo y su potencial para ser cultivados en condiciones controladas.



Figuras 1-9. Especies de peces y cefalópodos incluidas en el presente capítulo.

1. *Dicentrarchus labrax*
2. *Solea senegalensis*
3. *Sparus aurata*
4. *Mullus barbatus*
5. *Mullus Surmuletus*
6. *Solea solea*
7. *Sepia officinalis*
8. *Octopus vulgaris*
9. *Hippocampus* sp

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Capítulo
3

Crustacean amphipods from marsh ponds: a nutritious feed resource with potential for application in Integrated Multi-Trophic Aquaculture

Adaptado de: Jiménez-Prada P, Hachero-Cruzado I, Giráldez I, Fernández-Diaz C, Vilas C, Cañavate JP, Guerra-García JM. 2018. Crustacean amphipods from marsh ponds: a nutritious feed resource with potential for application in Integrated Multi-Trophic Aquaculture. PeerJ 6:e4194. DOI 10.7717/peerj.4194

Amphipod pictures taken from Arias and Drake (1994)

Resumen

La protección costera, el ciclo de nutrientes, el control de la erosión, la purificación del agua y el secuestro de carbono son servicios ecosistémicos proporcionados por las marismas. Además, las salinas ofrecen un hábitat de cría para peces y proporcionan abundantes invertebrados, como anfípodos, potencialmente útiles como recurso en acuicultura. La harina de pescado y el aceite de pescado son recursos alimentarios necesarios para mantener la acuicultura de especies carnívoras debido a sus ácidos grasos poliinsaturados de cadena larga omega-3 (n-3 LC-PUFA). Actualmente, la acuicultura depende de pesquerías y piensos limitados con niveles elevados de n-3 LC-PUFA, pero es necesario desarrollar fuentes de alimentos más sostenibles. Los anfípodos parecen ser un potencial recurso alimenticio alternativo de alta calidad para la acuicultura. Por lo tanto, se realizó un estudio nutricional para varias especies principales de anfípodos -*Microdeutopus gryllotalpa*, *Monocorophium acherusicum*, *Gammarus insensibilis*, *Melita palmata* y *Cymadusa filosa*- en esteros del sur de España. Estas especies mostraron un alto contenido de proteína (hasta 40%), altos niveles de PUFA n-3 y fosfolípidos, y altos niveles de fosfatidilcolina (PC), fosfatidiletanolamina (PE) y triacilgliceroles (TAG), siendo este último significativamente alto para *M. acherusicum*. *M. gryllotalpa* y *M. acherusicum* mostraron la mayor proporción de lípidos (19,15% y 18,35%, respectivamente). Isoleucina, glicina y alanina fueron los aminoácidos dominantes en todas las especies. Además, los anfípodos recolectados en estanques mostraron bajos niveles de metales pesados. Los perfiles bioquímicos de las cinco especies de anfípodos se compararon con otras presas alternativas. Por lo tanto, los anfípodos de esteros son buenos candidatos para ser utilizados como alimento y se proponen como un nuevo recurso sostenible y económico para ser utilizado en acuicultura. *G. insensibilis* puede ser la mejor especie para el cultivo intensivo como recurso alimenticio alternativo por sus características: 1) una composición adecuada de PUFA n-3 y PL; 2) altos niveles de glicina, alanina, tirosina, isoleucina y lisina; 3) altas densidades naturales; 4) cuerpo grande (≥1 cm) y 5) alta concentración de calcio. Más allá, una aquicultura combinada de anfípodos y peces parece una forma prometedora y ambientalmente sostenible de desarrollar Acuicultura Multitrófica Integrada (IMTA) en estos ecosistemas.

Abstract

Coastal protection, nutrient cycling, erosion control, water purification, and carbon sequestration are ecosystem services provided by salt marshes. Additionally, salt ponds offer coastal breeding and a nursery habitat for fishes and they provide abundant invertebrates, such as amphipods, which are potentially useful as a resource in aquaculture. Fishmeal and fish oil are necessary food resources to support aquaculture of carnivorous species due to their omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). Currently aquaculture depends on limited fisheries and feed with elevated n-3 LC-PUFA levels, but the development of more sustainable food sources is necessary. Amphipods appear to be a potential high quality alternative feed resource for aquaculture. Hence, a nutritional study was carried out for several main amphipod species -*Microdeutopus gryllotalpa*, *Monocorophium acherusicum*, *Gammarus insensibilis*, *Melita palmata* and *Cymadusa filosa*- in terrestrial ponds in the South of Spain. These species showed high protein content (up to 40%), high n-3 PUFA and phospholipid levels, and high levels of phophatidylcholine (PC), phosphatidylethanolamine (PE) and triacylglycerols (TAG), the latter being significantly high for *M. acherusicum*. *M. gryllotalpa* and *M. acherusicum* showed the highest proportion of lipids (19.15% and 18.35%, respectively). Isoleucine, glycine and alanine were the dominant amino acids in all species. In addition, amphipods collected from ponds showed low levels of heavy metals. Furthermore, the biochemical profiles of the five species of amphipods have been compared with other studied alternative prey. Therefore, pond amphipods are good candidates to be used as feed, and are proposed as a new sustainable economic resource to be used in aquaculture. *G. insensibilis* may be the best for intensive culture as an alternative feed resource because it shows: 1) adequate n-3 PUFA and PL composition; 2) high levels of glycine, alanine, tyrosine, isoleucine and lysine; 3) high natural densities; 4) large body size (≥1 cm), and 5) high concentration of calcium. Moreover, a combined culture of amphipods and fishes in these marsh ponds seems a promising and environmentally sustainable way to develop Integrate Multi-Trophic Aquaculture (IMTA) in these ecosystems.

Crustacean amphipods from marsh ponds: a nutritious feed resource with potential for application in Integrated Multi-Trophic Aquaculture.

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1.Introduction

Saltmarshes are aquatic systems with many ecosystem services arising from ecosystem processes and functions such as: coastal protection, nutrient cycling, erosion control, water purification, and carbon sequestration (Barbier et al., 2011). Nowadays, approximately 50% of salt marshes suffer deterioration due to human activity (Barbier et al., 2011). Interestingly, some salt marshes, such as those located in Southern Spain, require anthropogenic activities (hydraulic control, wall conservation, sediment removal, etc.) to be economically sustainable in the context of aquaculture (Arias and Drake, 1999). These modified saltmarshes provide, therefore, services as a coastal breeding and nursery habitat, and are used by locals for the practice of a kind of extensive aquaculture generating economic activity.

The saltmarsh zone located East and South of the Parque Natural Bahía de Cádiz (PNBC), in the South of Spain, is characterized by a complex system of tidal channels and creeks that supply seawater to saltmarsh fish-ponds situated along their course. Most of the saltmarsh ponds remain permanently flooded during the greater part of the year, and constitute a semi-natural lagoon ecosystem exploited for extensive and semi-intensive fish culture (Arias and Drake, 1994). These ponds are productive ecosystems thanks to their particular hydrology and morphology, allowing for optimal use of the available light and nutrients. The marshes' elevated primary productivity is thus associated to a

significant nutrient load and the flooding capacity in the adjacent lands (Cañavate et al., 2015). The macroinvertebrate saltmarsh community is subjected to intensive predation from fish and shorebirds, and is a main food source for non-intensively reared fish (Arias and Drake, 1994). Gastropods, amphipods and chironomid larvae dominate the macrofauna in terms of abundance (Arias and Drake, 1994).

Fish accounted for 16.7% of the global population's intake of animal protein and 6.5% of all protein consumed in 2010 (FAO, 2014; Tocher, 2015). Moreover, what is arguably of greatest importance to consumers in the developed world, fish and seafood are unique and rich sources of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), particularly eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids. An increasing proportion of fish are farmed, accounting for almost half of all fish for human food in 2012 (Tocher, 2015). Aquaculture of carnivorous species depends on n-3 LC-PUFA from fishmeal and fish oil but these come from limited, overexploited fisheries or from fish slaughter waste. Therefore, the continued growth of aquaculture will depend on the development of more sustainable feeds with alternative ingredients, generally derived from terrestrial agriculture, with important consequences for the supply of n-3 LC-PUFA (Tocher, 2015) or, as an alternative, finding new marine resources.

In that sense, amphipods could serve as an adequate alternative food resource for aquaculture. They are important low trophic position organisms which play a major role in the biological processing of algae inputs and facilitate the transfer of nutrients from the ocean to the coastline (Hamed et al., 2014). They are also regarded as important food for economically relevant fish species (chapter 2). Additionally, amphipods are abundant throughout the year in marsh zones, reach high densities (ca. 60.000 ind/m^2), and show adequate protein content and fatty acid profiles, with high levels of beneficial polyunsaturated fatty acids (DHA and EPA) (Kolanowski et al., 2007; Hyne et al., 2009; Baeza-Rojano et al., 2014). Previous studies have explored amphipods as alternative protein sources in experimental diets for farmed fish (Moren et al., 2006; Opstad et al., 2006; Suontama et al., 2007) and cephalopods (Baeza-Rojano et al., 2010; González et al., 2011; Baeza-Rojano et al. 2013a) obtaining promising results.

With the aim to select the more adequate amphipods from terrestrial ponds as alternative prey, we studied the composition (protein, carbohydrates, lipids and ashes) of the dominant species. Furthermore, lipid classes, fatty acids, amino acids and trace/major elements were also studied in depth. This allowed us to compare with other alternative preys used in aquaculture, in addition to evaluating their nutritional composition and potential to be used as alternative feed in aquaculture.

2. Materials and Methods

2.1 Sample collection

Amphipods were collected at terrestrial marsh ponds located at IFAPA "El Toruño" (Figure 1), El Puerto de Santa María, South Spain, within the "Bahía de Cádiz" Natural Park. Six ponds were selected (Figure 1A) for setting passive traps at shore areas during 1 week in December 2014. Salinity ranged from 23.6 to 31.8 and temperature from 12.0 to 15.4ºC. Passive traps, made up of filling oyster's cylindrical mesh structures with nylon mesh poufs, were used to collect amphipods (Figure 1B). Traps were fixed close to or inside algae patches of *Ulva* spp.

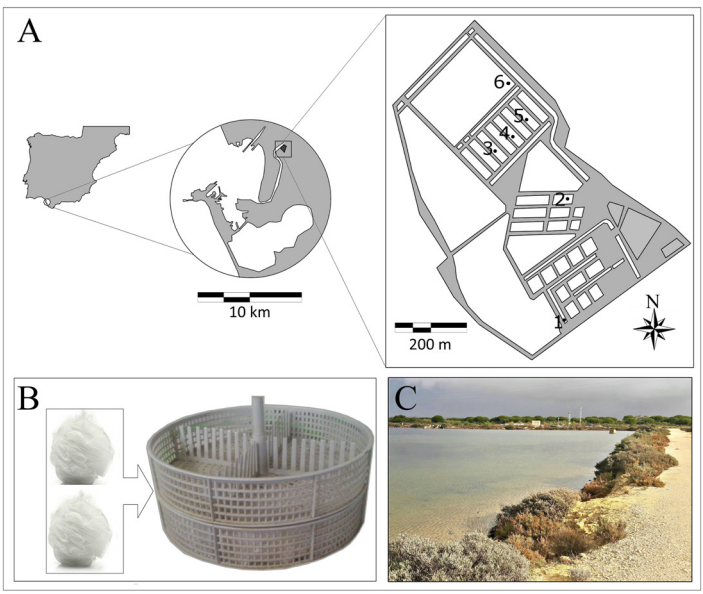

Figure 1. Ponds of IFAPA ``El Toruño''. (A) Map of ponds of IFAPA ``El Toruño'' with samples stations. (B) Traps were used to collect amphipods. (C) Ponds.

The studied species were those found at high densities in the traps, and which are common in macroalgae beds (mainly *Ulva* spp) at marsh ponds: *Gammarus insensibilis* Stock 1966, *Melita palmata* Montagu 1804, *Cymadusa filosa* Savigny 1816 *Microdeutopus gryllotalpa* Costa, 1853, and *Monocorophium acherusicum* Costa 1853 as described by Arias and Drake (1999). Details on date and collection sites together with size and additional information for each species are given in Table 1.

Because species' composition and density differed among ponds, in order to have enough biomass for analytical procedures, for each goal species two pools of specimens were selected from the two stations where this species was present at the highest densities. Results are shown as the mean value for the two pools at each station. Specimens were identified to the species level with a binocular stereomicroscope, cleaned with distillated water and directly frozen at -80ºC for later analysis.

Table1 Characteristic of the five studied species. Information of Arias and Drake (1999). Site numbers correspond with numbers of Fig.1A.

Species	*Gammarus insensibilis*		*Melita palmata*		*Cymadusa filosa*		*Microdeutopus gryllotalpa*		*Monocorophium acherusicum*	
Site	1	6	5	2	4	2	4	3	1	5
Collection date	15/12/ 2014	18/12/ 2014	22/12/ 2014	15/12/ 2014	22/12/ 2014	15/12/ 2014	22/12/ 2014	22/12/ 2014	15/12/ 2014	22/12/ 2014
Size	> 1cm		> 1cm		> 1cm		< 0.5 cm		< 0.5 cm	
Other characteristic	Associated to macroalgae, high density in spring (>3000 ind/m^2)		Large size range, max. density in winter (>1000 ind/m^2)		Associated to macroalgae, max. density in winter (300 ind/m^2)		High density in winter (>5000 in/m^2)		High density in winter (>14000 ind/m^2), create a fang tube to live	

2.2 Biochemical Analyses

2.2.1 Ash content

The ash content was gravimetrically determined after burning up at 550 °C for 4 h in a muffle furnace.

2.2.2. Carbohydrates

To estimate the total carbohydrate content 100 microliters of homogenised samples (5 mg/ml) were included in 900 microliters, 25 microliters of phenol (81%) and 2.5 ml of sulphuric acid (95-98%), shaken with vortex and maintained in darkness for 30 min; after this time, absorbance was measured at 485 nm (Dubois et al., 1956) following Kochert's techniques (Kochert, 1978).

2.2.3. Total protein and Amino acid composition

Total protein analyses were determined using the Lowry method (Lowry et al., 1951) increasing the concentration of NaOH (1.5 M) and heating at 100ºC for 60 min.

Acid hydrolysis was used to release individual amino acids from peptide and protein samples: 5–10 mg of homogenized samples of biota were placed in vials with 0.5 mL of 6 M HCl. Vials were flushed with N2, sealed with PTFE-tape and a heat-resistant cap, and placed in an oven at 150 °C for 70 min. After hydrolysis, the samples were dried in a heating block at 60 °C under a gentle stream of N2, and the remaining solids were re-suspended in 200 μL 0.1 M HCl and stored at -20 °C (Walsh et al., 2014). An aliquot (100 μL) of standard solution or sample was placed in a 2 mL vial, adding 400 μL of a water:ethanol:pyridine (60:32:8) mixture and 40 μL of ethyl chloroformate. It was capped and vigorously shaken using a vortex mixer for 30 s at room temperature. Gas evolution (carbon dioxide) usually occurs. Then, 200 μL of chloroform (containing 1% ECF) were added and the derivatives were extracted into the organic phase by striking the tube against a pad for about 30 s. The organic phase was dried with anhydrous sodium sulphate. The organic layer was transferred into a new vial with a 300 μL fixed insert. Aliquots (1 μL) of the derivatized extracts were injected into a Shimadzu GC-MS (GCMS-TQ8030) equipped with an Agilent HP-5MSfused silica capillary column (30m x 0.25mm i.d., 0.25μm film thickness). The gas chromatography system was equipped with a split/splitless injection port operating in Splitless mode. The oven temperature was programmed from 40 ºC (5min) to 270 ºC (20 min), increasing the temperature at a rate of 5 ºCmin-1. The transfer line was heated at 280 ºC. The carrier gas was helium with a constant flow of 1 mLmin-1 (mean velocity 36 cms-1). The mass spectrometer was performed with electron ionization (EI) at 70 eV, operating in scan mode (75-500 amu). Identification of derivative amino acids was achieved by comparing the gas chromatography retention times and mass spectra with those of the pure standard compounds. All mass spectra were also compared with the data system library (NIST 11).

2.2.4. Total lipids, Lipid classes and Fatty acids

Total lipids (TL) were extracted with chloroform:methanol (2:1 v/v) containing 0.01% of butylated hydroxytoluene (BHT) as an antioxidant (Christie, 2003). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically. Lipid classes were separated by one dimensional double development high performance thin layer chromatography (HPTLC) using methyl acetate/ isopropanol/chloroform/methanol/ 0.25% (w/v) KCl (25:25:25:10:9 by vol.), as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80:20:2 by vol.), as the neutral solvent system. Lipid classes were visualized by charring at 160 °C for 20 min after dipping in cupric acetate in 3% phosphoric acid (Olsen and Henderson, 1989). Final quantification was made by densitometry in a CAMAG scanner at a wavelength of 325 nm, and by comparison with an external standard (Sigma-Aldrich). To quantify fatty acids (FA) the TL extracts were subjected to acid-catalyzed transmethylation for 16 h at 50°C, using 1 ml of toluene and 2 ml of 1% sulphuric acid (v/v) in methanol. FAME were separated and quantified using a Shimadzu GC 2010-Plus gas chromatograph equipped with a flame ionization detector (280 °C) and a fused silica capillary column SUPRAWAX280 (15 m × 0.1 mm I.D.). Hydrogen was used as the carrier gas and the oven initial temperature was 100 °C for 0.5 min., followed by an increase at a rate of 20 °C min -1 to a final temperature of 250 °C for 8 min. Individual FAME were quantified by comparison with external standards (Sigma-Aldrich).

2.2.5. Major and trace elements

Approximately 10 mg of the sample was digested with 0.5 mL of conc. HNO3 and 1.0 mL of 30% H2O2. Then final solutions were made up to 5.0 mL in a volumetric flask with Milli-Q water. Trace and major elements concentrations were analyzed by ICP-MS (Agilent 7700) and ICP-OES (Jobin Yvon Ultima2), respectively, at the University of Huelva. Multi-element calibration standards were freshly prepared by dilution from certified stock solutions, standard solutions of ultrapure quality, and milliQ-water. The accuracy and precision of the measurements was greater than 3% RSD.

2.3 Statistical Analyses

The gross biochemical composition of the amphipod species (protein, lipid, ash, and carbohydrate) was expressed as the percentage on a dry weight basis.

To explore potential differences in the contribution of ashes, proteins, carbohydrates and total lipids, one-way ANOVA was used, including species as factor, with the two values of each station as replicates (n=2). Prior to the ANOVAs, the homogeneity of variances was tested with Cochran's test. Where variances remained heterogeneous, even with transformation, untransformed data was analysed, as ANOVA is a robust statistical test and is relatively unaffected by the heterogeneity of variances, particularly in balanced experiments (Underwood, 1997). In such cases, to reduce type I error, the level of significance was reduced to < 0.01. When ANOVA indicated a significant difference, the source of the difference was identified using the Student–Newman–Keuls (SNK) tests.

Principal component analysis (PCA) was conducted to lipid class, fatty acids, amino acids and trace/major elements matrices for the ordination of amphipod species. Additionally, permutation tests for multivariate analysis of similarity (PERMANOVA) were conducted to explore significant differences among species using the different matrices (lipid classes, fatty acids, amino acids and trace/major elements). Univariate analyses were conducted with GMAV, and multivariate analyses were carried out using PRIMER6 and PERMANOVA+ package (Clarke and Gorley, 2001).

3. Results

3.1. General composition

The five dominant species in the present study (Table 1) showed a similar composition of ash, proteins and carbohydrates, but differed slightly in total lipids (Table 2 and Figure 2). SNK tests revealed that *M. gryllotalpa* and *M. acherusicum* had a higher proportion of lipids (19.15% ± 0.48 and 18.35 ± 0.23, respectively, mean ± standard deviation) than the other three species, *G. insensibilis* (12.98 ± 2.01), *M. palmata* (15.9 ± 2.50) and *C. filosa* (13.42 ± 1.68). In general terms, all the species were

characterised by high levels of protein (30.85–39.58%) and ash (34.81–49.34%) and lower levels of carbohydrates (3.51–6.90%) and lipids (11.56–19.48%).

Table 2. Results of ANOVAs for general composition (Ash, Proteins, Carbohydrates and Total lipids). * indicates significant differences at p<0.05.

	Source of variation	df	MS	*F*	*P*	*F* versus
Ash	Species	4	9.1044	0.40	0.8023	Res
	Residual	5	22.765			
	Total	9				
	Cochran's C-test			C= 0.9276 (p<0.05)		
	Transformation			None		
Proteins	Species	4	17.5789	4.27	0.0716	Res
	Residual	5	4.1175			
	Total	9				
	Cochran's C-test			C= 0,8409 (Not significant)		
	Transformation			None		
Carbohydrates	Species	4	4.2886	1.52	0.3254	Res
	Residual	5	2.8289			
	Total	9				
	Cochran's C-test			C= 0.9238 (p<0.05)		
	Transformation			None		
Total Lipids	Species	4	15.5938	5.82	0.0402*	Res
	Residual	5	2.6809			
	Total	9				
				C= 0.4663 (Not significant)		
	Transformation			None		

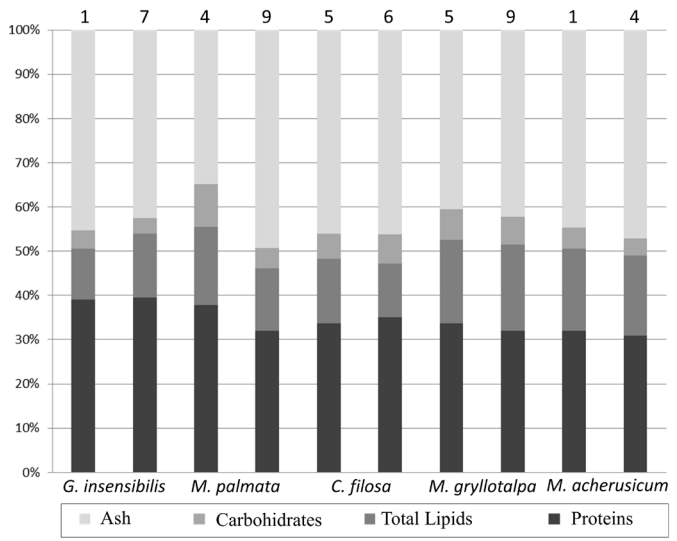

Figure 2. Dry weight percentage of proteins, carbohydrates, lipids and ash per species and station.

3.2. Amino acids

The 13 amino acids detected with acid extraction are shown in table 3. The seven most abundant amino acids accounted for about 75% of total amino acids. Among them, three are essential (isoleucine, lysine and valine), three are non-essential (glycine, alanine and aspartic acid) and one is a semi essential amino acid (tyrosine).

Table 3. Essential (E) and Not Essential (NE) amino acids (% of identified amino acids) of the five studied species. The values are a mean of two replicates. Each replicate consists of a pool of specimen.

Amino acid	E/NE	*G. insensibilis*		*M. palmata*		*C. filosa*		*M. grillotalpa*		*M. acherusicum*	
		1	6	5	2	4	2	4	3	1	5
Isoleucine	E	10.34	10.56	10.45	10.26	11.05	-	10.28	11.62	11.82	11.18
Leucine	E	7.86	6.96	7.98	7.32	6.87	-	7.09	6.77	5.56	5.47
Lysine	E	10.62	10.47	9.91	9.51	9.53	-	9.54	9.76	9.52	9.31
Methionine	E	2.73	2.10	2.36	2.21	1.45	-	2.14	2.68	2.35	2.26
Phenylalanine	E	6.63	6.24	6.40	6.22	6.35	-	6.35	6.48	6.24	6.42
Valine	E	8.41	8.15	8.54	8.21	8.42	-	8.04	8.54	8.33	7.81
Alanine	NE	10.89	11.19	10.74	10.79	11.41	-	10.77	12.05	12.43	12.46
Aspartic acid	NE	6.79	9.52	9.66	9.98	10.54	-	10.29	9.16	10.22	10.12
Glutamic acid	NE	2.43	2.43	3.20	4.20	3.78	-	4.64	1.50	3.01	3.34
Glycine	NE	13.38	14.05	12.75	13.39	13.90	-	12.79	14.38	13.98	14.79
Proline	NE	1.54	1.65	1.71	1.95	2.25	-	2.11	2.18	2.40	2.41
Serine	NE	5.43	4.68	5.40	5.38	5.15	-	5.88	4.95	4.49	5.07
Tyrosine	NE	12.95	12.00	10.90	10.57	9.31	-	10.07	9.93	9.64	9.37
Total E		46.59	44.48	45.63	43.73	43.67		43.43	45.85	43.82	42.44
Toral NE		53.41	55.52	54.37	56.27	56.33		56.57	54.15	56.18	57.56

PERMANOVA analysis did not show differences in amino acids among species (Pseudo-F=2,4224; P=0,061). Axis 1 of PCA analysis (Figure 3) explained 57.1% of total variation; tyrosine ($r=0.615$, $p<0.05$) and aspartic acid ($r=-0.502$, $p<0.05$) correlated with axis 1, and separated *G. insensibilis* from the remaining species by higher values of tyrosine and lower aspartic acid content. The axis 2 (29.8 % of total variation) correlated positively with glutamic acid ($r=0.612$, $p<0.05$), and separate *M. acherisicum* and *G. insensibilis* to *M. palmata* and *C. filosa* due to lower values of glutamic acid. Four amino acids showed differences (tyrosine ($F=29.905$, $p=0.001$), lysine ($F=14.415$, $p=0.006$), leucine ($F=10.774$, $p=0.013$) and proline ($F=29.271$, $p=0.001$)) among species in ANOVAs tests. SNK analysis separated *G. insensibilis* from the other four species with higher tyrosine and lysine, but lower proline contents; however, regarding leucine content, only *M. acherusicum* is separated due to its lower level.

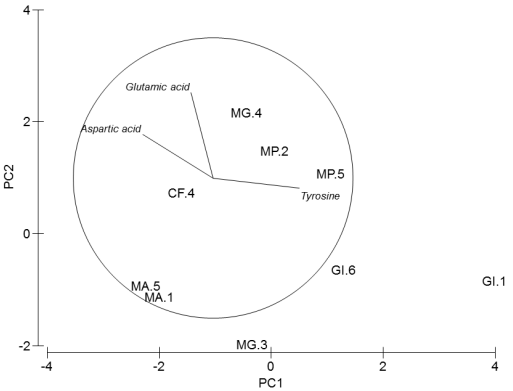

Figure 3. Principal Component Analysis (PCA) plot based on the Amino acid composition of the five amphipod species. GI: *Gammarus insensibilis*, MP: *Melita palmata*, CF: *Cymadusa filosa*, MG: *M. gryllotalpa*, MA: *Monocorophium acherusicum*. The numbers after the species indicate the collection station.

3.3. Lipid classes and fatty acids

Regarding lipid classes, all studied species showed high PC, PE and TAG levels (Table 4). Although PERMANOVA analysis showed differences in the lipid class profile among species (Pseudo-F =4.01, p=0.046), pair-wise tests did not show any differences. Axis 1 of the PCA analysis (Figure 4) explained 95.2% of total variance. TAG correlated significantly with this axis (r=-0.98, p<0.01) and separated *M. acherusicum*, with higher values of TAG, from the other species. Axis 2 explained 3.3% of total variance. PC correlated positively with this axis (r= 0.846, p<0.01) and separated *G. insensibilis*, with lower values of PC, from the other species. ANOVAs confirmed differences among species in PC (F=18.7, p=0.033) and TAG (F=5.47, p=0.045). In both lipids class, the SNK test did not show differences among species.

In connection with fatty acid composition, the most abundant fatty acids in all species were: the saturated 16:0, the monounsaturated 16:1n9 and 18:1n9, and the polyunsaturated 18:2n6, 20:5n3 (EPA) and 22:6n3 (DHA) (Table 5). PERMANOVA also reflected global differences in fatty acid composition among species (pseudo-F=9.97, p=0.001) despite no differences in species pair-wise comparisons being detected. These differences were also supported by the PCA analysis (Figure 5). Axis 1 explained 67.7% of total variance and axis 2 explained 22.7%. The fatty acid 22:6 n3 (DHA) correlated positively with axis 1 (r=0.657, p<0.05), and

Table 4. Lipid classes (µg/100µg of DW) per station of the five studied species per station. PC: Phophatidylcholine, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, PS: Phosphatidylserine, TAG: Triacylglycerols, Cho: Cholesterol. The values are a mean of two replicates. Each replicate consists of a pool of specimens.

	G. insensibilis		*M. palmata*		*C. filosa*		*M. grillotalpa*		*M. acherusicum*	
	1	6	5	2	4	2	4	3	1	5
PC	1.96	1.75	2.36	2.52	2.19	1.93	2.36	2.54	3.08	2.90
PE	1.27	1.17	1.58	1.66	1.52	1.29	1.52	1.49	1.72	1.50
PI	0.17	0.18	0.31	0.31	0.33	0.23	0.15	0.11	0.13	0.32
PS	0.26	0.36	0.40	0.46	-	0.31	0.43	-	-	0.36
TAG	3.01	2.93	3.87	2.44	2.76	1.86	1.97	3.97	5.58	7.19
Cho	0.32	0.18	0.35	0.25	0.30	0.35	0.24	0.25	0.332	0.30

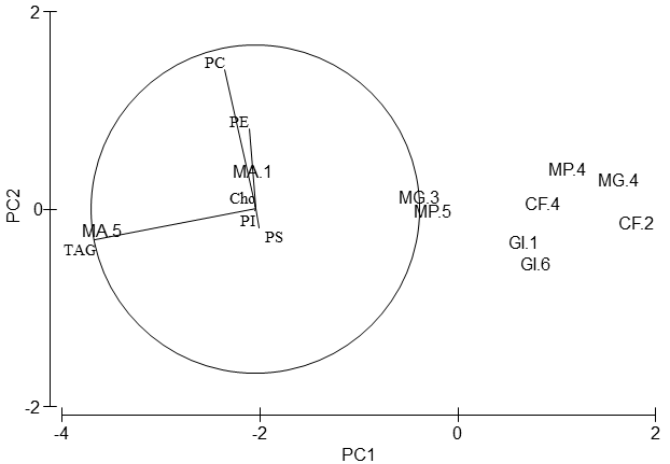

Figure 4. Principal Component Analysis (PCA) plot based on the Lipid Classes (LCs) composition of the five amphipod species. PC: Phophatidylcholine, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, PS: Phosphatidylserine, TAG: Triacylglycerols, Cho: Cholesterol. GI: *Gammarus insensibilis*, MP: *Melita palmata*, CF: *Cymadusa filosa*, MG: *M. gryllotalpa*, MA: *Monocorophium acherusicum*. The numbers after the species indicate the collection station. Only variables significantly correlated with axis 1 and 2 are represented.

separated *M. acherusicum* and *M. gryllotalpa* from the other species. 20:5n3 (EPA) correlated positively with axis 2 ($r=0.59$, $p<0.05$), and separated *M.gryllotalpa* from *M. acherisicum*. ANOVAs confirmed significant differences for these fatty acids among species (EPA, $F=6.03$ $p=0.0375$; DHA, $F=53.26$ $p=0.0003$). For both fatty acids the SNK test did not show differences among species.

Table 5. Fatty acids (μg/mg of DW) of the five species studied per station. ARA: Arachidonic acid; EPA: Eicosapentanoic acid; DPA: Docosapentanoic acid; DHA: Docosaheanoic acid. The values are a mean of two replicates. Each replicate consists of a pool of specimen.

	G. insensibilis		*M. palmata*		*C. filosa*		*M. grillotalpa*		*M. acherusicum*	
	1	6	5	2	4	2	4	3	1	5
Saturated (Sat)										
14:0	1.21	1.18	1.17	1.08	1.15	1.32	1.36	1.75	1.61	1.65
16:0	5.86	6.00	5.14	5.55	3.88	4.26	5.99	8.55	8.86	8.97
17:0	-	-	0.73	0.50	-	-	0.64	0.68	0.64	0.62
18:0	1.71	1.82	2.62	2.44	1.60	1.50	2.39	2.59	2.45	2.43
Total	8.79	9.01	9.66	9.56	6.63	7.08	10.37	13.56	13.55	13.66
Monounsaturated (MUFA)										
16:1n9	2.40	3.43	1.28	1.61	0.76	0.86	2.27	4.47	1.67	1.66
17:1	0.45	0.60	0.72	0.47	-	-	0.58	0.65	0.64	0.62
18:1n9	4.94	5.58	5.42	4.36	4.19	5.62	3.79	4.35	5.22	6.01
20:1n9	0.43	0.45	0.56	0.50	0.67	0.80	0.52	0.85	0.60	0.61
Total	8.22	10.05	7.99	6.95	5.61	7.28	7.16	10.32	8.13	8.91
Poliunsaturated (PUFA)										
18:2n6	4.75	2.16	1.73	2.23	2.71	2.61	1.72	1.65	2.27	2.23
18:3n6	0.44	0.48	-	-	-	-	0.66	0.85	-	-
18:3n3	0.57	1.17	0.81	0.65	1.25	2.01	0.69	0.63	1.82	1.74
20:2n6	0.77	0.49	0.52	0.54	0.90	0.78	-	-	0.63	0.65
20:4n6 ARA	1.40	1.47	2.08	2.28	1.71	1.47	1.99	1.94	1.41	1.43
20:3n3 DPA	0.36	0.41	0.36	0.40	0.62	0.92	0.39	0.41	1.02	1.03
20:5n3 EPA	5.02	6.03	5.41	7.03	3.47	3.63	7.26	9.98	6.47	6.77
22:6n3 DHA	3.25	3.59	5.68	4.41	2.04	1.97	4.04	4.86	8.71	8.79
Total	16.57	15.80	16.59	17.54	12.70	13.38	16.76	20.31	22.33	22.63

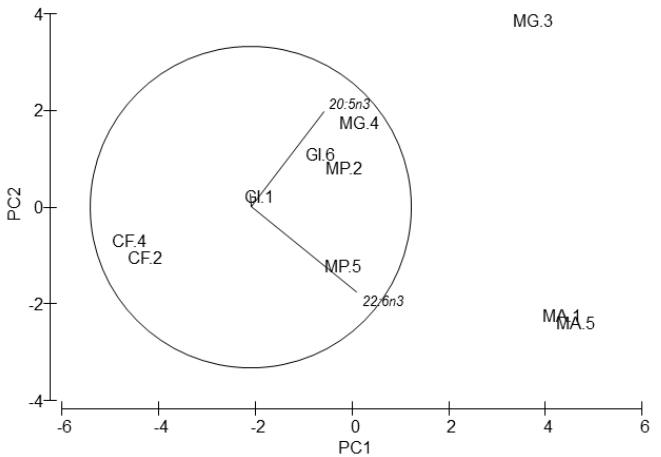

Figure 5. Principal Component Analysis (PCA) plot based on the Fatty acid composition of the five amphipod species. 20:5n3 (EPA), 22:6n3 (DHA). GI: *Gammarus insensibilis*, MP: *Melita palmata*, CF: *Cymadusa filosa*, MG: *M. gryllotalpa*, MA: *Monocorophium acherusicum*. The numbers after the species indicate the collection station. Only variables significantly correlated with axis 1 and 2 are represented.

3.4. Trace and major elements

Silver (Ag), arsenic (As), cadmium (Cd), cobalt (Co), nickel (Ni), lead (Pb) and selenium (Se) were not detected. Cr values ranged from 8.74 to 81.62 ppm, Cu from 36.42 to 162.63 ppm and Zn from 36.64 to 322.61 (Table 6). PERMANOVA results showed differences in the composition of trace and major elements among species (pseudo-F=9.36, p=0.007). In the PCA analysis (Figure 6), calcium (Ca) was significantly correlated (r=-0.98, p<0.001) with axis 1, which explained 96.7% of the variance; this axis separated *M. acherusicum* and *M. palmata* with lower Ca levels. Axis 2 explained 1.9% of the variance; Na (r=-0.60, p<0.05) and P (r=-0.56, p<0.05) were negatively correlated. ANOVA also indicated significant differences for Ca (F=20.31, p=0.0027). The SNK test separated two groups, where *M. acherusicum* and *M. palmata* were the group with a lower Ca level. Na (F=1.68, p=0.28) and P (F=0.19, p=0.93) did not present differences according to the ANOVA test.

Table 6. Trace and major elements (μg/g of DW) of the five studied species per station. The values are a mean of two replicates. Each replicate consists of a pool of specimen. Detection limit of trace elements = 33.33 μg/g of DW. Detection limit of Major elements = 83.33 μg/g of DW. Nd = under detection limit.

	G. insensibilis		*M. palmata*		*C. filose*		*M. grillotalpa*			*M. acherusicum*
	1	6	5	2	4	2	4	3	1	5
Trace elements										
Ag	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Co	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cr	16.23	12.30	8.74	15.10	8.98	9.27	27.11	81.62	14.28	14.08
Cu	87.04	133.73	38.76	126.92	134.90	52.88	78.02	162.63	52.11	36.42
Ni	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pb	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Se	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Zn	76.98	104.64	36.64	171.79	53.31	44.96	57.38	322.61	111.37	45.69
Major elements										
Al	6870	7209	11880	8103	6364	6755	6792	7019	9611	12236
Ca	247771	248136	195680	192075	223772	257195	256192	275224	158634	172823
K	17197	17327	9136	16778	10173	17700	15643	14008	9809	8474
Fe	1805	949	1909	1289	837	1316	2114	2220	1566	1814
Mg	13123	14098	15078	15811	17111	13852	18693	20532	13396	13944
Na	31793	36698	20066	27681	26060	36693	30951	37165	28885	21476
P	14240	21992	17343	19671	14073	26080	17002	16278	21949	18223
S	18251	18691	17992	20644	24265	20945	29232	26668	12616	12263

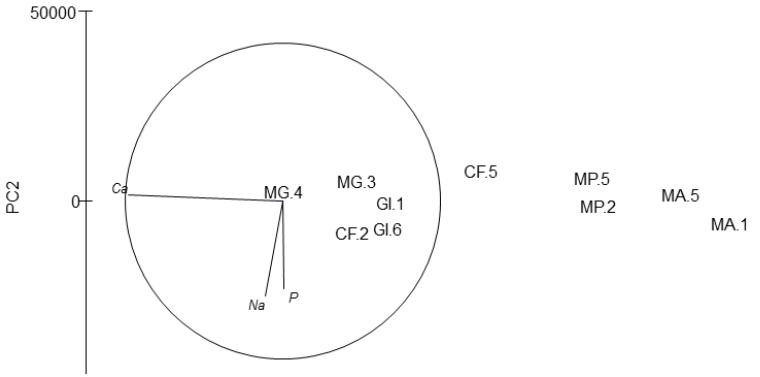

Figure 6. Principal Component Analysis (PCA) plot based on the Trace/Major elements composition of the five amphipod species. GI: *Gammarus insensibilis*, MP: *Melita palmata*, CF: *Cymadusa filosa*, MG: *M. gryllotalpa*, MA: *Monocorophium acherusicum*. The numbers after the species indicate the collection station. Only variables significantly correlated with axis 1 and 2 are represented.

4. Discussion

Aquaculture development requires sources of marine protein, n-3 LC-PUFA and micronutrients that are more sustainable than those currently in use, because, according to the FAO's (2016) report, fishery activity has stabilized around 90 million tons in the last 20 years, while aquaculture activity reached 106 million tons in 2015, exceeding fishery activity by 12.3 million tons. Because of that, it is necessary to investigate new marine feed sources to be used in aquaculture. Previous works have explored the use of alternative preys, like copepods, and traditional preys, like *Artemia* and rotifers (Tables 7, 8 and 9). Amphipods from marsh ponds could also be an alternative source. To date, amphipods have been used in meals for finfish with different results. *Themisto libellula* (Amphipoda: Hyperiidae) has been used in different studies: (1) Moren et al. (2006) found improved growth (Salmon) with a total substitution of fish meal for amphipod meal; (2), Suontama et al. (2007) described that a substitution lower than 60 % did not affect post-mortem pH and rigidity in trout; however, (3) Opstad et al. (2006) obtained negative results in meals with higher than 25% substitution for feeding Atlantic cod. Another amphipod, *Jassa marmorata*, was used as live prey in a cephalopod species (*Robsonella fontaniana*) achieving 20% higher growth than with *Artemia* (González et al., 2011). In Baeza-Rojano et al. (2010), *Sepia officinalis* had

a similar growth using amphipods or mysids, and, in Baeza-Rojano et al. (2012), marine gammarideans were better live prey than *Artemia* and freshwater gammarids for culture of *Octopus maya.* Moreover, Lari et al. (2013) showed that sturgeon species, *Acipenser gueldensuedtii* and *A. ruthenus*, preferred *Gammarus* to polychaetes of the genus *Nereis*.

4.1. General composition

In general terms, the studied amphipods from marsh ponds were characterised by high levels of protein and ash and low levels of carbohydrates and lipids. Protein values were similar to those found in copepods and mysids by other authors (Wang et al., 2014), but lower than those reported for other amphipods from the Bay of Algeciras (Baeza-Rojano et al., 2014). However, amphipods present a higher percentage of carbohydrates than other studied groups (e.g. Wang et al., 2014). Taking into account the National Research Council's (NRC, 2011) indications, no dietary requirement for carbohydrates has been demonstrated in fish. However, carbohydrates are important for sparing proteins and lipids for energy provision, and for the synthesis of important compounds derived from carbohydrates (NRC, 2011).

Regarding lipid content, values of 5.9 and 7.7% of DW have been measured in *Gammarus pulex* and *Carinogammarus roeselii* respectively (Geng, 1925); similar to the Strait of Gibraltar amphipods (Baeza-Rojano et al., 2014), but lower than total lipids measured in this study. However, higher lipid levels are normally found in deep-sea amphipod species (45% in populations of *Monoporeia affinis* (Lehtonen, 1996)) and in Arctic and Antarctic species (27% in *Onisimus affinis* and 53% in *Orchomonella plebs* (Percy, 1979; Pearse and Giese, 1966).

On the negative side, the high ash levels present in all the analysed amphipods species could be considered inadequate for feeding fish larvae (Moren et al., 2006; Opstad et al., 2006; Suontama et al., 2007).

4.2. Amino acids

Dietary supplementation with ingredients rich in specific amino acids is beneficial, due to the crucial roles in cell metabolism and physiology of these molecules. Amino acids have several functions in fishes, such as: (1) increasing the chemo-attractive property and nutritional value

of aquafeeds with low fishmeal inclusion; (2) optimizing efficiency of metabolic transformation in juvenile and subadult fishes; (3) reducing aggressive behaviours and cannibalism; (4) increasing larval performance and survival; (5) mediating timing and efficiency of spawning; (6) improving fillet taste and texture; and (7) enhancing immunity and tolerance to environmental stresses (Li et al. 2009).

The three most abundant amino acids in the analysed species are the non-essential amino acids glycine, alanine and aspartic acid. Although non-essential they have a significant role in stimulating feeding response and energy mechanisms in fishes (Li et al., 2009). Glycine participates in gluconeogenesis, sulphur amino acid metabolism, one-carbon unit metabolism and fat digestion (Fang et al., 2002), stimulates feed intake (Shamushaki et al., 2007), and has a critical role in the osmoregulatory responses of fishes and shellfishes (Li et al., 2009). Alanine and Aspartic acid are two of the major glucogenic precursors and important energy substrates for fish, and can also stimulate the feeding response (Li et al. 2009).

Isoleucine, leucine and valine are three of the most abundant essential amino acids in the analysed amphipods (EAA). These EAAs play important structural roles and are primarily deposited in body protein, notably in skeletal muscles (NRC, 2011). Phenylalanine and tyrosine, an essential and conditionally essential amino acid respectively, are also abundant in amphipod species (Table 3). The former can be converted to the latter, and dietary levels of both could profoundly influence pigmentation development, feed intake, growth performance, immunity, and survival of fish in natural environments (Pinto et al., 2008). Consequently, fishes' dietary requirements for phenylalanine and tyrosine increase substantially during metamorphosis (Pinto et al., 2008).

Analysed species show higher levels of the non-essential amino acids glycine, alanine and tyrosine, but lower levels of leucine, than other amphipods (Table 7). The high levels of glycine and alanine are of special interest for fish feeding, because these amino acids are feed intake stimulators (Shamushaki et al., 2007). Kasumyan and Mikhailova (2014) and Kasumyan and Marusov (2015) showed an increase in the fish's feed intake with alanine and glycine, respectively. Regarding essential amino acids, amphipods have higher levels of isoleucine and valine, but lower of

leucine (Table 7). These three AA are of interest for fish feeding because they play important structural roles and are primarily deposited in body protein, notably in skeletal muscles. In addition, Valine is involved in the synthesis of the myelin covering of the nerves (Cowey and Walton, 1989; Brosnan and Brosnan, 2006).

Table 7. Essential (E) and Non-Essential (NE) amino acids (% of identified amino acids) of other prey.

Taxa	Species	Amino acids													References
		Essencials						Non essencials							
		Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Valine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine	Tyrosine	
Amphipod	*Gammarus insensibilis*	10,45	7,41	10,55	2,41	6,44	8,28	11,04	8,16	2,43	13,72	1,60	5,06	12,47	Present study
Amphipod	*Melita palmata*	10,35	7,65	9,71	2,28	6,31	8,37	10,76	9,82	3,70	13,07	1,83	5,39	10,74	Present study
Amphipod	*Cymadusa filosa*	11,05	6,87	9,53	1,45	6,35	8,42	11,41	10,54	3,78	13,90	2,25	5,15	9,31	Present study
Amphipod	*M. gryllotalpa*	10,95	6,93	9,65	2,41	6,41	8,29	11,41	9,73	3,07	13,59	2,15	5,42	10,00	Present study
Amphipod	*M. acherisicum*	11,50	5,51	9,41	2,30	6,33	8,07	12,45	10,17	3,18	14,39	2,40	4,78	9,50	Present study
Copepod	*Pseudodiaptomas inopinus*	4,89	9,28	8,90	2,53	5,41	6,31	8,44	11,40	17,31	7,68	6,60	5,58	5,67	Yang & Hur (2014)
Copepod	*Paracyclopina nana*	5,26	9,69	8,94	0,62	5,98	6,48	7,98	12,17	17,82	6,40	6,79	5,67	6,22	Yang & Hur (2014)
Branchiop	*Artemia spp.*	6,09	10,07	10,97	1,97	5,70	6,30	7,24	10,76	16,07	6,26	7,97	6,99	3,60	Yang & Hur (2014)
Rotifers	*Brachionus plicatilis*	5,56	10,37	8,36	0,42	6,77	5,20	5,84	10,96	16,24	4,61	14,59	7,41	3,66	Yang & Hur (2014)
Copepod	*Pool of copepods*	4,40	7,60	7,40	2,30	4,10	5,30	7,10	9,60	13,60	7,50	5,30	5,30	4,60	Hamre *et al.* (2013)

4.3. Lipid classes and Fatty acids

Among lipid classes, triglycerides (TAG) are the predominant form of lipid reserve, and are always mobilized before phospholipids (PL) during starvation (Sargent et al., 1989; Hachero-Cruzado et al., 2014). The larvae presumably utilize the TAG to satisfy their energy demands while PL, which play an important structural role in the cell membrane, tend to be conserved (Rainuzzo et al., 1997) and increase the efficiency of the transport of dietary fatty acids and lipids from the gut to the rest of the body (Coutteau et al., 1997; Fontagne et al., 1998; Salhi et al., 1999; Tocher et al., 2008). Studied amphipods have a similar range of phospholipids (PL) but higher TAG dispersion, with *M. acherisicum* and *M. palmata* showing higher levels of PL and TAG than other preys (Table 8).

Table 8. Comparison of Lipid Classes (μg/100μg of DW). Taxa are organized according to increasing levels of Total Phospholipids (Total PL). PC: Phophatidylcholine. PE: Phosphatidylethanolamine. PI: Phosphatidylinositol. PS: Phosphatidylserine. TAG: Triacylglycerols.

Taxa	Specie	TAG	PC	PE	PI	PS	Total PL	References
Amphipod	*G. insensibilis*	2.97	1.86	1.22	0.17	0.31	3.56	Present study
Amphipod	*C. filosa*	2.31	2.06	1.41	0.28	0.15	3.90	Present study
Branchiopod	*Artemia franciscana*	17.84	1.74	1.43	0.42	0.32	3.91	Van der Mereen *et al*. 2008
Amphipod	*M. grillotalpa*	2.97	2.45	1.51	0.13	0.21	4.30	Present study
Rotifer (ME)	*Brachionus sp.*	-	2.50	1.90	-	-	4.40	Li *et al.* 2015
Rotifer (emulL)	*Brachionus sp.*	-	2.20	2.10	-	-	4.41	Li *et al.* 2015
Zooplankton	-	4.21	1.90	1.52	0.56	0.48	4.46	Van der Mereen *et al*. 2008
Amphipod	*M. palmata*	3.16	2.44	1.62	0.31	0.43	4.80	Present study
Copepods	-	2.63	2.00	1.99	0.39	0.55	4.93	Van der Mereen *et al*. 2008
Amphipod	*M. acherusicum*	6.38	2.99	1.61	0.22	0.18	5.01	Present study
Rotifer	*Brachionus plicatilis*	6.06	1.85	2.10	1.06	0.53	5.54	Van der Mereen *et al*. 2008
Copepod	*Acartia tonsa*	0.00	-	-	-	-	7.00	Olsen *et al.* 2014

Regarding fatty acids, DHA is one of the most important fatty acids for its key role in the formation and composition of nervous tissue and the retina (Mourente and Tocher, 1992; Bell et al., 1996; Coutteau and Mourente, 1997). Recent studies search for new feed sources with higher levels of n-3 and n-6 PUFA.

Table 9. Comparison of percentage in lipids (% of DW) and fatty acids (μg/mg of DW). Taxa are organized according to increasing levels of DHA. ARA: Arachidonic acid; EPA: Eicosapentanoic acid; DPA: Docosapentanoic acid; DHA: Docosaheanoic acid.

Taxa	Species	Lipids	ARA	EPA	DHA	Refrences
Copepod	Pool of three species*	3.20	-	0.63	0.80	Maehre *et al.* 2013
Rotifer	*Brachionus* sp.	10.00	-	0.44	1.01	Maehre *et al*. 2013
Rotifer	*Brachionus plicatilis*	15.40	0.29	1.09	1.91	Van der Mereen *et al*. 2008
Amphipod	*C. filosa*	13.42	1.59	3.55	2.00	Present study
Zooplankton	-	14.30	0.23	2.35	2.47	Van der Mereen *et al*. 2008
Rotifers	*Brachionus sp.*	9.80	0.72	3.20	3.00	Li *et al.* 2015
Copepod	*P. anmandeti*	16.00	0.24	1.47	3.09	Rayner *et al.* 2015
Amphipod	*G. insensibilis*	12.98	1.44	5.53	3.42	Present study
Copepod	-	11.10	0.09	1.93	3.80	Van der Mereen *et al*. 2008
Amphipod	*M. grillotalpa*	19.15	1.96	8.62	4.45	Present study
Branchiopod	*Artemia franciscana*	24.90	0.80	2.29	4.98	Van der Mereen *et al*. 2008
Amphipod	*M. palmata*	15.90	2.18	6.22	5.04	Present study
Amphipod	*M. acherusicum*	18.35	1.42	6.62	8.75	Present study
Rotifer	*Brachionus sp.*	12.70	0.60	4.00	10.00	Li *et al.* 2015
Copepod	-	7.07	-	5.17	11.76	Li *et al.* 2015
Copepod	*Acartia tonsa*	9.40	0.24	8.80	13.37	Olsen *et al.* 2014

* *Eurythemora affinis, Calanus finmarchicus* and *Microsetella norvegica* (Copepods).

Table 9 compares the amounts of essential fatty acids in some crustacean species and rotifers with the amounts in "El Toruño" ponds' amphipods. For example, rotifers studied by Li et al. (2015) and *Acartia tonsa* (copepod) studied by Olsen et al. (2014) have the highest values of DHA and high EPA values, but they have lower amounts of ARA and total lipids (7.0 and 9.4 %, respectively). Furthermore, *A. tonsa* is not easy to collect since a ship with trawls is necessary (Olsen et al., 2014). We must also take into account that the rotifers had been enriched with a lipid emulsion (Marol E, prepared by SINTEF Fisheries and Aquaculture, Norway), based on the marine oil DHASCO (Martek Biosciences, Columbia, MD, USA), which is used as a TAG source rich in DHA (Li et al., 2015). Both methods increase the economic cost. However, *M. palmata* and *M. acherusicum* are two species with high values of essential fatty acids; while *Cymadusa filosa*is is the species with the worst fatty acid profile of the five studied amphipod species.

4.4. Major and trace elements

Data of major and trace elements of amphipods from the "El Toruño" ponds can be compared with recent studies conducted in nearby areas with anthropogenic influence. In Algeciras Bay, Guerra-García et al. (2009) showed that there were elevated concentrations of heavy metals in the amphipods *Caprella penantis* and *Hyale schmidti* in the inner sites, higher than those obtained for amphipods of the PNBC's ponds.

Guerra-García et al. (2010b) studied the levels of trace elements in several species of peracaridean crustaceans (amphipods, isopods and tanaids) from different sites of Southern Spain, and reported similar concentrations of Cr and Cu, higher values of As, Ni and Pb, and a considerably higher concentration of Zn than in the present study's amphipods. In the same area, Guerra-García et al. (2010a) also studied some major elements in peracarideans and all taxa presented lower levels of K and Mg (except amphipods with lower values) and similar Ca levels (except caprellids with higher values).

Other prey, such as rotifers and copepods (Mæhre, Hamre and Elvevoll, 2013), are characterised by a similar concentration of major elements (Ca, Mg, P y Cu) as that of the amphipods studied in the present work, but lower Fe values were measured in rotifers (Unenriched and enriched).

Therefore, amphipods from ponds show better compositions of major and trace elements than other amphipods from nearby marinas (Guerra-García et al., 2010b), except for values of Fe. The elevated levels of Fe should be further investigated due to the effects of iron toxicity, including reduced growth, increased mortality, diarrhoea, and histopathological damage to liver cells in fish (NRC, 2011).

4.5. Nutritional requirements of aquaculture fishes

Marine fish larvae have a reduced ability to be fed prepared diets in early stages since they have lower digestion rates (Lauff and Hoffer, 1984; Kolkovsjki et al. 1993; 1997), low digestive enzyme activity and inadequate nutrition (Kolkovsjki et al., 1993; Teshima et al., 2000; Lazo et al., 2000) because the digestive apparatus of a larva is short and incomplete. In addition, the live food provides hormones, or their regulators, or growth factor (Lauff and Hoffer, 1984; Baragi and Lovell, 1986), and aids in digestion (Ronnestad et al., 2007). Considering this the amphipods (live or dry) could be better than a prepared diet.

Most of the research to understand nutritional requirements has focused on lipid requirements, concretely the level of phospholipids, DHA and EPA fatty acids. Marine fish larvae need an elevated protein percentage in their food to grow. Several studies of Atlantic salmon and many species of sea bass and sea breams have indicated that their larvae need around 50% of protein (NRC, 2011), and the marine fish larvae have a need for approximately 10 % of total lipids (Sargent et al., 1999). Regarding lipid requirements, phospholipids (principally PC) are the most necessary, with the optimal dietary level around 3%. Additionally, 1% of LC-PUFA n3 is required with a DHA:EPA 2:1 ratio (NRC, 2011). If studied amphipods are examined in the context of fish requirements we observe they have around 50% of protein, a range from 8 to 11 % of total

lipids, more than 3% of phospholipids (with an elevated PC level) and between 1% to 1.4% of LC-PUFA n-3, but they present a DHA:EPA 1:2 ratio, instead of the recommended 2:1 ratio.

4.6. Towards Integrated Multi-Trophic Aquaculture systems

During the last decade, there has been an increasing interest in the potential use of amphipods for aquaculture and ornamental aquariums. Woods (2009) conducted a comprehensive review examining aspects of the known biology and ecology of caprellid amphipods and their potential suitability as a novel marine finfish feed. In fact, he pointed out that caprellids could have a beneficial role to play in integrated coastal aquaculture, as a combined bioremediator and feed resource. Baeza-Rojano (2012) and Baeza-Rojano et al. (2013b) showed the suitability of amphipods to be included in Integrated Multi-Trophic Aquaculture (IMTA) programs; feeding on by-products of other cultivated species. Chapter 3 demonstrated experimentally that detritus (mainly composed of uneaten feed pellets and fish faeces released by cultured fish in fish farms and sea-cage structures) can be a nutritionally adequate and cheap feed for caprellid amphipods, providing a source of both omega-3 and omega-6 fatty acids. Therefore, these authors underlined the suitability of amphipods to be used in IMTA systems associated with the extensive culture of floating farms of fishes or molluscs, or with intensive cultures in terrestrial systems.

The present study reveals an interesting example of potential IMTA systems combining the extensive culture of fishes and amphipods associated to the marsh ponds of Southern Spain. The extensive culture of fishes (mainly *Sparus aurata* and *Dicentrarchus labrax*) carried out in these modified marsh ponds with aquaculture purposes, could be developed in the context of these IMTA systems. Amphipods are naturally cultured in high densities associated to algae and/or other substrates (such as traps or other artificial devices where amphipods can attach). They feed mainly on detritus (e.g. faeces) produced by the fishes growing in the ponds. Thus, a high and sustainable production of amphipods can be obtained. These amphipods could be useful (i) as natural food for fishes cultured in the marsh ponds, and (ii) as an additional resource to be used in aquaculture (alive or liophilized, and whole or integrated in fish feed). Taking into account that amphipods exhibit fast growth, quickly reach reproductive

maturity, have short interbrood periods, and are opportunistic feeders, the use of traps (such as those in Fig. 1), artificial meshes, cages, etc, could increase the available substrate for amphipods to cling on, reproduce and grow. Once these structures have been fully colonized by the amphipods (in a few weeks), they could be withdrawn and the amphipods removed using freshwater. The ponds are easily accessible from land; and algae, traps or other devices can be placed and replaced without great effort and with low costs. Marsh ponds are, consequently, promising locations to develop environmentally sustainable IMTA systems.

5. Conclusions

Among the studied species, *G. insensibilis* may be the best to be intensively cultured as an alternative feed resource because it shows: 1) adequate n-3 PUFA and PL composition; 2) high levels of glycine, alanine, tyrosine, isoleucine and lysine; 3) high natural densities; 4) large body size (≥1 cm) and 5) high concentration of Ca. *M. palmata* is also an option mainly due to its high n-3 PUFA and phospholipid levels. In contrast, *C. filosa* is the less suitable candidate because of its higher ash content, and lower protein and PUFA levels. While *M. acherusicum* and *M. gryllotalpa* present high lipid and DHA content, their size is considerably lower than other species so a higher number of specimens are required to reach the same biomass. Ponds of salt marshes from Southern Spain could be used as a sustainable source of amphipods, which are naturally growing in the macroalgae or sediments, and can be easily collected. The combination of stable amphipod populations, naturally inhabiting the ponds, with fish culture in ponds could allow for the establishment of multitrophic and sustainable aquaculture. In addition, it is necessary to investigate the optimal conditions for culturing gammarids in ponds or indoor tanks, and their economic profitability.

6. References

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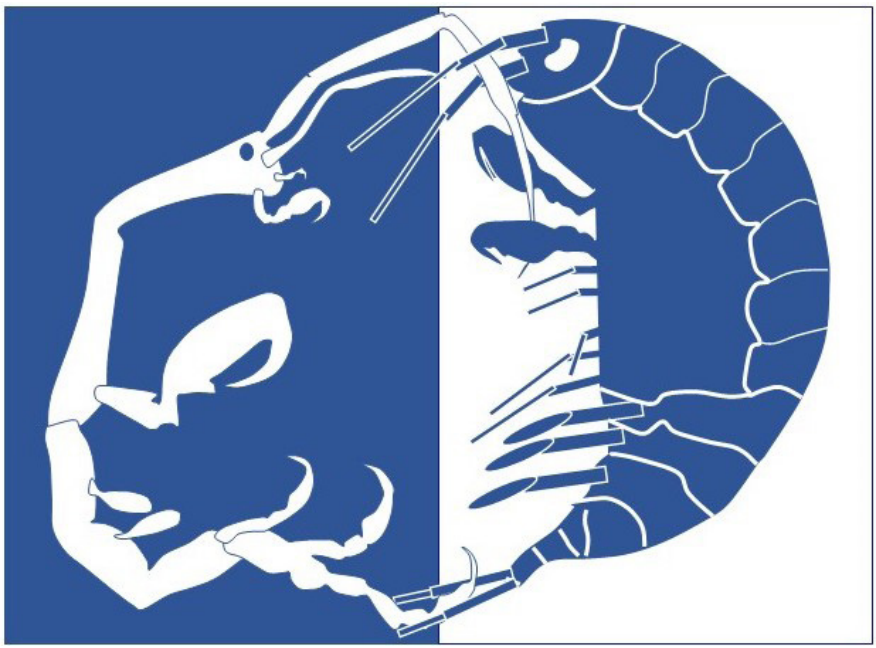
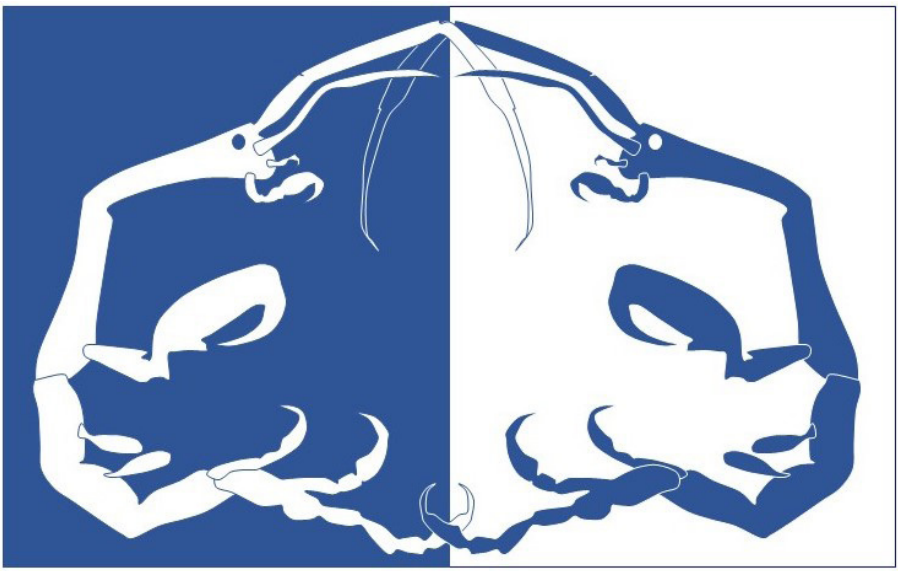
Capítulo
4

Testing diets for amphipods culture in Integrated Multi-Trophic Aquaculture



Capítulo 4.1 New findings for Integrated Multi-Trophic Aquaculture: Lessons from caprellids (Crustacea: Amphipoda)



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Resumen

La búsqueda de organismos alimentarios vivos alternativos y la progresión de la acuicultura multi-trófica integrada (IMTA) actualmente son una prioridad en las estrategias de la UE. Los caprélidos podrían ser un importante recurso potencialmente explotable en acuicultura debido a sus altos niveles de ácidos grasos poliinsaturados beneficiosos, crecimiento rápido natural y distribución global. Además, dado que son principalmente detritívoros, podrían ser excelentes candidatos para la integración en sistemas IMTA, podrían beneficiarse de las heces y pellets de alimento no consumido por peces cultivados en plantas de acuicultura y estructuras de jaulas marinas. A pesar de esto, faltan estudios experimentales para: (i) probar dietas económicas para caprélidos, como detritus, (ii) desarrollar técnicas de cultivo de caprélidos sostenibles e (iii) incluir caprélidos en sistemas IMTA. El objetivo principal de este estudio fue determinar si los detritus (D) en forma de heces de pescado proporcionaban una dieta adecuada para los caprélidos en comparación con otras dietas tradicionales, como nauplios de *Artemia* (A) o fitoplancton (P). Se demostró que la tasa de supervivencia de los adultos era significativamente más alta para los caprélidos alimentados con D. Por el contrario, las crías tenían la tasa de supervivencia más alta con A, aunque la tasa de crecimiento juvenil y el número de mudas eran similares en las tres dietas. Con respecto a la composición de lípidos, los caprélidos alimentados con A tenían concentraciones más altas de TAG y PC, mientras que los alimentados con P o D eran más ricos en ácidos grasos poliinsaturados, especialmente DHA. Curiosamente, los caprélidos alimentados con D también eran una fuente rica de LA, considerada como un ácido graso esencial en los vertebrados. Se encontró que los desechos basados principalmente en heces de pescado y gránulos de alimento no consumidos pueden considerarse un alimento adecuado para los caprélidos adultos, proporcionando una fuente de ácidos grasos omega-3 (DHA) y omega-6 (LA). Sin embargo, las crías parecen requerir una aporte adicional de TAG y PC durante las etapas juveniles para crecer adecuadamente.

Abstract

The search for alternative live food organisms and the progression of Integrative Multi-Trophic Aquaculture (IMTA) are currently being highly prioritised in EU strategies. Caprellids could potentially be an important exploitable resource in aquaculture due to their high levels of beneficial polyunsaturated fatty acids, fast growing nature and widespread distribution. Furthermore, since they are mainly detritivorous, they could be excellent candidates for integration into IMTA systems, potentially benefitting from uneaten feed pellets and faeces released by cultured fish in fish farms and sea-cage structures. Despite this, there is a lack of experimental studies to: (i) test inexpensive diets for caprellids, such as detritus, (ii) develop sustainable caprellid culture techniques and (iii) include caprellids in IMTA systems. The main aim of this study was to determine whether detritus (D) in the form of fish faeces provided an adequate diet for caprellids in comparison to other traditional diets, such as *Artemia* nauplii (A) or phytoplankton (P). Adult survival rate was shown to be significantly higher for caprellids fed with D. Conversely, hatchlings had the highest survival rate with A, although the juvenile growing rate and number of moults was similar in the three diets. With regard to lipid composition, caprellids fed with A had higher concentrations of TAG and PC while those fed with P or D were richer in polyunsaturated fatty acids, especially DHA. Interestingly, caprellids fed with D were also a rich source of LA, considered to be an essential fatty acid in vertebrates. It was found that detritus based mainly on fish faeces and uneaten feed pellets can be considered an adequate food for adult caprellids, providing a source of both omega-3 (DHA) and omega-6 (LA) fatty acids. Hatchlings however seem to require an additional input of TAG and PC during juvenile stages to properly grow.

New Findings for Integrated Multi-Trophic Aquaculture: Lessons from Caprellids (Crustacea: Amphipoda)

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1. Introduction

Aquaculture accounts for nearly 40% of fin- and shellfish consumed worldwide, reaching 62.7 million tonnes in 2011 (Alexander et al., 2015). To meet future demands, aquaculture production will need to more than double to 140 million tonnes by the year 2050 (Waite et al., 2014). The challenges facing aquaculture are increasingly recognised by the European Commission and are addressed through the EU Blue Growth Strategy and the reformed Common Fisheries Policy (Alexander et al., 2015). Additionally, aquaculture is given full consideration in all European strategies for Marine Biotechnology (Querellou et al., 2010).

In the framework of the innovative research programmes in aquaculture there are currently two areas of increasing interest: (i) the search for alternative live food organisms, and (ii) the progress in 'Integrated Multi-Trophic Aquaculture' (IMTA). (i) Many marine finfish aquaculture efforts, particularly for larval or juvenile finfish stages, utilise a limited range of live food organisms such as: *Artemia*, rotifers, copepods and mysid shrimp (Woods (2009) and references therein). Although formulated diets are being developed to replace these organisms (and thus reduce their production cost and support), live food organisms remain vital in aquaculture as better results are obtained from their use (Conceição et al., 2010; Hamre et al., 2013; Baeza-Rojano et al. 2014). Consequently, there is an urgent need to explore and investigate the potential of novel aquatic organisms as live feed in aquaculture. (ii) A number of methods to increase production levels in aquaculture have

attracted attention, such as offshore aquaculture installations (NOAA, 2008), recirculating aquaculture systems (Martins et al., 2010), and especially the innovative technology IMTA (Chopin et al., 2004). IMTA involves the integrated cultivation of fed species (e.g. finfish) together with extractive species (marine invertebrates and/or algae) which feed on detritus from the fed species (Alexander et al., 2015). IMTA allows species from two or more trophic levels to grow simultaneously in the same farm, with the waste of one feeding the other (Cruz-Suárez et al., 2010). It has been demonstrated that macroinvertebrate fauna present in fouling communities can take up sinking organic matter from fish farms and sea-cage structures, benefitting from uneaten feed pellets and faeces from the cultured fish (Madin et al., 2009; Gonzalez-Silvera et al., 2015). Along with improving effluent management, IMTA approaches reduce waste, diversify products, improve the economics, expand the range of suitable development sites and increase the biosecurity of farms (Samocha et al. (2015) and references therein). If proven to be less harmful to the environment, finfish produced within these systems could potentially be marketed as 'environmentally friendly' products (Alexander et al., 2015). In the future, IMTA could become an integral part of coastal regulatory and management frameworks, with the challenge being the establishment of safe and stable systems with an economically feasible output (Querellou et al., 2010).

Amphipod crustaceans are the most diverse group of crustaceans with respect to life styles, trophic types, habitats and sizes (De Broyer and Jazdzewaki, 1996). Amphipods inhabit a variety of marine environments and consequently show a high diversity of feeding habits (Guerra-García et al., 2014). Due to their nutritional characteristics, amphipods could serve as an adequate alternative live or dead feed resource for aquaculture (Kolanowski et al., 2007; González et al., 2011; Baeza-Rojano et al., 2014). During recent years, several promising results have been obtained by using marine amphipods as alternative prey for fishes and cephalopods (González et al., 2011; Holbrook and Schmitt, 1992; Moren et al., 2006; Page et al., 2006; Sountana et al., 2007; Baeza-Rojano et al., 2010; Baeza-Rojano et al., 2013a) and several amphipod species have recently been cultured in controlled conditions for potential use in aquaculture (e.g. Baeza-Rojano et al., 2013a). Woods (2009) examined aspects of known biology and ecology of caprellid amphipods and their potential

suitability as a novel marine finfish feed, concluding that caprellids were worthy of consideration for application in marine finfish aquaculture. The suggestion was based on the following characteristics: (i) they have a widespread global distribution, (ii) they form an important natural dietary component in a variety of coastal marine finfish, (iii) they contain high levels of beneficial polyunsaturated fatty acids, (iv) caprellids are relatively sedentary, readily colonise artificial structures (fouling communities) and under appropriate conditions can reach high biomass, especially around fish farms, (v) they exhibit fast growth with several generations per year, (vi) they are opportunistic feeders, (vii) some species show wide environmental tolerances, and (viii) recent studies have indicated the potential suitability of caprellids to larger scale culture (i.e.Baeza-Rojano et al., 2013b).

Despite the consensus in considering caprellids as a potential resource in aquaculture, field work and experimental approaches are very scarce in this regard. There is still a lack of studies (e.g. cuttlefish: Baeza-Rojano et al., 2011) in connection with the use of caprellids as alternative food (live or dehydrated) for fishes, mollusks or crustaceans. Provided that caprellids feed mainly on detritus Guerra-García and Tierno de Figueroa, 2009), they could be excellent candidates for integration into IMTA systems. Caprellids could have a beneficial role to play in IMTA as a combined bioremediator, feed resource and macroalgal enhancer (Woods, 2009). Caged mariculture typically releases particulate material (detritus mainly composed of faeces and uneaten fishfeed pellets) which can support caprellid populations (Gonzalez-Silvera et al., 2015; Cook et al., 2006). Marine fish farms provide substratum and nutrients for caprellids, and caprellids in turn may provide a bioremediation mechanism for fish farms, as well as an exploitable feed resource (Woods, 2009). There are no experimental studies exploring this potential however, and urgent outstanding issues are to test different diets for rearing caprellids (especially inexpensive ones such as detritus) and to develop and integrate economically sustainable caprellid culture techniques into IMTA systems. As a first step in understanding the usefulness of caprellids in IMTA, it is therefore mandatory to test the suitability of detritus in comparison with other diets (such as *Artemia* nauplii or phytoplankton) as an adequate food for caprellids in terms of nutritional support and the survival or growth rates achieved. Woods (2009) suggested the use of

Caprella equilibra Say, 1818 as one of the preliminary candidate species to be ideally suited to large-scale intensive land-based culture. This species was selected for the present study, together with *Caprella scaura* Templeton, 1836 (Fig. 1). These species are the most common caprellids associated with fouling communities along the Iberian Peninsula (see Ros et al., 2015). In the case of *Caprella scaura*, the large volume of organic detritus in its gut contents suggests that it may play an important role as a vector for carbon transfer from detritus to top predators (Ros et al., 2014a). *Caprella equilibra* has been established in the Mediterranean and the East Atlantic coast for hundreds of years and can be considered native in this region, although it can also be classified as cryptogenic based on the difficulty of determining its origin (Carlton, 1996; Guerra-García et al., 2015). In the Iberian Peninsula, *Caprella scaura* is a non-native species. It was recorded for the first time in 2005 by Martinez et al. (2008) in Girona. Currently, this species is spreading quickly along marinas of the Mediterranean and East Atlantic, reaching high densities and competitively displacing *C. equilibra* (Ros et al., 2015). Ros et al. (2014b) and Cabezas et al. (2014) found strong morphological and molecular evidence that *C. scaura* typica and *C. scaura* scaura correspond to the same "variety" and that this "variety" is the only one expanding its distribution range with a strong invasive capacity. This is the "variety" widely distributed in the Iberian Peninsula, which has been used as a target species in this study. Both species are easy to collect and can be found in high densities on artificial structures (floating pontoons, buoys, ropes, etc.). Recent studies have revealed that *C. equilibra* has a high nutritional value potentially significant for aquaculture purposes, with high contents of polyunsaturated fatty acids and polar lipids and proteins (Baeza-Rojano et al., 2014).

Lipid accumulation is the most widespread long-term energy storage strategy in aquatic crustaceans and their reproductive potential is largely dictated by lipid content (Clark et al., 1985). Of the Lipid Classes (LCs), Triacylglycerol (TAG) is the main storage lipid in benthic amphipods and fatty acids (FAs) are necessary for maintaining cell membrane integrity, lipid transport, pigmentation, and are the building blocks for many hormones (see Woods (2009) and references therein). A diet that does not provide adequate amounts of fatty acids can affect the life cycle of crustaceans (Arts et al., 2001). For all these reasons, lipid composition (LCs and FAs) was selected as a biomarker of nutritional quality in the

present study. As a growing number of studies use FAs as biomarkers at a community level, it has become increasingly important to improve our understanding of the effect of diet on FA profiles of a range of marine invertebrates (McLeod et al., 2013).

The main aim of this study was to determine if detritus (D) can be considered as an adequate diet for caprellids (*C. equilibra* and *C. scaura*) in comparison with traditionally used diets, such as *Artemia* nauplii (A) or phytoplankton (P). The general aim was addressed through specific objectives, which were: (i) to explore if the caprellids were able to ingest a variety of diets, including D; (ii) to compare the nutritional values of D with A and P by analysing lipid composition (LCs and FAs), (iii) to determine if the LC and FA composition of caprellids changed in response to different diets (A, P or D), (iv) to examine the influence of diet (A, P or D) on the survival and growing rates of adult and juvenile specimens.

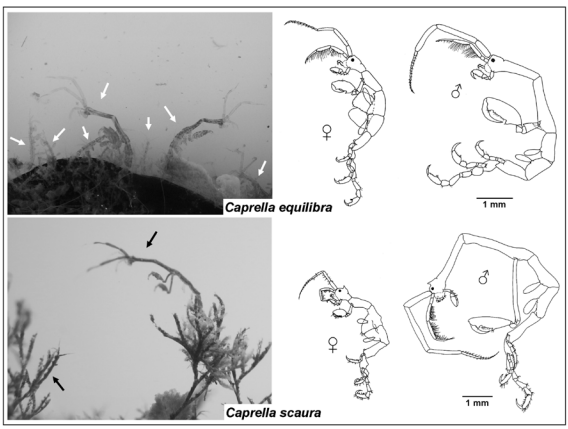

Figure 1.-Caprellid species used in the present study. Left: field populations inhabiting marinas of Southern Spain. Right: Lateral view figures of male and female specimens of each species.

2. Material and methods

2.1. Organisms, study sites and field sampling

Two caprellid amphipods were selected for the present study: *Caprella equilibra* and *Caprella scaura* (see introduction section for details of species). All the specimens used in the experiment and chemical analysis for this study were collected in June 2015 from two marinas located in Southern Spain. *Caprella equilibra* was sampled from Nuevo Portil Marina,

El Rompido, Huelva (37º12'46''N, 7º04'51''W) where it was the dominant amphipod, associated mainly to the bryozoans *Bugula neritina* and *Zoobotryon verticillatum*, and the hydroid *Ectopleura crocea*. *Caprella scaura*, absent in Nuevo Portil Marina, was collected from colonies of *B. neritina* from La Línea Marina -Club Marítimo Linense, Cádiz (36º09'36''N, 5º21'34''W). No specific permissions were required for collections in these marinas, and the field studies did not involve endangered or protected species. In previous samplings conducted in 2011, La Línea Marina was dominated by *C. equilibra* and *C. scaura* was absent (Guerra-García et al., 2015). In the last 4 years *C. scaura* has invaded this marina and reached high densities, displacing *C. equilibra*. Colonies of bryozoans and hydroids attached to floating pontoons, buoys and ropes of the marinas were handpicked. Preliminary caprellid sorting was conducted in situ, before the individuals were transported with aeration together with some bryozoan colonies to the laboratory (see Baeza-Rojano et al., 2013a).

2.2. Experimental designs in laboratory conditions

Experiment 1: Are caprellids able to feed on a variety of diets?

To test the feeding preferences of *C. equilibra* and *C. scaura* and to verify the ability of caprellids to properly ingest different food types under laboratory conditions, the following experiment was conducted. 80 adult specimens of each species were isolated in small, 120 ml glass containers with a diameter of 6.5 cm and a height of 6 cm (4 specimens per container). An equal amount of males and females were considered to avoid potential differences among sexes. A 1 mm plastic mesh was used as a substratum for attachment. Specimens were maintained without food for a duration of 24 hours to empty their guts and the water was then changed to remove faecal pellets. 16 specimens (4 glasses) of each species were then used for each different diet; *Artemia* nauplii (A), phytoplankton (P), detritus (D), the three items together to explore preferences (All) or no additional food (NF). Trace natural detritus would have entered all containers by attachment to specimens. *Artemia* nauplii were hatched from cysts maintained (1-2 days) in a Brine Shrimp Hatchery Hobby® with seawater at 25ºC. The phytoplankton consisted of a mixture of microalgae, made from lyophilized colonies of *Phaeodactylum, Tetraselmis* and *Nannochloropsis* (1:1:1). Detritus was obtained by scraping the bottoms of aquaculture tanks used for culture of meagre (*Argyrosomus regius*) at

the IFAPA center "El Toruño" experimental aquaculture station (Cádiz, Spain) and consisted primarily of fish faeces and also of uneaten fishfeed pellets used as food for meagre. Caprellids were fed ad libitum and after 24 hours were preserved in 90% ethanol. The caprellids were maintained at 20ºC with a photoperiod of 12 h light: 12 h dark. The seawater used for the culture was treated by filtration (through a 0.45 μm Milipore filter) and UV irradiation, and had a salinity of 35.5 psu. In addition to the specimens used in the 5 treatments of the experimental design, 16 adult specimens of each species were fixed directly after collection from the marinas to analyse the diet in field populations.

For the diet study, individuals were analysed following the methodology of Bello and Cabrera (1999) with slight variations, recently proposed to study the diet of amphipods (Guerra-García et al., 2014; Guerra-García and Tierno de Figueroa, 2009). Specimens of each species were placed in vials with Hertwig's liquid (consisting on 270 g of chloral hydrate, 19 ml of chloridric acid 1N, 150 ml of distilled water and 60 ml of glycerin) and heated in an oven at 65 ºC for 2 to 10 hours depending on the size of the specimens. After this, they were mounted on slides for study under a microscope. The percentage of the absolute gut content (at 40x or 100x), described as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 100x or 400x), described as the area occupied for each component within the total gut content, were estimated using a microscope equipped with an ocular micrometer.

Experiment 2: Does diet influence the lipid composition and survival of adults of *C. equilibra* and *C. scaura*? Is detritus an adequate food for them?

Provided that caprellids were able to feed on the three diets considered (see experiment 1), the same diets (A, P or D) were used to test the influence of food on the lipid composition of the caprellids and therefore their nutritional value for use as a resource for aquaculture. The present experiment was designed to last 12 days, as McLeod et al. (2013) reported changes in lipid composition of littoral amphipods in relation to diet after 12 days. 18 aquaria (20 cm x 13 cm x 13 cm) containing 3l of seawater (see experiment 1) were used; 3 for each treatment (A vs P vs D) of each species (*C. equilibra* vs *C. scaura*). In this experiment "All foods" and "no food" treatments were not included, mainly for two reasons: (i) in

this case, the objective was not to explore feeding selection among items; to clearly evaluate the effect of each diet on the lipid composition it was necessary to use each diet separately. (ii) The use of additional treatments such as a combination of foods or starvation was prevented due to the high amount of individuals required. The high number of specimens required is a common constraint limiting the number of experimental treatments possible. Forty adult specimens were added to each aquarium equating to a total of 360 specimens of *C. equilibra* and 360 specimens of *C. scaura*. Folded meshes, as proposed by Baeza-Rojano et al. (2013b) were used as substratum. Two small stones were used to prevent buoyancy of the meshes, maintaining their position at the bottom of the aquaria. The caprellids were fed daily and the water of the aquaria was replaced every two days. Any dead specimens, uneaten food or faecal pellets were removed daily. Aquaria were maintained with a continuous air supply, a water temperature of 20ºC, a photoperiod of 12 h light: 12 h dark, and a seawater salinity of 35.5 psu. Twelve days after the introduction of the caprellids in the aquaria, the plastic meshes were removed individually. The number of remaining adult specimens was counted, and the pool of specimens for each aquarium (considered as a replicate) was immediately frozen and stored at -80ºC for chemical analysis. Three replicates of the three food types used for feeding *C. equilibra* and *C. scaura* during the experiment (A, P and D) were also frozen for chemical analysis to characterise their lipidic nutritional value. In order to compare the treatments under laboratory conditions to the lipid composition of field populations, three replicates (three pools of specimens) for each species (*C. equilibra* vs *C. scaura*) were frozen immediately after collection from the marinas and stored at -80ºC for chemical analysis.

Experiment 3: Does diet influence the survival and growing rate of juveniles of *C. equilibra* and *C. scaura*? Is detritus an adequate food for them?

Thirty ovigerous females (15 of *C. equilibra* and 15 of *C. scaura*) taken directly from the marinas were isolated individually in small glass containers of 120 ml with a diameter of 6.5 cm and a height of 6 cm. A 1 mm plastic mesh, replaced every 5 days, was used as a substratum. This experiment was focused on the juvenile stage. As a reference, Baeza-Rojano and Guerra-García (2013) found the duration of the juvenile period in *C. equilibra* after emerging from the brood pouch to

be approximately 14-16 days at 20ºC, corresponding with instars I-III (from instar IV, sexes can be differentiated). Therefore, all the hatchlings which emerged from each female were monitored during the first 16 days of their life history, corresponding with the juvenile stage. Three treatments were also considered (A vs P vs D) and 5 replicas (consisting of the hatchlings produced by 5 females of each species) were used for each treatment. Juveniles were fed ad libitum every day, and water was also replaced daily. All of the juveniles were counted daily under a binocular microscope and observations were made of any signs of moulting or parental care. After 16 days, all of the juveniles were fixed in 90% ethanol. The number of articles of the flagellum of antenna 1, which is indicative of the number of moults (see Baeza-Rojano et al., 2011), were counted in each juvenile. Photographs of each specimen were taken with a Motic K-400L stereomicroscope and measures of the total body length were taken using the software Scion Image Alpha 4.0.3.2© (2000-2001 Scion Corporation).

2.3. Chemical analysis: lipid classes (LCs) and fatty acids (FAs)

Three replicates of the following samples from experiment 2 were used for chemical analysis: (i) the three food types the caprellids were subjected to (A, P and D), (ii) adult specimens of the two caprellid species collected from marinas and frozen immediately as reference values and (iii) adult specimens of the two caprellid species fed either A, P or D during 12 days. Each replicate consisted of a pool of specimens in order to reach the minimum quantity required for chemical analysis, which was 10 mg. Quantities used in other studies usually range from 10- 40 mg (Baeza-Rojano et al., 2014; McLeod et al., 2013; Guerra-García et al., 2004; Hyne et al., 2009) to 0.5-3 g (Gonzalez-Silvera et al., 2015). Unfortunately, the small weight of caprellids prevented the analysis of LCs and FAs in juveniles, since it was not possible to reach the minimum required weight for the chemical procedure, even when all the individuals of all the replicates of each treatment were pooled. LCs and FAs were therefore analysed only for adult specimens. Taking into account that Clark et al. (1985) found differences among sexes in some lipid classes, and that (Guerra-García et al., 2004) showed sex differentiation using fatty acid signatures, the same weight of male and female specimens were considered for each replicate to avoid the influence of sex in the results.

Samples were freeze-dried for 24 h at -50ºC. The lipid fraction was extracted according to the Folch-Lee method (Folch et al., 1957). Total lipid was extracted with chloroform:methanol (2:1v/v) containing 0.01% of butylated hydroxytoluene (BHT) as an antioxidant (Christie, 1982). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically. Lipid classes were separated by one-dimensional double development high-performance thin-layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9 by volume) as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80:20:2 by volume) as the neutral solvent system. Final quantification of lipid classes was made by densitometry in a CAMAG scanner at a wavelength of 325 nm, and by comparison with external standard (Sigma-Aldrich) (see Olsen and Henderson, 1989). For fatty acid analysis, total lipid extracts were subjected to acid catalysed transmethylation for 16 hours at 50 ºC, using 1mL of toluene and 2mL of 1% sulphuric acid (v/v) in methanol. The resulting fatty acid methyl esters (FAME) were separated and quantified using a Shimadzu GC 2010-Plus (Shimadzu) gas chromatograph equipped with a fame-ionisation detector (280 ºC) and a fused Tecnokroma – Suprawax-280TM (15 m x 0.1 mm I.D.). Hydrogen was used as carrier gas and the oven initial temperature was 100ºC, followed by an increase at a rate of 20 ºC min^{-1} to a final temperature of 250 ºC for 8 min. Individual FAME were identified by reference to authentic standards and to a well-characterised fish oil.

2.4. Statistical analysis

To determine whether the amount of ingested food varied among species and treatments (experiment 1), a two-way ANOVA was conducted with the following factors: 'Species' which was a fixed factor with two levels: *C. equilibra* (Eq) and *C. scaura* (Sc), and 'Treatment' which was a fixed factor orthogonal with 'Species' consisting of six levels: Marinas (caprellids taken directly from the marinas), A (caprellids fed with *Artemia*), P (caprellids fed with phytoplankton), D (caprellids fed with detritus), all foods (caprellids fed with the three diets simultaneously) and no food (caprellids given no food).

The differences in survival rates of adult males (experiment 2) and juveniles (experiment 3) among species and treatments were also tested using two-way ANOVA, with factor 'Species' (Eq vs Sc) and factor 'Treatment' (A vs P vs D).

For testing differences in the length of juveniles and number of flagellar articles in antenna 1 (experiment 3) among treatments, one-way ANOVAs were used for each species separately, with the single factor treatment (A vs P vs D) since the total mortality of juveniles of *C. equilibra* fed with D prevented the use of two-way ANOVAs.

Two-way ANOVAs were also used to compare the main lipid classes and fatty acids (experiment 3) among species (Eq vs Sc) and treatments (A vs P vs D). Previously one-way ANOVA was also conducted to explore differences in lipid composition among the three types of food used in the experiment for feeding caprellids.

Prior to ANOVAs, the homogeneity of variances was tested with Cochran's C-test. Where variances remained heterogeneous even after data transformation, untransformed data was still analysed, as ANOVA is a robust statistical test and is relatively unaffected by the heterogeneity of variances, particularly in balanced experiments (Underwood, 1997). In such cases, to reduce type I error, the level of significance was reduced to <0.01. Where ANOVA indicated a significant difference for a given factor, the source of difference was identified using Student-Newman-Keul (SNK) tests (Underwood, 1997).

Principal Component Analyses (PCAs) were carried out to show the relationship among treatments, foods (diets) and samples from marinas (reference values) according to the lipid classes and fatty acid matrices. Differences in fatty acid and lipid classes composition among species and diets were tested by the use of a permutational multivariate analysis of variance (PERMANOVA) with two factors: 'species', a fixed factor with two levels (Eq vs Sc) and 'treatment', a fixed factor orthogonal with 'species' consisting of three levels (A vs P vs D). Analysis was based on Euclidean distance measures and Monte Carlo tests were included. Significant P-values were obtained by computing 9999 permutations of residuals under a reduced model as this method gives the most accurate Type I error for complex designs (Anderson, 2005). Pairwise comparisons

were then used. Additionally, to test the dispersion among samples for the factors 'species' and 'treatments', a permutational analysis of multivariate dispersions (PERMDISP) was used.

Univariate analyses were conducted with GMAV5 (Underwood et al., 2002) and multivariate analyses were carried out using the PRIMER v.6 plus PERMANOVA package (Clarke and Gorley, 2001).

3. Results

3.1. *Caprella equilibra* and *C. scaura* as opportunistic feeders (Exp. 1)

Gut content of the specimens of *C. equilibra* and *C. scaura* which were directly fixed following collection in the marinas consisted mainly of detritus (more than 95% of the total), and included crustaceans (harpacticoid copepods) and phytoplankton (microalgae) (fig. 2). In laboratory conditions, when caprellids were offered a variety of diets, all items were observed in the gut, showing the ability of caprellids to ingest all three types of food (A, P and D). When the three food items were provided simultaneously, both caprellid species showed no feeding preferences and unselectively ingested the three of them (Fig. 2). Caprellids were able to actively prey on the live *Artemia* nauplii. In the control treatment 'No food', a small percentage of detritus (less than 5%) was registered in the gut, indicating that specimens isolated in the glass containers ingested the detritus attached to their body surface. When detritus was the single item provided, the amount of food ingested (measured as the total area occupied by the gut content in the whole digestive tract) was significantly higher than in other treatments (table 1, fig. 2). There were no significant differences in the quantity ingested among specimens fed with P, A or a combined diet of A+P+D (table 1).

Table 1. Results of the two-way ANOVA for the percentage of gut contents during the feeding experiment. ***P<0.001. Ma=Marinas, A=*Artemia*; P=phytoplankton; D=detritus; All=All food; NF=No food

Source of variation	df	MS	F	*P*	F versus
Species (Sp)	1	2352.00	3.28	0.0720	Res
Treatment (Tr)	5	5802.99	8.08	0.0000***	Res
Sp x Tr	5	998.02	1.39	0.2300	Res
Residual (Res)	180	717.98			
Cochran's test		C=0.16 n.s.			
Transformation		None			
SNK		D>(Ma=A=P=D=All)>NF			

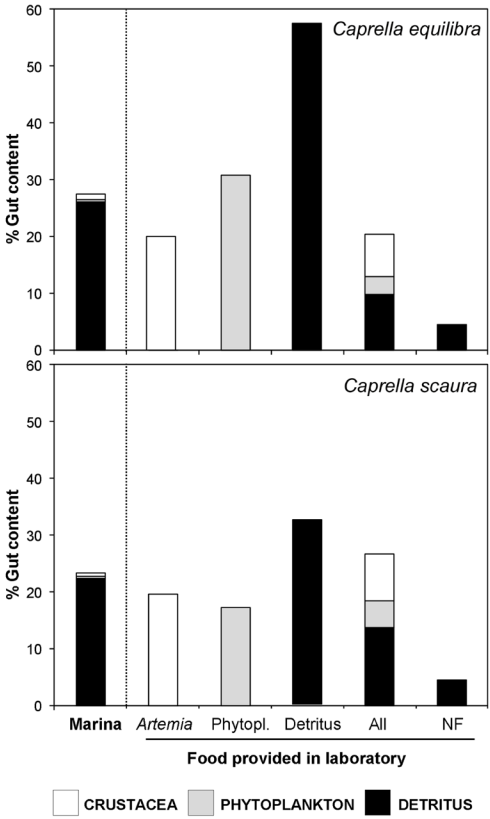

Figure 2. - Total area occupied by the gut content in the whole digestive tract of *Caprella equilibra* and *C. scaura*. 'Marina' includes data of the field populations directly fixed after collection. Treatments represent the different food provided in laboratory conditions: *Artemia* nauplii, Detritus, Phytoplankton, All food provided together (All), and no food provided (NF). The bars indicate the percentage of each item observed in the gut under the microscope (Crustacea, Phytoplankton and Detritus). The Crustacea are represented by copepods in specimens from the field populations of the marinas and *Artemia* in those maintained in laboratory conditions fed with *Artemia*.

3.2. Lipid composition of the diets used in the experiments

The three diets each had a distinct LC composition (table 2) and FA profiles (table 3). In connection with LCs, *Artemia* nauplii was characterised by a higher content in PC (one-way ANOVA, $F=322.1$, $p<0.001$) and TAG (one-way ANOVA, $F=1125.5$, $p<0.001$), while detritus was richer in ST (one-way ANOVA, $F=6.6$, $p<0.05$) and phytoplankton had a low content of most of the lipid classes analysed.

Table 2. Main lipid classes (μg/100 μg DW) of the three types of food used in the experiment as diets (A: *Artemia*, P: Phytoplankton, D: Detritus), the caprellids (Eq: *C. equilibra*, Sc: *C. scaura*) collected from marinas (used as reference values) and the caprellids fed either *Artemia*, phytoplankton or detritus during 12 days in laboratory conditions (treatments). Values are mean of 3 replicates. For caprellids, each replicate consisted on a pool of adult specimens. Dispersion among replicates can be observed in PCA (Fig. 3).

	FOOD			CAPRELLIDS							
	Diets			Marinas		Treatments					
	A	P	D	Eq	Sc	EqA	EqP	EqD	ScA	ScP	ScD
Phophatidylcholine (PC)	1.00	0.53	0.07	3.93	2.92	2.35	1.76	1.87	2.54	2.26	1.25
Phosphatidylethanolamine (PE)	0.40	-	0.15	1.45	1.13	0.95	0.73	0.85	0.94	0.98	0.58
Phosphatidylinositol (PI)	0.15	0.11	0.28	0.18	0.21	0.19	0.15	0.15	0.17	0.20	0.11
Phosphatidylserine (PS)	-	-	0.43	0.21	0.18	0.18	0.20	0.25	0.16	0.26	0.15
Triacylglycerols (TAG)	4.12	-	1.60	3.18	2.40	3.01	0.44	0.41	5.43	1.07	0.57
Sterols (ST)	0.04	0.01	1.68	0.27	0.28	0.15	0.29	0.19	0.22	0.21	0.12

In regard to FA, the saturated 16:0, the monounsaturated 16:1, OA, 18:1(n-7) and the polyunsaturated EPA were the dominant components in the three diets. However, the percentage of FA significantly differed among diets. Detritus had a higher content of 16:0 (one-way ANOVA, $F=307.1$, $p<0.001$), 18:0 (one-way ANOVA, $F=1454.4$ $p<0.001$), LA (one-way ANOVA, $F=1157.1$, $p<0.001$) and DHA (one-way ANOVA, $F=620.1$, $p<0.001$), but a lower content of 16:1 (one-way ANOVA, $F=3562.9$, $p<0.001$). *Artemia* nauplii were significantly richer in 18:1(n-7) (one-way ANOVA, $F=1125.5$, $p<0.001$) and EPA (one-way ANOVA, $F=4563.4$ $p<0.001$) while the phytoplankton had a significantly higher content of LNA than *Artemia* or detritus (one-way ANOVA, $F=3237.4$, $p<0.001$).

3.3. Influence of the diet on lipid composition and survival rate of adult specimens (Exp. 2)

Both caprellid species showed similar results in the experiment. LC and FA composition of the three treatments considered (A, F and D) are included in tables 2 and 3, together with the reference values obtained from specimens of field populations (marinas).

PCA results for LCs (fig. 3) showed differences among treatments, and these differences were also reflected by the PERMANOVA (table 4). Although an interaction between the factors 'Species' and 'Treatment' was detected, the pair-wise tests of the interaction reflected an identical pattern for both species: the caprellids fed with *Artemia* differed from those fed with phytoplankton or detritus, as shown by the PCA analysis. Axis 1 (fig. 3) explained 72% of the total variance and correlated with TAG. It clearly

Table 3. Fatty acid composition (expressed as % of the total identified fatty acids) of the three types of food used in the experiment as diets (A: *Artemia*, P: Phytoplankton, D: Detritus). Eq: *C. equilibra*, Sc: *C. scaura*. Values are mean of 3 replicates. For caprellids, each replicate consisted on a pool of adult specimens. Dispersion among replicates can be observed in PCA (Fig. 4). OA: Oleic acid; LA: Linoleic acid; LNA: Alpha linoleic acid; ARA: Arachidonic acid; EPA: Eicosapentanoic acid; DPA: Docosapentanoic acid; DHA: Docosaheanoic acid.

	FOOD			CAPRELLIDS							
	Diets			Marinas		Treatments					
	A	P	D	Eq	Sc	EqA	EqP	EqD	ScA	ScP	ScD
Saturated (SFA)											
14:0	1.84	4.06	2.42	1.82	3.17	0.93	0.70	0.53	0.81	0.63	0.62
15:0	0.43	1.20	0.68	0.53	0.65	0.28	0.85	0.46	0.39	0.75	0.60
16:0	12.33	19.44	27.58	22.06	19.58	13.80	20.06	18.96	12.60	17.71	17.85
17:0	0.92	-	0.93	1.37	1.55	0.99	1.73	0.98	1.05	2.14	1.27
18:0	4.91	0.64	9.44	6.10	5.31	4.52	5.56	4.94	4.01	5.29	4.91
20:0	-	-	1.10	0.12	0.53	-	-	0.09	0.15	0.19	0.17
22:0	0.34	-	0.98	0.05	0.05	0.19	-	0.09	0.18	-	-
23:0	-	-	-	-	-	0.19	-	-	0.43	0.94	0.89
24:0	-	0.77	0.97	0.09	-	-	0.21	-	0.02	-	-
Total SFA	20.77	26.11	44.09	32.15	30.84	20.90	29.10	26.05	19.65	27.63	26.31
Monounsaturated (MUFA)											
16:1	19.15	17.75	3.04	2.79	4.87	10.32	1.75	1.03	10.71	2.73	1.87
17:1	1.56	4.70	-	-	-	0.52	-	-	1.17	-	0.22
18:1(n-9) (OA)	17.58	3.45	20.49	9.41	10.08	21.76	14.06	18.17	20.52	11.68	18.34
18:1(n-7)	12.91	2.05	3.78	1.78	2.50	10.89	4.46	2.89	11.59	4.46	4.20
20:1(n-11)	-	-	0.11	0.08	-	-	-	-	-	-	-
20:1(n-9)	0.49	0.40	1.38	1.35	1.47	1.65	1.78	2.11	1.58	1.69	2.27
22:1(n-11)	-	-	0.82	-	-	-	-	-	-	-	-
22:1(n-9)	-	-	-	0.15	0.06	0.24	-	0.06	0.18	0.15	0.18
24:1	-	-	1.04	-	-	0.29	-	-	0.50	-	-
Total MUFA	51.69	28.37	30.66	15.56	18.97	45.68	22.05	24.26	46.25	20.70	27.08
Polyunsaturated (PUFA)											
16:2(n-4)	1.11	2.70	-	0.15	0.31	0.42	-	-	0.45	-	-0
16:3(n-4)	0.38	2.09	-	0.11	0.09	-	-	-	-	-	-
16:4(n-1)	0.06	0.79	-	-	0.11	-	-	-	-	-	-
18:2(n-6) (LA)	2.92	4.41	16.46	1.79	2.06	3.38	2.09	7.66	2.99	2.60	9.69
18:2(n-4)	0.45	-	-	-	0.03	0.14	-	-	0.22	-	-
18:3(n-6)	0.59	0.23	-	0.12	0.39	0.40	-	-	0.37	0.07	0.06
18:3(n-4)	0.52	-	-	-	-	0.31	-	-	0.43	-	-
18:3(n-3) (LNA)	4.27	20.79	1.95	1.41	1.09	3.87	1.32	0.41	3.07	2.41	0.76
18:4(n-3)	1.41	1.37	-	1.92	2.05	0.78	-	-	0.75	0.46	0.26
20:2(n-6)	-	-	-	1.06	0.77	0.42	0.64	1.50	0.33	0.97	1.78
20:3(n-6)	-	-	-	0.07	0.06	-	-	-	0.07	-	-
20:4(n-6) (ARA)	1.15	1.32	0.28	3.24	4.05	2.02	4.37	4.35	2.39	5.77	4.22
20:3(n-3)	-	0.54	0.15	0.69	0.87	0.66	1.38	1.02	0.73	1.91	0.74
20 4(n-3)	0.43	-	-	0.80	1.07	0.34	-	-	0.27	0.07	0.08
20:5(n-3) (EPA)	14.23	11.28	1.62	19.64	21.11	16.60	20.84	16.28	18.37	21.34	14.39
21:5(n-3)	-	-	-	0.39	0.38	-	0.26	-	0.03	0.18	-
22:4(n-6)	-	-	-	0.09	0.17	-	-	-	-	0.07	-
22:5(n-6)	-	-	-	0.55	0.51	-	0.32	0.64	0.04	1.08	0.89
22:5(n-3) (DPA)	-	-	0.26	0.78	0.82	0.12	0.74	0.91	0.16	0.78	0.59
22:6(n-3) (DHA)	-	-	4.52	19.50	14.25	3.97	16.89	16.91	3.42	13.94	13.13
Total (n-3)	20.34	33.98	8.50	45.13	41.64	26.35	41.43	35.52	26.82	41.09	29.96
Total (n-6)	4.67	5.96	16.75	6.90	8.01	6.21	7.42	14.16	6.19	10.58	16.65
Total PUFA	27.54	45.52	25.25	52.29	50.19	33.43	48.84	49.68	34.10	51.66	46.61

separated the samples of caprellids fed with *Artemia* (higher content in TAG) from those fed with phytoplankton and detritus (lower content in TAG). Axis 2 explained 22% of the total variance and correlated primarily with PC and secondarily with PE and ST. Interestingly, in only 12 days (duration of experiment), adult specimens of both species fed with the three diets (EqA, EqP, EqD, ScA, ScP, ScD) changed considerably in their LC composition (Eq, Sc). As expected, each treatment produced results close to the corresponding diet (A, P or D) at the end of the experiment, as can be observed in the PCA output (fig. 3). The two-way ANOVA analyses confirmed significant differences in the concentration of TAG and PC among treatments (table 5). Both variables showed the highest correlation with PCA axis 1 ($r=-0.93$, $p<0.001$) and axis 2 ($r=0.86$, $p<0.001$) respectively.

Table 4. Summary of the two-way PERMANOVA results examining the lipid classes and fatty acids of caprellids fed either *Artemia* (A), phytoplankton (P) or detritus (D) during 12 days in laboratory conditions. Star symbols indicates significant differences **P<0.01, ***P<0.001. PERMDISP results for the factors "Species" and "Treatment" are also included. MS=Mean Square; MC=Montecarlo.

Source of variation	**df**	**MS**	**Pseudo-F**	***P* (MC)**	**MS**	**Pseudo-F**	***P* (MC)**
		Lipid classes			**Fatty acids**		
Species (Sp)	1	5.17	12.28	0.0035**	28.92	7.03	0.0078**
Treatment (Tr)	2	27.29	64.81	0.0001***	636.94	154.85	0.0001***
Sp x Tr	2	2.74	6.52	0.0076**	14.25	3.46	0.0324**
Residual (Res)	12	0.42			4.11		
PERMDISP	(Sp)	F=7.52, *P*=0.0925 n.s			F=0.11, *P*=0.7829 n.s		
	(Tr)	F=10.10, *P*=0.0053**			F=8.67, *P*=0.0060**		
Pair-wise tests	Tr (Sp)				Tr (Sp)		
Sp x Tr:	*C. equilibra*: A≠P; A≠D; P=D				*C. equilibra*: A≠P; A≠D; P≠D		
	C. scaura: A≠P; A≠D; P=D				*C. scaura*: A≠P; A≠D; P≠D		

Regarding FA profiles, both PCA and PERMANOVA showed differences between treatments (fig. 4, table 4). In this case, although treatments also changed the initial FA composition, the phytoplankton treatment (EqP and ScP) showed higher similarities with the field populations inhabiting marinas (Eq and Sc). Unlike for LCs, in which each treatment was closer to its corresponding diet, for FAs the three diets (A, P and D) appeared clearly separated from the treatments. Although an interaction between the factors 'Species' and 'Treatment' was also detected in the PERMANOVA, the pair-wise tests of the interaction reflected an identical pattern for both species: significant differences

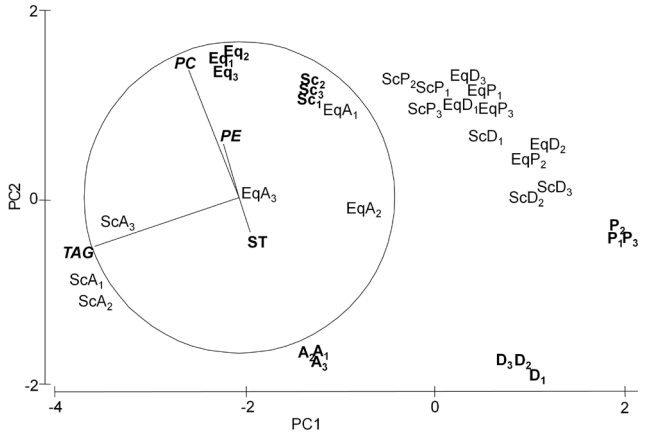

Figure 3. - Principal Component Analysis (PCA) plot based on the Lipid Classes (LCs) composition of the three types of food used in the experiment as diets (A: *Artemia*, P: Phytoplankton, D: Detritus), the caprellids. Eq: *C. equilibra*, Sc: *C. scaura*. Subscript numbers (1,2,3) correspond with each replicate value.

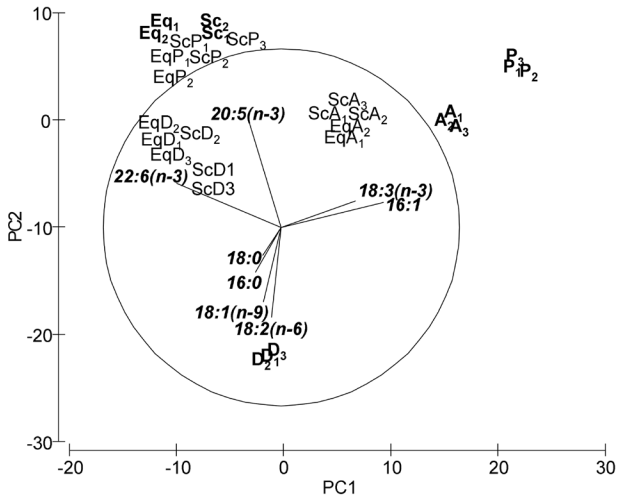

Figure 4. - Principal Component Analysis (PCA) plot based on the Fatty Acid (FAs) composition of the three types of food used in the experiment as diets (A: *Artemia*, P: Phytoplankton, D: Detritus). Eq: *C. equilibra*, Sc: *C. scaura*. Subscript numbers (1,2,3) correspond with each replicate value. Replicates Eq3, EqA3, EqP3 and Sc3 are not included since they were damaged during the chemical procedure and lost. For PERMANOVA analyses, the values of these replicates were filled with the mean values of the other two remaining replicates.

between the three treatments. PCA axis 1 (fig. 4) explained 47% of the total variance and correlated with 16:1, 18:3(n-3) (LNA) and 22:6(n-3) (DHA). Axis 2 explained 27% of the total variance and correlated with

16:0, 18:0, 18:1(n.9) (OA), 18:2(n-6) (LA) and 20:5(n-3) (EPA). The two-way ANOVA analyses confirmed significant differences of the selected fatty acids (those which correlated with axis 1 and 2 of the PCA) among treatments, except for 18:0 (table 5).

Table 5. Summary of the two-way ANOVA results examining the selected lipid classes and fatty acids (those which correlated with axis 1 and 2 of the PCA analysis). Caprellids were fed either *Artemia* (A), phytoplankton (P) or detritus (D) during 12 days in laboratory conditions. Star symbols indicates significant differences *P<0.05, **P<0.01, ***P<0.001.

Source of variation	**df**	**MS**	**F**	***P***	**MS**	**F**	***P***	**MS**	**F**	***P***
		Phosphatidylcoline (PC)			**Phosphatidylethanolamine (PE)**			**Triacylglycerols (TAG)**		
Species (Sp)	1	0.01	0.01	0.9061	0.00	0.01	0.9275	5.16	28.45	0.0002** *
Treatment (Tr)	2	1.18	7.09	0.0093**	0.08	1.40	0.2838	25.98	143.20	0.0000** *
Sp x Tr	2	0.49	2.98	0.0888	0.09	1.66	0.2313	2.14	11.84	0.0014**
Residual (Res)	12	0.16			0.06			0.18		
Cochran's test		C=0.26 n.s.			C=0.53 n.s.			C=0.57 n.s.		
Transformation		None			None			None		
SNK		A=P; A≠D; P=D			A=P=D			(Sp x Tr)	Eq:A≠P; A≠D; P=D Sc:A≠P; A≠D; P=D	
		Sterols (ST)			**16:0**			**18:0**		
Species (Sp)	1	0.00	0.59	0.4558	3.04	11.96	0.0047** 0.0000**	0.05	0.33	0.5740
Treatment (Tr)	2	0.00	0.17	0.8480	27.01	106.22	*	0.53	3.32	0.0711
Sp x Tr	2	0.02	4.34	0.0382	0.48	1.90	0.1919	0.21	1.33	0.3010
Residual (Res)	12	0.01			0.25			0.15		
Cochran's test		C=0.63 (p<0.01)			C=0.54 n.s.			C=0.78(p<0.01)		
Transformation		None			None			None		
SNK		A=P=D			A≠P; A≠D; P=D			A=P=D		
		16:1			**18:1(n-9)**			**18:2(n-6)**		
Species (Sp)	1	1.72	7.96	0.0154* 0.0000**	1.78	1.92	0.1911 0.0000**	2.61	3.13	0.1020 0.0000**
Treatment (Tr)	2	125.57	579.88	*	110.71	118.98	*	52.49	62.94	*
Sp x Tr	2	0.26	1.20	0.3342	2.69	2.90	0.0940	2.46	2.95	0.0980
Residual (Res)	12	0.21			0.93			0.83		
Cochran's test		C=0.37 n.s.			C=0.81 (p<0.01)			C=0.92 (p<0.01)		
Transformation		None			None			None		
SNK		A≠P; A≠D; P≠D			A≠P; A≠D; P≠D			A=P; A≠D; P≠D		
		18:3(n-3)			**20:5(n-3)**			**22:6(n-3)**		
Species (Sp)	1	0.14	3.05	0.1062 0.0000**	1.51	2.48	0.1411 0.0000**	11.58	24.42	0.0003** * 0.0000**
Treatment (Tr)	2	10.45	221.51	* 0.0000**	26.89	44.11	*	175.06	369.00	*
Sp x Tr	2	1.21	25.83	*	2.99	4.91	0.0277*	1.61	3.40	0.0677
Residual (Res)	12	0.04			0.60			0.47		
Cochran's test		C=0.60 n.s.			C=0.57 n.s.			C=0.40 n.s.		
Transformation		None			None			None		
SNK		(Sp x Tr)	Eq:A≠P; A≠D; P≠D Sc:A≠P; A≠D; P≠D		(Sp x Tr)	Eq:A≠P; A≠D; P≠D Sc:A≠P; A≠D; P≠D		A≠P; A≠D; P=D		

The survival rates of adults after 12 days significantly differed among treatments. For both species, the highest values for survival were obtained from caprellids fed with detritus (fig. 5, table 6).

Table 6. Results of the two-way ANOVA for the survival percentage of adult caprellids after the 12 days experiment. *P<0.05. A=*Artemia*; P=phytoplankton; D=detritus. See also Fig. 5.

Source of variation	df	MS	F	*P*	F versus
Species (Sp)	1	34.72	0.15	0.0703	Res
Treatment (Tr)	2	1554.38	6.78	0.0107*	Res
Sp x Tr	2	345.72	1.51	0.2603	Res
Residual (Res)	12	229.11			
Cochran's test		C=0.37 n.s.			
Transformation		None			
SNK		A=P; A≠D; P≠D			

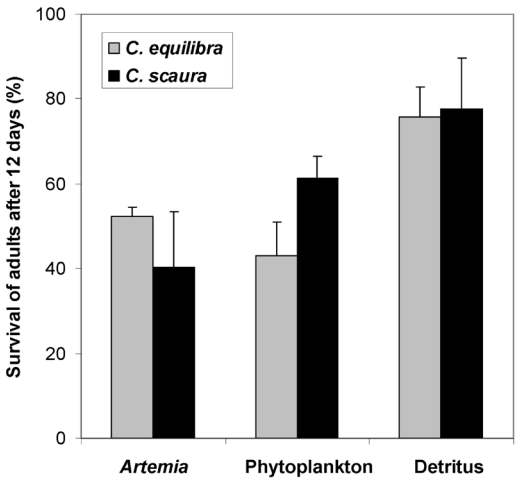

Figure 5. - Survival rate (%) of adult specimens of *Caprella equilibra* and *C. scaura* after 12 days of being fed with *Artemia*, phytoplankton or detritus.

3.4. Influence of diet on survival and growing rate of juveniles (Exp. 3)

In general terms, the invasive *Caprella scaura* showed higher values of survival than the native *C. equilibra* (fig. 6, table 7). For both species, the highest values of survival were obtained for hatchlings fed with *Artemia* nauplii (64.7% and 9.4% respectively), followed by those fed with phytoplankton (38.2% and 2.4%) and detritus (29.5% and 0%). The two-way ANOVA showed significant differences between the *Artemia* treatment and the other two (table 7). Despite the differences measured for survival rate, the juveniles which successfully reached the end of the experiment did not show any differences in body length among the three treatments (one-way ANOVA, *C. equilibra*: F=0.17, p=0.69, *C. scaura*: F=1.95, p=0.23) (Fig. 7) with mean values ranging from 1.7 to 2.6 mm. Similarly, no

significant differences among treatments were measured for the number of flagellar articles in antenna 1 (one-way ANOVA, *C. equilibra*: F=0.13, p=0.74, *C. scaura*: F=3.99, p=0.08) (Fig. 7) with mean values ranging from 4.0 to 5.5. The number of flagellar articles in the antenna 1 was always two in newly born juveniles, plus three basal articles at the peduncle. Juveniles increased one article in the flagellum before each moulting, thus the number of articles indicated the number of moults, which ranged from 2 to 4 in both species. During the experiment, most of the *C. scaura* juveniles were attached to the female body showing parental care, while this behaviour was not observed for juveniles of *C. equilibra*.

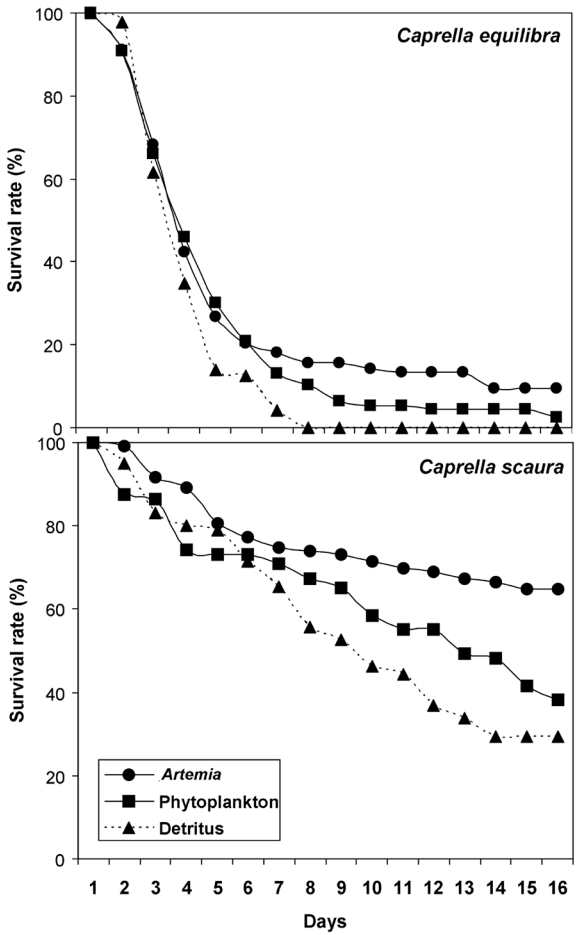

Figure 6. - Survival rate (%) of juvenile specimens newly emerged from the brood pouch of *Caprella equilibra* and *C. scaura* after 16 days of being fed with *Artemia*, phytoplankton or detritus.

Table 7. Results of the two-way ANOVA for the survival percentage of juvenile caprellids after the 16 days experiment. *P<0.05. A=*Artemia*; P=phytoplankton; D=detritus. See also fig. 6.

Source of variation	df	MS	F	*P*	F versus
Species (Sp)	1	10193.63	32.66	0.0000***	Res
Treatment (Tr)	2	1685.63	5.40	0.01166*	Res
Sp x Tr	2	658.43	2.11	0.1432	Res
Residual (Res)	24	312.13			
Cochran's test		C=0.34 n.s.			
Transformation		None			
SNK		A≠P; A≠D; P=D			

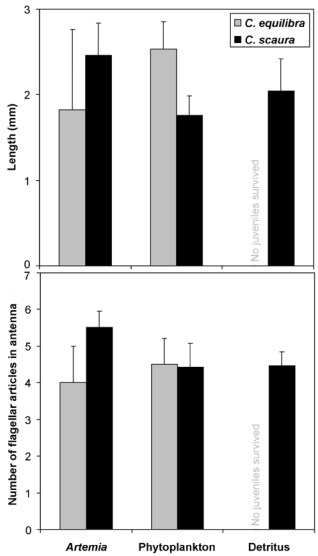

Figure 7. - Body length (mm) and number of articles in the flagellum of antennae 1 of juveniles of *Caprella equilibra* and *C. scaura* after 16 days of being fed with *Artemia*, phytoplankton or detritus. Data for detritus in *C. scaura* is not provided since all the juveniles died before the end of the experiment.

4. Discussion

4.1. The nutritional value of caprellids: an adequate resource in aquaculture?

Although there is little information available regarding the relative nutritional value of caprellids for marine finfish, recent efforts have been directed towards resolving this FA profiles have been characterised for several species (Woods, 2009; Baeza-Rojano et al., 2014; Gonzalez-Silvera et al., 2015; Guerra-García et al., 2004, Kawashima et al., 1999; Cook et al., 2010) and a complete nutritional characterisation has been conducted for

four common caprellids of the Strait of Gibraltar, including *C. equilibra* (Baeza-Rojano et al., 2014). These authors proposed that the shallow water amphipods from the Strait of Gibraltar were suitable for use as natural live feed since their protein, lipid and carbohydrate contents are adequate for normal fish, crustacean and mollusc growth. They also pointed out that, in connection with lipid content, marine amphipods, especially caprellids, turned out to be more adequate for aquaculture than freshwater gammarids, based on their higher PUFA levels and polar lipids. Our results, based on the lipid analysis of *C. equilibra* and *C. scaura* from marinas, also show an adequate nutritional level. Therefore, the suitability of caprellids as a food resource for fin- and shellfish in aquaculture, based on an adequate nutritional composition, is clear. Future research should be focused on how to obtain inexpensive diets to maintain sustainable caprellid cultures, and how these caprellids could be incorporated into IMTA systems, becoming a combined feed resource and bioremediation mechanism.

4.2. Detritus as food for caprellid culture

The present study produces evidence supporting the use of detritus (composed mainly of fish faeces, and secondarily of uneaten feed pellets) for caprellid culture. In natural habitats, although amphipods form a trophic continuum from primary herbivores to carnivores Mann (1998), Guerra-García and Tierno de Figueroa (2009) and Guerra-García et al. (2014) found that detritus is the main food item in the majority of species. The importance of detritus in benthic communities has often been reported in literature, and is considered to be highly nutritious after a short period of microbial colonization (Mann, 1998). Each of the three food types used as a diet for feeding caprellids during the present study showed a distinct composition; detritus had higher levels of ST and 16:0, 18:0, OA, LA or DHA, while *Artemia* nauplii was richer in PC, TAG, 18:1(n-7) and EPA, and phytoplankton was a source of LNA and PC. The lower concentrations of lipid classes measured for phytoplankton could be due to the presence of other polar lipids such as glucolipids, which were not quantified during the present study. High amounts of glucolipids in phytoplankton, and an abundance of free fatty acids in fish faecal faeces have also been measured (Hachero-Cruzado, unpublished data). In general terms, when compared with other diets described in literature for feeding amphipods or fishes (e.g. Gonzalez-Silvera et al., 2015; Hyne et al., 2009), detritus

can be considered a diet of relatively adequate nutritional value. It is a source of sterols (ST) 16:0, 18:0, and interestingly OA, LA and DHA. In fact, fragmented pellets and faeces might represent a new trophic source for fouling organisms, providing them with terrestrial fatty acids such as LA, which are unusual in marine environments (Gonzalez-Silvera et al., 2015). These authors found that the sediments associated with fish farms presented higher percentages of OA and LA than control sediments, due to the faeces contribution, and suggested that faeces could be used as a trophic resource. Indeed, LA is considered a dietary essential fatty acid in the diets of vertebrates (Hyne et al., 2009), since physiologically essential FAs, ARA (20:4n-6), EPA and DHA are obtained through diet or synthesised from LA or LNA via a desaturase-elongase system (Leonard et al., 2004). Gonzalez-Silvera et al. (2015) found that *Caprella equilibra* collected from fish farms were characterised by a higher concentration of PUFA(n-6) (mainly LA) than specimens from a control site. This data supports our results and the potential use of fish farm detritus as a resource. The FA profiles provided by Kawashima et al. (1999) for *C. mutica* from aquaculture facilities (salmon sea cages, shellfish longlines stocked with mussels and mooring lines from caged finfish aquaculture) do not differ very much from those obtained during the present study for *C. equilibra* and *C. scaura* fed with detritus, with the exception of the higher concentrations of LA measured in our study.

It has been well established in recent decades that long chain PUFAs, especially (n-3) PUFAs, have a vital role in human nutrition, disease prevention and health promotion (Kolanowski et al., 2007; Garofalaki et al., 2006 and references therein). They cannot be synthesised by fishes, meaning their levels depend upon dietary intake. Among these, the most important (n-3) PUFAs are EPA, DPA and DHA. These FAs cannot be produced by most crustacean species in sufficient quantities for metabolic functioning so they too must be obtained from the diet (Folch et al., 1957 and references therein). The present study has shown that detritus seems to be an adequate source of DPA and DHA for caprellids, however a poor source of EPA. Detritus proved also to contain low levels of PC and TAG while *Artemia* was an excellent source of these lipids (see table 2). Phospholipids, such as PC, located mainly in biological membranes, have an essential role in regulating biophysical properties, protein sorting and in cell signalling pathways (Coutteau et al., 1997; Lahdes et al., 2010). The

phospholipids of the majority of marine species including fish, molluscs and crustaceans are rich in PUFA, especially (n-3) (Coutteau et al., 1997). The principal storage lipids in marine organisms including amphipods consist of TAGs, with a sufficient amount of these compounds ensuring the survival of individuals over the non-productive season (Hyne et al., 2009). The percentage of TAGs in relation to the amount of total lipids can show an increase in energy demand allocated to gamete production and egg incubation (Dutra et al., 2007). A relatively high amount of TAG can indicate periods of starvation, where storage lipids may be important for survival (Graeve et al., 2001). During the present study, adults fed with detritus had a higher survival rate than those fed with *Artemia* or phytoplankton, but interestingly the survival rate of hatchlings during the first 16 days of development showed the opposite pattern. The higher survival rate obtained for caprellid hatchlings fed with *Artemia* is probably related with higher requirements of TAG and/or PC during the first stage of life history, which cannot be provided by detritus or phytoplankton. A highest level of TAG in amphipod females, increasing with the maturation of the ovary, has been reported by Clark et al. (1985) and it is expected that hatchlings also require higher TAG concentrations as a lipid reserve to properly face the first days of the life cycle. Clark et al. (1985) also pointed out that MUFAs are used in egg development, and in the present study *Artemia* proves to be a good source of MUFA, especially 16:1, in comparison with detritus. Curiously, Guerra-García et al. (2004) found higher percentages of 16:1 in caprellid females than in males, so this fatty acid is probably related with egg development and it is required in higher levels by hatchlings. For all these reasons, a supply of *Artemia* would be optimal in completing the requirements of TAG, PC, and MUFA during the early stages of development of caprellid cultures. In field populations, these requirements are probably provided by small crustaceans such as copepods or other amphipods, such as those observed in the guts of specimens from the marinas examined during the present study. The results of Ros et al. (2014a) seem to support this hypothesis since these authors found significantly higher amounts of prey (copepods) in juveniles of *C. scaura* than in adults. A diet shift during the development has also been observed in other amphipods (Olabarria et al., 2009). Ontogenetic shifts in diet may occur in order to overcome physiological constraints (Rossi et al., 2004). For instance, when juveniles have physiological limitation in the maximum rate of food uptake, they

might rely on higher quality sources of food to minimise the amount of food and maximise energy uptake (Hentschel, 1998). Hence, the juveniles could satisfy their higher requirements of TAG, PC and MUFA with an increased copepod intake.

4.3. Caprellid cultures and IMTA systems

The present study indicated that caprellids fed with detritus had an adequate lipid composition to be used as an aquacultural resource. Additionally, the survival rate for adult specimens was higher for caprellids fed with detritus than for those fed with *Artemia* or phytoplankton. In the case of hatchlings, the results are not as promising due to higher mortalities seen in specimens subject to a diet of detritus. Despite this, hatchlings which successfully completed the juvenile stage with detritus were seen to reach similar sizes as those fed with *Artemia* or phytoplankton. Detritus therefore seems not to negatively affect the growing rate of survivors. For caprellids cultured under laboratory conditions at small- or medium-scales (Baeza-Rojano et al., 2013b; Baeza-Rojano et al., 2011) it is therefore recommended that a mixed diet of detritus enriched with *Artemia*, especially during the first 15 days of culture, is used. If the culture is to be maintained offshore, the presence of a nearby supply of detritus would be recommended. Fish farms and sea-cage structures are interesting sources of detritus (Salvo et al., 2015) so a caprellid culture associated to these aquaculture facilities would be an excellent option. In this case, the availability of detritus for caprellids is guaranteed, and the additional TAG, PC and MUFA requirements of juveniles and reproductive females could be obtained by preying on copepods or other small planktonic crustaceans, which naturally inhabit fish farm ecosystems. Copepods are frequently found in the gut contents of caprellids in field populations (Guerra-García and Tierno de Figuero, 2009) and caprellids can prey directly on them, or ingest them accidentally while filtering (pers. obs.). Copepods are rich in lipids (e.g. Wang et al., 2014) and can be an important source of TAG (Cass et al., 2014). In fact, cultured copepods have been successfully used in the larviculture of various marine fish larvae, although the upscale of copepod cultures to commercial levels is still a challenge (Ajiboye et al., 2011).

The availability of detritus in fish farms, together with access to planktonic crustaceans naturally inhabiting the area, probably provide an optimum diet. This would explain the reason that the highest population densities for caprellids have been recorded in substrates of aquaculture facilities (see Woods (2009) for details). On the west coast of Scotland, densities of 319 000 ind/m^2 have been reported for *Caprella mutica* in a fish farm (Ashton et al., 2010). In Ireland and Scotland, *C. mutica* exhibits greater fecundity and abundance, size and population longevity at fish farm locations, with these results being attributed to organic enrichment from the fish farms (Tierney et al., 2004; Willis et al., 2004; Ashton et al., 2010). The present study confirms experimentally that detritus in the form of fish faeces is an adequate food, although also provides evidence that caprellids must have access to a source of TAG, PC and MUFA during the first stages of their life histories. From the environmental point of view the establishment of caprellid cultures associated to fish farms and sea cage structures seems to be justified, as caprellids would act as bioremediators. Encouraging the presence and abundance of bioremediating biota alongside net cage aquaculture has been proposed as a potential mechanism to ameliorate associated negative aquaculture impacts (see Woods (2009) and references therein).

In IMTA systems, the culture of caprellids alongside macroalgae is also advisable, since macroalgae provide substrate for caprellids. Caprellids may also have a direct benefit to macroalgae by reducing epiphytic overgrowth (e.g. Brawley and Fei, 1987; Duffy, 1990; see Woods (2009) for details). In addition to attaching to many different types of natural substrates (Guerra-García, 2001), caprellids easily use a range of artificial ones such as buoys, nets, plastic mesh, ropes and PVC panels. Baeza-Rojano et al. (2011) tested different kinds of plastic mesh with varying complexities for maintaining a culture of *C. scaura* and achieved very high densities (>10 000 ind/m^2) in all the substrates. Therefore, the culture of caprellids in IMTA could only require the use of additional artificial structures, easy to set and remove, located close to the fish farms. Previous studies of water currents would be desirable to find the most adequate placement locations in order to maximise efficiency in receiving the detritus from the fish faeces and uneaten food pellets. As pointed out by Woods (2009), framed mesh panels or a frame of string lines suspended within culture tanks could be used and once sufficient

biomass is reached, frames could then be transferred with their attached caprellids to fish culture tanks for fish to graze on at their leisure, closing the loop. Alternatively, caprellids from the frames could also be collected and dehydrated to be used as a component in the fabrication of fish feed, encased in microdiets, etc.

The two caprellid species used in the present study, *C. equilibra* and *C. scaura* can reach very high densities when associated with fouling communities of marinas in Southern Spain (Ros et al., 2013; Guerra-García et al., 2015; Ros et al., 2015). Recently, *Caprella scaura* has also been found on off-coast fish farm cages in the Mediterranean Sea (Fernández-González and Sánchez-Jerez, 2014). The life history of *Caprella equilibra* in small-scale laboratory conditions has been recently completed Baeza-rojano et al. (2013) and Woods (2009) considered *C. equilibra* as ideally suited to large-scale culture. A preliminary study of a medium-scale culture of *C. scaura* has also been conducted, showing promising results for this species (Baeza-Rojano et al., 2013b). In fact, during the present study hatchlings of *C. scaura* showed a significantly higher survival rate than those of *C. equilibra*. Consequently, *C. scaura* should also be a good candidate for large scale cultivation. However, it must be taken into account that *C. scaura* is a strongly invasive species which is quickly spreading across the Mediterranean and East Atlantic (Ros et al., 2014b) and seems to be ecologically displacing the native *C. equilibra* (Ros et al., 2015) along European coasts. Therefore, as pointed out by Woods (2009) any research elucidating the aquaculture potential of caprellid species should adopt a "precautionary principle" assuming nonindigenous species may have detrimental impacts on indigenous biota (unless proven otherwise) and concentrate on indigenous and/or already established caprellid species, such as *C. equilibra*.

Provided that detritus is an adequate diet and that fish farms are an excellent source of detritus, the next step is to experimentally develop caprellid culture at a large-scale under controlled conditions in order to optimise and evaluate the use of detritus in terms of caprellid survival rates and reproductive success. Pilot experiments with caprellid cultures associated to IMTA systems are also needed to evaluate the biomass production and the economic and ecological sustainability of these cultures.

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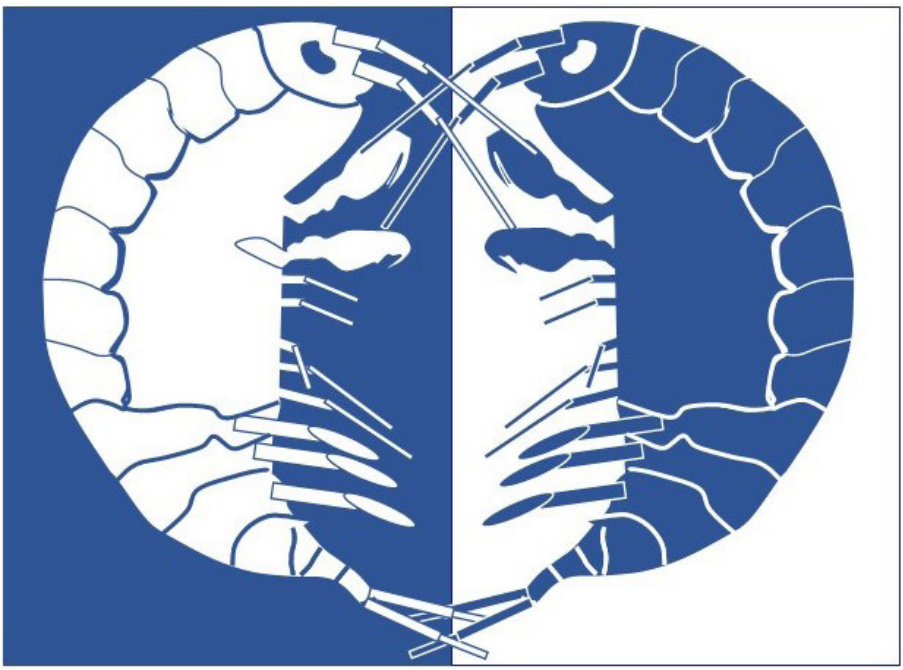
Capítulo
4.2

Evaluation of detritus and *Ulva* sp. as diets to culture the amphipod *Gammarus insensibilis*, a promising species for Integrated Multitrophic Aquaculture (IMTA).



Resumen

Los anfípodos son consumidos por los peces en la naturaleza y se han utilizado como presa alternativa para peces y cefalópodos. De los anfípodos de los esteros del sur de España, *Gammarus insensiblis* (Stock, 1996) reúne las características para ser una especie interesante para los sistemas IMTA. *G. insensibilis* fue cultivada durante 21 días con diferentes dietas; el experimento se replicó dos veces con 5 tratamientos, 4 dietas: *Artemia*, Detritus, Phytoplakton, *Ulva* sp. y el quinto tratamiento Ausencia de Alimento. Tanto el detritus como la *Ulva* generalmente se tratan como residuos en acuicultura, pero en este estudio se utilizaron como alimento consiguiendo buenas tasas de supervivencia. Los anfípodos criados con *Artemia* mostraron una alta supervivencia con altas concentraciones de triacilglicerol (TAG) y ácidos grasos saturados. Las dietas alternativas, detritus y *Ulva*, también mostraron tasas de supervivencia interesantes y contribuyeron a que los gammarideos presentaran altas concentraciones de ácido palmítico (16: 0), ácido oleico (18: 1n9), ácido araquidónico (20: 4n6) (ARA), ácido eicosapentaenoico (20: 5n3) (EPA) y ácido docosahexaenoico (22: 6n3) (DHA). Además, *Gammarus insensibilis* podría desempeñar un papel clave como biorremediador siendo cultivado con detritus y *Ulva* obteniendo una composición bioquímica adecuada para ser utilizada en acuicultura. Este anfípodo podría reemplazar parcial o totalmente las dietas formuladas consiguiendo la independencia del aceite de pescado y la reducción de costes económicos.

Abstract

Amphipods are consumed by fish in the nature and have been used as alternative prey for fishes and cephalopods. Among amphipods of South Spain ponds, *Gammarus insensiblis* (Stock, 1996) meet the characteristics to be an interesting species for IMTA. *G. insensibilis* was cultured during 21 days with different diets; the experiment was replicated twice with 5 treatments, 4 diets: *Artemia*, Detritus, Phytoplakton, *Ulva* sp. and the fifth was No Feed treatment. Detritus and *Ulva* are usually treated as residues in aquaculture but in this study were used as feed with good survival rates. Amphipods reared with *Artemia* showed high survival and high concentrations of triacylglycerol (TAG) and saturated fatty acids. The alternative diets detritus and *Ulva* also showed interesting survival rates and contributed to high concentrations in the gammaridean of palmitic acid (16:0), oleic acid (18:1n9), arachidonic acid (20:4n6) (ARA), eicosapentaenoic acid (20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA). Furthermore, *Gammarus insensibilis* could play a key role as biorremediator being cultured with detritus and *Ulva* getting an adequate biochemical composition to be used in aquaculture. This amphipod could replace partial or totally the formulated diet with two consequences, reduce dependence of fish oil and subsequently of economic costs.

Evaluation of detritus and *Ulva* sp. as diets to culture the amphipod *Gammarus insensibilis*, a promising species for Integrated Multi-Trophic Aquaculture (IMTA).

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1. Introduction

Fish are an important source of protein, 17% of the animal protein consumed in the world is provided by fish and even 50 % in Occidental Africa and East Asia, the geographical areas with the lower rent per capita (FAO, 2016). Aquaculture generate near 50 million of tonnes of finfish surpassing 160 $ millions (FAO, 2016). Aquaculture production is supported by high fish meal and fish oil consumption, so it consumes 75% of fish oil. According to FAO (2014) fish oil and fish meals are the only economical viable resource of n-3 HUFA to produce formulated diet. Consequently, there is an urgent need to explore and investigate the potential of novel aquatic organisms to replace formulated diet or as live feed in aquaculture.

Amphipod crustaceans are among the most diverse group of crustaceans with respect to life styles, trophic types, habitats and sizes (De Broyer and Jazdzewski, 1996). Amphipods inhabit a variety of marine environments and consequently show a high diversity of feeding habits (Guerra-García et al., 2014). Due to their nutritional characteristics, amphipods could serve as an adequate alternative live or dead feed resource for aquaculture (Baeza-Rojano et al., 2014, Kolanowski et al., 2007, Gonzalez et al. 2011). Amphipods are consumed by fish in the nature (chapter 2) and have been used as alternative prey for fishes (Moren et al., 2006, Sountana et al., 2007) and cephalopods (Baeza-rojano et al., 2010, 2013) with positive results too.

Residues generated by aquaculture, such as detritus or macroalgae can be used by others organism (e.g. amphipods) in the context of Integrated Multi-Trophy Aquaculture (IMTA) protocols. Chapter 3 showed that caprellid amphipods can breed with detritus obtained of aquaculture fishes and they could be excellent candidates for integration into IMTA systems. Detritus comes mainly from fish faeces and uneaten fishfeed pellets associated to tanks or ponds in aquaculture facilities. Moreover, it is also common that some macroalgae, such as *Ulva* sp, quickly grow and spread in these facilities reaching high biomass associated to the main cultures. The potential use of detritus and macroalgae as cheap diets for amphipods is therefore an interesting topic to be addressed.

In spite of the interest of amphipods as potential resource in aquaculture, there is a lack of studies focused on large scale culture testing different diets. For non-caprellids amphipods, most studies have dealt with freshwater species (see e.g. Kennedy et al., 2016) and for caprellid amphipods the first attempts have been conducted during the last years (see e.g. Baeza-Rojano et al., 2013). Therefore, the main aim of this study is to explore the viability of diets considered residues (such as detritus or macroalgae) to massive culture a marine amphipod potentially interesting as a resource in aquaculture. This would be the first step to further integrate this marine amphipod in IMTA systems

2. Material and Methods

The experiment lasted 21 days and was replicated twice being conducted in two different dates (09/2015 and 02/2016). Three 70 litres tanks were used for each treatment with 200 specimens of *Gammarus insensibilis*. The water (17-18ºC and 35 U of salinity) was renovated daily. *G. insensibilis* (Amphipoda: Peracarida) was collected from marine ponds in IFAPA "El Toruño" (South Spain) (see details of study area in chaoter 3) where inhabit mainly on *Ulva* sp. To simulate the wild conditions of this amphipod species a net with same size that *Ulva* seaweed was introduced in each tank. Both experiments had 5 treatments, 4 diets: *Artemia* (nauply of 0 or 1 day without enrichment), Detritus, Phytoplakton, *Ulva* sp. and the fifth was No Feed treatment, in which specimens were maintaining without any food. The phytoplankton used consisted of a mixture of freeze-dried microalgae (Easy Reefs1) containing Phaeodactylum,

Tetraselmis and Nannochloropsis (1:1:1, percentage in dry weight). To avoid the precipitation of Phytoplankton and Detritus, they were added in an accessory water container to adjust the continuous entry of food. In all treatments 100 mg per day of dry weight diet was supplied, except in *Ulva* treatment where the amphipods ate "ad libitum" the seaweed body.

At the end of the experiment, survival of amphipods in each tank was measured, and the remaining alive specimens were kept for chemical analysis of lipid classes and fatty acids. In addition, wild gammarids (taken directly from the field) were analysed to compare their biochemical composition with the four treatments. Detritus and Phytoplankton diet were the same than those used in chapter 3; in fact, data of biochemical composition was taken from them. For *Ulva* and *Artemia*, six replicates, three in each experiment, were taken and analysed in the present study.

2.1. Chemical analyses.

Three pools (ca. 15-20 individuals per pool) of the survivor specimens of each tank (3 tanks per treatment: *Artemia*, Detritus, Phytoplakton and *Ulva*) were used for the chemical analyses (lipid classes and fatty acids). Additionally, 3 pools of amphipods collected directly from the ponds (used as a control of the normal biochemical composition in natural conditions) were also analysed. The amphipod samples of each feed treatment were freeze-dried for 24h at -50°C. The lipid fraction was extracted according to the Folch-Lee method (Floch et al., 1957). Total lipid was extracted with chloroform:methanol (2:1v/v) containing 0.01% of butylated hydroxytoluene (BHT) as an antioxidant (Christie, 1982). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically. Lipid classes were separated by one-dimensional double development high-performance thin-layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9 by volume) as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80:20:2 by volume) as the neutral solvent system. Final quantification of lipid classes was made by densitometry in a CAMAG scanner at a wavelength of 325nm, and by comparison with external standard (Sigma-Aldrich) (see Olsen and Henderson, 1989). For fatty acid analysis, total lipid extracts were subjected to acid catalysed transmethylation for 16 hours at 50°C, using 1mL of toluene and 2mL of 1% sulphuric acid (v/v) in methanol. The resulting

fatty acid methyl esters (FAME) were separated and quantified using a Shimadzu GC 2010-Plus (Shimadzu) gas chromatograph equipped with a fame-ionisation detector (280°C) and a fused Tecnokroma–Suprawax-280 (15 m x 0.1 mm I.D.). Hydrogen was used as carrier gas and the oven initial temperature was 100°C, followed by an increase at a rate of 20°C min -1 to a final temperature of 250°C for 8 min. Individual FAME were identified and quantified using external standard (Sigma-Aldrich).

2.2. Statistic analyses

To explore differences in survival rates among treatments (including the No feed treatment as a control in this case) a two-way ANOVA was used. Prior to ANOVAs, the homogeneity of variances was tested with Cochran's test. The design included two factors: "Experiment" with two replications on the time, and "Diet" with 5 levels including the 5 treatmens (*Artemia*, Detritus, Phytoplankton, *Ulva* and No food). A Canonical Analysis of Principal Component (CAP) was conducted to show the relationship among Treatments (diets) according to the lipid classes and fatty acid matrices. Differences in fatty acid and lipid classes composition among treatments were tested by the use of a permutational multivariate analysis of variance (PERMANOVA) with two factors: "Experiment" with two replications on the time, and "Diet" with 5 levels, in this case, the four treatments (*Artemia*, Detritus, Phytoplakton and *Ulva*) and amphipods directly taken from the field (wild amphipod) as a control. In this case specimens of the no feed treatment were not included since they were not chemically analysed due to the lack of enough survivor specimens. Analysis was based on Euclidean distance measures and Monte Carlo tests were included. Significant P-values were obtained by computing 9999 permutations of residuals under a reduced model as this method gives the most accurate Type I error for complex designs (Anderson, 2005). Pairwise comparisons were then used. Data were transformed with function ln(x+1). Univariate analyses were conducted with GMAV5 (Underwood et al., 2002) and multivariate analyses were carried out using the PRIMER v.6 plus PERMANOVA package (Clarke and Gorley, 2001).

3. Results

3.1. Survival

The ANOVA test for survival rate presented interaction between Treatment and Experiment (table 1). In the first experiment, no significant differences among treatments were measured, while in the second experiment survival for *Artemia* treatment was statistically higher and for No Feed was statistically lower (table 1, figure 1) than treatments Detritus, Phytoplankton and *Ulva*.

Table 1. Two-way ANOVAs test for survival rate (%). Treatments: A= Artemia, D= Detritus, P= Phytoplankton, U= Ulva and NF= No Feed. *p<0.05, **p<0.001

	Survival				
	Two-way ANOVA				SNK
	df	MS	F	p	Experiment 1
Experiment	1	433.2	4.08	0.057	A=NF=D=P=U
Diet	4	1405.12	13.24	0.000**	Experiment 2
Exp*Diet	4	354.08	3.34	0.030*	A>D=P=U>NF
Residual	20	106.1			

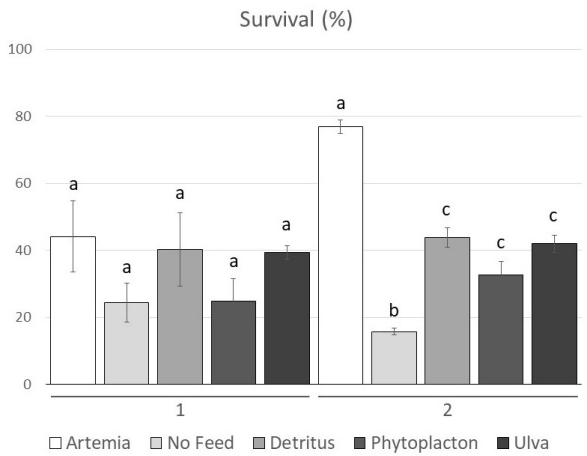

Figure 1. Means of survival percentage (and standard deviation) of three tanks per treatment of two experiment. Numbers indicated experiments. The letters indicate difference among treatment (p<0,01).

3.2. Lipid composition of the diets used in the experiments.

Biochemical composition of the different diets used in the experiment is included in table 2. Regarding the lipid classes, *Artemia* Diet (AD) had the highest amount of total lipids classes, triacylglycerols (TAG) and

Table2. Values of Lipid Classes (µg/100µg of DW) and Fatty Acids (% of total fatty acids) of used diets. AD= Artemia Diet, DD= Detritus Diet, PD= Phytoplankton Diet and UD= Ulva Diet. PC: Phophatidylcholine, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, PS: Phosphatidylserine, TAG: Triacylglycerols, Cho: Cholesterol. SFA: Saturated fatty acids, MUFA: Mono-unsatured fatty acids, PUFA: Poli-unsaturated fatty acids.

	Diets			
	AD	DD	PD	UD
Lipid Classes				
PC	2.31	0.07	0.53	0.12
PE	2.77	0.15	-	0.06
PI	-	0.28	0.11	0.02
PS	-	0.43	-	-
TAG	11.17	1.60	-	0.04
Cho	1.66	1.68	0.01	0.05
Fatty Acids				
SAT				
14:0	0.62	2.45	4.05	1.29
15:0	0.15	0.69	1.20	0.29
16:0	11.91	27.91	19.40	24.36
17:0	0.73	0.94	-	0.75
18:0	5.95	9.55	0.64	1.57
20:0	0.19	1.11	-	-
22:0	0.01	0.99	-	1.72
24:0	-	0.98	0.77	-
MUFA				
14:1	0.16	-	-	1.05
15:1	-	-	-	1.13
16:1n7	2.62	3.08	17.71	11.41
17:1	0.05	-	4.69	-
18:1n9	20.73	20.74	3.44	7.25
18:1n7	7.69	3.83	2.05	14.04
20:1n9	0.78	1.40	0.40	-
22:1n11	0.11	0.83	-	-
PUFA				
16:2n4	0.08	-	2.69	-
16:2n3	0.12	-	-	-
16:3n4	0.01	-	2.09	-
16:3n3	1.50	-	-	-
16:4n1			0.79	
16:4n1+18:0dma	0.02	-	0.79	-
18:2n6	5.39	16.66	4.40	2.08
18:3n6	0.46	-	0.23	-
18:3n3	31.02	1.97	20.74	8.43
18:3n4	0.08	-	-	-
18:4n3	4.50	-	1.37	15.91
18:4n1	-	-	-	-
20:2n6	0.23	-	-	-
20:3n6	0.11	-	-	2.06
20:4n6	0.83	0.28	1.32	0.58
20:3n3	0.94	-	-	0.96
20:4n3	0.75	-	-	0.58
20:5n3	1.59	1.64	11.25	2.08
22:5n3	-	0.26	-	2.46
22:6n3	0.03	4.57	-	-
∑ Sat	19.56	44.63	26.05	29.97
∑ MUFA	32.15	29.87	28.28	34.89
∑ PUFA	47.65	25.39	45.66	35.14

phospholipids PC and PE. In Detritus Diet (DD), TAG and sterols were the most abundant lipids, although the former was much lower than in AD. Regarding Phytoplankton Diet (PD) and *Ulva* Diet (UD), both had a low content of most of the lipid classes analysed

Regarding to fatty acid, all diets showed a high content of 16:0 (PA), 16:1n-7, 18:1n-9 (OA), 18:1n-7 and 18:2n-6 (LA). However, the percentage of FA significantly differed among diets. DD and AD were enriched in 18:1n-9 (OA), and AD and PD showed the higher content in 18:3n-3(LNA). Moreover, DD displayed the higher percentage of fatty acids 18:0, 18:2n-6 (LA) and 22:6n-3 (DHA), and PD of fatty acids 14:0, 16:1n-7, 17:1, 16:2n-4, 16:3n-4, 20:4n6 (ARA) and 20:5n3 (EPA). Finally, UD showed the higher content of 18:1n-7, 18:4n-3 and 22:5n-3 (EPA).

3.3. Lipid composition of treatments.

The five treatments and wild amphipods are represented in tables 3 and 4. Wild amphipods were included as positive control. Lipid classes and fatty acids PERMANOVAs tests (table 5) showed difference in the interaction Experiment with Diet. Despite the pair-wise test of interaction did not show clear consistent patters and reflected differences among experiments, the CAPs (Canonical Analysis of Principal Components) of lipid classes and fatty acids described clear groups, consistent for both experiments.

Table 3. Values of Lipid Classes (μg/100μg of DW) of amphipods of the 4 different treatments, values for wild amphipods are also used. A= Artemia treatment, D= Detritus treatment, P= Phytoplankton treatment, U= Ulva treatment, W= Wild amphipods. PC: Phophatidylcholine, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, PS: Phosphatidylserine, TAG: Triacylglycerols, Cho: Cholesterol. Each replicate consists on a pool of specimen.

	Experiment 1					**Experiment 2**				
	A	D	P	U	W	A	D	P	U	W
PC	1.66	1.23	1.22	1.61	1.74	2.07	1.61	1.84	2.02	1.25
PE	1.18	0.86	0.93	1.06	1.15	1.27	0.90	1.03	1.14	0.92
PI	0.08	0.06	0.06	0.04	0.06	0.08	0.05	0.09	0.09	0.06
PS	0.23	0.26	0.14	0.19	0.16	0.42	0.33	0.45	0.50	0.23
TAG	2.25	0.44	0.17	0.25	0.44	3.73	0.20	0.25	0.09	0.79
Cho	0.16	0.15	0.16	0.12	0.11	0.15	0.14	0.15	0.17	0.08

Table 4. Values of Fatty Acids (μg/mg of DW) amphipods of the 4 different treatments, values for wild amphipods are also indicated. A= Artemia treatment, D= Detritus treatment, P= Phytoplankton treatment, U= Ulva treatment, W= Wild amphipods, SFA: Saturated fatty acids, MUFA: Mono-unsatured fatty acids, PUFA: Poli-unsaturated fatty acids. The percentage of the dominant fatty acids with respect to total fatty acid composition is included in parenthesis. Each replicate consists on a pool of specimen. PA: palmitic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Alpha linoleic acid; ARA: Arachidonic acid; EPA: Eicosapentanoic acid; DPA: Docosapentanoic acid; DHA: Docosaheanoic acid.

	Experiment 1									
	A		**D**		**P**		**U**		**W**	
SAT										
15:0	0.07		0.09		0.08		0.16		0.15	
16:0 (PA)	3.64	(12%)	2.11	(15%)	2.08	(16%)	2.41	(17%)	4.45	(19%)
17:0	0.32		0.16		0.34		0.37		0.42	
18:0	1.17		0.44		0.48		0.51		0.84	
MUFA										
16:1n9	0.33		0.14		0.20		0.26		0.77	
18:1n9 (OA)	7.96	(25%)	3.10	(22%)	1.96	(15%)	2.39	(17%)	3.90	(16%)
18:1n7	1.96	(6%)	0.70	(5%)	0.73	(6%)	1.07	(7%)	2.28	(10%)
20:1n9	0.37		0.19		0.13		0.14		0.17	
PUFA										
18:2n6 (LA)	3.68	(12%)	1.45	(10%)	0.85	(7%)	0.80	(6%)	1.79	(8%)
18:3n6	0.15		0.01		0.01		0.01		0.02	
18:3n3 (LNA)	4.49	(14%)	0.18	(1%)	0.31	(2%)	0.35	(2%)	1.15	(5%)
18:4n3	0.49		0.03		0.06		0.16		0.83	
20:2n6	0.28		0.15		0.10		0.07		0.11	
20:3n6	0.16		0.05		0.04		0.03		0.10	
20:4n6 (AA)	1.11	(4%)	1.04	(8%)	1.25	(10%)	1.24	(9%)	1.28	(6%)
20:3n3	1.05		0.07		0.11		0.08		0.18	
20:4n3	0.49		0.09		0.08		0.12		0.35	
20:5n3 (EPA)	2.15	(7%)	2.10	(15%)	2.33	(18%)	2.44	(17%)	2.89	(12%)
22:5n3 (DPA)	0.30		0.28		0.31		0.48		0.78	
22:6n3 (DHA)	1.11	(4%)	1.45	(10%)	1.26	(10%)	1.35	(9%)	1.31	(6%)
∑ SFA	**5.20**	(17%)	**2.79**	(20%)	**2.98**	(23%)	**3.45**	(24%)	**5.86**	(25%)
∑ MUFA	**10.62**	(34%)	**4.12**	(30%)	**3.01**	(24%)	**3.86**	(27%)	**7.12**	(30%)
∑ PUFA	**15.46**	(49%)	**6.90**	(50%)	**6.73**	(53%)	**7.12**	(49%)	**10.80**	(45%)
Total	**31.29**		**13.81**		**12.71**		**14.43**		**23.78**	

Regarding lipid classes composition, CAP (figure 2) had a 50 % Misclassification error (delta_1^2: 0,91376; P: 0,0001) where *Artemia* and Wild treatments represent two distinct groups and Detritus, Phytoplankton and *Ulva* form a mixed group. In the axis 1, TAG was responsible of separation of amphipods fed with *Artemia* from the remaining treatments. Lipid class values confirmed this result, showing the amphipods fed *Artemia* the higher content in TAG (table 3).

Table 4 continued.

	Experiment 2									
	A		**D**		**P**		**U**		**W**	
SAT										
15:0	0.04		0.07		0.09		0.13		0.11	
16:0 (PA)	2.62	(13%)	1.96	(16%)	2.12	(17%)	1.69	(17%)	2.66	(19%)
17:0	0.19		0.15		0.28		0.30		0.31	
18:0	0.70		0.43		0.45		0.38		0.46	
MUFA										
16:1n9	0.21		0.30		0.30		0.23		0.41	
18:1n9 (OA)	5.25	(25%)	2.53	(20%)	2.01	(16%)	1.63	(16%)	2.17	(15%)
18:1n7	1.37	(7%)	1.04	(8%)	1.04	(8%)	0.76	(7%)	1.11	(8%)
20:1n9	0.23		0.13		0.11		0.08		0.10	
PUFA										
18:2n6 (LA)	2.43	(12%)	1.86	(15%)	0.91	(7%)	0.56	(5%)	1.26	(9%)
18:3n6	0.04		0.02		0.02		0.01		0.03	
18:3n3 (LNA)	2.76	(13%)	0.14	(1%)	0.34	(3%)	0.27	(3%)	0.59	(4%)
18:4n3	0.29		0.03		0.03		0.13		0.37	
20:2n6	0.18		0.11		0.10		0.06		0.11	
20:3n6	0.11		0.03		0.04		0.03		0.07	
20:4n6 (AA)	0.82	(4%)	0.82	(6%)	1.14	(9%)	0.87	(8%)	1.05	(7%)
20:3n3	0.67		0.07		0.09		0.07		0.11	
20:4n3	0.33		0.07		0.07		0.10		0.16	
20:5n3 (EPA)	1.63	(8%)	1.58	(13%)	2.20	(17%)	1.69	(17%)	1.77	(12%)
22:5n3 (DPA)	0.18		0.20		0.25		0.33		0.47	
22:6n3 (DHA)	0.85	(4%)	1.06	(8%)	1.19	(9%)	0.88	(9%)	0.85	(6%)
∑ SFA	**3.55**	(17%)	**2.62**	(21%)	**2.93**	(23%)	**2.51**	(25%)	**3.54**	(25%)
∑ MUFA	**7.06**	(34%)	**4.00**	(32%)	**3.47**	(27%)	**2.70**	(26%)	**3.80**	(27%)
∑ PUFA	**10.30**	(49%)	**5.98**	(47%)	**6.38**	(50%)	**4.99**	(49%)	**6.83**	(48%)
Total	**20.91**		**12.59**		**12.78**		**10.20**		**14.17**	

Table 5. PERMANOVAs tests of Lipid classes and Fatty acids composition of amphipods of the 4 difference treatments, values for wild amphipods are also used, and pair-wise test of interaction Experiment (Diet). A= Artemia treatment, D= Detritus treatment, P= Phytoplankton treatment, U= Ulva treatment, W= Wild amphipods. *p<0.05, **p<0.001

	PERMANOVAs					
	Lipid Classes			**Fatty Acids**		
	MS	Pseudo-F	p	MS	Pseudo-F	p
Experiment	0.274	5.945	0.003**	1.632	3.129	0.000**
Diet	1.596	3.459	0.000**	4.856	9.307	0.000**
Exp*Diet	0.148	3.211	0.003**	0.502	9.628	0.000**
Residual	0.046			0.052		
PAIR-WISE TESTs Experiment(Diet)						
Experiment 1	A≠(D=P=U=W)			A≠D≠P≠U≠W		
Experiment 2	A≠(D=P=U)≠W			A≠D≠P≠W; U≠A;U=D;U=P;U≠W		

According to the fatty acid composition, the CAP test (figure 3) separated the five treatments significantly (m= 14; 0% of Mis-classification error; delta_1^2: 0,99574; P: 0,0001). With 16:00 (PA), 18:0, 18:1n7, 18:1n9

(OA), 18:2n6 (LA), 20:1n9, 20:2n6, 20:3n6, 20:4n3 variables correlated positively and 15:0, 17:0, 20:4n6 (ARA), 22:5n3 (DPA) correlated negatively with CAP1; and 22:6n3 (DHA) variable correlated positively and 16:00 (PA), 17:0, 18:0, 16:1n9, 18:1n7, 18:3n3 (LNA), 18:4n3, 20:3n3, 20:3n6, 20:4n3, 22:5n3 (DPA) correlated negatively with CAP2.

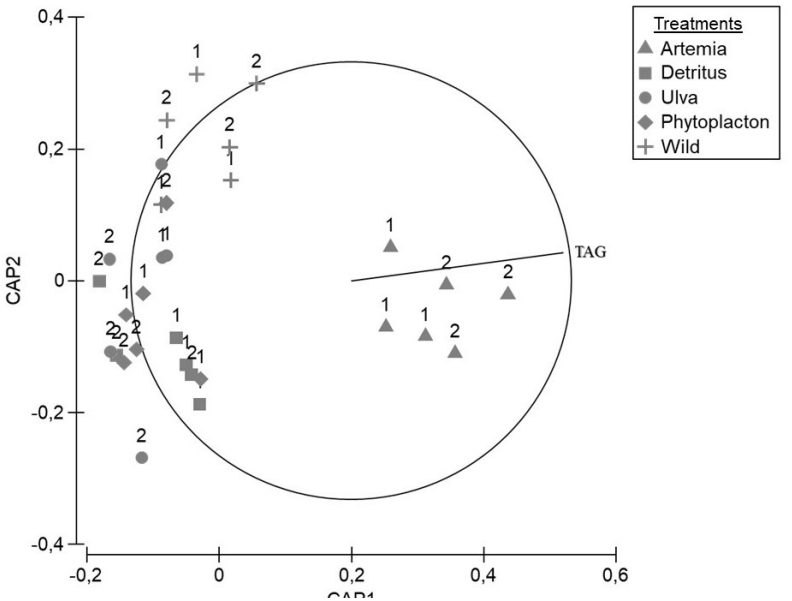

Figure 2. Lipid Classes's CAP of 5 treatments. Numbers indicates the experiment and colours the treatments.

Artemia treatment was characterized by the highest values of 18:1n9 (OA) and 18:2n6 (LA) (table 4). Both fatty acids were positively correlated with axis 1 of fatty acid's CAP (figure 3) and separated *Artemia* of the other treatments. Regarding the others dietary treatments, all were characterized by a low content in fatty acids (table 4), with the exception of essential fatty acids ARA, EPA and DHA. Specifically, DHA was slightly higher in gammarus fed detritus (table 4) and separated this treatment in the axis 2 of CAP (figure 3). In addition, ARA, EPA and DHA, did not present interaction between Experiment and Diet and there was no significant differences among treatments ($F=3.25$, $F=1.24$, $F=2.03$, respectively; with $p>0.05$ for all pairs-wise comparisons) Finally, wild amphipods displayed the highest content of 18:4n-3, 22:5n-3 and 16:1n-9, with separated this treatment in axis 2.

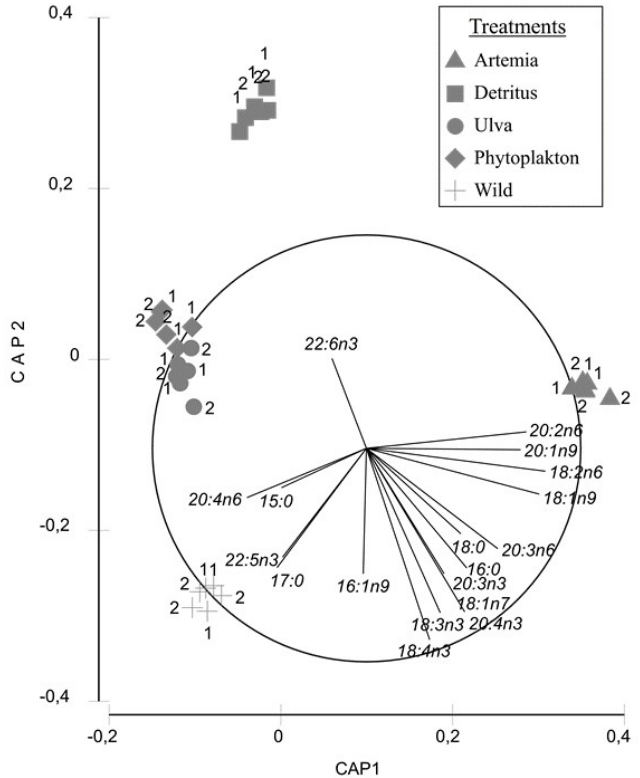

Figure 3. Fatty Acids's CAP of 5 treatments. Numbers indicates the experiment and symbols the treatments.

4. Discussion

Regarding the survival rate, higher values were measured in all diets than in the No Feed (negative control), being more perceptible in the second experiment especially for the *Artemia* treatment, which showed the highest rates. Detritus, *Ulva* and *Artemia* had elevated survival in the first experiment while *Artemia* had the higher survival in the second experiment. Although the most important patterns are similar in both experiments, some differences can be observed. This indicates the advisability of experiment replication before extracting further conclusions. The principal characteristic in both experiments are the high values of TAG of *Artemia* treatments and the fatty acid related with this lipid class (18:1n-9), but proportionally lack of essential fatty acid as EPA o DHA in this treatment (in percentage of total fatty acids). Phytoplankton was the less suitable treatment to culture the species because it was the

one treatment with cannibalism behaviour (personal observation) among specimens of *G. insensibilis*, that could be due to unfavourable food conditions and, although differences were not significant, phytoplankton had lower survival than other diets (see figure 1). Furthermore, it is an expensive diet and consequently, not recommended to maintain massive culture of the amphipods in cheap conditions.

Therefore, with detritus and with *Ulva*, this study shows that amphipods get a good chemical composition. They present higher values of essential fatty acid, ARA, EPA and DHA. Both treatments showed better values of ARA and EPA than other preys used in aquaculture like copepods (Rayner et al., 2015; Van der Mereen et al,. 2008) and higher values in the three essential fatty acids than rotifers (Maehre et al., 2013; Van der Mereen et al., 2008) and copepods used by Maehre et al. (2013).

Detritus and *Ulva* are used natural feed easily collected from the field, and, therefore, a cheaper treatment. Additionally, taking into account that these diets can be used to feed amphipods, they could be incorporated to IMTA systems, where the waste of some indoor and outdoor aquaculture cultures (such as detritus or macroalgae) can be use as feed for simultaneous cultures of amphipods. Detritus and *Ulva* are elements present in decantation and natural pools. Specifically, in the present study, wild amphipods showed fatty acids such as 18:1n7, 18:4n3 and 22:5n3 (table 3), that were also high in *Ulva* (table 2), indicating the importance of algae in the *G. insensibilis* diet. In wild condition, it is known that amphipods move to eat detritus (D'Avanzo and Valiela, 1990; Deegan et al., 1990). They can also be key links in the transfer of plant (macroalgae and seagrasses) production (Kneib et al., 1997) and therefore amphipods are a beneficial factor in extensive aquaculture. In addition, marine macrophytes, as *Ulva*, are used by macrograzers as both food and habitat (Duffy and Huy, 1991, 1994). In herbivore amphipods, nutrient content of the diet may be especially crucial for their growth, fecundity and fitness (Mattson, 1980; Cruz-Rivera and Hay, 2000a, b) because seagrass and seaweeds have a lower nutritional content than their own body (e.g., Sterner and Hessen, 1994; Gulati and DeMott, 1997). Consequently, herbivores exhibit different behavioural and physiological strategies in order to fulfill their nutritional requirements and provide it with a good biochemical composition (Duarte el al., 2010). For example, *G. insensibilis* has bacteria of the genus *Alteromonas* in the gut to cut a

polysaccharide, a long chain polymer called "Ulvan" (Coste et al., 2015); therefore, *Ulva* could not be eaten by organisms lacking the possibility of cutting this "Ulvan". Although it has not been tested in the present study, it is recommended the joint use of *Ulva* and detritus for the culture of *G. insensibilis*, considering that both diets are present in its habitat and provide it a good biochemical composition.

This experiment reinforces the used of this amphipod (replicable with any other amphipods) in IMTA. In chapter 3 was studied the biochemical composition of some gammarideans amphipods of Bahia of Cádiz and showed that they had good biochemical composition to be use as feed (live or dry). Among them, *Gammarus insensiblis* (Stock, 1996) meet the characteristics to be a novel aquatic organism to replace formulated diet or as live feed in aquaculture to be an interesting species to use:

1) Its medium size, lack of harder cuticle and good chemical composition: 5% total lipids, 10-15% carbohydrates, ≈40% proteins (Chapter 3).
2) A large distribution along Northeast Atlantic Ocean and Mediterranean Sea (see Costello and Bellan-Santini, 2017). This facilitates its culture in several countries to be used in aquaculture centres.
3) High density in nature (Arias et al., 1999).
4) It seems that the temperature and salinity oscillation do not affect *G. insensibilis* feeding rate and assimilation of food (Gates, 2006).
5) Capability of using aquaculture residues as feed, and this study amphipod feeds on detritus and *Chaeteromorpha* alga (Gates, 2006).
6) Suitability to be consumed by fishes in natural conditions (Chapter 2).

Although amphipods have been successfully reared under laboratory conditions in small scale (Myers, 1971; Takeuchi and Hirano, 1992; Baeza-Rojano and Guerra-Garcia, 2013), there is a lack of experiments in medium (Baeza-Rojano et al., 2013) or large scale. Baeza-Rojano et at. (2013) got suitable production levels using enriched *Artemia* nauplii and microalgae as food for *Caprella scaura*, as reported by Nakajima and Takeuchi (2008) for *Caprella mutica* feed on *Artemia* and diatoms in an exhibition tank. The price of *Artemia* cysts and phytoplankton is very high and is increasing

yearly, making this first large-scale caprellid culture study expensive and not economically sustainable. In comparison, this study use cheaper diets reducing the economic cost to reach sustainable productions level.

There are several possibilities to obtain these amphipods: 1) pick up them directly from the field, 2) rear the species in intensive culture or 3) rear it in extensive culture (ponds). However, it is necessary further research to study the viability and production capacity at large scale.

As conclusion, *Gammarus insensibilis* could play a key role as biorremediator being cultured with detritus and *Ulva* getting an adequate biochemical composition to be used in aquaculture. This amphipod could replace partial or totally the formulated diet reducing the economic costs.

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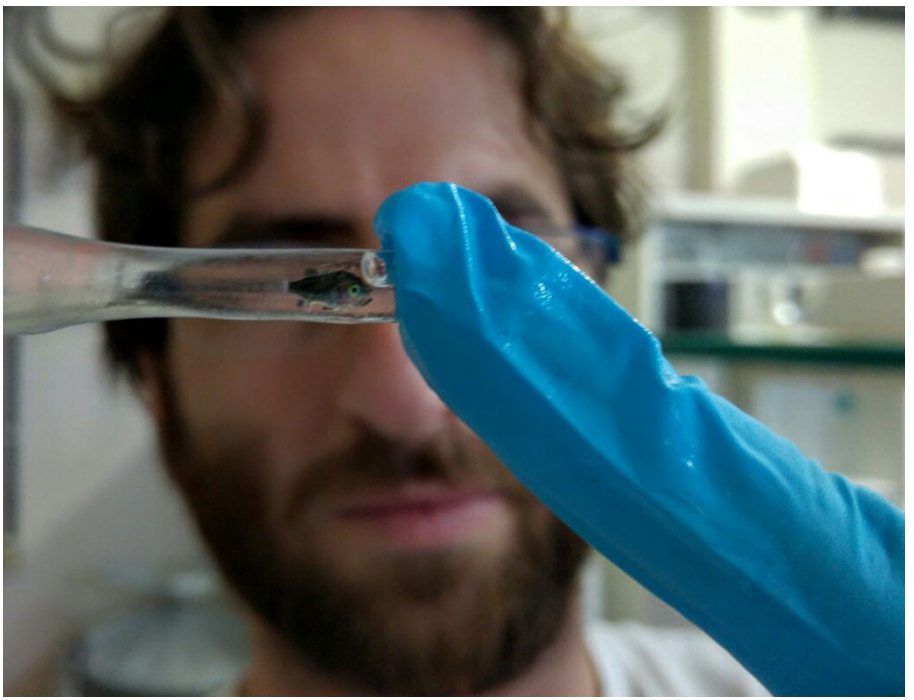
Capítulo 5

Effect of lyophilizate *Gammarus insensibilis* (Crustacea:Amphipoda) in growth, chemical composition and survival of *Seriola dumerili*.



Resumen

Tres de las áreas más relevantes de la investigación en acuicultura son: i) explorar e investigar el potencial de nuevos organismos acuáticos como alimento vivo, ii) progresar en 'Acuicultura Multitrófica Integrada' (IMTA) y iii) la diversificación acuícola en especies altamente apreciada por los consumidores. En el presente estudio, se realizaron dos experimentos con larvas de *Seriola dumerili* de 22 y 44 días después de la eclosión. Se alimentaron con el anfípodo *Gammarus insensibilis*, recogido de un estero, y con dieta formulada. Se estudió la supervivencia, biometría y perfil químico. En el primer experimento, el tratamiento con *G. insensiblis* mostró una mejor supervivencia, aunque los otros parámetros no presentaron diferencias. Por otro lado, en el segundo experimento la supervivencia fue similar, el crecimiento fue mejor con la dieta formulada y el tratamiento con *Gammarus insensibilis* tuvo el mejor perfil químico. El tratamiento con *G. insensibilis* mostró valores más altos de ARA (5,53 ± 0,18%) y DHA (19,07 ± 0,19%), bajo nivel de TAG (2,18 ± 0,5%) y una coloración similar a la de los juveniles silvestres *S. dumerili*. El objetivo de este estudio es mostrar el uso potencial de los anfípodos, como *G. insensibilis* en el cultivo de larvas de *Seriola dumerili* durante dos etapas distintas de este desarrollo.

Abstract

Three of the relevant areas to research in aquaculture: i) explore and investigate the potential of novel aquatic organisms as live feed, ii) The progress in 'Integrated Multi-Trophic Aquaculture' (IMTA) and iii) the aquaculture diversification in species highly appreciated by consumers. In the present study, two experiments were done with *Seriola dumerili* larvae of 22 and 44 days after hatchery. They were feed with the amphipod *Gammarus insensibilis*, recollected of a terrestrial pond, and one formulated diet. Survival, biometry measures and chemical profile were measured. In the first experiment, *G. insensiblis* treatment showed better survival, although the other parameters were no different. On the other hand, in the second experiment the survival was similar, the growth was better with formulated diet and *Gammarus insensibilis* treatment had the best chemical profile. *G. insensibilis* treatment showed higher values of ARA (5.53±0.18%) and DHA (19.07±0.19%), low level of TAG (2.18±0.5%) and a coloration similar to that in wild juveniles *S. dumerili*. The aim of this study is show the potential use of amphipod, such as *G. insensibilis* in the aquaculture rearing of *Seriola dumerili* larvae during two distinct stages of this development.

Effect of lyophilizate *Gammarus insensibilis* (Crustacea:Amphipoda) in growth, chemical composition and survival of *Seriola dumerili*.

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1. Introduction

Fishes are an important source of protein in the world, so 17% of the animal protein consumed in the world is provide by fish and even 50 % in Occidental Africa and East Asia (FAO, 2016). Aquaculture generates near 50 million of tonnes of finfish surpassing 160 $ millions, in addition, aquafeed resources production is one of the fastest expanding agricultural industries in the world, with growth rates more than 30 percent per year (FAO, 2016). In the framework of the innovative research programmes in aquaculture there are currently three areas of increasing interest (Querellou et al., 2010; Alexander et al., 2015):

a) Need to explore and investigate the potential of novel aquatic organisms as live feed in aquaculture.

Amphipods crustaceans are among the most diverse group of crustaceans with respect to life styles, trophic types, habitats and sizes (De Broyer and Jazdzewski, 1996). Amphipods inhabit a variety of marine environments and consequently show a high diversity of feeding habits (Guerra-García et al., 2014). Due to their nutritional characteristics, amphipods could serve as an adequate alternative live or dead feed resource for aquaculture (Baeza-Rojano et al., 2014; Kolanowski et al., 2007; Gonzalez et al., 2011). Amphipods are consumed by fish in the

nature (Chapter 2) and they have been used as alternative prey for fishes (Moren et al., 2006; Sountana et al., 2007) and cephalopods (Baeza-Rojano et al., 2010; Baeza-Rojano et al., 2013) with positive results.

b) The progress in 'Integrated Multi-Trophic Aquaculture' (IMTA):

IMTA involves the integrated cultivation of fed species (e.g. finfish) together with extractive species (marine invertebrates and/or algae) which feed on detritus from the fed species (Alexander et al., 2015). IMTA approaches reduce waste, diversify products and improve the economics (Samocha et al., 2015 and references therein). The present PhD shows that the amphipod *Gammarus insensibilis* (Stock, 1996) can breed with *Ulva* sp. and detritus obtained of aquaculture fishes and they could be excellent candidates for integration into IMTA systems.

c) Aquaculture diversification in species highly appreciated by consumers to get high prices on the market (Teletchea and Fontaine, 2014; Sicuro and Luzzana, 2016).

The greater amberjack (*Seriola dumerili* (Risso, 1810)) is an epipelagic fish (Carangidae family) which inhabit the circumglobal temperate area (Andaloro and Pipitone, 1997; Cummings et al.,1999; Thompson et al., 1999) with great interest to the aquaculture sector due to its excellent flesh quality, worldwide market availability and high consumer acceptability (Nakada, 2000). Spain (Grau, 1999) and Italy (Giovanardi et al., 1984; Pipitone and Andaloro, 1995; Lazzari et al., 2000) are the major producers of greater amberjack in the Mediterranean. It grows 1 kg in a year, and 6 kg in a period of 2.5 years (Jover et al., 1999; Mazzola et al., 2000; Mylonas and Robles, 2104). Culture of *Seriola dumerili* had two bottlenecks, the reproduction in captivity condition and the rear of larvae. Nowadays, protocols for artificial spawning of the species have been described (Roo et al., 2014; Fernández-Palacios et al., 2015; Sicuro and Luzzana, 2016), so the main problem is to know the nutritional requirements and find an appropriate food to larvae rearing.

The aim of this study is to rear larvae of *Seriola dumerili* of two different ages (22 dah and 44 dah; dah: days after hatching) with amphipods, *Gammarus insensiblis*, obtained from terrestrial ponds of an aquaculture centre.

2. Material and methods

The study was carried out in installation of Instituto Español de Oceanografía of Tenerife. Spawning of greater amberjack (*Seriola dumerili*) was get of broodstock reared in this centre. The same spawn was used in both experiment to minimize genetic difference factor. They were cultured in mesocosms tanks of 40m^3 and fed with rotifers and *Artemia* until 22 days, and after that fed with formulated diet A and B.

In the weaning experiment, the two treatments considered included Formulated diet A versus the freeze-dried amphipod *Gammarus insensibilis* (table 1). For each treatment, 200 mg per day (4 times/day) of their respective diet were used with a size lower than 100 μm. Specimens of *Gammarus insensibilis* used in the experiment were collected from the ponds of saltmarshes of "El Toruño", frozen and lyophilizate. Both diets were used to feed larvae with 22 dah (days after hatching) during 5 days; these larvae from the mesocosms had two previous days of acclimation (0.1 *Artemia*/ml and 2 gr per day of formulated A) in 12 litres tanks. The water (20ºC of temperature and 35 U of salinity) was renovated daily. Three replicates with 20 larvae per treatment were used. The duration was 5 days to avoid the completely mortality in two of three replicates of Formulated A treatment. To avoid fungi proliferation the tanks were disinfected twice with Aquacen Formaldehyde.

In the juvenile experiment the two treatments considered included Formulated diet B versus the freeze-dried amphipod *Gammarus insensibilis* (table 1). 500 mg per day in four times of freeze-dried *Gammarus insensibilis* and Formulated Diet B were used. Three replicates with 5 larvae of 44 dah per 100 litres tank were considered and this experiment lasted 12 days. The water (20ºC and 35 U) was renovated daily. Both diets, in this case, were used with a particle size higher than 1000 μm.

Before the beginning of both experiment, 5 larvae (Initial) of mesocosms tanks were taken to compare growth and chemical change. The biometry measures of Initial larvae and those which survived of each experiment were taken by a stereoscopic microscope coupled to a camera (59 x magnification, SMZ-10A; Nikon). Previously, larvae were sedated in

1% ethanol solution. To analyse their chemical composition, lipid classes and fatty acids profile a pool of 5 larvae were taken in the first experiment, however, each larva was analysed separately in the second one.

2.1 Chemical analyses

The lipid fraction was extracted according to the Folch-Lee method (Floch et al., 1957). Total lipid was extracted with chloroform:methanol (2:1v/v) containing 0.01% of butylated hydroxytoluene (BHT) as an antioxidant (Christie, 1982). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically. Lipid classes were separated by one-dimensional double development high-performance thin-layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9 by volume) as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80:20:2 by volume) as the neutral solvent system. Final quantification of lipid classes were visualized by charring at 160°C for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid, and dual-wavelength flying spot scanner Shimadzu CS-9001PC (Shimadzu, Duisburg, Germany (see Olsen and Henderson, 1989). To determine the fatty acid profiles, TL extracts were subjected to acid-catalysed transmethylation with 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were extracted using isohexane: diethylether (1:1 by volume) and purified by TLC using isohexane/diethyl ether/acetic acid (90: 10: 1, by volume) as developing system (Christie, 1982). Fatty acid methyl esters and DMA were separated and quantified using a TRACE-GC Ultra gas chromatograph (Thermo Electron Corp., Waltham, MA, USA). Individual FAME and DMA were identified by reference to authentic standards, and further confirmation of FAMEs and DMAs identity was carried out by GCMS (DSQ II; Thermo Electron Corp).

2.2 Statistic analysis

To explore differences in survival rates among treatments, long rank test was used in the first experiment . ANOVAS test were used to compare biometry measured, lipid classes and fatty acids. Prior to ANOVAs, the homogeneity of variances was tested with Cochran's test. A Principal Component Analyses (PCA) was conducted to show the relationship

among Treatments (diets) and Initial larvae according to the lipid classes and fatty acid matrices. Differences in fatty acid and lipid classes composition among treatments were tested by the use of a permutational multivariate analysis of variance (PERMANOVA) with one factor (diet). Analysis was based on Euclidean distance measures and Monte Carlo tests were included. Significant P-values were obtained by computing 9999 permutations of residuals under a reduced model as this method gives the most accurate Type I error for complex designs (Anderson, 2005). Pairwise comparisons were then used. Data were transformed with function Log(x+1). Univariate analyses were conducted with GMAV5 (Underwood et al., 2002) and multivariate analyses were carried out using the PRIMER v.6 plus PERMANOVA package (Clarke and Gorley, 2001).

3. Results

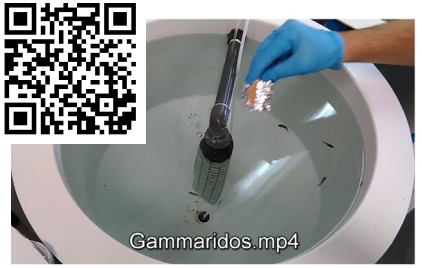
3.1 Biochemical profile and behaviour of diets

Chemical composition of the three diets was different (table 1). Regarding lipid classes, *Gammarus insensibilis* showed the higher values in PC (phosphatidylcholine)and PE, although both lipids showed the highest values of three diets; formulated A had the highest value of Cho but TAG and PE were its dominant lipids, FFA and TAG were the highest and the dominant in Formulated B. Regarding fatty acids, *Gammarus insensibilis* presented elevated values of percentage of PUFA (percentage about total fatty acids) as LNA, ARA, EPA and 22:5n3; formulated A had the highest value of LA and DHA, and Formulated B presented high values of 16:1n7 and saturated fatty acids as 16:0 and 18:0 (table 1).

The behaviour on water surface of formulated diets (A and B) and dry-frozen *Gammarus insensibilis* diet were different. Formulated diets presented ineffective floatability affecting their accessibility by fishes, but the floatability of *Gammarus insensibilis* was about 20-30 minutes. Furthermore, during the first experiment, the accumulation of formulated diet in bottom of tanks incremented the apparition of bacteria and fungi. On the other hand, larvae showed different behaviour during feeding depending on the diet; during the second experiment, larvae fed with formulated diet (video 1) showed more aggressive behaviour than with *Gammarus insensibilis* diet (video 2).

Table 1. Chemical profile of diets. SM: sphingomyelin, PC: Phophatidylcholine, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, PS: Phosphatidylserine, PG: Glycerophosphoglycerols, MAG: monoacylglycerols, DAG: diacylglycerols, FFA: Free fatty acids, TAG: Triacylglycerols, Cho: Cholesterol, SE: Sterol ester. SFA: Saturated fatty acids, MUFA: Mono-unsatured fatty acids, PUFA: Poli-unsaturated fatty acids. OA: Oleic acid; LA: Linoleic acid; LNA: Alpha linoleic acid; ARA: Arachidonic acid; EPA: Eicosapentanoic acid; DHA: Docosaheanoic acid.

	Diet		
	G. insensibilis	**Formulated A**	**Formulated B**
	Lipid classes		
SM	-	0.82	1.18
PC	42.73	13.19	9.72
PS	5.57	6.03	1.47
PI	1.69	0.52	0.92
PG	-	10.54	1.37
PE	29.57	14.42	5.59
MAG+DAG	-	5.40	3.26
CHO	2.75	9.45	8.17
FFA	-	7.56	23.13
TAG	17.69	24.59	42.54
SE	-	7.46	2.63
	Fatty acids		
SFA			
14:0	-	3.66	6.57
15:0	0.70	0.39	0.70
16:0	18.74	15.08	21.24
17:0	1.90	0.55	0.77
18:0DMA	-	-	-
18:0	3.42	2.59	4.21
Others	-	-	0.36
MUFA			
16:1n9	3.10	0.38	0.54
16:1n7	-	3.42	7.82
17:1	-	0.00	0.10
18:1n-9 (OA)	16.00	13.11	12.69
18:1n7	8.94	3.48	3.90
20:1n9	0.73	3.12	2.41
22:1n11+n9	-	4.84	3.49
Others	-	1.52	0.89
PUFA			
18:2n6 (LA)	8.06	12.68	3.61
18:3n3 (LNA)	4.58	1.84	1.39
20:4n6 (ARA)	6.14	0.89	1.01
20:4n3	1.34	0.38	0.52
20:5n3 (EPA)	12.30	8.15	11.72
22:5n6	-	0.27	0.33
22:5n3	3.30	2.38	1.30
22:6n3 (DHA)	5.69	18.54	8.73
Others	5.06	2.73	5.68



Video 1. Behaviour of *Gammarus insensibilis* on water surface and the effect in larvae. Use QR code to play the video.

Video 2. Behaviour of formulate diet on water surface and the effect in larvae. Use QR code to play the video.

3.2. Weaning experiment: Comparison between *Gammarus insensibilis* Treatment (W1) and Formulated Diet A Treatment (W2).

Survival, biometry measures and total lipid values of treatments are shown in Table 2. W1 presented higher survival than formulated treatment by Long-rank test (Chi square= 4.04; d= 1; p=0.04) but ANOVAs tests for biometry measures and total lipid value indicated no differences between treatments .

Table 2: Biometry measures, survival and total lipid of weaning experiment. FL: fork length, WL: wide length, EL: eye length, DW: Dry weight. Treatment are indicated by a letter (I= Initial, G= G. insensibilis treatment, F: formulated diet A). The numbers indicate the replicate.

	Biometry							Survival	Total Lipid
Treatment	FL (mm)	WL (mm)	EL (mm)	EL/FL	WL/FL	W/LT^3	DW (mg)	(%)	(%)
I	8.27	2.49	0.91	0.11	0.30	0.26	1.47	-	22
G1	9.43	2.93	1.13	0.12	0.31	0.24	2.20	70	19
G2	9.34	2.83	1.10	0.12	0.30	0.22	1.96	60	19
G3	9.57	2.90	1.11	0.12	0.30	0.21	1.88	55	19
F1	10.25	3.12	1.20	0.12	0.30	0.22	2.42	60	17
F2	9.61	2.86	1.12	0.12	0.30	0.28	2.50	25	18
F3	8.36	2.57	0.99	0.12	0.31	0.25	1.50	25	28
ANOVA F	0.01	0.05	0.03	0.47	0.61	1.88	0.14	-	0.14
p	ns	ns	ns	ns	ns	ns	ns	-	ns

Regarding lipid classes of the greater amberjack larvae (Table 3), PERMANOVA test showed no difference between treatments (Pseudo-F= 1.286, p (MC)= 0.3067). The treatments are compared with the Initial larvae in Table 3. Initial larvae (22 dah) showed higher values of triacylglyceride (TAG) and sum of monoacylglyceride (MAG) and diacylglyceride (DAG) than both treatments. In addition, W1 and W2 presented higher values of phosphatidylserine (PS), FFA and SE. Indeed, in the lipid classes's PCA

(Figure 1) , both treatments were separated clearly to Initial larvae by MAG+DAG correlated negatively with axis 1 (58,4% of total variance) and SE correlated negatively with axis 2 (29% of total variance).

Table 3: Values of Lipid Classes (percentage of total) of weaning experiment. SM: Sphingomyelin, PC: Phosphatidylcholine, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, PS: Phosphatidylserine, PG: Phosphoglycerols, MAG: monoacylglycerols, DAG: diacylglycerols, FFA: Free fatty acids, TAG: Triacylglycerols, Cho: Cholesterol, SE: Sterol ester. Each replicate consists on a pool of specimen.

		Treatment						ANOVA	
	Initial	*G. insensibilis* (W1)			Formulated A (W2)				
		1	2	3	1	2	3	F	p
SM	1.87	5.23	3.20	2.88	3.98	4.01	3.38	0.03	ns
PC	29.08	30.66	29.17	26.27	26.64	29.07	22.50	1.28	ns
PS	3.76	13.39	11.65	10.26	10.89	11.64	10.51	0.55	ns
PI	2.59	5.35	4.25	3.99	3.95	4.88	5.75	0.23	ns
PE	27.18	22.25	22.75	19.42	20.72	21.96	23.42	0.20	ns
MAG+DAG	7.84	2.05	2.14	2.26	1.99	2.06	5.06	0.68	ns
CHO	14.60	12.96	20.94	25.37	22.48	20.74	18.65	0.14	ns
FFA	-	2.58	2.40	2.52	2.57	3.24	4.75	2.84	ns
TAG	8.68	3.08	0.76	2.68	2.47	2.30	5.85	1.05	ns
SE	0.85	2.05	2.74	4.10	4.30	0.10	0.13	1.80	ns

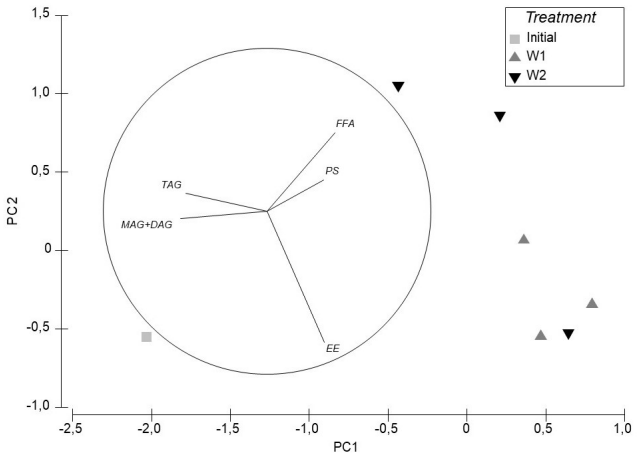


Figure 1. Lipid classes's PCA of weaning experiment.

Regarding fatty acids (table 4), PERMANOVA test showed no difference between treatment (pseudo-F= 0.6456, p (MC)= 0.5674). ANOVAs test of any fatty acids or coefficient in table 4 presented no difference either. The more relevant difference between Initial larvae and larvae of treatments (table 4) were a decrease of 18:3n3 (LNA) and an increase of 22:6n3 (DHA) values. This trend is supported by fatty

Table 4: Values of Fatty Acids (percentage of total) of weaning experiment. SFA: Saturated fatty acids, MUFA: Mono-unsatured fatty acids, PUFA: Poli-unsaturated fatty acids. OA: Oleic acid; LA: Linoleic acid; LNA: Alpha linoleic acid; ARA: Arachidonic acid; EPA: Eicosapentanoic acid; DHA: Docosaheanoic acid. Each replicate consists on a pool of specimen.

		Treatment					
	Initial	*Gammarus*			Formulated		
		1	2	3	1	2	3
SFA							
14:0	0.52	0.87	0.46	0.43	0.45	0.57	0.56
15:0	0.64	0.47	0.42	0.41	0.41	0.62	0.55
16:0DMA	0.00	0.41	0.40	0.00	0.00	0.43	0.00
16:0	14.64	16.73	15.56	15.81	16.11	17.36	17.66
17:0	1.42	1.36	1.39	1.46	1.42	1.50	1.57
18:0DMA	0.00	1.71	1.81	0.60	0.56	1.93	0.86
18:0	9.69	11.89	12.73	12.75	12.07	13.36	13.52
20:0	0.00	0.35	0.37	0.37	0.34	0.40	0.41
22:0	0.00	0.00	0.33	0.33	0.32	0.00	0.32
MUFA							
16:1n9	0.89	0.90	0.82	0.74	0.78	1.20	0.94
16:1n7	3.60	2.18	1.48	1.70	1.89	1.79	1.90
16:1n5	0.32	0.00	0.00	0.00	0.00	0.00	0.00
17:1	1.18	0.97	0.93	0.87	0.78	1.23	1.31
18:1n9DMA	0.00	0.39	0.40	0.00	0.00	0.42	0.00
18:1n7DMA	0.00	0.41	0.40	0.00	0.00	0.46	0.00
18:1n-11	0.44	0.00	0.00	0.00	0.00	0.00	0.00
18:1n-9	15.68	13.29	13.73	14.36	13.71	14.80	15.38
18:1n7	8.98	6.28	6.18	6.46	6.93	7.52	6.65
18:1n5	0.44	0.44	0.00	0.00	0.37	0.42	0.36
20:1n9	0.58	0.57	0.42	0.48	0.45	0.51	0.61
20:1n7	0.33	0.00	0.00	0.30	0.00	0.00	0.30
PUFA							
18:2n6	6.48	4.55	5.00	5.12	5.18	5.37	5.37
18:3n6	0.33	0.00	0.00	0.00	0.00	0.00	0.00
18:3n3	8.23	2.73	3.00	3.15	3.03	3.20	2.73
18:4n3	0.88	0.33	0.00	0.00	0.00	0.00	0.30
20:4n6	3.37	4.41	4.93	5.32	5.27	4.55	4.27
20:3n3	0.53	0.39	0.48	0.48	0.46	0.46	0.38
20:4n3	0.51	0.34	0.41	0.43	0.39	0.39	0.32
20:5n3	6.94	5.90	6.04	6.62	6.52	6.18	5.41
22:4n-6	0.00	0.00	0.00	0.32	0.31	0.00	0.00
22:5n6	0.40	0.78	0.77	0.73	0.67	0.45	0.58
22:5n3	1.45	2.13	2.34	2.34	2.55	2.01	2.02
22:6n3	11.56	19.23	19.17	18.11	19.03	12.87	15.75
∑16:1	4.81	3.08	2.31	2.43	2.67	2.99	2.84
∑18:1	25.53	20.02	19.91	20.81	21.00	22.74	22.38
∑20:1	0.91	0.57	0.42	0.78	0.45	0.51	0.91
SFA	26.90	31.66	31.25	31.57	31.13	33.81	34.58
MUFA	32.42	24.64	23.58	24.90	24.91	27.46	27.44
PUFA	40.67	40.79	42.16	42.94	43.41	35.49	37.12
DMA	0.00	2.91	3.01	0.60	0.56	3.24	0.86
n6 PUFA	10.58	9.75	10.70	11.81	11.44	10.37	10.22
n3 PUFA	30.09	31.04	31.45	31.13	31.98	25.12	26.91
n3 HUFA	20.98	27.99	28.45	27.99	28.95	21.92	23.88
n3/n6	2.84	3.18	2.94	2.64	2.80	2.42	2.63
DHA/EPA	1.66	3.26	3.17	2.73	2.92	2.08	2.91
ARA/EPA	0.48	0.75	0.82	0.80	0.81	0.74	0.79

acids's PCA (figure 2) where both treatments were separated than Initial by axis 1 that explain the 75, 4 % of total variance, with 16:1n7 and 18:3n3 (LNA) correlating positively, and axis 2 (13,8% of total variance) with 17:1 correlating positively and 22:6n3 (DHA) correlating negatively.

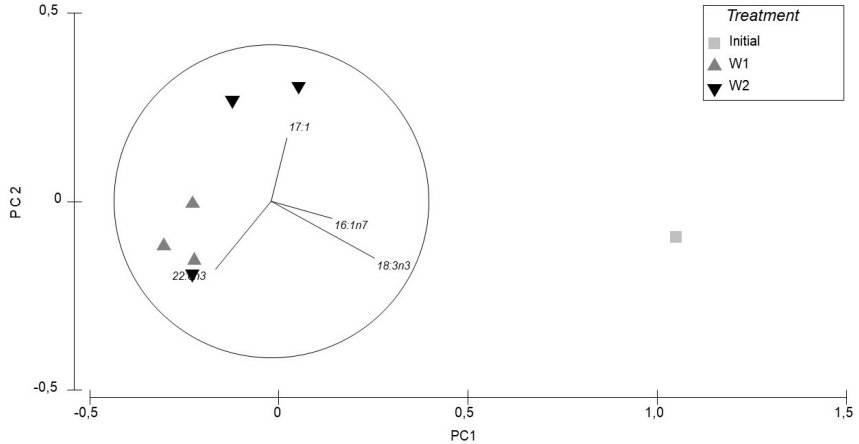


Figure 2. Fatty acids's PCA of weaning experiment.

3.3 Juveniles experiment: Comparison between *G. insensibilis* (J1) and formulated diet B treatments (J2).

Larvae of J1 (figure 3) look different than J2 (figure 4). The treatments with juveniles of greater amberjack presented clear difference in their biometry measures (Table 5) showing significantly difference in all of them, except width-large ratio. Survival and total lipid, however, did not show differences. It should be noted that although the size of the eye is greater in the J2 the relation of the eye with the length of the body is greater in the J1 (table 5).

Table 5: Biometry measures, survival and total lipid of juvenile experiment. FL: fork length, WL: wide length, EL: eye length, DW: Dry weight. Treatment are indicated by a letter (I= Initial, G= G. insensibilis treatment, F: formulated diet A). The numbers indicate the replicate.

	Biometry								Survival	Total Lipid
Treatment	FL (mm)	WL (mm)	EL (mm)	EL/FL	WL/FL	W/LT^3	DW (mg)	H%	(%)	(%)
I	26.45	8.46	2.88	0.11	0.32	0.63	108.38	-	-	12
G1	34.03	10.42	4.06	0.12	0.31	0.30	123.53	83.21	80	9
G2	30.50	9.43	3.90	0.13	0.31	0.34	99.24	83.48	100	10
G3	35.18	10.44	4.09	0.12	0.30	0.32	142.23	82.59	80	11
F1	47.68	13.95	4.50	0.10	0.32	0.39	484.40	77.44	80	11
F2	53.95	17.02	4.95	0.09	0.31	0.43	683.60	78.34	80	11
F3	47.50	14.38	4.65	0.10	0.32	0.42	468.74	78.42	100	12
ANOVA F	41.88	24.41	22.26	32.00	4.50	31.36	36.39	150.29	0.00	4.46
ANOVA p	0.00	0.01	0.01	0.00	ns	0.01	0.00	0.00	ns	ns

Figure 3. Picture of J1 juvenile of *Seriola dumerili.*

Figure 4. Picture of J2 juvenile of *Seriola dumerili.*

PERMANOVA test of lipid classes showed difference between treatments (Pseudo-F= 0.1006, P(MC)= 0.0052). ANOVAs tests indicated J1 had higher values of cholesterol (F= 35.49, p= 0.004) and sphingomyelin (F= 18.262, p= 0.0022) and lower values of TAG (F= 35.17, p= 0.0041) (Table 6). As shown by the lipid classes's PCA (figure 5) both treatments are separated for cholesterol and triacylglycerol, being the larvae feed with *G. insensibilis* clearly separated from the initial and those feed with formulated diet B Axis 1 explain the 78.8% of total variance with cholesterol negatively and triacylglycerides positively correlated, and Axis 2 (12% of total variance) had cholesterol positively correlated.

Table 6: Values of Lipid Classes (percentage of total) of juvenile experiment. SM: Sphingomyelin, PC: Phosphatidylcholine, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, PS: Phosphatidylserine, PG: Phosphoglycerol, MAG: monoacylglycerols, DAG: diacylglycerols, FFA: Free fatty acids, TAG: Triacylglycerols, Cho: Cholesterol, SE: Sterol ester. Each replicate consists on a pool of specimen.

	Initial	Treatment (62DAH)						ANOVA	
		G. insensibilis (J1)			Formulated B (J2)				
	(50 DAH)	G1	G2	G3	F1	F2	F3	F	p
SM	4.56	5.77	5.59	5.66	4.26	4.80	4.68	34.22	0.00
PC	31.55	28.81	27.53	31.47	25.84	27.48	31.10	0.37	ns
PS	6.83	8.12	6.72	7.23	6.84	6.12	4.86	0.75	ns
PI	5.08	4.96	5.24	4.82	3.02	4.21	6.50	0.34	ns
PG	5.25	5.75	5.23	5.45	5.47	4.51	5.54	0.69	ns
PE	21.69	24.41	23.03	22.06	21.43	18.44	21.74	4.14	ns
MAG+DAG	7.31	5.87	4.74	3.77	5.70	5.77	5.89	2.57	ns
CHO	2.24	8.46	12.72	10.21	3.80	3.01	1.90	35.49	0.00
FFA	4.97	4.39	4.77	4.74	5.28	6.76	5.01	4.06	ns
TAG	9.55	1.60	2.42	2.51	16.19	14.59	7.48	35.17	0.00
SE	0.96	1.86	1.99	2.10	2.16	4.32	2.62	3.08	ns

Regarding the fatty acids, PERMANOVA test indicated difference between treatments (Pseudo-F= 188.28, p (MC)=0.001). ANOVAs test of fatty acids (Table 7) showed that both treatments were completely different, with only the fatty acids 16:1n9 and 17:1 lacking differences. Additionally, the rate n3/n6, DHA/EPA and ARA/EPA indicated significantly difference. Furthermore, in fatty acids's PCA (figure 6) the treatments were separated

Table 7: Table 4: Values of Fatty Acids (percentage of total) of juvenile's experiments. SFA: Saturated fatty acids, MUFA: Mono-unsatured fatty acids, PUFA: Poli-unsaturated fatty acids. OA: Oleic acid; LA: Linoleic acid; LNA: Alpha linoleic acid; ARA: Arachidonic acid; EPA: Eicosapentanoic acid; DHA: Docosaheanoic acid. Each replicate consists on a pool of specimen.

	Initial	Juveniles treatment (62DAH)						ANOVA	
		G. insensibilis			Formulated B				
	(50 DAH)	G1	G2	G3	F1	F2	F3	F	p
SFA									
14:0	1.42	0.54	0.55	0.58	2.74	2.98	2.95	891.43	0.00
15:0	0.31	0.30	0.31	0.39	0.46	0.47	0.44	16.32	0.02
16:0	16.20	17.93	17.74	18.51	19.41	20.17	19.45	23.07	0.01
17:0	0.44	0.83	0.81	0.80	0.57	0.57	0.63	96.23	0.00
18:0DMA	0.75	2.32	1.87	2.34	0.69	0.37	0.33	74.43	0.00
18:0	5.82	10.24	10.35	10.51	6.77	6.41	6.32	457.33	0.00
Others	0.74	2.13	1.96	1.89	0.83	0.49	0.51	-	-
MUFA									
16:1n9	0.65	0.58	0.57	0.61	0.56	0.50	0.51	8.17	sn
16:1n7	2.78	1.86	1.77	1.85	4.63	5.10	4.96	806.08	0.00
17:1	0.31	0.46	0.66	0.47	0.39	0.44	0.37	3.89	sn
18:1n-9 (OA)	22.24	14.41	14.24	14.61	15.22	15.92	14.90	8.78	0.04
18:1n7	4.14	4.58	4.44	4.86	4.23	4.30	4.17	9.83	0.04
20:1n9	1.85	0.69	0.65	0.68	1.39	1.40	1.43	721.90	0.00
22:1n11+n9	1.43	-	-	-	0.85	0.83	0.97	757.85	0.00
Others	1.16	1.46	1.34	1.89	0.75	0.76	0.75	-	-
PUFA									
18:2n6 (LA)	11.63	5.79	5.43	5.42	6.59	7.09	6.44	27.13	0.01
18:3n3 (LNA)	2.60	0.75	0.77	0.75	1.43	1.71	1.47	114.90	0.00
20:4n6 (ARA)	1.36	5.34	5.69	5.56	1.71	1.51	1.80	616.32	0.00
20:4n3	0.43	0.13	0.12	0.07	0.52	0.58	0.54	270.31	0.00
20:5n3 (EPA)	4.79	5.79	5.83	5.49	8.23	8.17	8.53	268.74	0.00
22:5n6	0.39	0.71	0.77	0.71	0.46	0.40	0.50	56.94	0.00
22:5n3	1.40	2.85	3.03	2.95	2.20	2.16	2.19	241.97	0.00
22:6n3 (DHA)	15.81	19.16	19.93	18.11	17.10	15.13	17.29	8.14	0.05
Others	1.36	1.18	1.18	0.94	2.26	2.54	2.55	-	-
∑16:1	3.43	2.51	2.39	2.46	5.42	5.86	5.79		
∑18:1	26.74	19.31	18.99	19.81	19.79	20.60	19.42		
∑20:1	2.24	0.76	0.81	0.90	1.39	1.40	1.43		
SFA	24.61	31.10	31.04	31.99	30.35	30.84	30.10		
MUFA	34.15	23.03	22.85	23.89	27.84	29.13	27.98		
PUFA	39.76	41.69	42.75	40.01	40.53	39.29	41.30		
DMA	1.47	4.18	3.37	4.11	1.28	0.74	0.62		
n6 PUFA	13.88	12.86	12.91	12.64	9.08	9.27	8.97		
n3 PUFA	25.88	28.83	29.83	27.36	30.63	29.04	31.22		
n3 HUFA	22.55	27.93	28.92	26.61	28.22	26.27	28.70		
n3/n6	1.86	2.24	2.31	2.16	3.37	3.13	3.48	94.63	0.00
DHA/EPA	3.30	3.31	3.42	3.30	2.08	1.85	2.03	289.60	0.00
ARA/EPA	0.28	0.92	0.98	1.01	0.21	0.19	0.21	789.55	0.00

of Initial by axis 2 because both treatments presented higher values of ARA and less of 18:0 DMA. Moreover, J1 was separated by axis 1 to get higher values of EPA and lower LA than Initial and J2. Axis 1 of fatty acids's PCA (figure 6) explain the 82% of total variance and it presented positively correlated with 14:0 and negatively correlated with 18:0 DMA and 20: 4n6 (ARA). And, the axis 2 (13.8% of total variance) presented positive correlation with 18:2n6 (LA), and negatively with 14:0 and 20:5n3 (EPA).

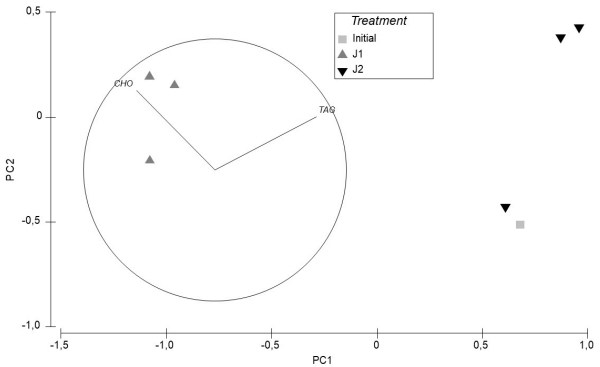


Figure 5. Lipid classes's PCA of juvenile experiment.

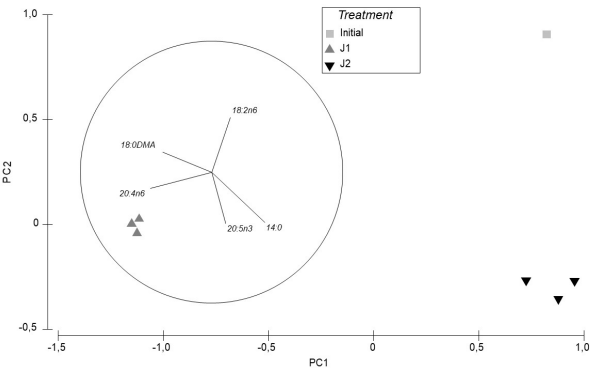


Figure 6. Fatty acids's PCA of juvenile experiment.

4. Discussion

Nowadays, there is a lack of information about chemical composition of greater amberjack larvae and the references are focused before weaning of larvae (Hamasaki et al., 2009; Matsunari et al., 2013; Mesa-Rodriguez et al., 2016), juveniles with larger size (Badalameti et al., 1995; Jover et al.,

1999; Skaramuca et al., 2001) and greater amberjack adults (Andaloro and Pipitore, 1997; Haouas et al., 2010; Rodriguez-Barreto et al., 2012; Saito, 2012; Rodriguez-Barreto et al., 2017).

One of the bottleneck of *S. dumerili* culture is the appropriate feed and the chemical composition of larvae. A very similar to our experiment was done by Yamamoto et al. (2008). Larvae of treatment W1 and W2 (as mentioned above, they are similar) showed higher values of LA, LNA and ARA than cultured larvae with formulated diet, and that higher than wild larvae analysed by them. However, EPA was similar in W1, W2 and wild larvae and they presented slight decrease than cultured larvae. About DHA, wild larvae had higher values than Yamamoto's cultured larvae and these higher levels than W1 and W2. In spite of that, the cultured larvae's fatty acids percentage of both studies can be considered similar. Fish survival is affected by multiple factors, the "internal" factors (physic and chemical factors) and "external" factors (genetical, etiological, biological and nutritional factors), where the most important survival factor is the nutrition of larvae (Hempel, 1979; Civera-Cerecedo et al., 2004). So, it seems that diet, at these stages, do not modify the composition of these fatty acids very much, however it is very important for survival. To reach higher survival in early stage, essential to large scale production of fishes, is the other bottleneck aspect. The first experiment lasted 5 days because two tanks of W2 reached less than 25% of survival. Survival was the only variable which showed significant differences. In the first experiment of this study the survival of *G. insensibilis* treatment (61.7%) was the double of Formulated A treatment (35%) while the study of Yamamoto et al. (2008) obtained a survival between 1.6 and 11.8 %, significantly lower than W1. If the treatments are compared with Initial larvae, W1 and W2 had lower TAG, and MAG+TAG, this could be an indicative that the larvae need more energetic resource, or the excess of TAG in the food (*Artemia*) of initial larvae (see table 1). In conclusion, W1 had a similar biochemical profile of W2 and other studies, but with a significant higher survival rate.

About the juvenile experiment, it is remarkable that larvae feed with *G. insensibilis* changed completely their chemical composition in 12 days (figure 5), although larvae had been feed with Formulated A and B until 44 dah in mesocosms tank and they had the handicap to adapt to the new food. However, larvae of J2 had similar chemical composition than Initial larvae (figure 5). Furthermore, from the second day, the intake

behaviour was different in both as shown by videos 1 and 2. Both videos (videos 1 and 2) showed one fish suffering dominance by the rest and the aggressive feed behaviour of J2 larvae (video 1). The higher floatability of lyophilizate *G. insensibilis* than formulated diets allowed for greater accessibility to food of non-dominant fishes. This behaviour of diet in the water surface end in so much lower standard deviation of biometric measure and chemical profiles in the juveniles of J1 than J2. This lower standard deviation was present in both between tanks (Tables 5, 6 and 7) and between larvae inside tanks (unpublished data).

Regarding fatty acids, J1 had slighter elevated values of PUFAs, where the essential fatty acids ARA and DHA marked the higher differences. EPA was higher in J2, and ARA and DHA were higher in J1. According to several studies compared wild stocks with cultured stocks of Seriola spp. the formulation of diet should be rich in phospholipid, ARA and DHA (Rodriguez-Barreto et al. 2012; O'Neil et al. 2015, Zupa et al. 2017). Elevate values of ARA is beneficial to many marine species but specially to greater amberjack (*Seriola dumerili*) (Civera-Cerecedo et al. 2004) and California yellowtail (*Seriola dorsalis*) (Rombenson et al., 2016) due to its function of production, reproduction and physiological condition (Bell and Sargent, 2003). It knows the key role of DHA in neural, visual and reproductive tissues (Mourente and Tocher, 1991; Sargent et al., 1999; Masuda, 2003; Glencross, 2009) in fishes. Moreover, the level of DHA in rich is related with the capacity of created schooling behaviour in *S. quinqueradiata* (Ishizaki et al. 2011), that lend another advantage to the treatment with *G. insensibilis* specially from point of view a large-scale aquaculture.

The indices n3/n6, DHA/EPA and ARA/EPA indicate the wellness status of fishes (Estévez 1996). The proportion n3/n6 was significantly higher in J2, but DHA/EPA and ARA/EPA better in J1. Numerous reports have suggested the dietary DHA/EPA ratio is critically important in marine fish nutrition (Sargent et al., 1999; Harel et al., 2002; Ding et al., 2009). In this experiment, the percentage of n3-LCPUFA is similar between treatment but the index DHA/EPA is higher than 3:1 in J1, and ARA/EPA was 1:1, five time higher than J2. Rodriguez-Barreto et al. (2012) and Zupa et al. (2017) analysed adult cultured females feed with formulated diet and wild females' adults where J1 present equivalent results than the wild fishes. Cultured greater amberjack with formulated diet had higher

values of LA and lower ARA, DHA/EPA and ARA/EPA than wilds or J1. So, it is possible that *Gammarus insensibilis* could supply the deficiency of formulated diets.

In connection with the chemical profile, J2 had significantly higher TAG values than J1. Probably, the elevated value is due to the 42% of TAG from Formulated B (table 1). And, J1 had significantly higher SM and sterols than J2 even being the percentage of those lipid classes lower in *G. insensibilis* (table 1). Sphingomyelin is higher in saltwater then fresh water, especially in gill tissues. Its function in gill are osmoregulation and active NaCl transport. In addition, SM catabolism liberates ceramide and/or free sphingosine, potential second messengers play an important role in controlling cell growth, differentiation and oncogenesis (El Babili et al., 1996). The elevated level of sphingomyelin and cholesterol are positively correlated with the brain size in fishes, furthermore, the biometry measures described that treatment (J1) had an index width eye/ size body significantly higher (EL/FL) (Table 5). So, J1 show a larger relative volume of whole brain. We have two possible reasons. First, the large relative volume is because the brain and eye have a constant growth, so, the smaller size of *S. dumerili* (feed with *G. insensibilis*) did the relation between them is elevated. And secondly, Ishizaki et al. (2001) showed an elevated relative volume of whole brain and tectum opticus in *S. quinqueradiata* fed with DHA-rich diet, so the elevated assimilation of DHA from dry-frozen *G. insensibilis* of the juveniles of greater amberjack could cause an increase of brain and eye.

Our results show that the use of *G. insensibilis* supply a better chemical composition in early stages of *S. dumerili* than currently used dry pellet. In addition, this amphipod could be used for others marine finfish species reared in aquaculture. Several species need a similar chemical composition with elevate values of essential fatty acids as ARA, DHA and EPA. For example, *Solea senegalensis* larvae had better survival and growth with elevate DHA-level diet (Navarro-Guillen- et al., 2014); *Argyrosomus regius* larvae growth was better with live food enrichment with DHA rich emulsions (Campoverde and Estévez, 2017); higher ARA level had a significant influence on the reproductive physiology of *Gadus morhua* adults female (Norberg et al., 2017), or moderate level of ARA (2.6 % of total fatty acids) increased growth and survival of *Siganus rivalatus* larvae (Nayak et al., 2017). The PUFAs higher values of J1 could be due to

the elevated PC and PE of them in comparison with Formulated B, since PUFAs from phospholipids can be used directly while it is not possible from triacylglycerides's PUFAs (Castell et al. 1994, Izquierdo et al. 2000).

In conclusion, J2 treatment presented a clear advantage, it had a better growth. In contrast, J1 presented a better nutritional composition rich in the essential fatty acids (ARA and DHA); a very similar appearance with wild greater amberjacks: juveniles with colour yellow-green, verticals dark body bands and a black lateral band from eye to anterior base of dorsal fin, excluding the neck (De la Gándara, 2006) Economically, *G. insensibilis* presented one more advantage: it is cheaper. *G. insensibilis* can be taken of decantation ponds where they fed detritus and *Ulva* sp. directly of natural environment and it is possible also to include them in Integrate Multi-Trophic Aquaculture system. Lyophilizate *G. insensibilis* is keep in the same condition than dry diet, and it can be ground to make a variety of sizes. Additionally, formulated diet generated more fungus in bottom of tank affecting to fish's gill. However, there is still a lack of studies dealing with large scale production to get enough amphipods to properly rare aquaculture fishes with commercial production. Therefore, *G. insensibilis* could be a new, cheaper and better source of food to the first stage of *Seriola dumerili* to get high survival (in weaning process) and a biochemical composition similar of the wild greater amberjack larvae and juveniles.

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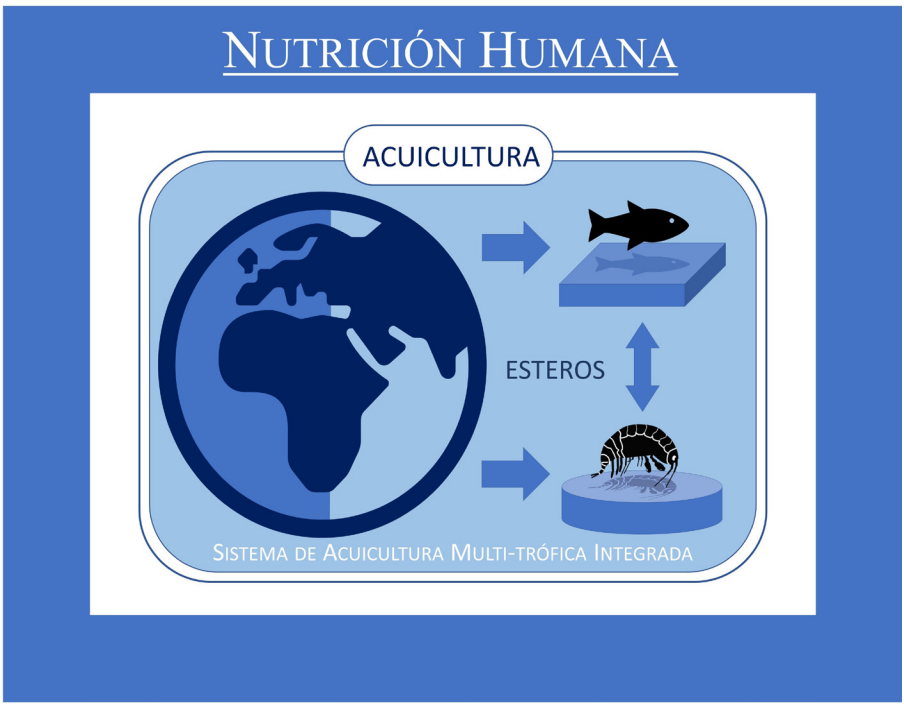
Capítulo
6

Discusión general



1. La importancia de los lípidos

El término "omicas" (p.e. genómica, transcriptómica, proteómica o peptiómica) está siendo cada vez más usado (Bogyo and Rudd, 2013; Simo et al., 2014; Debntah et al., 2010). Desde hace unos 15 años, dentro de la metabolómica, surgió la lipidómica cuyo progreso ha dependido directamente de los avances en las tecnologías analíticas, en particular la cromatografía de gases y la cromatografía líquida (Zárate et al. 2017). Los objetivos de la lipidómica son mapear, determinar y cuantificar todos los lípidos dentro de las células, tejidos u organismos sanos o alterados (Dennis, 2009; Brown, 2012)

Las funciones de los lípidos van desde la provisión de energía a diversas actividades biológicas como regulación de la estructura y función de la membrana, regulación de las vías de señalización intracelular, actividad del factor de transcripción junto con la expresión génica y regulación de la producción de mediadores lipídicos bioactivos. Por consiguiente, los lípidos, especialmente los ácidos grasos, afectan a la salud, el bienestar y al riesgo de desarrollar enfermedades (Calder, 2015). Es por ello, que el estudio de las necesitadas nutricionales y su composición en lípidos de los peces no es necesario solo para el correcto crecimiento de ellos o mayor producción en la acuicultura, sino que también es necesario para dar solución a enfermedades asociadas a los problemas alimenticios de la sociedad actual.

La importancia de los LC-PUFAS, particularmente de los ácidos grasos esenciales DHA, EPA y ARA, es formar parte como componentes principales del 75-88% de los fosfolípidos constituyentes de las membranas lipídicas de las células en los animales, jugando un papel central en casi todas las reacciones fisiológicas y bioquímicas de las células, por consiguiente, en el mantenimiento óptimo de las funciones celulares (Siriwardhana et al., 2012; Hussein, 2013). Zárate et al. (2017) describen la alimentación de los primeros humanos como una dieta con proporción 1:1 de ingesta de LC-PUFAS n6 y n3. Sin embargo, en las dietas occidentales actuales, que se caracterizan por una alta ingesta de grasas saturadas, ácidos grasos n-6, ácidos grasos "trans" perjudiciales y un elevado consumo de azúcares, la proporción ha aumentado a 20: 1 lejos del consumo óptimo de ácidos grasos n-3 saludables. Este desequilibrio favorece la aparición de obesidad, efectos pro-trombóticos y pro-inflamatorio (Simopoulos,

2013; Simopoulos, 2016), dando lugar a dolencias inflamatorias como artritis, cáncer, hipertensión, diabetes, asma, aterosclerosis, Alzheimer, Parkinson, alergias y muchas más (Calder, 2011; Calder, 2013; Fritsche, 2015). Para evitar este desequilibrio muchos de los cuerpos y agencias de salud, como la Organización mundial de la Salud (WHO), Organización de las Naciones Unidas para la Alimentación y Agricultura (FAO) y la Autoridad Europea de Seguridad Alimentaria (EFSA), recomiendan un consumo entre 250 y 1000 mg de EPA+DHA diarios para los adultos para el mantenimiento de la salud y el bienestar (SACN,2004; FAO, 2010; AEFS, 2010, GOED, 2015). El consumo de 2 o 3 raciones semanales de pescado (especialmente los grasos) serían suficientes para suplir estas recomendaciones.

En el presente estudio se refleja que los anfípodos presentan unos niveles nutricionales muy adecuados para ser utilizados como fuente de lípidos saludables. De hecho muchas especies de peces y moluscos de interés comercial se alimentan de anfípodos en el medio natural, como se refleja en el capítulo 2.

2. Estado Actual de la Acuicultura

Los recursos pesqueros extractivos se consideran en declive desde el 2015 (FAO, 2016), considerándose el 58.1% de los stocks marinos sobreexplotados, siendo imposible mantener el consumo medio de pescado que ascendió a 20 kg por persona y año alcanzado en dicha fecha. Por ello la FAO apuesta por una acuicultura sostenible y el uso de alimentos medioambientalmente "friendlies" que no comprometa los beneficios nutricionales del consumo de productos marinos (FAO, 2016), como puede ser sistemas de recirculación de agua (Martins et al., 2010) o reciclaje de los productos de desechos en los sistemas IMTA (Chopin y Robinson, 2004; Chopin et al., 2004,2012).

A diferencia de la ganadería y agricultura terrestre, especializada en un reducido número de especies muy domesticadas de animales y plantas, en el año 2015 se estaban criando en el mundo unas 400 especies acuáticas diferentes, entre peces, moluscos, crustáceos, algas y otros (APROMAR, 2017), de las cuales el 53.9% son especies de aguas marinas (FAO, 2016). De todas las especies acuáticas en producción 305 especies, entre plantas

y animales, son producidos en cantidades significativas (más de 100 toneladas anuales) (APROMAR, 2017). La continua diversificación de la acuicultura conlleva una investigación constante en la nutrición específica de cada especie y la búsqueda de nuevos recursos tróficos.

3. Los esteros como parte de la Acuicultura Multitrófica Integrada

La acuicultura multitrófica integrada reduce el impacto medioambiental generado en las granjas de acuicultura tanto por el pienso no comido como por las heces de los peces. Al introducir especies que lo consumen como invertebrados marinos o algas (Alexander et al., 2015) se convierten en productos con un valor económico añadido (Chopin y Robinson, 2004) aumentando la producción de las granjas. Por ello el sistema IMTA está en el punto de interés de las investigaciones por su doble función de eliminar desechos y aumentar la productividad (Soto, 2009; Troell et al., 2009; Chopin et al., 2012), además de aumentar la diversidad de productos marinos.

En el contexto de esta tesis doctoral, estas tres funciones ocurren de forma natural en los esteros y marismas, lugares con una alta producción primaria donde se practica la acuicultura de forma extensiva o semintensiva (Arias y Drake, 1994), y se generan altas densidades de algas como la *Ulva*, la cual también asume el rol de biorremediador y puede ser usada como alimento (Shpigel et al.,2017) y sustrato para invertebrados; además de albergar altas concentraciones de invertebrados, como los anfípodos, que adquieren perfiles nutricionales óptimos para ser usado como suplemento alimenticio en las plantas de cultivo cercanas, o generar un nuevo tipo de alimento para ser usado en las primeras etapas de vida de los peces cultivados o para peces ornamentales en el mundo de la acuariofilia. El Capítulo 2 muestra la composición nutricional de los anfípodos recogidos en los esteros de la Bahía de Cádiz, presentando un elevado contenido de proteínas (40%) y de lípidos total (10-12%), con valores mayores al 3% de fosfolípidos y cercanos o superiores al 1% de LC-PUFAS, requisitos mínimos para ser usado como alimento para larvas de peces (NRC, 2011), teniendo en cuenta que los anfípodos se alimentan del detritus y algas generado por la actividad acuicultura de la zona, los anfípodos pueden ser usado como un engranaje más de un sistema IMTA. Trabajos recientes

también defienden la posibilidad de recolectar anfípodos alrededor de las jaulas en mar abierto de acuicultura, adquiriendo densidades altas, y un valor nutricional con altos valores de proteínas y lípidos, aunque no los suficientes para servir de alimento a las etapas tempranas de peces de cultivo (Fernández-González et al., 2018).

4. Aplicabilidad de los anfípodos

La definición de los requerimientos de ácidos grasos esenciales de larvas de peces marinos es complicada debido a su pequeño tamaño y generalmente tubo digestivo poco desarrollado, lo que dificulta la elaboración de microdietas o uso de presas vivas (Izquierdo et al., 2000; Cahu and Zambonino-Infante,2001; Koven et al., 2001a; Robin and Vicent, 2003; Kvale et al., 2006; Conceição et al., 2007, 2010). Actualmente los recursos vivos usados son *Artemia*, rotíferos, copépodos y misidáceos (e.g. Domingues et al., 2001; Dhont and Van Stappen, 2003; Lubzens and Zmora, 2003; Støttrup, 2006). Los copépodos tienen un valor nutricional superior a las alternativas actuales, pero presentan problemas técnicos para ser cultivados lo que conlleva un coste elevado (Støttrup, 2000). En contraste, rotíferos y *Artemia*s tiene un valor nutricional muy pobre y tienen que ser enriquecidos con LC-PUFAS, especialmente DHA (Conceinção et al., 2000), donde, además, aparecen problemas asociados con la oxidación durante el enriquecimiento, como la retroconversión y oxidación de ácidos grasos debido al metabolismo endógeno de los LC-PUFAS del organismo (Sargent et al. 1997 y 1999b).

En cuanto a los anfípodos, en la presente tesis se ha demostrado el alto valor nutricional de estos y su posibilidad de ser extraídos de los esteros asociados a plantas de cultivos de acuicultura, funcionando como biofiltradores y eliminando sustancias de desechos procedente de la acuicultura reduciendo el impacto medioambiental (Capítulo 4.1, Capitulo 4.2). Los capítulos 4.1 y 4.2 demuestran que la viabilidad de usar detritus procedentes de una planta de acuicultura (constituidos principalmente por restos de heces de peces cultivados así como pienso sobrante) como alimentos para anfípodos, proporcionándoles valores nutricionales aceptables. Los caprélidos adultos alimentados con detritus (Capítulo 4.1) presentaron una supervivencia mayor en comparación con los alimentados con *Artemia*s y fitoplancton, además presentaron los niveles

más alto de DHA, LA y OA con un sumatorio de los PUFA casi del 50% del total de ácidos grasos. En el cultivo de *Gammarus insensibilis* (Capítulo 4.2), donde se añadió *Ulva* como dieta, los individuos alimentados con detritus y *Ulva* presentaron valores altos de ARA, EPA y DHA, cercanos o superiores al 1% del peso seco por cada ácido graso esencial, superando al 3% de fosfolípidos recomendado, ambas proporciones esenciales para un buen crecimiento en las primeras etapas de peces (NRC, 2011). Acorde con ello, diversos estudios han demostrado el cambio en la composición bioquímica de los anfípodos que se alimentan de detritus (González-Silvera et al., 2015) aumentando sus valores de PUFAS debido al alto contenido de estos en los piensos usados para los peces.

En cuanto la composición bioquímica general de los anfípodos, sus perfiles lipídicos y de aminoácidos, el contenido de micro y macroelementos (Baeza-Rojano et al., 2014; Fernández-González et al, 2018; Capítulo 3), y formar parte de la dieta habitual de las etapas tempranas de los peces (Capítulo 2), convierten a los anfípodos en un nuevo recurso potencialmente adecuado para el uso en la acuicultura. Estudios previos obtuvieron resultados prometedores usando anfípodos como alimento vivo en peces (Parsons et al., 1985; Moren et al., 2006; Baeza-Rojano et al., 2013a) y cefalópodos (Baeza-Rojano et al., 2010,2013b; González et al., 2011). Además, tener un ciclo de vida y tasa de reposición rápidos (Baeza-Rojano et al., 2013a), hacen posible que se puedan cultivar a gran escala y no afectar negativamente a las poblaciones (en caso de ser recolectados directamente del medio) de anfípodos.

5. Necesidades fisiológicas de las primeras etapas de los peces

Es comúnmente aceptado que las larvas necesitan una alta proporción de proteínas y lípidos, así como una baja concentración de hidratos de carbono y cenizas. Recientemente se está prestando más atención a los ácidos grasos esenciales debido a sus implicaciones en el desarrollo de las larvas. Aunque realmente los únicos ácidos grasos que no producen los vertebrados, incluidos los peces, son LA y LNA (Sprecher et al., 1995; Nakamura and Nara, 2004; NRC, 2011), la incapacidad de producir ácidos grasos más largos e insaturados, hace que sea necesaria la incorporación de ellos mediante la ingesta (Turchini et al., 2009).

En peces, los PUFAS son necesarios para la correcta estructural y funcionabilidad de las membranas celulares, como elementos integrantes de los fosfolípidos fundamentales en la bicapa lipídica (Gylfason et al., 2010). DHA en particular tiene un rol funcional y estructural importante en todas las membranas, con especial relevancia en las membranas neuronales (Feller, 2008; Wasall and Stillwell,2008) importante para el correcto desarrollo de los tejidos neuronales en larvas de peces pudiendo afectar incluso comportamientos etológicos como la agrupación de larvas en *S. quinqueradiata* (Ishizaki et al. 2011). Los ácidos grasos ARA y EPA son precursores de eicosanoides con un amplio efecto fisiológico pues en casi todos los tejidos se producen eicosanoides: coagulación de la sangre, respuesta inmune y antinflamatoria, tono cardiovascular, funciones renales y neuronales, y reproducción (Schmitz and Ecker, 2008). Particularmente, en peces planos se presta atención a los niveles de ARA en la dieta por su relevancia en el proceso de pigmentación y migración del ojo (Lund et al., 2007,2008).

Aunque no se ha analizado la concentración de los diferentes ácidos grasos en cada una de las clases lipídicas, los altos niveles de PC de los anfípodos estudiados (Capítulo 3) hacen que la asimilación de los PUFAS en los peces sea más efectiva, ya que los ácidos grasos esenciales contenidos en fosfolípidos tienen una mayor digestibilidad que los contenidos en triglicéridos (Sargent et al, 1997; Tocher, 2008), debido a que las etapas tempranas de los peces, por su limitada síntesis de lipoproteínas, no tienen la capacidad de transportar y asimilar lípidos desde el intestino (NRC, 2011).

6. Ensayo del uso de anfípodos en acuicultura: *Seriola dumerili* como modelo de estudio

Se eligió *Seriola dumerili* como especie objetivo por ser una especie epipelágica (familia Carangidae) con área de distribución global en zonas templadas (Andaloro y Pipitone, 1997; Cummings et al., 1999; Thompson et al., 1999) con gran interés al sector de la acuicultura debido a su excelente calidad (Nakada, 2000), enmarcada dentro del proyecto DIVERSIFY (Mylonas et al., 2016) para la diversificación de cultivo de nuevas especies en acuicultura. También tiene una gran aceptación en el mercado con precios (17 euros/kg) muy superiores a los de dorada

(5,78 euros/kg) y lubina (5,67euros/kg) (APROMAR, 2017), principales especies de acuicultura vendidas en España, por lo que sería una especie económicamente muy rentable de cultivar.

Los resultados obtenidos en el cultivo de *Seriola* con el anfípodo *Gammarus insensibilis* en la época de destete (Capítulo 5) mostraron que las larvas alcanzan altos valores de PUFAs (LA, LNA, ARA, EPA y DHA), con un incremento en la supervivencia y reducción de problemas asociados a la contaminación de los tanques de cultivos. Respeto a los resultados obtenidos en el cultivo de juveniles, el crecimiento fue menor en aquellos que fueron alimentados con gammáridos liofilizados, pero obteniéndose un perfil nutricional mejor que aquellos alimentados con piensos comerciales sin efectos en la supervivencia. Además, presentaron una coloración similar a la de los juveniles salvajes y como muestran los videos anexos a esta tesis doctoral un comportamiento menos agresivo hacia la comida.

Teniendo en cuenta estos resultados podríamos decir que la aplicación en otros peces, como peces planos, seria idónea en la época de metamorfosis (Lund et al., 2007,2008) por los valores altos de EPA y ARA que han aportado a *S. dumerili*. También es posible que pudieran ser usados en adultos de peces ornamentales, ya que el pequeño tamaño en fase adulta permitiría poder alimentarlos directamente con los anfípodos, aportándoles una mejora en la coloración y una mejor capacidad reproductora, pues en el estudio observamos que los peces adquieren altos valores de ARA, precursor de los eicosanoides biológicamente activos, como las prostaglandinas y los leucotrienos, que están implicados en muchos aspectos de la reproducción de los peces (Norambuena et al., 2013; Asil et al., 2017; Norberg et al., 2017; Peng et al., 2017; Xu et al., 2017).

7. Proyección de futuro

Las necesidades actuales para que la utilización de los anfípodos como recurso trófico sea posible son tecnológicas no nutricionales. Es necesario la investigación de técnicas de extracción del medio que permita la obtención de grandes cantidades para poder ser usado a escala industrial, y/o incrementar la investigación en el cultivo en condiciones controladas de laboratorio asociados a plantas de acuicultura para poder

tener un suministro constante de detritus. Desarrollar el cultivo bajo condiciones controladas aporta ventajas como controlar la calidad del agua, así como las fluctuaciones naturales de salinidad y temperatura, pero sobre todo la posibilidad de poder seleccionar diferentes tallas (juveniles o adultos) que se adapten mejor a la necesidad de la especie objetivo que queramos alimentar.

Solo en España se utilizaron 121,000 toneladas de pienso en la acuicultura. Aunque normalmente estos piensos se utilizan para etapas adultas vemos que existe un nicho de mercado en las etapas tempranas que los anfípodos podrían cubrir, además del mercado de la acuariofilia (Olivotto et al., 2018) cada vez más emergente donde la coloración llamativa es necesaria.

En síntesis, la adecuada calidad nutricional de gammarideos y caprélidos así como su viable posibilidad de cultivo, les hace potencialmente interesantes para numerosas aplicaciones en acuicultura y acuariofilia. Es necesario explorar el uso industrial potencial de estos anfípodos como complemento alimenticio en acuariofilia para especies ornamentales, así como su uso como integrantes de piensos comerciales para cría de juveniles o como complemento para adultos en especies de interés comercial en acuicultura tanto en cultivos intensivos como extensivos. En este sentido esta tesis muestra que es interesante promover su uso y explorar futuras iniciativas en el contexto de la Acuicultura Multitrófica Integrada, donde los anfípodos parecen ser un recurso muy adecuado, pues puede combinarse su uso como biofiltros y una producción a gran escala con un coste muy reducido a partir de los desechos de cultivos asociados

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Capítulo
7

Conclusiones generales

Conclusiones generales

- **Las especies de interés acuícola en Andalucía consumen frecuentemente anfípodos** en el medio natural, aunque el consumo puede variar a lo largo del desarrollo y estacionalmente.
- **Los esteros del Parque Natural de Cádiz son una fuente sostenible de anfípodos**, los cuales crecen de forma natural en algas y sedimento, además de ser fácilmente recolectables.
- **Los anfípodos** de los esteros de la Bahía de Cádiz, así como de otros ambientes naturales y artificiales, **presentan una composición nutricional optima, con un porcentaje alto de proteínas, nivel de lípidos superiores a los mínimos requeridos**, especialmente altos en fosfolípidos y ácidos grasos esenciales, aunque los niveles de quitina y ceniza pueden afectar negativamente a la digestibilidad.
- ***Gammarus insensibilis*** **es la especie más indicada para ser usada como recurso alternativo** porque muestra: niveles adecuados de fosfolítpidos y ácidos grasos poliinsaturados de cada larga, niveles altos de aminoácidos y de calcio, altas densidades en el medio natural y tamaño corporal grande.
- **El detritus es una dieta adecuada para poder cultivar anfípodos** siendo una fuente de DPA y DHA (aunque pobre en EPA) en caprélidos, y de ARA, EPA y DHA en gammarideos.
- ***Ulva*** **sp.**, además de ser sustrato natural de *Gammarus insensibilis* en los esteros de la Bahía de Cádiz, como alimento le **aporta valores altos de ácidos grasos poliinsaturados de cadena larga**.
- **El detritus y la *Ulva* son alimentos que pueden ser recolectados fácilmente del medio natural y de instalaciones de acuicultura, y por tanto pueden resultar un recurso muy económico**. Ambos alimentos son considerados como desechos en cultivos de peces

y podrían incorporarse al sistema de Acuicultura Multitrófica Integrada. En estos cultivos los anfípodos pueden alimentarse de los desechos, y estos a su vez servir de alimento a las primeras etapas de desarrollo de peces o moluscos de interés comercial

- ***G. insensibilis* puede sustituir por completo a los piensos usados actualmente en *Serioa dumerili*.** El liofilizado de este anfípodo usado como pienso aumenta significativamente la supervivencia en la etapa de destete y, otorgan una mejor calidad nutritiva y valores más altos de ácidos grasos poliinsaturados, especialmente DHA y ARA, que los piensos comerciales en la etapa juvenil de *S. dumerili.* También proporciona una coloración similar a la encontrada en la naturaleza, además de reducir la competencia por el alimento en los juveniles.