UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivo.

M. Besay Ramírez Bordón

Doctorado en Acuicultura: Producción controlada de animales acuáticos.

Co-directores: **Dr. Ricardo Haroun Tabraue (Grupo de Investigación en Biodiversidad y Conservación - BIOCON) – I.U. ECOAQUA**

y **Dr. Daniel Montero Vítores (Grupo de Investigación en Acuicultura - GIA) – I.U. ECOAQUA**

ÍNDICE GENERAL

INDICE DE FIGURAS

ÍNDICE DE TABLAS

LISTA DE ACRÓNIMOS

ANOVA: Analysis Normal Variance

ALA: Alpha-linolenic acid

ARA: Arachidonic acid

CI: Fulton's K condition index

DHA: Docosahexaenoic acid

DNA: Deoxyribonucleic acid

EPA: Eicosapentaenoic acid

FA: Fatty Acid

FAO: Food and Agriculture Organization of the United Nations

FADs: Fish Aggregating Device

HSI: Hepatosomatic index

ICCM: Canary Institute of Marine Sciences

n-3 HUFA: n-3 series Highly Unsaturated Fatty Acid

LA: Linoleic acid

OA: Oleic acid

nm-MDS: Non metric multidimensional scaling

M.O: Materia Orgánica

VHS: Septicemia Viral Hemorrágica

RNA: Ribonucleic acid

S.L.: Sociedad limitada

PERMANOVA: Permutation Analysis of Normal Variance

PA: Palmitic Acid

PCA: Principal components analysis

PUFA: Poli Unsaturated Fatty Acid

SIMPER: Similarity percentages

SD: Desviación estándar

M: Measure

TL: Total Length

AGRADECIMIENTOS

Me gustaría dedicar esta Tesis a mi familia, en agradecimiento al apoyo que siempre me han dado. A mis padres por haberme brindado los estudios y formación necesarios, y transmitirme el esfuerzo y tesón como directrices para la vida. A María por levantarme en momentos de bajón y hacerme ver las cosas más claras después de una charla suya. A mi hijo Besay que fue, es y será la fuerza que me empuja y saca lo mejor de mí. A mis hermanos Jonás y Héctor con los que he compartido una vida, hogar y vivencias.

A mi director el Dr. Ricardo Haroun que vio algo en aquel chico que quería cultivar lapas. Confiaste en mí dándome trabajos desde el principio y abriéndome un camino que de otra manera nunca habría existido para mí, guiando mis pasos desde el principio hasta el final de esta aventura. Te debo el poder haber trabajado y dedicado estos años a la ciencia, un gran sueño. GRACIAS

A mi codirector el Dr. Daniel Montero, mi corrector más duro. Sus consejos y críticas constructivas me hicieron aprender y mejorar con rapidez. No solo se conformó con enseñarme, corregirme papers, etc… además fue el responsable de que retomara la tesis en el tramo final, en un momento que quise abandonar, sacando para ello un tiempo que no tenía. En estos últimos meses encontré más a un amigo que un codirector en las reuniones de la tesis. GRACIAS

A el Dr. Fernando Tuya, un ídolo de masas. Un amigo, siempre dispuesto a echar una mano desinteresada. El profesor que todos deberíamos tener. Me hizo comprender de manera sencilla la estadística, consiguiendo además que me gustase. GRACIAS por tus correcciones de los papers, tu ayuda estadística y apoyo personal.

A todas las personas que me han ayudado en muestreos y/o logística: Mis amigos Juan Luis Sánchez, Airam Pérez, Roberto Betancor, Lionel Artiles, Juan Estefanel, Antonio Mesa. Mis hermanos Héctor y Jonás. A Rafa Guirao y su equipo: Soto, Lucas, etc… A Finn Økland

y Uglem Ingebrigt del Norwegian Institute of Nature Research. A David Jiménez y Leonor Ortega compañeros de campañas.

RESUMEN

La acuicultura marina es una industria en auge que implica cierto impacto en el medio. Esta tesis está centrada en el estudio de los escapes de peces de jaulas de cultivos marinos. Se diseñaron cuatro experiencias con el fin de: 1) estudiar la entrada de piensos en la ictiofauna asociada; 2) determinar la validez de bio-indicadores como el cambio de perfil lipídico o la morfología externa en peces escapados; y 3) evaluar su comportamiento tras un escape. El primer experimento demostró que las bogas (*Boops boops*) asociadas a jaulas de cultivo un perfil lipídico distinto con respecto a congéneres salvajes, que estas diferencias desaparecen a escasa distancia de las jaulas y que puede contaminarse por otras entradas. El segundo mostró que las lubinas escapadas van cambiando su perfil lipídico, distanciándose de los individuos cultivados así como su capacidad para sobrevivir en el medio litoral canario. A nivel morfométrico, se encontraron escasas diferencias entre los peces cultivados y los escapados. El cuarto experimento demostró que los escapados de lubina y corvina permanecen alrededor de las jaulas una semana, observándose además, una disminución exponencial del número de individuos durante esta primera semana. Con estos resultados podemos concluir que las jaulas oceánicas afectan al perfil lipídico de la ictiofauna asociada por aumento de ácidos grasos de origen terrestre, aunque este efecto se ve enmascarado por otras fuentes de entrada de lípidos en la zona costera. En las lubinas escapadas, el perfil lipídico no cambia con la distancia a las jaulas, sino con el tiempo transcurrido tras el escape. El perfil lipídico no es un bioindicador de peces escapados, en cambio, la morfometría si podría usarse con ese fin. Un bajo porcentaje de lubinas escapadas tienen capacidad de sobrevivir en el litoral canario y sus poblaciones acaban desapareciendo con el tiempo.

Palabras claves: Acuicultura, Escapes, Biomarcadores, Ácidos Grasos, Morfometría, Telemetría, Adaptación, Comportamiento, Lubina, Corvina.

ABSTRACT

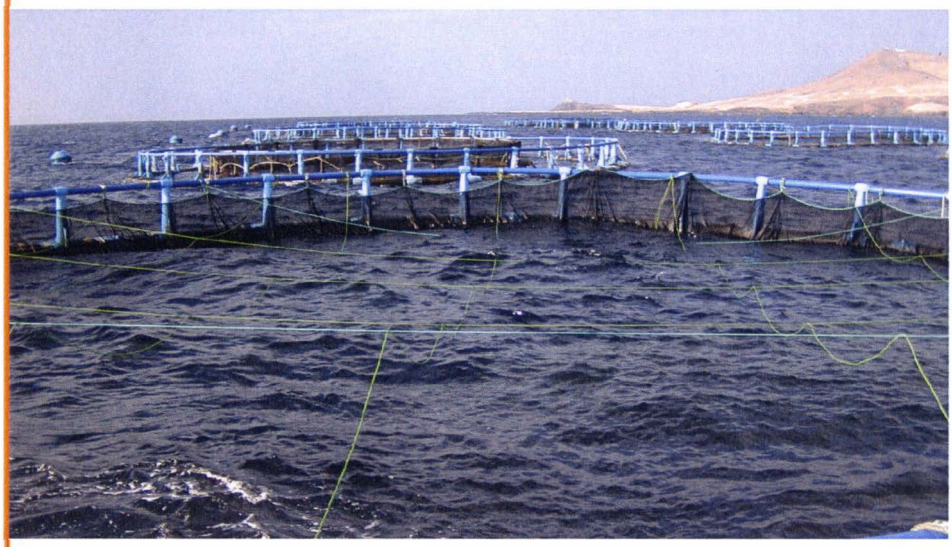
The production of marine organisms is an increasing industry, which implies certain impacts in the environment. Throughout this thesis we have focused on the study of fish escapees from off-shore fish-farms. Four experiments were designed in order: 1) to study the aquafeeds effects in the associated ichthyofauna; 2) the potential use of lipid profile and morphology as biomarkers for escapees; and 3) to evaluate the fish behaviour after an escape event. The first experiment showed that cages associated fish (bogue – *Boops boops*) changed their lipid profile respect to those non-associated wild conspecifics, however that difference disappeared in a short distance away from the aquaculture cages and can be contaminated by other coastal lipid sources. The second experiment showed that the lipid profile of escaped sea bass was changing as they were adapting to environment, differing from those cultured; also, we observed some degree of environmental adaptation in the Canarian coast. In third experiment we found few morphometric differences between cultivated and escaped sea bass. The fourth experiment indicated that sea bass and meagre remain approximately one week around cages after an escape event, with an exponential decrease of individuals throughout that week. We concluded that the aquafeeds affect the lipid profile of associated fish, increasing fatty acids typically associated to terrestrial oils, nevertheless, others input sources of these terrestrial oils were identified on the coast. Escaped sea bass do not change lipid profile with the distance to aquaculture cages, it changes with time after escape. The lipid profile is not a bioindicator of escaped fish, however the morphometry could be used so. A low percentage of escape sea bass survived survive capacity in the Canarian coast, but populations tends to disappear along time.

Keywords: Aquaculture, Escapees, Biomarkers, Fatty Acids, Morphometry, Telemetry, Establishment, Behaviour, Sea Bass, Meagre.

1

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

INTRODUCCIÓN GENERAL



1.1. Situación actual de la acuicultura

La acuicultura puede definirse como el cultivo de organismos acuáticos mediante técnicas encaminadas a hacer más eficiente su producción. Aunque tiene una historia de 4.000 años, ha sido a partir de los últimos 50 años cuando se ha convertido en una actividad socioeconómica relevante, dando empleo a más de 12 millones de personas en el mundo (APROMAR 2012). Abarca diversos sistemas de cultivo en aguas continentales, costeras y marítimas, utilizando y produciendo una amplia variedad de especies. Los sistemas de cultivo varían desde algunos muy sencillos, como los estanques familiares en varios países asiáticos, hasta otros de alta tecnología como los sistemas de circuito cerrados de producción intensiva (FAO 2014a). En general, el cultivo de especies animales conllevan una serie de fases: mantenimiento de reproductores, hatchery (huevos y larvas), nursery (post-larvas) y engorde de juveniles. En el caso de la fase de engorde, la tendencia mayoritaria es que por razones económicas, ésta tenga lugar en jaulas marinas, circulares o rectangulares, fondeadas a una cierta distancia de la costa Cada una de estas fases se ha ido desarrollando y especializando, e implica diferentes sistemas y tecnologías de cultivo. En este sentido y por lo que respecta a la presente tesis doctoral, cuando hablemos del cultivo, nos referiremos a la fase de engorde de juveniles de lubina y corvina en jaulas de cultivo marinas, por ser la fase del cultivo que entraña un riesgo real de escapes.

Ante la complicada realidad que estamos viviendo en este comienzo de siglo (problemas financieros, cambios climáticos y escasez de recursos), propiciada por la superpoblación del planeta, la humanidad debe afrontar el reto que se nos presenta pensando como especie y no como las distintas naciones que nos separan. La FAO apunta que uno de los principales problemas del siglo XXI será la producción de alimentos para abastecer este incremento de población. Esta producción deberá aumentar en un 70% entre 2010 y 2050

(cuando llegaremos a 9,6 billones de personas). Según la FAO, de la superficie total de la Tierra tan solo es aprovechable para la agricultura y ganadería el 38% (52.129.685 km^2). Teniendo en cuenta que la mayor parte de la superficie del planeta la conforman los océanos y el agotamiento de los recursos terrestres, parece obvio que este aumento de la producción primaria tendrá lugar en los mares y océanos. Además, una de las principales ventajas que presenta la producción marina es que no se necesita emplear agua dulce, cuyo agotamiento es otro de los principales problemas de este siglo. Dentro del sector de producción de alimentos, la acuicultura es la actividad que más rápidamente ha crecido en los últimos años, alcanzando las 97,201,872 t (146,229,575,654 €) y convirtiéndose en el principal productor de alimentos de origen acuático (50.87 %), de un total de 191,063,087 t en 2013 (FAO 2015b). Dentro de esta producción acuícola se incluye el cultivo de especies de peces de origen marino (1,788,164 t), que también ha experimentando un importante crecimiento durante los últimos años (FAO 2015b).

1.2 Características biológicas generales

1.2.1 Lubina

La lubina (*Dicentrarchus labrax* Linnaeus 1758) es un pez teleósteo que pertenece a la clase Actinopterigia, orden Perciformes, familia Moronidae, género *Dicentrarchus* y especie *D. labrax* (Fig. 1.1*)*. Este pez se distribuye desde Noruega a Cabo Blanco, incluyendo el Mediterráneo y el Mar Negro (Moretti et al., 1999) (Fig. 1.2). Su rango natural de distribución incluye las Islas Canarias orientales: Fuerteventura y Lanzarote (Brito 1991), aunque en la actualidad la podemos encontrar en otras islas del archipiélago Canario (Gran Canaria, Tenerife y La Palma) (Fig. 3.1) como consecuencia de escapes de acuicultura (Carrillo and Castillo, 2001; Toledo et al., 2009; Toledo et al., 2012) (Fig. 1.3). En su hábitat natural se encuentra en las aguas costeras, aunque también están presentes a mayor profundidad, llegando a los 100 m. Posee un amplio rango de distribución observándose en estuarios,

canales, lagunas e incluso ríos. En Canarias, Toledo et al. (2009) observaron, en lubinas escapadas, una preferencia de costas con sustratos formados por pequeñas rocas o cantos.

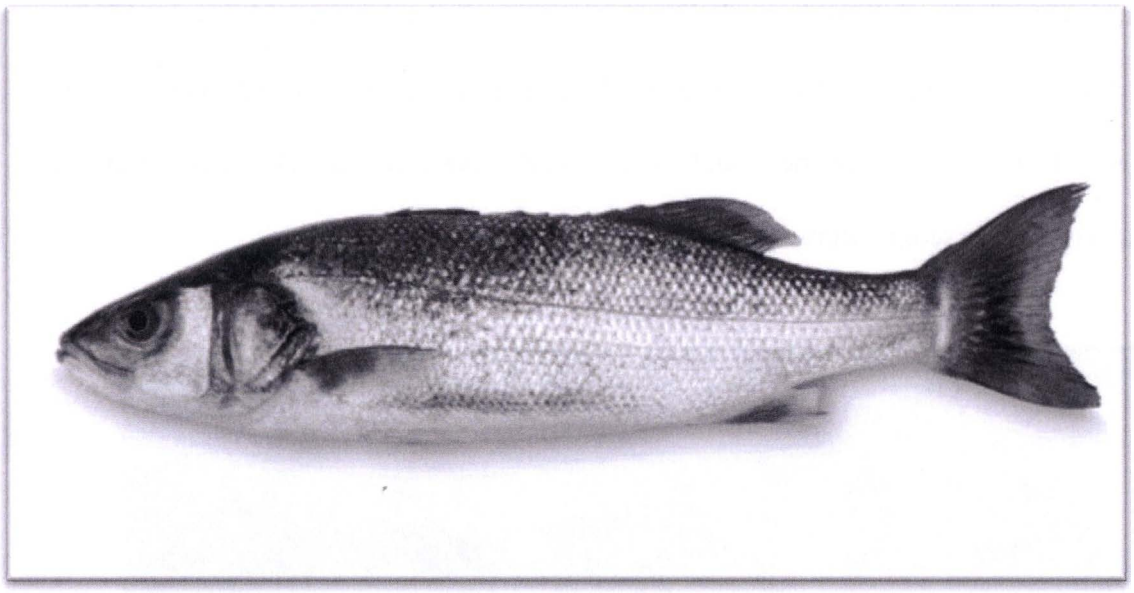


Figura 1.1. Lubina (*Dicentrarchus labrax*) adulta.

La lubina es una especie gonocórica (Arias A., 1980; González A., 2003) de elevada fecundidad (150.000 – 200.000 huevos por kg/hembra). La proporción de sexos es prácticamente 1:1 entre machos y hembras durante los tres primeros años de vida, llegando a 4:1 a favor de las hembras en el 4º y 5º año (Arias A., 1980). En cautividad suele ser 3:1, siempre favorable a machos (Carrillo et al., 1995; Colombo et al., 1997). La reproducción tiene lugar entre los meses de diciembre a marzo (Do Chi and Hoai Thong, 1971; Barnabé G., 1976; Arias A., 1980; González A., 2003). La talla de primera madurez de esta especie es de 32,3 cm (con un rango 23 -46 cm) (Froese and Pauly 2006). Laffaille et al., (2001) sitúan la época de reproducción entre febrero y abril en la costa Británica. Algunos estudios demuestran un pico reproductivo a finales de julio en el Atlántico. Para que comience la gametogénesis esta especie requiere salinidades inferiores al agua de mar (< 35 ‰) y los últimos pasos del desarrollo gonadal una salinidad de 35 ‰. (FAO, 1999). La temperatura

mínima y máxima para que se produzca la gametogénesis es 9-18 º C respectivamente (FAO, 1999) y el rango óptimo para la puesta está entre 13-15 º C (Moretti et al., 1999). La supervivencia de las larvas aumenta considerablemente al disminuir la salinidad. Numerosos autores han encontrado un reclutamiento de la especie en medios estuarinos (Arias A., 1980; Serrano L., 1989; Laffaille et al., 2001). El rango de temperatura óptima de incubación de la puesta va desde 13ºC a 17º (Devauchelle and Coves, 1988; Saka et al., 2001), considerándose este el límite superior de temperatura óptima de incubación.

Figura 1.2. Mapa de la distribución de la lubina (*D. labrax*) en el Atlántico Oriental (Froese and Pauly 2006)

El periodo de larva tiene una duración de 46 días a 16,5ºC (Houde and Zastrow, 1993). La dispersión puede durar 3 meses (huevos y larva pelágica. Además existen otros factores ambientales que afectan a la reproducción de la especie, tales como fotoperiodo, hidrodinámica, predadores, disponibilidad de alimentos, etc.

Es un predador que se alimenta principalmente de crustáceos, moluscos y peces (Tortonese 1986; Laffaille et al., 2001; Leitao et al., 2008; Toledo et al., 2009). Su dieta cambia a lo largo de su ciclo vital, dependiendo del tamaño del individuo (Arias, 1980; Kennedy and Fitzmaurice, 1972). En estadios juveniles se alimentan de crustáceos y pequeños peces (Tortonese, 1986; Laffaille et al., 2001; Leitao et al., 2008; FAO 1999). Los animales de más de 20 cm consumen camarones y cangrejos principalmente (FAO, 1999). Esta especie en el medio natural tiene un crecimiento lento (aproximadamente 4 años para alcanzar 1 kg de peso medio). Puede alcanzar los 100 cm de longitud y los 12 Kg de peso (FAO, 1999). Los machos crecen menos y más lentamente que las hembras (Arias, 1980; Carrillo et al., 1995), además viven menos. Arias (1980) no encontró ningún macho con más de cinco años de edad entre 1013 lubinas muestreadas.

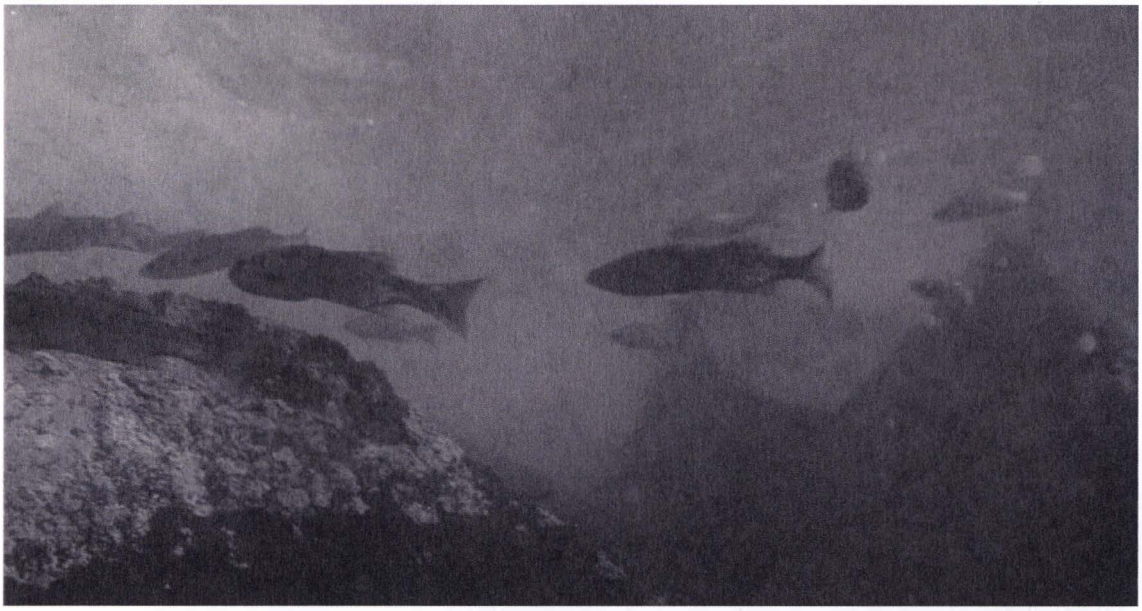


Figura 1.3. Grupo de lubinas escapadas en la escollera de Santa Cruz de la Palma (Santa Cruz de Tenerife, España)

El crecimiento de estos peces es mayor en poblaciones que penetran en lagunas litorales (Arias, 1980). Chervinski (1975) registró un mayor crecimiento en agua dulce frente agua de mar. En la tabla 1.1 se muestran los resultados obtenidos por algunos autores en distintas regiones geográficas.

Tabla 1.1 Talla (mm) por clase de edad (años) en *Dicentrarchus labrax* para diferentes autores en distintas áreas geográficas. 1 Arias, 1980. 2 Gravier, 1961. 3 Labourg and Stequert, 1973. 4 Bernabé 1973; 5 Serrano, 1989. Tabla de Arias (1980), añadiéndose datos de Serrano (1989).

Zona	Edad (años)						
	1	2	3	4	5	6	7
1. Cádiz (España, Atlántico)*	180,9	288,5	378,5	458,8	528,6	598,3	645,0
2. Marruecos (Mediterráneo)	150	220	290	340	400	450	500
3. Arcachon (Francia, Atlántico)*	110	210	260	300	320	350	390
4. Thau (Francia, Mediterráneo)*	174	283	382	486	539	576	621
5. Aveiro (Portugal, Atlántico)*	166	213	-	-	-	-	-

* Estudios realizados en estuarios o lagunas.

1.2.2 Corvina

La corvina es un pez teleósteo que pertenece a la clase Actinopterigia, orden Perciformes, familia Sciaenidae, género *Argyrosomus* y especie *A. regius* (Fig. 1.4).

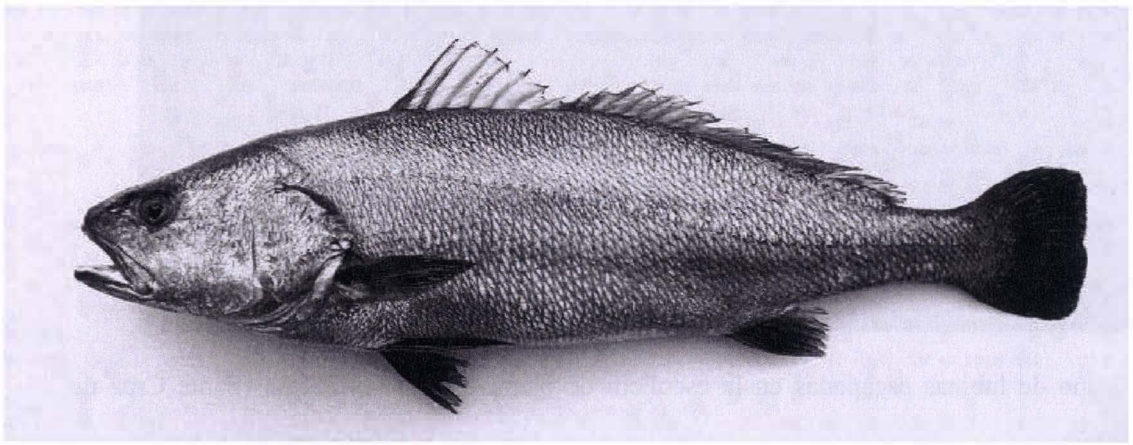


Figura 1.4. Corvina (*Argyrosomus regius*) adulta.

Este pez se distribuye por el Atlántico Oriental desde Francia hasta Senegal, incluyendo el Mediterráneo Occidental (Quéméner 2002). El rango de distribución incluye las Islas Canarias (Dooley et al., 1985; Lloris et al., 1991) (Fig. 1.5). Esta especie está presente en

aguas costeras, asociadas a plataforma continental, son peces bentónicos, aunque se le puede encontrar en toda la columna de agua (Froese and Pauly 2006). Estos animales pueden alcanzar los 2 metros de envergadura y 50 kg de peso. La temperatura del agua es el factor más importante que determina la migración trófica y la reproducción de la corvina. Tanto los adultos como los juveniles realizan migraciones inducidas por los cambios de temperaturas. (Froese and Pauly 2006; Stipa and Angelini 2005).

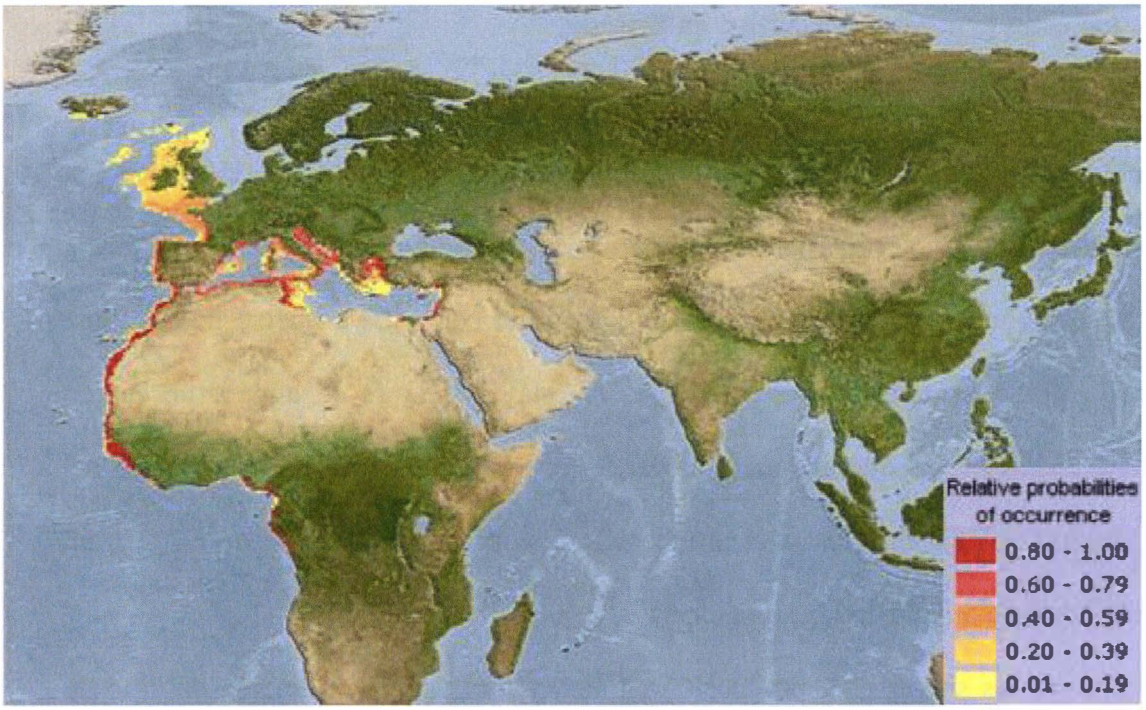


Figura 1.5. Mapa de la distribución de la corvina (*Argyrosomus regius*) en el Atlántico Oriental (Froese and Pauly 2006)

La talla de primera madurez se ha establecido en 61.6 cm para los machos y entre 70-110 cm para las hembras. Entre marzo y agosto las corvinas adultas se aproximan a la costa, penetrando en los estuarios para desovar. Las larvas y juveniles se desarrollan en ese medio estuarino hasta que al final del verano migran a aguas costeras. Estos juveniles se incorporan al stock reproductor a los 6 años de edad (González-Quirós et al., 2011). Las condiciones más favorables para el crecimiento y desarrollo se dan entre 17 y 21 ºC, aunque se pueden adaptar

a valores de 14-23 ºC (Cárdenas, 2010). Una hembra de 1,2 m produce alrededor de 800 000 huevos y el desove ocurre entre 17-22 °C. Se ha capturado juveniles bentónicos de 3,7 cm, indicando que la vida pelágica es bastante corta. Las larvas necesitan temperaturas de 20-21 °C para desarrollarse (Stipa and Angelini 2005). Cabral and Ohmert (2001) encontraron, en la zona estuarina del Tajo, que los juveniles se alimentaban de misidáceos y pequeños cangrejos (*Crangon crangon*); y conforme aumentaban de talla, la dieta se componía de peces y crustáceos decápodos.

1.3 Aspectos generales del cultivo en Europa

En Europa para el 2013, la FAO estimó la producción de lubina (*Dicentrarchus labrax*) de cultivo en 77.057 t (546.346.000 €) representando el 47,84 % de la producción acuícola mundial de esta especie. El cultivo de lubina es un área de especial importancia dentro de la acuicultura europea. Distinto es el caso de la corvina, ya que se trata de una especie cuyo cultivo es relativamente reciente. A esta especie se le augura un rápido crecimiento a medio plazo, incluso puede llegar a ser una de las principales especies del mercado (Monfort, 2010). En Europa la producción de corvina en 2013 fue de 1.722 t, un 25,85 % de la producción mundial, generando 11.316.000 € (FAO 2015b).

En general, los sistemas de producción en acuicultura varían en función del tipo de aguas (continentales o marinas), densidad de cultivo (extensiva, semi-intensivo e intensiva), número de especies que se cultivan (monocultivo o policultivo), lugar donde se encuentren las instalaciones (tierra, costa u offshore) y flujo de agua (abierto, semi-cerrado y cerrado). En la actualidad, las instalaciones en tierra son menos rentables debido a la escasez y elevado precio de terrenos en la costa (en muchos casos en competencia directa con el turismo u otras actividades costeras) y, por otro lado, los elevados costes energéticos (bombeo de agua) para mantener condiciones óptimas de cultivo. Por todo ello, en la Unión Europea se ha experimentado un importante aumento de la implantación de jaulas flotantes por parte de las

empresas de acuicultura, hasta el punto de que hoy por hoy, el engorde de peces marinos en la acuicultura europea (incluido estas especies) se realiza mayoritariamente de manera intensiva en jaulas flotantes off-shore (monocultivo marino de flujo abierto) (Fig. 1.6). En este tipo de instalaciones los alevines son engordados hasta talla comercial (300-500 gr.). La cantidad de peces que hay en cada jaula puede estar en torno a 70,000 individuos.

Figura 1.6. Jaulas flotantes off-shore en Tufia (Gran Canaria, España), granja marina propiedad de la empresa Canexmar S.L.

En Canarias todas las empresas existentes realizan el engorde en jaulas flotantes off-shore. Holmer (2010) clasificó las instalaciones de engorde en jaulas en 'coastal', 'off-coastal' y 'off-shore' (Tabla 1.2), incluyendo a Canarias en el cultivo off-shore aunque conceptualmente no se cumple todas las características. Las especies cultivadas en Canarias son la dorada (*Sparus aurata*) y la lubina. Como ya adelantamos en el caso de la lubina, aunque nos encontramos dentro de su rango de distribución, tampoco la dorada presenta

poblaciones autóctonas numerosas, siendo una especie esporádica en las islas orientales. Por esta razón se hace aún más necesario comprender como están interactuando con el medio, estos peces cuando escapan de las jaulas de engorde.

Taba 1.2. Clasificación y ejemplos de los principales países con actividad acuícola costera, off-coast y off-shore según condiciones físicas e hidrodinámicas. (Holmer, 2010).

	Acuicultura Costera	Acuicultura Semi-costera	Acuicultura Off-shore
Entorno Físico	<500 m de la costa <10 m de profundidad A la vista de la costa	500 m – 3 km de la costa 10-50 m de profundidad Normalmente a la vista desde la costa	>3 km de la costa >50 m de profundidad Sobre plataforma continental No visible desde la costa
Exposición meteorológica	Olas de < 1 m Vientos locales Corrientes locales Fuertes corrientes de mareas Protegido 100% accesibilidad	Olas de hasta 4m Vientos y corrientes localizados Corrientes de mareas débiles Algo protegido 90% accesibilidad	Olas de más de 5 m Vientos y oleajes oceánicos Sin corrientes de mareas Expuestos totalmente >80 % accesibilidad
Definiciones legales	Dentro de las líneas de base costeras Aguas nacionales	Dentro de las líneas de base costeras Aguas nacionales	Fuera de las líneas de base costeras Aguas nacionales o internacionales
Ejemplos de principales países productores	China Chile Norway	Chile Norway Mediterráneo	USA (Hawaii) España (Canarias)

1.4 Implicaciones medioambientales de la acuicultura

1.4.1 Implicaciones medioambientales generales

El rápido crecimiento de la acuicultura sólo se podrá mantener si éste es viable desde un punto de vista social, económico y medioambiental. Es por esta razón que esta actividad, sobre todo en zonas costeras, ha incentivado la investigación de las interacciones con el medio (Naylor et al., 2000; Black, 2001; Hargrave, 2005; Holmer et al., 2003; Holmer et al., 2010). La acuicultura se enfrenta actualmente a una serie de retos medioambientales como consecuencia de las implicaciones que esta conlleva para con la biodiversidad marina a nivel de variabilidad genética, interacciones intra e interespecíficas y alteración de ecosistemas (CBD 2004).

El principal impacto es el producido por **el alimento excedente y las descargas fecales**, que consiguen alterar y modificar las características físico-químicas bajo las instalaciones. En la actualidad la mayor parte del esfuerzo en investigación pasa por entender el efecto que produce el desarrollo de esta actividad sobre el ecosistema bentónico asociado. La concentración de sulfuro en sedimento es inversamente proporcional a la biodiversidad de la infauna bentónica (Chang et al., 2011). Como efectos de la acuicultura costera, los autores han descrito: aumento de materia orgánica (anoxia y pérdida de producción secundaria), impacto negativo sobre las praderas de algas, empobrecimiento de las condiciones en el sedimento por incremento de concentración de sulfuros y pérdida de productividad y biodiversidad de fauna en ambientes eutróficos (Holmer and Kristensen 1992; Molina-Domínguez et al., 2001; Holmer et al., 2003; Hall-Spencer et al., 2006; Mente et al., 2006; Kutti et al., 2007a, 2007b, 2008; Hargrave et al., 2008). Sin embargo es interesante apuntar que las instalaciones off-shore (alejadas de la costa, mayor profundidad y dinámica marina) reducen considerablemente las afecciones descritas, debido al aumento de la dispersión de material orgánico disuelto y particulado (Holmer, 2010).

La presencia de las instalaciones **modifican la comunidad ictiológica**, agregando a las diferentes especies a su alrededor tanto por el efecto FADs (Fish Aggregating Device), como por el incremento de alimento disponible (Carss, 1990; Machias et al., 2004; Dempster and Taquet 2004; Dempster et al., 2005; Tuya et al., 2006). Esta agregación se puede convertir en una trampa ecológica, concentrando peces en un hábitat inapropiado, desviándolos de las rutas migratorias naturales y/o haciéndolos más susceptibles de ser capturados (Battin, 2004)

Dentro de la problemática de introducción de **productos químicos** al medio (fertilizantes, compuestos para tratar el agua y sedimentos, aditivos alimenticios, hormonas, etc.), Rico et al. (2012) señalan a los desinfectantes, pesticidas y antibióticos como los más peligrosos por su alta toxicidad, además de por la capacidad de bioacumulación a lo largo de la cadena trófica. Añade que poseen la capacidad de afectar a la biodiversidad y funcionalidad de los ecosistemas acuáticos. Zheng et al. (2012) demostraron que la existencia de actividad acuícola intensiva, aumentaba la concentración de antibióticos (eritromicina, sulfametoxazol y sulfadimidina) en el medio, provocando un riesgo para algunas especies como *Pseudokirchneriella subcapitata* (microalga) o *Synechococcus leopoliensis* (cianobacteria). En los siete principales países productores de Asia (principal productor acuícola mundial) se encontraron un total de 36 tipos de antibióticos distintos (Rico et al., 2012). Cabello (2006) afirma que el uso indiscriminado y en grandes dosis de antibióticos en la acuicultura moderna, no solo produce un decremento medioambiental, sino también en la salud humana. Demuestra que esta práctica ha creado bacterias resistentes, incrementado la resistencia a los patógenos (que a su vez pueden trasladar esta resistencia a otros microorganismos terrestres, incluidos aquellos patógenos para el ser humano) que afectan a los peces (tanto cultivados como salvajes) y se altera la flora bacteriana de la columna de agua y de los sedimentos afectados por las descargas acuícolas. Los principales desinfectantes utilizados en la acuicultura son el

formaldehido, permanganato de potasio, cloro y derivados de este. Pueden ser desde moderada hasta extremadamente tóxicos para organismos planctónicos y macroinvertebrados (Rico et al., 2012). Los pesticidas usados, como los fungicidas orgánicos e inorgánicos, sulfato de cobre, insecticidas (organoclorados, organofosfatos), piscicidas (rotenona y saponina); son tóxicos para especies a las que no van dirigidos estos tratamientos: especies de invertebrados acuáticos, peces, micro y macro algas, organismos planctónicos e incluso afecciones directas a la salud humana por bioacumulación de estos compuestos (Ling, 2003; De Oliveira-Filho et al., 2004; Qin and Dong 2004; Li et al., 2005; Maltby et al., 2009).

Los peces cultivados tienen mayor riesgo de ser infectados por organismos patógenos y parásitos debido a las altas densidades de cultivo y el estrés por confinamiento (Grau and Crespo 1991; Nowak and La-Patra 2006). Existen diversas evidencias de **transferencia horizontal y vertical de agentes patógenos** y parásitos entre animales cultivados con salvajes. Mladineo et al. (2013) demostraron la transferencia del parásito *Furnestinia echeneis* desde poblaciones de dorada (*Sparus aurata*) salvajes hacia doradas cultivadas. Diamant et al. (2000) encontraron que entre el 20 y el 30% de la población salvaje muestreada de *Siganus rivulatus* se encontraban infectada por *Mycobacteryum marinum;* bacteria llegada al Mar Rojo con el cultivo de lubina. Los peces cultivados pueden transferir virus como el VHS (septicemia viral hemorrágica) y provocar elevadas mortalidades en peces salvajes incluso de otras especies (Munro et al., 2014). Además el efecto de algunos parásitos encontrados como *Anisakis simplex* en salmones cultivados (Mo et al., 2014), puede ser perjudicial para el propio ser humano.

Iribarren et al. (2010) estudiaron la actividad productiva marina en Galicia (pesca costera, acuicultura extensiva, pesca de altura y acuicultura intensiva de *Psetta maxima*) y concluyeron que la acuicultura intensiva fue la segunda actividad en el ranking de huella de carbono (~20 kg CO_2/kg producido), solo superada por la pesca de altura de la langosta con

nasas. Dentro de las modalidades de acuicultura, el engorde de peces en instalaciones off-shore tiene una de las mayores **huellas de carbono**, ya que se aumenta el consumo energético para transportar alimentos, materiales y los propios peces, hasta las instalaciones En cambio, la acuicultura extensiva (*Mytilus galloprovincialis*) fue la actividad con menor huella de carbono con un valor de 0.08 kg CO_2/kg producido.

1.4.2 Escapes

Tradicionalmente la definición de 'escapes' de acuicultura solo englobaba a peces, tanto juveniles como adultos. Más recientemente, Jørstad et al. (2008) descubrieron que se estaban liberando huevos fecundados desde las jaulas, con lo que se ha redefinido el término "escapes" para incluir esta introducción de huevos fecundados en el medio, por parte de los peces cultivados. En el tipo de instalaciones off-shore encontramos dos tipos de escapes: crónicos, aquellos que ocurren con relativa periodicidad y bajo número de individuos, cuya principal causa son los agujeros en la red ocasionados por mordidas por predadores y los propios peces cultivados (Fig. 1.7); y los escapes masivos (> 10,000 individuos) ocasionados principalmente por roturas de las anillas de amarre (Fig. 1.8) (Jackson et al., 2015). Según Jensen et al. (2010), estos escapes masivos en la actualidad son poco frecuentes en la acuicultura off-shore Noruega. Representan el 19 % de los incidentes de escapes en cultivo de salmón y bacalao, pero suponen el 91% en el número total de individuos escapados.

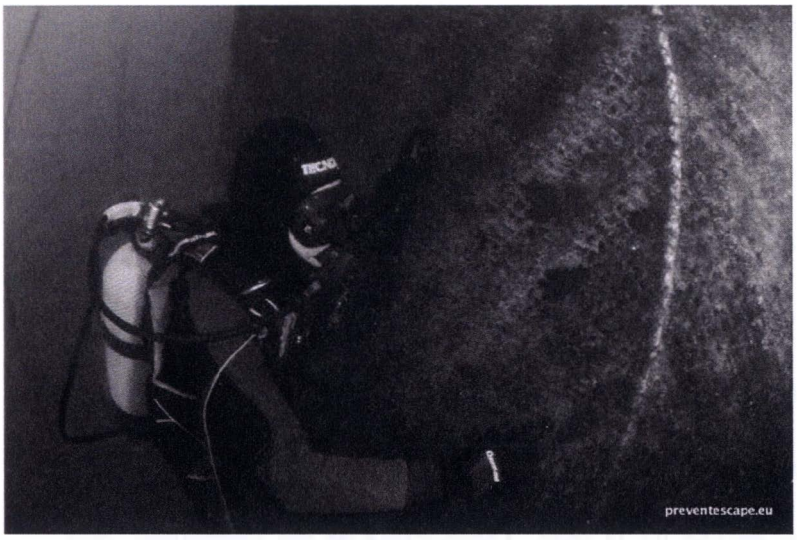


Figura 1.7. Buzo reparando pequeñas roturas en la red de una jaula de engorde.

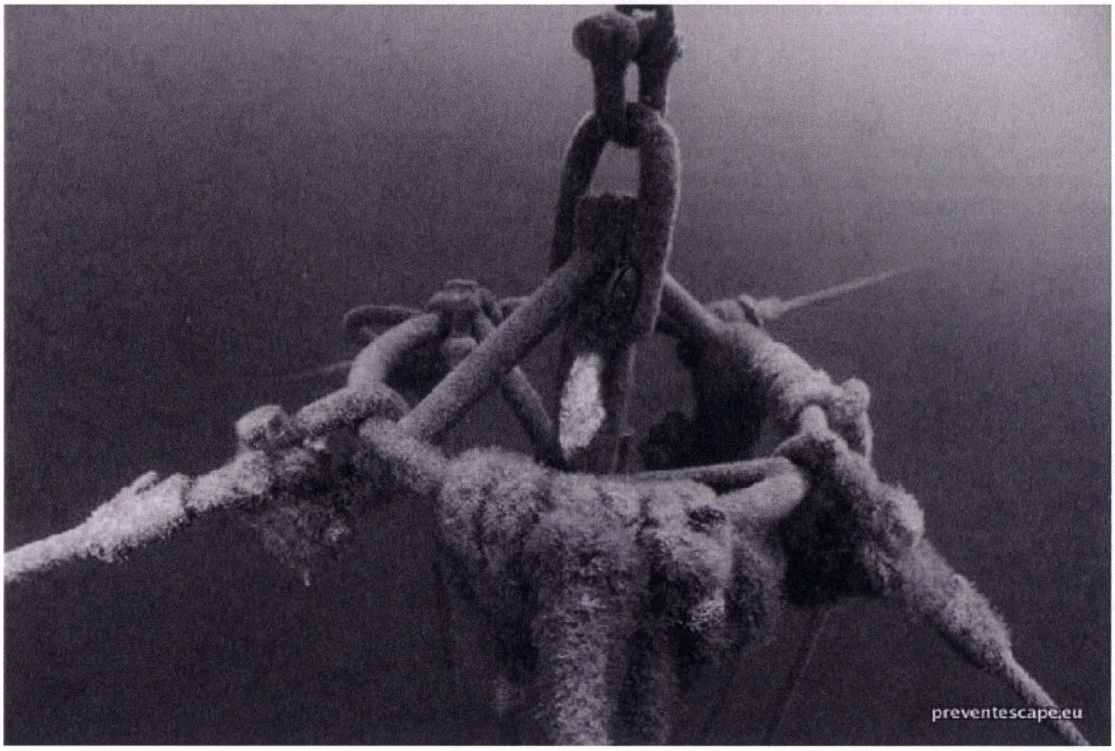


Figura 1.8. Anilla de amarre.

Jackson et al. (2015), además de coincidir con lo expuesto por Jensen et al. (2010), describen las principales causas de los escapes en la acuicultura europea (Figs. 1.9 y 1.10):

1. Fallos en los puntos de amarre y sujeción de las jaulas. Esto normalmente se debe, a su vez, a una combinación de 3 factores: desgaste del material, falta de revisión-reemplazamiento y aparición de condiciones marítimas adversas (temporales, mar de fondo, tormentas, etc.).
2. Predadores. Animales que tienen capacidad de realizar roturas en la red, con la intención de alimentarse de los peces que se cultivan en el interior de las jaulas.
3. Uso de materiales inapropiados en las jaulas.
4. Eventos tormentosos y de mal estado de la mar. Temporales de tal intensidad, que aunque el material de la instalación (cabos, muertos, redes, anillos, etc..) es el adecuado, estos sufren roturas por sobrecarga.

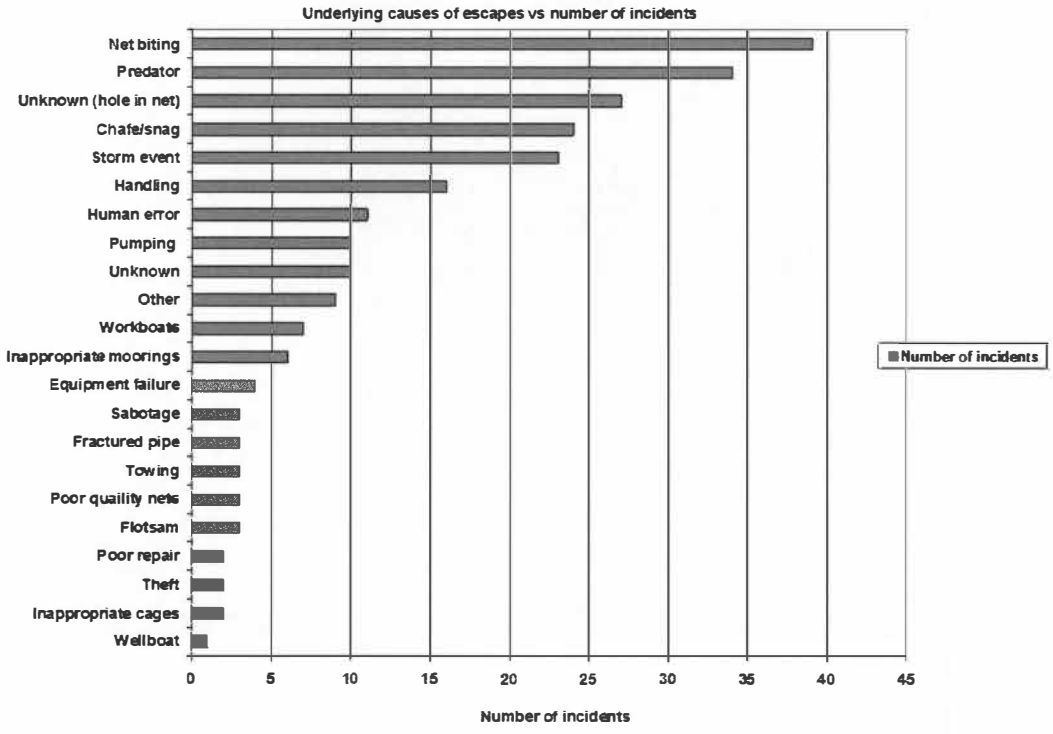


Figura 1.9. Ranking de las principales causas de escapes, según el número de escapes reportados (Jackson et al., 2015).

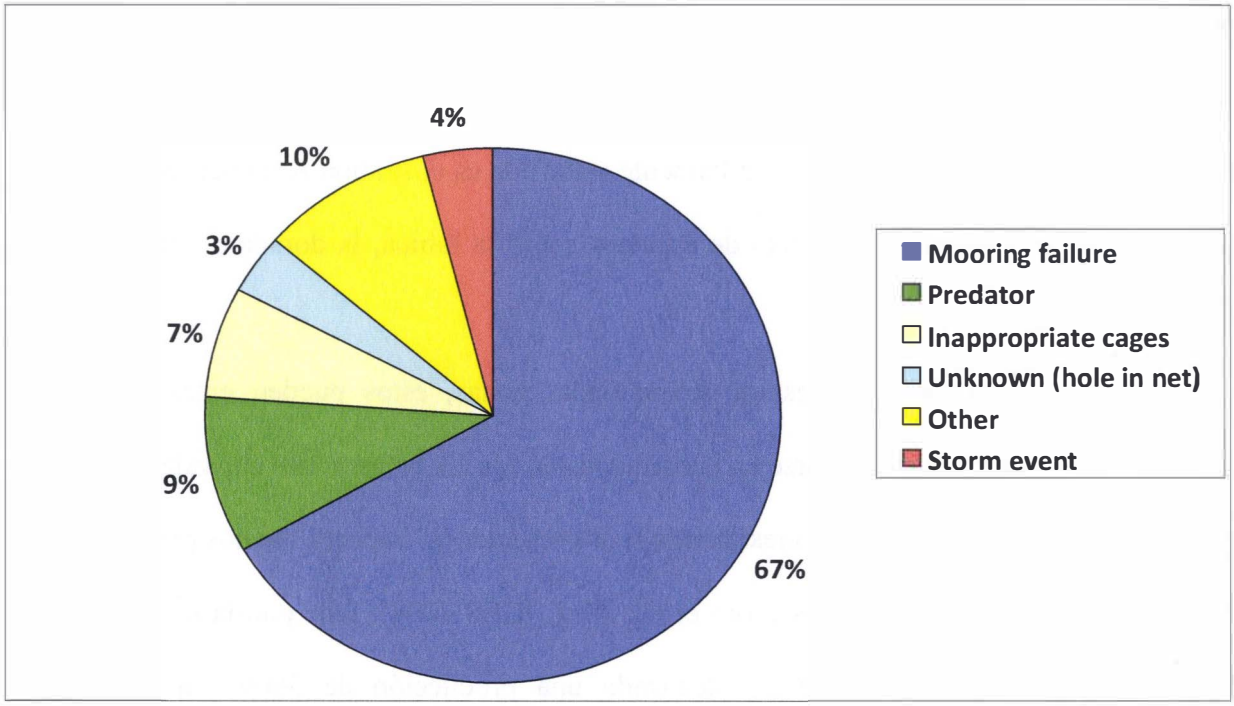


Figura 1.10. Ranking de las principales causas de escapes, según el número de peces escapados (Jackson et al., 2015).

1.4.3 Impacto de los escapes

Solo en Noruega, las jaulas existentes pueden contener unos 325 millones de salmones en cada ciclo de engorde; mientras que los salmones salvajes que entran a desovar en estos ríos cada año, apenas llegan a 1 millón de individuos. Según Naylor et al. (2005), los escapes se presentan como un problema para la sostenibilidad de la acuicultura. Los escapes representan problemas por mezcla genética de poblaciones separadas geográficamente, alteración de la cadena trófica, introducción de patógenos y enfermedades, competencia intra e interespecífica o introducción de especies exóticas (CBD, 2004; Molina and Vergara 2005; Naylor et al., 2005; Vergara et al., 2005; Jensen et al., 2010; Grigorakis and Rigo 2011). Además, en términos económicos los escapes representan un total de pérdidas de 47.5 millones de euros al año (Jackson et al., 2015). Se han descrito escapes de jaulas marinas para especies como el salmón Atlántico, el bacalao Atlántico (*Gadus morhua*), trucha arcoíris (*Oncorhynchus mykiss*), trucha Alpina (*Salvelinus alpinus*), halibut (*Hippoglossus*

hippoglossus), dorada (*Sparus aurata*), lubina, corvina o serviola (*Seriola lalandi*) (Soto et al., 2001, Naylor et al., 2005, Gillanders and Joyce 2005, Moe et al., 2009). Dentro de esta línea de investigación, la especie más ampliamente estudiada es el salmón Atlántico, mientras que el conocimiento acerca del impacto de especies como la lubina, la dorada o corvina es muy limitado o inexistente.

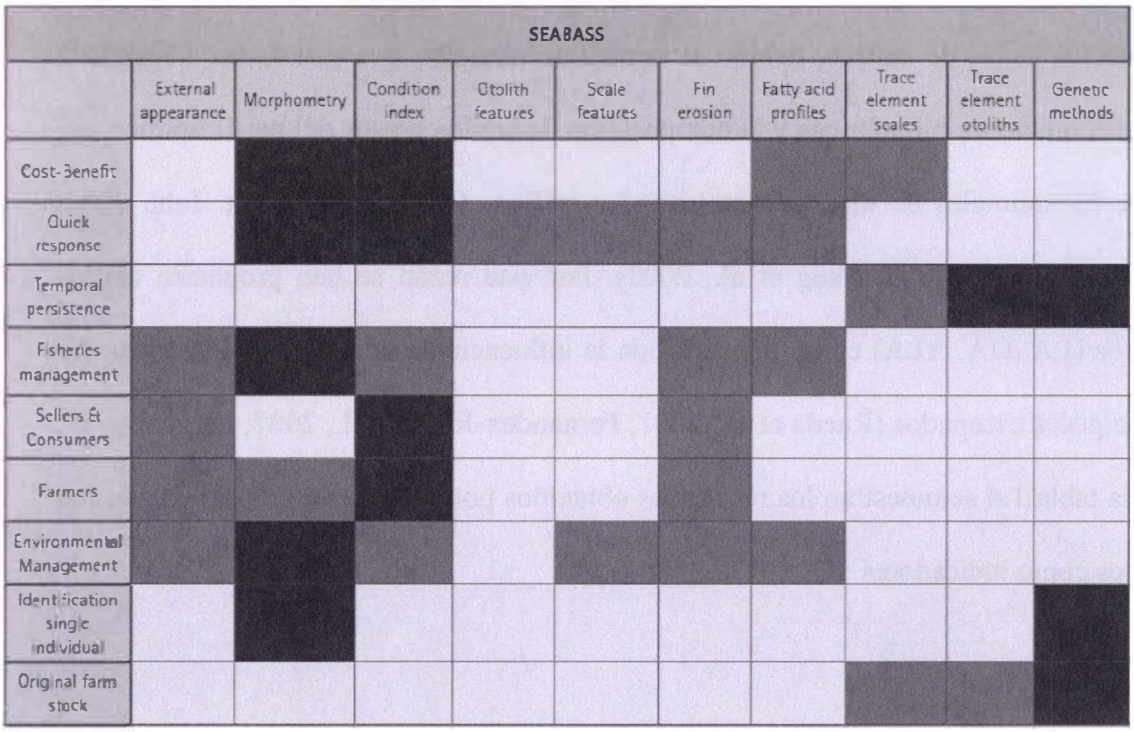
Cuando se producen escapes en determinadas zonas, estos pueden entrañar una problemática particular por encontrarse en ecosistemas donde las poblaciones naturales de las especies cultivadas sean pequeñas o inexistentes, por presentar un impacto menos predecible y unas pérdidas económicas mayores (Soto et al., 2001; ICES 2004). Este podría ser el caso de Canarias donde en el 2012 se ha alcanzado una producción de 3600 t de lubina (JACUMAR 2014) y solo han sido descritas pequeñas poblaciones autóctonas en las islas orientales (Fuerteventura y Lanzarote) (Brito, 1991), (donde único sería posible la mezcla de poblaciones). Además varios trabajos han concluido que no existen evidencias de la reproducción de esta especie (poblaciones de peces escapados) en las demás islas (Carrillo and Castillo 2001; Toledo et al., 2009). Estos mismos trabajos señalan que la dieta de las lubinas escapadas es similar a la de otras especies locales, llegando competir por el recurso (Carrillo and Castillo 2001; Toledo et al., 2009).

1.5 Bioindicadores

Para poder comenzar a estudiar los escapes de acuicultura, con el fin de entender cómo estos pueden interactuar y/o afectar al entorno, es imprescindible poder diferenciar, tanto en el medio marino como una vez capturados, individuos escapados de aquellos salvajes. Los investigadores han propuesto varios métodos como la diferenciación genética (Gwak et al., 2003; Glover et al., 2009), cambios en el perfil lipídico (Grigorakis et al., 2002; Fernández-Jover et al., 2007;), elementos traza-morfología en escamas y otolitos (Lund and Hansen 1991; Katayama and Isshiki 2007; Adey et al., 2009; Person-Le Ruyet and Le Bayon 2009),

trazas biomoleculares (Megdal et al., 2009), isótopos estables (Weber et al., 2002), diferencias morfológicas (Loy et al., 1999; Murta, 2000; Cramon-Taubadel et al., 2005; ; Ellis et al., 2009; Uglem et al., 2011; Arechavala-López et al., 2013b). Cada uno conlleva una serie de ventajas e inconvenientes, como queda reflejado en la tabla 1.3, donde Jackson et al. (2015) han recopilado gran número de ellos. En el presente trabajo se han testado de forma más exhaustiva los bioindicadores relacionados con los cambios de perfil lipídico y diferencias morfológicas, al considerar que nos pueden suministrar información científica de interés relacionada con las interacciones de los peces escapados con el medio ambiente.

Tabla 1.3. Resumen del uso de indicadores para identificar lubinas (*Dicentrarchus labrax*) escapadas. La escala de idoneidad va desde el negro (máxima) al blanco (menos recomendable) (Jackson et al., 2015).



1.5.1 Perfil lipídico

Muchos autores proclaman el perfil lipídico como un indicador de la salud medioambiental (Dunn et al., 2008; Hu et al., 2008; Maazouzi et al., 2008) y en estos últimos años se le ha prestado especial atención debido a los continuos cambios que sufren los piensos para acuicultura. Tradicionalmente los peces cultivados se alimentaban con piensos hechos a base de harina y aceite de pescado, pero el encarecimiento de esta la materia prima ha obligado a sustituirlos en parte, sobre todo el aceite de pescado por aceite de origen vegetal (Tacon and Metian 2008). Su uso está limitado por la falta de n−3 HUFA, a pesar de la alta concentración de ácidos grasos como linoleico (LA) (18: 2n−6) y linolénico (ALA) (18: 3n−3) (Turchini et al., 2009). Estos ácidos grasos son incorporados a los tejidos de los peces cultivados, aunque también pueden transferirse a los peces salvajes que se encuentran alrededor de las jaulas de cultivo, debido al exceso de alimento que sale fuera; llegando a cambiar las condiciones biométricas y la composición de ácidos grasos del perfil lipídico, así como la de los animales de los siguientes niveles tróficos (Dalsgaard and St. John 2004; Fernández-Jover et al., 2007; Skog et al., 2003). Por esta rezón se han propuesto ciertos ácidos grasos (LA, OA, ALA) como indicador de la influencia de la acuicultura en el medio marino y de peces escapados (Rueda et al., 2001, Fernández-Jover et al., 2007; Megdal et al., 2009). En la tabla 1.4 se muestran los resultados obtenidos por algunos autores utilizando los ácidos grasos como indicadores

Tabla 1.4. Resumen de los resultados en estudios previos. Se muestran los ácidos grasos estudiados que mostraron un aumento al comparar peces cultivados/'asociados a las instalaciones (fuera de las jaulas)' y peces cultivados/salvajes.

Autor	Species	Tissue	Wild	Into Cage	Around Cage
Rueda et al., 2001	***Diplodus puntazzo***	**Músculo**	**↑ 22:5n-3**	**↑ 18:2n-6** **18:1n-9** **20:5n-3**	
		Hígado	**↑ 20:5n-3** **22:6n-3**	**↑ 18:2n-6** **18:1n-9**	
Grigorakis et al., 2002	***Sparus aurata***	**Músculo**		**↑ 18:1n-9** **18:2n-6** **18:3n-3**	

Skog et al., 2003	***Pollachius virens L.***	**Músculo**	**↑ n-3/n-6**		**↑ 18:1n-9** **18:3n-3** **18:2n-6**
Mnari et al., 2007	***Sparus aurata***	**Músculo**	**↑ 18:1n-9**	**↑ 22:6n-3** **n-3/n-6** **18:2n-6** **18:3n-3**	
		Hígado	**↑ 18:1n-9**	**↑ 18:3n-3** **n-3/n-6** **22:6n-3** **20:5n-3**	
Fernandez-Jover et al., 2007	***Trachurus mediterraneus***	**Músculo**	**↑ 22:6n-3** **n-3/n-6**		**↑ 18:2n-6** **18:1n-9**
Martinez-Rubio	***Boops boops***	**Músculo**	**↑ 22:6n-3**		**↑ 18:2n-6**

et al., 2009			**18:1n-9**
	Hígado	**↑ 22:6n-3**	**↑ 18:2n-6**
			18:1n-9
Megdal et al., 2009	***Salmo salar***	**Músculo**	**↑ 18:2n-6**

1.5.2 Cambios morfológicos

La descripción cuantitativa, análisis e interpretación de la morfología son herramientas fundamentales en el campo de la biología en general. Las técnicas morfológicas se basan, a rasgos generales, en el análisis de las distancias entre una serie de puntos situados estratégicamente a lo largo del cuerpo de la especie a estudiar. Los grupos de individuos de una misma especie pueden mostrar diferencias morfológicas (Fig. 1.11) como consecuencia de las diferencias genéticas y medioambientales (Barlow, 1961; Kinnison and Hendry 2004; Solem et al., 2006), llegando a poderse utilizar estas diferencias para identificar el stock de origen (Hurlbut and Clay 1998; Murta, 2000; Solem et al., 2006) o servir para diferenciar peces que fueron cultivados de otros salvajes (Loy et al., 1999; Murta, 2000; Cramon-Taubadel et al., 2005; Arechavala-López et al., 2011; Uglem et al., 2011). Los cambios morfológicos de especies cultivadas está relacionada con los primeros estadíos de desarrollo

(Loy et al., 1999, 2000), ya que es en ese momento cuando aparecen el mayor porcentaje de anomalías esqueléticas en comparación con individuos salvajes (Boglione et al., 2001) y además la baja mortalidad que sostiene el cultivo controlado (Fleming et al., 1994), permitiendo que larvas y juveniles, que en medio natural no llegarían a sobrevivir, consigan llegar a adultos. En el caso concreto de la lubina, Roncarati et al. (2001) encontraron diferencias morfológicas entre juveniles criados en tanques tradicionales y cultivados en mesocosmos. Rinaldi et al. (2005) demostraron diferencias en las branquias como respuesta a la disponibilidad de oxígeno. Person-Le Ruyet and Le Bayon (2009), observaron distintos grados de erosión en las aletas según las densidades de cultivo. Estudios previos han mostrado diferencias morfológicas entre peces salvajes y cultivados en especies como el salmón Atlántico (Solem et al., 2006), bacalao (*Gadus morhua*) (Uglem et al., 2012), barbo Índico (*Tor putitora*) (Patiyal et al., 2014), dorada (Rogdakis et al., 2011) o lubina (Arechavala-López et al., 2011) entre otras. Mención aparte merece el estudio de Rogdakis et al. (2011), quienes compararon criados, salvajes y liberados-recapturados. Estos peces criados, que se liberan y recapturan pasado un determinado tiempo, nos proporciona una información vital ya que podemos asumir que son peces escapados. De los resultados publicados por Rogdakis et al. (2011), se puede destacar que la distancia entre el labio superior y la primera espina dorsal no presenta diferencias entre aquellos peces cultivados y liberados (escapados); pero a su vez, estos (cultivados y liberados) se diferencian de los salvajes.

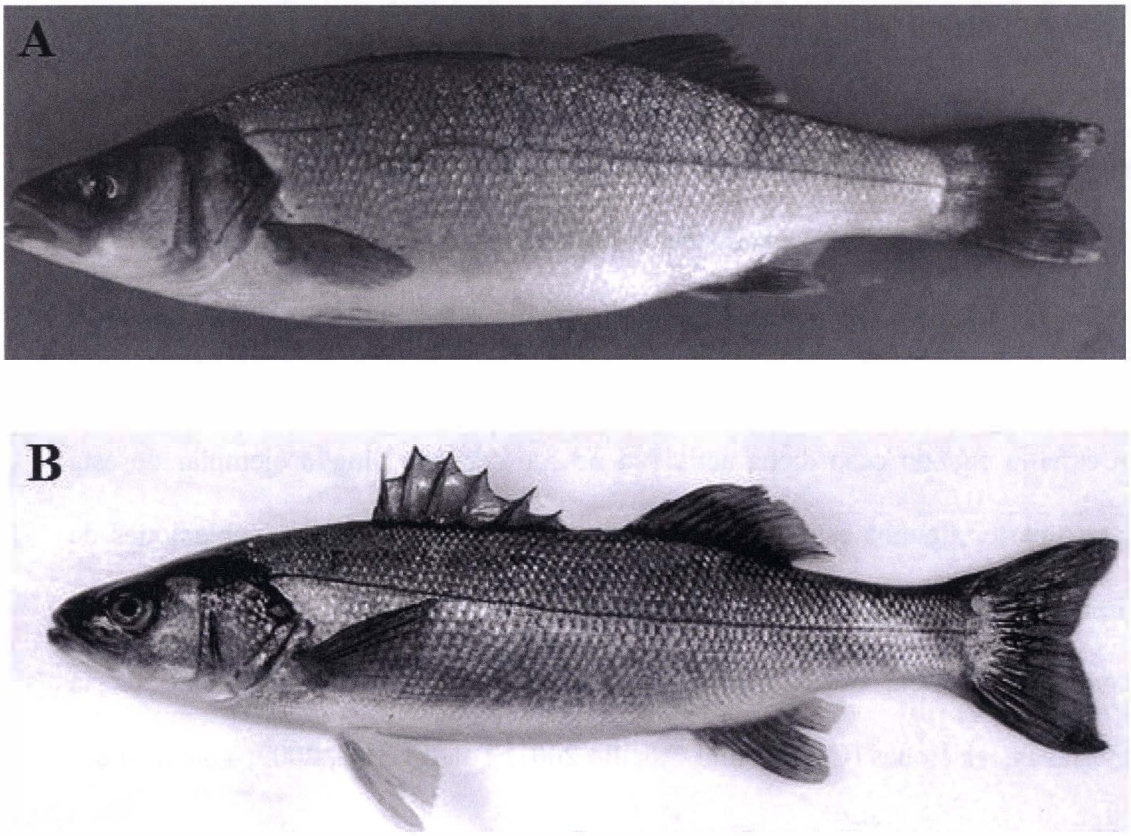


Figura 1.11. Ilustración comparativa de la morfología externa entre lubina cultivada (A) y lubina salvaje (B).

1.6 Establecimiento de poblaciones de peces escapados

Como ya se ha adelantado, la problemática de los escapes puede tener dos vertientes: especies que una vez se escapan son capaces de adaptarse y sobrevivir en el medio, y especies que además son capaces de reproducirse. Independientemente del caso de estudio, una vez conocida la problemática de los escapes y contando con herramientas para conocer si los peces que nos encontramos son salvajes o escapados; el siguiente paso es el estudio del asentamiento de las poblaciones de peces escapados en el medio natural. Para ello debemos estudiar en qué tipo de ecosistemas se establecen, comportamiento, alimentación y capacidad de reproducción, entre otros. Algunas técnicas usadas con este fin son los censos visuales, telemetría, contenidos estomacales y desarrollo gonadal, respectivamente.

1.6.1 Censos visuales

Los censos visuales submarinos son ampliamente usados para estudiar la variabilidad de poblaciones de peces litorales, en aguas costeras poco profundas. El impacto de esta técnica es mínimo. La literatura existente donde se usa esta técnica, ya sea por medio de video o por buceadores, es extensa. Tuya et al. (2006) observaron doradas escapadas alrededor de las jaulas de engorde y en las zonas de control mientras la granja objeto de estudio mantenía actividad; en cambio cuando cesó dicha actividad no fue censado ningún ejemplar de esta especie. Por otra parte, algunos estudios similares, concluyeron que existen poblaciones de lubina en Gran Canaria, La Palma y Tenerife (islas oceánicas donde no estaba descrita esta especie), como consecuencia de la actividad acuícola y dependen directamente de la existencia de estas instalaciones (Carrillo and Castillo 2001; Toledo et al., 2009; Toledo et al., 2012, 2014b).

1.6.2 Telemetría

La telemetría es una tecnología que se basa en el envío de información (de distinta clase, aunque en este trabajo hablaremos de desplazamientos de los individuos marcados), de manera inalámbrica (en nuestro caso) entre un transductor (Fig. 1.12) y un receptor (Fig. 1.13). Esta información se transmite en el medio a través de ondas electromagnéticas. Para los estudios que se llevan a cabo en el medio acuático, la alta conductividad del medio, impide el uso de telemetría basada en ondas de radio, por lo que en este medio se usa la telemetría acústica. Es una potente herramienta que nos permite estudiar especies en su hábitat. A modo general, dentro de los estudios ecológicos, existe telemetría activa (técnicas que nos provee de datos de manera continua y simultánea permitiendo visualizar los movimientos de los ejemplares prácticamente a tiempo real) y pasiva (en la que se va almacenando la información, de los movimientos de los peces liberados en este caso, en un dispositivo receptor, para posteriormente descargar y visualizar los datos).

Figura 1.12. Transmisor (TAG) de telemetría acústica usado en la presente Tesis.

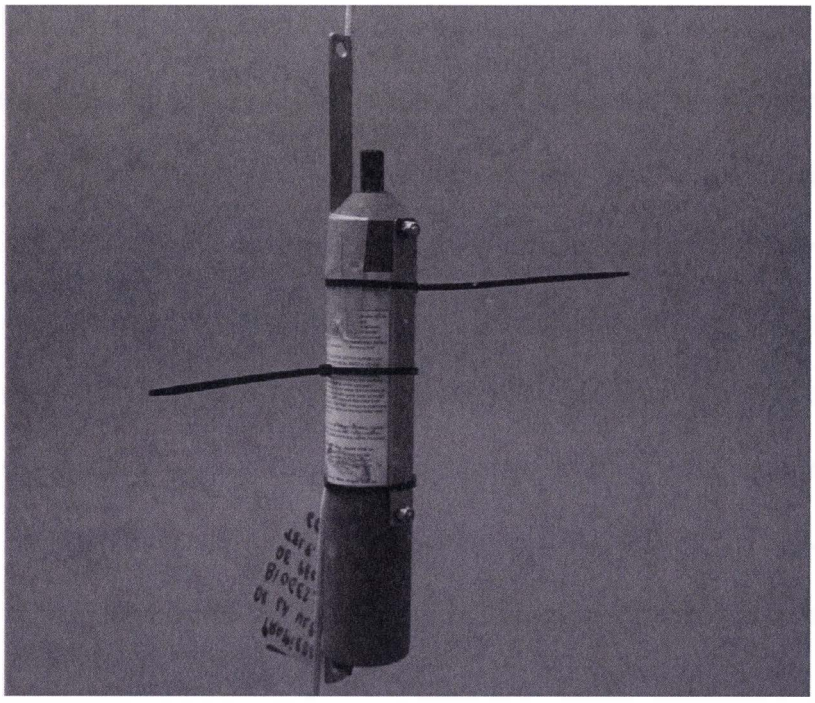


Figura 1.13. Receptor de telemetría acústica instalado durante uno de los experimentos de la presente Tesis.

En las dos últimas décadas se ha extendido el uso de esta última técnica para monitorizar el comportamiento de las especies objeto de cultivo, una vez se escapan de las instalaciones de engorde. Con este fin se liberan controladamente individuos con

transmisores. Arechavala-López et al. (2012) mostraron un alto porcentaje de dispersión y mortalidad (>60%) para la dorada en el Mediterráneo, aunque alguno de estos individuos permanecieron alrededor de las instalaciones de cultivo durante un largo periodo (> 4 semanas). Otros resultados muestran que la lubina escapada se mueve relativamente rápido y repetidamente a través de varias instalaciones acuícolas, además presenta una alta mortalidad en el medio natural (Arechavala-López et al., 2012).

1.6.3 Contenidos estomacales

Una parte esencial en el estudio ecológico de cualquier especie, es la composición de la dieta. Aunque en la actualidad se están usando técnicas más modernas (p.e. isótopos estables, Gil et al., 2013), el análisis de contenido estomacal sigue siendo el método más ampliamente usado en el estudio de la dieta de una especie. Mediante esta técnica, se ha observado que la mayoría de las especies cultivadas son capaces de alimentarse de los recursos existentes en el medio una vez escapan de las jaulas de cultivo (Lorenzen et al., 2012). En el caso concreto de la lubina esto se corrobora incluso en zonas fuera de su rango de distribución, como es el caso de algunas islas del archipiélago Canario: Gran Canaria, Tenerife y La Palma (Carrillo and Castillo 2001; Toledo et al., 2009, 2014a). No obstante el cambio de hábitos alimenticios puede provocar un largo periodo de inanición, como se ha descrito para la corvina en el Mediterráneo, donde los animales tardaron más de uno y dos meses tras la suelta en comenzar a alimentarse de recursos disponibles en el medio (Gil et al., 2014).

1.6.4 Desarrollo gonadal.

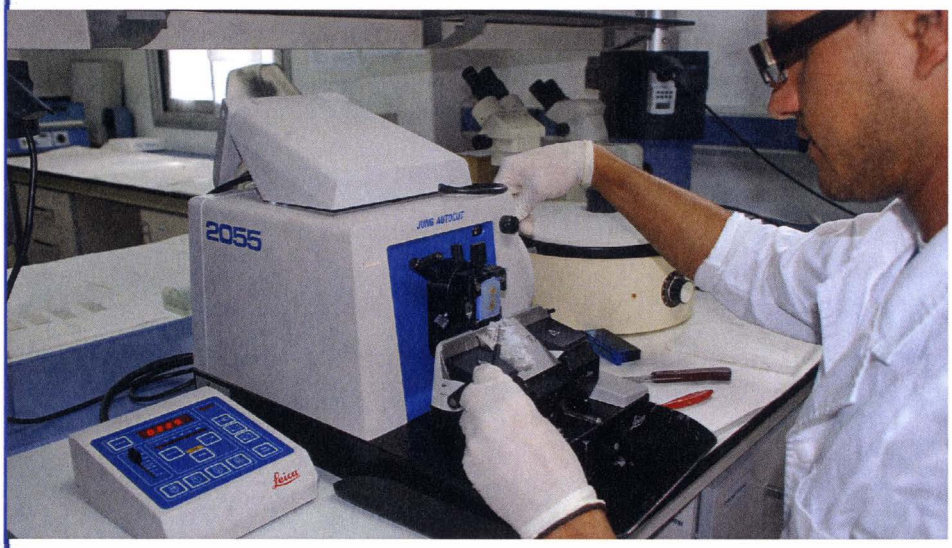
Como ya hemos adelantado, el estudio de los mecanismos reproductivos animales es uno de los campos más importantes en la investigación ecológica. En los peces se han descubierto un amplio rango de características sexuales y estrategias reproductivas. Además, el conocimiento acerca de la reproducción de una determinada especie es imprescindible para

su producción controlada mediante técnicas acuícolas (Chen et al., 2013). Ya habíamos subrayado que dentro de la problemática de los escapes, está la posibilidad de que estos se reproduzcan entre sí o con sus congéneres en el medio natural (Hansen et al., 2000; Naylor et al., 2001; Jonsson and Jonsson 2006; Hewit et al., 2006; Uglem et al., 2008; Jensen et al., 2010; Arechavala-López et al., 2011; Grigorakis and Rigo 2011; Arechavala-López et al., 2012). El caso del cultivo de la lubina no está exento de este problema, ya que existe la posibilidad de reproducción en el Mediterráneo, además de todos aquellos lugares de cultivo, incluidos en su rango de distribución. Más complejo resulta cuando nos encontramos fuera de cualquier área reproductiva descrita para la especie, pues habrá que comprobar que dicha especie está reproduciéndose. El primer paso a seguir con este objetivo, sería pues, estudiar el desarrollo gonadal de esta especie no nativa. Estos estudios se pueden llevar a cabo tanto macroscópicamente (*a visu*), como microscópicamente (estudios histológicos). Para ambos casos hay infinidad de técnicas y escalas definidas. Los estudios llevados a cabo en Canarias sostienen una escasa probabilidad de reproducción de la lubina en el medio natural (Carrillo and Castillo 2001; Toledo et al., 2009, 2014a).

2

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

HIPÓTESIS Y OBJETIVOS



La hipótesis general de la presente tesis es que (1) el perfil lipídico de los peces (fauna asociada y peces cultivados) bajo influencia acuícola cambia con respecto a la distancia a la que se encuentren de las instalaciones de cultivo; aunque, por otro lado, (2) este perfil puede estar influenciado por otras fuentes de ácidos grasos de origen terrestre, con lo que (3) el perfil lipídico no es una herramienta efectiva para determinar con rotundidad el origen del animal. (4) Existen otras herramientas como la morfometría que si lo son. (5) Estos peces escapados (lubina y corvina) tienen capacidad de sobrevivir en el medio, aprovechando los recursos existentes, aunque sin llegar a reproducirse. Con el fin de validar estas hipótesis se definieron los siguientes objetivos:

- Conocer el efecto del excedente de piensos usados en las jaulas de engorde, sobre el perfil lipídico de la comunidad ictiológica asociada
- Estudiar la viabilidad del uso de determinados ácidos grasos como biomarcadores de acuicultura, usando como modelo una especie cosmopolita que se encuentra generalmente asociada a sistemas de cultivo y otra especie cultivada con poblaciones de peces escapados a lo largo de la costa de una isla oceánica.
- Estudiar la viabilidad de otros parámetros como indicadores de peces de origen acuícola: Morfometría
- Analizar la variación espacio-temporal de peces cultivados, escapados tras un escape masivo alrededor de una isla oceánica o modelizando el escape mediante una suelta controlada; así como conocer el grado de asentamiento de estas poblaciones (alimentación y potencial reproductor).

3

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

MATERIAL Y MÉTODOS

3.1 Organigrama de la tesis

La fase experimental de esta tesis doctoral comenzó en agosto del 2009 y se prolongó con diferentes experimentos hasta febrero del 2012.

3.2 Zona de estudio

La totalidad de los experimentos llevados a cabo durante la presente tesis doctoral, fueron realizados en el archipiélago Canario (España). Esta región se encuentra entre los 27º 37' hasta los 29º 15' N; y 13º 24' hasta los 18º 11' W (Fig. 3.1). Se trata de un archipiélago oceánico, situado en el Atlántico Centro-Oriental. Su situación cercana al continente Africano (a 100 km de Tarfaya, Marruecos), un régimen prácticamente constante de vientos alisios (generador de micro afloramientos) junto a la existencia de una corriente fría ("Corriente Fría de Canarias"), una alta biodiversidad marina, existencia de 7 islas donde se encuentran gran variedad de ecosistemas marinos (con 3 reservas marinas integrales), islas muy pobladas frente a otras de escasa población, gradientes naturales entre islas, etc... hacen que nos encontremos ante lo que podemos considerar como uno de los mejores laboratorios marinos naturales del planeta.

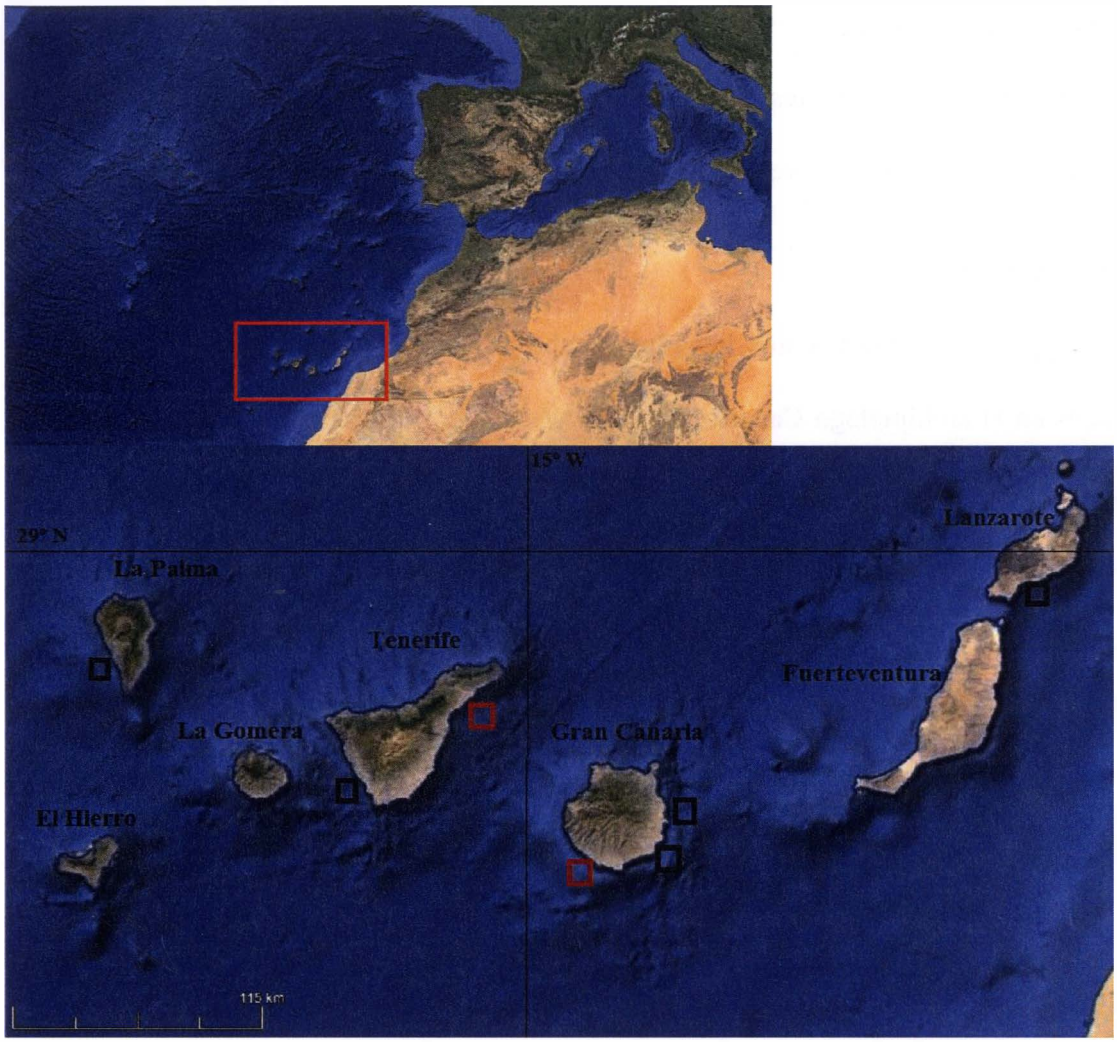


Figura 3.1. Mapa de la zona de estudio (Islas Canarias, España). Las áreas en negro indican zonas con presencia de instalaciones de engorde 'offshore'. Las áreas en rojo, en cambio, indican zonas donde existían instalaciones pero en la actualidad su actividad ha cesado.

3.3 Captura y transporte de las muestras

Los peces salvajes que se precisaron durante los experimentos, se capturaron mediante pesca recreativa (a caña y pesca submarina) y pesca profesional (cerco y nasas). La técnica a utilizar dependió de la especie objeto, zona de pesca, características del experimento y, sobre todo, las posibilidades logísticas (mirar Material y Métodos de cada experimento). La pesca a caña consiste en una línea de nailon o hilo sintético, de gran resistencia y enrollada en un carrete manual, que pasa por unas anillas fijas a la caña. Una vez liberada dicha línea, es recogida a través del carrete; el bajo de línea (parte del arte que realmente ejerce la pesca) se

utilizó un palangre vertical consistente en un plomo (para que la línea llegue al fondo), y tres anzuelos en vertical separados 20 cm del plomo y entre sí. Como carnada para los anzuelos se usó langostino (*Penaeus notialis*). La pesca submarina consiste en la inmersión en apnea (sin equipo de respiración autónomo), equipado como mínimo de gafas, tubo, aletas y fusil submarino, que libera un arpón metálico con el que se captura el pez (Fig. 3.2). Los peces capturados mediante esta técnica no fueron seleccionados bajo ningún criterio (tamaño, coloración, comportamiento, zona, etc), para evitar un sesgo en el muestreo.

Figura 3.2. El autor capturando lubina escapada en el litoral de Gran Canaria.

La nasa para peces es un armazón, generalmente de forma circular, revestido de una red o forro, cuya malla tiene forma hexagonal regular, con una o dos entradas y una puerta. Las entradas tienen forma de embudo, quedando hacia el interior la parte más estrecha, de tal forma que permite la entrada de los peces pero no su salida. La malla es degradable y tiene una luz de malla mínima de 50,8 mm en las nasas grandes y de 31,6 mm en las pequeñas (no

superan 1 m de diámetro). El arte de cerco tradicional en Canarias recibe el nombre de "traíña". Consiste en una red rectangular sustentada por flotadores y mantenida verticalmente por pesos, con la que se rodea o cerca a las especies. Está provista de un cabo o jareta que cierra el arte por la parte inferior, una vez realizado el cerco, quedando los peces embolsados en él. La luz de malla mínima de la traíña será de 10 mm. Sus dimensiones máximas son de 350 m de longitud y 80 m de altura. Los peces cultivados, por su parte, se obtuvieron aleatoriamente, el día del despesque, del total extraído por la empresa que los engordaba. Esta operación se realiza mediante un cerco dentro de la jaula (Fig. 3.4).

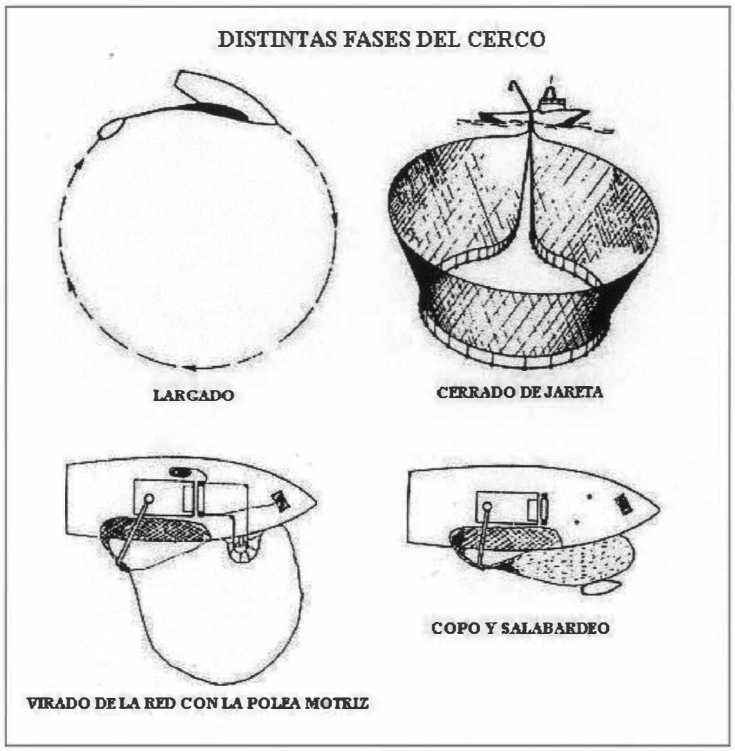


Figura 3.4. Representación de arte de cerco y maniobra de pesca

Una vez capturados, los especímenes se mantuvieron en hielo (0ºC) hasta la llegada al laboratorio, donde se procesaban y/o congelaban según procediera en cada ocasión. Este protocolo se realizaba siempre y cuando las muestras llegaran al laboratorio dentro de las 5 horas tras la captura. En caso que no llegaran antes de este tiempo, se procedería a congelar en

alguna cámara frigorífica más cercana, para un posterior transporte ya congeladas; asegurando de esta manera mantener una correcta cadena de frío que evitaría la degeneración de tejidos y/o bioquímica.

3.4 Disección de los peces

Todos los peces se pesaron (peso total y peso eviscerado; precisión de 0.1 gr) y midieron (longitud total; precisión de 0.1 cm). En el caso de las lubinas utilizadas para el experimento de morfometría, antes de ser eviscerados, cada individuo se limpió, preparó y colocó sobre la superficie dispuesta para tomar las fotografías que servirían para el posterior análisis de imagen (Fig. 3.5). El resto de peces fueron eviscerados sin fotografiar. Las gónadas fueron examinadas macroscópicamente (Fig. 3.6) según la escala propuesta por Holden and Raitt (1974) (Tabla 3.1). En caso de duda se procedió a conservar una muestra en formol tamponado para su posterior examen histológico (Fig. 3.7) y clasificación según la escala propuesta por Wallace and Selman (1981). El hígado y el filete del lado derecho se conservaba al vacío y en frio (-80ºC) para el posterior análisis de lípidos totales, humedad, cenizas y ácidos grasos.

Tabla 3.1. Estadíos de desarrollo gonadal para examen macroscópico. (Holden and Raitt, 1974)

I. **Inmaduro.** La gónada es pequeña y firme, ocupando cerca de la tercera parte de la longitud de la cavidad abdominal. Los ovarios y los testículos son transparentes o de color claro. Los ovocitos son invisibles a simple vista y los sexos no son diferenciables a simple vista.

II. **Reposo o crecimiento lento**. La gónada tiene un aspecto firme, ocupando cerca de la mitad de la longitud de la cavidad abdominal. Los ovarios son de color rosados o translúcidos y los testículos son blanquecinos, más o menos simétricos. Los ovocitos son invisibles a simple vista.

III. **Maduración, prefreza o prepuesta**. La gónada presenta un aspecto grueso, ocupando cerca de dos terceras partes de la longitud de la cavidad abdominal. Los ovarios son más o menos cilíndricos, de color anaranjado y los testículos son más o menos romboidales, blanquecinos o de color crema. Los ovocitos son visibles a simple vista a través de la membrana ovárica, dando un aspecto granular a la superficie del ovario.

IV. **Maduro, freza o puesta**. La gónada es más gruesa, ocupando cerca de dos terceras partes de la longitud de la cavidad abdominal. Los ovarios poseen un color naranja rosado con vasos sanguíneos superficiales, mientras que los testículos tienen aspecto lechoso y brillante. Los óvulos son maduros y transparentes, de gran talla y perfectamente visibles a simple vista, con una membrana ovárica muy fina. Los productos sexuales son expulsados a la menor presión ejercida sobre el abdomen del individuo.

V**. Postfreza o postpuesta**. La gónada presenta un aspecto contraído, ocupando cerca de la mitad de la longitud de la cavidad abdominal. Las paredes gonadales tienen aspecto de saco vacío. Los ovarios están completamente colapsados, son muy flácidos,

son de color rojo debido a una gran vascularización. Los testículos presentan un aspecto oscurecido. Los ovocitos en vías de necrosis, pudiendo quedar algunos maduros residuales.

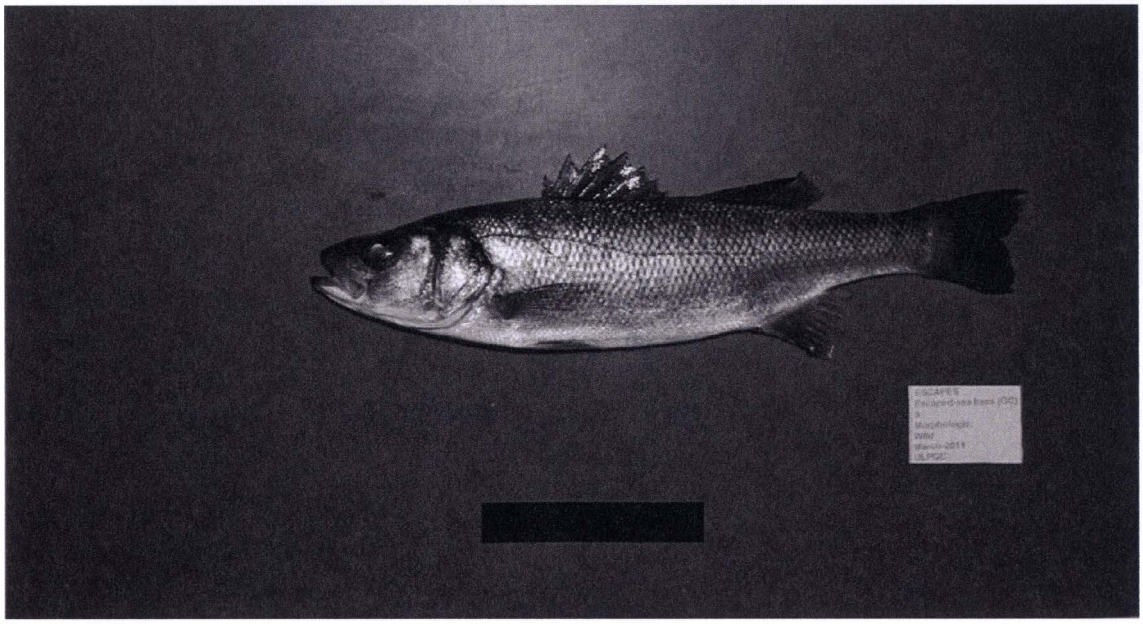


Figura 3.5. Lubina escapada colocada sobre superficie diseñada para el posterior análisis morfométrico

Figura 3.6. Examen macroscópico gonadal de un ejemplar de corvina (*Argyrosomus regius*)

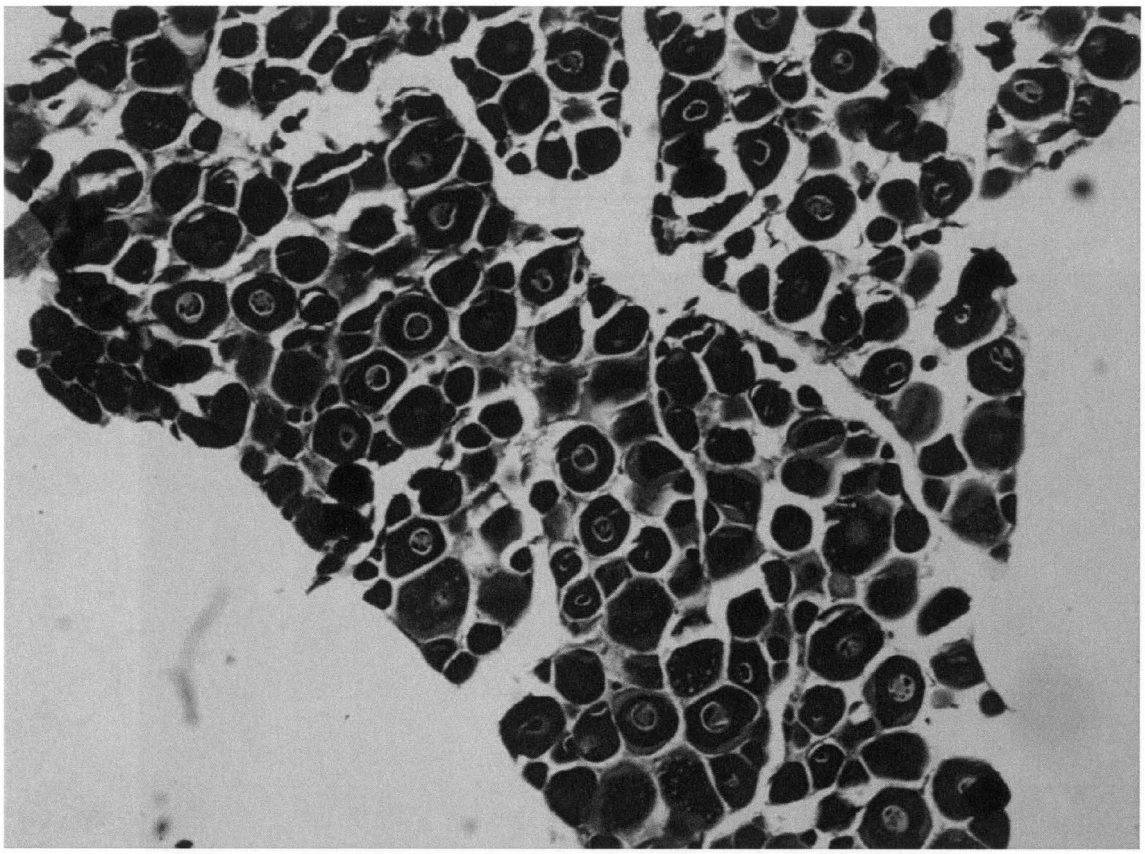


Figura 3.7. Corte histológico de gónada de hembra de lubina (*Dicentrarchus labrax*) escapada. Estado II (inmaduro tardío) según clasificación de Wallace and Selman (1981).

3.5 Análisis bioquímico

La composición bioquímica de los peces fue analizada siguiendo el procedimiento estandarizado AOAC (2000). Contenido en cenizas fue determinado por combustión en horno-mufla a 600ºC durante 12 horas. La humedad se calculó secando la muestra a 105ºC, hasta observar que el peso de la misma permanecía constante. Los lípidos totales fueron extraídos siguiendo los procedimientos propuestos por Folch et al. (1957).

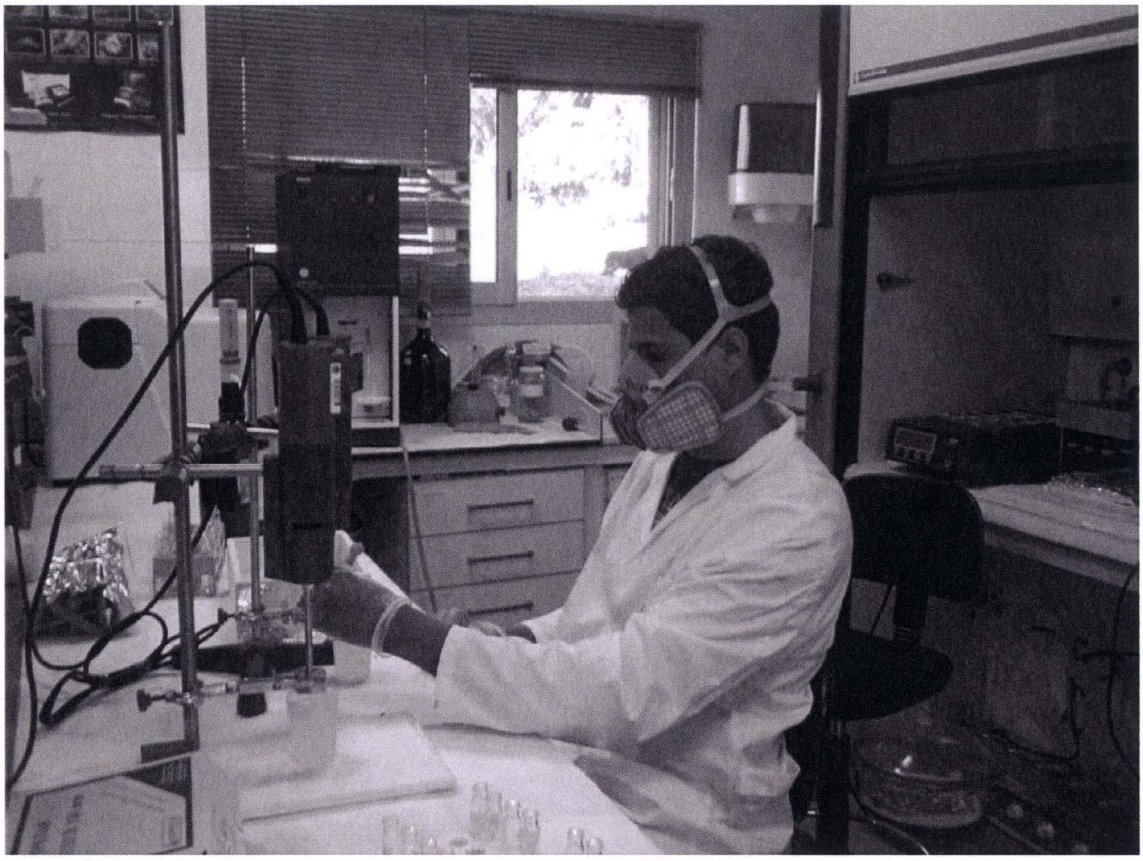


Figura 3.8. Análisis bioquímico en el laboratorio analítico del Grupo de Investigación en Acuicultura, Instituto Universitario de Sanidad Animal (IUSA).

Finalmente, los ácidos grasos fueron obtenidos de los lípidos totales (que habían sido conservados en atmósfera de nitrógeno a -80ºC) mediante la transmetilación descrita por Christie (1982) y los metil ésteres de ácidos grasos, separados por cromatografía de gases bajo las condiciones descritas por Izquierdo et al. (1990). Todos estos análisis se realizaron por triplicado.

3.6 Establecimiento de poblaciones

Como ya adelantamos, con el fin de estudiar el establecimiento de las poblaciones de peces escapados, se realizaron censos visuales, estudios de telemetría pasiva, contenidos estomacales y desarrollo gonadal. Cada una de estas técnicas está ampliamente detallada en su respectivo capítulo de tesis.

4

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

ÁCIDOS GRASOS COMO BIOINDICADORES

4 "AQUAFEED IMPRINT ON BOGUE (*Boops boops*) POPULATIONS AND THE VALUE OF FATTY ACIDS AS INDICATORS OF AQUACULTURE-ECOSYSTEM INTERACTION: ARE WE USING THEM PROPERLY?

Besay Ramírez[a,b], Daniel Montero[a], Marisol Izquierdo[a], Ricardo Haroun[b]

[a]Grupo de Investigación en Acuicultura. Universidad de Las Palmas de Gran Canaria and Instituto Canario de Ciencias Marinas. P.O. Box 56. 35200. Telde, Las Palmas, Canary Islands, Spain.

[b]BIOGES, Marine Sciences Faculty, Campus Tafira, Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas de G.C., Canary Islands, Spain.

4.1 Abstract

The increasing aquaculture production in coastal areas has resulted in different interactions with the environment. Thus, studies on the escape of farmed fish into the ecosystem have been recently increased for different reasons such as competition with wild populations, genetic pollution, among others. Morphological and physiological indicators have been proposed as biomarkers for escapees, including biometrical parameters, morphology of scales and otholits, microchemistry of scales, RNA/DNA ratios or fatty acid content of muscle. Fatty acid profile have been received increasing attention due to the changes of ingredients and feeding strategies occurring within aquafeeds in the last years, with an increased use of terrestrial ingredients to substitute marine ingredients. This study evaluates 1) the effect of wasted food on fatty acid of a farm-associated and 2) the suitability of fatty acid profile as a bioindicator of aquaculture-ecosystem interactions, using the bogue (*Boops boops*) as a model. This species is an opportunistic fish usually associated to –or even within- sea farms, and with a high natural occurrence in Mediterranean and Atlantic coasts,

which integrates in its body composition the feeding strategies arising from the different habitats in which this species is located.

Keywords: aquaculture-ecosystem interaction, wasted aquafeeds, fatty acid indicator

4.2 Introduction

Aquaculture constitutes the fastest growing food production sector and the main contributor of marine food to satisfy the demand of the unceasingly increasing human population (Tacon and Metian 2009). Nevertheless, this fast growth can only be maintained if it is sustainable under a social, economic and, particularly, environmental point of view. Thus, the fast development of aquaculture production in coastal areas, particularly when it is sea-based, has promoted the research on its interactions with the environment, (Naylor et al., 2000; Black, 2001; Hargrave, 2005; Holmes et al., 2008). Aquaculture activity may affect marine biodiversity in relation to genetic variability, species-species interaction or ecosystem alteration (CBD, 2004). The most direct effect of aquaculture on the environment, and the most studied, is that coming from wasted food and, secondly, faecal discharges, that may modify the characteristics of the sediment under the fish cages (Molina-Dominguez et al., 2001; Mente et al., 2006). Besides, the sea cages presence may alter the icthyological community around them (Carss, 1990; Machias et al., 2004; Tuya et al., 2006; Dempster et al., 2005). Nevertheless, other types of impacts could relate to the addition of anti-fouling treatments, transfer of parasites or exotic species, discharge of toxic therapeutic products or fish escapes (Hewitt et al., 2003; IUCN, 2007; Fernández-Jover et al., 2010).

Escapes of fish from sea-cage aquaculture have been reported for many aquaculture species including Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*) Arctic char (*Salvelinus alpinus*), halibut (*Hippoglossus hippoglossus*), gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrachus labrax*), meagre (*Argyrosomus regius*) or kingfish (*Seriola lalandi*) (Soto et al., 2001, Naylor et al. 2005,

Gillanders and Joyce, 2005, Moe et al., 2007). The nature of the escapes is multi-factorial but is mainly related to farming equipments and its operations. Massive fish escapes (more than 10.000 fish) are very rare and represent only 19% of the escape incidents reported in salmon or cod, but they account for 91% of the total number of escaped fish (Jensen et al., 2010). When escaped fish belong to an exotic species, the environmental impact is less predictable and is consider as one of the major threats of aquaculture activities to ecosystems from both a biological or an economic perspective (Soto et al., 2001). If the escapes belong to a native species, the interactions escaped-wild fish are difficult to precise and may include the mutual transfer of diseases and pathogens, competition for food or interbreeding (Jensen et al., 2010), despite both types of fish differ on behavioral ecology and life history depending on the two niches: wild and aquaculture (Gross, 1998).

To identify those animals coming from aquaculture in order to better understand these interactions, it is necessary to develop an appropriate methodological approach. Several morphological and physiological indicators have been proposed to be useful to identify escapees, including biometrical parameters (Uglem et al., 2011; Ellis et al., 2009), scales and otholits morphology (Person-Le Ruyet and Le Bayon 2009; Katayama and Isshiki 2007), scales mineral contents (Adey et al., 2009) RNA/DNA ratios (Gwak et al., 2003) or muscle fatty acid composition (Grigorakis et al., 2002; Fernández-Jover et al., 2007). Regarding the latest, the fatty acid profile has been claimed to be a good bio-indicator of ecosystem health (Dunn et al., 2008; Hu et al., 2008; Maazouzi et al., 2008). The fatty acid profile has received an increasing attention due to the changes in aquafeeds ingredients during the last years. Traditionally, fish were fed diets based on fishmeal and fish oil as mean ingredients for its adequate price and content in some essential fatty acids for fish, such as docosahexaenoic acid (DHA) (22:6n-3), eicosapentaenoic acid (EPA) (20:5n-3) and arachidonic acid (ARA) (20:4n-6). These fatty acids, which have a wide plethora of very important functions, are consider

essential for marine fish since these species have not the ability to bio convert shorter fatty acids into these fatty acids due to the very low activity of delta 6 and delta 5 desaturase in these fish (Izquierdo and Koven 2011). However, within the last decade, the world production of fish oil has become stagnant, global fish oil costs increased and fish oil inclusion in compound aquafeeds has been substituted by more sustainable vegetable oils (Tacon and Metian 2008). The use of vegetable oils in marine fish is constrained by their lack of n-3 HUFA, despite the high abundance of the 18:C fatty acid precursors linoleic acid (LA) (18: 2n-6) and alpha-linolenic acid (ALA) (18: 3n-3) (Turchini et al., 2009). These precursor fatty acids are incorporated into the tissues of aquaculture fish, but can be also transferred to wild fish. Fish around the cages may feed on waste pellets from farms, what leads to changes in body condition and fatty acid profiles making their body composition and that of other organisms in different trophic levels more similar to that of cultivated fish (Skog et al. 2003; Dalsgaard et al., 2003; Fernández-Jover et al., 2007). For this reason, the presence of certain fatty acids, such as LA and oleic acid (OA) (18: 1n-9), in wild organisms has been proposed as an indicator of the influence of aquaculture on marine ecosystems (Rueda et al., 2001; Fernández-Jover et al., 2007; Megdal et al., 2009). Nevertheless, the presence of each of these fatty acids in fish tissues may be affected by different factors that should be considered such as the metabolic utilization of dietary fatty acids, their selective retention or the period required to deplete them from the different tissues (Izquierdo et al., 2005). Moreover, the presence of those fatty acids in wild fish could be also related to a different origin than aquaculture feeds, such as other human activities (Quemeneur and Marty 1992; Sargent et al., 2002; Wong et al., 2008). Thus, the objective of the present study was 1) to evaluate the effect of wasted food on the fatty acid composition of a farm-associated and 2) the suitability of fatty acid profile as a bioindicator of aquaculture-ecosystem interactions, i.e., the potential value of some fatty acids as aquafeed tracers on escaped fish. The bogue was used as a model,

since this species is an opportunistic fish frequently associated to sea farms (Dempster et al., 2005), but also with a high natural occurrence in Mediterranean and Atlantic coasts. The location of the study in an oceanic Archipelago (Canary Islands) composed of several islands with a very different degree of development of coastal aquaculture or human activity, has allow to compare isolated sites with very different characteristics to better define the relative importance of aquaculture activities to the fatty acid profile of sea-cage associated fish communities.

4.3 Materials and Methods

4.3.1 Sampling points

To determine the potential area of influence of aquaculture activities on wild fish fatty acid profiles and compare it with those of fish from areas free of aquaculture production but having or not other human activities, seven sampling points were selected.

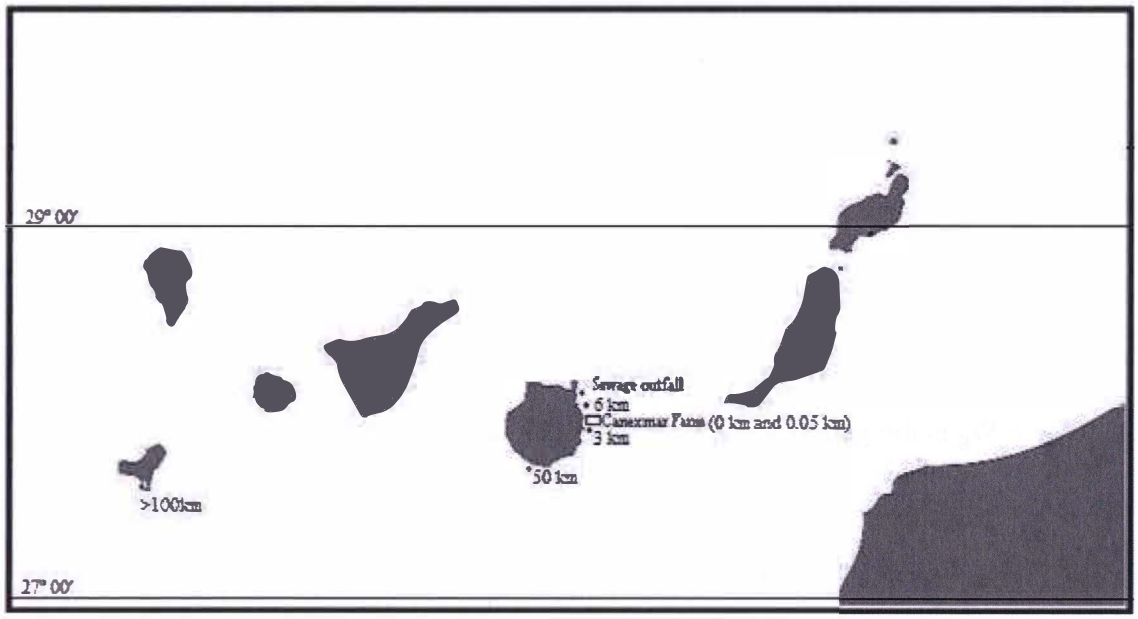


Figure 4.1. Location of stations for sampling points at Canary Islands, in Central East Atlantic Ocean.

The first sampling point was located inside a cage of a fish farm at Tufia (0 km), east coast of Gran Canaria Island, were wild bogue have entered and fed almost exclusively on aquafeeds. The second one was located near the cages (0.05 km). The third was 3 km away from the fish

farm at Gando, east coast of Gran Canaria (3 km). A fourth sampling point was located at 6 km from the fish cages (6 km). The fifth was in Arguineguín, south west coast of Gran Canaria Island (50 km), where there is no aquaculture, but animals coming from aquaculture could be also present. The sixth sampling point was located in an aquaculture free island, El Hierro Island, were a marine reserve is located (>100km) (Fig. 4.1). Finally, the seventh sampling point was at the vicinity of sewage outfall from Las Palmas de Gran Canaria city that fulfills all the legal requirements of Spanish and European Union legislations for this type of outfalls.

4.3.2 Fish samples

Fish samples were obtained from the different sampling points defined. At least 20 individuals from each sampling point were obtained within same week by fishing or netting, both during summer (August) and winter (February). All individuals were sacrificed after sampling and kept in ice until dissection in the lab, where whole fish and their livers were weighed and measured. From each sampling point, 9 fish were used for whole fish analysis, whereas liver and muscle were dissected from another 9 individuals, all the samples being packed under vacuum and kept at -80ºC until analysis. Fulton´s K condition index (CI) was calculated as an indicator of general well-being of individuals. This morphometric index is based on the assumption that heavier fishes for a given length are in better condition: $K = 100*(W_g/L_t^3)$, where W_g is the gutted body weight (g) and L_t is the total length (cm), and was calculated for all the animals captured. Hepatosomatic index (HSI) was calculated as $HSI=(\text{liver weight} \times \text{bodyweight}^{-1}) *100$.

4.3.3 Biochemical and fatty acid analysis

Biochemical compositions of fish were analyzed following standard procedures (AOAC, 2000). Ash content was determined by combustion in a muffle furnace at 600 °C for 12 h; moisture content was determined by drying the sample at 105 °C until it achieved a

constant weight; finally, crude lipid content was extracted following the method of Folch et al. (1957). Fatty acids from total lipids (stored under nitrogen atmosphere at−80 °C) were prepared by transmethylation as described by Christie (1982) and fatty acid methyl esters separated by gas chromatography under the conditions described by Izquierdo et al. (1990). All analyses were conducted in triplicate.

4.3.4 Statistical analysis

To test whether condition index, HSI, fat content and the main FAs varied among the proximity and season, an analysis of variance (ANOVA) was used, which incorporated the factors – season (fixed) with two levels (summer and winter) and proximity (fixed) with 6 levels (localities): 0km, 0.05km, 3km, 6km, 50km and >100km. Fish weight and liver weight (in liver analysis) were covariates. Variance was not homogeneous and data transformations did not get homogeneity, then significance level at 0.01 ($P<0.01$) was defined, and increased confidence level (99%). (Underwood, 1997). Principal components analysis (PCA) was used as the ordination method. Variables that had more influence on similarities within groups and dissimilarities among groups of locations were calculated using the SIMPER (similarity percentages) procedure (Clarke, 1993). Inside sea cages (0km) were compared with sewage outfall. A permutation test (PERMANOVA) was used to assess the significance of the overall fatty acid composition among the considered sources of variation (Anderson, 2004).

4.4 Results

Those fish inside sea cages showed aquaculture-related increases of CI and HSI. CI was significantly ($P<0.01$) higher for bogues inside the fish cage (0km). Besides, HSI was significantly ($P<0.01$) higher inside sea cages when compared to values obtained at 6 and 50 km stations (Table 4.1). Proximate composition analysis showed that there were also influences of aquaculture on biochemical composition of fish inside sea cages. Lipid content

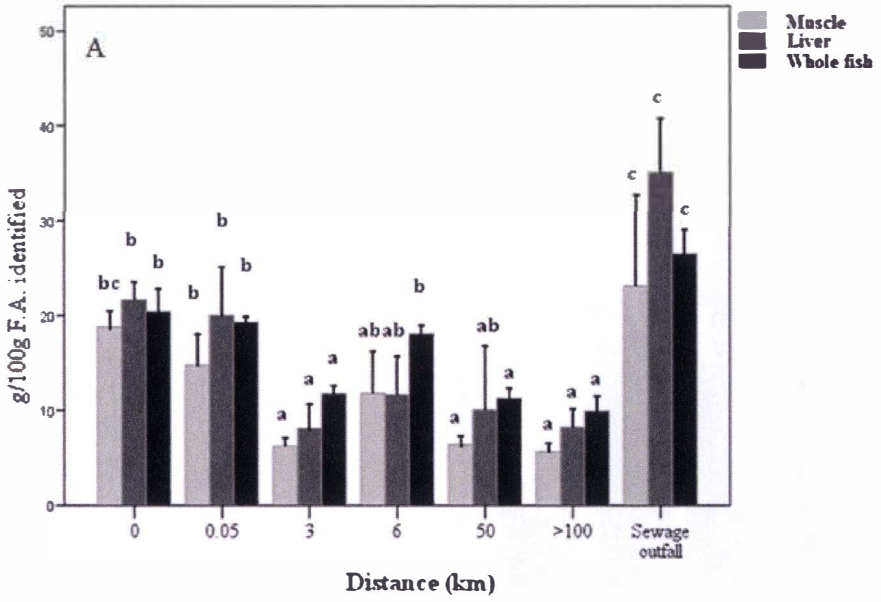
of fish inside the farm cage (0 km) showed significantly (P<0.01) higher lipid content in muscle and whole fish, followed by that of bogue at 0.05 km (Table 4.1).

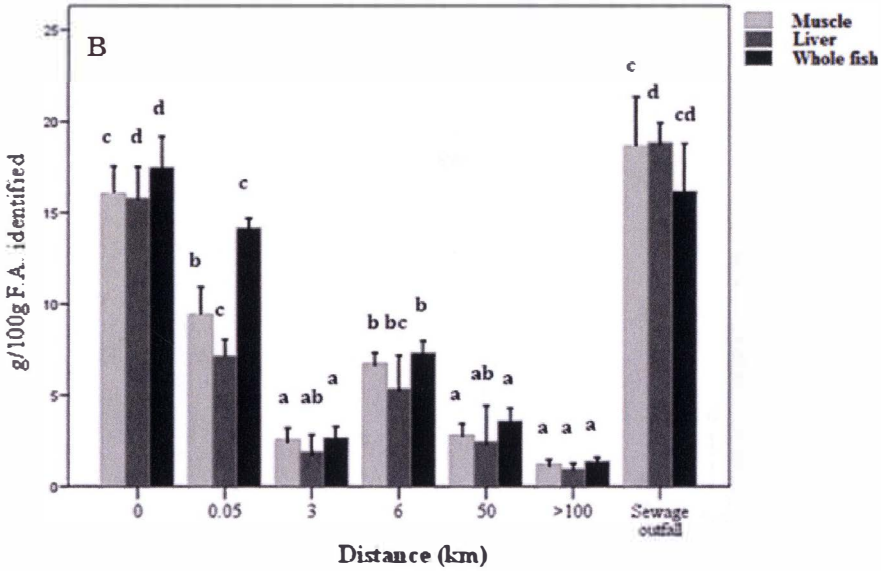
Table 4.1. Crude lipid, moisture and ash content of muscle, liver and whole fish, and condition index and HSI of bogue (*Boops boops*) from different sampling points. (Mean ± SD)

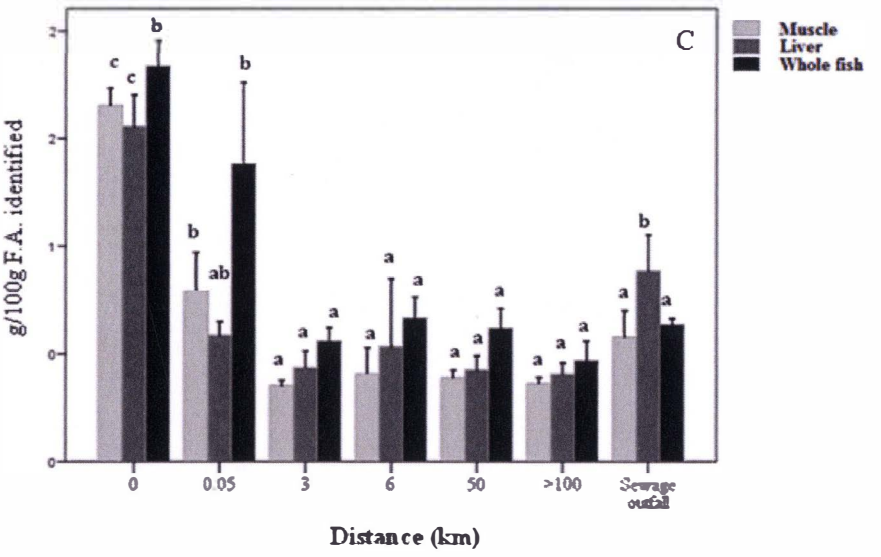
	0km	**0.05km**	**3km**	**6km**	**50km**	**>100km**	**Sewage outfall**
Crude Lipid (% d.w)							
Muscle	18.8 ± 2.2^{b}	8.3 ± 3.7^{a}	4.9 ± 1.3^{a}	5.6 ± 1.8^{a}	4.0 ± 0.5^{a}	4.6 ± 0.3^{a}	5.8 ± 0.6^{a}
Liver	58.8 ± 10.7^{c}	28.2 ± 13.7^{ab}	16.1 ± 2.4^{a}	29.1 ± 9.3^{ab}	19.2 ± 2.0^{a}	14.8 ± 3.5^{a}	41.6 ± 5.4^{bc}
Whole fish	40.8 ± 5.4^{c}	22.9 ± 2.1^{b}	7.5 ± 2.6^{a}	13.6 ± 2.1^{a}	8.8 ± 4.4^{a}	9.2 ± 1.1^{a}	7.0 ± 2.7^{a}
Moisture (%)							
Muscle	72.1 ± 0.7^{a}	75.0 ± 0.9^{b}	77.4 ± 0.4^{d}	77.7 ± 0.7^{d}	77.5 ± 1.4^{d}	75.5 ± 0.6^{cb}	77.3 ± 0.5^{dc}
Liver	52.4 ± 5.8^{a}	65.7 ± 5.0^{cb}	73.4 ± 2.5^{c}	69.1 ± 6.7^{c}	72.0 ± 1.7^{c}	73.5 ± 1.1^{c}	56.5 ± 3.0^{ba}
Whole fish	61.3 ± 5.4^{a}	69.8 ± 0.8^{b}	76.2 ± 1.3^{c}	75.0 ± 1.5^{cb}	76.8 ± 1.9^{c}	76.2 ± 0.7^{c}	75.0 ± 0.7^{cb}
Ash content (% d.w.)							
Muscle	1.2±0.3	1.8±0.7	1.5±0.2	1.4±0.3	1.4±0.1	1.3±0.3	1.6±0.1
Whole fish	2.1±0.7	4.6±1.1	4.5±1.1	3.3±0.9	2.7±1.7	2.2±1.0	5.1±0.7
CI **	1.8 ± 0.3^{b}	1.1 ± 0.1^{a}	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}	1.1 ± 0.0^{a}	1.1 ± 0.1^{a}
HSI	0.8 ± 0.4^{b}	0.7 ± 0.3^{ab}	0.5 ± 0.2^{ab}	0.2 ± 0.1^{a}	0.3 ± 0.2^{a}	0.5 ± 0.1^{ab}	0.4 ± 0.2^{ab}

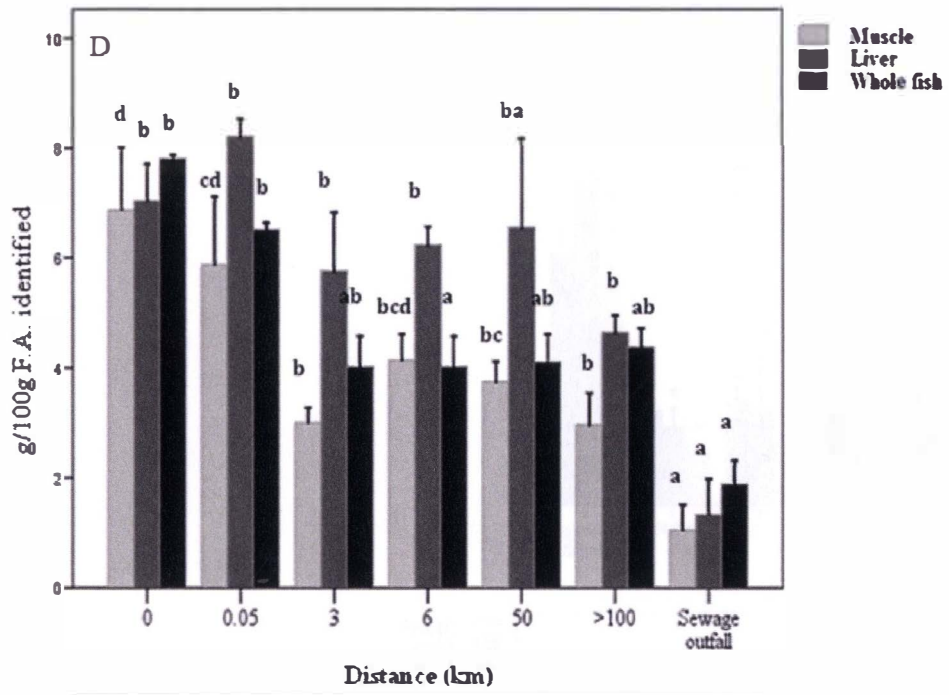
Different letters within a row denotes significant differences (P<0.01) among sampling points. N=18. ** n=40

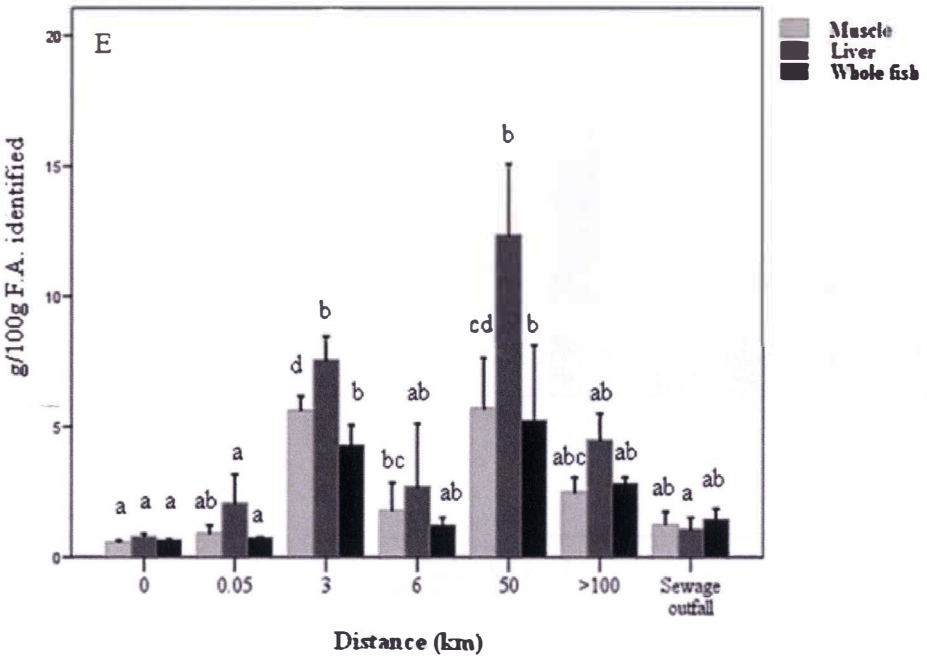
In the liver, the higher (P<0.01) lipid content was also found in fish inside sea cages, but this value did not differ from that of fish coming from the sewage outfall. Moisture content was inversely related to lipid contents, being significantly (P<0.05) lower in liver, muscle and whole fish of bogue inside the cage (0km) (Table 4.1). No significant differences were found for ash content.


A
Muscle
Liver
Whole fish
g/100g F.A. identified
0
10
20
30
40
50
bc b b b b b a a a ab ab b a ab a a a c c c
0
0.05
3
6
50
>100
Sewage outfall
Distance (km)


B
Muscle
Liver
Whole fish
g/100g F.A. identified
0
5
10
15
20
25
c d d b c c a ab a b bc b a ab a a a a c d cd
0
0.05
3
6
50
>100
Sewage outfall
Distance (km)


C
Muscle
Liver
Whole fish
g/100g F.A. identified
0
1
2
c c b b ab b a a a a a a a a a a a a b a
0
0.05
3
6
50
>100
Sewage outfall
Distance (km)


D
Muscle
Liver
Whole fish
g/100g F.A. identified
0
2
4
6
8
10
d b b
cd b b
b ab a
bcd b a
bc ba ab
b b ab
a a a
0
0.05
3
6
50
>100
Sewage outfall
Distance (km)


E
Muscle
Liver
Whole fish
g/100g F.A. identified
0
5
10
15
20
a a a
ab a a
d b b
bc ab ab
cd b b
abc ab ab
ab a ab
0
0.05
3
6
50
>100
Sewage outfall
Distance (km)

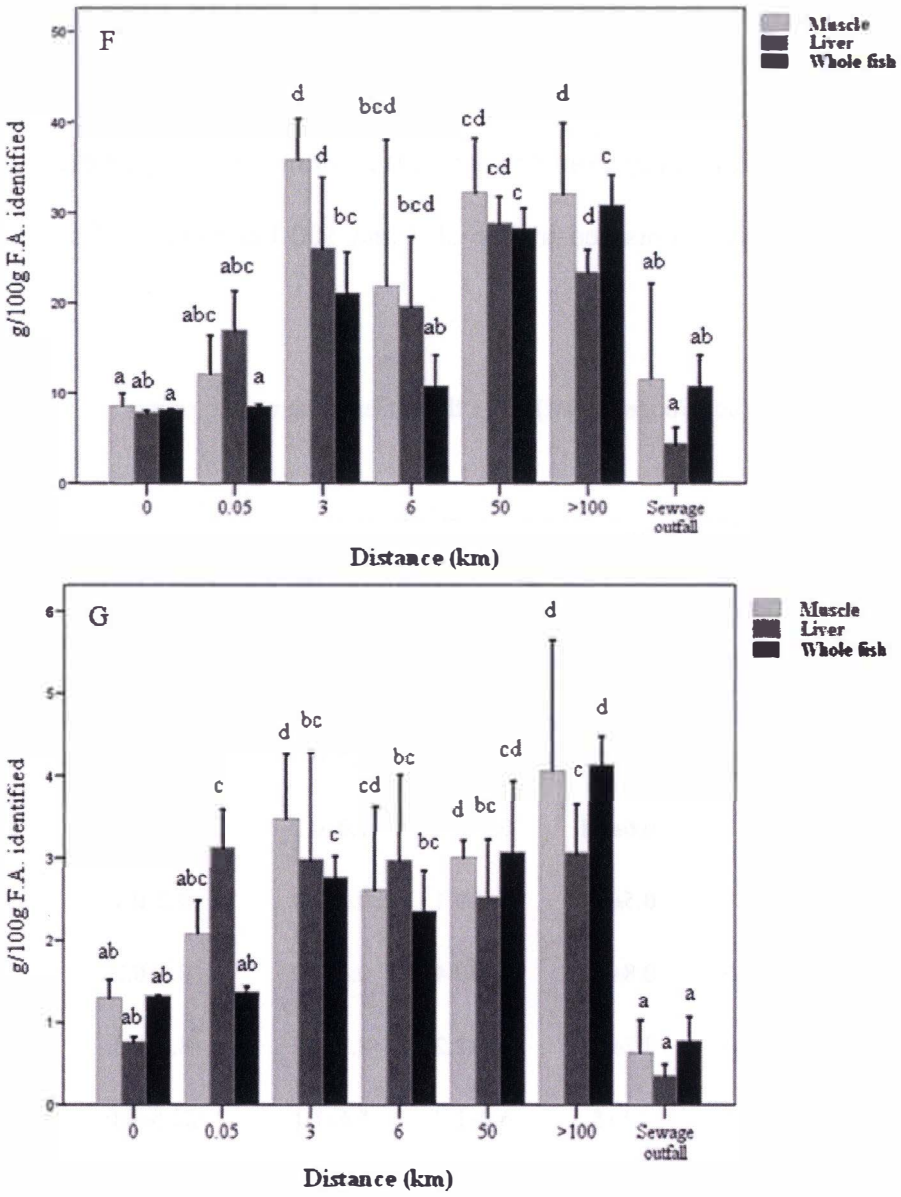


Figure 4.2. Effect of seacages farm and sewage outfall on selected fatty acids in bogue (*Boops boops*) at different sampling points. A) Oleic acid (18:2 n-9); B) Linoleic acid (18: 2n-6); C) Linolenic acid (18:3n-3); D) Eicosapetaenoic acid (20:5n-3); E) Arachidonic acid (20: 4n-6); F) Docosahexaenoic acid (22: 6n-3); G) ratio n-3/n-6.

Many minor fatty acids did not statistically differ among the different samples and were removed from the tables. In general, regardless the tissue studied, palmitoleic (16:1n-7) and linolenic (18:3n-3, ALA) acids tend to be higher in fish inside the cage and at 0.05km than in the other sampling points (Tables 4.2, 4.3 and 4.4). Besides, linoleic acid (18:2n-6, LA) tended to be higher and arachidonic (20: 4n-6, ARA) and docosahexaenoic (22: 6n-3,

DHA) acids tended lower in fish coming from either inside the cage, at 0.05km or in the sewage outfall, than in the other sampling points (Tables 4.2, 4.3 and 4.4). More in detail, OA was significantly ($P<0.01$) higher in fish coming from sewage outfall samples in any of the tissues studied, whereas lowest values were obtained in fish at 3 and 100 km from the fish farm (Fig. 4.2a).

Table 4.2. Muscle Fatty Acids profile of bogue (*Boops boops*) from the different sampling points (mean ± SD). N= 18.

	Muscle						
	0 km	**0.05 km**	**3 km**	**6 km**	**50 km**	**>100 km**	**Sewage outfall**
14:0	3.8±1.0	3.3±1.2	1.0±0.3	2.1±1.4	1.1±0.6	1.2±0.7	1.3±0.4
16:1n-7	5.6±1.3	4.7±1.7	1.6±0.5	3.4±1.9	1.9±0.5	1.5±0.6	2.4±0.7
16:2n-4	0.7±0.2	0.6±0.1	0.6±0.1	0.6±0.1	0.9±0.2	1.0±0.2	0.3±0.0
17:0	0.7±0.2	0.6±0.2	0.2±0.1	0.5±0.2	0.3±0.1	0.3±0.0	0.2±0.1
16:4n-3	0.5±0.3	0.5±0.2	0.7±0.2	0.8±0.2	0.8±0.4	0.4±0.0	0.6±0.2
18:0	6.3±1.6	7.3±1.1	7.6±0.4	7.7±0.9	9.2±2.2	8.3±1.2	8.2±0.5
18:1n-9	18.8±2.1	14.8±4.0	6.2±1.1	11.8±5.5	6.0±1.2	5.6±1.1	22.6±6.0
18:1n-7	3.0±0.8	3.1±0.4	1.7±0.9	2.7±0.4	2.4±0.6	2.1±0.2	2.7±0.2
18:2n-6	16.0±1.9	9.4±1.8	2.6±0.8	6.7±0.7	2.8±0.8	1.2±0.3	18.6±1.7
18:3n-3	1.7±0.2	0.9±0.2	0.4±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.6±0.1
18:4n-3	0.9±0.3	0.5±0.3	0.2±0.1	0.3±0.1	0.2±0.1	0.1±0.1	0.1±0.0
18:4n-1	0.2±0.1	0.1±0.1	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.1±0.0
20:1n-9+n-7	1.0±0.1	1.3±0.3	2.0±1.2	1.1±0.5	0.7±0.1	1.5±0.4	0.8±0.2
20:1n-5	0.2±0.0	0.2±0.1	0.1±0.1	0.2±0.1	0.1±0.0	0.1±0.0	0.1±0.0
20:4n-6	0.6±0.1	1.3±0.5	5.2±0.6	3.0±1.4	4.4±1.8	2.5±0.3	1.3±0.3
20:3n-3	0.1±0.0	0.1±0.0	0.3±0.1	0.1±0.0	0.2±0.0	0.2±0.1	0.1±0.0

20:4n-3	0.5±0.1	0.4±0.1	0.3±0.0	0.3±0.0	0.3±0.1	0.2±0.1	0.1±0.0
20:5n-3	6.1±1.2±	5.4±0.9	3.5±0.6	4.7±0.6	4.0±0.7	3.2±0.4	1.1±0.3
22:5n-6	0.2±0.0	1.0±0.5	2.8±0.3	1.9±0.7	2.8±0.6	3.3±0.6	1.2±0.6
22:5n-3	1.8±0.2	2.1±0.5	1.0±0.2	2.3±0.5	1.0±0.3	0.9±0.2	0.5±0.2
22:6n-3	8.0±1.3	17.1±6.6	35.9±5.2	26.0±10.0	32.3±8.1	38.4±8.5	12.0±6.6

Table 4.3. Liver fatty acid profile of bogue (*Boops boops*) from the different sampling points (mean ± SD). N=18

	Liver						
	0 km	**0.05 km**	**3 km**	**6 km**	**50 km**	**>100 km**	**Sewage outfall**
14:0	3.4±0.3	2.5±0.9	1.3±0.2	1.4±1.1	2.0±0.7	1.6±0.3	2.6±0.2
16:1n-7	5.9±0.4	4.6±1.1	1.9±0.5	3.2±2.0	2.6±1.0	2.1±0.6	3.9±0.6
16:2n-4	0.7±0.1	0.7±0.2	0.9±0.4	0.8±0.3	1.0±0.6	1.6±0.2	0.3±0.1
17:0	0.6±0.1	0.5±0.2	0.2±0.1	0.3±0.2	0.3±0.1	0.3±0.2	0.2±0.0
16:4n-3	0.3±0.2	0.2±0.1	0.1±0.1	0.1±0.0	0.3±0.1	0.0±0.0	0.0±0.0
18:0	5.9±0.9	8.8±1.2	7.8±0.6	9.1±2.7	9.5±0.9	8.5±1.8	5.0±0.1
18:1n-9	21.7±2.3	20.0±6.2	8.1±3.1	11.7±4.9	10.0±8.3	8.3±2.4	35.1±3.1
18:1n-7	3.2±0.3	3.2±0.5	1.6±0.2	2.7±0.7	2.0±0.4	1.6±0.4	2.5±0.2
18:2n-6	15.8±2.1	7.1±1.1	1.9±1.1	5.3±2.3	2.5±2.4	1.0±0.4	18.8±0.6
18:3n-3	1.6±0.2	0.7±0.1	0.4±0.1	0.4±0.2	0.5±0.1	0.3±0.1	0.9±0.1
18:4n-3	0.6±0.3	0.4±0.2	0.3±0.1	0.3±0.2	0.2±0.1	0.1±0.0	0.3±0.0
18:4n-1	0.2±0.1	0.2±0.1	0.0±0.0	0.0±0.1	0.0±0.0	0.0±0.0	0.1±0.1
20:1n-9+n-7	1.2±0.0	1.5±0.3	2.9±1.0	0.9±0.2	1.2±0.7	2.6±0.8	0.7±0.1
20:1n-5	0.3±0.0	0.3±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.0	0.1±0.0
20:4n-6	0.6±0.2	1.5±0.9	8.1±0.9	5.1±3.0	7.9±5.3	4.5±0.6	1.1±0.2

20:3n-3	0.1±0.0	0.1±0.0	0.6±0.1	0.1±0.0	0.2±0.0	0.4±0.1	0.0±0.0
20:4n-3	0.6±0.2	0.6±0.1	0.6±0.1	0.4±0.2	0.4±0.1	0.4±0.1	0.1±0.0
20:5n-3	5.6±1.7	6.4±2.0	5.9±0.7	5.2±1.4	4.8±2.3	5.6±1.1	1.3±0.4
22:5n-6	0.2±0.0	0.4±0.1	1.5±0.3	0.9±0.3	1.2±0.4	2.0±0.5	0.3±0.0
22:5n-3	2.2±0.4	2.8±0.6	1.4±0.2	2.2±1.2	1.2±0.3	1.9±0.6	0.4±0.2
22:6n-3	6.1±1.9	12.0±5.9	26.6±4.7	19.2±4.3	23.0±9.4	28.1±5.9	4.4±1.0

Table 4.4 Whole fish fatty acid profile of bogue (*Boops boops*) from the different sampling points (mean ± SD). N=18.

	Whole fish						
	0 km	**0.05 km**	**3 km**	**6 km**	**50 km**	**>100 km**	**Sewage outfall**
14:0	4.2±0.4	4.3±0.2	2.8±0.4	4.3±1.5	2.5±1.5	2.3±0.2	2.4±0.0
16:1n-7	6.5±0.5	6.0±0.2	3.4±0.5	6.1±1.3	2.9±1.3	2.8±0.1	3.3±0.3
16:2n-4	0.7±0.3	0.7±0.1	1.0±0.4	0.9±0.0	1.1±0.5	1.4±0.2	0.3±0.1
17:0	0.8±0.2	0.8±0.1	0.4±0.1	0.8±0.2	0.5±0.1	0.3±0.1	0.3±0.0
16:4n-3	0.6±0.3	0.6±0.1	0.7±0.4	0.6±0.3	0.7±0.7	0.2±0.0	0.7±0.1
18:0	4.9±0.3	5.6±0.4	8.6±1.1	6.7±1.0	8.7±1.0	8.4±1.1	7.1±0.8
18:1n-9	19.9±2.9	19.4±0.8	11.8±1.1	18.1±1.2	11.2±1.3	9.6±2.0	26.6±1.6
18:1n-7	3.3±0.6	3.0±0.7	2.8±0.2	3.6±0.5	2.5±0.3	2.2±0.5	2.2±0.2
18:2n-6	17.1±2.1	14.1±0.7	2.8±0.8	7.2±0.9	3.4±0.9	1.4±0.3	16.4±1.7
18:3n-3	1.7±0.3	1.6±0.1	0.6±0.2	0.7±0.1	0.8±0.2	0.4±0.1	0.6±0.0
18:4n-3	1.1±0.5	0.9±0.1	0.5±0.2	0.8±0.2	0.5±0.3	0.3±0.1	0.3±0.0
18:4n-1	0.2±0.1	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
20:1n-9+n-7	1.1±0.1	1.7±0.3	3.5±1.2	1.7±0.3	1.3±0.6	3.0±1.2	1.1±0.2
20:1n-5	0.2±0.0	0.3±0.0	0.3±0.1	0.3±0.1	0.2±0.1	0.3±0.2	0.1±0.0

	Whole fish						
20:4n-6	0.6±0.1	0.8±0.1	4.0±0.9	2.0±1.1	4.1±2.4	2.6±0.4	1.4±0.2
20:3n3	0.1±0.0	0.1±0.0	0.3±0.1	0.1±0.0	0.2±0.1	0.3±0.0	0.1±0.0
20:4n-3	0.5±0.1	0.5±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.3±0.0	0.2±0.0
20:5n-3	6.5±1.8	5.9±1.0	4.2±0.5	5.8±1.2	4.1±0.4	4.0±0.5	1.9±0.3
22:5n-6	0.2±0.0	0.4±0.1	1.5±0.3	0.6±0.2	1.9±0.5	2.5±0.4	0.9±0.3
22:5n-3	1.8±0.3	2.3±0.5	1.1±0.2	2.1±0.4	1.1±0.3	1.1±0.1	0.5±0.1
22:6n-3	6.6±2.1	8.3±0.9	19.6±3.4	10.4±2.6	23.6±6.6	27.0±5.6	10.4±2.2

Muscle, liver and whole fish LA content were significantly ($P<0.01$) higher in fish inside sea cages, but not different from those coming from the sewage outfall (Fig. 4.2b). Alpha linolenic acid was significantly ($P<0.01$) highest in fish inside the cage or at 0.05km (Fig. 4.2c). Interestingly, EPA values obtained for muscle 0km was significantly ($P<0.01$) higher than the values obtained for the rest of the sampling points except from those coming from 0.05km and 6 km point. Sewage outfall fish had the lowest ($P<0.01$) content in EPA in muscle (Fig 4.2d). Arachidonic acid values varied markedly among the different samples, without a clear pattern, denoting the essentiality of this fatty acid and suggesting a potential relation with the different dietary regimes. Nevertheless, values were significantly ($P<0.01$) lower for fish at 0km, 0.05km and sewage outfall, without significant differences with 100 km samples (Fig. 4.2e). Similarly, DHA also varied among samples and were significantly ($P<0.01$) lowest at 0km, 0.05km and at the sewage outfall (Fig. 4.2f). Since n-6 fatty acids were higher in fish inside the sea cages, at 0.05 km and the sewage outfall and long chain n-3 fatty acids were lower, the ratio n-3/n-6 was significantly lowest for those fish (Fig. 4.2g).

The PCA analysis of fatty acids contents showed two different groups of samples: those from fish obtained inside the sea cages (0km), at 0.05km together or in the sewage

outfall in one hand and those located at 3km, 50km and >100km in the other hand (Fig. 4.3). The data obtained in the 6km group remained between both groups. PERMANOVA analysis showed that these differences in fatty acid composition were significant ($P<0.01$). A whole SIMPER analysis of pooled data from the three tissues showed dissimilarity between samples of fish inside the cage, at 0,05km or the sewage outfall and samples at 3km, 6km, 50km and 100km, was high 34.07. This analysis showed that the main differentiating FAs were DHA, OA and LA (Table 4.5), followed by palmitic acid (16:0).

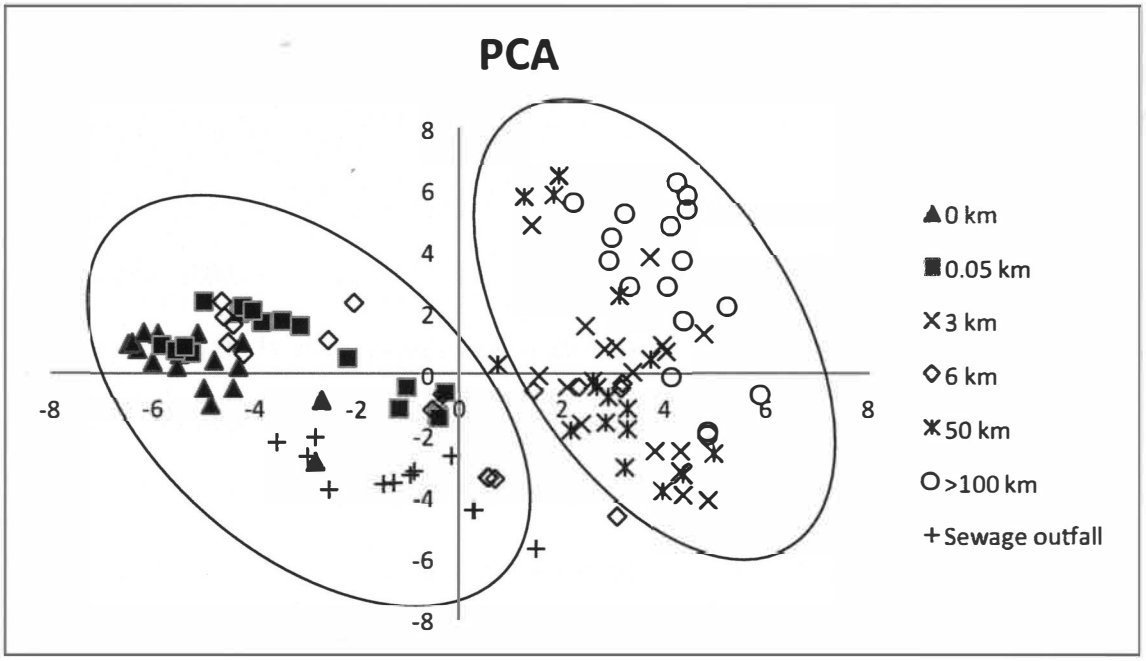


Figure 4.3. Principal Component analysis (PCA) of all fatty acids within the different sampling points.

Besides, dissimilarity given by SIMPER analysis between samples from animals inside sea cage (0km) and the sewage outfalls was low: 19.35. This analysis showed that the main differentiating FAs were OA, EPA and DHA (24.43, 12.30 and 10.32 % of contribution respectively). Other fatty acids such as LA contributed with 6.79, whereas ARA contributed with 1.64% (Table 4.6).

Table 4.5. SIMPER analysis. Contribution of the main fatty acids in different tissues (muscle liver and whole fish) to overall dissimilarities between human input (aquaculture-related sampling points plus sewage outfall) and the rest of sampling points away from cages (3km, 6km, 50km, >100km), and percentage to the cumulative dissimilarity.

Groups Human input and Sampling points away from cages.
Average dissimilarity = 34.07

	Human input	Sampling points away from cages				
Fatty acids	Average abundance	Average abundance	Average Dissimilarity	Dissimilarity/ SD	Contribution%	Cumulative%
22:6n-3	9.43	26.17	8.68	1.78	25.47	25.47
18:1n-9	22.09	9.71	6.4	1.75	18.8	44.27
18:2n-6	14.7	3.21	5.76	2.48	16.92	61.19
16:0	20.0	23.01	1.85	1.41	5.42	66.6
20:4n-6	1.03	4.52	1.77	1.31	5.18	71.78
16:1n-7	4.79	2.71	1.27	1.64	3.74	75.52
20:5n-3	4.5	4.56	1.15	1.56	3.39	78.91
18:0	6.57	8.35	1.14	1.43	3.35	82.26
14:0	3.09	1.92	0.83	1.54	2.43	84.69
22:5n-6	0.51	1.93	0.74	1.61	2.17	86.86
20:1n-9+n-7	1.17	1.82	0.48	1.04	1.41	88.27
22:5n-3	1.61	1.46	0.47	1.47	1.39	89.66
18:1n-7	2.91	2.29	0.44	1.33	1.28	90.94

4.5 Discussion

It has been widely described that sea cages farms aggregate wild fish around the floating structures, altering their natural occurrence, schooling behaviour and feeding behaviour even when the cages are empty (Machias et al., 2004; Tuya et al., 2006; Dempster et al., 2005; Arechavala-López et al., 2011). Sea cages may mimic the role of Marine

Protected Areas (Dempster et al., 2004; 2005) as consequence of both natural prey concentration or the existence of wasted food in the water column from aquaculture activity (Tuya et al., 2006; Mente et al., 2008). About 80% of the particulate organic matter may be consumed before it settles on the sediment (Vita et al., 2004). In this sense, farm-associated fish help to remove wastes produced and may enhance local fishing (Vita et al., 2004; Dempster et al., 2005; Dimitriou et al., 2007).

However, fish associated to these structures may be influenced by the composition of the aquafeeds wastes as a consequence of the abundance of a highly energetic food. In the present study, tissue lipid contents of bogue found far from the sea cages were close to those previously described for this species in the Mediterranean (Ozogul and Ozogul 2007). In comparison, bogues found inside the fish farm showed an increased lipid content in muscle and whole fish, whereas in fish surrounding the cages the values showed intermediate values. This fact, together with the higher CI, may be related to the continuous availability of the highly energetic aquafeeds (up to 27% lipid content, Sargent et al., 2002) and the reduction in the bogue energy expenditure for predatory effort. Aquaculture-associated fish have been found to have higher lipid content in muscle (Skog et al., 2003; Fernandez-Jover et al., 2007). Indeed, the lipid content in fish muscle changes in relation to the species, geographical origin, season and, especially diet (Rasoarahona et al., 2005). Other studies have shown that liver lipids are higher in farmed than in wild sea bream (Grigorakis et al., 2002; Mnari et al., 2007) and sharpsnout sea bream *Diplodus puntazzo* (Rueda et al., 2001). In the present study, despite lipid contents were also higher in liver of bogues associated to the farms, their values were similar to those found close to the sewage outfall and could not be considered as a good indicator of aquaculture influence.

Although the balance between energy intake and expenditure markedly affects tissue lipid contents and CI, the diet may also markedly affect also the tissue fatty acid profile as a

consequence of the type of dietary lipids ingested. Thus, the fatty acid profile in the different fish tissues is characteristic of the different dietary oils (reviewed by Turchini et al., 2009). At present aquafeeds include vegetable oils and meals that increase the fish content in fatty acids characteristics from terrestrial sources (Turchini et al., 2009). Thus, some fatty acids such as 18:C n-6 or 18:C (mainly LA and OA) are present in aquafeeds in a higher proportion than in the natural marine environment, due to the inclusion of soybean, sunflower or canola oils (Brown and Hart 2010; Turchini and Mailer 2010). The type of dietary fatty acids affects different metabolic pathways and the expression of genes encoding many different proteins including those involved in lipid metabolism (Izquierdo and Koven 2011). For instance, vegetable oils increase lipids storage as triacylglicerols, especially in the liver, as well as the type of fatty acids used to obtain energy by modulating the beta-oxidation capacity (Torstensen and Tocher 2010).

Table 4.6. SIMPER analysis. Contribution of the main fatty acids in different tissues (muscle liver and whole fish), to overall dissimilarities between fish inside sea cages (0 km) and fish around sewage outfall, and percentage to the cumulative dissimilarity.

Groups: Inside sea cages and Sewage outfall
Average dissimilarity = 19.35

	Inside sea cages	Sewage outfall				
Fatty acids	Average abundance	Average abundance	Average Dissimilarity	Dissimilarity/SD	Contribution%	Cumulative%
18:1n-9	19.88	28.54	4.73	1.67	24.43	24.43
20:5n-3	6.18	1.41	2.38	3.31	12.3	36.73
22:6n-3	7.08	8.65	2	1.1	10.32	47.05
16:1n-7	6.01	3.26	1.38	2.34	7.14	54.2

18:2n-6	16.21	18.03	1.31	1.54	6.79	60.98
16:0	19.12	20.22	1.15	1.13	5.92	66.9
14:0	3.81	2.11	0.87	2.04	4.5	71.4
18:0	5.64	6.67	0.85	1.38	4.38	75.78
22:5n-3	1.93	0.46	0.73	3.74	3.8	79.58
18:3n-3	1.71	0.71	0.5	3.62	2.59	82.17
18:1n-7	3.13	2.44	0.38	1.45	1.99	84.15
18:4n-3	0.88	0.24	0.32	1.84	1.67	85.83
20:4n-6	0.62	1.25	0.32	2.07	1.64	87.46
22:5n-6	0.2	0.74	0.27	1.06	1.39	88.85
17:0	0.75	0.25	0.25	3.41	1.29	90.14

Fernandez-Jover et al. (2007) related higher values of LA and OA to farm-associated fish, together with a reduction in Highly Unsaturated Fatty Acids (HUFAs) in comparison to non-associated fish. Similarly, Martinez-Rubio et al. (2009) found higher OA and LA contents together with lower DHA, ARA, ARA/EPA and n-3/n-6 in muscle of bogue sampled around a fish farm. In Norwegian fiords, LA and ALA were higher in saithe (*Pollachius virens L.*) found near to a fish farm and decreased with the increasing distance from the farm, suggesting a dilution gradient effect of aquaculture activities (Skog et al., 2003). The results of the present study, agree well with those observations studies, being LA, OA, ALA percentage higher in bogues influenced by aquaculture, and ARA and DHA lower. This aquaculture-influenced fatty acid profile was diluted by the distance to the sea cages farm and disappeared at 3 km from the farm. Mente et al. (2006) proposed that some fish species from Scotland, including saithe, effectively move from distances larger than 2 km far away from sea cages to feed aquafeeds pellets. In our study, the dominant currents in the farm zone, east of Gran

Canaria Island, run north to south, but no aquaculture wasted-feed effect could be detected at 3 km.

Interestingly, EPA values are increased in muscle for aquaculture-influenced fish in agreement with those results obtained by Rueda et al., (2001) for sharpsnout sea bream. On the other hand no differences on EPA have been reported among wild and cultured Atlantic salmon (Megdal et al., 2009).

Based on those described changes of fatty acids due to aquaculture influence, some authors have proposed certain fatty acids (and even general FA changes) as biomarkers or indicators in studies of the structure and dynamics of fish food webs around Mediterranean marine fish farms (Fernández-Jover et al., 2010). However Megdal et al. (2009) proposed that not all the fatty acids can be used as indicators, LA being a reliable indicator for Atlantic salmon. For sea bream, HUFAs have been proposed as indicators being EPA more abundant in cultured fish, but ARA more abundant in wild (Rueda et al., 2001). Both LA and ALA have been described to be presented at higher concentrations in cultured fish than wild fish (Mnari et al., 2007). However, few of this studies took into account other parameters and events that can be affecting fatty acid composition of wild and aquaculture fish other than aquafeeds. Firstly, fatty acids of the different tissues reflect in a general way the fatty acid profile of the diet, due to the accumulation of fatty acids as triacylglicerols (Torstensen and Tocher 2010) and, although 18:C fatty acids are increased in aquafeeds-fed fish, these fatty acids tend to be eliminated or washed-out progressively from the fillet and other tissues as soon as those animals stop to feed aquafeeds (Izquierdo et al., 2005; Torstensen et al., 2004). Secondly, 18:C fatty acids, and specially LA, is also abundant in the marine trophic chain, being algae and other organisms rich on this fatty acid (Meziane and Tsuchiya 2000, 2002; Ortiz et al., 2006), all of these organisms being candidates to be predated by escaped fish and of natural occurrence both around farm structures and on the wild. Thirdly, aquafeeds

formulae are continuously changing depending on the ingredients availability and prices, and consequently fatty acid profile of aquafeeds is in a continuous evolution (Gunstone, 2010), and thus, farm associated animals and aquaculture fish are not receiving always the same fatty acid composition. Fourthly, although aquaculture wastes are important inputs of these "terrestrial" fatty acids, other human activities increase the presence of these fatty acids in the marine environment, through sewages and agriculture activities (Quemeneur and Marty 1992; Seguel et al., 2001).

Indeed, sewage outfall is a variable source of different fatty acids that directly change fatty acid profiles of organisms living around them (Yep, 2006), being this effect disseminated within large distances (Seguel et al., 2001). For instance, urban waste waters have been shown to alter fatty acid profile of sediments and marine organisms (Yip, 2006; Dunn et al., 2008). Wong et al. (2008) found a relationship of trophic linkage between mussel fatty acids (*Perna viridis*) and fatty acid profile from suspended particulate matter, affected by domestic sewage. These authors, as well as others (Yip, 2006), defined an increase of LA and OA in animals around sewage outfalls. In agreement, in the present study bogue sampled around a sewage outfall showed the accumulation of LA and OA, denoting that statistically the fatty acid profile of fish living in the vicinity of a sewage outfall is similar to those associated to a fish farm. Nevertheless, they only slightly differed on the levels of LA and EPA. Moreover, samples obtained at 6km point, in the vicinity of Las Palmas de Gran Canaria city, showed higher values of those "terrestrial" fatty acids than samples with no human effects the 100km sampling point. Besides, not only urban waste water can be inducing an important input of LA or OA in the marine environment, but also other human activities such as agriculture wastewater. For instance, agriculture wastewater modifies the fatty acid profiles of the marine crab (*Uca vocans*) or the mollusc (*Terebralia sulcata*) (Meziane and Tsuchiya 2002). Mangrove detritus input (Meziane and Tsuchiya 2002), and

uncontrolled input of different chemicals and contaminants, such as diesel oil, have been also seen to alter the fatty acid composition of the gastropod *Littorina littorea* (Grahl-Nielsen and Barnung 1985). Therefore, the presence of those so-called "terrestrial" fatty acids in the marine trophic webs is due to multiple sources and not only due to aquafeeds.

In conclusion fish farms have a direct effect on the CI, muscle and whole lipid content of bogue inside or around the sea cage, as a consequence of continuous availability of food that completely disappeared at 3 km from the cages. Despite aquafeeds also affected the bogue fatty acid profiles by increasing linoleic and oleic acids and reducing DHA, these profiles were very similar to those of bogue sampled close to a sewage outfall. Therefore, the fatty acid profile does not seem to be a completely reliable biomarker of aquaculture activities, since other human activities including urban waters may induce similar changes in the linoleic acid, oleic acid and DHA contents.

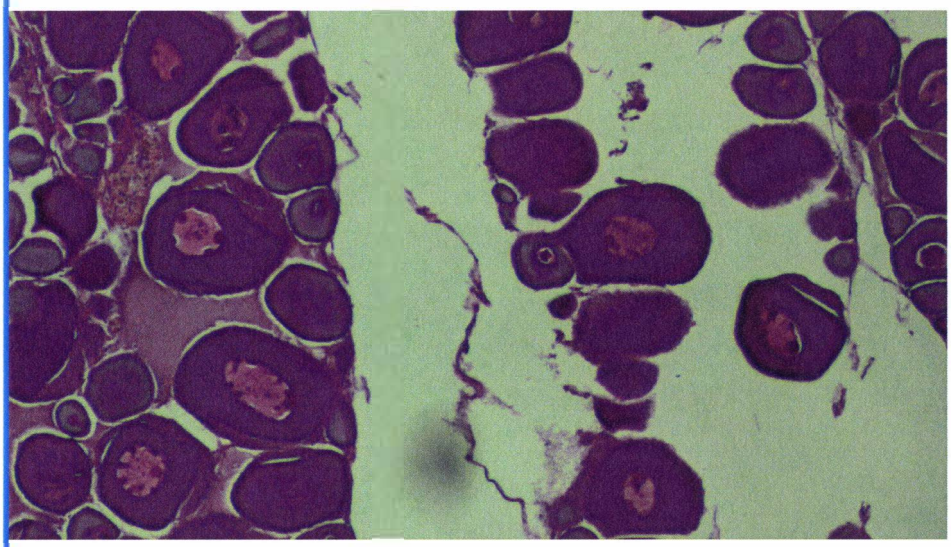
4.6 Acknowledgements

Authors would like to thanks Rafael Guirao, Hector Ramírez, Jonás Ramírez, Juan Luis Sánchez, and Cristobal Ruano for assistance during sampling, and the staff of the Canexmar fish farms for their help. We are grateful for the assistance of Fernando Tuya in statistical analysis

5

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

MONITOREO ESCAPE



5 "MONITORING A MASSIVE ESCAPE OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) AT AN OCEANIC ISLAND: POTENTIAL SPECIES FERALIZATION

Besay Ramírez[a], Leonor Ortega[a,b], Daniel Montero[c], Fernando Tuya[a], Ricardo Haroun[a]

[a] *Grupo de Investigación en Biodiversidad y Conservación, Centro de Biodiversidad y Gestión Ambiental, Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas, Spain.*

[b] *Philippe Cousteau "Union of the Ocean" Foundation, C/ General Oraá 26, 28006 Madrid, Spain*

[c] *Grupo de Investigación en Acuicultura. Universidad de Las Palmas de Gran Canaria, P.O. Box 56, 35200 Telde, Spain.*

5.1 Abstract

The post-escape behavior of aquaculture escapees is a growing topic of research. We monitored a massive escape event of the European sea bass, *Dicentrarchus labrax*, which occurred at a sea-cage fish farm off the oceanic island of La Palma, Canary Islands, eastern Atlantic. To assess the degree of post-escape feralization, stomach contents and gonadal development of escapees were analyzed from two islands (Gran Canaria and La Palma). We also tested (at both islands), the suitability of fatty acid profiles as biomarkers of aquaculture escapes, processing recaptured escaped fish at a range of distances away from aquaculture facilities. Escaped European sea bass concentrated within breakwaters and decreased in abundance through time after the massive escape at La Palma. Decapod crustaceans (particularly *Percnon gibbesi* and *Rhynchocinetes sp*) were the main diet constituents of escapees, followed by fishes (mainly the parrotfish, *Sparisoma cretense*). No fully developed

gonads were found. Crude lipid, oleic acid, linoleic acid, linolenic acid, eicosapentaenoic acid, ∑n-9 fatty acids and ∑monounsatured fatty acids showed higher values in cultured or escaped individuals near cages relative to fish far away from farms. Arachidonic acid, docosahexaenoic acid, ∑n-3, satured fatty acids, ∑n-3/∑n-6 ratio and Palmitic acid showed the opposite pattern. Our data showed that escaped European sea bass is able to exploit natural recourses, altering their fatty acid profiles relative to farmed conspecifics. The usefulness of fatty acids as biomarkers is, however, limited to a short period of time after escape events.

Keywords: escapees, stomach contents, fatty acids, gonadal development, Canary Islands

5.2 Introduction

Global production of fish from aquaculture has grown substantially in the past decade, reaching more than 90 million tons in 2012. Aquaculture continues to be the fastest-growing animal food producing sector and currently accounts for nearly half (49.4%) of the world's food fish consumption (FAO, 2014). The production of European sea bass, *Dicentrarchus labrax*, reached 70,147 tons in 2012, contributing to 33.6% of the total European marine fish production and 45.8% of the global European sea bass production (153,182 tons). European sea bass culture is a consolidated and economically important area of the European aquaculture (FAO, 2014).

Among those environmental issues facing aquaculture, escapes are widely regarded as a major problem in the marine environment, including genetic interactions through inbreeding, transfer of pathogens, prey predation, introduction of alien species, habitat alteration, etc. (CBD, 2004; Molina and Vergara 2005; Naylor et al. 2005; Vergara et al., 2005; Jensen et al., 2010; Grigorakis and Rigo 2011; Lorenzen et al., 2012; Toledo et al., 2012; Arechavala-López et al., 2013a, 2014). Escapes are, indeed, regarded as a problem for

the future sustainability of sea-cage aquaculture (Holmer, 2010). However, the number of studies evaluating interactions between escapees and the environment are still not sufficient to be conclusive.

The European sea bass, *Dicentrarchus labrax*, is a voracious predator, feeding mainly on crustaceans, molluscs and fishes (Tortonese 1986; Laffaille et al., 2001; Leitao et al., 2008; Toledo et al., 2009; Toledo et al., 2014a), and its diet changes with fish size (Kennedy and Fitzmaurice, 1972; Arias, 1980; Toledo et al., 2014a); for escaped individuals, the diet also depends on the time at liberty (Toledo et al., 2014a). European sea bass first maturation is reached at a range of length between 23-46 cm (Froese and Pauly, 2006) and reproduction occurs between December and March (Do Chi and Hoai Thong, 1971; Barnabé, 1973; Arias, 1980; González, 2003). Importantly, this species requires low salinities (<35‰) in its natural habitat to trigger gametogenesis; in turn, the last phase of gonadal development require a *ca.* 35‰ of salinity and, concurrently, larval survival increases at low salinities. Spawning optimal thermal range is 13-15ºC (Moretti et al., 1999). Recruitment is typical in estuarine habitats across its distribution range (Arias, 1980; Serrano, 1989; Laffaille et al., 2001; Dufour et al., 2009). During eggs incubation, the optimal temperature range is between 13º-17ºC (Devauchelle and Coves, 1988; Saka et al., 2001) and larval development lasts 46 days at 16.5 ºC (Houde and Zastrow, 1993).

Our knowledge on how European sea bass escapes might affect ecosystems is limited. The long-term data available on escapes of the European sea bass indicate that, in the Mediterranean Sea, escaped populations maintain distinct populations from wild populations (Bahri-Sfar et al., 2005). Escapes at some locations may be particularly problematic, principally where local populations are reduced, or in areas outside the species' natural distribution range (Lorenzen et al., 2012). Populations of the European sea bass in the central and westernmost islands of the Canarian Archipelago (Fig. 5.1), where no native populations

have existed, are related to aquaculture escapes (Carrillo and Castillo, 2001; Toledo et al., 2009; Toledo et al., 2012). In these islands, for example, escaped European sea bass diet overlaps with other top predators and may become a new competitor for local species (Carrillo et al., 1995; Toledo et al., 2009; Toledo et al., 2014a), though significant correlations between the number of escapees and abundances of other fish species were not found in the Canary Islands (Toledo et al., 2014a). Yet, there is no reported evidence of reproduction of escaped European sea bass from this archipelago (Carrillo et al., 1995; Toledo et al., 2009), although developed gonads have been found (Toledo et al., 2012). Inbreeding would be exclusively possible at the eastern islands, where small wild populations exist (Brito 1991).

Several morphological and physiological indicators have been proposed as useful tools for the identification of escapees, based on biometrical parameters (Ellis et al., 2009; Uglem et al., 2011), scales and otholits morphology (Katayama and Isshiki, 2007; Person-Le Ruyet and Le Bayon, 2009), scale mineral contents (Adey et al., 2009), RNA/DNA ratios (Gwak et al., 2003) or muscle fatty acid (FA) composition (Grigorakis et al., 2002; Fernández-Jover et al., 2007, 2011; Arechavala-López et al., 2013b). Regarding the latter, the FA profile has been claimed to be a good bio-indicator of species interactions (Dunn et al., 2008; Hu et al., 2008; Maazouzi et al., 2008). The FA profile has received increasing attention due to changes in aquafeed ingredients over recent years. Traditionally, fish were fed with diets based on fishmeal and fish oil as the main ingredients to ensure a competitive price and adequate content in some essential FAs for fish, such as docosahexaenoic acid (DHA) (22:6n−3), eicosapentaenoic acid (EPA) (20:5n−3) and arachidonic acid (ARA) (20:4n−6). These FAs, which have a plethora of very important functions, are considered essential for marine fish, since these species do not have the ability to bio-convert shorter FAs into these FAs, due to the very low activity of delta 6 and delta 5 desaturase in these fish (Izquierdo and Koven, 2011). Given that the world production of fish oil is stagnated with the consequent increase in

cost, there is a strong trend for the use of vegetable oils as fish oils substitutes (Tacon and Metian, 2008). The use of vegetable oils in the feeds of marine fish is problematic, because of their lack of long chain n−3 PUFA, and the abundance of the 18:C FAs precursors, e.g. linoleic acid (LA) (18: 2n−6) and alpha-linolenic acid (ALA) (18: 3n−3) (Turchini et al., 2009). These FAs precursors are incorporated into the tissues of farmed fish, and can also be transferred to wild fish (Fernández-Jover et al., 2011). Wild fishes around offshore aquaculture cages may feed on pellets released from farms, resulting in changes in body condition and FA profiles, bringing their body composition and that of other organisms in different trophic levels closer to that of cultivated fish (Skog et al., 2003; Fernández-Jover et al., 2007, 2011). For this reason, the presence of certain FAs, such as LA, ARA and oleic acid (OA) (18: 1n−9), in wild organisms has been proposed as an indicator of the influence of aquaculture on marine ecosystems (Rueda et al., 2001, Fernández-Jover et al., 2007; Fernández-Jover et al., 2011; Arechavala-López et al., 2013b).

The goal of this paper was, firstly, to analyze the spatio-temporal variability in the population structure of escaped European sea bass after a massive escape (approximately 1,500,000 fish, 400,000 kg, 30-45 cm of total length at the moment of escape) that occurred on February 2010 at La Palma Island (Canary Islands, eastern Atlantic). We secondly determined whether escaped European sea bass diet, reproductive potential and FA profile changed with distance from aquaculture cages. We hypothesize that: (1) escaped fish can re-distribute around the entire island perimeter after a massive escape, (2) being able to adapt to the wild by consuming local prey, (3) lacking reproduction and (4) altering their FA profiles depending on distance from the source of escapees.

5.2 Materials and methods

5.2.1 Sampling design and study locations

To monitor the massive escape of European sea bass that occurred between the 20 and 26th February 2010 at La Palma Island, we selected six locations throughout the entire island perimeter (Fig. 5.1). One location was immediately adjacent to the escape point (a sea-cage fish farm off Tazacorte) and the rest were selected at different distances away from this point, northward and southward around the entire island perimeter. One location (El Remo) was set within a marine reserve (*Reserva Marina Isla de La Palma*). No location was selected in the north face of the island due to prevailing swells from the NW that impedes regular sampling. This protocol was repeated six times (i.e. sampling campaigns): August 2010, September 2010, October 2010, November 2010, August 2011 and October 2011. Locations encompassed a range of habitats at shallow water (*ca.* 5-10 m depth).

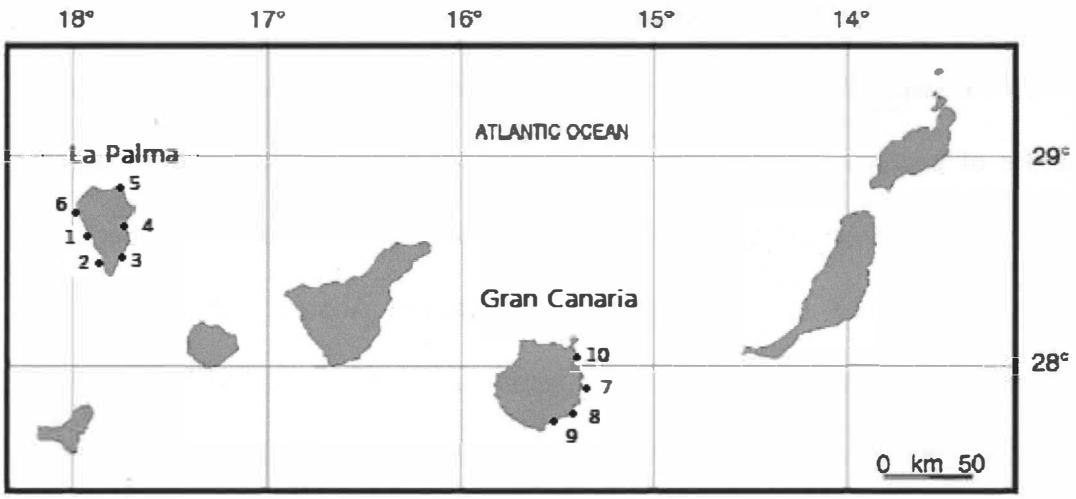


Figure 5.1. Map of the study area detailing sampling locations at La Palma Island: **1** Tazacorte (Acuipalma S.L. farm); **2** El Remo (marine reserve); **3** La Salemera; **4** Santa Cruz de la Palma; **5** Puerto Talavera; **6** Puntagorda; and Gran Canaria Island: **7** Melenara (ADSA S.L. and Canexmar S.L. farms); **8** Arinaga; **9** Castillo del Romeral (ADSA S.L. and Playa Vargas S.L. farms); **10** (San Cristobal).

To study stomach contents, gonad development and FAs profile, escaped European sea bass were captured by spear-fishing throughout the entire study, i.e. from August 2010 to October 2011, at both La Palma and Gran Canaria. Although some of them presented unusable tissues due to shaft impacts, this was the most appropriate and effective capture method because local fishermen do not fish close to the surf, where European sea bass are often grouped (Toledo et al. 2009). Catches were kept in ice until processed in the laboratory, where individuals were weighed (accuracy to 0.1 g) and measured (accuracy to 0.1 cm). The gonads, stomachs, muscle tissue and livers were removed from each individual; the gonads were preserved in formaldehyde and the stomachs preserved in 70% ethanol; muscle tissue and livers were frozen for subsequent lipid analysis. Samples for lipid analyses were categorized according to 3 distances from aquaculture facilities: 'Cages': 0 km (inside cages), 'Near cages': <10 km and 'Far away from cages':> 10 km. In addition to samples collected at La Palma Island (i.e. where the escape event took place), samples were simultaneously taken at Gran Canaria Island (Fig. 5.1) to assess whether patterns with distance from aquaculture cages for a range of descriptors (see below) were consistent between the two islands. By increasing the spatial replication, the robustness of the study is enhanced if results were consistent between both islands. In La Palma Island, there is only one off-shore farm, while at Gran Canaria there are four off–shore fish farms located at two locations (Fig. 5.1).

5.2.2 Fish surveys

At each location and time, fish were counted by means of visual census techniques through four replicated 50 m long transects, which were haphazardly laid out during daylight hours. The abundance and size of fish was recorded on waterproof paper by a SCUBA diver within 2 m of either side of transects, according to standard procedures implemented in the study region (Tuya et al. 2004). We also recorded the type of habitat under each transect (%), which was categorized according to 'big boulders' (> 2 m of diameter), 'small boulders' (< 1 m

of diameter), 'sand', 'breakwaters' (i.e. artificial man-made constructions) and 'bare rock' (Tuya et al., 2004).

5.2.3 Stomach contents

To analyze the diet composition of escapees, stomachs were weighed and the content removed. Total food items from each stomach were placed on filter paper to eliminate excess moisture and subsequently weighed. Prey items were identified to the lowest possible taxonomic level; the total number of prey items were counted and weighed for each stomach. The percentage composition by number and weight was then calculated for each prey category to calculate the indices of importance by number (IN), wet mass (IW) and global importance (IG).

IN = $[(\%\ composition\ by\ number)\ X\ (\%\ occurrence)]^{1/2}$

(Windell, 1971; Vesin et al., 1981).

IW = $[(\%\ wet\ mass)\ X\ (\%\ occurrence)]^{1/2}$

(Castro, 1993).

IG = $(IN\% + IW\%) / 2$

(Moreno and Castro 1995)

5.2.4 Gonadal development

For each fish, gonads were macroscopically examined for sex differentiation to establish the stage of gonadal development, by using a visual scale of five stages of maturity based on color and the relative size of gonads: I undeveloped; II developing; III mature; IV spawn; V post-spawn (Holden and Raitt, 1974).

5.2.5 Lipid and FA profiles

A total of 60 livers and 60 muscles samples were analyzed. 10 for each distance ('Cages', 'Near Cages', and 'Far away from Cages') from both La Palma and Gran Canaria

islands (n = 10). Biochemical assays followed standard procedures (AOAC, 2000). Moisture content was determined by drying the sample at 105 °C, until achieving a constant weight. Crude lipid content was extracted following the method of Folch et al. (1957). FAs from total lipids (stored under nitrogen atmosphere at −80 °C) were prepared by transmethylation, as described by Christie (1982) and FA methyl esters separated by gas chromatography following Izquierdo et al. (1990). All analyses were conducted in triplicate.

5.2.6 Data analysis

Fish survey data and FA profile composition were analyzed by means of ANOVA (Underwood, 1997). We tested for differences in escapees abundance between locations at varying distances from the escape point around La Palma Island and the sampling times through a 2-way ANOVA that incorporated the factors: (1) "Locality" (fixed factor with six levels, corresponding to the 6 locations) and (2) "Time" (fixed factor with six levels and orthogonal to the previous factor); "Time" was considered fixed, as sampling dates were equidistantly separated. A χ^2 tested for differences in diet composition (by considering the percentages of the different prey items) between islands, and a Wald-Wolfowitz test contrasted differences in fish length between islands. To test for differences in FA profile composition (total lipid, OA, LA, ALA, palmitic acid (PA) (16:0)), ARA, EPA, DHA, ∑satured, ∑monounsatured, ∑n-9, ∑n-6, ∑n-3 F and ∑n-3/∑n-6 ratio) with varying distance from cages, 2-way ANOVAs incorporated the factors: (1) "Island" (fixed factor with two levels: La Palma and Gran Canaria); (2) "Distance" (fixed factor with three levels corresponding to the three distances from the aquaculture sea farms: 'Cages', 'Near cages' and 'Far away from cages'. Before the analyses, the Cochran's test was used to check for homogeneity of variances. If the test detected heterogeneous variances (Cochran's test, $P <$ 0.01), data transformation was performed. In some cases, variances remained heterogeneous despite transformations; the significance level was then set at the more conservative 0.01

value instead of the conventional 0.05 level to decrease a type I error (Underwood, 1997). If ANOVA detected significant differences, further analyses were performed by using the SNK *a posteriori* multiple comparison test (Underwood, 1997). When data did not achieve homogeneous variances, the Games-Howell *post hoc* test was used. The SIMPER analysis identified the main contributors to differences in FA profiles between islands at varying distance from cages. A Chi square (X^2) tested for departures from a 1:1 sex ratio. Non-metric multidimensional scaling (nm-MDS) ordination plots were implemented to visualize differences in the FA profiles (OA, LA, ALA, PA, ARA, EPA, DHA, ∑satured, ∑monounsatured, ∑n-9, ∑n-6, ∑n-3 FAs and ∑n-3/∑n-6 ratio) from muscle and liver tissue between islands and the three distances from the aquaculture sea farms: 'Cages', 'Near cages' and 'Far away from cages'. The SPSS (v. 15.0), PRIMER (v. 5.2.4) and PERMANOVA (v.1.6) software were used in these statistical analyses.

5.3 Results

5.3.1 Spatial-temporal distribution of escaped European sea bass after the escape event

The mean abundance of escaped European sea bass at La Palma Island was 4.9 ± 14.3 ind 100 m^{-2} (mean ± SE, n = 144 transects) during the study period, which varied between 0 fish in several transects to a maximum of 100 ind 100 m^{-2}. All locations had escaped European sea bass, at least one individual during the study period (Fig. 5.2). The ANOVA (Table 5.1) demonstrated that escaped European sea bass densities decreased significantly ($p<0.01$) through the study period, particularly in October 2010; and that Tazacorte (i.e. the point of massive release) had significantly ($p<0.01$) higher densities (17.0 ± 29.1 ind 100 m^{-2}) than the other locations (Fig. 5.3).

Table 5.1. ANOVA results of the effect of 'Time' and 'Locality' on the mean abundance of sea bass (*Dicentrarchus labrax*) at La Palma Island.

Source of variation	df	MS	F	P
Time	5	204.909	79.096	0.0002
Locality	5	150.132	57.951	0.0004
Time x Locality	25	29.859	11.526	0.2918
Residual	108	25.907		

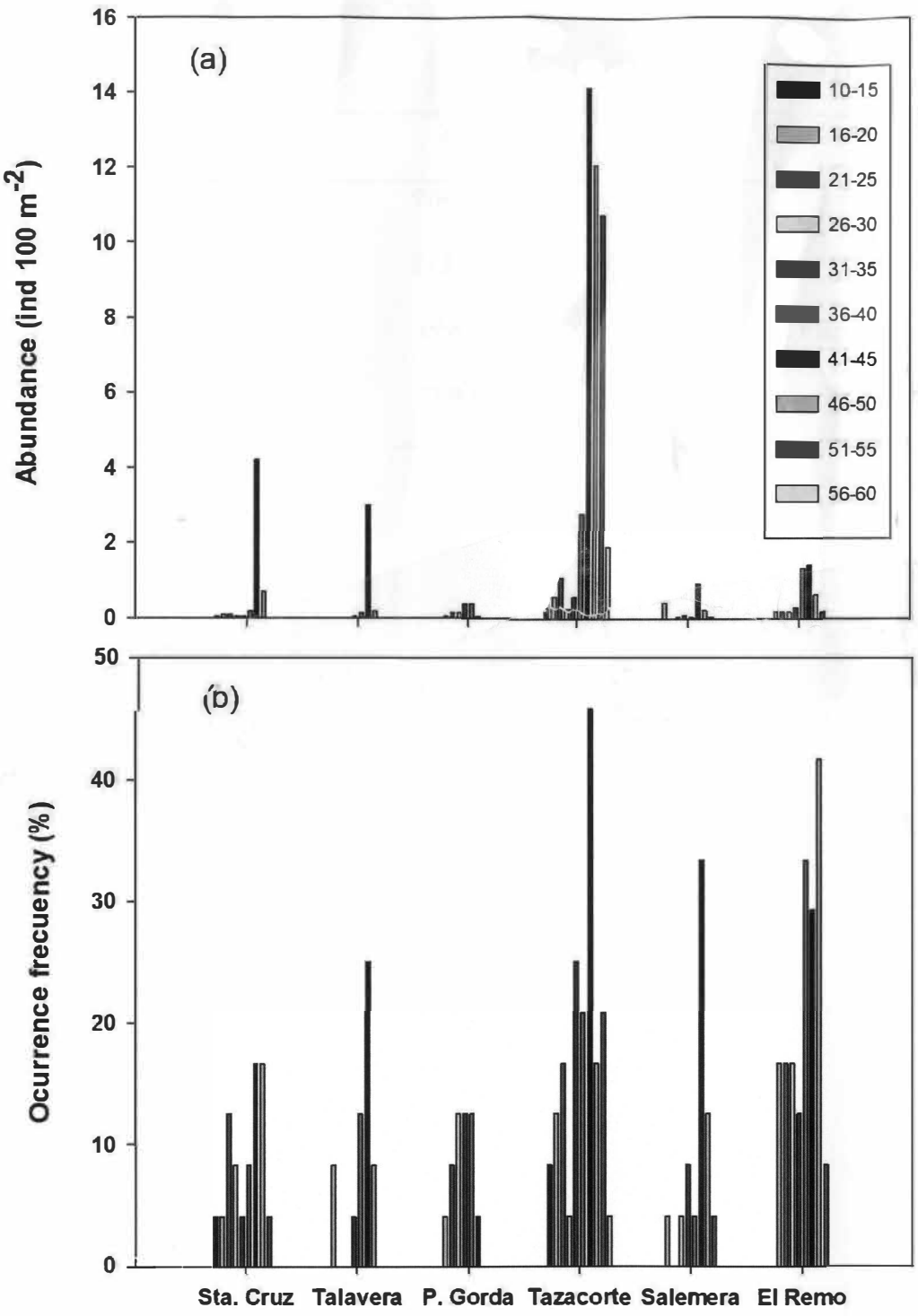


Figure 5.2. Size-class (a) abundances and (b) frequency of occurrence of escaped European sea bass, *Dicentrarchus labrax*, at each location around La Palma Island. Data were pooled for each location through the 6 sampling times.

The mean abundance of escaped European sea bass at La Palma Island was 4.9 ± 14.3 ind 100 m^{-2} (mean ± SE, n = 144 transects) during the study period, which varied between 0 fish in several transects to a maximum of 100 ind 100 m^{-2}. All locations had escaped European sea bass, at least one individual during the study period (Fig. 5.2).

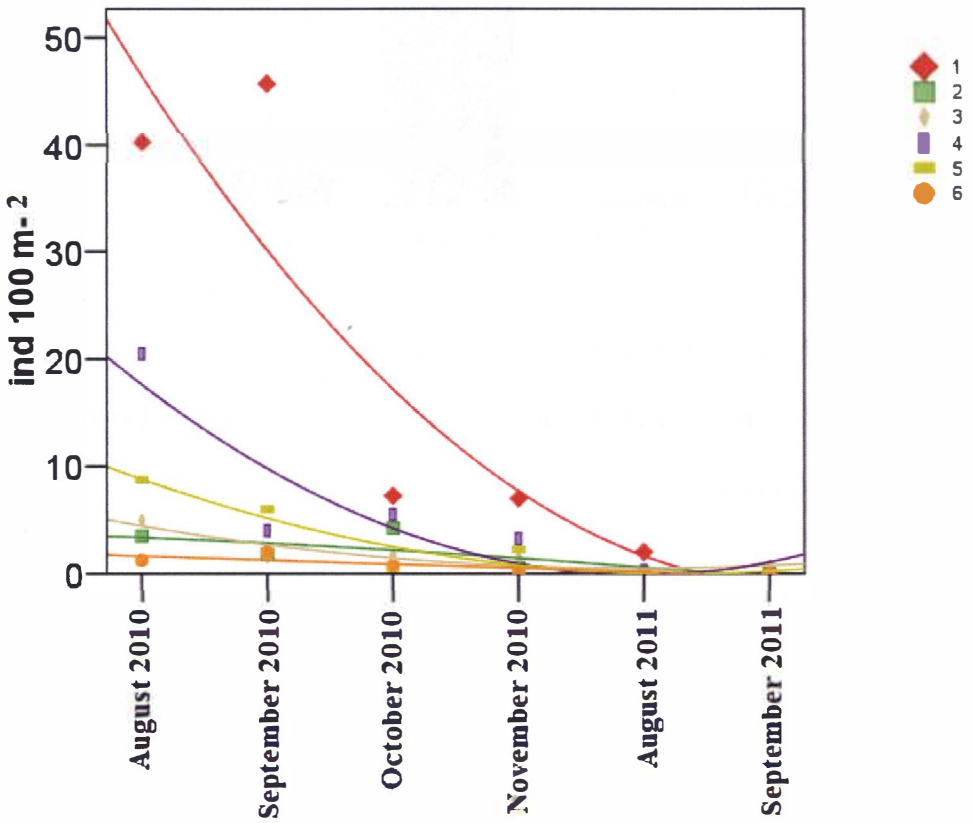


Figure 5.3. Mean abundances (ind 100m^{-2}) of escaped sea bass, *Dicentrarchus labrax*, at each time and location at La Palma Island. **1** Tazacorte (Acuipalma S.L. farm); **2** El Remo (marine reserve); **3** La Salemera; **4** Santa Cruz de la Palma; **5** Puerto Talavera; **6** Puntagorda

The largest densities of escaped European sea bass from La Palma were observed in breakwaters (Game-Howell test, $p<0.05$, Fig. 5.4). The length range of observed European sea bass varied between 15 and 60 cm (Fig. 5.5); the majority of fish, however, were between 35 and 50 cm (Fig. 5.2).

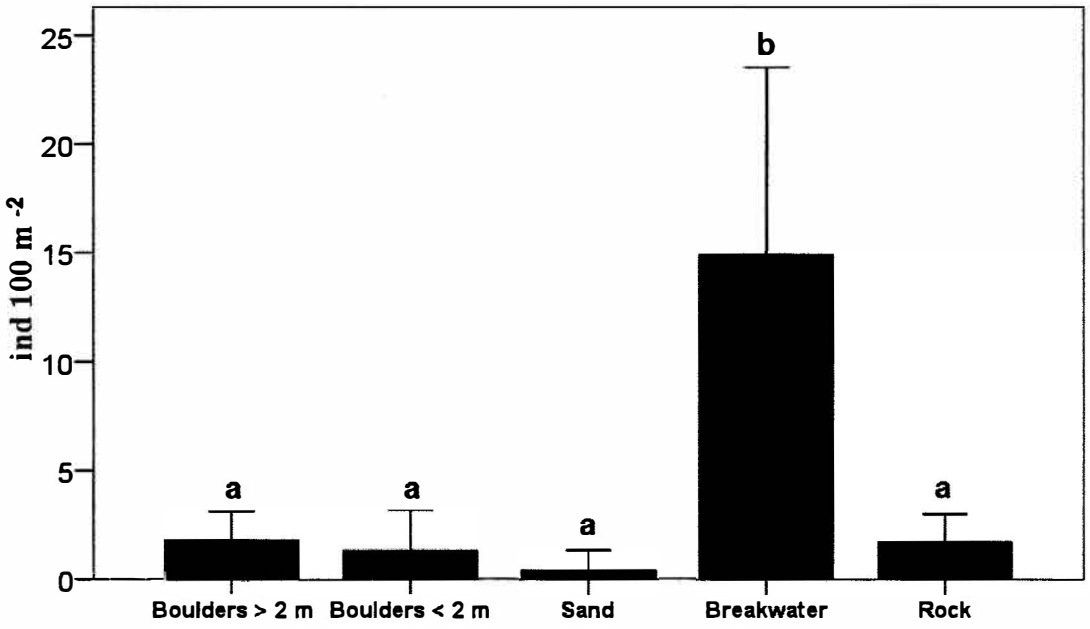


Figure 5.4. Mean abundances (ind $100m^{-2}$) of escaped sea bass, *Dicentrarchus labrax*, at different substratum types at La Palma Island (data pooled from the different locations). Alphabetic superscripts indicate significant differences.

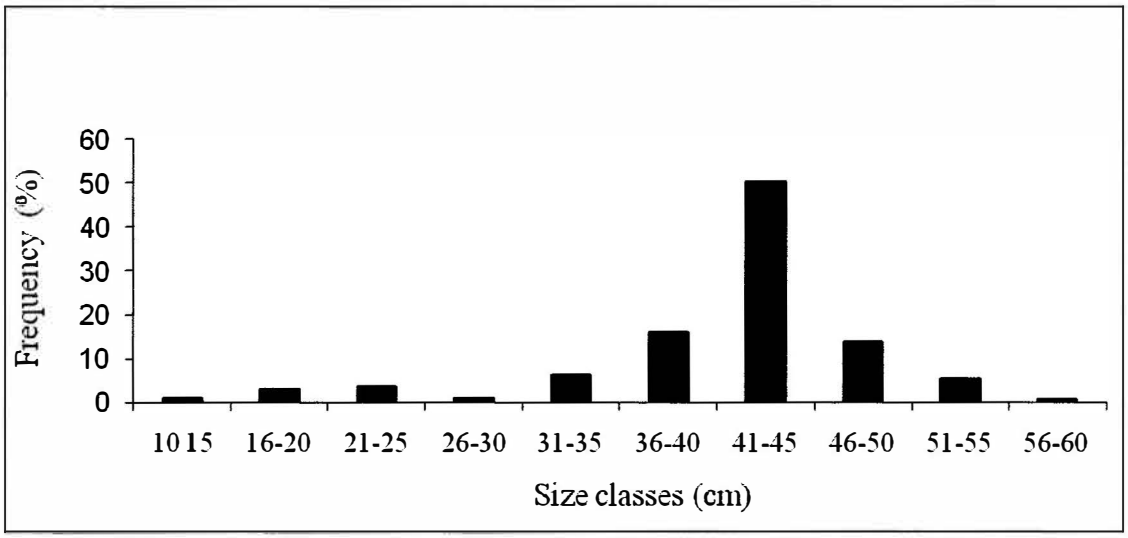


Figure 5.5. Size-frequency distributions of sea bass, *Dicentrarchus labrax*, escaped in La Palma on February 2010 (data pooled from the different locations).

5.3.2 Stomach contents of escaped European sea bass

A total of 101 individuals were collected (Table 5.2). A total of 77 stomachs were analyzed (24 stomachs were broken by the spear impact during field collections);

Table 5.2 Length and weight of each capture fish

Island	Weight	Length
La Palma	895	44,5
La Palma	605	37,6
La Palma	550	39
La Palma	2475	63,1
La Palma	555	35,2
La Palma	1020	44,9
La Palma	550	37,8
La Palma	480	38,9
La Palma	430	38,1
La Palma	535	39,5
La Palma	425	36,2
La Palma	2145	57,3
La Palma	765	42,6
La Palma	660	37,8
La Palma	213,9	29,6
La Palma	171,7	28
La Palma	247,7	29,5
La Palma	347,8	32,5
La Palma	178,8	28,3
La Palma	462,4	39
La Palma	339,4	34,8
La Palma	311,4	34,8
La Palma	464,2	38,4
La Palma	342,5	35,4
La Palma	731,7	41,8
La Palma	601,4	41,2
La Palma	596,4	41,9
La Palma	355,4	33,6
La Palma	521,4	39,6
La Palma	776,8	44,5
La Palma	730,7	42,3
La Palma	807,5	44,5
La Palma	668,4	42,3
La Palma	782,8	43,5
La Palma	286,8	32,4
La Palma	636,4	42,2
La Palma	217,11	29,6
La Palma	345,9	33,7

La Palma	773,9	42,5
La Palma	818	43,8
La Palma	1001,1	46,9
La Palma	702	42,1
La Palma	846,4	44,5
La Palma	436,8	36,5
La Palma	634,7	41,5
La Palma	700	42,7
La Palma	743,2	42,6
La Palma	897,1	42,4
La Palma	1058,4	45
La Palma	366	33,6
La Palma	312,6	33,6
La Palma	614,6	41,7
La Palma	473,9	38,2
La Palma	626,4	39,1
La Palma	736,8	42,9
La Palma	316,2	34,1
La Palma	468	36
La Palma	527,7	39,9
La Palma	351,8	35,9
La Palma	2214,5	56,7
La Palma	622,2	41,4
La Palma	571,9	41
La Palma	541,1	40,2
La Palma	682,8	43,7
La Palma	571,1	41,6
La Palma	453,6	38,9
La Palma	316,5	31,7
La Palma	752,9	43
La Palma	662,4	41,2
La Palma	1364,7	52,8
La Palma	1168,9	49
La Palma	582,3	37,5
La Palma	535,5	37
La Palma	1118,3	47,7
La Palma	680,3	40,8
La Palma	780	39,5
La Palma	1540	57
La Palma	1420	52
La Palma	2100	58,5
La Palma	3460	67,5
La Palma	1400	47,8
Gran Canaria	572,8	38,7
Gran Canaria	3240,6	65,4
Gran Canaria	728,7	39,9
Gran Canaria	944	42,9
Gran Canaria	895,4	43,3

Gran Canaria	1194,5	46,5
Gran Canaria	463,9	33,9
Gran Canaria	691,7	40,1
Gran Canaria	650,9	39,3
Gran Canaria	2310,2	60,9
Gran Canaria	928,1	43,9
Gran Canaria	259,7	28,7
Gran Canaria	1143,5	45,9
Gran Canaria	473,1	31,5
Gran Canaria	227,6	28,9
Gran Canaria	436,1	37,8
Gran Canaria	848,8	44,8
Gran Canaria	285,9	31,4
Gran Canaria	451,1	35,1
Gran Canaria	507,1	36

twenty stomachs (25.97%) were empty and 19 stomachs (24.67%) contained unidentifiable items. Decapod crustaceans were the main prey, followed by osteichthyes and inorganic matter. The most commonly preyed crustaceans were *Percnon gibbesi* and *Rhynchocinetes sp*, while the main fish species was the parrotfish, *Sparisoma cretense* (Fig. 5.6).

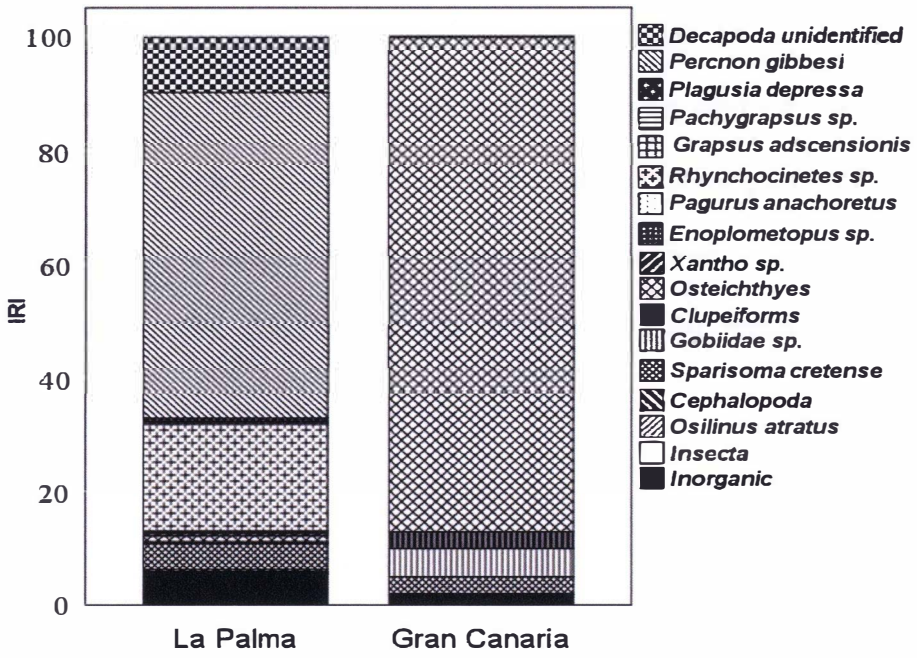


Figure 5.6. Percentage Index of Relative Importance (% IRI) of different prey in the diet of sea bass, *Dicentrarchus labrax*, escaped from La Palma and Gran Canaria islands.

Stomachs from La Palma (n=54) mainly contained crustaceans, while stomachs from Gran Canaria (n=20) were dominated by unknown osteichthyes. This resulted in significant differences in diet between La Palma and Gran Canaria (χ^2 = 100.2421, d.f. = 16, p< 0.0001), despite a lack of significant differences in fish length between islands ($Z_{adjusted}$ = -2.5266, p = 0.8407). However, similar percentages of *Sparisoma cretense* and inorganic matter (sand and plastics) were presented in fish stomachs from both islands.

5.3.3 Gonadal development of escaped European sea bass

A total of 87 escaped European sea bass were examined; gonads from 14 individuals were not analyzed due to spear impact during collection. The sex ratio did not deviate from a theoretical 1:1 ratio (χ^2 =2.400, d.f. = 1, p = 0.121). About 20.7% of escaped European sea bass were sexually undefined. The 87.5% of males and 91.7% of females presented immature gonads (stages I and II); only 8.3% (considering both males and females) were mature (stage III) and exclusively 1 male were in a spawning state (state IV). No females were found in the IV stage. No fish (both male and female) were found in stage V.

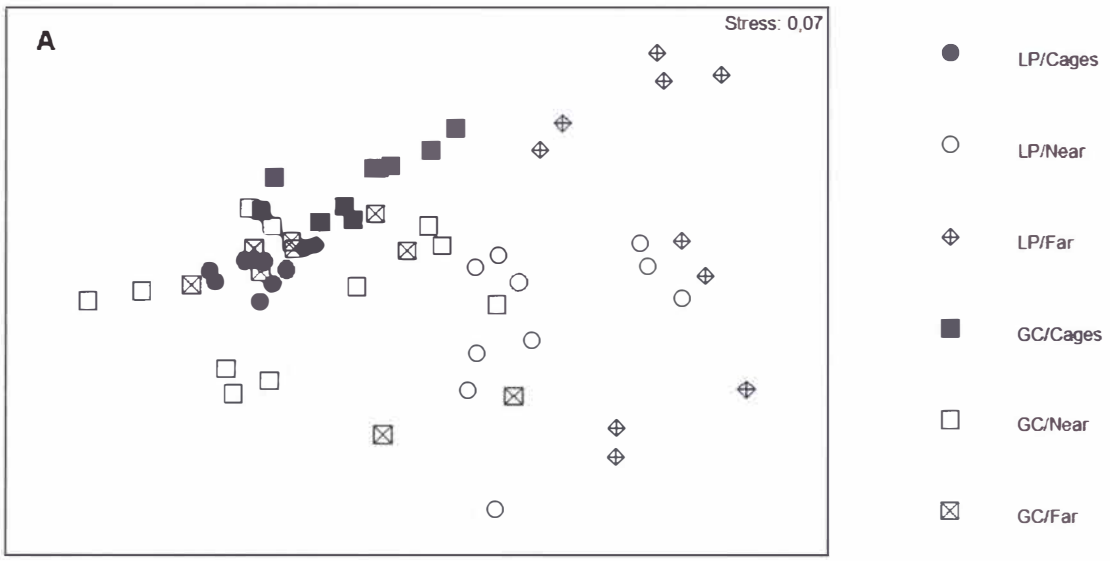
5.3.4 FA profiles of escaped European sea bass

For Gran Canaria Island, the nMDS plot did not show a separation of individuals according to distance from cages for both muscle and liver tissues. In contrast, at La Palma Island, individuals separated according to their distance from cages (Figs. 5.7a and 5.7b). This resulted in an inconsistent pattern in FA profiles among groups at varying distance from cages between islands (MANOVA, significant 'Island x Distance' interaction. Table 5.3).

Table 5.3. Result of multivariate analysis of variance (MANOVA) testing for differences in the fatty acids profiles (including liver and muscle tissue) between island and varying distance from farms. LP: La Palma, GC: Gran Canaria.

Source of variation	df	MS	F	P	Pairwise tests
Island (Is)	1	6.875.190	220.042	0.0002	
Distance (D)	2	5.596.024	179.102	0.0002	
Is x D	2	5.857.735	187.478	0.0002	LP: 'Cages' ≠ 'Near' ≠ 'far away' GC: 'Cages' = 'Near' = 'far away'
Residual	54	312.450			

The SIMPER analysis showed that, for muscle tissue, the main differentiating variables between localities at varying distance from the cages were: total lipid, DHA, LA, ∑satured, ∑monounsatured and ∑n-3 FAs. For liver tissue, the main contributors to dissimilarities were total lipid, DHA, OA, ∑satured, ∑monounsatured and ∑n-3 FAs.



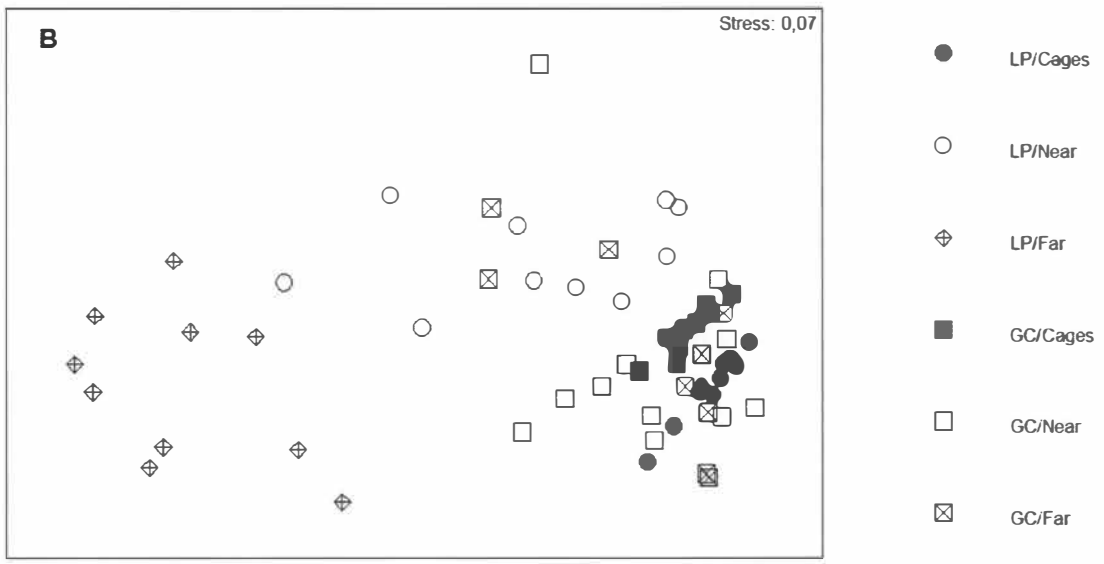
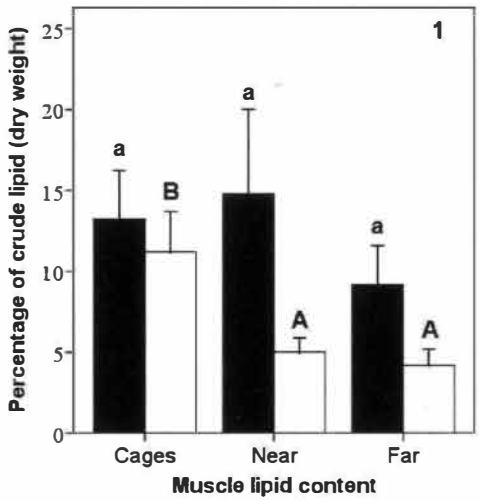
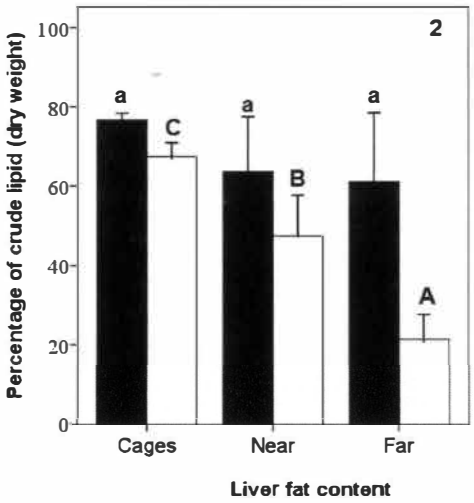


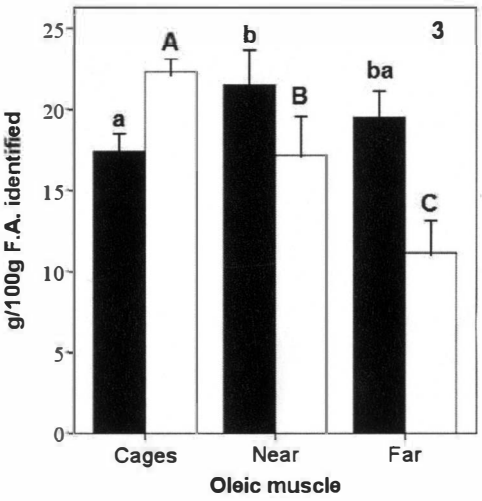
Figure 5.7. Non-metric multi-dimensional scaling ordination of (A) muscle and (B) liver fatty acids profiles of sea bass, *Dicentrarchus labrax*, from 'Cages', 'Near' (<10 km away from the cages) and 'Far' (>10 km away from the cages) at La Palma (LP) and Gran Canaria (GC) islands.

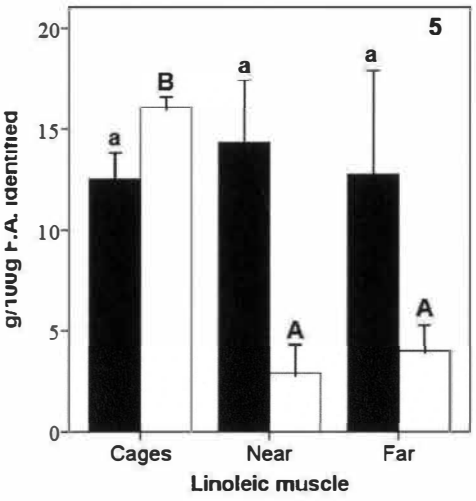
The majority of FAs showed a similar pattern with varying distance from cages at both islands (Tables 5.4 and 5.5). At La Palma Island, we typically detected significant differences in the percentage of FA with varying distance from the farm; this pattern, however, was minored at Gran Canaria Island, where we exclusively detected significant differences with varying distance from cages in the percentage of ∑n-3/∑n-6 ratio (Figs. 5.8_13 and 5.8_14), muscle total lipid (Fig. 5.8_1), OA from muscle (Fig. 5.8_3) and EPA from muscle (Fig. 5.8_9). *A posteriori* SNK tests demonstrated that crude lipid from individuals captured at La Palma Island, including both muscle (5.8_1) and liver (Fig. 5.8_2) tissues, had significantly larger values in samples coming from 'cages' than 'far away' from cages; 'Near' samples did not differ from 'far away' samples for muscle tissue (Fig. 5.8_1). At La Palma Island, the concentration of OA was significantly higher in samples from 'cages' than 'near' cages' or 'far away', for both muscle (Fig. 5.8_3) and liver (Fig. 5.8_4) samples. Cultured ('cages') European sea bass from La Palma had significantly higher LA (Figs. 5.8_5 and 5.8_6) and

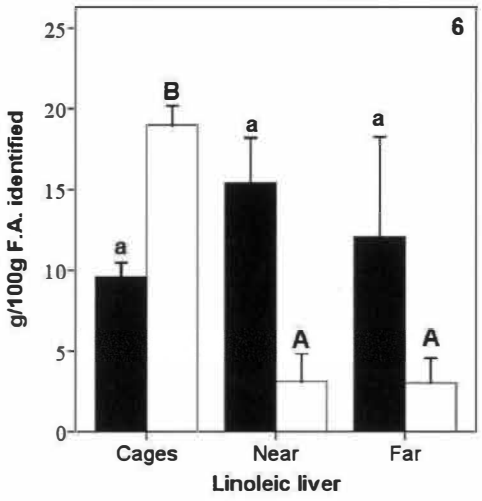
ALA (Figs. 5.8_7 and 5.8_8) than 'near' and 'far away' samples, for both muscle and liver; fish from 'near' and 'far away' distances did not show significant differences. The percentage of EPA in muscle was larger at 'cages' relative to 'near' and 'far away' samples at Gran Canaria (Fig. 5.8_9); at La Palma, however, there was no difference between 'cages' and 'far away' samples (Fig. 5.8_9). The percentage of EPA in liver tissue (Fig. 5.8_10) differed between 'near cages' and 'far away' samples collected from La Palma. ∑n-9 and ∑Monounsatured FAs from La Palma followed the same pattern: significantly larger values for cultured fish than 'far away', for both muscle (Figs. 5.8_15 and 5.8_17, respectively) and liver (Figs. 5.8_16 and 5.8_18, respectively) tissue samples.

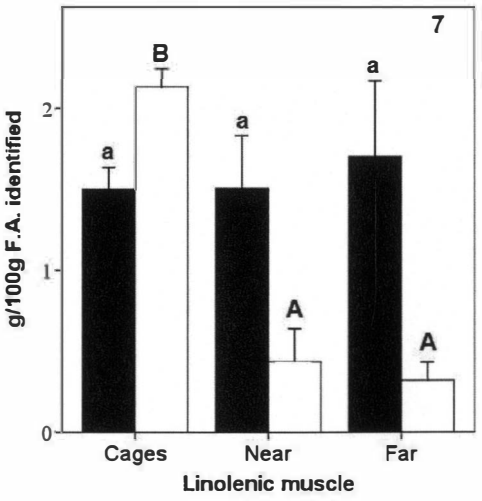

1
Percentage of crude lipid (dry weight)
25
20
15
10
5
0
a
B
a
A
a
A
Cages
Near
Far
Muscle lipid content

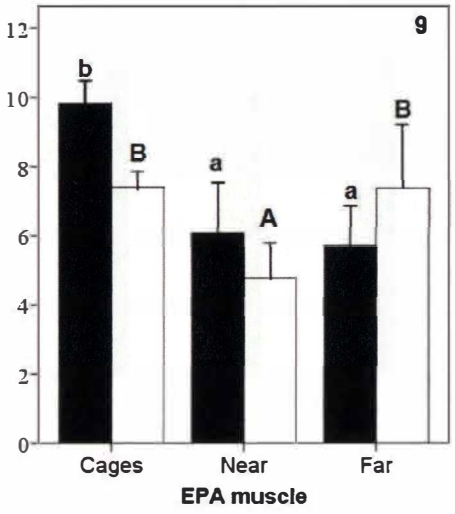

2
Percentage of crude lipid (dry weight)
100
80
60
40
20
0
a
C
a
B
a
A
Cages
Near
Far
Liver fat content

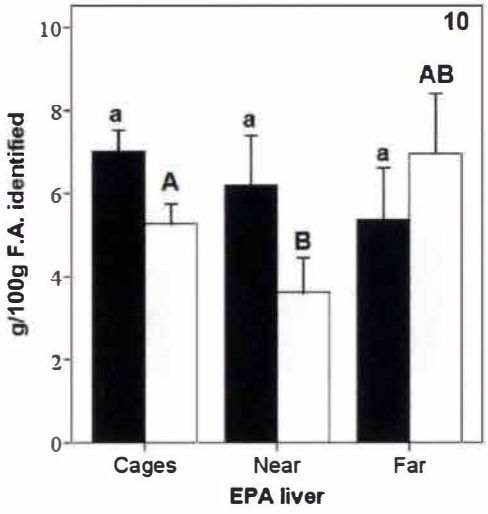

3
g/100g F.A. identified
25
20
15
10
5
0
a
A
b
B
ba
C
Cages
Near
Far
Oleic muscle

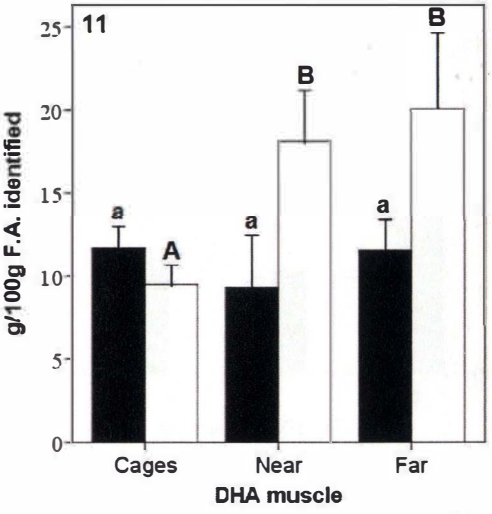

5
g/100g F.A. identified
20
15
10
5
0
a
B
a
A
a
A
Cages
Near
Far
Linoleic muscle

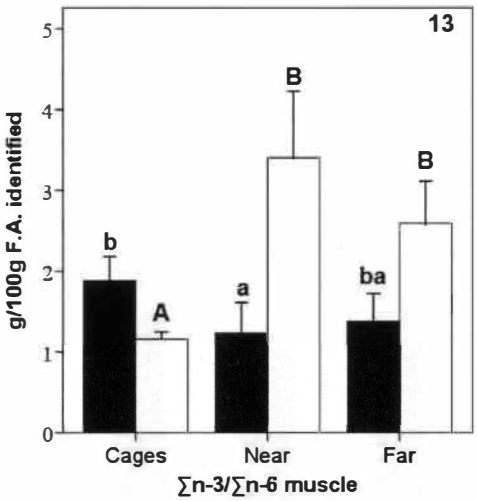

6
g/100g F.A. identified
25
20
15
10
5
0
a
B
a
A
a
A
Cages
Near
Far
Linoleic liver

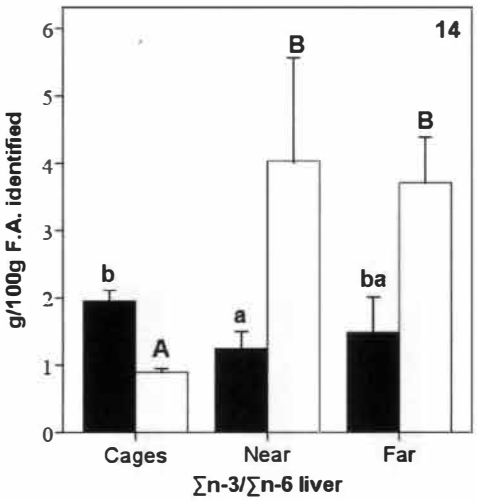

7
g/100g F.A. identified
2
1
0
a
B
a
A
a
A
Cages
Near
Far
Linolenic muscle

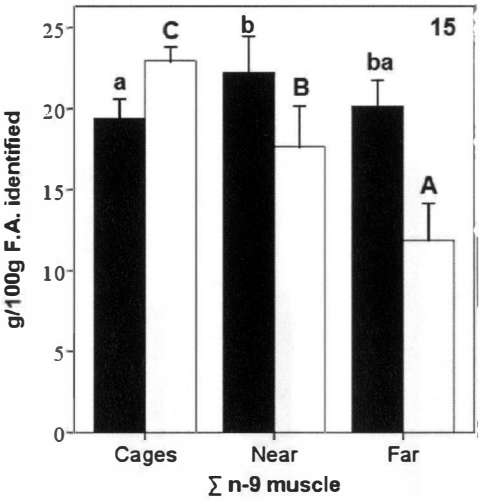

9
12
10
8
6
4
2
0
b
B
a
A
a
B
Cages
Near
Far
EPA muscle

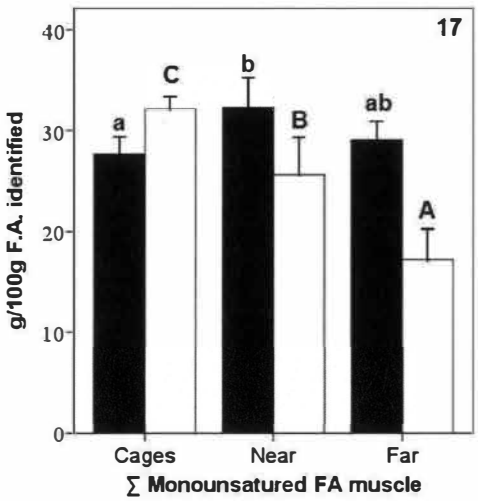

10
g/100g F.A. identified
10
8
6
4
2
0
a
A
a
B
a
AB
Cages
Near
Far
EPA liver

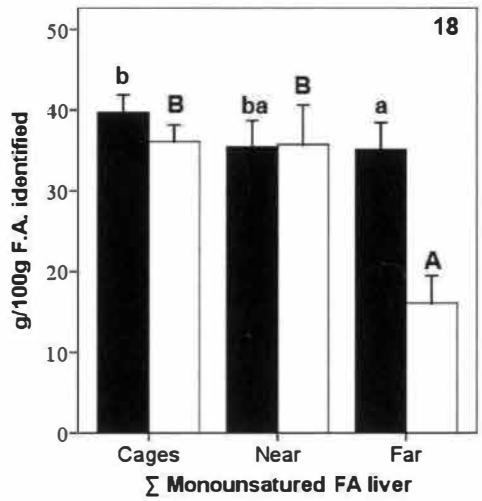

11
g/100g F.A. identified
25
20
15
10
5
0
a
A
a
B
a
B
Cages
Near
Far
DHA muscle

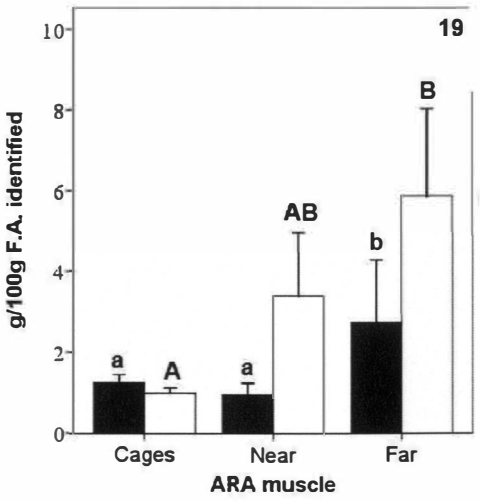

13
g/100g F.A. identified
0
1
2
3
4
5
b
A
a
B
ba
B
Cages
Near
Far
∑n-3/∑n-6 muscle

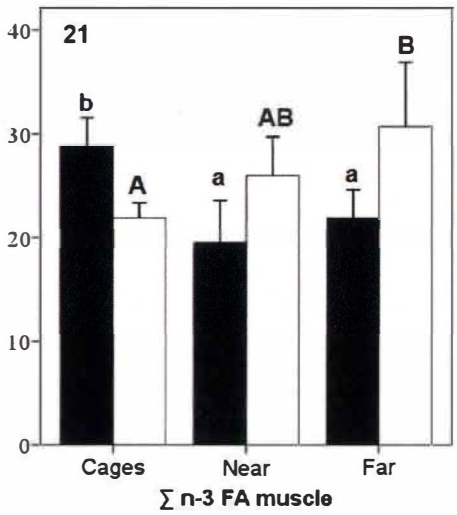

14
g/100g F.A. identified
0
1
2
3
4
5
6
b
A
a
B
ba
B
Cages
Near
Far
∑n-3/∑n-6 liver

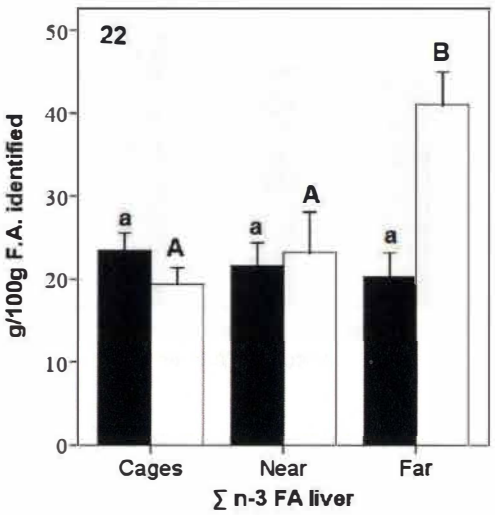

15
g/100g F.A. identified
0
5
10
15
20
25
a
C
b
B
ba
A
Cages
Near
Far
∑ n-9 muscle

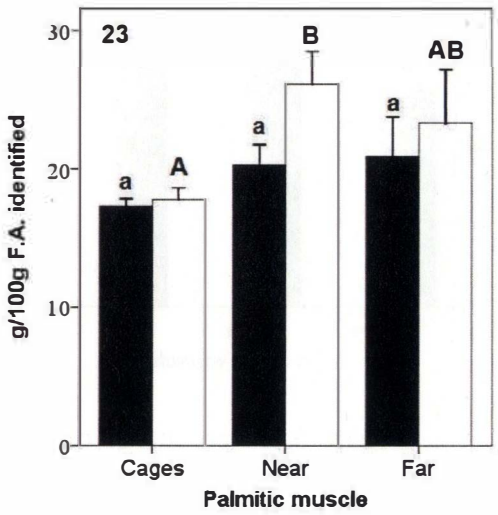

17
g/100g F.A. identified
0
10
20
30
40
a
C
b
B
ab
A
Cages
Near
Far
∑ Monounsatured FA muscle


18
g/100g F.A. identified
0
10
20
30
40
50
b
B
ba
B
a
A
Cages
Near
Far
∑ Monounsatured FA liver


19
g/100g F.A. identified
0
2
4
6
8
10
a
A
a
AB
b
B
Cages
Near
Far
ARA muscle


21
40
30
20
10
0
b
A
a
AB
a
B
Cages
Near
Far
∑ n-3 FA muscle


22
50
40
30
20
10
0
g/100g F.A. identified
a
A
a
A
a
B
Cages
Near
Far
∑ n-3 FA liver


23
30
20
10
0
g/100g F.A. identified
a
A
a
B
a
AB
Cages
Near
Far
Palmitic muscle

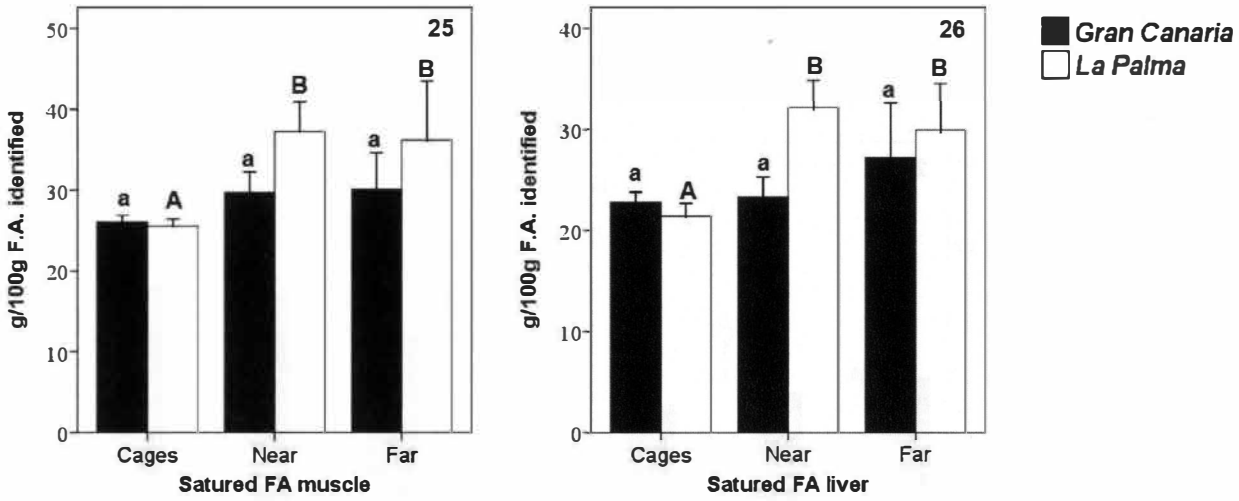


Figure 5.8. Percentage of lipid content and fatty acids in muscle and liver of escaped sea bass, *Dicentrarch* away from the cages) and 'far' (>10 km away from the cages). Alphabetic superscripts denote significant farms. **1)** muscle lipid content; **2)** liver fat content; **3)** muscle oleic acid (18:2n−9); **4)** liver oleic acid (18:2n− liver linoleic acid (18:2n−6); **7)** muscle linolenic acid (18:3n−3); **8)** liver linolenic acid (18:3n−3); **9)** musc liver eicosapentaenoic acid (20:5n−3); **11)**; muscle docosahexaenoic acid (22:6n−3); **12)** liver docosahexa n−3/n−6; **14)** liver ratio n−3/n−6; **15)** muscle ∑ n-9 FA; **16)** liver ∑ n-9 FA; **17)** muscle ∑ monounsatured muscle arachidonic acid (20:4n−6); **20)** liver arachidonic acid (20:4n−6); **21)** muscle ∑ n-3 FA; **22)** liver ∑ n **24)** liver palmitic acid (16:0); **25)** muscle ∑ satured FA; **26)** liver ∑ satured FA.

In contrast, several FAs showed an overall increase in their concentration with distance away from cages. For example, ARA showed higher values in 'far away' than 'cages' and 'near' samples, for both muscle (Fig. 5.8_19) and liver (Fig. 5.8_20) for La Palma, and exclusively for muscle tissue at Gran Canaria (Fig. 5.8_19). At La Palma, the percentage of DHA was significantly higher in 'far away' and 'near' samples than cultured fish ('cages') for muscle samples (Fig. 5.8_11). For liver samples (Fig. 5.8_12), however, there was no difference between 'near' and 'cages'. The concentration of ∑n-3 FAs from La Palma, for both muscle (Fig. 5.8_21) and liver (Fig. 5.8_22), were larger in 'far away' than 'cages' samples. At Gran Canaria, we detected a larger concentration of ∑n-3 FAS for muscle samples at 'cages' (Fig. 5.8_21). No difference in the level of PA was registered between 'cages' and 'far away' samples from both islands, for both muscle (Fig. 5.8_23) and liver (Fig. 5.8_24). The ∑n-3/∑n-6 ratio and satured FAs from La Palma, for both muscle (Figs. 5.8_13 and 5.8_25, respectively) and liver (Figs. 5.8_14 and 5.8_26, respectively), were significant higher from 'near' and 'far away' than 'cages' samples.

Finally, it is worth noting that the percentage of all FAs of cultured fish (i.e. 'cages') differed between islands (Fig. 5.8, Tables 5.4 and 5.5), except the fat percentage, DHA, satured, ARA and PA in muscle tissue, and monounsatured, ∑n-9, satured, OA and PA in liver tissue.

Table 5.4 Percentage value (mean ± SD) of different fatty acids identified in muscle of sea bass with varying distance from sea cage fish farms.

	MUSCLE					
	Cultured		Near		Far	
	Gran Canaria	La Palma	Gran Canaria	La Palma	Gran Canaria	La Palma
Lipid	13.3 ± 4.7	11.2±4.0	14.8±8.2	5.0±1.4	9.2±3.8	4.2±1.6
n-9	19.4±1.7	23.0±1.2	22.2±3.1	17.7±3.5	20.1±2.2	11.9±3.2
n-6	15.6±1.6	19.0±0.8	17.0±4.2	8.2±2.6	17.3±4.9	12.0±2.4
n-3	28.9±3.8	21.9±2.0	19.5±5.7	25.9±5.3	21.9±3.7	30.7±8.7

Saturated	26.0±1.2	25.6±1.3	29.7±3.6	37.3±5.1	30.2±6.3	36.2±10.2
Monounsaturated	27.7±2.4	32.1±1.8	32.3±4.1	25.6±5.1	29.1±2.6	17.2±4.2
n-3/n-6 ratio	1.9±0.4	1.2±0.1	1.2±0.5	3.4±1.1	1.4±0.5	2.6±0.7
14:0	3.0±0.4	3.0±0.3	3.1±0.9	1.8±0.6	2.4±0.6	1.3±0.6
14:1n-7	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
14:1n-5	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
15:0	0.3±0.0	0.3±0.0	0.3±0.1	0.4±0.1	0.3±0.1	0.3±0.1
15:1n-5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
16:0ISO	0.0±0.0	0.0±0.0	0.1±0.0	0.6±0.8	0.1±0.0	1.0±0.9
16:0	17.3±0.9	17.8±1.4	20.3±2.3	26.1±3.8	20.9±4.5	23.3±6.1
16:1n-7	4.6±0.5	4.3±0.4	4.7±1.1	3.7±1.6	4.1±0.5	2.1±0.8
16:1n-5	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.2±0.0	0.1±0.1
16:2n-6	0.0±0.0	0.5±0.1	0.3±0.2	0.3±0.2	0.1±0.1	0.1±0.1
16:2n-4	0.4±0.1	0.2±0.0	0.3±0.1	0.6±0.2	0.3±0.1	0.4±0.2
17:0	0.4±0.1	0.3±0.1	0.2±0.0	0.2±0.2	0.2±0.0	0.2±0.1
16:3n-4	0.2±0.0	0.2±0.1	0.1±0.1	0.3±0.1	0.1±0.1	0.1±0.1
16:3n-3	0.1±0.0	0.1±0.0	0.1±0.1	0.2±0.0	0.1±0.0	0.2±0.1
16:3n-1	0.2±0.1	0.2±0.1	0.2±0.2	0.8±0.4	0.4±0.3	1.4±0.4
16:4n-3	0.4±0.1	0.4±0.1	0.4±0.2	0.6±0.3	0.3±0.1	0.8±0.3
16:4n-1	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.1±0.0	0.3±0.1
18:0	4.9±0.5	3.9±1.5	5.5±1.3	8.5±2.1	6.1±2.2	11.0±4.3
18:1n-9	17.4±1.5	22.4±1.1	21.5±3.0	17.2±3.4	19.5±2.3	11.2±2.8
18:1n-7	2.9±0.1	2.7±0.1	2.9±0.3	2.4±0.2	3.0±0.5	2.4±0.3
18:1n-5	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1
18:2n-9	0.2±0.0	0.2±0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.0
18:2n-6	12.5±1.8	16.1±0.7	14.3±4.3	2.9±2.0	12.8±7.2	4.0±1.7
18:2n-4	0.3±0.0	0.2±0.0	0.2±0.0	0.1±0.1	0.2±0.0	0.1±0.1
18:3n-6	0.2±0.0	0.2±0.0	0.2±0.1	0.1±0.1	0.2±0.1	0.2±0.1
18:3n-4	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0
18:3n-3	1.5±0.2	2.1±0.2	1.5±0.5	0.4±0.3	1.7±0.6	0.4±0.1
18:3n-1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
18:4n-3	0.9±0.1	0.6±0.1	0.6±0.2	0.7±0.8	0.5±0.2	0.2±0.1
18:4n-1	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0	0.2±0.2
20:0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.1
20:1n-9+n-7	1.4±0.2	1.5±0.1	1.8±0.6	1.4±0.5	1.4±0.2	0.8±0.4
20:1n-5	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.1
20:2n-9	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0
20:2n-6	0.7±0.1	0.7±0.0	0.7±0.1	0.4±0.1	0.7±0.1	0.7±0.1
20:3n-9	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
20:3n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.1±0.1
20:4n-6	1.3±0.3	1.0±0.2	1.0±0.4	3.4±2.2	2.7±2.2	5.9±3.0
20:3n-3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.1
20:4n-3	0.5±0.0	0.4±0.0	0.3±0.1	0.3±0.2	0.3±0.1	0.2±0.1
20:5n-3	9.8±0.9	7.4±0.7	6.1±2.0	4.8±1.4	5.7±1.6	7.3±2.6
22:1n-11	0.6±0.1	0.5±0.1	0.8±0.7	0.2±0.1	0.3±0.1	0.2±0.1
22:1n-9	0.2±0.0	0.2±0.0	0.2±0.1	0.2±0.1	0.2±0.0	0.1±0.1
22:4n-6	0.4±0.0	0.1±0.0	0.2±0.1	0.1±0.1	0.2±0.2	0.1±0.1
22:5n-6	0.4±0.1	0.3±0.0	0.3±0.1	0.9±0.3	0.5±0.2	1.0±0.3

22:5n-3	1.9±0.2	1.4±0.1	1.2±0.3	0.8±0.3	1.6±0.6	1.5±0.9
22:6n-3	13.7±3.1	9.5±1.6	9.3±4.4	18.1±4.3	11.5±2.6	20.1±6.4

Table 5.5 Percentage value (mean ± SD) of different fatty acids identified in liver of sea bass with varying distance from sea cage fish farms.

LIVER						
	Cultured		Near		Far	
	Gran Canaria	La Palma	Gran Canaria	La Palma	Gran Canaria	La Palma
Lipid	76.7±2.6	67.5±5.7	63.7±21.7	47.4±16.4	61.1±27.5	21.4±9.9
n-9	30.3±2.8	28.2±2.7	25.7±3.6	25.6±6.1	25.6±3.9	10.1±3.5
n-6	12.1±1.4	21.7±1.8	18.1±4.1	6.8±2.7	15.9±7.1	11.6±2.4
n-3	23.5±2.9	19.4±2.8	21.6±3.9	23.2±6.8	20.3±4.0	41.1±5.5
Saturated	22.8±1.4	21.4±1.8	23.3±2.8	32.1±3.8	27.2±7.6	29.9±6.5
Monounsaturated	39.7±3.0	36.1±2.8	35.5±4.5	35.7±6.8	35.1±4.7	16.1±4.7
n-3/n-6 ratio	2.0±0.2	0.9±0.1	1.2±0.4	4.0±2.1	1.5±0.7	3.7±0.9
14:0	1.9±0.2	1.8±0.6	2.4±0.7	1.9±0.4	2.2±0.6	1.7±0.7
14:1n-7	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
14:1n-5	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.1±0.0
15:0	0.3±0.0	0.2±0.0	0.3±0.1	0.4±0.1	0.3±0.1	0.5±0.1
15:1n-5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
16:0ISO	0.1±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1
16:0	16.6±1.1	15.7±1.7	16.4±1.8	23.7±2.6	19.8±5.9	20.0±4.2
16:1n-7	5.0±0.3	3.9±0.2	4.9±1.2	5.0±0.9	5.2±0.7	3.3±1.6
16:1n-5	0.1±0.0	0.1±0.0	0.2±0.0	0.3±0.1	0.2±0.1	0.2±0.1
16:2n-6	0.0±0.0	0.2±0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.1
16:2n-4	0.2±0.0	0.2±0.0	0.3±0.2	0.5±0.3	0.3±0.1	0.4±0.2
17:0	0.2±0.0	0.2±0.1	0.3±0.1	0.3±0.4	0.3±0.1	0.1±0.1
16:3n-4	0.5±0.0	0.2±0.1	0.1±0.1	0.7±1.0	0.1±0.1	0.2±0.1
16:3n-3	0.1±0.0	0.1±0.0	0.1±0.0	0.9±2.4	0.1±0.0	0.1±0.1
16:3n-1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.2	0.3±0.2

16:4n-3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.1	0.1±0.1
16:4n-1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.1	0.1±0.0
18:0	3.6±0.4	3.3±0.4	3.8±1.4	5.6±1.3	4.5±2.2	7.6±2.7
18:1n-9	27.9±2.7	27.4±2.7	24.9±3.5	24.9±6.1	24.9±3.8	9.0±3.1
18:1n-7	3.9±0.2	2.7±0.1	3.1±0.6	3.3±0.7	3.0±1.2	2.2±0.6
18:1n-5	0.2±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.1
18:2n-9	0.4±0.1	0.4±0.1	0.3±0.1	0.3±0.1	0.4±0.3	0.0±0.0
18:2n-6	9.6±1.2	19.0±1.6	15.4±3.9	3.1±2.4	12.1±8.7	3.0±2.2
18:2n-4	0.3±0.0	0.2±0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.1
18:3n-6	0.3±0.0	0.6±0.1	0.3±0.1	0.2±0.1	0.3±0.1	0.2±0.2
18:3n-4	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0
18:3n-3	1.2±0.1	2.2±0.3	1.8±0.5	0.6±0.3	1.9±1.0	0.5±0.2
18:3n-1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
18:4n-3	0.7±0.1	0.5±0.1	0.5±0.1	0.7±0.6	0.5±0.2	0.4±0.4
18:4n-1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.3	0.1±0.0	0.0±0.0
20:0	0.2±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.1±0.0	0.1±0.1
20:1n-9+n-7	1.6±0.1	1.2±0.1	1.5±0.5	1.6±0.7	1.2±0.4	0.9±0.4
20:1n-5	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1
20:2n-9	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0
20:2n-6	0.6±0.1	0.8±0.1	0.8±0.2	0.4±0.2	0.6±0.1	0.9±0.4
20:3n-9	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
20:3n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.1	0.2±0.1
20:4n-6	0.7±0.1	0.5±0.1	0.8±0.5	1.9±1.4	2.2±2.2	6.4±3.4
20:3n-3	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.1±0.0	0.2±0.1
20:4n-3	0.5±0.1	0.4±0.1	0.5±0.1	0.5±0.3	0.4±0.1	0.3±0.2
20:5n-3	7.0±0.8	5.3±0.7	6.2±1.9	3.6±1.3	5.4±2.0	7.0±2.3
22:1n-11	0.6±0.1	0.3±0.0	0.5±0.3	0.1±0.1	0.2±0.1	0.10.1
22:1n-9	0.2±0.0	0.1±0.0	0.2±0.1	0.2±0.1	0.1±0.0	0.1±0.1
22:4n-6	0.4±0.1	0.1±0.0	0.3±0.1	0.3±0.3	0.2±0.2	0.2±0.1
22:5n-6	0.3±0.1	0.3±0.0	0.3±0.1	0.6±0.2	0.3±0.1	0.7±0.2

22:5n-3	2.0±0.3	1.6±0.3	1.8±0.4	1.2±0.6	1.8±0.4	2.2±1.5
22:6n-3	11.7±1.8	9.2±1.6	10.6±2.7	15.4±7.3	10.0±1.8	30.3±7.3

5.4 Discussion

Populations of European sea bass in the studied islands are related to aquaculture escapes (Carrillo and Castillo, 2001; Toledo et al., 2009; Toledo et al., 2012). Our results agree with this idea, because European sea bass density over time was much higher adjacent to the sea-cage fish farm at Tazacorte, as also demonstrated by Toledo et al. (2014b), than at the other locations, where most fish disappear through time after the massive release. Moreover, the size class distribution of European sea bass from La Palma clearly indicated that the majority of individuals are aquaculture escapees, as also indicated by Toledo et al. (2014a, 2014b) for the same escape event. The European sea bass may reach any location around the islands perimeter after a massive escape, showing therefore a great capacity of dispersion as proposed by previous studies (Carrillo and Castillo, 2001; González-Lorenzo et al., 2005; Tavares and González 2010; Toledo-Guedes 2014a, 2014b). Small, chronic, escapes, however, have not caused a high dispersion of escapees; escaped fish are typically located around cages or in the near coast (Carrillo and Castillo, 2001; Toledo et al., 2009; Arechavala-López et al., 2011b; Ramírez et al., under review).

Over time, escaped fish tend to approach shallow waters (Carrillo and Castillo, 2001; Tavares and González 2010; Arechavala-López 2014; Toledo et al., 2014a, 2014b). Despite Toledo et al. (2009) showed a preference by the European sea bass for bottoms covered by boulders on shallow waters, our study demonstrated that escaped European sea bass prefers breakwaters; in particular, higher European sea bass densities were observed in those locations (Tazacorte and Santa Cruz) where breakwater do exist. In this sense, Santa Cruz was the second location with a higher density of fish, in spite of being far away from the escape point (Fig. 5.1). This result contrasts with those observations reported by Toledo et

al. (2014b), which demonstrated lower densities in the east face of the island (contrary to the escape location) and so a clear correlation between the distance from the fish farm and European sea bass density. It is noteworthy that this result was despite our sampling and that performed by Toledo et al. (2014a, 2014b) were carried out within a similar temporal window.

Previous studies have concluded that escaped European sea bass is able to exploit natural resources in the Canary Islands (Carrillo and Castillo, 2001; Toledo et al., 2009; González-Lorenzo et al., 2005; Toledo et al., 2014a) The principal preys identified in all of these studies are common on subtidal bottoms of the Canary Islands. We corroborated this idea, showing an overlap with the diet indicated by Toledo et al. (2014a) for the same escape event, which highlighted *Percnon gibessi* as the principal prey. However, these authors did not found *Rhynchocinetes sp* as a relevant diet constituent of escaped fish, although much of escaped fish were caught practically in the same locations and time. These differences could be due to variations in sample size (Kennedy and Fitzmaurice, 1972; Arias, 1980; Toledo et al., 2014a), and, of course, the somehow opportunistic feeding behavior of the European sea bass (Laffaille et al., 2001). Nonetheless, our overall diet results agree with previous studies (Arias 1980; FAO, 2012) that showed crustacean decapods and osteichthyes as the principal prey of wild European sea bass.

Toledo et al. (2014a) showed that the diet of fishes from massive escape events typically differs as a result of the 'time at liberty'. Previous studies (Arechavala-López et al., 2012; Toledo et al., 2014a) highlighted that these differences in escaped fish diet could reflect a 'hunting learning' period, i.e. recent escapees predate mainly over crustaceans that are less mobile and 'more time escaped fish' predate over fish. Lorenzen et al. (2012) concluded that there are critical uncertainties on the effects of different domestication strategies on the fitness of cultured fish in the wild. We observed that diets of escaped

European sea bass differed between La Palma and Gran Canaria; *Percnon gibessi* was the main prey for escapees from La Palma and osteichthyes for Gran Canaria. However, in the light of FA results, 'more time escaped fish' (those from La Palma) mainly fed over crustaceans, while recently escaped fish (those from Gran Canaria) principally fed over fish. These preys are common in both islands, so prey availability does not seem to be the cause of these differences. In any case, we may overestimate this issue, since we have not analyzed a high number of stomachs from Gran Canaria, due to difficulties to find escaped fish.

Escaped European sea bass diet includes a high percentage of inorganic items (Carrillo and Castillo, 2001; Toledo et al., 2009). Carrillo and Castillo (2001) and Toledo et al. (2009) showed a mean 82.63 % and 50 % (respectively) of stomach vacuity, proving evidence of a low adaptation to wild conditions of escaped individuals. Yet, we recorded a mean stomach vacuity of 25.97 %, suggesting a high variability of this parameter. In any case, inorganic matter (sand and plastics) was the third main item in the stomachs of escaped European sea bass in our study, highlighting the difficulties of escaped fish to wild feeding conditions. Toledo et al. (2014a) reported a similar value for long-term escaped fish (33.9% of vacuity), while the value was lower for recently escaped fish (12.5% vacuity); as a result, this contradicts the previous idea and suggests that the European sea bass is able to actively exploit available resources in natural habitats.

The reproduction of the European sea bass often occurs between December and March (Do Chi and Hoai Thong, 1971; Barnabé, 1973; Arias, 1980; González, 2003). In our study, only a very low number of individuals, including both males and females, showed mature gonads; this contrasts with a larger number of mature gonads found by Toledo et al. (2012). However, both studies have not found evidence of reproduction. This is noteworthy, because individuals were in their sexual maturity period. In addition, only one male at a

spawning state was observed. This could be because the range of temperature and salinity necessary for gonadal development (FAO, 2012) is not present in the studied islands (including higher temperatures). Even if the European sea bass would spawn, neither incubation of eggs conditions, nor term-haline conditions for larval development (Devauchelle and Coves, 1988; Houde and Zastrow, 1993; Saka et al., 2001), are present in the Canary Islands. The sex ratio (1:1) was, moreover, not optimal (4:1 according to Arias, 1980) and no juvenile European sea bass had been reported (Carrillo and Castillo, 2001; Toledo et al., 2009; Toledo et al., 2014a). Therefore, there is no evidence of European sea bass reproduction at La Palma and Gran Canaria islands, as similarly concluded by Toledo et al. (2009) and Carrillo and Castillo (2001). In any case, further and more specific studies would be necessary in order to know escaped European sea bass capacity of reproduction in Canary Islands.

After the mass escape event, the number of European sea bass decreased through time, almost disappearing, providing evidence that no more escape events occurred at La Palma Island. A similar conclusion was highlighted by Toledo et al. (2009; 2014b). This decrease is principally due to a high fishing pressure, failure to adapt (starvation, deformities) and predation by large-sized fish (e.g. *Seriola sp, Sphyraena viridensis, Pomatomus saltatrix*) (Vergara et al., 2005; Sanchez-Jerez et al., 2008; Toledo et al., 2009; Arechavala-López et al., 2010; Tavares and González, 2010;Arechavala-López et al., 2011b; Arechavala-López et al., 2014; Toledo et al., 2014b; Ramírez et al., under review). Nevertheless, Arechavala-López et al. (2014) showed low recapture rates by local fishermen on European sea bass inform the Mediterranean Sea (including both recreational and professional captures). As previous studies, we also recognize fishing pressure as a significant driver of the progressive decay in fish abundances through time. About 63 tons of escaped European sea bass were fished by this professional (artisanal) fleet during the study

period in La Palma ('Consejería de Agricultura y Pesca del Gobierno de Canarias', pers. comm.) and recreational fishermen captures have been reported as larger than 100 fish, or 100 kg, per angler in just one day in the study region (Tavares and González, 2010). If we assume that recreational captures were larger than those performed by professional fishermen, the overall capture was far from reaching *ca.* 400 tons of escaped European sea bass. We then hypothesize that remaining fish were predated or non-adapted. Moreover, the great decrease occurred during October, matching the arrival at coastal areas of migratory, large-sized, predators (tuna, yellowtails, *Sphyraena viridensis*, dolphins and sharks) (Bas et al. 1995). Beside this, it is plausible that escaped European sea bass may compete with native species, such as *Dicentrarchus punctatus*, juveniles of *Sphyraena viridensis*, *Synodus sp.*, *Serranus sp.*, which have a similar ecological niche and feeding habits, as concluded by González-Lorenzo et al. (2005), Toledo et al. (2009) and Tavares and González (2010).

In the light of overall FA results, it seems clear that escaped fish from Gran Canaria were recently escaped, because no difference existed with varying distance from aquaculture cages. In particular, the 18:C FAs would tend to be eliminated or washed-out progressively from the muscle, as soon as those animals finish to feed aquafeeds and begin to feed on wild prey (Torstensen et al., 2004; Izquierdo et al., 2005). This emphasizes the rapid dispersion that European sea bass presents after an escape event as we observed at La Palma by means of visual census. FA profiles of escapees changes over time after an escape by wash-out (Arechavala-López et al., 2013b; Ramírez et al., 2013), so it seems clear that escaped European sea bass at La Palma have been more time escaped than at Gran Canaria. Our initial hypothesis aimed to relate distance with time (more distance, more time to disperse, more wash out), but we somehow failed to detect this, mainly because this species has a quick dispersion behavior. The FA composition of muscular tissue is often related to dietary FA composition (Montero et al., 2005), so some FAs can be used as bioindicators of escaped

fish (Rueda et al., 2001; Bell et al., 2002; Blanchet et al., 2005; Fernández-Jover et al., 2007, 2011; Mnari et al., 2007; Megdal et al., 2009). Normally, reared European sea bass present higher proportions of OA and LA and lower proportions of PA, ARA, EPA, and DHA than wild European sea bass (Alasalvar et al., 2002; Fernández-Jover et al., 2011; Arechavala-López et al., 2013b). Additionally, percentages of total saturated, as well as the n-3/n-6 ratio, are higher in wild than cultured European sea bass (Alasalvar et al., 2002; Fernández-Jover et al., 2011); these results match the outcomes of this study. FA composition, however, may not be reliable biomarkers of aquaculture activities since other human activities, including discharges of urban waters, may induce similar changes (Ramírez et al., 2013), directly by feeding or due to the relatively high conservation of FA composition throughout the food web (Fernández-Jover et al., 2011). This is particularly relevant for European sea bass, as a result of its quick dispersion behaviour. We also observed differences in the profile of FAs between cultured fish from Gran Canaria and La Palma; this is a consequence of continuous changes in aquafeeds formulae that depends on the ingredients availability and fluctuating market prices (Gunstone, 2010). DHA, OA and LA seem to be selectively retained in European sea bass muscle (Montero et al., 2005). When fish that has been fed with vegetable oil containing diets is subjected to a period of fish oil re-feeding, the amount of 18:C FAs (particularly LA in those fish fed previously with soybean oil containing diet) remains higher and EPA lowers (Montero et al., 2005).

To determine if a FA is a good aquaculture biomarker, it would be necessary to perform a study at the same region, where the FA composition should be in similar from both culture and escaped fish and distinct from wild fish. In the present study, however, there was no wild fish, so we were able to exclusively assess if FA composition changed with varying distance from farms. Contrary to Fernández-Jover (2011), FA profile cannot be used as biomarkers, because there were significant differences between distance groups ('cages'-

'near'-'far') in this study. Moreover there is an inconsistency for FAs highlighted among several studies (Grigorakis et al., 2002; Mnari et al., 2007; Arechavala-López et al., 2011a; Ramírez et al., 2013; Arechavala-López et al., 2013b), so the usefulness of FAs as biomarkers for escaped fish is doubtable. We found different FAs than those proposed by Arechavala-López et al. (2013b) (i.e. LA and ARA) as possible biomarkers. The current study could suggests PA (liver) and EPA (both at liver and muscle) as the candidates as possible biomarkers, because these FAs were not different with varying proximity from the farms; however further studies are necessary to confirm its reliability. This matches the result showed by Montero et al. (2005), where EPA values did not become to control diet (fish oil) after a re-feeding period in the laboratory. In the Canary Islands, Ramírez et al. (2013) concluded that ALA is a possible aquaculture biomarker for bogue, *Boops boops*, a zoo-planktivorous and opportunistic fish that aggregates around sea-cage fish farms in the Canary Island and the Mediterranean; this is because samples taken around sewage discharge points did not increase the ALA percentage. However, this study demonstrated that ALA cannot be used as a biomarker for escaped European sea bass. FAs could be used to identify escaped fish when matching similar FA profiles from cultured fish. However, if FA profiles do not match those of cultured fish, it is impossible to work out whether a fish has been born in the wild or, alternatively, is an old escapee that has progressively suffer a wash-out.

In summary, despite escaped fish were able to exploit natural resources, the density of escapees decreased through time. Those fish able to adapt and use natural resources altered their FA profiles in comparison to cultured fish, denoting the poor usefulness of FA profile as a good bio-indicator, limiting their potential to a very short period of time after escape events.

5.5 Acknowledgements

Authors would like to thank María López Ruano for her help to produce this document. Our thanks also go to Tamia Brito, manager of La Palma Island Marine Protected Area. The staff at Acuipalma S.L. for their collaboration. David Jiménez Alvarado and Yamilet Cárdenes are acknowledged for their assistance during field sampling. This work was partially funded by Prevent Escape project (no. 226885).

6

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

DIFERENCIAS MORFOLÓGICAS

6 "MORPHOLOGICAL DIFFERENCES BETWEEN FARMED AND ESCAPED SEA BASS (*Dicentrarchus labrax*)"

Besay Ramírez[a,b], Daniel Montero[a], Ricardo Haroun[b]

[a]*Grupo de Investigación en Acuicultura. Universidad de Las Palmas de Gran Canaria and Instituto Canario de Ciencias Marinas. P.O. Box 56. 35200. Telde, Las Palmas, Canary Islands, Spain.*

[b]*BIOGES, Marine Sciences Faculty, Campus Tafira, Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas de G.C., Canary Islands, Spain.*

6.1 Abstract

In order to evaluate the effectiveness of morphometry to distinguish farmed from escaped sea bass (*Dicentrarchus labrax*), twenty individuals of both farmed and escaped from Gran Canaria and La Palma Island (Canary Islands, Spain) were analyzed. We established thirteen landmarks which defined 28 morphological measurements that were processed by means of "Image-Pro Plus" software. ANOVA test showed differences only in two morphological measures from head region. Most of morphological measures matched between escaped and reared fish in Sea bass, pointing out that morphometry could be used as escape indicator because escaped fish practically did not differ from those cultured.

6.2 Introduction

Global production of fish from aquaculture has grown substantially in the past decade, reaching 78.9 million tons in 2010. Aquaculture continues to be the fastest-growing animal food producing sector and currently accounts for nearly half (46.8 percent) of the world's food fish consumption (FAO 2012). FAO estimated sea bass (*Dicentrarchus labrax*)

European production about 55710 tons in 2010, contributing in 29.91% of total European marine fish production and 44.24% of global sea bass production (125901 tons). Sea bass is an important commercial marine fish species along the Mediterranean and Eastern Atlantic coastline both for aquaculture and fisheries.

Aquaculture has important implications for marine and coastal biodiversity at the level of genetic variability, species-species interaction and alteration within ecosystems (CBD 2004). The main impacts considered, and the most studied, are the effect of wasted food and faecal discharges, that alters and modify the sediment characteristics under fish cages (Molina-Dominguez et al. 2001; Mente et al. 2006), and the presence of sea cages that modifies the icthyological community around them (Carss 1990; Machias et al. 2004; Tuya et al. 2006; Dempster et al. 2005). Nevertheless, other sources of impacts have been studied such as addition of anti-fouling products, parasite transfers, exotic species, input of therapeutic products, visual impact or fish escapes (Hewitt et al. 2006; Fernandez-Jover et al. 2010).

Escapees could present environmental impact such as genetic interaction by interbreeding, transfer of pathogens, prey predation alteration, introduction of new alien species, habitat competition or/and alteration, etc. (CBD, 2004; Molina and Vergara 2005; Naylor et al. 2005; Vergara et al., 2005; Jensen et al., 2010; Grigorakis and Rigo 2011). Escapees can have detrimental genetic and ecological effects on populations of wild conspecifics, and the present level of escapees is regarded as a problem for the future sustainability of sea-cage aquaculture (Naylor et al., 2005). For sea bass, sea bream and meagre, knowledge regarding how escapes might affect ecosystems is limited or inexistent. To study this, is important to identify individuals that escape from sea-cages. Authors had suggested different ways to identify escaped fish as genetic (Glover et al. 2009), fatty acids (Fernández-Jover et al. 2007), scales and otholits elementary profiles (Adey et al. 2009),

bimolecular trace (Megdal et al. 2009) or scales morphology (Lund and Hansen 1991). Intraspecific fish groups can show morphological differences due to environmental differences coupled with adaptative genetic changes (Barlow G. 1961; Kinnison and Hendry 2004; Solem et al. 2006). Some authors had demonstrated that body morphology can be used as stock identifier (Hurlbut and Clay 1998; Murta 2000; Solem et al. 2006; Swatripiyanka et al. 2011) or escape indicator (Loy et al. 1999; Murta 2000; Cramon-Taubadel et al. 2005; Arechavala-López et al. 2011; Uglem et al. 2011).

The aim of this study was to evaluate the suitability of morphological variation as escapes indicator of Mediterranean aquacultured fish, identifying these body measures which match between escaped and reared fish in Sea bass.

6.3 Material and Methods

Ten reared Sea bass were randomly sampled from each two fish farm placed in two different islands at Canarian Islands: Gran Canaria (Canexmar S.L.) and La Palma (Acuipalma S.L.) (Fig. 6.1). Escaped sea bass (n=20) were spearfished around Gran Canaria and La Palma. Samples were caught randomly between September 2010 and November 2011. A larger escape event was reported for Acuipalma, in La Palma, seven months before study sampling began. In Gran Canaria no large escape event during sampling were recorded. Logistics of sampling required the frozen of samples at -40 ºC. Fish were de-frozen after dissection and then all samples were weighted (accuracy of 0.1 gr.) and measured (accuracy of 0.1 cm). Following, points were placed throughout fish body, to help to identify the landmarks that we used in the posterior analysis. Morphological landmarks were selected to give a precise definition of the fish morphology (Fig. 6.2). Properly calibrated coordinates of morphometric locations, or 'landmarks', are generally more efficient and precise than manual distance measurements. We measured distances between selected landmarks in the pattern of a graph called a *truss* (Strauss and Bookstein 1982), by

means of Image-Pro Plus (Media Cybernet, Silver Spring, MD, USA) software. 'Truss networks' of distances between landmark coordinates provide more comprehensive coverage of form for greater discriminating power. Strauss and Bookstein (1982) and Swatipriyanka et al. (2011) demonstrated the possibility of discrimination between populations by means of this technique. A total of 13 landmarks that defined 28 morphological measurements were defined (Table 6.1). Morphometric measurements were standardized to the overall mean total length, the standardized measure being given by: $Mc=Mx\ (\frac{\overline{TL}}{TL})^b$ (Hurlbut and Clay 1998; Ihssen et al. 1981); where TL is the total length, M is the original measurement, $\overline{TL}$ is the overall mean total length and b is the slope, within areas, of the geometric mean regression (Ricker 1973) on the logarithms of M and TL. Normalized measurements were statistically analyzed. Data were balanced, presented normal distribution ($n > 30$) and homogeneity of variances (Cochran´s test; $p>0.05$). Non metric-multidimensional scaling (nm-MDS) was used as the ordination method. A permutation test (PERMANOVA) was used to assess the significance of the overall body measurements among the considered sources of variation (factors) (Anderson 2004). Then, variables that had more influence on dissimilarities among origin factor were calculated using the SIMPER (similarity percentages) procedure (Clarke 1993). Finally an analysis of variance (ANOVA) was used to test differences of factors among each variable. If ANOVA detected significant differences, further analyses were performed by using the SNK *a posteriori* multiple comparison test (Underwood, 1997). Both for PERMANOVA and ANOVA the model incorporated the following experimental design: (1) Origin (fixed factor with 2 levels corresponding to cultivated and escaped origin); (2) Island (random and orthogonal factor with 2 levels corresponding to 2 different islands: Gran Canaria and La Palma). The SPSS (v. 15.0), PRIMER (v. 5.2.4) and PERMANOVA (v. 1.6) software were used for statistical analysis.

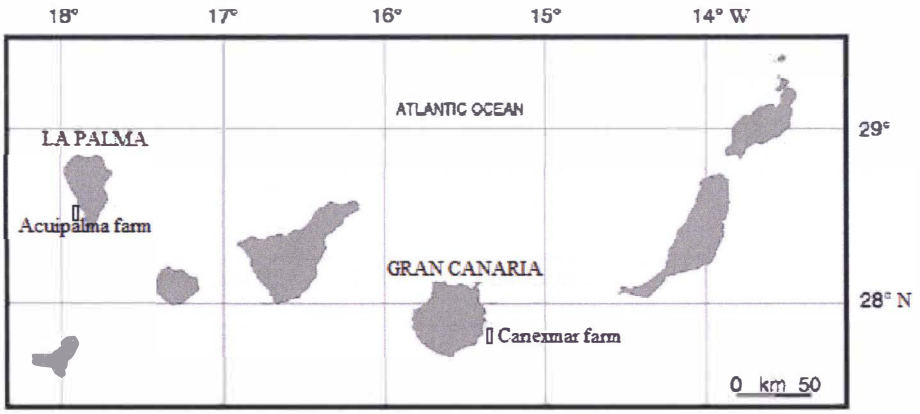


Figure 6.1. Map of study area detailing sampling farms in La Palma and Gran Canaria.

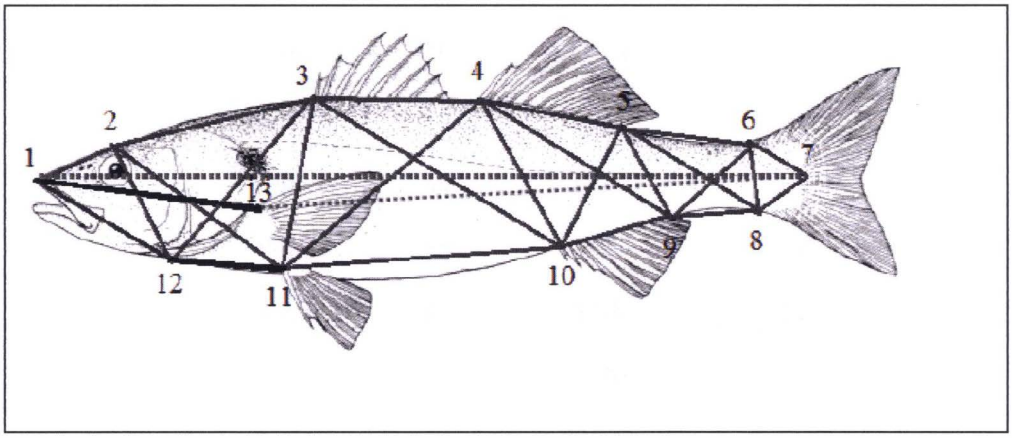


Figure 6.2. The 13 landmarks and the distance measured between them, which were used for body shape study of sea bass. Black lines show significant differences between cultured and escaped fish. Landmarks were located as follows: 1 snout; 2 point of maximum curvature in the head profile curve; 3 origin of the first dorsal fin; 4 origin of the second dorsal fin; 5 posterior end of the second dorsal fin; 6 dorsal point at least depth of caudal peduncle; 7 posterior extremity of the lateral line; 8 ventral point at least depth of caudal peduncle; 9 posterior insertion of anal fin; 10 origin of anal fin; 11 origin of pelvic fin; 12 insertion of the operculum on the profile; 13 origin of pectoral fin.

Table 6.1. Codes of morphological measurements with corresponding landmarks (Fig. 6.2). Landmarks were: 1 snout; 2 point of maximum curvature in the head profile curve; 3 origin of the first dorsal fin; 4 origin of the second dorsal fin; 5 posterior end of the second dorsal fin; 6 dorsal point at least depth of caudal peduncle; 7 posterior extremity of the lateral line; 8 ventral point at least depth of caudal peduncle; 9 posterior insertion of anal fin; 10 origin of anal fin; 11 origin of pelvic fin; 12 insertion of the operculum on the profile; 13 origin of pectoral fin

Code	Landmark	Code	Landmark
a1	1-2	c5	4-11
a2	2-12	d1	4-5
a3	1-12	d2	5-9
b1	2-3	d3	9-10
b2	3-11	d4	4-9
b3	11-12	d5	5-10
b4	2-11	e1	5-6
b5	3-12	e2	6-8
c1	3-4	e3	8-9
c2	4-10	e4	5-8
c3	10-11	e5	6-9
c4	3-10	f1	6-7
f2	7-8		
f3	1-13		
f4	7-13		

6.4 Results

The mean weights of cultivated fish were 434.8 and 347.6 in Gran Canaria and La Palma respectively. For those escaped were 679.3 and 1115.0 in Gran Canaria and La Palma respectively (Table 6.2).

Table 6.2. Weight (mean±SD) and length (mean±SD) of cultivated and escaped fish from both studied island.

Origin	Island	Weight (gr.)	Length (cm.)
Cultivated	Gran Canaria	434.8±76.7	32.7±2.5
	La Palma	347.6±34.6	31.3±1.0
Escaped	Gran Canaria	679.3±619.2	38.2±9.8
	La Palma	1115.0±486.5	46.3±7.3

Non metric-multidimensional scaling (nm-MDS) did not showed clear differentiation between treatments (Fig. 6.3).

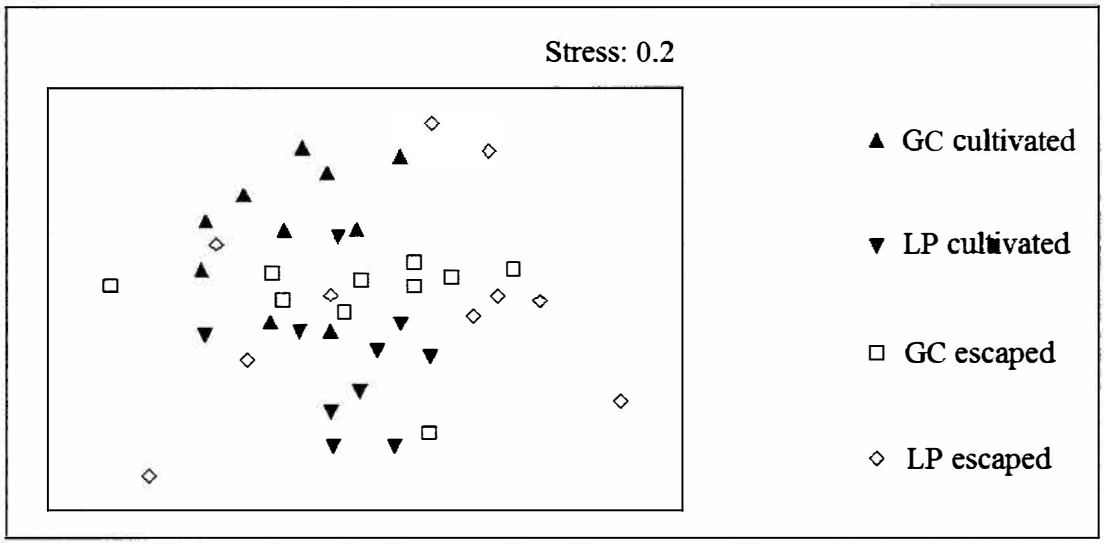


Figure 6.3. Non-metric multi-dimensional scaling plots of body shape measurements of cultured and escaped fish coming from both Gran Canaria (GC) and La Palma (LP).

PERMANOVA analysis (Table 6.3) showed that body shape did not differ among origin factor because no significant differences were found ($p>0.05$). Differences exists between island and "origin x island" factors. Post hoc analysis among "origin x island"

factor demonstrated that exist differences between cultivated and escaped fish only in Gran Canaria, moreover body shape of cultivated fish varied between Gran Canaria and La Palma.

Table 6.3. Results of PERMANOVA analysis of body shape measurements among defined factors: Origin, Island and Origin x Island interaction.

Source	df	SS	MS	F	P(perm)
Or	1	80.821	80.821	0.4562	0.6206
Is	1	180.212	180.212	34.096	0.0018
Or x Is	1	177.175	177.175	33.522	0.0030
Residual	36	1.902.744	52.854		
Total	39	2.340.952			

Besides this, dissimilarity given by SIMPER analysis between cultured and escaped samples was very low: 3.38. This analysis showed that no variable (body shape measurement) provides greater differentiation than the rest (Table 6.4).

However when studied each variable through ANOVA analysis (Table 6.5) we observed that two morphological measures (b3 and f3) differed significantly between cultured and escaped fish. These measures are distances between: anterior insertion of pelvic fin and insertion of the operculum on the profile (b3); tip of the maxillary and dorsal insertion of pectoral fin (f3) (Fig. 6.2). Traits a2, a3, b3, b5, c1, c4, e3 and e5 differed between islands. Studying interaction between origin and island factor, we observed that were b2, d4 and f1 these measures that presented significant differences between cultivated and escaped only in Gran Canaria; and c2, c4 and f2 showed differences in both island. Among cultivated fish b2, c2, c4, d4, f1 and f2 showed differences between islands.

Table 6.4. SIMPER analysis. Contribution of the body measurements to overall dissimilarities between cultured an the cumulative dissimilarity. Groups Cultivated and Escaped. Average dissimilarity = 3.38

	Cultivated	Escaped		
Body measurements	Average Abundance	Average Abundance	Average Dissimilarity	Dissimilarity/SD
A3	7.06	7.31	0.21	1.38
F3	9.01	9.27	0.19	1.55
B3	4.54	4.39	0.19	1.41
F4	25.12	24.84	0.18	1.51
C3	12.68	12.64	0.18	1.38
D1	6.3	6.08	0.16	1.24
E1	4.74	4.63	0.14	1.2
B2	8.51	8.45	0.14	1.34
E4	6.37	6.2	0.14	1.3
C5	12.78	12.82	0.14	1.28
A2	5.97	6.11	0.14	1.33
C1	8.21	8.42	0.12	1.36
C2	8.34	8.2	0.12	1.45
B5	9.63	9.52	0.12	1.32
D4	9.37	9.11	0.11	1.49
B4	9.49	9.52	0.11	1.36
C4	13.66	13.66	0.11	1.42
B1	9.08	9.08	0.11	1.37
E3	4.13	3.95	0.1	1.41
A1	3.25	3.45	0.1	1.27
E5	5.63	5.51	0.09	1.35
F2	3.49	3.5	0.09	1.4
F1	3.4	3.4	0.09	1.45

Table 6.5. Significant differences of variables showed by ANOVA analysis, among studied factors (* = $p<0.05$)

	Origin	Island	Origin x island
a1			
a2		*	
a3		*	
b1			
b2			*
b3	*	*	
b4			
b5		*	
c1		*	
c2			*
c3			
c4		*	*
c5			
d1			
d2			
d3			
d4			*
d5			
e1			
e2			
e3		*	
e4			
e5		*	
f1			*
f2			*
f3	*		
f4			

6.5 Discussion

Farmed fish species are genetically manipulated through breeding regimes and owning to domestication attain commercially desirable attributes such as higher growth rates (Grigorakis and Rigos 2011). Farmed fish have higher growth rate than wild fish (Weber and Fausch 2003; Ferguson 2004). Growth rate apparently determines body shape by altering the timing of transition from one growth stanza to another (Huxley 1932; Martin 1949). For instance growth changes in early life are particularly efficacious in modifying the shape of the fish (Martin 1949), so morphological differences caused by the culture process might be a relatively permanent and directly related with the duration of the cultivation period (Loy et al. 2000; Craig et al. 2007; Uglem et al. 2011). In most of cases are caused by skeletal anomalies in the cephalic region, lordosis, kyphosis, etc. (Loy et al. 2000). This supports that escaped fish show a body shape similar to those cultured, as observed in present results where no remarkable differences exist between farmed and escaped sea bass.

Present study shows that escaped fish only presented significant longer traits in head (Fig. 6.2) (as demonstrated by Fleming et al. (1994) for wild Atlantic salmon and unlike results showed by Rogdakis et al. (2011) for hatchery-released sea bream) and pectoral region (Fig. 6.2) than those cultured. Arechavala-López et al. (2011) showed differences between cultured and wild sea bass in the same morphological measurements both Spain as Greece population. Moreover f3 (tip of the maxillary to dorsal insertion of pectoral fin) was the measurement that major percentage of variance presented for Greece population (wild-cultured). It could indicate that the first morphological changes that occur after escapes are in this zone (including f3 and b3). Differences in head morphology (f3) can be explained by difference in feeding habitat (Skúlason et al. 1989; Keeley et al. 2005; Keeley et al. 2007) and this, together with density, are the greatest changes that occur when fish escape. Moreover Arechavala-López et al. (2011) highlights the possibility that an escapee that

survives over time could resemble a wild individual due to the changes on habitat and food. This phenotypic plasticity had been well documented by authors for different species (Currens et al. 1989; Beacham 1990; Robinson and Parsons 2002; Wintzer and Motta 2005); Released gilthead sea bream after a 6 to 7 month period in a wild habitat configured a wild-like shape (Rogdakis et al. 2011), even juvenile brown trout and Atlantic salmon changed shape within a month in response to altered water velocities (Pakkasmaa and Piironen 2000). Fleming et al. (1994) demonstrated that some environmental induced differences due to juvenile hatchery rearing persisted but many disappeared.

Differences between islands (Table 6.3) were due to these differences among cultivated fish and within those escaped fish, due to time elapsed after escape event. Cultivated fish differ between islands (b2, c2, c4, d4, f1 and f2) due culture conditions (Sarà et al. 1999): differences in stock density, aggressivity, stress, accumulation of perivisceral fat, hepatic hyperplasia and the type and quality of food (Favaloro et al. 2002; Favaloro and Mazzola 2003; Sara et al. 1999), swimming performance (Basaran et al. 2007), fish mobility (Hanson et al. 2007), physical environmental condition and/or broodstock families resulting in genetic differences (as proposed by Solem et al. (2006) within Atlantic salmon juveniles).

On the other hand differences between cultured and escaped sea bass in Gran Canaria correspond to height measurement. It shows cultured higher bodies (b2, c2, c4 d4, f1 and f2), i.e. more elongated bodies for escaped sea bass, matching with results showed by previous authors between wild and cultured fish (sea bream: Grigorakis et al. 2002; Eurasian perch: Mairesse et al. 2005; sea bass: Arechavala-López et al. 2011); and between cultured and escaped (hatchery-released) sea bream (Rogdakis et al. 2011). Wild individuals are characterized by a more elongated body shape when compared to those farmed (Mairesse et al. 2005). However results from La Palma were contrary, but only in 3 traits (c2, c4 and f2), so escaped bodies were higher. This could due to less escaped time, consequently has not

been time to morphological changes. Besides this, analyzed escaped sea bass from La Palma could coming from a cultured stock that might be phonotypical/genotypical different (higher bodies) than this current cultured analyzed.

Summarizing previous authors is clear that body morphology is an interesting tool that permit to distingue between cultured and wild fish, including sea bass in Mediterranean (Arechavala-López et al. 2011), moreover seem possible to distingue even between cultured, hatchery-released and wild fish (Rogdakis et al. 2011). Our results supports that morphology can be used as escape indicator too, because escaped fish practically did not differ from those cultured in Gran Canaria and La Palma (were no autochthonous populations existed), however this conclusion should be considered cautiously due to its potential depend to the period of time after escape events since morphologic changes need time to occur. Further studies will be necessary taken into account the time elapsed since escape event.

6.6 Acknowledgements

Authors would like to thank Acuipalma S.L. and Canexmar S.L. for their wide availability to get us the samples. Mónica Betancor for her assistance with the software and Victor Tunset for provide us his knowledge in this issue. The study was partially financed by the EU-project Prevent Escape.

7

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

COMPORTAMIENTO POST-ESCAPE

7 "POST-ESCAPE BEHAVIOR OF CULTURED EUROPEAN SEA BASS, *Dicentrarchus labrax*, AND MEAGRE, *Argyrosomus regius*"

Besay Ramírez[a], Finn Økland[b], Fernando Tuya[a], Ingebrigt Uglem[b], Daniel Montero[c], Ricardo Haroun[a]

[a] *Grupo de Investigación en Biodiversidad y Conservación, Centro de Biodiversidad y Gestión Ambiental, Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas, Canary Islands, Spain.*

[b] *Norwegian Institute of Nature Research, Tungasletta 2, 7485 Trondheim, Norway*

[c] *Grupo de Investigación en Acuicultura. Universidad de Las Palmas de Gran Canaria, P.O. Box 56, 35200 Telde, Canary Islands, Spain.*

7.1 Abstract

Escapes from fish farms may involve considerable economic losses for aquaculture industry and cause negative interactions with the environment. The aim of this work was to study the movements of farmed sea bass, *Dicentrarchus labrax*, and meagre, *Argyrosomus regius*, following an induced escape, to understand the post-escape activity and behavior of escaped fish, as well as to suggest ways to mitigate escape events. Twenty five individuals of both sea bass and meagre were equipped with acoustic transmitters and released immediately adjacent to a fish farm in the eastern coast of Gran Canaria Island (Canary Islands, Spain). In addition, another 25 externally tagged conspecifics of each species were also released. Fish movements around this farm were recorded by ten stationary receivers during three months. Recapture rates by fishermen were low, including only 1 sea bass and 2 meagre. Recaptured fishes showed signs of interaction with predators (biting attacks). The

number of fish detected decreased exponentially during the first week after the release. Only nine fishes (two meagre and seven sea bass) were detected at the study zone one month after the release. Apart from a tendency towards meagre being observed more often in the nearby coastal area, sea bass and meagre appeared to have similar post-escape movement patterns; most fish did not totally leave the farm through the first week, so we propose to focus recapture efforts around cages during the first week after an escape event.

7.2 Introduction

Global production of fish from aquaculture has grown substantially in the past decade, reaching more than 90 million tons in 2012 (FAO 2014). Aquaculture continues to be the fastest-growing animal food producing sector and currently accounts for nearly half (49.4%) of the world's food fish consumption (FAO, 2014). FAO estimated that European sea bass production reached 70,147 tons in 2012, contributing in 33.6 % of total European marine fish production, 3.1% of European fish (included marine, freshwater and diadramous species) and 45.8% of global sea bass production (153,182 tons). The culture of other species, e.g. meagre, is expected to grow fast in the next few years. Indeed, meagre has the potential to become a mass market species (Monfort 2010) and it is a feasible candidate for the diversification of European aquaculture (Gil et al 2013). European meagre production was estimated in 1,865 tons in 2012, representing 0.9 % of the total European marine fish production, 0.08% of European fish (included marine, freshwater and diadramous species) and 11.7 % of the global meagre production (13,742 tons) (FAO 2014).

Sea bass, *Dicentrarchus labrax*, is distributed from Norway to Cabo Blanco, including the Mediterranean and Black Sea (Moretti et al. 1999). Its natural range includes the easternmost islands of the Canarian Archipelago: Fuerteventura and Lanzarote (Brito 1991), but it has also been recently found at Gran Canaria, Tenerife and La Palma, as a

consequence of escape events from offshore aquaculture facilities (Carrillo and Castillo, 2001; Toledo et al., 2009). Sea bass typically has a wide range of distribution at local scales; it is frequent along inshore waters, inhabiting estuaries, canals, coastal lagoons and even the final part of rivers. This species can grow up to 1 m and reach 12 kg. The mean length at first maturity is 32.3 cm (range between 23-46 cm) (Froese and Pauly 2006). Sea bass feed mainly on crustaceans, molluscs and osteichthyes (Tortonese 1986; Laffaille et al. 2001; Leitao et al. 2008; Toledo et al. 2009).

Meagre, *Argyrosomus regius*, is a fish distributed from France to Senegal, including the eastern Mediterranean (Quéméner 2002) and Canary Islands (Dooley et al., 1985; Lloris et al., 1991). Meagre is present at all depths along inshore and shelf waters (Froese and Pauly 2006). Both adults and juveniles are migratory in response to temperature changes (Froese and Pauly 2006; Stipa and Angelini 2005). Fish can grow up to 2 m and reach more than 50 kg. First maturity is estimated at 61.6 cm for males and within the 70–110 cm range for females; this species spawns in estuaries from March to August (González-Quirós et al 2011). Meagre feed mainly on crustaceans and osteichthyes (Moretti et al 1999; Gil et al 2014).

Aquaculture has important implications for coastal biodiversity at the level of genetic variability, species interactions, and alterations within ecosystems (CBD 2004). The main impacts are the effect of wasted food and faecal discharges, which alters and modify the sediment characteristics under fish cages (Molina et al. 2001; Mente et al. 2006), and the aggregation of wild fishes around cages (Carss 1990; Machias et al. 2004; Dempster et al. 2005; Tuya et al. 2006). Other sources of impacts include the addition of anti-fouling products, parasite transfers, the input of therapeutic products, etc. (Hewitt et al. 2006; IUCN 2007; Fernandez-Jover et al. 2010).

Offshore aquaculture of marine fish suffers from chronic and mass escapes, which may cause additional environmental impacts, such as genetic interaction by interbreeding with natural populations, transfer of pathogens, competition for food resources, introduction of exotic species, habitat competition or/and alteration, etc. (CBD 2004; Molina and Vergara 2005; Naylor et al. 2005; Vergara et al. 2005; Jensen et al. 2010; Grigorakis and Rigo 2011).

Escapes could be particularly problematic in those zones where wild, native, populations are small, or zones outside from their natural distribution ranges. In the Canary Islands, for example, escaped sea bass and meagre diet may overlap with other top predators. Carrillo and Castillo (2001) and Toledo et al (2008) highlight that sea bass may become a new competitor for local species. No evidence of reproduction of escaped sea bass in the Canary Island has been reported however (Carrillo and Castillo 2001; Toledo et al. 2008; Ramírez et al. under review), and interbreeding (between wild and escaped fish) is exclusively possible in the easternmost islands: Fuerteventura and Lanzarote islands; because these are the only islands where wild, but reduced, populations exist (Brito 1991). While there is limited knowledge on how escaped sea bass interact with the environment, there is a total lack of insight regarding the potential interaction between escaped meagre and the environment

The objective of this study was to describe the post escape behavior of both sea bass and meagre, by describing their spatial and temporal distribution around sea cages in coastal waters immediately after an escape. For this purpose, twenty five individuals of both sea bass and meagre acoustically tagged were released and movements recorded. In addition, to evaluate the feasibility of recapturing escaped fish by the local fishery, another twenty five externally tagged conspecifics of each species were also released. Finally, we aimed to evaluate the degree of establishment (feralization) of escaped fish in the wild by studying the stomach contents of recaptured fish.

7.3 Materials and methods

The study was carried out around a sea-cage fish farm located at Tufia (27° 57' N, 15° 22' W), on the east of Gran Canaria (Canary Islands, Spain); this is an area with a steep bathymetric slope (Fig. 7.1). The farm (Canexmar S.L.) cultures sea bream, *Sparus aurata*, as well as sea bass and meagre. Cages are located between 22 and 31 meters depth. During the study, both recreational (spear, hook and line) and commercial fisheries (mainly via fish traps) occurred in the area surrounding the fish farm.

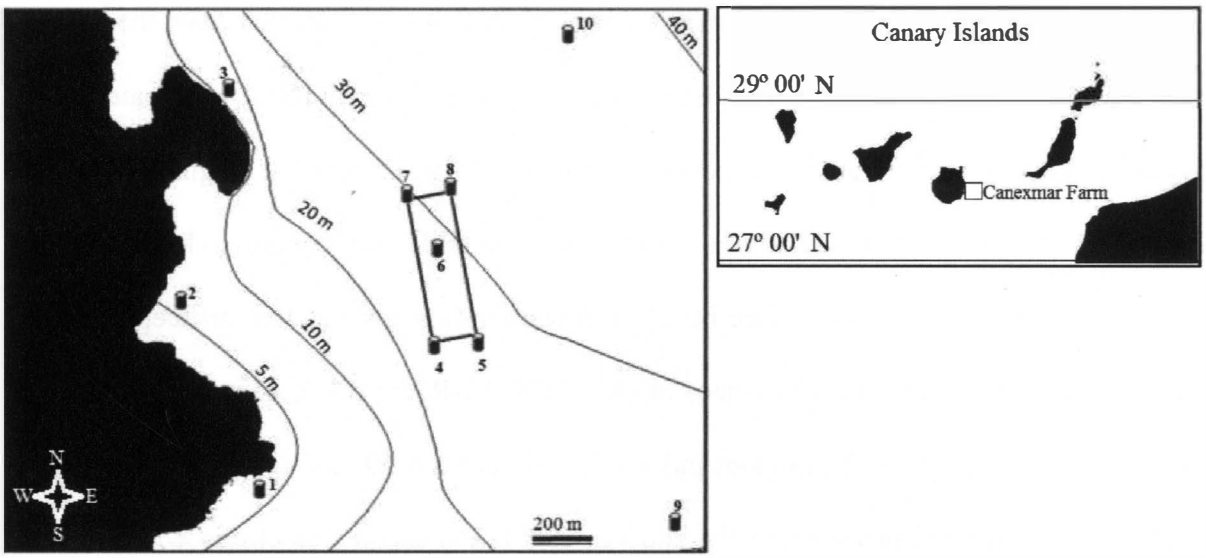


Figure 7.1. Map of the study area in the eastern coast of Gran Canaria, including receiver locations ( ) and farm perimeter . The 'Near shore' zone included receivers: 1, 2 and 3; the 'Around farm' zone included receivers: 4, 5, 6, 7 and 8; the 'Off shore' zone included receivers: 9 and 10. Radial detection range of receivers was between 80 and 100 m.

Fifty adult Sea bass (mean weigh ± SD = 829.73 ± 155.23 g) and fifty meagre (mean weigh ± SD = 966.75 ± 300.47 g) were obtained from the Canexmar S.L. reared stock, on the 7th and 12th April 2010, respectively; this was 24 hours before tagging with acoustic transmitters. These individuals were transported, via a transportation tank (500 l) with constant oxygen delivery, from the sea farm at Tufia to the Canary Institute of Marine

Sciences (ICCM), located ~ 5 km away from the fish farm. The fishes were then distributed in 5 tanks of 500 l with a density of 0.02 ind l^{-1}. Twenty five individuals of meagre and twenty five sea bass were tagged with acoustic transmitters on the 8th and 13rd April 2010, respectively. Ten of these acoustic transmitters recorded fish swimming depth. Five depth recorder transmitters were used for each species. In addition, 25 conspecifics were marked with an external anchor tag (Avery Dennison Company) showing the name 'ICCM' and a numeric code to enable individual identification in case of recapture. Immediately before tagging with acoustic transmitters and external tags, each fish was collected from the tanks using a hand net and then anaesthetized by immersion in a small tank (100 l) with water and clove oil (130 ppm; mean immersion period 4:15 min, T^a=19 °C). During tagging, the fish were placed on their ventral side onto a V-shape surgical table. An incision (~1.5 cm) was made on the ventral, posterior, surface between the pelvic fins and the anal orifice, using a scalpel. Each coded transmitter (Vemco, model V13P-1L-R256, 13 x 45 mm, weight in water: 6 g, frequency: 69 kHz, random pulse interval between 60 and 180 s) was inserted through the incision and pushed into the body cavity. The incision was closed with 2 or 3 independent silk sutures (2/0, Ethicon, Perma-Hand). Gills were in contact with salt water during surgery (mean handling time ~ 2:00 min). Prior to each incision, the surgical equipment was rinsed with 70% ethanol and allowed to dry. All handling and tagging was conducted according to the Spanish regulations for the treatment and welfare of animals (Real Decreto 1201/2005, published in BOE num. 252, 21st October 2005). After tagging, the fishes were transferred to a recovery tank (mean recovery time 3:06 ± 0:29 min), before being transferred to larger tanks in which they were held for 24 h until release. We assumed that fish were recovered when regained control of equilibrium and attained a vertical position (Munday and Wilson 1997). No fish died during transport, surgery and/or recovery after surgery. Tagged individuals with acoustic tags, and another 25 conspecifics equipped

with external anchor tags, were transported, via a transportation tank (500 l) with constant oxygen delivery, from the research center (ICCM) to Canexmar sea farm (Tufia) and released on the 9th April 2010 (meagre) and on the 14th April 2010 (sea bass). It was logistically impossible to release both species on the same day. The releases were accomplished by immersion (by means of a crane) to minimize stress.

Movements of tagged fishes were recorded using 10 individual acoustic receivers (Vemco, VR2 Model: VR2-069.0k-1.03-2-1432-S, SN2621) deployed on anchored ropes at 3 zones, so-called: 'around cages' (4 receivers), 'near shore' (3 receivers) and 'off shore' (2 receivers) (Fig. 7.1). The depth at 'around cages' was 25-30 m; depth at 'near shore' varied between 6 m (beach) and 20 m (cliff); 'off shore' depth was 30-35 m. Receivers were attached to the ropes 3 m above the bottom. Through a pilot assay, maximum detection ranges of the receivers were detected to vary in a radius between 80 and 100 m around the receivers. We then designed the spatial allocation of the receivers to avoid overlapping theirs detection ranges. Detection of a fish by at least one of the receivers within the 3 different zones was defined as a presence within that particular zone. To exclude false signals generated by environmental noise, single detections within 1 h were considered as erroneous, unless there was a clear indication that detection was valid (frequent detection during the same day and also by nearby receivers) (Uglem et al., 2008; Uglem et al., 2010). The date of fish release was defined as 'day' 1 to standardize the data sets. We considered that a fish had left a certain zone if it was not detected during 24 hours within this zone.

Local, professional and recreational, fishermen were noticed to report the capture of tagged fishes by offering a reward. Local fishermen boats are typically between 7 and 12 meters long and use fish-traps that place throughout the area of study, except a non-permitted perimeter of 200 m around cages. Traps are normally circular with a radius of 2 m; traps are assembled using wire mesh around a metallic frame. For those recaptured

individuals, we analyzed the diet composition; stomachs were dissected and the content analyzed according to standard procedures (Hyslop 1980).

We tested for differences in fish abundance between 'zones' at different times after release (1, 2 and 3 weeks, respectively) using contingency tables that were analyzed using a two-tailed Fisher exact test. To test for differences between species in the time taken to leave the farm, or to reach the coast for the first time, and to test for differences between swimming depths, we used a Student´s test in each case. When data did not achieve homogeneous variances, the Mann-Whitney *U*-test was alternatively used.

7.4 Results

Two Meagre (4% of released individuals) and one Sea bass (2%), out of 50 released fish of each species were recaptured by fishermen; two days (11th April 2010), eleven days (20th April 2010) and two days (16th April 2010) after releases respectively. Meagre were captured by fish-traps, while the Sea bass was spear-fished. All recaptured fishes had predator attack signals (i.e. biting attacks), although Meagre were the only large-sized predator in the traps (personal communication by professional fishermen), so it is unlikely that both recaptured Meagre suffered any attack inside the traps. All recaptured fishes presented empty stomachs.

There was no difference between Meagre and Sea bass with respect to the number of fish present at the farm 24 h (two-tail Fisher exact test, $p = 0.07$, Fig. 7.2) and 48 h (two-tail Fisher exact test, $p = 0.78$) after the release. Furthermore, there was no difference between species with respect to the time when they left the farm for the first time (Student´s t-test, $p = 0.22$).

Table 7.1. *P* values of two-tail Fisher exact test contrasting the presence of tagged sea bass and meagre between zones

Pairwise	Sea bass			Meagre		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Around farm *vs*. Off-shore	0.0001	0.0738	1	0.0016	0.0488	1
Around farm *vs*. Near-shore	0.0001	0.0738	1	0.002	1	1
Near-shore *vs*. Off-shore	1	1	1	1	1	1

Similarly, no significant difference in the time to reach the coast for the first time was detected between both species (Student's t-test, p = 0.30). Records of meagre moving both onshore and offshore were larger than those of sea bass (two-tail Fisher exact test, p = 0.0005 and 0.0006, respectively). During the first week, meagre were present in the coast in higher number than sea bass (two-tail Fisher exact test, p = 0.0186; Fig. 7.3). However, after the first week, both species did not show differences between zones (two-tail Fisher exact test, Table 7.1). On the other hand, both species presented significantly higher (two-tail Fisher exact test, Table 7.1) number of fish around the farm during the first week (Fig. 7.3).

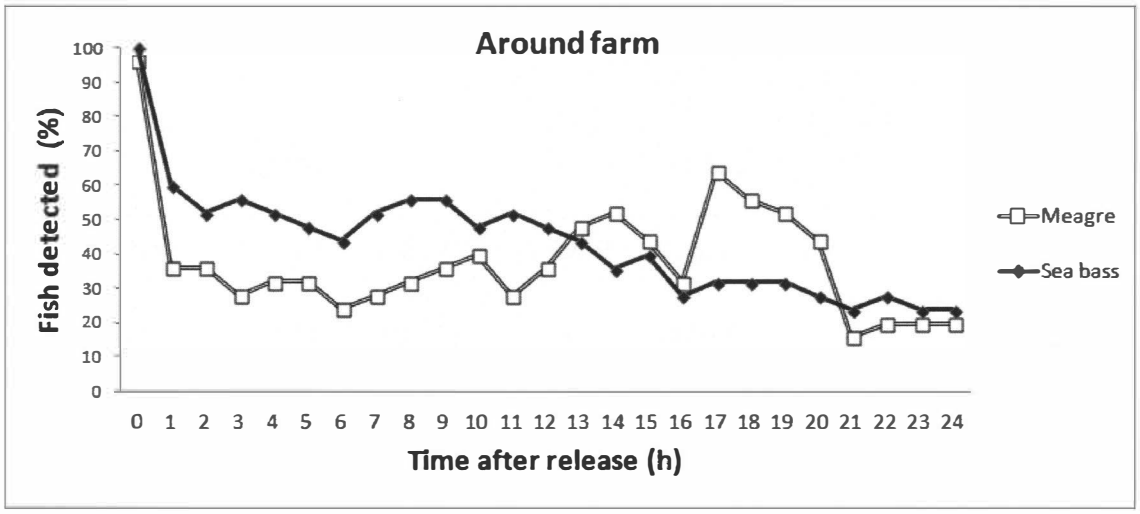
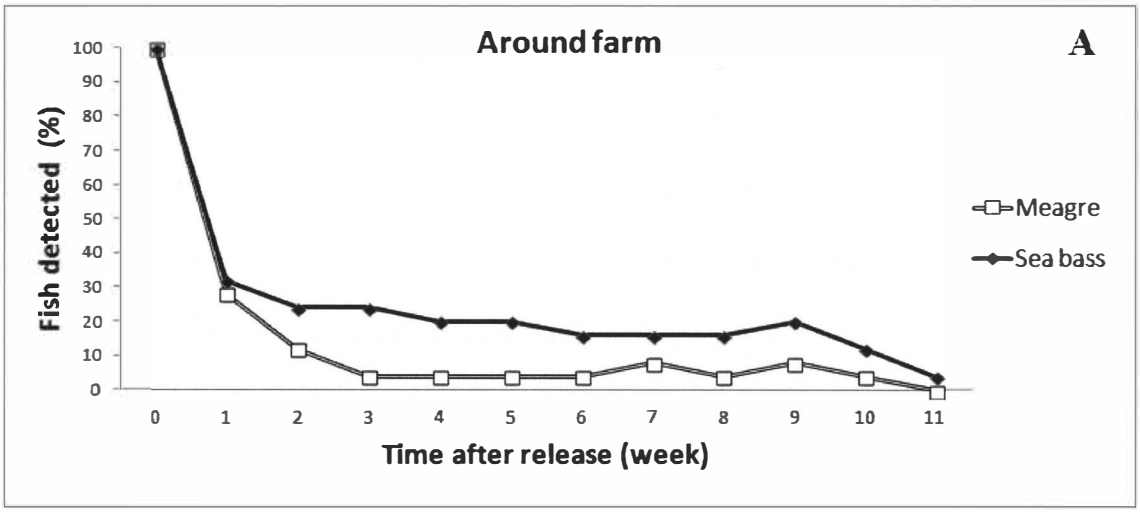
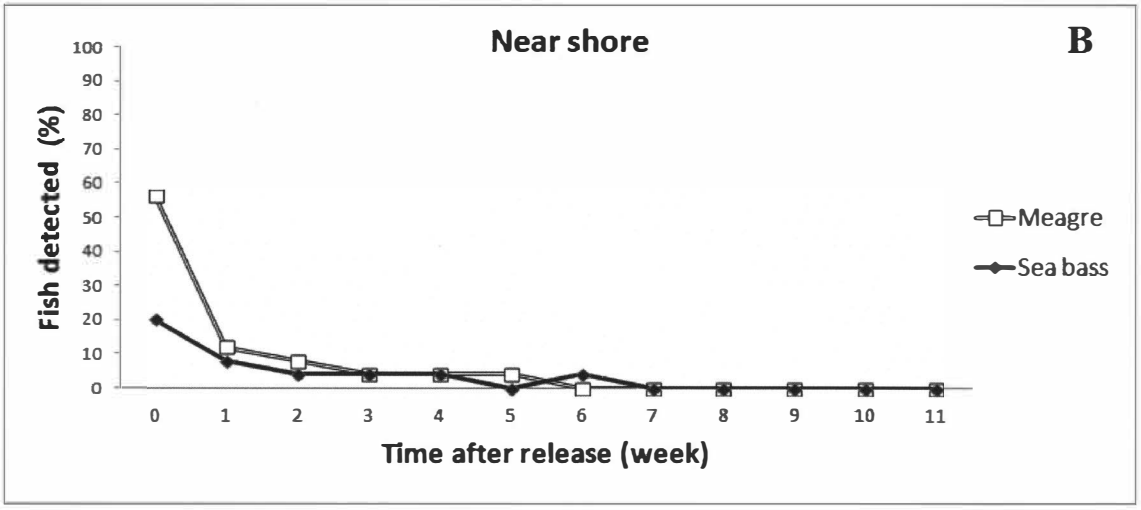


Figure 7.2. Detection of meagre (*Argyrosomus regius*; n=25) and sea bass (*Dicentrarchus labrax*; n=25) after release at the "Around farm" zone during 24 hours.





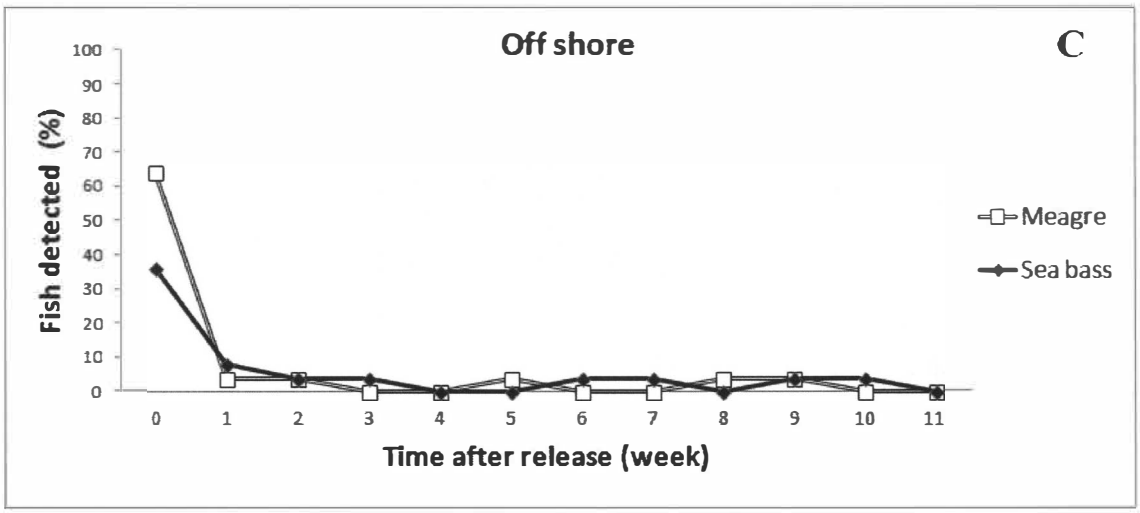


Figure 7.3. Detections of meagre and sea bass for the entire period of study: "Around farm" (A), "Near-shore" (B) and "Off-shore" (C).

During the first 2 months after release, all 'zones' recorded any fish, but only 9 fishes (2 meagre and 7 sea bass) were detected after the first month. After two weeks, there was no significant difference in the total number of tagged individuals between zones (two-tail Fisher exact test, $p > 0.05$, Table 7.1). Overall, our data showed that meagre tended to move onshore (northwards) (Mann-Whitney *U*-tests, p=0.07). Sea bass did not show any particular movement pattern (Mann-Whitney *U*-tests, p=0.25). Meagre (n=5) swimming depth (19.34 ± 9.58 m) was significantly higher (Student´s t-test, p=0,001) than that of sea bass (n=4) (11.75 ± 8.67) (Fig. 7.4). Depth data from sea bass 5 was not recorded by any receiver.

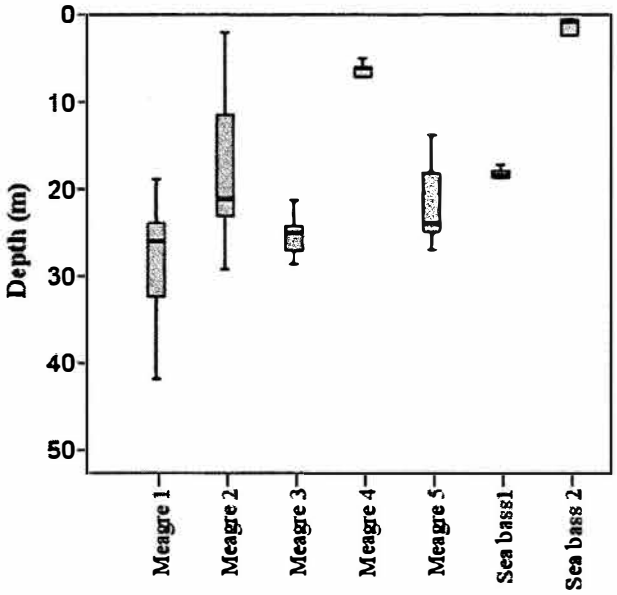


Figure 7.4. Box plot of swimming depth (m) of 7 released individuals (sea bass and meagre). Sea bass 3 and sea bass 4 are not represented because very low depth data (n=1 and n=3 respectively) were collected. Box plots represent the mean of depth detections from each individual tagged with a depth-record tag.

Ten meagre (40%) and fifteen sea bass (60%) moved, at least once, from the farm zone to either the onshore or offshore zone; this difference was, however, not significant (two-tail Fisher exact test, p = 0.257). Nine of these meagre (90 %) and eleven of these sea bass (73.3 %) returned to the farm zone; the difference between species was not significant (two-tail Fisher exact test, p = 0.626). These fifteen sea bass presented a total of 56 'lefts'; in 92.85% of these cases, individuals returned to the farm. The 10 meagre presented a total of 19 'lefts'; in 94.73 % of the cases, individuals returned to the farm.

7.5 Discussion

This study has demonstrated that both meagre and sea bass presented a similar spatio-temporal distribution after an escape (release) event, showing a tendency to remain around the fish farm the first week; this was also annotated by Carrillo and Castillo (2001), Arechavala-Lopez et al. (2011) and Toledo et al. (2008) for sea bass. A percentage of

escaped fishes (16% of sea bass and 40% of meagre) dispersed away since they escaped from the farm; this was also concluded by Bégout-Anras et al. (1998), Tavares and Gonzalez (2009) and Arechavala-Lopez et al. (2011). Although sea bass and meagre initially left the farm, individuals returned in most cases (73.3 % and 90 %, respectively), therefore showing a high farm fidelity. This seems to support the idea that the disappearance of fishes through the experimental period was due to mortality, although other causes could be possible as well, as sudden losses of receiver detection ranges, and quick migratory responses of fish towards uncovered zones (Froese and Pauly 2006; Stipa and Angelini 2005).

Although differences in the timing of release/escape could cause differences in dispersal patterns between sea bass and meagre, both species were released with only five days of difference, a narrow time scale that lacks any seasonal difference. Indeed, individuals of both species were released at the same time of the day and under the same oceanographic conditions (e.g. tide, wind, swell, etc...), so potential differences in dispersion between both species were not confounded by differences in the timing of release. Nevertheless, it is possible that the behaviour of tagged fishes may be altered as a result of tagging and transportation relative to those fishes that are escaping through holes in the nets of cages.

Both species took a similar time to leave the farm for the first time or to reach the coast. However, during the first week after release, meagre showed a slightly different behavior, including a higher tendency to move onshore and offshore, which suggests that escaped meagre had a larger movement capacity than sea bass.

Fishermen only recaptured 4% of released meagre and 2% of released sea bass during the first week after the experimental escape. Recaptures of both species were low compared with those reported by Uglem et al. (2008; 2010) for Atlantic cod (40 % and 33.3%, respectively), but match with those obtained by Mariño et al. (2009) for turbot

(*Scophthalmus maximus*) in northern Spain (1-3%), Skilbrei (2010) for Atlantic salmon (1%), Arechavala-Lopez et al. (2012) for sea bream in Mediterranean coasts (3.5 and 8.9% in two release episodes), Bishop (1957) for white sea bass (*Atractoscion nobilis*) in the Canton Reservoir, Oklahoma (5.5 %) and Gil et al. (2014) for meagre in the Mediterranean Sea (1.2 %). Meagre recapture rate was high compared with a 0.46 % for mulloway (*Argyrosomus japonicus*) in Australia (Taylor and Piola 2008). These results suggest that for most aquaculture-released species, recaptures rates are low and, as a result, mitigation through local fisheries seems irrelevant, particularly after some days after release. In our case, however, the results are not showing the potential of local fisheries because fishing gears (e.g. traps) were not placed immediately under cages. In this sense, a high number of meagre recaptures has been annotated when traps are placed illegality under cages (personal communication by professional fishermen). This matches the observations of Monfort (2010), who indicated that meagre may be easily recaptured after an escape, which may reduce the risk of altering resident fish assemblages. The fact that meagre tended to swim deeper than sea bass make meagre more susceptible to fish traps, which are typically deployed at depths below 18 m depth. Further studies would be necessary to determine recapture rates of escaped fishes through intensive fishing efforts immediately under, or right near, cages.

Sanchez-Jerez et al. (2008) demonstrated that bluefish (*Pomatomus saltatrix*) around sea-cages consumed pelagic species such as *Sardinella aurita* and *Trachurus mediterraneus*, while they predated on sea bream once they incurred into cages. Arechavala-Lopez et al. (2011, 2012) reported high predation on escaped sea bass and sea bream, as possible causes of mortality of escaped fishes. Arechavala-Lopez et al. (2011) concluded that long-term ecological impacts of such escape incidents might be relatively low due to high mortalities of escapees. Our results somehow agree with this idea, because all recaptured fishes showed

predator attack signals; nevertheless, recaptures were low, so further studies are necessary. In this sense, the high densities of predators around sea-cage fish farms, typically of the Canary Islands (Boyra et al. 2004; Dempster et al. 2005; Tuya et al. 2006), might contribute to largely mitigate chronic escape events through direct consumption of escapees. This is another reason to guarantee healthy populations of top predatory fish around sea-cage fish farms (Dempster et al. 2006).

When escaped from sea-cage fish farms, some individuals are able to survive in the new environment (e.g. via consumption of wild prey) (ICES 2005; Becker et al. 2007; Blanchfield et al. 2009; Arechavala-López et al. 2012), e.g. mulloway in Australia (Taylor and Mazumder, 2010), or sea bass in the Canary Islands (Toledo et al. 2008; Ramirez et al. in review); still most escapees probably do not survive. Although meagre may take more than 1 or 2 months after their release to consume natural prey (Gil et al 2014), we lack data to determine whether escape fish may, or may not, have consumed wild prey. We only analyzed a few stomachs, particularly from individuals captured after a few days after the release, so we largely underestimate their potential feeding activity.

In summary, this study supports the idea that both sea bass and meagre remained around the cages during the first week after release, progressively disappearing afterwards. In the light of post-escape movement patterns, we propose to focus recapture efforts around cages under authority's supervision, as soon as possible after the escape event.

7.6 Acknowledgements

Authors would like to thank Canexmar S.L. staff, especially to Rafael Guirao, for their help and assistance during sampling. The study was partially financed by the EU-project Prevent Escape.

8

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

DISCUSIÓN GENERAL

Según los distintos estudios referenciados en este documento (Tacon and Metian 2008), la acuicultura es una actividad en expansión de vital importancia en las próximas décadas como consecuencia de la sobreexplotación de las pesquerías, necesidad de crecimiento empresarial (diversificando sectores) y el aumento de la población que a su vez demanda consumo de pescado. Como en cualquier actividad humana, es evidente que existe una interacción con el medio ambiente que rodea a las instalaciones de acuicultura. De ahí la relevancia del estudio de las interacciones y posibles impactos que pudiera presentar esta industria en un medio acuático especialmente sensible a cambios y alteraciones físico-químicas. Varios autores han descrito previamente los efectos (de la acuicultura) sobre la biodiversidad, variabilidad genética y ecosistemas en general (CBD, 2004). Numerosos autores coinciden en que el principal problema ambiental asociado a la acuicultura es la inclusión de M.O. (materia orgánica) al medio, en modo de descargas fecales y exceso de pienso, modificando las características del sedimento bajo las jaulas (Molina-Domínguez et al., 2001; Mente et al., 2006; Holmer, 2010). En los planes de gestión de jaulas oceánicas se ha detectado como un punto crítico la distribución del alimento en función de la dinámica de la población de peces en sí y de la masa de agua, siendo este punto crítico motivo de preocupación en la gestión general de la instalación, focalizándose varios estudios en la optimización de la distribución del alimento para minimizar las pérdidas de alimento

Si parte del pienso que se está distribuyendo en las jaulas está saliendo del sistema y poniéndose en disposición de las poblaciones de animales que rodean la jaula, ¿está afectando este pienso en cantidad y calidad a las poblaciones asociadas a las jaulas?

Actualmente, la expansión de la acuicultura implica un incremento en la producción de piensos de engorde. Tradicionalmente, los piensos de engorde para peces marinos han

dependido directamente de harinas y aceites de pescado, dependiendo directamente de las pesquerías y generando presión sobre los stocks pesqueros (Naylor and Burke, 2005, Naylor et al., 2009, Tacon and Metian, 2008). En los últimos años se ha tendido a sustituir esos aceites y proteínas de origen marino, por otros de origen terrestre, especialmente vegetales, debido a la disminución de los stocks pesqueros por a) problemas climáticos, b) sobrepesca, c) el incremento de la demanda de los productos pesqueros por la propia demanda de la acuicultura y de otras industrias (Tacon and Metian 2008). La inclusión de estos ingredientes terrestres ha creado una posible controversia sobre si este input de ácidos grasos mayoritariamente presentes en ingredientes terrestres puede provocar una incorporación permanente en los ecosistemas marinos, siendo el exceso de pienso proveniente de las jaulas de acuicultura o la predación de animales escapados el vehículo de entrada en el ecosistema marino el (Skog et al., 2003; Dalsgaard et al., 2003; Fernández-Jover et al., 2007). Los resultados de esta tesis demuestran que, efectivamente, los peces asociados a las instalaciones acuícolas se alimentan del excedente de pienso (en el caso de estudio la boga), llegando incluso a cambiar su composición lipídica y perfil de ácidos grasos, aumentando las proporciones de ciertos ácidos grasos mayoritariamente presentes en aceites vegetales de origen terrestre, como los ácidos Oleico, linoleico y linolénico. No obstante, éste es un efecto muy local, observado en la zona inmediatamente alrededor de las jaulas, ya que a escasos 3 km alejados de las instalaciones acuícolas, el perfil de ácidos grasos no difiere del de una boga capturada en la isla del Hierro, donde no existe actividad acuícola.

Si se produce un aumento de ácidos grasos característicos de aceites vegetales de origen terrestre, ¿puede usarse este parámetro como bioindicador de animales asociados a acuicultura, bien escapados de las instalaciones o bien influenciados por la acuicultura?

Algunos autores asocian el incremento tisular en peces de ácidos grasos de característicos de aceites de origen terrestres exclusivamente a la acuicultura (Rueda et al., 2001; Bell et al., 2002; Blanchet et al., 2005; Fernández-Jover et al., 2007, 2011; Mnari et al., 2007; Megdal et al., 2009). No obstante, la utilidad de los ácidos grasos como biomarcadores de acuicultura está limitada a un espacio de tiempo relativamente corto en el caso de los escapados, pues como demuestran Montero et al. (2005) o Benedito-Palos et al., (2009), existe un lavado de ácidos grasos en los tejidos de los peces, que depende de la dieta de los mismos y que dependerá de cada especie e individuo. Una vez que el pez se escapa y es capaz de alimentarse en el medio, su perfil de ácidos grasos iría cambiando consecuentemente, al reflejarse el perfil de la dieta y lavarse progresivamente el perfil marcado por la anterior dieta, y pareciéndose cada vez más aquel de sus congéneres salvajes. Los resultados de esta tesis concuerdan con estos autores, pues hemos observado diferencias en el perfil de ácidos grasos entre peces escapados y cultivados, demostrando que existe un lavado de estos ácidos grasos.

Por otro lado, la entrada al medio marino de estos los ácidos grasos propuestos como biomarcadores, como hemos demostrado, no es exclusiva del excedente de pienso de peces cultivados. En este caso se describe un emisario de aguas residuales urbanas que produce un incremento de ácido linoleico en las especies capturadas en las inmediaciones de este emisario. Cualquier otra fuente de materia orgánica de origen terrestre, como aguas agrícolas, industria etc, es una potencial entrada masiva de estos ácidos grasos en la cadena trófica marina. Así pues, los ácidos grasos característicos de aceites vegetales de origen terrestre no pueden ser usados como bioindicadores de interacciones acuicultura/ecosistemas marinos a largo plazo.

Las condiciones de cultivo en las que se desarrollan estos peces ¿pueden cambiar la morfología externa de los peces. ¿puede entonces usarse este efecto como bioindicador de animales escapados de instalaciones de acuicultura?

Estudios previos demuestran que la morfología de un individuo está afectada por la tasa de crecimiento, sobre todo en los primeros estadíos de desarrollo (Huxley 1932; Martin 1949). Existe una serie de factores ambientales que repercuten en la morfología de un individuo (incidencia de luz, tipo de presas, velocidad de la corriente, etc…); por lo tanto un ambiente de cultivo causará diferencias morfológicas importantes frente a sus congéneres salvajes (Loy et al., 2000; Craig et al., 2007; Uglem et al., 2011). Hay varios estudios que han propuesto la viabilidad de la morfometría como biomarcador de acuicultura (Arechavala-López et al., 2011; Rogdakis et al., 2011). Nuestros resultados estarían en concordancia con estos autores, ya que al menos dos parámetros morfométricos (cefálico/pélvico) se mantuvieron invariables al comparar poblaciones de acuicultura con poblaciones de animales escapados, lo que denota la estabilidad de estos parámetros morfométricos en poblaciones escapadas alejadas de la influencia de la acuicultura. No obstante, no se encontraron diferencias en el resto de medidas estudiadas (n=13), lo cual indica que existe un cambio morfológico posterior al escape y este hecho demanda cautela a la hora de establecer la morfometría como bioindicador inequívoco.

Cuando hay un escape masivo de peces de una jaula de acuicultura, ¿cómo se comportan estos individuos en el ecosistema circundante?

Se ha especulado mucho sobre esta cuestión y poco es el conocimiento científico del que disponemos. En el caso de las especies estudiadas durante esta tesis, la lubina y la corvina, nos encontramos con especies de rápida dispersión tras el escape o suelta (Carrillo

and Castillo, 2001; González-Lorenzo et al., 2005; Tavares and González 2010;Gil et al., 2014; Toledo-Guedes 2014a, 2014b) y con capacidad de adaptación al medio una vez escapan o son liberados (Toledo-Guedes 2009; Gil et al., 2014). Sin embargo, hemos observado que una gran proporción de los individuos de ambas especies, una vez liberados, se mantienen en cardúmenes alrededor de las jaulas durante una semana, siendo vulnerables a ciertos artes de pesca como las nasas. Pasado este primer periodo alrededor de las jaulas, tanto la lubina como la corvina se acercan a costa donde además de los artes profesionales, aumentan las capturas por pescadores deportivos . Este hecho debe ser tomado en cuenta a la hora de elaborar cualquier plan de contingencia ante escapes masivos, pues facilitaría la recaptura mediante pescas controladas. Por otro lado, aunque vemos que una proporción de los individuos llegan a adaptarse al medio, no encontramos ninguna evidencia de la posibilidad de un establecimiento de poblaciones, coincidiendo con Toledo et al. (2009), ya que por un lado demostramos como las densidades de lubinas escapadas disminuyen notablemente con el paso del tiempo, llegando a desaparecer en la mayoría de localizaciones; y por otro, no existen factores biológicos ni ambientales que permitan la reproducción de la lubina o la corvina en las islas estudiadas en la actualidad. Aunque la proporción de las lubinas que llegan a adaptarse y sobrevivir en la costa es baja (debido a la predación, deformidades, inadaptabilidad, actividad pesquera, etc...), el número de individuos que podríamos llegar a tener en la costa en los primeros meses del escape puede ser elevado, ya que se han reportado escapes masivos de 1 o 2 millones de individuos, y es este primer momento de dispersión el que dispara las críticas desde la opinión pública y sectores contrariados con la actividad acuícola.

Aún así, cuando se produce un escape masivo, pueden estos individuos escapados alterar las poblaciones de invertebrados y otros peces de las zonas colindantes a las jaulas de acuicultura?

Aunque está descrito la ingestión de un alto porcentaje de alimento no orgánico por parte de las lubinas escapadas, reflejando este hecho el proceso de adaptación al medio y que conlleva la muerte de la mayoría de estos peces que han ingerido grandes piedras, plásticos o incluso envases de aluminio (Carrillo and Castillo 2001; Ramírez et al., 2015), queda claro a la vista de los resultados y los trabajos anteriores, que la lubina que es capaz de adaptarse tras el escape y alejamiento de las jaulas de acuicultura se alimenta de un rango limitado de especies, incluyendo principalmente *Rhynchocinetes sp, Percnon gibessi, Sparisoma cretense y Thalassoma pavo*. De esta manera, la lubina puede figurar como competidor con otros predadores en las islas estudiadas tal y como sugieren estudios previos (Toledo et al., 2009, 2014). Este aspecto ha suscitado debates sociales y políticos acerca del efecto que esto ha provocado sobre las especies autóctonas (tanto potenciales presas como competidores), pero lo cierto es que no se observa ninguna correlación en los datos de las pesquerías de ninguna especie posible competidora o presa de la lubina en los años sucesivos a grandes escapes masivos. En 1999 se produjo un escape de más de un millón de lubinas en la bahía de Melenara (Gran Canaria). Sin embargo, las tasas de pesca de las especies potenciales presas de esta especie, como la vieja o el guelde no se vieron afectadas por este escape masivo durante ese año y años sucesivos, según se puede observar en los informes de evolución de las principales especies desembarcadas por la flota pesquera canaria con base en Canarias de la Consejería de Agricultura, Ganadería, Pesca y Alimentación del Gobierno de Canarias. En estos informes se puede observar que la captura de especies potenciales presas de la lubina, como la vieja se mantuvieron el tiempo pese al

escape masivo de lubinas (238, 334, 344, 311 toneladas desembarcadas en los años 1999, 2000, 2001 y 2002 respectivamente), denotándose que un escape masivo no produjo un efecto directo en las poblaciones de otras especies en esa zona.

9

Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

CONCLUSIONES GENERALES

A partir de los resultados obtenidos en los diferentes experimentos, muestreos de campo y analíticas realizadas a lo largo de esta investigación, se pueden extraer las siguientes conclusiones:

1. **El contenido en lípidos así como el perfil de ácidos grasos de los peces asociados a jaulas de acuicultura (como es el caso de la boga) cambia con respecto a sus congéneres no asociados a este tipo de instalaciones, aunque este efecto desaparece con la distancia.**
2. **Las jaulas oceánicas de acuicultura tienen un efecto directo en el perfil lipídico en poblaciones de peces asociadas a las jaulas como la boga, que se ve reflejado en un aumento de ácidos grasos característicos de aceites vegetales de origen terrestre.**
3. **En el ambiente costero existen otras fuentes de incremento de ácidos grasos característicos de aceites vegetales de origen terrestre, como son los emisarios urbanos, que incrementan el ácido linoleico en las poblaciones de peces asociadas a dichos emisarios, como sucede en el caso de la boga.**
4. **Cuando se produce un escape de lubina, el perfil lipídico de los individuos recién escapados no cambia con la distancia, pero si lo hace con el tiempo transcurrido tras el escape.**
5. **Dada la variabilidad en el perfil de ácidos grasos en las distintas poblaciones de peces asociadas a jaulas o a emisarios submarinos y al efecto de lavado (wash-out) que tiene lugar con el transcurso del tiempo tras el escape, consideramos que este parámetro no es un bioindicador**

válido para diferenciar peces escapados ni de influencia exclusiva de acuicultura.

6. Por otra, parte, la morfometría corporal de las poblaciones de peces, tanto de los escapados como de los asociados a jaulas y salvajes, presenta unos patrones estables de variación, por lo que consideramos que es un bioindicador válido de peces escapados de instalaciones acuícolas.
7. Un pequeño porcentaje de lubinas escapadas tiene capacidad de sobrevivir en el medio aprovechando los recursos existentes, tal como indica el contenido estomacal, aunque sus poblaciones tienden a disminuir paulatinamente y a desaparecer con el tiempo.
8. El comportamiento de las lubinas y corvinas después de un escape de jaulas de acuicultura está caracterizado por la permanencia de los individuos alrededor de las jaulas durante la primera semana. Posteriormente, los individuos escapados tienden a migrar hacia zonas costeras.

10 Evaluación de interacciones ambientales de peces escapados de jaulas de cultivos.

BIBLIOGRAFÍA

Adey, A.D., K. D. Black, K.D., Sawyer, T, Shimmield, T.M., Trueman, C.N. 2009. Scale microchemistry as a tool to investigate the origin of wild and farmed *Salmo salar*. *Marine Ecology Progress Series* 390, 225-235.

Adhikari, S., Lal, R., Sahu, B.C. 2013. Carbon footprint of aquaculture in eastern India. *Journal of Water and Climate Change* 4, 410-421.

Alasalvar, C., Taylor, K., Zubcov, E., Shahidi, F., Alexis, M. 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. *Food Chemestry* 79, 145-150.

Anderson, M.J. 2004. PERMANOVA_2 factor: a FORTRAN computer program for permutational multivariate analysis of variance (for any two-factor ANOVA design) using permutation test. Department of Statistics, University of Auckland, New Zealand.

AOAC (Association of Official Analytical Chemists) 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

APROMAR (Asociación empresarial de productores de cultivos marinos) 2012. La acuicultura marina de peces en España. Informe Apromar, 84 pp.

Arechavala-Lopez, P., Uglem, I., Sanchez-Jerez, P., Fernandez-Jover, D., Bayle- Sempere, J. T., Nilsen, R. 2010. Movements of grey mullets (*Liza aurata* and *Chelon labrosus*) associated with coastal fish farms in the western Mediterranean Sea. *Aquaculture Environment Interactions* 1, 127-136.

Arechavala-Lopez, P., Sanchez-Jerez, P., Bayle-Semper, J., Fernandez-Jover, D., Martinez-Rubio, L., Lopez-Jimenez, J.A., Martinez-Lopez, F.J. 2011a. Direct interaction between wild fish aggregations at fish farms and fisheries activity at fishing grounds: a case study with *Boops boops*. *Aquaculture Research* 42, 996-1010.

Arechavala-Lopez, P., Uglem, I., Fernández-Jover, D., Bayle-Sempere, J., Sánchez-Jerez, P. 2011b. Immediate post-escape behaviour of farmed European sea bass (*Dicentrarchus labrax*) in the Mediterranean Sea. *Journal of Applied Ichthyology* 27, 1375-1378.

Arechavala-Lopez, P., Uglem, I., Fernandez-Jover, D., Bayle-Sempere, JT., Sanchez-Jerez, P. 2012a. Post-escape dispersion of farmed sea bream (*Sparus aurata L.*) and recaptures by local fisheries in the Western *Mediterranean Sea. Fisheries Research* 121/122,126-135.

Arechavala-Lopez, P., Sanchez-Jerez, P., Bayle-Sempere, J.T., Sfakianakis, D.G., Somarakis, S. 2012b. Morphological differences between wild and farmed Mediterranean fish. *Hydrobiologia* 679, 217-231.

Arechavala-Lopez, P., Sánchez-Jerez, P., Bayle-Sempere, J., Uglem, I., Mladineo, I. 2013a. Reared fish, farmed escapees and wild fish stocks - a triangle of pathogen transmission of concern to Mediterranean aquaculture management. *Aquaculture Environment Interactions* 3, 153-161.

Arechavala-López. P., Fernández-Jover, D., Black, K., Ladoukakis, E., Bayle-Sempere J., Sánchez-Jerez, P., Dempster, T. 2013b. Differentiating the wild or farmed origin of Mediterranean fish: a review of tools for sea bream and European sea bass. *Reviews in Aquaculture* 5, 137-157.

Arechavala-Lopez, P., Izquierdo-Gómez, D., Sánchez-Jerez, P., Bayle-Sempere J. 2014. Simulating escapes of farmed European sea bass from Mediterranean open sea-cages: low recaptures by local fishermen. *Journal of Applied Ichthyology* 30, 185-188.

Arias, A. 1979. Biología y cultivo experimental de la dorada *Sparus aurata* L., y del róbalo *Dicentrarchus labrax* L., de estero y estudio de las salinas de Cádiz en relación con su aprovechamiento en cultivos marinos. PhD Thesis, Universidad de Sevilla. Sevilla. España.

Arias, A. 1980. Crecimiento, régimen alimentario y reproducción de la dorada (*Sparus aurata* L.) y del róbalo (*Dicentrarchus labrax* L.) en los esteros de Cádiz. *Investigación Pesquera* 44, 59-83.

Bahri-Sfar, L., Lemaire, C., Chatain, B., Divanach, P., Hassine, O.K.B., Bonhomme, F. 2005. Impact of aquaculture on the genetic structure of Mediterranean populations of *Dicentrarchus labrax*. *Aquatic Living Resources* 18, 71–76.

Barnabé, G. 1976. Contribution a la connaissance de la biologie du loup *Dicentrarchus labrax* (L.) (Poisson Serranidae). Thèse de Doctoral d'etat, mention sciences, Université Sciences et Techniques du Languedoc, Montpellier. France. 426 pp.

Barlow, G.W. 1961. Causes and significance of morphological variations in fishes. *Systematic Zoology* 10, 105-117.

Bas, C., Castro, J., Hernández-Gracía, V., Lorenzo, J.M., Moreno, T., Pajuelo, J., Ramos, A., 1995. *La pesca en Canarias y áreas de influencia*. Cabildo Insular de Gran Canaria, Las Palmas de Gran Canaria. 331 pp.

Basaran, F., Ozbilgin, H., Ozbilgin, Y.D. 2007. Comparison of the swimming performance of farmed and wild gilthead sea bream, *Sparus aurata*. *Aquaculture Research* 38, 452-456.

Battin, J. 2004. When good animals love bad habits: ecological traps and the conservation of animal populations. *Conservation Biology* 18, 1482-1491.

Beacham, T.D. 1990. A genetic analysis of meristic and morphometric variation in chum salmon (*Oncorhynchus keta*) at three different temperatures. *Canadian Journal of Zoology* 68, 225-229.

Becker, L., Pascual, M., Basso, N.G. 2007. Colonization of the Southern Patagonia Ocean by exotic Chinooksalmon. *Conservation Biology* 2, 11347-1352.

Bégout-Anras, M., Lagardère, J. 1998. Variabilité météorologique et hydrologique. Conséquences sur l'activité natatoire d'un poisson marin. *Comptes rendus de l'Académie des Sciences, Sciences de la vie* 321, 641-648.

Bell, J.G., Henderson, R.J,, Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R.P,. Sargent, J.R.. 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. *Journal of Nutrition* 132, 222-230.

Benedito-Palos, L., Navarro, J., Bermejo-Nogales, A.,Saera-Vila, A., Kaushik, S., Pérez-Sánchez J., 2009. The time course of fish oil wash-out follows a simple dilution model in gilthead sea bream (*Sparus aurata*) fed graded levels of vegetable oils. *Aquaculture* 288, 98-105.

Bishop, H. 1957. Marking white bass with spaghetti tags. *Proceedings of the Oklahoma Academy of Science* 38, 68-70.

Black, K. D. 2001. *Environmental Impacts of Aquaculture*. Sheffield Academic Press, Sheffield, UK, 214 pp.

Blanchet, C., Lucas, M., Julien, P., Morin, R., Gingras, S., Dewailly, E. 2005. Fatty acid composition of wild and farmed Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Lipids* 40, 529-531.

Blanchfield, P., Tate, L., Podemski, C. 2009. Survival and behavior of rainbow trout (*Oncorhynchus mykiss*) released from a experimental aquaculture operation. *Canadian Journal of Fisheries and Aquatic Sciences* 66, 1976-1988.

Boglione, C., Gagliardi, F., Scardi, M., Cataudella, S. 2001. Skeletal descriptors and quality assessment in larvae and post-larvae of wildcaught and hatchery-reared gilthead sea bream (*Sparus aurata* L.1758). *Aquaculture* 192, 1-22.

Boyra, A., Sanchez-Jerez, P., Tuya, F., Espino, F., Haroun, R. 2004. Attraction of wild coastal fishes to Atlantic subtropical cage fish farms, Gran Canaria, Canary Islands. *Environmental Biology of Fishes* 70, 393-401.

Brito, A. 1991. Catálogo de los peces de las Islas Canarias. Editorial F. Lemus, 230 pp. La Laguna.

Brown, P.B., Hart, S.D. 2010. Soybean oil and other n-6 polyunsaturated fatty acid-rich vegetable oils. In: Turchini G, Ng W, Tocher D. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Cambridge, UK, pp. 133-160.

Busack, C., Knudsen, C.M., Hart, G., Huffman, P. 2007. Morphological differences between adult wild and first-generation hatchery upper Yakima river spring Chinooksalmon. *Transactions of the American Fisheries Society* 136, 1076-1087.

Cabello, F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology* 8, 1137-1144.

Cabral, H., Ohmer, T.B. 2001. Diet of juvenile meagre, *Argyrosomus regius*, within the Tagus estuary. *Cahiers de Biologie Marine* 42, 289-293.

Cárdenas, S. 2010. Crianza de la Corvina (*Argyrosomus regius*). En Cuadernos de Acuicultura 3. Fundación Observatorio Español de Acuicultura, Madrid.97 pp.

Carrillo, J., Castillo, R. 2001. Estudio del Impacto producido por la introducción de las lubinas (*Dicentrarchus labrax* Linnaeus, 1758) y doradas (*Sparus aurata*, Linnaeus, 1758), escapadas de los cultivos en jaulas flotantes, en el ecosistema marino litoral de Gran Canaria.Viceconsejería de Pesca del Gobierno de Canarias. Informe inédito. 72 pp.

Carrillo, M., Zanuy, S., Blazquez, M., Ramos, J., Piferrer, F., Donaldson, E. 1995. Sex control and ploidy manipulation in European sea bass. In: OCDE Secretary-General (Ed.), *Environmental Impacts of Aquatic Biotechnology*. Publ. OCDE, Paris, pp. 125–143.

Carss, D. N. 1990. Concentrations of wild and escaped fishes immediately adjacent to fish farm cages. *Aquaculture* 90, 29-40.

Castro, J. 1993. Feeding ecology of chub mackerel (*Scomber japonicus*) in Canary Island area. *South African Journal of Marine Science* 13, 323-328.

CBD (Secretariat of the Convention on Biological Diversity), 2004. Solutions for sustainable mariculture avoiding the adverse effects of mariculture on biological diversity. *CBD Technical Series* no. 12. ISBN: 92-807-2406-1. 52 pp.

Chang, B.D., Cooper, J.A., Page, F.H., Losier, R.J., McCurdy, E.P., Reid, J.C.E. 2011. Changes in the benthic macrofaunal community associated with sediment sulfide levels under salmon farms in southwestern New Brunswick, Bay of Fundy. *Aquaculture Association of Canada Special Publication* 17, 21-23.

Chen, J., Hu, W., Zhu, Z.Y. 2013. Progress in studies of fish reproductive development regulation. *Chinese Science Bulletin* 58, 7-16.

Chervinski, J. 1975. Sea Basses, *Dicentrarchus labrax* (Linne) and *D. punctatus* (Block) (Pisces, Serranidae) a control fish in fresh water. *Aquaculture* 6, 249-266.

Christie, W.W. 1982. Lipid Analysis, 2nd ed., Pergamon Press, Oxford, pp. 52-56.

Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *American Journal of Ecology* 18, 117-143

Colombo, J.C., Silverberg, N., Gearing, J.N. 1997. Lipid biogeochemistry in the Laurentian Trough. II. Changes in composition of fatty acids, sterols and aliphatic hydrocarbons during early diagenesis. *Organic Geochemitry* 26, 257-274.

Cramon-Taubadel, N.V., Ling, E.N., Cotter, D., Wilkins, N. P. 2005. Determination of body shape variation in irish hatchery-reared and wild Atlantic salmon. *Journal of Fish Biology* 66, 1471-1482.

Currens, K.P., Sharpe, C.S., Hjort, R., Schreck, C.B., Li, H.W. 1989. Effects of different feeding regimes on the morphometrics of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). *Copeia* 1989, 689-695.

Dalsgaard, J., St. John, M. 2004. Fatty acid biomarkers: Validation of food web and trophic markers using super (13) C-labelled fatty acids in juvenile sandeel (*Ammodytes tobianus*). *Canadian Journal of Fisheries and Aquatic Sciences* 61, 1671-1680.

De Oliveira-Filho, E.C., Lopes, R.M., Paumgartten, F.J.R. 2004. Comparative study on the susceptibility of freshwater species to copper-based pesticides. *Chemosphere* 56, 369-374.

Dempster, T., Taquet, M. 2004. Fish aggregation device (FAD) research: gaps in current knowledge and future directions for ecological studies. *Reviews in Fish Biology Fisheries* 14, 21-42.

Dempster, T., Fernandez-Jover, D., Sanchez-Jerez, P., Tuya, F., Bayle-Sempere, J., Boyra, A., Haroun, R. 2005. Vertical variability of wild fish assemblages around sea-cage fish farms: Implications for management. *Marine Ecology Progress Series* 304, 15-29.

Devauchelle, N., Coves, D., 1988. The characteristic of European sea bass (*Dicentrarchus labrax*) eggs: descripction, biochemical composition and hatching performances. *Aquatic Living Resources* 1, 223-230.

Diamant, A., Banet, A., Ucko, M., Colorni, A., Knibb, W., Kvitt, H. 2000. Mycobacteriosis in wild rabbitfish (*Siganus rivulatus*) associated with cage farming in the Gulf of Eilat, Red Sea. *Diseases of Aquatic Organisms* 39, 211- 219.

Dimitriou, E., Katselis, G., Moutopoulos, D.K., Akovitiotis, C., Koutsikopolous, C. 2007. Possible influence of reared gilthead sea bream (*Sparus aurata*, L.) on wild stocks in the area of the Messolonghi lagoon (Ionian Sea, Greece). *Aquaculture Research* 38, 398-408.

Do Chi, T., Hoai, T. 1971. Croissance differentielle de *Dicentrarchus labrax* (Linné 1758). Etude préliminaire du phénomène dans región des Sbles d'Olonne (Vendée). *Trav. Lab. Biol. Halieutique, University Rennes* 5, 29-43.

Dodge, Y. 2008. Chi-Square Test. The Concise Encyclopedia of Statistics. Springer, 77-79.

Dooley, J., Van Tasell, J., Brito, A. 1985. An Annotated Checklist of the Shorefishes of the Canary Islands. *American Museum of Natural History* 2824, 1-49.

Dufour, V., Cantou, M., Lecomte, F. 2009. Identification of sea bass (*Dicentrarchus labrax*) nursery areas in the north-western Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* 89, 1367-1374.

Dunn, R. J. K., Welsh, D. T., Teasdale, P. R., Lee, S. Y., Lemckert, C. J., Meziane, T., 2008. Investigating the distribution and sources of organic matter in surface sediment of Coombabah Lake (Australia) using elemental, isotopic and fatty acid biomarkers. *Continental Shelf Research* 28, 2535-2549.

Ellis, T., Hoyle, I., Oidtmann B., Turnbull, J.F., Jacklin T.E., Knowles, T.G. 2009. Further development of the "Fin Index" method for quantifying fin erosion in rainbow trout. *Aquaculture* 289, 283-288.

FAO (Food and Agriculture Organisation) 1999. Manual on Hatchery Production of Sea bass and Gilthead Sea bream. Volume 1. FAO, Rome. Disponible en: http://www.fao.org/docrep/005/x3980e/x3980e00.htm

FAO (Food and Agriculture Organisation) 2014a. 'Aquaculture topics and activities'. Tecnología de la acuicultura. En: Departamento de Pesca y Acuicultura de la FAO. Rome. Actualizado 27 Agosto 2014. Disponible en:http://www.fao.org/fishery/topic/2801/es

FAO (Food and Agriculture Organisation) 2014b. Fish finder. Species fact sheets. FAO, Rome. Disponible en: http://www.fao.org/fishery/species/2291/en

Favaloro, E., Lopiano, L., Mazzola, A. 2002. Rearing of sharpsnout sea bream (*Diplodus puntazzo*, Cetti 1777) in a Mediterranean fish farm: monoculture versus polyculture. *Aquaculture Research* 33, 137-140.

Favaloro, E., Mazzola, A. 2003. Shape change during the growth of sharpsnout sea bream reared under different conditions in a fish farm of the southern Tyrrhenian Sea. *Aquacultural Engineering* 29, 57-63.

Ferguson, A. 2004. The importance of identifying conservation units: brown trout and pollan biodiversity in Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy* 104B, 33-41.

Fernandez-Jover, D., Lopez Jimenez, J., Sanchez-Jerez, P., Bayle-Sempere, J., Gimenez Casalduero, F., Martinez Lopez, F., Dempster, T. 2007. Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) associated to sea cage fish farms. *Marine Environmental Research* 63, 1-18.

Fernandez-Jover D., Faliex E., Sanchez-Jerez P., Sasal P., Bayle-Sempere J.T. 2010. Coastal fish farming does not affect the total parasite communities of wild fish in SW Mediterranean. *Aquaculture* 300, 10-16.

Fernández-Jover, D., Arechavala-López, P., Martínez-Rubio, L., Tocher, D., Bayle-Sempere, J., López-Jiménez, J., Martínez-López, F., Sánchez-Jerez, P. 2011. Monitoring the influence of marine aquaculture on wild fish communities: benefits and limitations of fatty acid profiles. *Aquaculture Environment Interactions* 2, 39-47.

Fleming, I.A., Jonsson, B., and Gross, M.R. 1994. Phenotypic divergence of sea-ranched, farmed, and wild salmon. *Canadian Journal of Fisheries and Aquatic Sciences* 51, 2808-2824.

Folch, J., Lees, M., Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemestry* 226, 497-509.

Froese, R., Pauly, D., 2006. Fish base. Available at http://www.fishbase.org/

Gil, M., Grau, A., Basilone, G., Ferreri, R., Palmer, M. 2013. Reproductive strategy and fecundity of meagre *Argyrosomus regius* Asso, 1801 (Pisces: Sciaenidae): implications for restocking programs. *Scientia Marina* 77, 105-118.

Gil, M., Palmer, M., Grau, A., Deuderos, S., Alconchel, J., Catalán, I. 2014. Adapting to the wild: the case of aquaculture-produced and released meagres *Argyrosomus regius*. *Journal of Fish Biology* 84,10-30.

Gillanders, B.M., Joyce, T.C. 2005. Distinguishing aquaculture and wild yellowtail kingfish via natural elemental signatures in otoliths. *Marine Freshwater Research* 56, 693-704.

Glover, K.A., Hansen, M.M., Skaala, Ø. 2009. Identifying the source of farmed escaped Atlantic salmon (*Salmo salar*): Bayesian clustering analysis increases accuracy of assignment. *Aquaculture* 290, 37-46.

González, A. 2003. Actividad citocromo P450 Aromatasa en la lubina (*Dicentrarchus labrax*). PhD Thesis. Facultad de Biología. Departamento de Fisiología. Universitat de Barcelona. Barcelona. Spain.

González-Lorenzo, G., Brito, A., Barquín, J. 2005. Impactos provocados por los escapes de peces de las jaulas de cultivos marinos en Canarias. *Vieraea* 33, 449-454.

González-Quirós, R., del Árbol, J., García-Pacheco, M.M., Silva-García, A.J., Naranjo, J.M., Morales-Nin, B. 2011. Life history of the meagre *Argyrosomus regius* in the Gulf of Cádiz (SW Iberian Peninsula). *Fisheries Research* 109, 140-149.

Grahl-Nielsen, O., Barnung, T. 1985. Variations in the fatty acid profiles of marine animals caused by environmental and developmental changes. *Marine Environmental Research* 17, 218-221.

Grau, A., Crespo, S. 1991. Epitheliocystis in the wild and cultured amber-jack, *Seriola dumerili* Risso: Ultrastructural observations. *Aquaculture* 95, 1-6.

Gravier, R. 1961. Les Bars (Loups) du Maroc Atlantique *Morone labrax* (Linné) et *Morone punctata* (Bloch). *Revue des Travaux de l'Institut des Peches Maritimes* 25, 281-292.

Grigorakis, K., Alexis, M. N., Anthony Taylor, K. D., Hole, M. 2002. Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. *International Journal of Food Science and Technology* 37, 477-484.

Grigorakis, K., Rigos, G. 2011. Aquaculture effects on environmental and public welfare; The case of Mediterranean mariculture. *Chemosphere* 855, 899–919

Gross, M.R. 1998. One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. *Canadian Journal of Fisheries and Aquatic Sciences* 55, 131-144.

Gunstone, F.D. 2010. The world's oil and fats. In: Turchini G., Ng W., Tocher D. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Cambridge, UK, pp. 61-98.

Gwak, W., Tanaka, Y., Tsusaki, T., Tanaka, M. 2003. Field evaluation by RNA/DNA ratios on post-release nutritional status of released and wild Japanese flounder (*Paralichthys olivaceus)* juveniles. *Journal of Experimental Marine Biology and Ecology* 293, 107-124.

Hall-Spencer, J., White, N., Gillespie, E., Gillham, K., Foggo, A. 2006. Impact of fish farms on maerl beds in strongly tidal areas. *Marine Ecology Progress Series* 326, 1-9.

Hansen, M., Ruzzante, D.E., Nielsen, E.E., Mensberg, K.D. 2000. Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). *Molecular Ecology* 9, 583-594.

Hanson, K.C., Hasler, C.T., Suski, C.D., Cooke, S.J. 2007. Morphological correlates of swimming activity in wild largemouth bass (*Micropterus salmoides*) in their natural environment. *Comparative Biochemistry Physiology (Part A)* 148, 913-920.

Hargrave, B. 2005. *Environmental Effects of Marine Finfish Aquaculture*. The Handbook of Environmental Chemistry, Vol. 5, Water Pollution, Part M. Springer-Verlag, Berlin, Germany. 467 pp.

Hargrave, B.T., Holmer, M., Newcombe, C.P. 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. *Marine Pollution Bulletin* 56, 810-824.

Hewitt, C.L., Campbell, M.L., Gollasch, S. 2006. Alien Species in Aquaculture. Considerations for responsible use. IUCN, Gland, Switzerland and Cambridge, UK. VIII, 32 pp.

Holden, M.J., Raitt, D.F.S. 1974. Manual of fisheries science. 2. Methods of resource investigation and their application. F A O Fisheries Technical Paper, 115, Rev. 1: 211 pp.

Holmer, M., Kristensen, E. 1992. Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. *Marine Ecology Progress Series* 80, 191-201.

Holmer, M., Perez, M., Duarte, C.M. 2003. Benthic primary producers: A neglected environmental problem in Mediterranean maricultures? *Marine Pollution Bulletin* 46, 1372-1376.

Holmer, M. 2010. Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. *Aquaculture Environmental Interactions* 1, 57-70.

Houde, E., Zastrow, C. 1993. Ecosystem and taxon-specific dynamic and energetics properties of fish larvae assemblages. *Bulletin of Marine Sciences* 53, 290-335.

Hu, J., Zhang, G., Li, K., Peng, P., Chivas, A.R. 2008. Increased eutrophication offshore Hong Kong, China during the past 75 years: evidence from high-resolution sedimentary records. *Marine Chimestry* 110, 7-17.

Hurlbut, T., Clay, D. 1998. Morphometric and meristic differences between shallow- and deep-water populations of white hake (*Urophycis tenuis*) in the southern Gulf of St Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences* 55, 2274-2282.

Huxley, J.S. 1932. Problems of relative growth. Dial New York, 276 pp.

Hyslop, E.J. 1980. Stomach contents analysis, a review of methods and their application. *Journal of Fish Biology* 17, 411-429.

ICES (International Council for the Exploration of the Sea), 2005. Report of the Working Group on Environmental Interactions of Mariculture (WGEIM), 11–15 April 2005, Ottawa, Canada. CM 2005/F:04. 112 pp.

Ihssen, P.E., Evans, D.O., Christie, W.J., Reckahn, J.A., DesJardine, R.L. 1981. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of late whitefish (*Coregonus clupeaformis*) in the Great Lakes region. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 1790-1807.

IUCN 2007. Guide for the Sustainable Development of Mediterranean Aquaculture I. Interactions between Aquaculture and the Environment. Gland, Switzerland and Málaga, Spain.

Iribarren, D., Vázquez-Rowe, I., Hospido, A., Moreira, M.T., Feijoo, G. 2010. Estimation of the carbon footprint of the Galician fishing activity (NW Spain). *Science of the Total Environment* 408, 5284-5294.

Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T., Kitajima, C. 1990. Optimum EFA levels in Artemia to meet the EFA requirements of red sea bream (*Pagrus major*). In: Takeda, M., Watanabe, T. (Eds.), The Current Status of Fish Nutrition in Aquaculture. Tokyo Univ. Fisheries, Tokyo, pp. 221-232.

Izquierdo, M.S., Montero, D., Robaina, L.E., Caballero, M.J., Rosenlund, G., Ginés, R. 2005. Alteration in fillet fatty acid profile and flesh quality in gilthead sea bream (*Sparus aurata*) fed vegetable oils for a long period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* 250, 431-44.

Izquierdo, M.S., Koven, W. 2011. Lipids. In: Holt, J. (Ed.), Larval Fish Nutrition. Wiley- Blackwell, John Wiley and Sons Publisher. Pp 47-82.

Jackson, D., Drumm, A., McEvoy, S., Jensen, O., Dempster, T., Mendiola, D., Gabiña, G., Borg, J.A., Papageorgiou, N., Karakassis, I. Black, K.D. 2015. A pan-European valuation of the extent, causes and cost of escape events from sea cage fish farming. *Aquaculture* 436, 21-26.

Jensen, O., Dempser, T., Thorstad, E.B., Uglem, I., Fredheim, A. 2010. Escapes of fish from Norwegian sea-cage aquaculture: causes, consequences and preventions. *Aquaculture Environment Interactions* 1, 71-83.

Jiménez, M.T., Pastor, E., Grau, A., Alconchel, I., Cárdenas, S. 2005. Revisión sobre el cultivo de scienidos en el mundo, con especial atención a la corvina (*Argyrosomus regius*). *Boletín del Instituto Español de Oceanografía* 21, 169- 176

Jonsson, B., Jonsson, N. 2006. Cultured Atlantic salmon in nature: a review of their ecology and interaction with wild fish. *ICES Journal of Marine Science* 63, 1162-1181.

Jørstad, K.E., Karlsen, Ø., Svåsand, T., Otterå, H. 2006. Comparison of growth rate among different protein genotypes in Atlantic Cod, *Gadus morhua,* under farmed conditions. *ICES Journal of Marine Science* 63, 235-245.

Jørstad, K.E., Van Der Meeren, T., Paulsen, O.I., Thomsen, T., Thorsen, A., Svåsand, T. 2008. 'Escapes' of eggs from farmed cod spawning in net pens: recruitment to wild stocks. *Reviews in Fisheries Science* 16, 285-295.

Katayama, S., Isshiki, T. 2007. Variation in otolith macrostructure of Japanese flounder (*Paralichthys olivaceus*): a method to discriminate between wild and released fish. *Journal of Sea Research* 57, 180-186.

Keeley, E.R., Parkinson E.A., Taylor E.B. 2005. Ecotypic differentiation of native rainbow trout (*Oncorhynchus mykiss*) populations from British Columbia. Canadian *Journal of Fisheries and Aquatic Sciences* 62, 1523-1539.

Keeley, E.R., Parkinson, E.A., Taylor, E.B. 2007. The origins of ecotypic variation of rainbow trout: A test of environmental vs. genetically based differences in morphology. *Journal of Evolutionary Biology* 20, 725-736.

Kennedy, M., Fitzmaurice, P. 1972. The biology of the bass, *Dicentrarchus labrax* (Linné 1758), in Irish waters. *Journal of the Marine Biological Association of the United Kingdom* 3, 39-68.

Kinnison, M.T., Hendry, A.P. 2004. Tempo and mode of evolution in salmonids. In Evolution Illuminated: Salmon and Their Relatives (Hendry, A. P. and Stearns, S. C.,eds), pp. 208-231. New York: Oxford University Press.

Kutti, T., Ervik, A., Hansen, P.K. 2007a. Effects of organic effluents from a salmon farm on a fjord system I. Vertical export and dispersal processes. *Aquaculture* 262, 367-381.

Kutti, T., Hansen, P.K., Ervik, A., Hoisaeter, T., Johannessen, P. 2007b. Effects of organic effluents from a salmon farm on a fjord system II. Temporal and spatial patterns in infauna community composition. *Aquaculture* 262, 355-366.

Kutti, T., Ervik, A., Hoisaeter, T. 2008. Effects of organic effluents from a salmon farm on a fjord system III. Linking deposition rates of organic matter and benthic productivity. *Aquaculture* 282, 47-53.

Labourg, P.J., Stequert, B. 1973. Régime alimentaire du bar *Dicentrarchus labrax* L. des réservoirs poissons de la région d'Arcachon. *Bulletin d Ecologie* 4, 187-194.

Laffaille, P., Lefeuvre, J.C., Schricke, M., Feunteun, E. 2001. Feeding ecology of 0-group sea bass, *Dicentrarchus labrax*, in salt marshes of Mont Saint Michel Bay (France). *Estuaries and Coasts* 24, 116-125.

Leitao, F., Santos, M., Erzini, K., Monteiro, C. 2008. The effect of predation on artificial reef juvenile demersal fish species. *Marine Biology* 153, 1233-1244.

Li, N., Zhao, Y,L., Yang, J. 2005. Accumulation, distribution and toxicology of copper sulfate in juvenile giant freshwater prawns, *Macrobrachium rosenbergii. Bulletin Environmental Contamination and Toxicology* 75, 497-504.

Ling, N. 2003. Rotenone – a review of its toxicity and use for fisheries management. New Zealand Department of Conservation. Science for conservation Report 211. ISSN 1173-2946

Lloris D, Rucabado J, Figueroa H (1991) Biogeography of the Macaronesian ichthyofauna (The Azores, Madeira, The Canary Islans, Cape Verde and the African enclave). *Boletim do Museu Municipal do Funchal* 43,191–241

Lorenzen, K., Beveridge, M., Mangel, M. 2012. Cultured fish: integrative biology and management of domestication and interactions with wild fish. Biological *Reviews of the Cambridge Philosophical Society* 87, 639-660.

Loy, A., Boglione, C., Cataudella, S. 1999. Geometric morphometrics and morphoanatomy a combined tool in the study of sea bream (*Sparus aurata*, Sparidae) shape. *Journal of Applied Ichthyology* 15, 104-110.

Loy, A., Boglione, C., Gagliardi, F., Ferrucci, L., Cataudella, S. 2000. Geometric morphometrics and internal anatomy in sea bass shape analysis (*Dicentrarchus labrax* L.), Moronidae. *Aquaculture* 186, 33-44.

Lund, R.A., Hansen, L.P. 1991. Identification of wild and reared Atlantic salmon, (*Salmo salar* L.), using scale characters. *Aquaculture and Fisheries Management*, 22, 499-508

Maazouzi, C., Masson, G., Izquierdo, M.S., Pihan, J.C. 2008. Chronic copper exposure and fatty acid composition of the amphipod *Dikerogammarus villosus*: Results from a field study. *Environmental Pollution* 156, 221-226.

Machias, A., Karakassis, I., Labropoulou, M., Somarakis, S., Papadopoulou, K. N., Papaconstantinou, C. 2004. Changes in wild fish assemblages after the establishment of a fish farming zone in an oligotrophic marine ecosystem. *Estuarine, Coastal and Shelf Science* 60, 771-779.

Mairesse, G., Thomas, M., Gardeur, J.N., Brun-Bellut, J. 2005. Appearance and technological characteristics in wild and reared Eurasian perch, *Perca fluviatilis* (L.). *Aquaculture* 246, 295-311.

Maltby, L., Brock, T.C.M., Van den Brink, P.J. 2009. Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action, and exposure regime. *Environmental Sciences and Technology* 43, 7556-7563.

Mariño, J., Pérez, G., Rodríguez, J., Cid, E., Fernández, B., Graña, M. 2009. Repoblación de rodaballo (*Scophthalmus maximus*, L.) en la costa de Galicia. Actas del XII congreso Nacional de Acuicultura, (Madrid, 24-26 de Noviembre de 2009), Cicegraf Artes Gráficas. 708–709 pp.

Martin, W.R. 1949. The mechanics of environmental control of body form in fishes. Univ. Toronto, Stud., Biol. Ser., No. 58; Publ. Ontario Fish. Research Lab., No. 70, 91 pp.

Megdal, P. A., Craft, N. A., Handelman, G. J. 2009. A simplified method to distinguish farmed (*Salmo salar*) from wild salmon: Fatty acid ratios versus astaxanthin chiral isomers. *Lipids* 44, 569-576.

Mente, E., Pierce, G. J., Santos, M. B., Neofitou, C. 2006. Effect of feed and feeding in the culture of salmonids on the marine aquatic environment: A synthesis for European aquaculture. *Aquaculture International* 14, 499-522.

Mente, E., Pierce, G. J., Spencer, N. J., Martin, J. C., Karapanagiotidis, I. T., Santos, M. B., Wang, J., Neofitou, C. 2008. Diet of demersal fish species in relation to aquaculture development in Scottish sea lochs. *Aquaculture* 277, 263-274.

Meziane, T. Tsuchiya, M. 2000. Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. *Marine Ecology Progress Series* 200, 49-57.

Meziane, T., Tsuchiya, M. 2002. Organic matter in a subtropical mangrove-estuary subjected to wastewater discharge: Origin and utilisation by two macrozoobenthic species. *Journal of Sea Res*earch 47, 1-11.

Mladineo, I., Šegvić-Bubić, T., Stanić, R., Desdevises, Y. 2013. Morphological Plasticity and Phylogeny in a Monogenean Parasite Transferring between Wild and Reared Fish Populations. *Plos One* 8, 1-10.

Mnari, A., Bouhlel, I., Chraief, I., Hammami, M., Romdhane, M. S., El Cafsi, M., Chaouch, A. 2007. Fatty acids in muscles and liver of Tunisian wild and farmed gilthead sea bream, *Sparus aurata*. *Food Chemestry* 100, 1393-1397.

Mo, T.A., Gahr, A., Hansen, H., Hoel, E., Oaland, Ø., Poppe, T.T. 2014. Presence of *Anisakis simplex* (Rudolphi, 1809 det. Krabbe, 1878) and *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda; Anisakidae) in runts of farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 37, 135-140.

Moe, H., Gaarder, R.H., Olsen, A., Hopperstad, O.S. 2009. Resistance of aquaculture net cage materials to biting by Atlantic Cod (*Gadus morhua*). *Aquacultural Engineering* 40, 126-134.

Molina-Domínguez, L., Lopez-Calero, G., Vergara-Martín, J.M., Robaina-Robaina, L.E. 2001. A comparative study of sediments under a marine cage farm at Gran Canaria Island (Spain): Preliminary results. *Aquaculture* 192, 225-231.

Molina, L., Vergara, J. 2005. Impacto ambiental de jaulas flotantes: estado actual de conocimientos y conclusiones prácticas. *Boletín del Instituto Español de Oceanografía* 21, 75-81.

Monfort, M.C. 2010. Present market situation and prospects of meagre (*Argyrosomus regius),* as an emerging species in Mediterranean aquaculture. Studies and Reviews. General Fisheries Commission for the Mediterranean. No 89. Rome, FAO. 28pp.

Montero, D., Robaina, L., Caballero, M.J., Gines, R., Izquierdo, M.S. 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets

containing vegetable oils: a time-course study on the effect of a re-feeding period with a 100% fish oil diet. *Aquaculture* 248, 121-134.

Moreno, T., Castro, J. 1995. Community structure of the juvenile of coastal pelagic fish species in the Canary Islands waters. *Scientia Marina* 59, 405-413.

Moretti, A., Pedini, Fernandez-Criado, M., Cittolin, G., Guidastri, R. 1999. Hatchery production procedures. In: Manual on hatchery production of sea bass and gilthead sea bream (Vol.1), 194 p. FAO, Rome, Italy. Available at http://www.fao.org/docrep/005/x3980e/x3980e06.htm

Munday, P., Wilson, S., 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. *Journal of Fish Biology* 51, 931-938.

Munro, E.S., McIntosh, R.E., Weir, S.J., Noguera, P.A., Sandilands, J.M., Matejusova, I., Mayes, A.S., Smith, R. 2014. A mortality event in wrasse species (Labridae) associated with the presence of viral haemorrhagic septicaemia virus. *Journal of Fish Diseases*, doi:10.1111/jfd.12237.

Murta, A.G. 2000. Morphological variation of horse mackerel (*Trachurus trachurus*) in the Iberian and North African Atlantic: implications for stock identification. *ICES Journal of Marine Science* 57, 1240-1248.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effects of aquaculture on world fish supplies. *Nature* 405, 1017-1024.

Naylor, R., Burke, M., 2005. Aquaculture and ocean resources: raising tigers of the sea. *Annu Rev Environ Resour* 30, 185-218

Naylor, R., Hindar, K., Fleming, I.A., Goldburg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D., Mangel, M., 2005. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. *Bioscience* 55, 427–437.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., 2009. Feeding aquaculture in an era of finite resources. *Proc Natl Acad Sci USA* 106, 15103-15110

Nowak, B.F., LaPatra, S.E. 2006. Review: Epitheliocystis in fish. *Journal of Fish Diseases* 29, 573-588.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernandez, J., Bozzo, C., Navarrete, E., Osorio, A., Ríos, A. 2006. Dietary fiber, amino acids, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chemestry* 99, 98-104.

Ozogul, Y., Ozogul, F. 2007. Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black seas. *Food Chemestry* 100, 1634-1638.

Patiyal, R.S., Mir, I.J., Sharma, R.C., Chandra, S., Mahanta, P.C. 2014. Pattern of Meristic and Morphometric Variations Between Wild and Captive Stocks of Endangered *Tor putitora* (Hamilton 1822) Using Multivariate Statistical Analysis Methods. *Proceedings of the National Academy of Sciences of India* 84, 123-129.

Pakkasmaa, S., Piironen J. 2000. Water velocity shapes juvenile salmonids. *Evolutionary Ecology* 14, 721-730.

Person-Le Ruyet, J., Le Bayon, N., 2009. Effects of temperature, stocking density and farming conditions on fin damage in European sea bass (*Dicentrarchus labrax*). *Aquatic Living Resources* 22, 349-362.

Qin, J.G., Dong, P. 2004. Acute toxicity of trichlorfon to juvenile yabby *Cherax destructor* (Clark) and selected zooplankton species. *Aquaculture Resea*rch 35, 1104-1107.

Quéméner, L. 2002. Le maigre, *Argyrosomus regius* (Asso) (Pisces, Sciaenidae) en Mediterranee occidentale. *Bulletin de la Socíeté Zoologique de France* 114, 81- 89.

Quemeneur, M., Marty, Y. 1992. Sewage influence in a macrotidal estuary: fatty acid and sterols distributions. *Estuarine, Coastal and Shelf Science* 34, 347-363.

Ramírez, B., Montero, D., Izquierdo, M., Haroun, R. 2013. Aquafeed imprint on bogue (*Boops boops*) populations and the value of fatty acids as indicators of aquaculture-ecosystem interaction: Are we using them properly? *Aquaculture* 414-415, 294-302.

Ramírez, B., Ortega, L., Montero, D., Tuya, F., Haroun, R. 2015. Monitoring a massive escape of European sea bass (*Dicentrarchus labrax*) at an oceanic island: potential species feralization. *Journal of Aquaculture Research and Development* 6, 339

Rasoarahona, J., Barnathan, G., Bianchini, J., Gaydou, E. M. 2005. Influence of season on the lipid content and fatty acid profiles of three tilapia species (*Oreochromis niloticus, O. macrochir* and *Tilapia rendalli*) from Madagascar. *Food Chemestry* 91, 683-694.

Ricker, W.E. 1973. Linear regressions in fishery research. *Journal of the Fisheries Research Board of Canada* 30, 409-434.

Rico, A., Satapornvanit, K., Haque, M., Min, J., Nguyen, P.T., Telfer, T.C., Van den Brink, P.J. 2012. Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review. *Reviews in Aquaculture* 4, 75-93.

Rinaldi, L., Basso, P., Tettamanti, G., Grimaldi, A., Terova, G., Saroglia, M., De Eguileor, M. 2005. Oxygen availability causes morphological changes and a different VEGF/Flk-1/HIF-2 expression pattern in sea bass gills. *Italian Journal of Zoology* 72, 103-111.

Robinson, B.W., Parsons, K.J. 2002. Changing time, space, and faces: test and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 59, 1819-1833.

Rogdakis, Y.G., Koukou, K.K., Ramfos, A., Dimitriou, E., Katselis, G.N. 2011. Comparative morphology of wild, farmed and hatchery-released gilthead sea bream (*Sparus aurata*) in Western Greece. *International Journal of Fisheries and Aquaculture* 3, 1-9.

Roncarati, A., Meluzzi, A., Melotti, P., Mordenti, O. 2001. Infuence of the larval rearing technique on morphological and productive traits of European sea bass (*Dicentrarchus labrax* L.). *Journal of Applied Ichthyology* 17, 244-246.

Rueda, F.M., Hernández, M.D., Egea, M.A., Aguado, F., García, B., Martínez, F.J. 2001. Differences in tissue fatty acid composition between reared and wild sharpsnout sea bream, *Diplodus puntazzo* (Cetti, 1777). *British Journal of Nutrition* 86, 617-622.

Sanchez-Jerez, P., Fernandez-Jover, D., Bayle-Sempere, J., Valle, C., Dempster, T., Tuya, F., Juanes, F. 2008. Interactions between blue fish (*Pomatomus saltatrix*.) and coastal sea-cage farms in the Mediterranean Sea. *Aquaculture* 282, 61-67.

Sánchez-Jerez, P., Arechavala-López, P., Uglem, I., Fernández-Jover, D., Black, K., Somarakis, S., Ladoukakis, M., Haroun, R., Borg, J., Dempster T. 2013. Recommendations for identification of escapees and conclusions from risk assessment analysis. In PREVENT ESCAPE Project Compendium. Chapter 2. Commision of the European Communities, 7th Framework Program. www.preventescape.eu

Sara, M., Favaloro, E., Mazzola, A. 1999. Comparative morphometrics of sharpsnout sea bream (*Diplodus puntazzo* Cetti, 1777), reared in different conditions. *Aquacultural Engineering* 19, 195-209.

Sargent, J.R., Tocher, D.R., Bell, J.G. 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition* 3rd ed. Academic Press, San Diego, CA, USA, pp. 181-257.

Saka, S., Firat, K., Kamaci, H. 2001. The development of European sea bass (*Dicentrarchus labrax* L., 1758) eggs in relation to temperature. *Turkish Journal of Veterinary and Animal Sciences* 25, 139-147.

Seguel, C.G., Mudge, S.M., Salgado, C., Toledo, M. 2001. Tracing sewage in the marine environment: altered signatures in Concepcion Bay, Chile. *Water Research* 35, 4166-4174.

Sen, S., Jahageerdar, S., Jaiswar, A.K., Chakraborty, S.K., Sajina, A.M., Dash, G.R. 2011. Stock structure analysis of *Decapterus russelli* (Ruppell, 1830) from East and West coast of India using truss network analysis. *Fisheries Research* 112, 38-43.

Serrano, L. 1989. Age, Growth and sexuality of sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) (Perciformes, Moronidae) from Aveiro lagoon, Portugal. *Scientia Marina* 53, 121-126.

Shabana, N., El Rahman, S., Al Absawy, M., Assem, S. 2012. Reproductive biology of *Argyrosomus regius* (Asso, 1801) inhabiting the south eastern Mediterranean Sea, Egypt. *Egyptian Journal of Aquatic Research* 38, 147-156

Skilbrei, O.T. 2010. Adult recaptures of farmed Atlantic salmon post-smolts allowed to escape during summer. *Aquaculture Environment Interactions* 2, 147-153.

Skog, T., Hylland, K., Torstensen, B. E., Berntssen, M. 2003. Salmon farming affects the fatty acid composition and taste of wild Saithe *Pollachius virens* L. *Aquacultue Research* 34, 999-1007.

Skùlason, S., Noakes, D.L.G., Snorrason, S.S. 1989. Ontogeny of trophic morphology in four sympatric morphs of Arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. *Biological Journal of the Linnean Society* 38, 281-301.

Solem, Ø., Berg, O.K., Kjøsnes, A.J. 2006. Inter and intra population morphological differences between wild and farmed Atlantic salmon juveniles. *Journal of Fish Biology* 69, 1466-1481.

Soto, D., Jara, F., Moreno, C. 2001. Escaped salmon in the inner seas, Southern Chile: facing ecological and social conflicts. *Ecological Applications* 11, 1750-1762.

Stipa, P., Angelini, M. 2005. *Argyrosomus regius*. Cultured Aquatic Species Information Programme. In: FAO (Food and Agriculture Organisation) Fisheries and Aquaculture Department. Disponible en: http://www.fao.org/fishery/culturedspecies/Argyrosomus_regius/

Strauss, R.E., Bookstein, F.L. 1982. The truss: body form reconstruction in morphometrics. *Systematic Zoology* 31, 113-135.

Tacon, A.G.J., Metian, M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture* 285, 146-158.

Tavares, D., González, N. 2009. Caracterización de eventos de escape de *Sparus aurata* y *Dicentrarchus labrax* en Gran Canaria mediante entrevistas a pescadores deportivos. En: Actas del XII congreso Nacional de Acuicultura (ed. by D. Beaz, M. Villarroel y S. Cárdenas), Cicegraf Artes Gráficas, Madrid. pp. 730-731.

Taylor, M., Piola, R.F. 2008. Scale stocking checks to differentiate between hatchery-reared and wild mulloway (*Argyrosomus japonicus). Fisheries Management and Ecology* 15, 211-216.

Taylor, M., Mazumder, D. 2010. Stable isotopes reveal post-release trophodynamic and ontogenetic changes in a released finfish, mulloway (*Argyrosomus japonicus*). *Marine and Freshwater Research* 61, 302-308.

Tlusty, M., Andrew, J., Baldwin, K., Bradley, T. 2008. Acoustic conditioning for recall/recapture of escaped Atlantic salmon and rainbow trout. *Aquaculture* 274, 57-64.

Toledo, K., Sánchez-Jerez, P., González-Lorenzo, G., Brito, A., 2009. Detecting the degree of establishment of a non-indigenous species in coastal ecosystems: sea bass *Dicentrarchus labrax* escapes from sea cages in Canary Islands (Northeastern Central Atlantic). *Hydrobiologia* 623, 203-212.

Toledo-Guedes, K., Sánchez-Jerez, P., Benjumea, M., Brito A. 2014a. Farming-up coastal fish assemblages through a massive aquaculture escape event. *Marine Environmental Research* 98, 86-95.

Toledo-Guedes, K., Sánchez-Jerez, P., Brito, A. 2014b. Influence of a masive aquaculture escape event on artisanal fisheries. *Fisheries Management and Ecology* 21, 113-121.

Torstensen, B.E., Froyland, L., Ornsrud, R., Lie, O., 2004. Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. *Food Chemistry* 87, 567-580.

Torstensen, B.E., Tocher, D.R. 2010. The effect of fish oil replacement on lipid metabolism of fish. In: Turchini G, Ng W, Tocher D. (Eds.). Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Cambridge, UK, pp. 133-160.

Tortonese, E. 1986. Moronidae. In: Fishes of the North-Eastern Atlantic and the Mediterranean (ed. by P.J.P. Whitehead, M.L. Bauchot, J.C. Hureau, J.Nielsen and E. Tortonese), pp. 793–796. UNESCO, Paris.

Turchini, G.M., Torstensen, B.E., Ng, W.K. 2009. Fish oil replacement in finfish nutrition. *Reviews in Aquaculture* 1, 10–57.

Turchini, G.M., Mailer, R.J. 2010. Rapeseed (Canola) oil and other monounsaturated fatty acid-rich vegetable oils. In: Turchini G, Ng W, Tocher D. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Cambridge, UK, pp. 161-208.

Tuya, F., Boyra, A., Sánchez-Jerez, P., Haroun, R.J., Barberá, C. 2004. Relationships between rocky-reef fish assemblages, the sea urchin *Diadema antillarum* and macroalgae throughout the Canarian Archipelago. *Marine Ecology Progress Series* 278, 157-169.

Tuya, F., Sanchez-Jerez, P., Dempster, T., Boyra, A., Haroun, R. J. 2006. Changes in demersal wild fish aggregations beneath a sea-cage fish farm after the cessation of farming. *Journal of Fish Biology* 69, 682-697.

Uglem, I., Bjørn, P., Dale, T., Kerwath, S., Økland, F., Nilsen, R., Aas, K., Fleming, I., McKinley, R. 2008. Movements and spatiotemporal distribution of escaped farmed and local wild Atlantic cod (*Gadus morhua* L.) *Aquaculture Research* 39, 158-170.

Uglem, I., Bjørn, P., Mitamura, H., Nilsen, R. 2010. Spatiotemporal distribution of coastal and oceanic Atlantic cod (*Gadus morhua*) sub-groups after escape from a farm. *Aquaculture Environment Interactions* 1, 11-19.

Uglem, I., Berg, M., Varne, R., Nilsen, R., Mork J., Bjørn, P.A. 2011. Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern. *ICES Journal of Marine Sciences* 68, 1928-36.

Underwood, A.J. 1997. *Experiments in ecology. Their logical design and interpretation*. Cambridge University Press, UK.

Vergara, J.M., Haroun, R., González, M.N., Molina, L., Briz, M.O., Boyra, A., Gutiérrez, L., Marañón, M., Ballesta, A. 2005. Evaluación de Impacto Ambiental de Acuicultura en Jaulas en Canarias. (ed. by J.M. Vergara, R. Haroun and N. González), Editorial Oceanográfica, Telde. 110 pp.

Vesin, J., Leggett, W., Able, K. 1981. Feeding ecology of capelin (*Mallotus villosus*) in the estuary and Western Gulf of St. Lawrence and its multispecies implications. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 257-267.

Vita, R., Marin, A., Madrid, J. A., Jimenez-Brinquis, B., Cesar, A., Marin-Guirao, L. 2004. Effects of wild fishes on waste exportation from a Mediterranean fish farm. *Marine Ecology Progress Series* 277, 253-261.

Wallace, R.A., Selman, K., 1981. Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoology* 21, 325–343.

Weber, P.K., Hutcheon, I.D., McKeegan, K.D., Ingram, B. 2002. Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life history. *Canadian Journal Of Fisheries and Aquatic Sciences* 59, 587-591.

Weber, E.D., Fausch, K.D. 2003. Interactions between hatchery and wild salmonids in streams: Differences in biology and evidence for competition. *Canadian Journal of Fisheries and Aquatic Sciences* 60, 1018-1018.

Windell, J. 1971. Food analysis and rate of digestion in: Methods for assessment of fish production in fresh waters. (ed. by W.E. Ricker), pp. 215-226. Blackwell Scientific Publications ltd, Oxford and Edinburgh.

Wintzer, A.P., Motta, P.J. 2005. Diet-induced phenotypic plasticity in the skull morphology of hatchery-reared Florida largemouth bass, *Micropterus salmoides floridanus*. *Ecology of freshwater Fish* 14, 311-318.

Wong, W.H., Gao, Q.F., Cheung, S.G., Shin, P. 2008. Field observations on correlation of fatty acid profiles between suspended particulate matter and green-lipped mussels in subtropical waters of Hong Kong. *Marine Pollution Bulletin* 57, 662-671.

Yip, K.M. 2006. Fatty acid profile in green-lipped mussel (*Perna viridis*) as environmental markers in marine ecosystems. City University of Hong Kong. Ph.D. Thesis. 255pp

Zheng, Q., Zhang, R., Wang, Y., Pan. X., Tang, J., Zhang, G. 2012. Occurrence and distribution of antibiotics in the Beibu Gulf, China: Impacts of river discharge and aquaculture activities of river discharge and aquaculture activities. *Marine Environmental Research* 78, 26-33.